Studies On The Microbial Flora Found In The Intestine Of *Heteroclarias* Specie

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ABSTRACT: Studies of the microbial flora of H*eteroclarias* fish was carried out 48hrs prior in which the fish was starved. The enumeration of bacterial found in the intestine of H*eteroclarias* fish was done through serial dilution and agar plating techniques. A nutrient agar at 45c and O.1ml of the suspension was poured into a petridish. The isolation was done using various biochemical tests. The various micro organisms which were isolated were mainly, *Escherichia coli, pseudomonas fluorescence's cereus*, *staphyloccus aureus, klebsiella aerugenes, salmonella*, *entritidis, bascillus cereus, proteus vulgaris* and various fungal species. The bacteria gotten from this isolation were mostly gram negative with E. coli having the highest number of growth often occurred in concentration ranging between 10 and 10 colony forming unit per ml of the fish content.
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INTRODUCTION

In Africa, especially in Nigeria, the species mostly cultured are *Clarias gariepinus*, *heterobranchus* species and their hybrids. The reasons for their culture are based on their fast growth rate, diseases resistance, high stocking density, aerial respiration, high speed conversion efficiency among others. Aquaculture in Nigeria is in the developing stage, because it has not been able to meet the demand and supply of the ever –increasing population, catfish are cultured conveniently under mono and polyculture systems (Reich, 1975).

However, with the intensification of tank system where fish culturists rely solely on artificial feed as the only food resource of closely related species of the same family and of the same feeding habits, this type of system, there is the culture of only one single specie known as monoculture. Most cat fish culturists in Africa especially in Nigeria have practiced any of these culture systems without knowing the best culture for their fish. These farmers believe that culturing different cat fish together or separately have little or no effect in their growth performance as well as their survival. The major preliminary condition in setting up a polycultured system is to identify an ideal stocking ratio which takes into consideration the intensity of species interaction and utilization of different ecological strata's and a better valorization of the water body (Billad, 1980).

In a catfish/tilapia polyculture system, stocking tilapia at densities equal to or greater than 25% of the weight of stocked catfishes (Hash, 1980). The positive effect of polyculture with predatory fish species in an additional source of food which is later represented by tilapia larvae (Pompa, 1978). Different combination of fish species in polycultured systems have been practiced throughout the world (Elmendo, 1980). Studies on the growth performance and survival of fish especially salmon species under the mono and duo culture systems have been reported. Salmon species in duo culture system had better growth than those in monoculture system. (Mork, 1982; Nor, dvedt and Holm 1991) reported that salmon species in duo culture system had better than those in the mono culture system.

However, salmon reared in duo culture did not grow significantly better than those reared in monoculture, no difference in growth increments between monoculture of one species and polyculture of several species within the same period (Shepherd, 1988).however one species might affect the environment to prove the growth condition of the other species, these increased stocking density will increase inter-specific and intra-specific competition and fish production will slow down the body weight at harvest of catfish (160-190g) was twice those of tilapia (50-70g) range (Alan, 1994). Experimental studies on the hybridization of *Heterobranchus longifilis* and *Clarias gariepinus,* which lead to hybrids with valuable characteristics for culture (Heent and Lublenkhot, 1985).

Hybrid morphology was intermediate to that of the parents and had a faster growth and survival (Legendre et al., 1991). Intra-specific hybridization of fish has been considered to combine valuable traits from two or more species to obtain hybrids that exceed both parents' species (Pan and Zeng, 1986).

The Nile Tilapia (*Oreochromis Niloticus)* generally is good for polyculture traits because it

does not affect the growth and production of most of the species (Cruz, 1980). Observation shows that, the highest stocking ratio (clarias manganese/ tilapia were 1:4 and 1:8). Had a higher but lower individual weight gains (sunset and Bayne, 1978). The production in a tilapia monoculture system was lower than in polyculture with macrobrachium (Guerrero et al., 1977). An individual species could be used as a predator for recruitment control under different stocking ratio (Bedaroi, 1985). The aim of catfish/tilapia polyculture system is to increases productivity based on the availability of tilapia larvae (Stainer, 1979). Most of the commercial feed millers in Nigeria are poultry based, fish feed production remain negligible and often incidental through the methodology of producing fish feed is not quite different from poultry ; it consumes more time and money than poultry. Many of the machines required are not even available within the country and where they can be improved local fabrication, the funds become a problem to the medium scale farmers. Fish body is mainly protein especially animal sources (fish meal) is always canvassed (Lovell, 1980).

