

UNIVERSITY OF NAMIBIA

PARASTIC INFESTATION ON FRESH WATER AQUACULTURE PONTENTIAL FISH SPECIES

(C.garipinus, C. ngamensis Omossambicus, O. andersonii, T.rendali, and C.carpio) IN NAMIBIA.

NAME: ALDRIN MWILIMA

STUDENT #:200823256

Faculty of Agriculture and Natural Resources

A report submitted to the Department of Fisheries and Aquatic Sciences, Faculty of Agriculture and Natural Resources, University of Namibia, 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. Martin Tjipute.

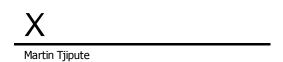
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Declaration

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of Mr Martin Tjipute on PARASTIC INFESTATION ON FRESH WATER AQUACULTURE POTENTIAL FISH SPECIES IN NAMIBIA and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly and appropriately acknowledged.

Certification

This is to certify that this 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.



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Abstract

The study was conducted to investigate the parasites found on Culturable fish species in Namibia (Kavango River and Hardap dam). A total of 64 fishes that belong to six different fish species: Clarias ngamensis, Clarias garipinus, Oreochromis mossambicus, Oreochromis andersoni, Tilapia rendali and Cyprinus carpio were examined for parasites. Nine parasite species were found in the examined fishes; Contracaecum sp (Nematotode), Gyrodactylogyrus (Monogenean), Dactylogyrus (Monogenean), two unidentified nematode species, ranarum (Dolop), Argulus sp (Crustacean), and two unidentified trematode species. Among all six fish species, C. ngamensis and C. garipinus were the most prone to parasites and T.rendali was the least infected with parasites. Among the parasites, Contracacum sp were the most frequent infect ants of C. garipinus and C.ngamensis and Gyrodactylus was most coomom in O. andersonii. Parasite diversity among the different fishes of Kavango River was high in C. garipinus (9.646) and was low in C. ngamensis (0.364). There was no parasite diversity in T. rendali. Among Hardap dam fishes, O. Mossambicus (0.472) had the highest diversity. There was zero diversity in C. garipinus and C. carpio. Primer 5 was used to determine parasite diversity among different fish species. There was a significant difference in parasite count between fish species (P=0.001<0.05) but there was no significant difference in parasite count between fish length (P=0.884<0.05) of Kavango fishes. There was a significant difference in parasite count between fish length (P=0.005<0.05) but there was no significant difference in parasite count between fish species (P=0.263>0.05) FOR Hardap dam fishes.

CHAPTER ONE

Introduction

1.1. Background

Aquaculture "is ranching and farming of aquatic organisms" (Namibia Aquaculture Act no. 18 of 2002). Clarias ngamensis and Clarias garipinus, Oreochromis mossambicus and Oreochromis andersonii, Tilapia rendali, and Cyprinus carpio are generally considered as commercial tropical freshwater fishes in Africa (Akinisanya, 2005). These fish species inhibit calm fresh waters (dams, lakes, streams and rivers). The above mentioned are good candidates for aquaculture in Namibia. Therefore more research on aquatic parasites has to be done in order to improve Aquaculture production.

Aquaculture production refers to output from aquaculture activities which are designed for final harvest for consumption. The current world aquaculture production for fish and crustaceans is 52 546 205 tones of which 940 440 tones was produced in Africa and 46 687 046 tones was produced by Asia, (FAO, 2008). The main contributors to world aquaculture production are: China (32 735 tones); Vietnam (2461 700 tones); and Thailand (1374 024 tones). The figures in Africa are very low compared to the ones for Asia for example China and this implies that little work has been done on fish parasites and their impacts on aquacultures and low technology employed in Aquaculture.

Aquaculture in Namibia started in the 1980s, therefore it is considered young. Inland aquaculture was started with the introduction of cat fish (*Clarias garipinus*) and Tilapias

(*Orechromis mossambicus* and *andersonii*) by stocking them in dams, (Namibia's Aquaculture Strategic Plan 2001). The ministry of fisheries and marine resources of Namibia is interested in developing fresh water aquaculture in Kavango, Caprivi, and Omusati region because fish is economical and socially important for the Namibian nation.

Tilapia (O. Mossambicus and O.andersonii) and cat fish (C.garipinus) are produced at Hardap dam. Roughly 15 tons is produced per annum, FAO (2004). Inland Aquaculture Centers (KIFI, Omahenene/Onavivi) are involved in the production of fingerlings of (C.garipinus, O.andersonii, T. rendali) to small scale fish farmers. Commercial marine aquaculture is mostly involved on oysters' production (Crassosostria.gigas and Ostrea edulis).

It is evident that the development of aquaculture in Namibia is having great potential.

Namibia has seen a steady aquaculture development due to the abundance of natural resources, vast uninhabited coastlines and proactive government support.

The outbreaks of established and emerging diseases, however, present a threat to the survival and sustainability of the developing aquaculture. Infectious diseases are a continual threat to consistent industry growth. With increasing intensification, the incidence of diseases is also expected to increase proportionately. The importance of containing the threats of these diseases in aquaculture is a matter of regional concern, especially with increased trade and increased transboundary movements of goods which include live fish and other aquatic organisms. Proper and timely identification and treatment of epidemics not only guarantees the survival of the species cultivated, but also ensures food security and hygiene.

1.2. General introduction

Parasites cause parasitic diseases e.g. white spot disease which is caused by a parasite called *Ichthyophthirius multifilii*. Fish parasites share a common characteristic which is that they are all associated with fish. The type of association differs among the different taxonomic groups of parasites. Fish parasites are found in different parts of the body of fish.

The purpose of this report is to document fish parasites found in six fresh water aquaculture species. This report also documents parasite species specific infestation and the microhabitat of the parasite.

There are two major distinct groups of parasites and these are ecto and endo parasites. Ecto parasites are parasites that occur on body surface (outside) and endo are those that occur inside the body. Gill and skin parasites are ecto and muscle and stomach parasites are endo.

1.3. Impact of parasites on fish

Parasites are more pronounced in enclosed set ups and areas with higher temperatures (Barlas *et al*, 2008). Fish parasites are very dangerous to fishes that are found in ponds, dams, hatcheries, and aquariums (Khan *et al*, 2003). Parasites increase fish mortality, cause weight loss, reduction in reproductive activities, reduction in growth (Khan *et al*, 2003). Olofintoye (2006) revealed that pathological conditions caused by fish parasites leads to nutritive devaluation of fish. Parasites produce waste products which cause allergies in fish consumers and this is unwanted by the public (Olofintoye, 2006). The above mentioned parasite impacts on fish occur on cultured fish species in Namibia.

