



Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 2(12) pp. 315-321, December, 2013 Special Anniversary Review Issue.

Available online <http://garj.org/garjas/index.htm>

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Full Length Research Paper

Detection of fish saprolegniasis in thomas dam and challawa commercial fish pond.

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Accepted 12 December, 2013

The incidence of saprolegniasis in Thomas dam and challawa commercial fish ponds was studied in the 2008 wet season. Alongside this, the physicochemical parameters of the two ponds were also determined. During the study, two common fish species; *Claria* and *Tilapia* were considered. The length and weight of each specimen were determined. Occurrence of disease/infection due to saprolegniasis was also determined using simple pathogenecity test. Results of the study have shown that the incidence of saprolegniasis was highest in Thomas dam, a natural water reservoir than Challawa fish pond and female fishes were more affected than the male fishes and Clarias were more infected than the tilapias. Paired T-test revealed that there were significant difference ($P>0.05$) between the disease incidences (%) in the two study sites as well as between the fish species

Keywords: Saprolegniasis, disease incidence (%), Thomas dam, Challawa fish ponds

INTRODUCTION

Water moulds, *Saprolegnia* are responsible for devastating infections on fish in aquaculture. Members of the genus *Saprolegnia* causes a disease called "*Saprolegniasis*" (Bakers et al., 1994, Roberts, 1989). The Oomycete *Saprolegnia* is economically one of the most important fish pathogen especially on catfish species (Bala, 2007). It is often considered an opportunistic pathogen that is saprophytic and necrotrophic (Bruno and Wood, 1999). However, it has become apparent that some *Saprolegnia* strains are highly virulent and are able to cause primary infections on catfishes (Roberts, 1989). *Saprolegnia* is

ubiquitous in freshwater ecosystems and is the main genus of water mould responsible for significant fungal infections of fresh-water fish and eggs. Almost every freshwater fish is exposed to at least one species of the fungus during its life time (Neish, 1991; Noga, 1996) especially from the egg stage through simultification (Bruno and Wood, 1999; Pickering, 1994). On fishes, *Saprolegnia* invades epidermal tissues, generally beginning on the head or fins (Neish, 1991, Wi1loughby, 1994) and can spread over the entire surface of the body. Visible as white or gray patches of filamentous mycelium (Bruno and wood, 1999; Beakers et al., 1994), *Saprolegnia* is characterized by an external cotton-like appearance that radiates out in a circular crescent-shaped or whorled pattern. Pickering and Willoughby (1982) suggest that, there are differences in the

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patterns of infection between hatchery fish and wild fish. *Saprolegnia* also infects moribund eggs by adhesion to and penetration of the egg membrane (Willoughby, 1994), and can spread from dead eggs via positive chemotaxis (Bruno and Wood, 1999). In catfishes, the physiological state of the fish generally determines if a fungal infection will be successfully established (Neish, 1991, Snieszko, 1994). *Saprolegnia* generally invades fish that have been stressed or otherwise have weakened immune systems (Bruno and Wood, 1999; Pickering, 1994). Since the fungus is almost always present in fresh-water, it is assumed that some changes in the fish occurs which allows a *saprolegnia* infection to take hold (Bruno and Wood, 1999). Neish (1991) suggests that immuno suppression provides a mechanisms that causes a transformation of normally non-pathogenic organisms, including *Saprolegnia* to become pathogenic. *Saprolegnia* has a fairly wide range of temperature tolerance, from 3°C to 33°C, which appears to reflect the thermal preference of the host (Pickering and Willoughby, 1982). However, sudden changes in temperature can make fish vulnerable to *saprolegniasis* due to the increased physiological stress (Bruno and Wood, 1999; Willoughby, 1994). Conditions that render fish susceptible to *Saprolegniasis* include but are not limited to the following; physical activity on spawning beds (Bruno and Wood, 1999), sexual maturity (Pickering and Willoughby, 1982), water temperature change (Bruno and Wood, 1999; Howe et al., 1999), water quality (Pickering, 1994), pathogens and parasites, (Meyer, 1991), handling (Hatai and Hoshiai, 1994), pollution (Snieszko 1994) and crowded hatchery conditions (Whister, 1996). When these conditions are met especially in commercial ponds with natural sources, the disease (*saprolegniasis*) prevails and may reach epidemic proportion and as a result, greater part of the profit is lost resulting into a complete failure in commercial fish production, on which most developing countries depends on. This article reports the occurrence of *saprolegniasis* in two water reservoirs; Thomas dam and challawa commercial fish ponds in Kano State.

