THE RELATIONSHIP BETWEEN TUBIFICID WORMS ABUNDANCE AND PHOSPHATES AND NITRATES CONCENTRATION ALONG THE SHORELINE OF GOREANGAB DAM.

By

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A research proposal submitted to the Department of Fisheries and Aquatic Sciences, Faculty of Agriculture and Natural Resources, in partial fulfilment of the requirements for the award of the degree of Bachelor of Science in Fisheries and Aquatic Sciences of the University of Namibia.

SUPERVISOR: Mr. L. Kandjengo

Declaration

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

Tobias Muyeu Nghwada (200521632)

Certification

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

External examiner:

Internal examiner:

Supervisor:

Head of the Department:

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Table of Contents
Page

List of tables **Page**

List of figures **Page**

Abstract

Tubificid worms are known to be one of the dominant zoobenthos in eutrophic lakes and influence lake ecosystems through increasing nutrient release to water from the bottom sediments. The effects on the nutrient dynamics are recognized to be responsible for the bioturbation of tubificid worms.

Water samples and sediment was collected from the shoreline of Goreangab dam and analyzed in laboratory. The study was conducted mainly to determine how much the shoreline of Goreangab dam is polluted through the use of tubificid worms as a water quality indicator and determine the abundance of tubificid worms of the sites sampled and the relationship it has with phosphates and nitrates concentration at the two sites sampled. The study showed that the abundance of tubificid worms at site 1 is 1600 individual/m^2 and site 2 is 4600 individual/m^2. The relationship between tubificid worms and nitrates concentration was found to be a negative relationship and the relationship between tubificid worms abundance and phosphate concentrations was positive. Base from the result of tubificid worms abundance the study was concluded that Goreangab dam shoreline is moderately polluted.

Keywords:

Bioturbation, Nitrification, Denitrification, tubificid worms

Chapter 1

1.1. Introduction

Studies on the effect of bioturbation by marine infauna on nitrification and denitrification processes have shown that the particular feeding and burrowing strategies of the various animals can lead to variable stimulation of aerobic respiration, nitrification and denitrification (Kristensen et al. 1991, Pelegri et al. 1994, Pelegri & Blackburn 1995).

Tubificid worms (aquatic oligochaetes) are known to be some of the dominant zoobenthos in eutrophic lakes. In polluted freshwaters, tubificid worms are among the dominant components of the benthic community (Risnoveanu et al., 2004). Population densities can be as high as millions of individuals per m^2 (Palmer 1968).

The bioturbation of tubificid worms is recognized to have an effect on nutrient dynamics. Tubificid worms live typically and feed head down in the sediment. Some portion of the posterior of the worms may project above the sediment and water interface. The worms selectively ingest silt and clay particles at depth and digest the attached microflora, primarily bacteria (Davis, 1974). Fecal pellets are deposited at the sediment-water interface, where they may form a pelletized layer. The nutrient like phosphate is released both into the water column and sediments and from sediments, phosphate may reach the water column by molecular diffusion or through channels created from its feeding process while nitrate is taken up (Rıˆsnoveanu et al., 2001). This type of feeding is called conveyor belt feeding (Rhoads, 1974). The release of phosphorus in sediments and take up of nitrate influences ecosystems of lakes and paddy fields (Simpson et al., 1993).

1.2. Literature review

In their study on environmental factors affecting the distribution and abundance of *Myxobolus spp.,* Koprivnikar *et al.* (2002) found that a high percentage of tubificids occurred in the detritus and muddy substrate.

The density of tubificid worms influence the phosphates concentration in the way that when tubificid worms density increases the phosphates release rate increases also. This was observed by Fukuhara and Sakamoto (1987). The study of nitrification done by tubificid worms was studied by Pelegri and Blackburn (1995) and they found that nitrification was stimulated at low worm density but inhibited at higher worm density. All the result of tubificid worms excreting nitrates and phosphates was also shown in Mermilod-blondin *et al,* (2004) who found that tubificid worms increased the release of NH_4^+, PO_4^{3-}, and dissolved organic carbon. They concluded that Tubificid worms significantly increased the organic matter mineralization and the release of nutrients from storm water sediments.

1.3. Statement of the problem

Goreangab dam has been used by people as a recreational area, fishing, by some as washing place for laundry. The Gammams Reclamation Plant also uses Goreangab dam as a reservoir. All this activities has an impact on the aquatic ecosystem.

1.4. Main objective

To determine how much Goreangab dam shoreline is polluted.

1.5. Objectives

- To determine the abundance of tubificid worms of the sites sampled.
- To investigate the link between the abundance of tubificid worms and the phosphates and nitrates concentration.

