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STUDIES ON COLUMNARIES DISEASE AMONG INTENSIVELY CULTURED NILE TILAPIA (OREOCHROMIS NILOTICUS) REARED IN CONCRETE PONDS

(With 4 Fig. & 3 Tables)

By

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دراسات عن مرض الكولمنارز في البلطي النيلي المستزرع في الأحواض الاسمنتيه

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عرفه العطـــــار

قامت هذه الدراسه لمعرفة نسبة الاصابه والتشخيص الدقيق وكذلك مقاومة مرض الكولمنارز فى أسماك البلطى النيلى المستزرعه بكثافه عاليه فى الاحواض الاسمنتيه ولذلك تم تجميع ٣٠٠ سمكة بلطى نيلى خلال مواسم الفحص الدورى (الشتاء ، الربيع ، الصيف ، الخريف – ٧٥ سمكه لكل موسم)

وجد أن نسبة الاصابه بمرض الكولمنارز هى صفر ، ٧ ر٦ ، ٧ ر٢ ، ٧ ر١٠ ٪ فى المواسم الاربعه على التوالى .

وأهم الاعراض المرضيه التى شوهدت على الاسماك المريضه هى إحتقان فى الخياشيم ، تأكل الزعانف وتساقط القشور وتقرحات بالجلد . بينما كانت دكانة اللون والتدمير الكامل لمنطقة البدنكل الخلفيه وكذلك تأكل الزعنفه الذيليه والشرجيه غير دائمة المشاهده . وقد وجد أن الفلكسيبكتر كولمنارز هو الميكروب المسبب للمرض حيث استطاع هذا الميكروب إحداث المرض بالعدوى الصناعيه فى أسماك البلطى الصحيحه من خلال الخياشيم بعد احداث الجروح بها ، وكذلك من خلال الخياشيم الصحيحه فى درجات الحراره العاليه .

وقد تم القضاء على المرض معملياً باضافة السلفاميرازين الى العليقه وبرمنجانات البوتاسيوم الى ماء الاحواض .

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SUMMARY

The study was carried out to investigate the prevalence, proper diagnosis and control of colmunaris disease among Nile tilapia *(O.niloticus)* intensivelly cultured in concerete ponds. A total of 300 *O.niloticus* collected throughout the seasonal diagnostic services (winter, spring, summer and autumn, 75 fish/season) revealed that the prevalence of columnaris disease were 0.0, 6.7, 2.7 and 10.7% respectivelly. The most common lesions of the disease were gill congestion, fin rot, scale detachment and skin ulceration. Darkeness of the fish body and complete destruction of caudal peduncle including tail and anal fins were of unusual observation. The causative agent of the disease was identified as *Flexibacter columnaris.* The organism was able to produce the disease in apparently healthy *O.niloticus* through injured gills, but uninjured gills also appeared to be attacked at comparativelly high water temperature. The disease was controlled in the lab. by application of sulphamerazine in the fish diet and pot. permenganate to aquarium water.

Keywords: Clumnaries disease, cultured nile fish,concrete bonds.

INTRODUCTION

Columnaries disease is an acute to chronic bacterial infection that affects all species of warm water fishes. The disease was first described by *DAVIS (1922)* and the causative agent was first isolated by *ORDAL and RUCKER (1944)* and named Chondrococcus columnaris. Subsequently, the organism was known as *Flexibacter columnaris (LEADBETTER, 1974)* which only invades fishes in the freshwater environment. However, marine fishes also suffer from columnaris-like disease and had lesions similar in appearance to those of freshwater fishes *(SAWYER, 1976; WAKABAYASHI* et al., *1986 and WAKABAYASHIA, 1993).*

The present study was planned to investigate the prevalence and proper diagnosis of columnaris disease among nile tilapia *(O.niloticus)* intensivelly cultured in concrete ponds, as well as the lab. Trials to centrol the disease in artificially infected *O.niloticus.*

MATERIAL and METHODS

A total of 300 *O.niloticus* were collected from the concerete ponds of Fish Research Center, Suez canal Univ. throughout the seasonal diagnostic services (75 fish/season). The water temperature was recorded at each sampling collection. All fish were subjected to full clinical and postmortem examination *(LUCKY, 1977)*.