MATERIALS AND METHOD

Study Sites

Two sites were used for the purpose of this research and these were Thomas Darn along Kano-Danbatta road and Challawa Commercial Fish Ponds along Panshekara Road. Both the sites are been used for fishing activities and they were designated A and B, respectively. Both are found in Kano state located between 16°47 North and 2°24 East as reported by (Ajayi, 1999).

SITE A: Thomas Dam

Thomas was established in 1973 during the Late Alhaji

Audu Bako Administration the Darn was named "Thomas" derived from the name of the stream. The Dam is about 585 square metres, while the depth is about 30m. The dam is now sited near Danmarke village under Dambatta Local Government Area of Kano State, some 30 km away from the ancient Kano city.

Sandy- loam and clay – loam are the soil types of the area of which they are rich in nutrients and other minerals. The vegetation of the area is of Sudan Savannah type. Rainy season occurs during the warm summer months (May to September). The agro-metrological station nearest to the area shows the estimated average rainfall as 385 mm during months of July and August. The total annual free water evaporation was calculated as 248 mm from the station, and monthly evaporation varies from 171m in December to 270 m in May (Mukhtar, 2000).

SITE B: Challawa Commercial Fish Pond

The Challawa Commercial Fish pond is located along Panshekara road in Kumbotso Local Government Area. The pond was bounded to Hisbah Command Office by the north, the new Abattoir building by the south, Challawa Industrial area by the East and the main road leading to Panshekara by the West. The pond has an area of 350 square metre and the pond is designed for commercial fish production.

Sample Collection

The following protocol were adopted from Rehulka (2008).

Isolation

The fishes were sampled out randomly from the water (Dam/pond) and transported to the laboratory immediately. The fungus was obtained from the fresh fish by taking inoculum from the suspected tissue onto agar medium using a sterile/dissecting needle. The surface was surface disinfected to prevent secondary contamination by airborne spores. This was done by dipping in 1% formaldehyde 1-5 minutes.

Cultivation

The purpose of cultivation is to obtain pure cultures for studying the fungus that is their variability and condition of growth; and for investigating the whole biogenesis. This was done in synthetic agar media to produce its progeny. The strains produced multi-spores that fused to form growth.

Culture Media

This was cultivated on natural substrate media, and of these, the potato dextrose agar (PDA) was used (40g glucose, 10g peptone, 15g agar, 2000ml distilled water).

Microscopy

This was adopted from the Koi and water garden society of Central New York (2008). This technique was used to examine the fresh water fish. It is probably the most common technique used in fish medicine and applicable to any hobbyist as there is only a minimal amount of apparatus required.

Preparation of the Apparatus

The key to taking a good smear was that of taking the sample as quickly and painlessly as possible so that the patient (fish) would have a minimal amount of stress and the infection would not be worsened. This was done by preparation of all the surfaces and the essential equipments. The bench was cleaned and the microscope was set, ensuring that all of its components are working correctly. The smear was placed on a smoothed, and non absorbent surface on to the bench, and this was cleaned with disinfectant. A moistened paper, towel long enough for the fish to be placed was then placed on the surface. Another moistened towel was ready to cover the fish head (as for (hued microscopy). A new clean slide and cover slip was obtained at hand. A bath was prepared to hold the fish before the actual sample is to be taken. To prepare the bath, a vessel that was suitable for the fish size was used and placed enough water into it so that the fish is comfortable. Finally, a plastic glove was put to protect the fish during handling. The slide preparation was examined at a low magnification of x 40.

Pathogenicity Test

The non infected fishes plus one of the infected fishes were kept in a laboratory aquarium for observation keeping the physico-chemical parameters for fresh water fish culture constant (e.g. temperature, pH, transparency, and dissolved oxygen).

Collection of water Sample

Two water samples were collocated using 1000 dm³ (1 liter) bottle which was lowered into the water, and some parameters were measured immediately before transporting the sample to the laboratory (i.e. the temperature, pH and transparency) for the sample needed for dissolved oxygen determination, 2ml of manganous

chloride and 2ml of Winkler's reagent were added immediately.

RESULTS AND DISCUSSION

The results of the work showed some changes in the physico-chemical and biological factors from the two study sites, A (Thomas Dam) and B (Challawa Commercial Fish Pond) both in Kano State. The variations in these parameters were probably due to the fact that site B got treatment at intervals together with regular changing of the water in the pond.