1.6. Research hypotheses

1. H_0: There is no significant difference in abundance of tubificid worms between the two sites sampled.

   H_0: There are significant differences in abundance of tubificid worms between the two sites sampled.

2. H_0**:** There is no significant relationship between the abundance of tubificid worms and phosphates concentration at two sites sampled.
   H_1: There is a significant relationship between the abundance of tubificid worms and phosphates concentration at two sites sampled.

3. H_0: There is no significant relationship between the abundance of tubificid worms and nitrates concentration at two sites sampled.
   H_1: There is a significant relationship between the abundance of tubificid worms and nitrates concentration at two sites sampled.

Chapter 2

2.1. Materials and methods

Field sampling was carried out at the Goreangab dam (Fig. 1) shoreline and two sites were sampled. Site one is close to the inlet and site two away from inlet. The sites were chosen based on the fact that inflow of water which comes from gammas reclamation plant will make the abundance of tubificid worms, concentration of water to be different at the stations sampled. Other reason why the sites chosen because site two is close to the recreational area and site one away from recreational area will make the abundance of tubificid worms, nitrate and phosphates concentration to be different between the sites sampled. The two sites are about 0.7 km away from each other. The Van Veen grab that is normally used for this type of studies was not used because when tried to use it, it collected very little sediment. Therefore aquatic grasses were pulled out from the water, the roots bound or attached to the sediments was collected in plastic bottles for each site and preserved in a 10% formalin solution. Once in the laboratory the samples were washed through a set of 500µm sieves and the tubificid worms were separated from other invertebrates using a dissecting microscope and for identification an identification key by Quigley (1977) was used.

Approximately 0.005m^2 of sediment was collected for each sample. Three plastic bottles were used for collecting water samples at each site for phosphates and nitrates analysis in the laboratory. Water was collected without sediments because nutrients are released both into the water column and sediments (Fukuhara & Sakamoto, 1987). Three bottles were used for invertebrates sampling and three bottles were used for water sampling for each site. Therefore

twelve sampling bottles were used. Sampling was done twice every week from 23 September until 14 October 2010 at the end of sampling making 60 samples (30 for sediment samples and another 30 samples for water).