1.4. Target species

Table 1: Fish family, genus, species, and feeding habits and distribution of the target fish species

Family	Genus	Species	Feeding habit	Distribution
Cichlidae	Tilapia	rendalli	Omnivore	Kavango
				river
Cichlidae	Oreochromis	andersonii	Herbivore &	Kavango
			detrivore	river
Cichlidae	Oreochromis	mossambicus	Herbivore &	Hardap dam
			detrivore	
Cyprinidae	Cyprinus	Carpio	Omnivore	Hardap dam
Claridae	Clarias	garipinus	Omnivore	Hardap dam
				& Kavango
				river
Claridae	Clarias	ngamensis	Omnivore	Kavango
				river

1.5. Significance of the study

The study will research and determine the diversity, species specific infestation and species of parasites found on six species of fresh water fishes that are cultured in Namibia. The results will be used as a source of information by documenting the parasite species found in

each fish species. The study also provides the pathology and impact of parasites on Aquaculture. Fish farmers will be able to sale health and high value fish. Fish farmers will also be able to improve on fish production since the impact of parasites will be minimised e.g. fish mortalities will be reduced.

1.6. Statement of the Problem

Due to limited information for Namibia on parasite occurrence, parasite species diversity and species specific infestation of fish parasites that are very important to fish farmers, it is difficult for fish farmers to produce a lot of fish due to lack of knowledge on how to minimise parasitic infection. The most common parasites reported on the five commercial fresh water fishes are: *Gyrodactylus sp, Argulus acuta, Trichodina acuta, Itchyobodo, Emeria sp, Procamallamus laevioconchus, Contracaecum and Lerniae*. The above mentioned fresh water fish parasites are very important in commercial fresh water fishes but little is known about them in Namibia. Fresh water parasites lead to low quality fish due to diseases caused by parasites for instance white spot disease. Parasites devaluate nutritive value of fish and this results in low quality fish which is hardly acceptable by consumers. This study will investigate parasite species found on cultured fish species in Namibia, species diversity, and species specific infestation of fresh water fish parasites on *C. garipinus, C. ngamensis, O. mossambicus, T. rendali*, and *C. carpio*. The questions research questions are:

- 1) Is there a significant difference in parasite count between fish species and length classes?
- 2) Which fish species are more vulnerable to parasites?

3) What parasite species are found in *C. garipinus*, *C. ngamensis*, *T.rendali*, *O. mossambicus*, *C.carpio* and *O.andersonii* (Aquaculture fish species).

1.7. Aims and objectives

The main objective of this project was to examine parasite species found on fresh water Aquaculture potential fish species in Namibia. The specific objectives were: (a) to identify fish parasite species; (b) compare the diversity of fish parasites among the different fish species (*C. garipinus*, *C. ngamensis*, *T. rendali*, *O. mossambicus*, *C. carpio and O. andersonii*); (c) look at species specific infestation of fish parasites on six cultured commercial fresh water fishes, (e) determine if parasites count differs between length groups and fish species.

1.8. Research hypothesis

Only the null hypotheses was stated. The following hypothesis was tested for each study area.

f). There is a significant difference in parasite count between different fish species and length classes(A,B.C) for Kavango River and A,B,C, and D for Hardap dam. The length classes were grouped as follows:

1.9. Literature Review

Numerous studies on fish parasites have been carried out worldwide by scientists in the past. A study on parasite profile was carried out in different fresh water fishes in Meinhart and Mangia mini dams of Potohar region, in Pakistan by Mahammad *et al* (2003). The study investigated fish parasites on five different fish species in carp (*Cyprinus carpio*,

Hypophthalmicthys molitrix, Ctenooharryngodon idella, Cirrhinus mrigala and Labio rohita) and nine parasites species were recovered from the 78 examined fish. The highest prevalence of parasites was found in Cyprinus carpio and the lowest was found in Ctenooharryngodon idella. This implies that C. carpio is more vulnerable to parasite infections. A study on Protozoan parasites was reviewed by Durbrow (2003) at the Southern Regional Aquaculture Center. In the study *Ichthyophthirus multifillis* was found and this is the causing agent of the white spot disease which is also known as ich. Other important protozoan parasites that were found during the study are: trichodona, ambimphyra, apisoma, chilonella, and epistyles, heteropolaria, and Myxobolus cerebralis. A study on community of helminth parasites in Rita rita (Dhaka), Bangladesh was done by (Khannum et al. (2008). Based on their results, they concluded that fish parasites destroy the value of fish and they further stated that parasites activities damage tissues that are lining the intestine, bile, and liver. The study investigated infestation of helminth parasites in Dhaka. A sample of 100 was collected from the river and careful examinations were carried out in the laboratory and was found that 50 of the 100 fish were infected. In the infected fish, 148 parasites species were recovered of 3 trematodes (Phyllodistomum folium, Horatrema pristipomatis, and Opistorchis gomtii), one nematodes (Cucullanus dogeili) were collected from R.rita. Prevalence of P. Folium and O. gmtii recorded (26%) and P. Folium had high intensity (2.2). The lowest prevalence (1.33%) was record for H. Pristiposmatis. The infestation of C, doieli was 10% and intensity was 1,5.

Studies of fish parasites have also been done in Africa. Parasite fauna in some fresh water fish species in Ekiti, Nigeria was studied by Olafintoye (2006). Nematode parasites of Clarias garipinus was reviewed in South Africa by Barson and Avenant (2006). Durring this study, 617 fishes were examined and the species under study were *T. zilli, Clarias garipinus*, and *Clarias anguillaris*. A nematode (*Cuculanus*) was recorded to have the highest prevalence of

40,4% during the period of study. Olofintoye (2004) determined that the prevalence of infection in fish species increase with standard length and body weight of the fish. Three taxonomic groups of parasites were recovered (two nematodes, two cestoda and one acanthocephalan). Barson (2004) carried out research on the occurance of Contracaecum larvae (nematode) in Clarias garppinus from lake Chivero, Zimbabwe. A total 202 Clarisa garipinus were sampled of which 86(42.6%) were infected with Contaracaecum larvae. The mean intensity of this parasite was 2.2 worms per fish. Prevalence in relation to sex was also examined and there was no significant difference between sexes in prevalence infection.