The bacteriological examination was carried out on the samples collected from gills, fins, liver, kidneys, spleen and musculature of clinically diseased *O.niloticus*. The samples were streaked on trypticase soy agar, Cytophaga agar (CA) medium *(ANACKER and ORDAL, 1959)*, as well as on a selective medium for *Flexibacter columnaris* isolation *(BULLOCK et al., 1986)*. The cultures were incubated at 28°C and examined daily for 3 days. The colonies on CA and F. columnaris selective medium were picked up and used for studying the morphological, cultural and biochemical characters of the bacterial isolates *(BOOTSMA and CLERX, 1976 and AUSTIN and AUSTIN, 1987)*.

For pathogenicity test, a total of 120 apparently healthy *O.niloticus* each with 50±5g body weight were divided into 12 groups, each contained 10 fish. The fish were placed in glass aquaria supplied with declorinated tap water. The water temperature was maintained at 16±1°C in 6 aquaria and 25±1°C in the other 6 aquaria. The fish groups at each water temperature were exposed to challenge with *F.columnaris* by contact method, with and without mechanical injury of gills, and interaperitoneal injection (I/P) according to the method adopted by *KUO et al. (1981)* (Table 3).

1- Contact challenge with gill injury:

Mechanical injury on one side of the gill was done by scraping with test tube brush. The fish were allowed to bath for 60 min in a 1 : 12 dilution of the bacterial broth culture [$2x10^6$ colony forming unit (CFU)/ml]. The fish of control groups were exposed to mechanical injury of the gill and bathed in sterial Cytophaga broth at the same dilution and for the same time.

2- Contact challenge without gill injury:

The fish were bathed in the diluted bacterial broth culture for the same time but without gill injury. The fish of control groups were bathed in diluted sterilized Cytophaga broth for the same time and without gill injury.

3- Injection challenge:

The fish were injected I/P with 1.0 ml of undiluted bacterial broth culture ($2.4x10^7$ CFU/ml). The fish of control

groups were injected with 1.0 ml of sterilized Cytophaga broth.

The mortalities and clinical signs of the challenged *O.niloticus* were recorded in all groups during the experimental period. Re-isolation of *F.columnaris* from the dead and experimentally infected fish was also performed.

The disease control was established in the lab. on artificially infected *O.niloticus* by using pot. permenganate *(ROGERS, 1971)* and sulphamerazine *(WAKABAYASHI, 1991)*. Three groups of *O.niloticus* each contained 20 fish with 50±5 g body weight for each fish were challenged with *F.columnaris* by contact method with gill injury. Six days post-inoculation, *O.niloticus* of group 1 were treated externally by addition of 4 ppm pot. permenganate to the aquarium water. *O.niloticus* of group 2 were treated by oral adminstration of sulphamerazine, in fish diet, at a rate of 220 mg/Kg/day for 10 successive days. *O.niloticus* of group 3 were received the combined external pot. permenganate and the oral sulphamerazine.

RESULTS

Clinical examination of naturally diseased *O.niloticus* revealed gill congestion, detachment of scales, skin ulceration (Fig. 1) and tail rot (Fig. 2). Some cases revealed slight darkness all over the body, complete destruction of tail fin and peduncle region (Fig. 3 and 4). P.M. examination revealed no pathological alterations occured in the internal organs.

The bacteriological examination revealed that, on CA and *F.columnaris* selective medium, the isolated bacteria produced yellow pigmented colonies with convoluted center, rhizoid edges and tend to adhere to the medium. The other morphological, cultural and biochemical characters of the isolated *F.columnaris* were documented in Table (1).