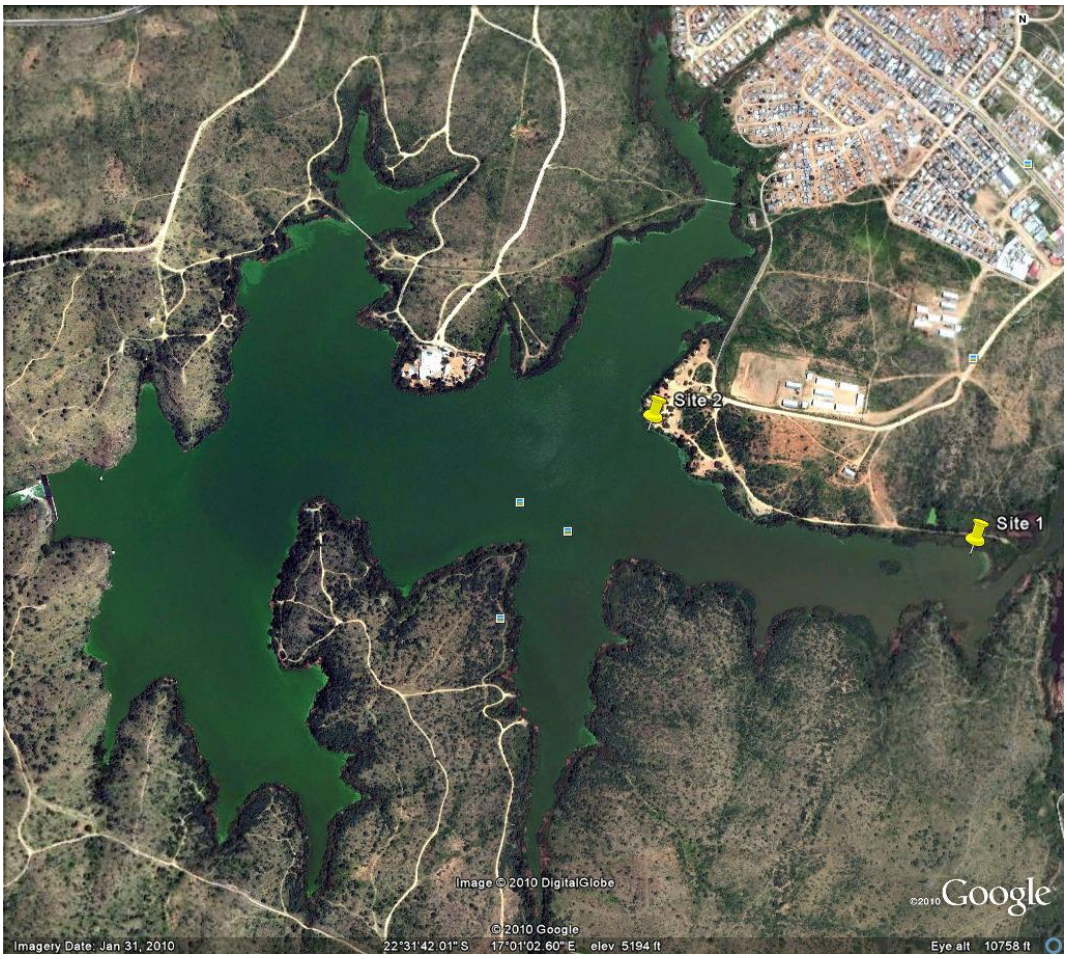

Figure 1: Goreangab dam sampling sites (Site 1 and Site 2)

Nutrients determination

Nutrients analysis was done at the University of Namibia's Fisheries laboratory.

Methods for nitrate determination

Samples of distilled water blank and standard were prepared in a test tube rack. In the same rack two rows of test tubes was prepared, one for nitrite analysis and one for nitrate analysis. Row one for nitrate analysis 5ml of water samples was placed in the test tubes for nitrate analysis. Row two for nitrite analysis 25ml of water sample was placed in test tubes and0.5ml of concentrated NH_4CL solution was added to each water samples in test tubes. The water

sample was passed through the Cadmium column activator (nitrate bomb) with a concentration of 100µg-at.litre, which is a reduction column. To each test tube (blank, standard, nitrate sample and nitrite samples) sulfanilamide of 0.1ml was added, mixed and allowed to react for 2 -8 minutes. To each sample again, 0.1ml of NEDI was added and mixed. After ten minutes, the absorbencies were taken using the spectrophotometer at a wavelength of 885 nm, which is first calibrated using the water blank.

To get the nitrate concentration the formula below was used:

Nitrate concentration = (corrected sample absorbance/ corrected standard absorbance)*10µg-at/liter.
Subtract row 1 readings from row 2 readings to get the nitrate concentration for each sample.

Methods for phosphate determination

In a test tube rack 5ml of distilled water blank and standard was prepared. In six test tube 5ml of sample water was fill in. 5ml of water sample was filled in each test tube. Fill a test tube for each sample with 5ml of sample water. 0.5ml of mixed reagent was pipetted into the blank, standard and samples then mixed and left for a minimum of 5minutes and a maximum of 2-3 hours. The spectrophotometer was set at the wave length of 543nm to read the absorbance. The blank absorbance was subtracted from standard and samples absorbance.

To get the concentration of phosphates in the sample the formula below was used:

Concentration of phosphates in sample= (corrected sample absorbance/ corrected standard absorbance)*3µg –at/liter.

2.2. Data analysis

A Kruskal-Wallis one way ANOVA was used at 95% significance level to test for significant differences in abundance of tubificid worms between the two sites. Regression analysis of variance was used at 95% significant level to test (determine) the relationship between the abundance of tubificid worms and phosphates and nitrates concentration at the two sites. The experiment was replicated three times.

The analysis was carried out using GenStat software release 7.22 TE

Chapter 3

3.1. Results

As indicated in Table 1 below, there is a significant difference in the abundance of tubificid worms between the two sites (p=0.001). The results show that central has high mean abundance of tubificid worms compared to inlet (Table 1). Central was 23 and inlet 8 in terms of tubificid worms abundance.

Table 1: Kruskal-Wallis one way analysis of variance results

Sample	Site 1	Site 2
Sample size	15	15
Mean Rank	8.00	23.00
Degrees of freedom	1	
Chi-square p-value	0.001	

From 30 samples mean abundance of tubificid worms were 11. The highest abundance of tubificid worms in 30 samples was 22.00 and the lowest was 2.000 as shown in Table 2 below.

Table 2: Summary statistics for tubificid worms abundance of both stations

Identifier	n-Sample size	Minimum	Mean	Maximum
Abundance	30	2.000	11.00	22.00

When the data was analyzed in genstats the result showed that there was a significant relationship between tubificid worms abundance and nitrates concentration at two sites sampled ($P<0.05$) shown in table3.