LITERATURE REVIEW

According to Fagbenro and Davis (2003) although only two species of anaerobic bacteria, namely *Clostridium botulinum* and *eubacterbium tarantellae*, have been implicated as fish pathogens, and it shows that through biological investigation anaerobes cause more widespread problems than has been hitherto realized (Davies, 1969). In the first place, diagnostic laboratories do not normally use anaerobic methods. Therefore, it is unlikely that isolation of an offending anaerobic pathogen would ever be achieved. Consequently, the cause of disease may not be recognized, or maybe attributed to an aerobic secondary invader.

Although there is no evidence that anaerobic pathogens have been missed, there are puzzling causes of mortalities among fish populations for which the aetiological agent has never been isolated. Of course, this could reflect the use of inappropriate methods. It is recognized, however, that anaerobes occur in aquatic sediments (Davies, 1969; Rouhbakhsh-khaleghdoust, 1975) and in the gastro-carried out in hatcheries with hormonal induction. Farmers have found that homoplastic pituitary gland cheaper, practical and more highly reliable than the imported synthetic hormonal analogues.

The *C. gariepinus* broostock weight used for artificial breeding ranges between 0.3kg and 2kg (Olaleye, 2005). Despite the breakthrough with use of hormones in induced spawning; fry survival is still beset with a number of biotic and abiotic factors (Sylvester, 2005). The biotic factors include cannibalism, heavy predation by frog/aquatic, insects and the abiotic factors include water temperature, dissolved oxygen (>4.5mg/L-1), levels of ammonia. During the first week after stocking, the most critical factors for the successful nursing of the catfish larvae are the availability of zooplanktons.

According to Ayinla (2008), feeds and feeding of the larvae, fry and fingerlings of the catfish have been most studied and shown to influence the growth and survival of the fish. Studies have revealed that live zooplankton is the preferred larvae food. Many small holdings merely rear larvae to fingerling size in organically fertilized ponds as a density of between 30-1000 larvae/m2 (Olaleye, 2005). Fingerlings are stocked into rearing ponds at a rate of 50-75 fish/m3 under good management. Because of the cannibalistic nature, multiple sorting is essential (Eyo et al., 2001). For outdoor fry/fingerlings rearing, screening of the tanks with mosquitoes nets is recommended to prevent dragonfly and other predatory insects from breeding in the ponds. Poly-culture of *Clarias gariepinus* and tilapia species is practiced. A poly-culture of *Clarias gariepinus* and *Oreochromis niloticus*, intergraded with poultry with some supplementary feeding had been shown to be viable. Feed and feeding of catfishes in grow outs ponds are perhaps the most documented in literature. Various efforts have been made to establish the crude protein and amino acid requirement of c. gariepinus. Ayinla (2008) recommended 35% and 40% crude protein (CP) for raising table size and brood stock respectively.

Of the 10 essential amino acids (EAA) required by warm water fish species, only 3 EAAs studied have been documented and these are arginine, methionine and lysine (Fashakin and Balogun, 1996). In order to formulate and compound aqua feeds that will meet the nutritional requirement of the catfish at affordable cost, several conventional and non-conventional animal by-products and plant residues have been tested to substitute or replace fishmeal (Ayinla, 2008). Feeding development has moved from the use of single ingredients, broadcasting un-pelleted meal to pelleting and in fact the use of pelleted floating feed which has made a big difference to aquaculture development in Nigeria as *C. gariepinus* is being raised to maturity within 6 months. The yearnings of farmers and scientists to have a farmed catfish that combines the fast growth traits of *Heterobranchus* spp and early maturing traits of C. gariepinus led to the development of a hybrid '*Heteroclarias*'spp. The technology was widely accepted as it gave 58%

internal rate of return (IRR) on investment (Adeogun et al., 1999).

Oresegun et al., (2007), stated that early fish farmers in Nigeria raised their fish in burrow pits, abandoned minefields and in earthen ponds on extensive production system. The introduction of concrete tanks allows for manageable pond size and modification of the environment through a water flow-through system and supplementary feeding thus allowing for higher fish yield. The advent of the indoor water re-circulatory system (WRS) has ushered in a new prospect for aquaculture.