Table (2) shows the prevalence of columnaris disease in cultured *O.niloticus* throughout the four seasons of the year. It reveals that the percentage of total diseased fish to the total number of examined fish in winter, spring, summer and autumn seasons were 5.3, 16, 24 and 20 respectivelly. The percentage of columnaris diseased fish to total diseased fish were 0.0, 41.7, 11.1 and 53.3 in the 4 seasons respectivelly. While, the percentage of columnaris diseased fish to the total examined fish were 0.0, 6.7, 2.7 and 10.7 in the 4 seasons respectivelly.

The pathogenicity of *F.columnaris* to *O.niloticus* with different routes of challenge and under different water temperature was documented in Table (3). The results revealed that the mortality rate of bath challenge with gill injury at

16±1°C and 25±1°C were 20 and 90% respectivelly, while the mortalities of bath challenge without gill injury at the same water temperatures were 0.0 and 40% respectivelly. The mortality rate of I/P challenge at both water temperatures were 10 and 60% respectivelly. Meanwhile, *F.columaris* produced the same clinical signs and P.M. lesions observered in natural infection and was re-isolated from all dead and moribund fish.

Application of pot. permenganate to aquarium water in combination with sulphamerazine to the fish diet succeded to ontrol the artificially induced disease in lab. while the usage of each drug alone failed to control the disease completely.

DISCUSSION

The clinical pathology of columnaris disease in Nile tilapia *(O.niloticus)* and in other scaled fish *(CHUN* et al., *1985; BULLOCK* et al., *1986; ALVARADO* et al., *1989 and BERNOTH and KORTING, 1989)* begained at the outer margins of the fins and spread inward toward the body. The most common lesions were fin rot, scales detachment, skin ulceration and gill congestion. Fish body darkness and complete destruction of tail fin and caudal peduncle musculture were of unusual observation.

The morphological, cultural and biochemical characters of the bacterial isolates isolated from naturally infected *O.niloticus* were similar to those of *Flexibacter columnaris* as described by many authores *(BOOTSMA and CLEVX, 1976; CHUN* et al., *1985; AUSTIN and AUSTIN, 1987, ALVARADO* et al., *1989; BERNARDET, 1989 and BERNOTH and KORTING, 1989).* The bacterium forms yellow colonies characterized by convoluted center, rhizoid edges and tend to adhere to CA and *F.coulmnaris* selecive medium. This type of colony has not been encountered in other Flexibacter pathogenic to fish *(BULLOCK, 1972 and BULLOCK* et al., *1986).*

Regarding to the pathogenicity of *F.columnaris* it was found that, the organism attacks *O.niloticus* only at comparatively high water temperature as the disease was discovered with high prevalence during the autumn monthes and the high mortality rate (90%) of artificial infection was recorded at 25 C°. The relation ship between water temperature and columnaris disease in steelhead trout and salmon species revealed no deathes occured at temperatures of 9.4C° and below while the mortality increased progressively with increasing temperature to 100% at 20.5C° *(HOLT* et al. *1975).* The optimum water temperature for *F.columnaris* infection was reported between 20 and 30 C° while the mortalities seldom occur at temperature 15C° *(WAKABAYASHI 1991).* Meanwhile, *F. columnaris*

infection to *O.niloticus* and other fish species *(KUMAR et al. 1986 and WAKABAYASHI 1991)* was occured when one of the natural barriers of the fish body was injured. On the other hand, *O.niloticus* and Oriental weatherfish *(WAKABAYASHI and EGUSA 1972)* of uninjured tissue also appeared to be attacked at high water temperature.