Table 3: F- test from regression analysis of variance for tubificid worms abundance and nitrates concentration.

Source of variation	d.f	s.s	m. s.	v. r.	F pr
Regression	1	0.02854	0.028537	16.11	<.001
Residual	28	0.04960	0.001771		
Total	29	0.07813	0.002694		

In fifteen samples at the site 1 the mean nitrates concentration was 1.185mg/l. The highest nitrates concentration in the samples was 1.226mg/l and lowest concentration was 1.170 mg/l.

Table4: Summary statistics for nitrate concentration for site 1

Identifier	n-Sample size	Minimum	Mean	Maximum
Nitrates	15	1.170	1.185	1.226

In fifteen samples at site 2 the mean concentration was 1.146mg/l. The highest nitrates concentration in the samples was 1.305mg/l and lowest concentration was 1.068 mg/l.

Table5: Summary statistics for nitrate concentration for site 2

Identifier	n-Sample size	Minimum	Mean	Maximum
Nitrates	15	1.068	1.146	1.305

The relationship between tubificid worms abundance and nitrates concentration was demonstrated graphically and it showed that when tubificid worms increases the nitrate concentration decreased for all the site 1 and site 2 (Fig. 2 and 3).

The relationship between tubificid worm abundance and nitrate concentrations have is non linear.

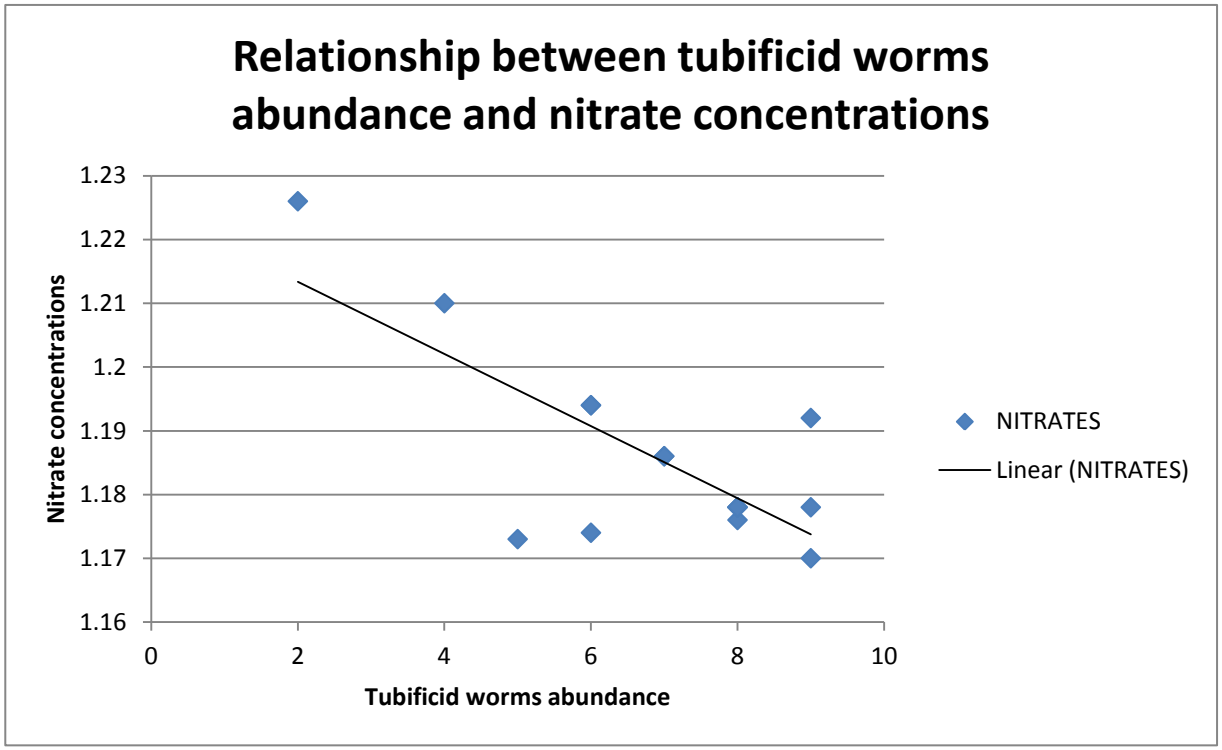

Figure 2: Relationship between tubificid worms abundance and nitrates concentration at site 1