The introduction of WRS has created a turning point in the production of catfish in Nigeria. The story of aquaculture in Nigeria is essentially the story of catfish culture and the hope of fish supply in Nigeria hangs on its development and culture. Recent trends all over the world, points to a decline in landing from capture fisheries, an indicator that fish stocks have approached or even exceeded the point of maximum sustainable yield. Aquaculture therefore remains the only viable alternative for increasing fish production in order to meet the protein need of the people. It was observed that of the over 30,000MT of various freshwater and brackish water fish caught in the year 2000, catfishes were more abundant next to tilapia. FAO (1993) reported that 27, 488MT of catfishes produced in 1990 were consumed locally. This implies that there is still great need for higher production for both local and international markets. Fagbenro et al., (2003), a number of problems confront the production of catfish. Prominent among these are: poor management skills, scarcity of good quality seed, lack of capital, high cost of feed, faulty data collection, lack of environmental impact consideration and marketing of products. Many people who are currently engaged in catfish farming lack management skill. Although there has been a lot of research work on the production of catfish feed and feeding, the use of cheap feedstuffs to replace or substitute fishmeal catfish farmers still rely on the costly, mostly imported pellet floating feed (Adewunmi.2005).

The success of the industries for channel catfish, rainbow trout and the salmonids in the USA is due mainly to the availability of pelleted diets formulated based on the results obtained from the nutritional studies of fishes over many years (Absoloom et al., 1999). There is urgent need for coordination of such research work and feed manufacturers' access to the relevant data for quality and relatively cheap feed production. The federal/ state governments' public/ private partnership initiative programmes and the various private concerns establishing standard hatcheries are gradually yielding results to solve the problem of seed scarcity. However, to produce good quality seed, aquaculture needs to explore the potential of genetics. As at today, most teaching institutions do not have well equipped genetic laboratories where research can be carried out on the production of genetically improved catfish species (Omitoyin, 2007). Considerable effort had been devoted to the study and production of *Clarias* and *Heterobranchus sp* in Nigeria.

MATERIALS AND METHODS

SAMPLE COLLECTION

Ten samples of *Heteroclarias* fish (five male and five female) were obtained from Ajima fish farm Kuje Abuja, and transported to the aquaria in the department of biological sciences, University of Abuja. The fishes being starved for 48hrs in order to allow feed taken to be digested and to empty the gut content in preparation for easy dissection.

Ten samples of *Heteroclarias* fish consist of five male and five female is being differentiated from the female by the means of the reproductive organ in that the male has the papilla which is like the penis in man and the female has the oviduct close to the anus and red in color.

The aquaria used for this project was well aerated and also covered with mosquito net to prevent the fishes from jumping out and also to prevent intrusion of insect and other foreign bodies e.g. lizard, geckos, etc. Fresh water was used throughout the experiment .The fishes used were mainly adult sizes.

ISOLATION OF BACTERIA FROM THE DIGESTIVE TRACT OF FISH

After 48 hours, the fishes were washed with sterile distilled water to remove unwanted particles a period of fasting for 48hrs allows the isolation of bacteria from the enteric content to be easy leading to the suggestion that the tract becomes effectively sterile soon after emptying (Margolis, 1993), thus the feeding history of the organism before its investigation appears to be a primary influence over the qualitative and quantitative nature of its micro flora.

The micro-organisms presence in fish gastro-intestinal tract arise from either the surrounding water or on food particles. In experiment run on fresh water, the bacteria expelled on faecal particles are capable of extensive proliferation. this is possible due to their association with the concentrated organic in the faeces and subsequent distribution into surrounding water. These observations suggest that the bacteria are adapted for existence in the enteric habitat and that this association may be significant

source of luminous cells in the fresh water environment.

Media used for this research include Nutrient agar (NA), MacConkey agar (McA), Yeast extract agar (YEA), Potatoes Dextrose agar (PDA), Salmonella Shigella agar (SSA) Eosin methyline Blue agar, (EMB) and this agar were all prepared according manufacturers instruction.

PREPARATION OF SERIAL DILUTION FOR THE FISH SAMPLE

After 48hrs the fishes were being washed with distilled water, the fishes were also dissected to remove the entire gastro intestinal tract with sterile knife .The digestive tract surface was being homogenized in the same sterile distilled water for centrifugation, each intestine was immediately dissected with sterile knife to open the intestine and also a rubber spatula was used to scrap out the content found in the intestine of the fish and it is being placed into a sterile container (Ten sterile container)and these content of the gastro-intestinal tract was homogenized using sterile distilled water and each were being transferred to ten test tube for centrifugation which took place for l5mins at l500rpm(rotation per minutes) after which the supernatant part of the fluid was being collected separately into another test tube there by separating it from the decant.

Then 9m1 of sterile distilled water was being placed into 5 test tubes each and the homogenized solution of Male 1,2,3,4,5 and female 1,2,3,4,5 also were serially diluted (that is 1ml of the homogenized supernatant solution)into test tube of $l0^{-1}$, 10^{-2}, $l0^{-3}$, 10^{-4},10^{-5} dilution. These dilution were pour plated on nutrient agar, MacConkey agar, EMB agar, potatoes dextrose agar, salmonella shicrel1a agar, yeast extract agar.