Control of columnaris disease with copper sulfate and nitrofurans was established for many years until their use as therapeutic agent was restricted in most countries because copper sulfate accumulate in the fish tissue and nitrofurans were suspected of being carcinogenic *(WAKABAYASHI 1991)*. On the other hand, sulphamerazine is authorized as one of fishery medicines in the U.S.A. and was used to control columnaris disease. Pot. permenganate was also used *(ROGERS 1971, AVAULT 1985, BULLOCK et al. 1986 and MARZOUK 1991)* to control columnaris disease. In the present study, sulphamerazine added to the fish diet was succeeded to control columnaris disease when used with pot. permenganat in aquarium water. The usage of each drug alone failed to control the disease completely as pot. perminganate was effective only when the disease primarly affects external surface of fish while sulphamerazine was effective when used at the early stage of the disease before the fish lose their ability to feed. These results supported those of *MARZOUK (1991)* who succeeded to control columnaris disease in O.niloticus by combination of pot. permenganate and streptomycin antibiotic.

It could be concluded that:

1- Columnaris disease among Nile tilapia usually occured with high prevalence throughout the monthes of comparatively high water temperature.
2- *F.columnaris* infection to fish was occurred through injured tissues but uninjured tissue was also attacked at high water temperature.
3- Successful control of the disease was established on the combination effect of sulphamerazine in fish diet and pot. permenganate in aquarium water.

REFERENCES

Alvarado V.; Stanislawski D.; Boehm K.H. and Schlotfeldt H.J. (1989): First isolation of flexibacter columnaris in eel (Anguilla anguilla) in northwest Germany (Lower Saxony). Bull. Europ. Ass. of Fish Pathol. 9, 96-99.

Anacker, R.L. and Ordal, E.J. (1959): Studies on the myxobacterium Chondrococcus. columnaris. I. Serological typing. J. Bacteriol., 78: 25-32.

Austin, B. and Austin, D.A. (1987): Gram-ngative pigmented rods. In: Bacterial fish Pathogens: Disease in Farmed and Wild Fish (Austin B. and Austin D.A. ed.) pp. 225-249. Ellis Horwood Ltd. New York.

Avault, J.W. (1985): Pond treatment with potassium permanganate. Aquacult. 11 (1): 45-46.

Bernardet, J.F. (1989): Flexibacter columnaris: first description in France and comparison with bacterial strains from other origin. Diseases of Aquatic Organisms 6, 37-44.

Bernoth, E.M. and Korting, W.(1989): First report on Flexibacter colunaris in tench (Tinca tinca L.) in Germany. Bull. Eur. Ass. Fish Pathol.9(5), 125-127.

Bootsma, R. and Clerx, J.P.M. (1976): Columnaris disease of cultured carp, Cyprinus carpio L.,: Characteristic of the causative agent. Aquacul. 7,371-384.

Bullock, G.L. (1972): Studies on selected myxobacteria pathogenic for fishes and on bacterial gill disease in hatchery reared salmonids. U.S. Wildl. Serv., Tech. Pap. 60. 30pp.

Bullock, G.L.; Hus, T.C. and Shotts, E.B. (1986): Columnaris disease of fishes. U.S. Fish Wlel. Fish Dis. Leaflet 72, 1:9.

Chun, S.K.; Park, C.Y. and Lee, S.K. (1985): Characteristics of Flexibacter columnaris isolated from tilapia (Tilapia sp.). Korean Fish. Soc. 18, 369-373.

Davis, H.S. (1922): A new bacterial disease of freshwater fishes. U.S. Bureau Fish. Bull. 38, 261-280.

Holt, R.A.; Sanders, J.F.; Zinn, J.L.; Fryer, J.L. and Pilcher, K.S. (1975): Relation of water temperature to Flexibacter columnaris infection in steelhead trout (Salmo gairdneri), Coho (Oncorhynchus kisutch) and chinook (O.tshawycha) salmon. J. Fish. Res. Canada 32, 1553-1559.

Kumar D.; Suresh, K.; Dey, R.H.; and Mishra, B.K. (1986): Stress-mediated columnaris disease in rohu, Labeo rohita (Hamilton).J. Fish Dis., 9, 87-89.

Kuo, S.; Chung, H.Y. and Kou, G.H. (1981): Studies on artificial infection of the gliding bacteria in cultured fishes. Fish pathol. 15, 309-314.