Few studies of fish parasites of fresh water fishes have been done in Namibia. The latest list of fish parasites of the Kavango river was published in 2005 by Christison *et al*. A total of seventeen species of *Gyrodactylus* were identified and known today in the fresh waters of Africa. Finally, nematode parasites of *Clarias garipinus* has been done in Africa by Barson (2006). *Contracaecum* and *Procamallamus laeviconcus* were identified on *Clarisa garipinus*. A study on Endo-parasite infection of cichlids in Kavango river, Namibia was carried out by Kosmas, (2010). The species that were under study are (*Tilapia ruweti, Tilapia rendali* and *Oreochromis Andersonii*).

CHAPTER TWO

Research Methods and Materials

2.1. Study areas on which research was conducted

The study areas were Kwetze and Hardap dam. Both study areas are situated in Namibia but in different regions. These study areas have different characterics where by Kwetze is a natural set up with strong water currents and Hardap dam is a static system that was built by humans. The two study areas were chosen because they house aquaculture commercial fish species farmed in Namibia. Hardap dam is a home to *C.garipinus*, *C. carpio*, and *O. Mossambicus* and *C. garipinus*, *C. ngamensis*, *T. rendali*, and *O. andersonii* inhibit the waters of Kavango River.

Kwetze is a channel of the Kavaogo river which originates from the central high lands of Angola and seeps in the Kavango delta in Bostwana. This channel is within Mahango game park. Kwetze is located in the latitude measured in degrees and minutes: 18°, 13' S and longitude also measured in the same unts: 21°, 45' E. The site is 1 to 2,5 meters deep and it is vegeted with a lot of reeds and water lilies.

Hardap dam is situated in Mariental at latitude: 24° S and longitude 17° E. The dam was constructed to store run off the fish river. The dam has maximum depth of 33 meters, (Schewe, 1998).

2.2. List of materials used

The following materials were used during the research period.

- Four by four vehicle
- Research boat for setting nets in the water body (Kwetche and Hardap dam)
- Cooler box for containing water from where fish was caught with fish in it
- Slides for smear preparation
- Beakers, Petri dishes and
- Marker for labelling
- Hot plate for heating formalin
- Microscopes (Compound and Dissecting)

2. 3. Sampling procedures

Six commercial fresh water fish species (*C.ngamensis, C.garipinus, O.andersonii*, and *T.renadli*) were selected for this study. Selection criteria were based on the availability of fish and commercial importance. The technique used for fish sampling was very simple, little technology involved. Fish sampling was done using monofilament nets of one inch mesh size, multifilament gillnets of varying mesh sizes and drag nets. A minimum of two gill nets were soaked in water for 12 hours. The gill nets were set every evening between 5:00 p.m. and 6:00 p.m. and hauling was done at between 6:00 a.m. and 7:00 a.m. Mulitfillament and monofilament gill nets were both used to sample in Kwetche. Drag nets and multifilament gill nets were used for sampling in Hardap dam.

2.4. Laboratory procedures

Live fish samples were transported for examination in the laboratory in water from their natural habitat and in good condition .To avoid parasites from escaping, fish was examined soon after capture. The first step in the laboratory was to fish identification using keys by Paul Skeleton (2001). After fish identification, Total length was then measured using measuring board, weighed with a balance and then sex determination followed. Sex was determined by looking at the gonads.

2.5. Parasitological examination

Ectoparasites were carefully examined on skin surfaces and under fins with the help of magnifying glass. Wet smears were prepared from the skin and gills of fish to locate for gill and skin parasites using a compound microscope. All collected parasites were quantified, identified using morphological with the help structures and photographs were taken using a digital camera. Endo parasites were located by preparing wet smears from gut walls and incising fish muscles.

Nematodes and Trematodes

Adult nematodes were recovered from muscles of the fish. Muscle parasites (Nematodes and Trematodes) were traced by incising and scraping with forceps. Nematodes found in head

cavity were located by cutting through the mouth to the stomach. The Nematodes and Trematodes were preserved in 10 % hot formalin.

Monogeaneans

Monogeneans were examined by preparing wet smears from gills and skin of fish. The specimens were preserved in 70 % ethanol.

Crustaceans

Crustaceans (Dolops) found on the skin were recovered by careful examination with the help of magnifying glass and *Argulus* was located by viewing prepared wet smears under the compound microscope.

CHAPTER THREE

Results

3.1. Data analysis

The collected data was analysed separately according to the sampling areas. Data was analysed separately because the two study areas are different. Hardap dam is an enclosed area and Kavango is a river which have different characteristics from Hardap dam. The two different study areas were chosen because some culturable fish species are only in Haradap dam and other in Kavango River so for one to look at a complete list of fresh water aquaculture species in Namibia, both areas have to be considered. Data was analysed in terms of prevalence (% of infestation), species diversity, and parasite counts.

Prevalence (%) =
$$\frac{\text{Number of fish infested with parasites}}{\text{Total number of fish examined}} \times 100$$

Infection% = No of fish infected by single parasite species/ Total No of fish examined* 100

A two-way ANOVA was conducted to examine the effect of fish length and fish species on parasite count. The dependent variable, parasite count was normally distributed for the groups formed by the combination of the levels of fish length (A, B, and C) for Haradap and (A, B, C, D) for Kwetche fish species assessed by One sample K-S.

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Length classes were grouped as follows:

7-29: Class A

31-51: Class B

52-74: Class C

74<: Class D

3.2. Kavango River

Thirty nine fish samples were collected from Kavango River of which seventeen belonged to the Claridae family which comprised of Seven *ngamensis and* Ten garipinus. The 39

samples comprised of 22 Cichlids that included 16 O. andersonii and 6 T.renadli.

A total of seven parasite species was found in the examined fish. Two species monogeaneans

(Gyrodactylus and Dactylogyrus), two nematode species (Contracaecum sp and one

unidentified), one trematode species (unidentified), and two Crustaceans (Argulus and

ranarum) were found from the fish that were examined and this is summarised in Table2.

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Table 2: Prevalence of parasites in different fishes of Kwetche

		No of examined	No of infected		
No	Host	fish	fish	Prevalence(%)	Parasite found
1	C.ngamensis	7	7	100	Contracaecum sp
					Gyrodactylus
					Dactylogyrus
					Argulus
2	C.garipinus	10	10	100	Contracaecum sp
					Gyrodactylus
					Dactylogyrus
					Dolop(ranarum)
3	O.andersonii	16	10	62.5	Gyrodactylus
					Dactylogyrus
					Trematode unidentified
					Nematode(unidentified)

4	T.rendali	6	1	16.67	Dactylogyrus sp
	Total	39	28	71.79	

Parasite prevalence was high *in C. ngamensis* and *C. garipinus* (100%) and this is plainly shown in Table 2. *T.rendali* scored the lowest (16.67) parasite prevalence. Among the 39 examined fish 28 fish were infested with parasites and this gives a prevalence score of 71.79 %.