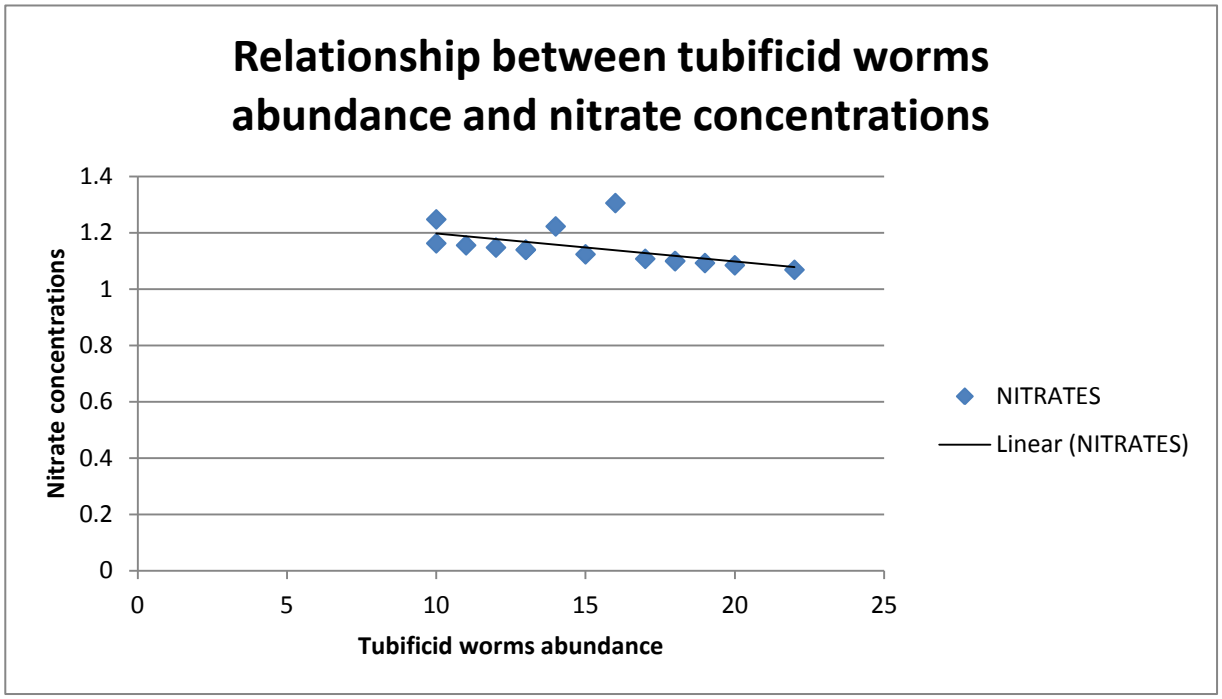

Figure 3: Relationship between tubificid worms abundance and nitrates concentration at site 2

As shown in Table 5 below, the there is a significant relationship between the abundance of the tubificid worms and the phosphates concentration. The p value was 0.001 less than the level of significance.

Table 6: F- test from regression analysis of variance for tubificid worms abundance and phosphates concentration.

Source of variation	d. f	s. s	m. s	v. r	F pr
Regression	1	0.029537	0.0295366	129.35	<.001
Residual	28	0.006394	0.0002283		
Total	29	0.035930	0.0012390		

The relationship was demonstrated graphically (fig. 4 and 5) which shows that when abundance of tubificid worms increases phosphates concentration increases also.