Leadbetter, E.R. (1974): Chapter on genus flexibacter. In: Buchanan R.E. and Gibbons N.E. (Co-Editors), Bergey's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore, Md., 8th edition, pp. 105-107.

Lucky, Z. (1977): Methods for diagnosis of fish diseases. Amerind Publ. Co. PVT, Ltd., New York, 1st Ed.

Marzouk, M.S.M. (1991): Attempts to control columnaris disease in cultured Oreochromis niloticus in Egypt. J. Egypt. Vet. Med. Ass. 51, No. 1&2, 119-129.

Ordal, E.J. and Rucker, R.R. (1944): Pathogenic myxobacteria. Proceeding of the Society of Experimental Biology and Medicine 56, 15-18.

Rogers, W.A. (1971): Principal diseases of catfish: how to identify identify and fight them. fish Farming Ind, 2: 20-26.

Sawyer, E.S. (1976): An outbreak of myxobacterial disease in coho (Oncorhynchus kisutch) reared in a marine estuart. J. Wildl. Dis. 12, 575-578.

Wakabayashi, H. (1991): Effect of environmental conditions on the infectivity of Flexibacter columnaris to fish. J. Fish Dis. 14, 279-290.

Wakabayashia, H. (1993): Columnaris disease. In: Bacterial Diseases of Fish. (Inglis V., Roberts R. and Bromage N., eds.). Chapter 2: pp. 23-39. Black kwell Scientific Publication, London.

Wakabayashi, H. and Egusa, S. (1972): Preliminary experiments on environmental factors influencing the prevalence of columnaris disease. Fish Pathol. 7, 58-63.

Wakabayashi, H.; Hikida, M. and Masumura, K. (1986): Flexibacter maritimus sp. nov, a pathogen of marine fishs. International J. of Systemic Bacteriology 36, 396-398.

Table (1). Morphological, cultural and biochemical characters of isolated Flexibacter columnaris.

Test	Response
Gram staining	Gram - negative
Shape	Long thin bacillus
Growth at 5 C°	+
Growth at 35 C°	+
Growth at 40 C°	+
Growth on tryptic soy agar (5 days)	(+)
Oxidase	+
Catalase	+
Voges - Proskauer	-
Methyl red	-
Indole	-
O / F glucose	O/-
H_2S production	+
Lysine decarboxylation	+
Ornithine decarboxylation	+
Nitrate reduction	+
Aesculin hydrolysis	-
Gelatin hydrolysis	+
Starch hydrolysis	-
Urease	+
Acid production from :-	
Glucose	-
Lactose	-
Mannitol	-
Arabinose	-
Salicin	-

(+) Weakly positive

Table (2).Seasonal prevalence of columnaris disease in O. niloticus intensively cultured in concrete ponds.

Time of diagnostic service	Water temp	Total No. of fish	No. of diseased fish	%	No. of disea-fish with columnaris	%	% of columnaris to total No. of fish
Winter	14±2	75	4	5.3	-	-	-
Spring	21±2	75	12	16	5	41.7	6.7
Summer	29±3	75	18	24	2	11.1	2.7
Autumn	23±2	75	15	20	8	53.3	10.7

Table (3): Result of pathogenicity of F. columnaris to O. niloticus with different routes of challenge and at different water temperature.