Table 3: Individual parasite prevalence on different fishes of Kwetche

		No of		Infection (%)
		examined	No of infected	
No	Parasite	fish	fish	
1	Contracaecum sp	39	5	12.82
2	Gyrodactylus sp	39	11	28.21
3	Dactylogyrus sp	39	17	43.59
4	Argulus	39	2	5.13
5	Dolop (ranarum)	39	2	5.13
6	Trematode(uniedmntified)	39	1	2.56
7	Nematode(unidentified)	39	1	2.56
	Total	39	37	94.87

Results in Table 3 show that *Dactylogyrus sp* infected a higher number of fish. The parasites *Dactylogyrus sp and Gyrodactylus sp* were the most frequent and were found in all four fish species excluding *T. reandali* which was only infected by *Dactlogyrus sp*. Contracaecum sp was also most frequent in *C. ngamensis and C. garipinus*.

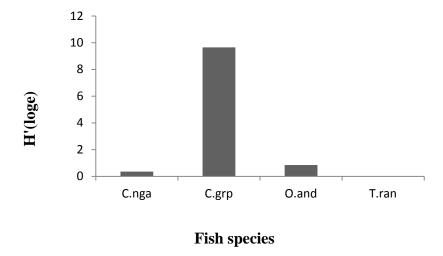


Figure 1: Diversity among four fish species of Kwetche

According to figure 1, parasite diversity was highest in *C. garipinus* (9.646) as it was determined by Primer 5 using Shonons diversity index. Diversity was lowest in *C. ngamensis* and there was no diversity of parasites in *T. rendali*.

Table 4:Summery table from SPSS16

	Type III Sum of				
Source	Squares	df	Mean Square	F	Sig.
Corrected Model	6200.615 ^a	5	1240.123	13.633	.000
Intercept	7450.738	1	7450.738	81.911	.000
Fish	1799.513	3	599.838	6.594	.001
Length	22.556	2	11.278	.124	.884
Fish * Length	.000	0	•		•
Error	3001.744	33	90.962		
Total	16003.000	39			
Corrected Total	9202.359	38			

There was a significant difference between the assessed groups by Levene's test for equality of error of variances. There was no significant interaction between the effects of length and fish species levels on parasite counts. There was a significant difference in parasite count

between fish species (P=0.001<0.05) but there was no significant difference in parasite count between fish length (P=0.884<0.05).

3.2. Hardap dam

Twenty five fish samples were collected from Hardap dam. The Twenty five fishes were composed of ten *C. garipinus*, twelve *O. mossambicus*, and three *C. carpio*.

Two nematode species (*Contracaecum sp* and one unidentified), one trematode species (unidentified), and one Monogenean (*Dactylogyrus*). The parasites *Dactylogyrus* was found only found on gills of *O. mossambicus* and *Contreacaecum* worms were only recovered from the stomach of *C. garipinus*. The recovered Trematode was found in only one fish species, *O. mossambicus*.

Table 5: Parasite prevalence in different fishes of Hardap dam

		No of fish	No of infected	Prevalence	
No	Host	examined	fish	%	Parasites found
1	C. garipinus	10	6	60	Contracaecum sp
2	O. mosssambicus	12	9	75	Nematode
					Dactylogyrus sp
					Trematode
3	C. carpio	3	3	0	
	Total	25	18	72	

Table 5 Shows that *O. mossambicus* was the fish species infested with parasites and *C. Carpio* was not infested with any parasites. High parasite infections in *O.mossabicus and C. garipinus* suggest that they are more vulnerable to parasites, especially Nematodes. As we can see from the table that three different parasites species were found in *O. mossambicus*, it is an indication that *O.mossambicus* is less resistant to parasites.

The zero infection for *C.carpio* implies that is less vulnerable to parasites and this means that it is more resistant to parasites.

Table 6: Individual parasite prevalence of different fishes of Hardap dam

		No of	No of infected	Infection
No	Parasite	examined fish	fish	(%)
1	Datylogyrus sp	25	4	16
2	Trematode(unidentified)	25	1	4
3	Nematode(unidentified)	25	9	36
4	Contracaecum sp	25	6	24
	Total	25	20	80

The unidentified nematodes were the most infected a lot of fish from the sampled fish and it was only specific in *O. mossambicus*. Contracaecum sp scored the highest counts and it was only specific in *C. garipinus*.

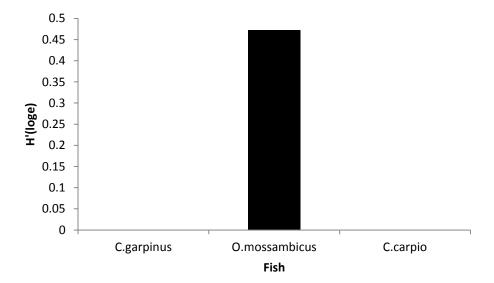


Figure 2: Diversity between Hardap dam fishes

Figure 2 plainly shows that there was no parasite diversity in *C. garipinus* and *O. mossambicus* but has parasite diversity of 0.47.

Table 7: ANOVA table from SPSS

	Type III Sum of			-	r.
Source	Squares	Df	Mean Square	F	Sig.
Corrected Model	43748.460 ^a	8	5468.558	7.144	.000
Intercept	12468.898	1	12468.898	16.289	.001
Length	14254.199	3	4751.400	6.207	.005
Fish	2227.691	2	1113.845	1.455	.189
Length * Fish	4329.985	3	1443.328	1.886	.106
Error	12247.300	16	765.456		
Total	72074.000	25			
Corrected Total	55995.760	24			

There was no significant difference (P=0.53>0.05) between the assessed groups by Levene's test for equality of error of variances. There was no significant interaction (P=0.173>0.05) between the effects of length and fish species levels on parasite counts. This is clearly seen in table 7 that the significance value is greater than 0.05. As seen in Table 7 that there was a significant difference in parasite count between fish length (P=0.005<0.05) but there was no significant difference in parasite count between fish species (P=0.263>0.05).