INOCULATION AND GROWTH

The five (5) male and five (5) female stock culture were placed inside a sterile container and about 5ml of the stock culture were placed in a test tube and centrifuge for about 15 min for about 1500 rpm and immediately the serial dilution was done up to five (5) dilution using different sterile pipette, aliquote of 0.lnil of each dilution was inoculated into MacConkey agar, Nutrient agar, Salmonella Shigella agar, yeast extract agar, EMB agar, Potatoes dextrose agar.

Pour plate method was used mainly in this period and the plate were allowed to dry together with the main culture at dry room temperature of 25°C to 30°C. Then the plates were incubated in an inverted position for 24 hours, exceptional cases were that of potatoes dextrose agar which was kept for 72hours (3 days) and in some case more than that to ensure that there was growth.

It was observed also that the yeast extract agar there was no growth even all several pour plating. The total number of colonies formed was recorded at different intervals, tabled in the result. After the growth of the colonies, discrete colonies were aseptically sub cultured with Nutrient agar (NA), MacConkey agar (McA), Eosin methylene Blue agar, Salmonella Shigella agar and thus the plate were further incubated in an inverted position for 24hours further sub culturing way done by streaking onto nutrient agar plate, EMB, SSA, MacConkey, FDA agar and incubated at 37°C for 24hours.

MAINTENANCE OF STOCK CULTURE

Stock culture of the isolated organisms gotten from both male and female were maintained on nutrient agar, slope at temperature of 4°C after incubation at 37°C.More so the stock culture kept in the sterile container was kept in the fridge for preservation but before then Ethanol was added to prevent it smelling and from spoiling.

PREPARATION OF STANDARD INOCULUMS

Organism from stock culture were sub-cultured into nutrient agar start and kept in the fridge for preservation. The preserves culture served as the standard inoculum for various morphological cultural biochemical test.

IDENTIFICATION OF ISOLATES

Cultural Characteristics

The cultural characteristics of the isolates was observed after incubation for 24hours at 37°C on Nutrient agar, EMB agar, SSA, MacConkey agar while potatoes dextrose agar was more than 24hours and different types of isolates identified.

Gram Staining

Smears of isolates were first prepared by puffing a drop of water on the slide and picking an inoculum, air dry and heat for according to Willey et al., 2008. Each smear was then stained for 1 min using crystal violet. This was washed in gentle running tap water and then flooded with gram's iodine solution. It was again washed and decolorized with 95% alcohol and counter stained with safranin for 30 seconds finally it was washed, blow dried and observed under oil immersion objective.

Mount Staining

This list was preferred as described by Willey et al., 2008 that spore surface growth of the isolate from the midway between the centre and the periphery of the colony was made on a clean slide using a straight

needle. This spore which fluffy growth was gently tensed apart in lactophenol blue on a microscopic slide using an inoculating pin and inoculating loop and then cover slip was applied and examined microscope. This test was done in order to identify different fungi isolates.

BIOCHEMICAL TEST

Catalase Test

Two drops of hydrogen peroxide solution (3%) was placed in a glass slide. A loopful of the test organisms was added to the hydrogen peroxide solution. Presence of gas bubble in the culture shows positive test result. This was done according to Chessbrough, 2000.

Motility Test

The medium used was a semi-solid agar (Willey et al., 2008), it was prepared by adding 4g of nutrient agar to 15g of nutrient both in 1 litre of decolorized water. Heat was applied to dissolve the agar and 10ml amount were dispensed into test tube and sterilized by autoclaving. The test tubes and allowed to set in a vertical position. Inoculation was done by making a single stab down the centre of the test rube to about half the depth of the medium using a sterile stabbing rod. The test tube were incubated at room temperature and growth examined after about hours. Motile swarms gave a diffused growth that was visible to the naked eyes.

Indole Test

This test was done according to Chessbrough 2000. Pure culture of the organism were inoculated in to physiological saline and incubated at 37oC for 48 hours. Indole production was tested using 3 drops of KOVAL REAGENT made up of alcohol of 15ml, p-dimethyl amino benzaldehyde 1g and concentrated hydrocholoric acid 15ml, culture in which red colour developed are positive while absence of red colour is negative.