Physico-chemical parameters

The result shows a lower mean temperature at site B (25°C) and higher mean temperature at site A (26°C). Similar observations were made by Alabaster and Lind, (1980) that the tropical temperature range for normal fish growth ranges between 18-30°C. Also Lind (1992) added that any value outside the range will make the fish vulnerable to poor feeding, parasitic development and also develop vectors of parasites. The water temperature also shows positive correlation with pH, transparency, BOD and dissolved oxygen. Pickering and Willoughby (1982) also added that *Saprolegnia* can tolerate a temperature range of 3°C to 33°C, which appears to reflect the thermal preferences of the host. However, sudden changes to temperature can make fish vulnerable to *Saprolegniasis* (Bruno and Wood, 1999; Willoughby 1994).

The pH of site A was observed to be 7.5 and the value slightly increased to 7.8 at site B. The results showed that both the two sites were slightly basic. Similar report was observed by Rabi'u (2008). Also Brain and Corosfield (1993) demonstrated that the pH range for a better fresh water fish growth ranges from 6.6 to 9.3 for stream water and 6.5 - 8.5 were recorded for pond. Decrease in transparency was observed from site A (64 cm) to site B (45 cm). Low Secchi visibility was recorded at Site A, that agrees with the findings of Rabi'u (2008) that turbidity was high during the early part of the rainy season due to increase in surface run-off water flood from the catchment which brings dissolved substances and re-suspend of dissolved materials. And the lowest turbidity values was observed at Site B which could be as a result of prevailing conditions of less surface run-off and absence of flood.

Biological oxygen demand (BOD) was higher in Site A (1.26 mg l⁻¹) than site B with the concentration of (1.11 mg l⁻¹). This coincided with the period of oxygen-consumption by decomposers (Fungi and bacteria) (Rabi'u, 2008). This was also similar to the work of Akpa (2001), and Hassan et al. (2013). Table 1 shows an inverse correlation with the dissolved oxygen. There was also a difference between the sites in the dissolved oxygen (DO)

Table 1. Monthly mean physico-chemical parameters of Challawa and Thomas dam in 2008 rainy season

Parameters	Months in Thomas dam, site A				Months in Challawa pond, site B			
	June	July	August	September	June	July	August	September
Water Temp. ($^{\circ}$ C)	26	26	26	25	22	22	25	25
Transparency (CM)	240	246	252	290	360	381	735	238
pH	7.5	7.5	7.5	7.4	7.8	7.8	7.8	7.8
Alkalinity (Mg/l)	148	152	150	81	82	83	120	139
Conductivity (μ s/cm)	213	214	214	600	600	601	370	385
Dissolved oxygen (Mg/l)	12.6	12.7	12.7	9.60	9.50	9.40	27.0	22.0
BOD ₅ (Mg/l)	1.26	1.26	1.25	1.26	1.11	1.11	1.11	1.11

Table 2. Incidence Of Saprolegniasis and Some Physical Parameters of *Tilapia* sp. of Site A

S/N	Sex	Length	Weight	Disease Occurrence
1.	M	0.35	0.412	*
2.	M	0.33	0.412	*
3.	F	0.32	0.405	**
4.	F	0.33	0.411	**
5.	F	0.35	0.432	**
6.	M	0.30	0.418	*
7.	F	0.32	0.419	**
8.	M	0.31	0.423	*
9.	M	0.29	0.402	*
10.	M	0.30	0.400	*
	Mean	0.33	0.413	*
	LSD	0.110	0.120	

*= low infection, **= high infection, LSD=Least significant difference

Table 3. Infection and Some Physical Parameters of *Claria* spp of Site A

S/N	Sex	Length	Weight	Disease Occurrence
1.	F	0.41	0.506	**
2.	F	0.40	0.513	**
3.	F	0.31	0.508	**
4.	M	0.45	0.405	N
5.	F	0.40	0.430	**
6.	M	0.32	0.530	N
7.	M	0.39	0.490	*
8.	F	0.40	0.502	**
9.	M	0.23	0.470	*
10.	M	0.40	0.487	*
	Mean	3.71	0.484	
	LSD	0.23	1.320	

Table 3. Infection and Some Physical Parameters of *Claria spp* of Site B

S/N	Sex	Length	Weight	Disease Occurrence
11.	F	0.44	0.516	**
12.	F	0.43	0.523	**
13.	F	0.38	0.508	**
14.	M	0.45	0.475	N
15.	F	0.40	0.520	**
16.	M	0.45	0.510	N
17.	M	0.39	0.480	*
18.	F	0.40	0.502	**
19.	M	0.44	0.490	*
20.	M	0.46	0.497	*
	Mean	0.424	0.502	
	LSD	0.130	0.340	

N= No infection, *= low infection, **= high infection, LSD=Least significant Difference

Table 4. Infection and of Sic 13 some Physical parameters of *Tilapia spp* of site B