3.3. Parasites specimens for both Hardap dam and Kavango River



Figure 3: Nematode



Figure 4: Dolop (ranarum)



Figure 5: Dactylogyrus sp



Figuere 6: Gyrodactylus sp



Figure 7: red spot indicates Gyrodactylus sp on fin



Figure 8: Contracaecum sp



Figure 9: Contracaecum in stomach of C. garipinus

CHAPTER FOUR

Discussion, Conclusion and Contribution to Knowledge

4.1. Statistics

In Table 4 the results showed that there was no significant difference (P=0.884<0.05) in parasite count between length classes (A, B, and C). The observed results shown are not in agreement with results from SPSS (Table 4) and this difference could be due to different fish species because parasite occurrence depends on the biology of the fish species.

There was a significant difference (P=0.001<0.05) in parasite count between fish species from Kvango River and this is shown in Table 4. This is due to the fact that some fish species are more vulnerable to parasites .e.g. cat fish which was recorded to have more parasite counts than other fish species.

Table 7 shows that there a significant difference (P=0.005<0.05) in parasite count between different length groups of fish sampled from Hardap dam and this could have been a result of different fish species that were compared. There was no significant difference (P=189>0.05) in counts between species and this could be due to the feeding habits of fish.

4.2. Parasite species found

A total of nine parasite species were found in all six different aquaculture potential fish species and these were (*Contracaecum sp, Gyrodactylus, Dactylogyrus sp, Argulus*, Dolops, two unidentified trematode, and two unidentified nematodes species).

In this study, seven parasite species were recorded on host fishes from both Kavango River. As we can see in Table 2 that the parasites that were found in four different fish species of Kavango River were: *Contracaecum sp, Gyrodactylus, Dactylogyrus sp, Argulus*, Dolops, unidentified trematode, unidentified nematodes.

Four different parasite species were recorded in Hardap dam in 25 fishes that were examined. This is shown in Table 5. The parasite species found were *Contracaecum sp*, undefined trematode, *Dactylogyrus* and unidentified nematode.

4.2a.1. Parasite diversity

Results in Figure 1 shows that parasite diversity was highest in *C. garipinus* and lowest in *T. rendali*. There was zero diversity in *T. rendali* because only one parasite species was found in this fish species. Four different parasite species were recorded in *C. garipinus* therefore there was high diversity in *C. garipinus* and this could be due to the feeding habits of *C. garipinus* and its soft muscle which may make a very suitable host for parasites.

As seen in Figure 2 that the highest parasite diversity in among all fish examined fish species sample in Hardap dam was in *O. mossabicus*. High diversity in *O. mossambicus* suggests that it is more vulnerable to different parasites. There were more different parasite species on *O. mossambicus* than other two fish species and this could be due to host preferences of the parasites.

4.2a.2. Prevalence

In this study, prevalence between fish species of the Kavango River was highest(100 %) in *C. garipinus* and *C. ngamensis*. Parasite prevalence was lowest(16.67 %) in *T. rendali*. C. *garipinus and C. ngamensis* had high prevalence because it is due the fact parasite occurrence depends on what feeding habits and the biology of fish. As it is it indicated in Table 1 that Clarias species are predatory, this means that they easily get infected by parasites by feeding on other fish species which may be already infested with parasites. Feeding on other fish species which may be infected with parasites will result into catfish getting infected and highly infested with different parasite specoies. High parasite prevalence in Clarisa species could also be a result of a week defence system. This observation suggests that *C. garipinus* and *C. ngamensis* are more vulnerable to parasites. *O. andersonii* also had parasite prevalence of 62.5 % and since *O. andersonii* feeding habits muscle are similar to that of *T. rendali*, the high prevalence in *O. andersonii* could be justified by the parasite preferences on hosts.

In Hardap dam, Table 5 shows that prevalence of parasites was high (75 %) in *O. mossabicus* and then followed by *C. garipinus* with prevalence of 60 %. High prevalence in *O. mossambicus* and *C. garipinus* is an indication that they less resistant to parasites.

4.2a.3. Infection

In this study, total percentage infection for Kavango river (94.87%). Percentage parasite infection is shown in Table 3 for Kavango river and Table 6 for Hardap dam. High Parasite infection was caused by *Dactylogyrus* with an infection of 43.59 % (Table 3) in fish species sampled from Kavango River and this was due to the fact that *Dactylogyrus* occurs in many fish species (Barlas *et al*, 2008) and this is in nagreement with the observed results, see Table 2 and Table 5.

Results of Hardap dam shows that the unidentified nematode species infected a lot of fish. It its infection was 36 % and this parasite was only recorded in *O. mossambicus*. It was specific to smaller *O. mossambicus* fish and this could be justified by their feeding habits (Barlas *et al*, 2008). The unidentified trematode which was recovered from the gut wall of *O. mossabicus* was only recorded in one fish sample.

4.2b.1. Monogenea

Impact on Aquaculture

The recovered monogeneans from the investigated fish were *Dactylogyrus sp* and *Gyrodactylus sp*. Monogeneans need a direct contact for them to be transferred to other fishes. The parasite *Gyrodactylus* cause irritation and skin damage which cause ulcers and lesions. Figure 7 shows a colouration caused by *Gyrodatylus*. Ulcers and lesions leads to the infected fish to become more vulnerable to secondary infection such as Epizootic Ulcerative Syndrome (Abowei and Ezekiel, 2011).

In fish farms, *Dactylogyrus* and *Gyrodactylus* sp may be highly pathogenic, contributing to high fish mortalities and economic losses. There can cause a mass kill of fish in fish farms because there is high contact of fish and this makes the transfer rate of parasites between fish very high and faster. Heavy infestation of monogeneans caused mass mortalities in carp fry during spawning season in breeding and nursery ponds in Israel (*Barlas et al*, 2003). *Dactylogyrus vastator* caused so much damage to gill filaments of *Carps* in California hatcheries (Shamall and Abdullah, 2009).

Occurrence and Specificity

The study results in (Table 2) show that most common parasites were *Dactylogyrus sp*. The parasite *Dactylogyrus* (Figure 5) was found on gills in five different fish species (*C. garipinus, C. ngamensis, T. rendali, O. andersonii and O. mossambicus*) as indicated in (Table 2 and Table 5) and this is because *Dctylogyrus* is known to parasitize many fresh water fish species (Barlas *et al*, 2008). This parasite was found in both areas that were studied (Hardap dam and Kavango River). *Dactylogyrus* infection is influenced by fish size and maturity because larger fish provide more attachment area for *Dactylogyrus* (Barlas et al, 2008). This present study is in agreement with the above mentioned statement, *Dactylogyrus* counts were higher in bigger fish than in smaller fish.

The skin monogenean, *Gyrodactylus* (Figure 6, page) was also found in the sampled fish species. This species was only found in fishes from Kavango River and it was only found in two species (*C. garipinus* and *O.andersonii*). The prevalence of this parasite was less pronounced compared to *Dactylogyrus*.