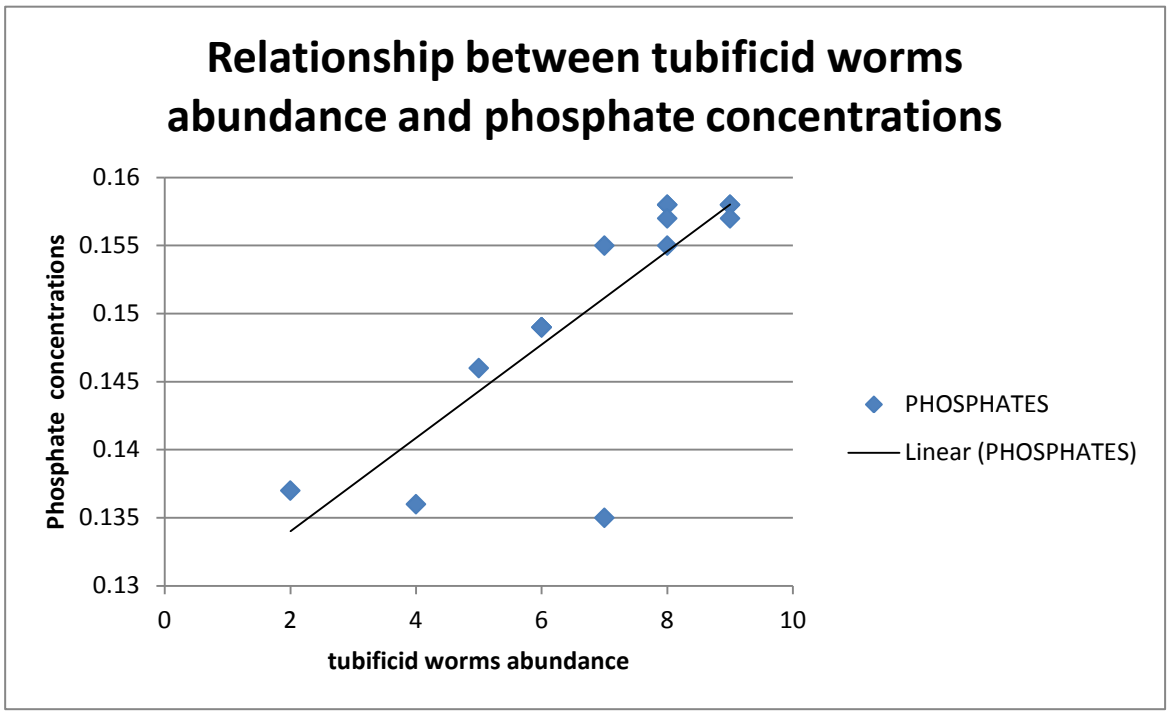

Figure 4: Relationship between tubificid worms and phosphates concentration at site 1

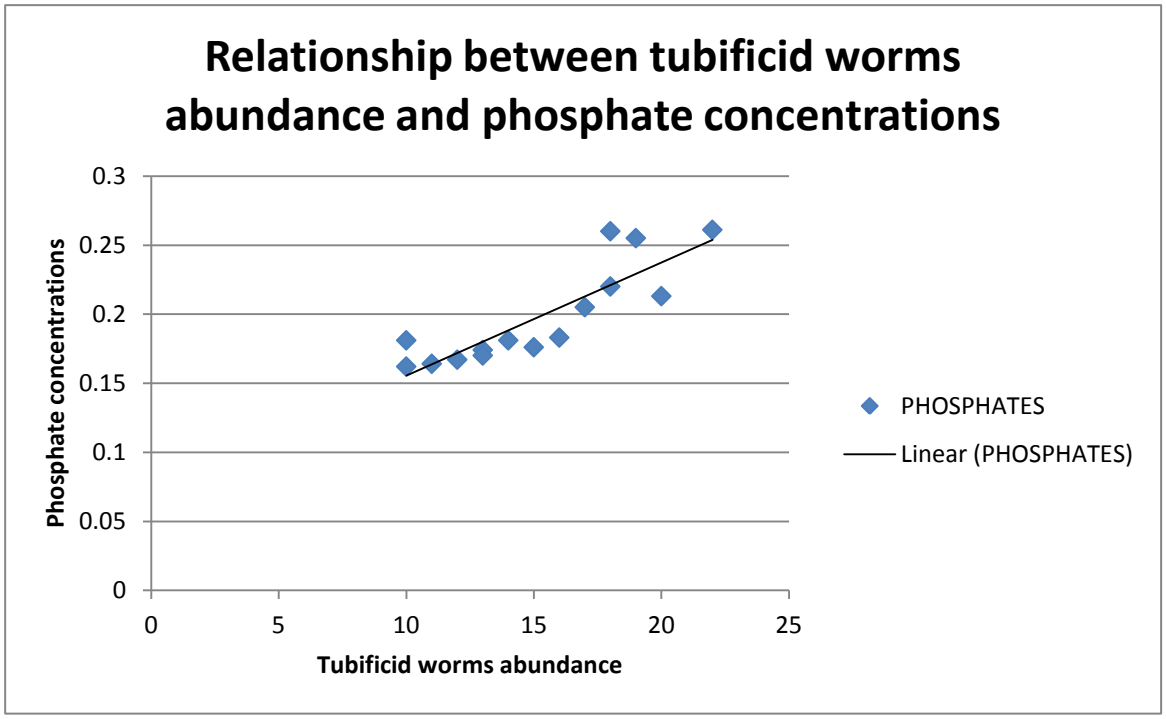

Figure 5: Relationship between tubificid worms and phosphates concentration at site 2

In fifteen samples at site 1 the mean phosphates concentration was 0.1505mg/l. The highest nitrates concentration in the samples was 0.1580mg/l and lowest concentration was 0.1350mg/l.