Oxidase Test

As stated in Chessborough, 2000, oxidase reagent containing %10 (w/v) tetramethyl-p-phenyldiamine dihydrocholoric acid was prepared by dissolving 0.1g of this compound in 10ml of deionized water strip of the filter paper was placed in a petri dish, 2-3 drops of the prepared oxidase reagent was added, smears of the isolates from the pure culture was made in the oxidase paper strip using glass or a piece of stick and not an oxidized wire loop and observation for color change was blue to purple or violet. For oxidase-negative bacteria, there is usually no colour change. This change of colour is due to the possession of cytochrome.

Hydrogen Sulphide Production Test

As described in Chessborough, 2000, this was performed using triple sugar iron (TSI) agar. The TSI was sterilized using autoclave and allowed to cool to 45oC. 9ml of TSI was disposed in test help in slant position and allowed to solidify. A loopful of the organisms was then streaked and stabbed on it and incubated at 37oC for 24-48hours. The presence of darkened colouration was positive for this production.

Lactose Sugar Fermentation

This test was carried out to know the ability of an isolate to metabolize lactose sugar (glucose, sucrose, lactose, mannitol etc) with the resultant production of acid and gas or either into 1 liter of 1 % (w/v) peptone water added to 3m1 of 0.2% (w/v) bromo cresol purple and dispensed in 9ml amount in to test tube that contained inverted Durham tubes.

The medium was then sterilized by autoclaving . the sugar solutions (glucose, sucrose and lactose) were each prepared at %10 (w/v) and sterilized. 1ml of each sugar was dispensed aseptically in to the test tube. The medium was then inoculated with the appropriate isolate and the culture incubated at 37°C for 48hours and examined for acid and gas production. A colour change from purple to yellow indicated formation while gas production was assessed by the presence of bubble in the inverted Durham's tubes.

Coagulase Test

As stated in Cheesbrough, 2000 this test is use to identify this organism that produce the enzymes coagulase. A drop of distilled water was placed on each end of the slide. This was emulsified by a colony of the test organism. A drop of plasma was added to one of the suspension and mixed gently, clumping of the organism within 10 seconds indicated a positive result.

TOTAL VIABLE COUNT

The total viable count of each samples of the fishes was done by dividing the incubated plates which had colonies into four parts equally and pick a particular part of the plate multiply it by a total number of divided plate and also multiply it by the reciprocal of the number of test tubes used for serial dilution (five test tube) which is 10^5.

RESULTS

The result of the identification of micro-organism population in fish in different samples, colonial morphology, cultural characteristics unit and biochemical reaction are shown in table 1, Nine

bacteria isolate and four fungal isolate were isolated. For the bacteria isolate for the male fish include; *Escherichia coli, Pseudomonas fluorescence*, *Staphylococci aureus, Klebsiella aerogenes, Salmonella enteridis*, *Aspergillus niger* while for the female, they include *Pseudomonas fluorescence, Escherichia coli, Staphylococci aureus*, *Bacillus cereus, Klebsiella aerogenes*, *Actinomycete spp, Aspergillus niger, Penicillum digitatum, Enterococcus spp, Mucor* spp*, Proteus vulgaris, Enterobacter spp.*

The identification of *Pseudomonas flourecence* was based on aerobic gram-negative rod, indole negative, formation of fluorescent green on MacConkey agar and oxidase positive.

Escherichia coli was gram negative when stained facultative anaerobes and also aerobes, non-motile, oxidase negative, indole positive, H_2S negative and shows greenish metallic sheen an Eosin methylene blue. *Klesiella aerogenes* was lactose fermentation positive and do not produce Hydrogen Sulphide (H_2S) producing mucoid pink colonies on MacConkey agar.

Staphylococcus aureus was gram positive cocci, oxidase negative, catalase positive (effervescence positive) coagulase positive.

Samonella enteridis inform was gram negative rods, indole negative, lactose negative, gas was produced from glucose fermentation. Blackening in the medium was due to hydrogen sulphide.*Bacillus cereus* was motile, non-lactose fermenting and it is mainly gram-positive rod.*Proteus vulgaris* was non-lactose fermenting but it was able to ferment glucose and sucrose. It was also positive for indole test and also for motility test and they is also gram negative.

Enterococcus spp are aerobic organisms, they were able to ferment lactose, they were negative to catalase test, produced a small dark red magenta color on MacConkey agar, they are non-motile. *Enterobacter spp* has gram negative motile rod and was able to ferment both lactose and glucose and also grows purple on Emb agar. *Penicillium digitatum* had folded growth with a grey fluffy mycelium. It possessed chains of spherical conidia and also has hyphen on a straight line. *Mucor spp* had white fluffy growth on the Petri dish (i.e. potatoes dextrose agar). *Aspergillus niger* had whitish fluffy spread growth after some days and also had balls of sporangium and lots of hyphen attached to it after checking it under microscope.