4.2b.2. Nematodes

Impact on Aquaculture

Three nematode species were found in all fish that were investigated from both Hardap dam and Kavango River. Only one species was identified (*Contracaecum sp*) and the other two species were not identified. The impact of nematodes parasites on aquaculture will only be focussed on *Contracaecum sp* since it was the only identified nematode and the unidentified species will not be discussed so as to avoid documenting wrong information.

Contracaecum sp have no effect on fish though the intensity can be very high in an aquaculture environment (Barson ,2004). Though Contracaecum sp does not affect fish, this

parasite species may render the unsuitable sight for human consumption, especially when encysted in fish muscles as was the case with the Kavango cat fishes (*C. garipinus and C. ngamensis*). *Contracaecum sp* sight on fish affect the marketability of commercial fresh water fish, thus raising a lot of public health concerns.

Occurrence and Specificity

As we can see from Table 2 and 5 that *Contracaecum sp* (Figure 8 and 9) was both recorded in Hardap dam and Kavango River. This parasite is a common fresh water parasite and this observation can be justified by the fact that *Contracaecum* life cycle which involves migratory bird species makes it one of the most common fresh water fish parasites (Barson, 2006).

Contracaecum was only found in Clarias species of both Hardap dam and Kavango River. The occurrence of Contracaecum in cat fish and not in other fish species could be related to the feeding habits of catfish, feeding on smaller fishes and copepods. Intensity of parasites is related to fish size and maturity which was the case in this study. More than 30 (see appendix) Contracaecum counts was observed in larger fish and less than 20 Contracaecum worms were recorded in smaller fish of C. garipinus in Kavango River. In Hardap dam, there was more than 100 Contracaecum per fish in larger fish and no Contracaecum were recorded in smaller C. garipinus (see appendix).

One unidentified nematode species was found in *O. andersonii* and the other in *O. mossambicus*. The nematode that was recorded in *O. mossambicus* is shown in Figure 3. The unidentified nematode recorded in Kavango in the month of May was only found in one fish throughout the research period. This observation could be a result in variation between seasons (winter and summer). The unidentified nematode in *O. mossambicus* was most

frequent throughout the research period. This nematode was only affecting smaller fish of *O*.

mossambicus (see appendix). This phenomenon can be justified by their feeding habits.

4.2b.3. Trematodes

Impact on Aquaculture

Two different trematode species were recorded during the research period but they were not identified beyond the phylum level due to lack of resources. Though the trematodes were not identified, their impact on aquaculture will be discussed based other studies that were done on other trematodes. Treamatodes are pathogenic to fish and they can cause fish mortalities. A study by Terhune *et al*, (2003) documented that a trematode identified as Bolbophorus sp caused high mortalities and decreased production in channel cat fish in Luisiana.

Occurrence and Specificity

Trematodes are prevalent in many fish species and are common in cultured fish in areas with a lot of fish-eating birds (Terhune *et al*, 2003) which was not the case in this study. Only one diginean species was recorded in *O. andersonii* and it was only recorded in one fish in May. In Hardap dam, one trematode species was recorded in the gut wall of *O. mossabicus*.

4.2b.4. Crustaceans

Impact on Aquaculture

The recorded crustaceans were Dolop (*ranarum*) and *Argulus* sp. Dolops have two large hooks that are used for attachment on skin and gills of the host fish. Theses hooks cause mechanical damage to the fish skin and the damaged skin becomes a sight for secondary infection caused by bacteria and fungi. Secondary infection may weaken all parasitized fish and cause dearth, especially in an aquaculture environment. Transfer rate of the skin parasite

(Dolop) is high in aquaculture systems than in natural systems since dolops are more mobile on fish skin. Dolops are even much faster to be transferred to other fish in aquaculture because there is so much contact of fish in aquaculture set up than in natural systems and this may cause mass kill of fish in aquaculture.

Occurrence and Specificity

Crustaceans were less frequent in this study. Among the nine parasites that were found, two different Crustacean species. The recovered were *Argulus* spp and Dolop (Figure 4) which are shown in Table 2. These parasites were only recorded in Kavango River and not Hardap dam. *Argulus* was only recorded in *C. ngamensis* and Dolops were only recorded in *C.garipinus*. Both *Argulus* and Dolops were less infectious and had the same infection of 5.a3 %, see Table 3. The low infection could be due to the fact that these parasites leave the host as soon the host dies (Khan et al, 2003). The absence of these crustaceans could be due to the fact parasite occurrence is related to temperature and oxygen concentration (Barlas, 2008). The observed results in this study are in agreement with the above statement.

Argulus and Dolops are related (Van, 2004). Khan et al, (2003). This parasite is responsible for eating health problems in confined areas like hatcheries and ponds. Dolop (ranarum) have hooks that it uses for attachment and these hooks can puncture the fish skin which may result in secondary infection by fungi and bacteria (Van, 2004).

4.3. Contribution to Knowledge

This study contributes to reduction of information deficit for some fish parasites in Namibia (Hardap dam and Kavango River). This study documents two monogenean parasite species (*Gyrodatylus* and *Dactylogyrus*), Nematodes (Contracaecum and two unidentified species), Crustaceans (*Argulus* and Dolops) and Diginean trematode species in six commercial fresh water fish species. The study also documents the impact of parasite species on Aquaculture and Human health.

4.4. Conclusion

The findings of the study conclude that the occurrence of nine different parasites was a great diversity, although the frequency was not so high. The presence of more parasites in *C. garipinus* suggests its low resistance to parasite infection; especially *Contracaecum* infection which was high in both study areas. Among the parasites studied, *Dactylogyrus* was the commonly found parasite and was also the most infectious parasite in Kavango River. The unidentified nematode that was found in Hardap dam fish were most infectious and frequent parasite in *O. mossamibicus*. The least infectious parasites were the unidentified nematode of Kavango and unidentified trematodes of Kavango River and Hardap dam.

The results showed that here that there was a significant difference in parasite counts between different length groups and fish species.