Table 7: Summary statistics for phosphates concentration at site 1

Identifier	n- sample size	Minimum	Mean	Maximum
Phosphates	15	0.1350	0.1505	0.1580

In fifteen samples at central the mean phosphates concentration was 0.06917mg/l. The highest nitrates concentration in the samples was 0.09720mg/l and lowest concentration was 0.02800 mg/l.

Table 8: Summary statistics for phosphates concentration at site 2

Identifier	n- sample size	Minimum	Mean	Maximum
Phosphates	15	0.02800	0.06917	0.09720

Chapter 4

4.1. Discussion

Tubificid worms abundance was found to be low for both site 1 (having 1600 individual/m^2) and site 2 (having 4600 individual/m^2) compared to the density of worms Palmer (1968) found which was high as a millions of individuals per m^2. This could be because of the way worms were sampled as the sampling was done by pulling out the aquatic plants that's found on the shallow site instead of Van Veen grab that is normally used for this type of studies. When the data was analyzed to determine if there is a significant difference in tubificid worms abundance between sites sampled, the result shows that there is a significant difference. Site 2 was having high abundance of worms (4600 individual/ m^2) compared to

site 1 having 1600 individual/ m^2. The abundance was high at site 2 because it is the area where a lot of activities take place. People drink alcohol and do barbeques when they are drunk some urinate in the water and some may even defecate close to the water which makes site 2 to be more polluted. While site 1 has no anthropogenic inputs and the water at site 1 has less polluted compared to site 2. This has lead to the reduction of tubificid worms abundance at site 1. For the entire site 1 and 2 are polluted they are only different in the population density. Another reason the abundance of worms was low at site 1 is when the clean water comes in the dam from the Gammams Reclamation plant at site 1 the water is diluted and mixed well making it less polluted and less suitable environment for tubificid worms. On the other hand at site 2 the mixing is not well because is 0.7 km away from site 1. According to Wright (1955) tubificid worm densities of 100-999 per square meter indicate light pollution, moderately polluted areas supports 1000-5000 worms/m^2 and density of worms exceeding 5000 per m^2 represents heavily polluted waters. With the result found of 1600 of worms/ m^2 at site 1 and 4600 of worms/m^2 at site 2 the shoreline of Goreangab dam is moderately polluted.

Figure 4 and 5 shows that there is a positive correlation between tubificid worms abundance and phosphates concentration. This is because of the feeding behavior tubificid worms have of excreting nutrients into the sediment and overlying water. The mean concentration of phosphate at site 2 (have 0.1981mg/l) is greater than the mean concentration of phosphate at site 1 (have 0.1505mg/l). Because abundance of tubificid worms at site 2 is higher than site 1. Since tubificid worms are less at site 1 and responsible for excretion of phosphates during its feeding, the phosphate concentration will be less also. The relationship between the tubificid worms abundance and nitrates concentration was a inverse relationship. When tubificid worms abundance increases the nitrate concentration decreases. Because most of the feeding and burrowing strategies of the various animals can lead to variable stimulation of aerobic respiration, nitrification and denitrification (Kristensen et al. 1991, Pelegri et al. 1994, Pelegri & Blackburn 1995).

4.2. CONCLUSION

Since the dam is a recreational area the anthropogenic activities has polluted the shoreline of Goreangab dam due to irresponsible of people like littering, urinating in the water, defecating close to the water and the washing clothes in the dam. The result that was found of tubificid worms population density of the site sampled the shoreline of the dam can be classified as a moderately polluted. People who do fishing in the dam cannot be recommended to continue fishing because the dam is polluted. Therefore the fish is not fit for human consumption. Gammas reclamation plant has a positive impact on the dam because is pumping clean water in Goreangab dam making the dam to be not heavily polluted.

4.3. Recommendation

In the future when someone wants to do a study on the benthic invertebrate I recommend that he or she has to use Van Veen grab to collect enough sediment samples.

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6.0. APPENDICES

Appendix .6.1.Reagents methods for nitrate determination:

Concentrated Ammonium chloride solution

- 25g of NH_4Cl was weighed and dissolved in 100ml of distilled water to prepare Ammonium chloride solution.