Fish group	Total No. of fish	Route of inoculation	Water temp.	Time of mortality in days 1	2	4	7	14	Dead fish No.	Dead fish %	Morbid fish No.	Morbid fish %
1	10	Bathing the fish with gill injury in diluted bacterial cultured broth.	16±1C°	-	-	-	-	2	2	20	1	10
3	10		25±1C°	-	-	-	3	6	9	90	1	10
5	10	Bathing the fish without gill injury in diluted bacterial cultured broth	16±1C°	-	-	-	-	-	-	-	-	-
7	10		25±1C°	-	-	-	-	4	4	40	1	10
9	10	I/P injection of undiluted bacterial cultured broth (1.0 ml).	16±1C°	-	-	-	-	1	1	10	1	10
11	10		25±1C°	-	-	-	1	5	6	60	2	20
2	10	Bathing the fish with gill injury in sterile Cytophaga broth.	16±1C°	-	-	-	-	-	-	-	-	-
4	10		25±1C°	-	-	-	-	-	-	-	-	-
6	10	Bathing the fish without gill injury in sterile Cytophaga broth.	16±1C°	-	-	-	-	-	-	-	-	-
8	10		25±1C°	-	-	-	-	-	-	-	-	-
10	10	I/P injection of undiluted sterile Cytophaga broth (1.0 ml).	16±1C°	-	-	-	-	-	-	-	-	-
12	10		25±1C°	-	-	-	-	-	-	-	-	-

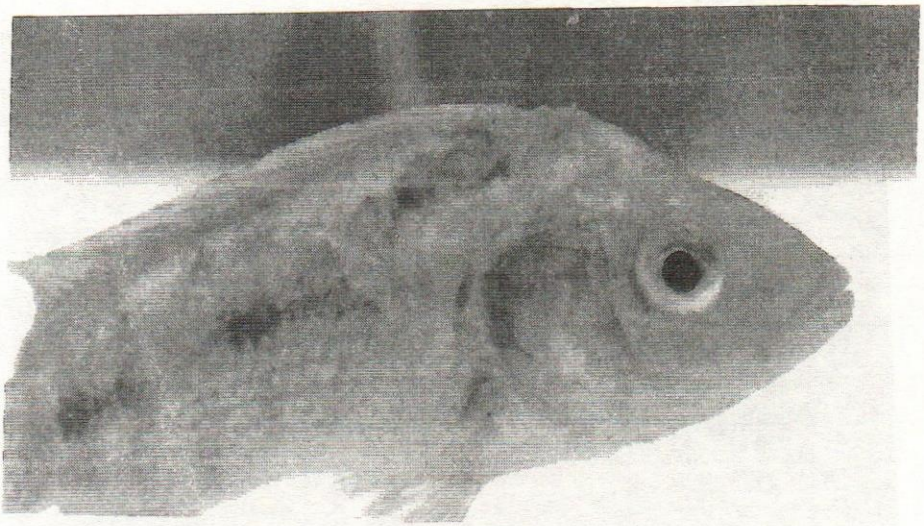

Fig. (1): O. niloticus showing detachment of scales and skin ulceration.

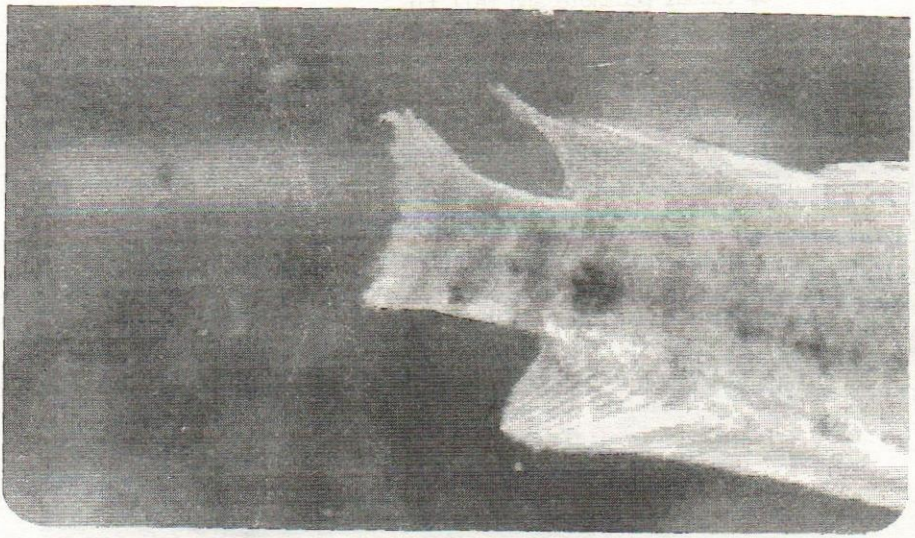

Fig. (2): O. niloticus showing tail rot.