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APENDICES

HARDAP DAM

Between-Subjects Factors

Both con Gabjooto i actoro		
-	-	N
Length	Group A	10
	Group B	8
	Group C	2
	Group D	5
Fish	C.carpio	3
	C.garipi	10
	O.mossam	12

Descriptive Statistics

Length	Fish	Mean	Std. Deviation	N
Group A	C.carpio	.00		1
	C.garipi	.00		1
	O.mossam	2.25	1.488	8
	Total	1.80	1.619	10
Group B	C.carpio	.00		1
	C.garipi	.00	.000	3
	O.mossam	4.50	5.447	4
	Total	2.25	4.301	8
Group C	C.carpio	.00		1
	C.garipi	109.00		1
	Total	54.50	77.075	2
Group D	C.garipi	97.80	55.097	5
	Total	97.80	55.097	5
Total	C.carpio	.00	.000	3
	C.garipi	59.80	63.322	10
	O.mossam	3.00	3.275	12
	Total	25.36	48.303	25

Levene's Test of Equality of Error Variances^a

Dependent Variable:Count

F	df1	df2	Sig.
2.551	8	16	.053

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Length + Fish + Length * Fish

Tests of Between-Subjects Effects

Dependent Variable:Count

	Type III Sum of				
Source	Squares	df	Mean Square	F	Sig.
Corrected Model	43748.460 ^a	8	5468.558	7.144	.000
Intercept	12468.898	1	12468.898	16.289	.001
Length	14254.199	3	4751.400	6.207	.005
Fish	2227.691	2	1113.845	1.455	.263
Length * Fish	4329.985	3	1443.328	1.886	.173
Error	12247.300	16	765.456		
Total	72074.000	25			
Corrected Total	55995.760	24			

a. R Squared = .781 (Adjusted R Squared = .672)

Estimated Marginal Means

1. Length

Dependent Variable:Count

			95% Confidence Interval	
Length	Mean	Std. Error	Lower Bound	Upper Bound
Group A	.750	13.444	-27.749	29.249
Group B	1.500	11.604	-23.100	26.100
Group C	54.500 ^a	19.563	13.027	95.973
Group D	97.800 ^a	12.373	71.570	124.030

a. Based on modified population marginal mean.

2. Fish

Dependent Variable:Count

			95% Confide	ence Interval
Fish	Mean	Std. Error	Lower Bound	Upper Bound
C.carpio	-3.733E- 14 ^a	15.973	-33.862	33.862
C.garipi	51.700	11.009	28.362	75.038
O.mossam	3.375 ^a	8.471	-14.583	21.333

a. Based on modified population marginal mean.

3. Length * Fish

Боронаон	- variable.Count	1	7	<u>-</u>	_
				95% Confide	ence Interval
Length	Fish	Mean	Std. Error	Lower Bound	Upper Bound
Group A	C.carpio	-2.842E-14	27.667	-58.651	58.651
	C.garipi	-2.798E-14	27.667	-58.651	58.651
	O.mossam	2.250	9.782	-18.486	22.986
Group B	C.carpio	-2.842E-14	27.667	-58.651	58.651
	C.garipi	-5.596E-14	15.973	-33.862	33.862
	O.mossam	4.500	13.833	-24.826	33.826
Group C	C.carpio	-5.329E-14	27.667	-58.651	58.651
	C.garipi	109.000	27.667	50.349	167.651
	O.mossam	a			
Group D	C.carpio	a •			
	C.garipi	97.800	12.373	71.570	124.030
	O.mossam	a			

a. This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Post Hoc Tests

Length

Multiple Comparisons

Count

Tukey HSD

Tultoy 1102						
-	-	Mean Difference			95% Confide	ence Interval
(I) Length	(J) Length	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Group A	Group B	45	13.124	1.000	-38.00	37.10
	Group C	-52.70	21.431	.106	-114.01	8.61
	Group D	-96.00 [*]	15.154	.000	-139.36	-52.64
Group B	Group A	.45	13.124	1.000	-37.10	38.00
	Group C	-52.25	21.873	.120	-114.83	10.33
	Group D	-95.55 [*]	15.773	.000	-140.68	-50.42
Group C	Group A	52.70	21.431	.106	-8.61	114.01
	Group B	52.25	21.873	.120	-10.33	114.83
	Group D	-43.30	23.148	.279	-109.53	22.93
Group D	Group A	96.00 [*]	15.154	.000	52.64	139.36
	Group B	95.55 [*]	15.773	.000	50.42	140.68
	Group C	43.30	23.148	.279	-22.93	109.53

Based on observed means.

The error term is Mean Square (Error) = 765.456.

^{*.} The mean difference is significant at the .05 level.

Homogeneous Subsets

Count

Tukey HSD

		Subset	
Length	N	1	2
Group A	10	1.80	
Group B	8	2.25	
Group C	2	54.50	54.50
Group D	5		97.80
Sig.		.056	.139

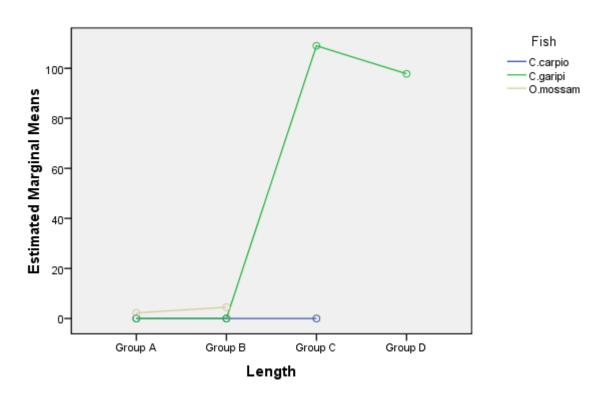
Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 765.456.

Profile Plots

Estimated Marginal Means of Count



Non-estimable means are not plotted

One-Sample Kolmogorov-Smirnov Test

		Count
N	-	39
Normal Parameters ^a	Mean	13.90
	Std. Deviation	16.186
Most Extreme Differences	Absolute	.247
	Positive	.247
	Negative	195
Kolmogorov-Smirnov Z		1.544
Asymp. Sig. (2-tailed)		.017

a. Test distribution is Normal.