- Dilute ammonium chloride
  - 10ml of concentrated NH_4Cl was diluted in 400ml of distilled water and was stored in a plastic or glass bottle.
- Column of copperized cadmium
- Sulfanilamide solution
  - 1g of sulfanimide was dissolved in a mixture of 10ml of concentrated HCl and 60ml of distilled water then diluted 100ml with distilled water
- N-ethylenediamine dihydrochloride solution (NEDI)
  - 0.1g of NEDI was dissolved in 100ml of distilled water and kept at room temperature
- Nitrite standard (10^{μ}g-at./litre).
  - Anhydrous, analytical grade sodium nitrite was dried at 110^0C for one hour and 0.345g was dissolved in 1000ml of distilled water.
- Cadmium column activator (nitrate bomb)
  - 0.5055g of dry KNO_3 was dissolved in 500ml distilled water. Thiosphate solution had a concentration of $10000^{\mu g - at/litre}$. 1ml of the solution was diluted in 100ml of distilled water that gave a concentration of 100 $\mu g - at/litre$

Spectrophotometer was used in the measurement of color intensity of the blue solution. A wavelength of 885 nm was used.

Appendix .6.2. Reagents methods for phosphates determination

- Ammonium molybdate solution. 7.5g of analytical grade ammonium paramolybdate (NH4)6MO7O24.4H2O in 250ml of distilled water. Store in a plastic bottle away from direct sunlight. The solution was stable.

- Sulfuric acid solution. Add 70ml of concentrated (sp. Gr. 1.82) analytical reagent quality sulfuric acid to 450ml of distilled water. The solution was allowed to cool and store it in glass bottle.

- Ascorbic acid solution. 13.5g of ascorbic acid was dissolved in 250ml distilled water and stored the solution in a plastic bottle frozen solid in the freezer. Solution was stable.

- Potassium antimonyl-tartrate solution. 0.34g of potassium antimonyl-tartrate (tartar emetic) was dissolved in 250ml of distilled water.

- Mixed reagent. Mix together:

  10ml of Ammonium molybdate

  25ml of Sulfuric acid

  10ml of Ascorbic acid

  5ml of Potassium antimonyl-tartrate

- Phosphate standard: 0.816g of anhydrous potassium dihydrogen phosphate (KH2PO4) was dissolved in 1 litre of distilled water. Store in dark bottle with 1ml of chloroform. Take 0.1ml of concentrated standard and make up to 200ml. This is the phosphate standard and has a concentration of 3.00µg-at/litre.

Appendix 6.3. Data collection form for site 1

BOTTLE	SITES	ABUNDANCE	PHOSPHATES (mg/l)	NITRATES (mg/l)
1	1	6	0.149	1.174
2	1	8	0.155	1.176
3	1	5	0.146	1.173
4	1	9	0.158	1.192
5	1	6	0.149	1.194
6	1	6	0.149	1.194
7	1	7	0.155	1.186
8	1	8	0.158	1.178
9	1	9	0.158	1.178
10	1	2	0.137	1.226
11	1	4	0.136	1.21
12	1	7	0.135	1.186
13	1	9	0.157	1.17
14	1	8	0.158	1.178
15	1	8	0.157	1.178

Appendix 6.4. Data collection form for site 2

BOTTLE	SITES	ABUNDANCE	PHOSPHATES	NITRATES
1	2	10	0.162	1.162
2	2	11	0.164	1.155
3	2	13	0.174	1.139
4	2	12	0.167	1.147
5	2	15	0.176	1.123
6	2	13	0.17	1.139
7	2	17	0.205	1.107
8	2	20	0.213	1.084
9	2	18	0.22	1.099
10	2	19	0.255	1.092
11	2	22	0.261	1.068
12	2	18	0.26	1.099
13	2	14	0.181	1.222
14	2	10	0.181	1.247
15	2	16	0.183	1.305

Appendix 6.5. Data collection form for both sites.

BOTTLE	SITES	ABUNDANCE	PHOSPHATES (mg/l)	NITRATES (mg/l)
1	1	6	0.149	1.174
2	1	8	0.155	1.176
3	1	5	0.146	1.173
4	2	10	0.162	1.162
5	2	11	0.164	1.155
6	2	13	0.174	1.139
7	1	9	0.158	1.127
8	1	6	0.149	1.194
9	1	6	0.149	1.194
10	2	12	0.167	1.147
11	2	15	0.176	1.123
12	2	13	0.17	1.139
13	1	7	0.155	1.186
14	1	8	0.158	1.178
15	1	9	0.158	1.178
16	2	17	0.205	1.107
17	2	20	0.213	1.084
18	2	18	0.22	1.092
19	1	2	0.137	1.226
20	1	4	0.136	1.21
21	1	7	0.135	1.186
22	2	19	0.255	1.092
23	2	22	0.261	1.068
24	2	18	0.26	1.099
25	1	9	0.157	1.17
26	1	8	0.158	1.178
27	1	8	0.157	1.178
28	2	14	0.181	1.222
29	2	10	0.181	1.247
30	2	16	0.183	1.305