Actinomycetes spp has a black folded growth and also when checked under microscope it had a unique conidia structure.

Table 2, shows the biochemical reactions of some enteric bacteria and other enteric organisms found in the experiment, such properties were used to know the population of bacteria found in fish.

Table 3, showed the total viable count (TVC) found in plate after incubation of twenty hours by serial dilution method.

The result in table 4, represent the weight and length of each fish from the pond, But it should be noted that most bacterial isolated from the samples werepredominantly gram negative and some were predominantly fungus species. The total counts of the suspension are obtained by multiplying the number of colonies per plate by dilution factor which is the reciprocal of the dilution. The changes in respective values of the total count during the study period are presented in table 5; it shows a continuous increase in the total viable count. This was as a result of the organism having the ability in the medium as they utilize the nutrient component of the nutrient of the nutrient sugar as a silo source of carbon and energy. Bacterial isolated, recovered from the intestine of Heteroclarias were identified to its adult level where possible.

TABLE 2: BIOCHEMICAL REACTIONS OF SOME *ENTEROBACTERIA* AND OTHER ENTERIC ORGANISM.

Species	Lact	Glu	Suc	Mot	Indo	H2S	Gas
Escherichia coli	+	+	D	+5	+2	_	+2
Shigella species	_	+	_	_	d	_	-3
Salmonella typhi	_	+	_	+	_	+weak	_
Most other							
Salmonella	_	+	_	+	_	+2	d
Klebsiella							
Aerugenes	+	+	+	+	-3	_	+
Enterobacter sp	+	+	+	_	-3	_	+
Proteus vulgaris	_	+	+	+	+	+	d

KEY: Lact=lactose, Glu=glucose, suc=sucrose, mot=motility, indo=indole test, H2s= hudrogen sulphide(blackening), d=different strains give different results

TABLE 3: Total viable count (TVC) 10^{-5} dilution

Species of fish	**Types of agar**	**No of colonies**	**Cful/ml**	
MALE 1	NA	80	8.0×10^{6}	
2	NA	128	1.28×10^{7}	
3	NA	112	1.12×10^{7}	
4	NA	96	9.6×10^{6}	
5	NA	72	7.2×10^{6}	
FEMALE 1	NA	50	5.0×10^{6}	
2	NA	100	1.00×10^{7}	
3	NA	36	3.6×10^{6}	
4	NA	68	6.8×10^{6}	
5	NA	28	2.8×10^{6}	
Species of fish	**Types of agar**	**Number of colonies**	**Cfu/ml**	
MALE 1	McA	36	3.6×10^{6}	
2	McA	156	1.56×10^{7}	
3	McA	164	1.64×10^{7}	
4	McA	160	1.60×10^{7}	
5	McA	84	8.4×10^{6}	
FEMALE 1	McA	88	8.8×10^{6}	
2	McA	224	2.24×10^{7}	
3	McA	180	1.80×10^{7}	
4	McA	232	2.32×10^{7}	
5	McA	56	5.6×10^{6}	

Species of fish	**Type of agar**	**Number of colonies**	**Cfu/ml**
FEMALE 1	Emb	80	8.0×10^{6}
2	Emb	124	1.24×10^{7}
3	Emb	208	2.08×10^{7}
4	Emb	260	2.60×10^{7}
5	Emb	104	1.04×10^{7}
MALE 1	Emb	84	8.4×10^{6}
2	Emb	140	1.40×10^{7}
3	Emb	96	9.6×10^{6}
4	Emb	100	1.00×10^{7}
5	Emb	40	4.0×10^{6}
Species of fishes	**Type of agar**	**Number of colonies**	**Cfu/ml**
MALE 1	SSA	48	4.8×10^{6}
2	SSA	80	8.0×10^{8}
3	SSA	76	7.6×10^{6}
4	SSA	124	1.24×10^{7}
5	SSA	212	2.12×10^{7}
FEMALE 1	SSA	252	2.52×10^{7}
2	SSA	224	2.24×10^{7}
3	SSA	188	1.88×10^{7}
4	SSA	120	1.20×10^{7}
5	SSA	72	7.2×10^{6}

TABLE 4. Bacterial load, mean_+ standard deviation (cfu g-1) in the intestine heteroclarias fish in different types of agar using Number of colonies.