One-Sample Kolmogorov-Smirnov Test

	-	Count
N		39
Normal Parameters ^a	Mean	13.90
	Std. Deviation	16.186
Most Extreme Differences	Absolute	.247
	Positive	.247
	Negative	195
Kolmogorov-Smirnov Z		1.544
Asymp. Sig. (2-tailed)		.017

KAVANGO RIVER

Between-Subjects Factors

	_	N
Length	A	17
	В	9
	С	13
Fish	C.grp	10
	C.nga	7
	O.and	16
	T.rnd	6

Descriptive Statistics

Length	Fish	Mean	Std. Deviation	N
А	O.and	2.73	3.036	11
	T.rnd	1.17	2.858	6
	Total	2.18	2.984	17
В	C.grp	30.50	14.549	4
	O.and	2.20	3.899	5
	Total	14.78	17.591	9
С	C.grp	27.50	13.019	6
	C.nga	25.71	14.863	7
	Total	26.54	13.488	13
Total	C.grp	28.70	12.928	10
	C.nga	25.71	14.863	7
	O.and	2.56	3.204	16
	T.rnd	1.17	2.858	6
	Total	13.21	15.562	39

Levene's Test of Equality of Error Variances^a

Dependent Variable:Count

F	df1	df2	Sig.
4.540	5	33	.003

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Length + Fish + Length * Fish

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6200.615 ^a	5	1240.123	13.633	.000

Intercept	7450.738	1	7450.738	81.911	.000
Length	22.556	2	11.278	.124	.884
Fish	1799.513	3	599.838	6.594	.001
Length * Fish	.000	0			
Error	3001.744	33	90.962		
Total	16003.000	39			
Corrected Total	9202.359	38			

a. R Squared = .674 (Adjusted R Squared = .624)

Estimated Marginal Means

1. Length

Dependent Variable:Count

			95% Confidence Interval		
Length	Mean	Std. Error	Lower Bound	Upper Bound	
А	1.947 ^a	2.420	-2.977	6.871	
В	16.350 ^a	3.199	9.842	22.858	
С	26.607 ^a	2.653	21.209	32.005	

a. Based on modified population marginal mean.

2. Fish

			95% Confidence Interval		
Fish	Mean	Std. Error	Lower Bound	Upper Bound	
C.grp	29.000 ^a	3.078	22.737	35.263	
C.nga	25.714 ^a	3.605	18.380	33.048	

O.and	2.464 ^a	2.572	-2.769	7.697
T.rnd	1.167 ^a	3.894	-6.755	9.088

a. Based on modified population marginal mean.

3. Length * Fish

Dependent Variable:Count

				95% Confidence Interval	
Length	Fish	Mean	Std. Error	Lower Bound	Upper Bound
А	C.grp	a			
	C.nga	a			
	O.and	2.727	2.876	-3.123	8.578
	T.rnd	1.167	3.894	-6.755	9.088
В	C.grp	30.500	4.769	20.798	40.202
	C.nga	a			
	O.and	2.200	4.265	-6.478	10.878
	T.rnd	a			
С	C.grp	27.500	3.894	19.578	35.422
	C.nga	25.714	3.605	18.380	33.048
	O.and	a •			
	T.rnd	a			

a. This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Post Hoc Tests

Fish

Multiple Comparisons

		_	Mean Difference			95% Confide	ence Interval
	(I) Fish	(J) Fish	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	C.grp	C.nga	2.99	4.700	.920	-9.73	15.70
		O.and	26.14 [*]	3.845	.000	15.74	36.54
		T.rnd	27.53 [*]	4.925	.000	14.21	40.86
	C.nga	C.grp	-2.99	4.700	.920	-15.70	9.73
		O.and	23.15 [*]	4.322	.000	11.46	34.84
		T.rnd	24.55 [*]	5.306	.000	10.19	38.90
	O.and	C.grp	-26.14 [*]	3.845	.000	-36.54	-15.74
		C.nga	-23.15 [*]	4.322	.000	-34.84	-11.46
		T.rnd	1.40	4.566	.990	-10.95	13.75
	T.rnd	C.grp	-27.53 [*]	4.925	.000	-40.86	-14.21
		C.nga	-24.55 [*]	5.306	.000	-38.90	-10.19
		O.and	-1.40	4.566	.990	-13.75	10.95
LSD	C.grp	C.nga	2.99	4.700	.530	-6.58	12.55
		O.and	26.14 [*]	3.845	.000	18.32	33.96
		T.rnd	27.53 [*]	4.925	.000	17.51	37.55
	C.nga	C.grp	-2.99	4.700	.530	-12.55	6.58
		O.and	23.15 [*]	4.322	.000	14.36	31.94
		T.rnd	24.55 [*]	5.306	.000	13.75	35.34
	O.and	C.grp	-26.14 [*]	3.845	.000	-33.96	-18.32
		C.nga	-23.15 [*]	4.322	.000	-31.94	-14.36
		T.rnd	1.40	4.566	.762	-7.89	10.68
	T.rnd	C.grp	-27.53 [*]	4.925	.000	-37.55	-17.51
		C.nga	-24.55 [*]	5.306	.000	-35.34	-13.75
		O.and	-1.40	4.566	.762	-10.68	7.89

Based on observed means.

The error term is Mean Square (Error) = 90.962.

^{*.} The mean difference is significant at the .05 level.

Homogeneous Subsets

Count

Count					
	-		Subset		
	Length	N	1	2	3
Tukey HSD ^a	Α	17	2.18		
	В	9		14.78	
	С	13			26.54
	Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

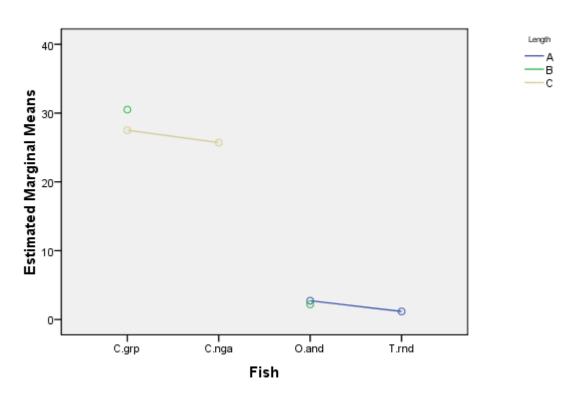
Based on observed means.

The error term is Mean Square(Error) = 90.962.

a. Uses Harmonic Mean Sample Size = 12.153.

Profile Plots

Estimated Marginal Means of Count



Non-estimable means are not plotted

NORMALITY TEST

One-Sample Kolmogorov-Smirnov Test

C.1.5 Gap.15 :	tominogorov omininov reet	
	•	Count
N	-	25
Normal Parameters ^a	Mean	21.44
	Std. Deviation	40.174
Most Extreme Differences	Absolute	.419
	Positive	.419
	Negative	297
Kolmogorov-Smirnov Z		2.094
Asymp. Sig. (2-tailed)		.000
a Test distribution is Norma		

a. Test distribution is Normal.

One-Sample Kolmogorov-Smirnov Test

	-	Count
N	-	25
Normal Parameters ^a	Mean	21.44
	Std. Deviation	40.174
Most Extreme Differences	Absolute	.419
	Positive	.419
	Negative	297
Kolmogorov-Smirnov Z		2.094
Asymp. Sig. (2-tailed)		.000