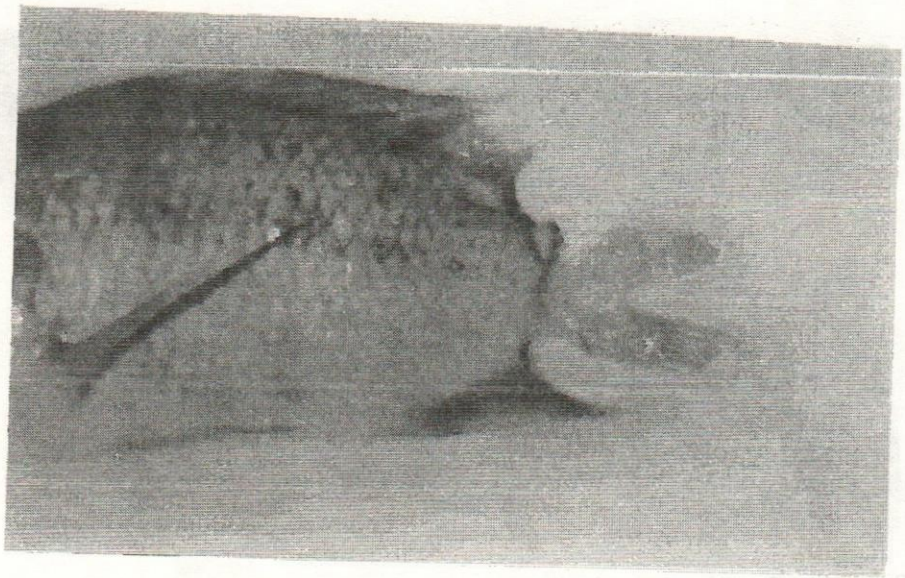

Fig. (3): O. niloticus showing slight darkness all over the body, destruction of caudal peduncle and tail fin.

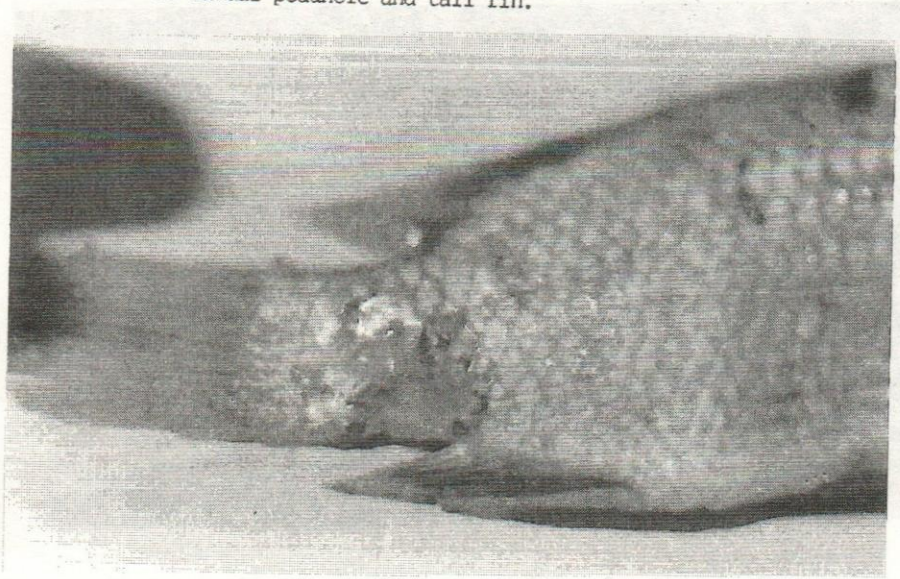

Fig. (4): O. niloticus showing destruction in the muscle of caudal peduncle from the ventral side.