SEX OF SAMPLE	WEIGHT F FISH	NUTRIENT AGAR	MACCONKEY AGAR	EMB AGAR	SSA AGAR
MALE1	34.1+- 1.0				
2	32.7+-1.0				
3	33.6+_1.7	$9.76+\_4.1\times10^{6}$	$3.36+\_2.6\times10^{7}$	$3.00+\_2.5\times10^{7}$	$4.70+\_2.8\times10^{6}$
4	37.4+_2.4				
5	35.2+_1.5				
FEMALE1	36.5+_1.8				
2	35.4+_1.2				
3	34.7+_1.2	$4.00+\_2.3\times10^{6}$	$4.20+\_2.6\times10^{7}$	$4.88+\_3.5\times10^{6}$	$2.80+\_2.1\times10^{7}$
4	35.8+_1.4				
5	32.5+_1.3				

TABLE 5: The change in respective value of total viable count during incubation.

Day	*p.flourescence*	*E.coli*	*S.aureus*	*B.cereus*	*k.aergenes*	*enterc×occus*	*p.vulgaris*	*enterobacter*
0	$\mathbf{64\times10^{6}}$	$\mathbf{5.4\times10^{6}}$	$\mathbf{4.9\times10^{6}}$	$\mathbf{5.6\times10^{6}}$	$\mathbf{4.6\times10^{7}}$	$\mathbf{8.6\times10^{6}}$	$\mathbf{9.6\times10^{7}}$	$\mathbf{3.2\times10^{8}}$
7	$\mathbf{7.5\times10^{6}}$	$\mathbf{8.2\times10^{6}}$	$\mathbf{4.7\times10^{7}}$	$\mathbf{3.2\times10^{6}}$	$\mathbf{1.5\times10^{6}}$	$\mathbf{5.0\times10^{6}}$	$\mathbf{6.2\times10^{6}}$	$\mathbf{4.8\times10^{8}}$
14	$\mathbf{6.5\times10^{7}}$	$\mathbf{3.0\times10^{7}}$	$\mathbf{5.4\times10^{6}}$	$\mathbf{7.5\times10^{7}}$	$\mathbf{7.2\times10^{6}}$	$\mathbf{4.8\times10^{6}}$	$\mathbf{5.6\times10^{6}}$	$\mathbf{4.2\times10^{6}}$
21	$\mathbf{5.4\times10^{6}}$	$\mathbf{5.4\times10^{6}}$	$\mathbf{9.4\times10^{6}}$	$\mathbf{2.5\times10^{6}}$	$\mathbf{8.4\times10^{6}}$	$\mathbf{7.2\times10^{7}}$	$\mathbf{5.2\times10^{7}}$	$\mathbf{6.1\times10^{6}}$

DISCUSSSION

The intestine of fish contains a great diversity of bacterial and fungal isolates withmany of the organisms belonging to a particular group (Margolis, 1993).it is widely postulated that enteric tract of fresh water fishes, do not contain autochthorous micro flora, but instead serve as enrichment vessels for bacteria ingested on or in their food (Horsely, 1997). After a period of extended fasting, the isolation of bacteria from the enteric content is difficult, leading to the suggestions that the trait become effectively sterile soon after emptying (Margolis, 1993). Thus the feeding history of the organism before its investigation appears to be a primary influence over the qualitative and quantitative nature of its micro flora. The E. coli isolated in this work is an indication that there is faecal contamination in the water where the fishes are living or feed with which they are being fed, since E. coli is an indicator organism. This calls for caution in the consumption of such fishes or where necessary, the cooking must be done properly to destroy this potential pathogen. Also, for the *Salmonella enteritidis* that was involved precaution must be taken by monitoring the kind of feed given to the fishes and also the fresh water used should be changed at specific period. Due to the fact that one of the ultimate goals of this study was to determine the presence and identify micro organism under usage of zooplankton for feeding *Heteroclarias* fish was chosen.

Unless otherwise noted, the specimen utilized in this study were dissected after 48hours of collection, it was observed that the presence of food material in the alimenting tract often correlate with high concentration of enteric micro flora, however no systematic study of this relationship was made. The appearances on the colonies on serial dilution (10^{-1}, 10-^{2},10^{-3}, $10^{-4'}$, $10\text{-}^{5)}$ on the nutrient agar (N.A) plastic were raised, flat, flat convex and raised, in elevation while in colour were white,dirty yellow, creamy1 white, white respectively, while on the other hand for MacConkey Petri dish was raised, flat and on the other hand in colour it was yellow1 brown with large colony like, and also there was formation of fluorescent green and pinkish growth while on the Eosin methylene blue agar Petri dish was mostly flat colony and few raised and colour were mostly greenish metallic sheer,shining pinkish growths and red colour respectively and for the salmonella Shigella agar was flat mostly and in door it was Mostly black and all round due to the hydrogen

sulphide it Produced. On potatoes dextrose agar was white, fluffy shape and some were black and also round in shape.