S/N	Sex	Length	Weight	Disease Occurrence
1.	M	0.28	0.410	*
2.	M	0.31	0.423	*
3.	F	0.30	0.430	*
4.	M	0.41	0.432	*
5.	M	0.29	0.420	*
6.	M	0.28	0.411	N
7.	F	0.28	0.419	**
8.	F	0.30	0.422	**
9.	M	0.31	0.417	*
10.	F	0.32	0.431	**
	Mean	0.28	0.4215	
	LSD	0.41	0.210	

N= No infection, *= low infection, **= high infection, LSD=Least significant Difference

with site B showing higher concentration (27.0 mg^{-1}) and A showing lower concentration. This could be attributed to the peak time of the biochemical oxygen demand due to bacteria and other decomposers uptake as observed by Rabi'u (2008). The higher concentration also coincided with time of lower temperature (25°C) observed in Site B. The higher the temperature the lower the dissolved oxygen, and the lower the temperature, the higher the dissolved oxygen (Hassan et al. 2013; Kutama et al., 2011). Earlier on, Boyd and Lichtkoppler (1979) observed that, for a better fish growth, the dissolved oxygen has to be from time concentration of 4.0 mg^{-1} to above, but when the value is continuously below 4.0 mg^{-1} , there will give an adverse effect on fishes even at levels which do not cause

mortality, making the fishes more susceptible to parasites and diseases.

Incidence of *Saprolegniasis*

There were also variations in the disease incidence (%) that were observed from the two sites of the study; A and B. There was a significant ($P>0.05$) difference in the incidence (%) with regards to the sex of the fishes. Out of the 40 samples representing the sample size, 20 were males and 20 were females. Six fishes were infected from the two sites (A and B) out of which (5), five were females and only one (1) was male. Similar report was observed by

Neish (1991), and Noga (1996) that the main genus of fresh water moulds is responsible for the significant fungal infection of fresh water fishes and eggs. Fishes usually got infected during spawning (Wolke, 2005). Scott and O'Bier (1992) also added that fishes giving parental care are liable to get infected through contaminated eggs.

Results of this work show that, the heaviest fish was 0.532kg from Site A and the lightest fish was 0.400kg in weight. The six (6) fishes that were found to be infected had weights; ranging from 0.513kg, 0.516kg, 0.517kg, 0.523kg, 0.524kg and 0.532kg from both site A and B. The results were similar to the findings of Bala, (2007) who reported that the disease causes chronic losses and usually affects harvestable fish size (>pound) equivalent to 0.450 kg, where a very high mortality can occur.

The highest length found from the results of this work was 0.52 m and the lowest 0.28 m. Length of fish and weight of fish follows isometric growth pattern (uniform), that is, as the length increases, the weight also increases (Bichi, 2007).

Pathogenicity Test

The pathogenicity test revealed that, out of the (60%) non infected *Clarias* from site A that were inoculated, only 20% *Clarias* (10% males and 10% females) became infected and the remaining 40% still remains non infected. From the 80% *Clarias* from site B, that were inoculated, only 10% became infected, and 70% still remained not infected. In general, out of the nine (22.5%) fishes that were infected, from both the two study sites with 15% and from the pathogenicity test with 7.5%, about 7 (17.5%) were females and only 2 (5.0%) were males. This is similar with the findings of Neish (1991) and Noga (1996), that the main genus of the fresh water mould is responsible for the significant fungal infection of fresh water fishes and eggs. Also Wolke (2005) added that fishes usually got infection during spawning. Since normally only female fishes that spawns then the two reports looks similar although the males can get infection as it reaches maturity stage, (Bruno and Wood, 1999; Pickering, 1994), also water temperature changes (Bruno and Wood, 1999; Howe et al., 1999).

Site A had the highest incidence than Site B and this is probably because, site B received treatment and a regular changing of water, and that helps to reduce contaminations and infections unlike site A with the highest incidence, it does not usually receive any treatment, instead, a lot of effluents are discharged to the water body and that is what raised the BOD concentration to 1.26 mg^l-¹ less than that of site B. Also female fishes were more infected, although a number of males were infected majority of which attain sexual maturity and weighted greater than 1 Pound.

CONCLUSION

Conclusively, fungal infections are among the most common disease seen in tropical fish. As fungal Spores are found in all fish tanks, they can quickly colonies and create problems in a stressed, injured or diseased fish. And poor water quality can also lead to an increase in infection in an otherwise healthy fish population. And mostly, infection are usually associated with a pre existing infection or injury and this is why two part treatment is often necessary to completely cure these infected fish.

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