All the selected strains were identified either as gram positive or gram negative organism. They are either cocci or rod in shape. All the strains were oxidase negative except *Pseudornonas flourescence* which is positive. The result obtained from the biochemical and carbohydrate fermentation are stated in table 1.

According to Bergey's manual of systemic bacteriology the above table 1) biochemical and carbohydrate fermentation tests indicated that the characters represented by plate of $10^{-1,}$ lO^{-2}, $10^{-3,}$*1O*$^{-4}$*,10*$^{-5}$ *are mostly Staphylococci aureus and Bacilli cereus* and the Ones found on MacConkey were *Pseudornonas spp, Kiebsiella spp,proteus spp*, *Enterococcus spp* and on the Eosin methylene blue (EMB) Petri dish was mostly *Enterobacter, Kiebsiella* and *Escherichia Coli* and on the potatoes dextrose agar plate was mostly *Aspergillus niger* and *ActinomyceteS* due to the sporangium and hyphae seen in microscopy and whitish fluffy colour seen on the plate.

From the result in the table 5.0, the analysis on bacteria Carried out on a day shows lesser microbial load, followed by day seven analysis on the bacteria in fish which shows a higher microbial load than that of day one and finally, the analysis on bacteria on day twenty one shows the highest microbial load. This data of microbial load present in the Petri dish was discovered to be due to sorne factors leading to the multiplication of organism on nutrient agar, Eosin methylated blue agar,MacConkey agar, Salmonella shigella agar.

The report gotten from the study establishes important points concerning the ecology of fresh water bacteria.

1. They are commonly present in the gastro intestinal tracts of many fresh water fishes.

2. Their presence in fish gastro intestinal tract can arise from ingestion of bacteria (from the surrounding water or on food particles).

3. In experiments run in sterilized fresh water, the bacteria expelled on faecal particles are capable of extensive proliferation (possibly due to their association with the concentrated organism in the faeces) and subsequent distribution into surrounding water.

These observations suggest that luminous bacteria are well adapted for existence in the enteric habitant and that this association may be a significant source of luminous cells in the fresh water environment. In addition, the same species of bacteria are generally present both in the entenc tract.

In this way the contribution that these microbes make to the degradation and mobilization of organic compound can be estimated.

CONCLUSION

In conclusion, the result that showed that bacterial load in The intestine of Heteroclarias fish varies in its gender (i.e male and female), the bacterial load in fish might be increased with the increase of water temperature (Fernades et al., 1997; Hasseen et al., 1999). By monitoring the bacterial load in fish from growing pond, which will affect the storage life and quality of the fishery product the quality of fish being produced can be measured.

The predominant bacterial flora consisted of gram negative rods. The bacterial flora detected in the intestine of Heteroclarias fish varied in its gender. *Pseudomonas fluoresces, E. coli, S. aureus and Salmonella* was dominated fl all bacterial isolates examined from intestine of Heteroclarias fish.

The high prevalence of *Pseudomonas fluoresces, Escherichis coli* and *salmonella spp*, throughout the study period suggest that these bacteria may be common bacteria commensal in Heteroclarias intestine. Ruane et al., (1997) as observed in significantt coliform, including E. coli in fish culture ponds at 24-29°C, it may be expected that the bacterial flora in Heteroclarias intestine would also vary.

The bacterial presence or the particular species complex detected in the intestine of Heteroclarias fish probable had effect on fish disease prevalence. None of the fish sampled was dead (that is it may be due to cannibalism). No pathogenic bacteria are species for Heteroclarias, but some affect them more severely than they do in other cultured fishes. In this present study isolation of *E..coli, P. fluoresces, Salmonella spp, Staph. aureus*, which are facultative pathogen or agent of food poisoning is of utmost importance. The present result indicated that the commensal bacterial flora included facultative pathogens which under conditions of stress could give rise to fish epizootics (Billard, 1980).

This information will help in controlling the storage life quality of the fishery product arid if considerable effort will be devoted to the study and production of *Heteroclarias* fish, cat fish farming will attract private sector initiative compared to earlier public or government - sponsored programmes (Balogun et al.,1992). If the associated problems of reductions especially the issue of feed production and fingerling supply are tackled, Nigeria will soon become a world exporter of cat fish.

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