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COMPARATIVE FECUNDITY ESTIMATION OF CULTURED FEMALE FISHES WITH MYXOSPOREAN AND FUNGAL INFECTIONS

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ABSTRACT

This is the first trial so far in Egypt through upgrading the fish production by revealing specific ovarian myxozoan and fungal infections that affect their fecundity. Fecundity comparison was held between noninfected and infected *Oreochromis niloticus* and *Cyprinus carpio* with either *Myxobolus* spp. or *Icthyophonus hoferi*.

The myxosporean parasite, *Myxobolus* spp. (with higher prevalence in both species) appeared as numerous white large rounded spores embedded in the ovarian tissue causing excessive fibrosis. The fungus *I. hoferi* inspite of its lower prevalence among the 2 species, the white capsulated cysts and separate colonies caused ovarian atresia and fibrosis with inflammatory oedema.

Drop of fecundity was represented by significant decrease in relative and absolute fecundity, total protein, gonadosomatic and hepatosomatic indices ranging from highly with *O. niloticus* to moderately with *C. carpio*. In *O. niloticus* (more sensitive) *I. hoferi* reflected a positive correlation with ovarian weight and gonadosomatic index, while *Myxobolus* spp. recorded a negative correlation between ovarian weight and the body parameters (weight, length & depth).

The results revealed that infection takes place among apparently healthy fish proving that gonadal periodical examination is the only way for detecting such infections.

INTRODUCTION

Improvement in knowledge of factors affecting productivity is of valuable impact for further success of fish culture. The common carp *Cyprinus carpio*, which was imported from Far East, is

nowadays widely distributed in most Egyptian fish farms. The Nile tilapia *Oreochromis niloticus* as well is becoming increasingly prominent in freshwater aquaculture alover Egypt. Unfortunately, the reproductive cycles of tilapia females within a breeding group are not synchronized although each fish breeds upto 12 times yearly (Macintosh, 1985). These two fish species represent valuable protein food, as the most popularly consumed fish for millions of people. The increasing demand necessitates that culture operations shouldn't only be optimized but also expand to meet requirements.

Myxospora are essentially and widely distributed parasites among freshwater fishes. Infection is usually subclinical and undetectable unless fish die. Ovarian infection in female Cichlids from Lake Victoria remained focal in the interstitial tissue while that occurring in Cichlids from Lake George enlarged the ovary displacing the entire ovigerous tissue, evidently causing casteration (Paperna, 1996). Landsberg & Lom (1991) counted 450 species of the Genus *Myxobolus* common in Cichlids and Cyprinids.

Icthyophoniasis is a systemic fungal uncurable and uncontrolled disease. Besides, being economically significant in both fish cultivation as well as wild fisheries, it has wide host and geographical distribution. *Icthyophonus hoferi* is the most obligate destructive fungal organism affecting fish.

In spite of the increase of fish hatcheries everywhere yet insufficient work has been concerned with fecundity impairment due to either parasitic, fungal, bacterial or environmental factors.

On the way for upgrading fish fecundity this work aimed, to examine the drastic causative agents inhibiting fecundity: that is parasitic and fungal diseases. It attempts to establish baseline data by comparing females fecundity of the two fish species.

MATERIAL AND METHODS

Fish Collection:

A group of mature females (120) *O. niloticus* and *C. carpio* of average weights (150-250 gm) and (150- 200 gm) respectively between 1-2 years old were netted from the Central Laboratory Aquaculture Research at Abbassa and transported alive to Fish Disease Department in Animal Health Research Institute. Acclimatization was held for 2 weeks in glass aquaria at temperature 18 ± 0.5 °C with on feeding commercial pellets with 30% protein at a ratio of 3% of the biomass twice daily.

Fish Parameters:

For each fish the following parameters were recorded: body length (B.L.) - body weight (B.Wt.) - body depth (B.D.) - liver weight (W_H) and ovarian weight (W_G).

Microscopical Examination:

Fishes were clinically examined to make sure they were free from abnormalities. Fish were sacrificed, postmortum examination was done and any macroscopic changes were detected. Squash preparations from ovary and abnormal cysts or nodules in internal organs were taken to detect presence of any infective pathogens. Positive impression smears from ovary for Myxospora spores were air dried, fixed in methanol and stained with Giemsa for microscopic examination and identification (Shulman, 1984). The ovary samples were aspetically cultured in (MEM-10) minimum essential medium (Sigma M 0643) supplemented with 10% faetal bovine serum (Gibco,011062904) and 1% glucose. The culture was incubated at 15°C examined for 10-15 days for fungal infection (Spanggaard,1994).

Identification of the fungal growth was performed by wet mount preparation and stained on slides with lactophenol cotton blue (Spanggaard *et al.* 1995).

Fecundity Estimation:

Relative fecundity was estimated through the following relations:

(Babiker & Ibrahim, 1979)

+ Relation between fecundity and body length " B.L."

$F = 2.895\ L^{2.017}$

+ Relation between fecundity and body weight "B.Wt."

$F = 16.12\ W^{0.83}$

+ Relation between fecundity and ovarian weight " W_G"

$F = 380 + 204\ W_G$

+ Absolute fecundity (Ab.F.) which is (Total Egg No.)

$F = 1/2\ N1/W1 + N2/W2 \times W_G$

W1&W2 : weight of the 2 subsamples from the ovary.

N1& N2 : number of eggs in W1&W2 respectively.

W_G: weight of ovary

+Relation between fecundity & body depth (B.D.):

$F = 2849.36 + 1155.55\ D$ (Danzie & Wangila 1980)

+ Hepatosomatic index: $I_H = 100\ W_H / (W - W_H)$

+ Gonadosomatic index: $I_G = 100\ W_G / (W - W_G)$

(Coward & Bromage, 1998)

Histopathological Examination:

According to El Ashram 1997 the specimens of gonads were trimed and fixed in 10 % phosphate buffered formalin, washed and dehydrated for longer periods to preserve intact sections of large eggs. It was then embedded in paraffin wax, cut in sections of 5u stained with H&E then examined and photographed under a compound microscope.

Total serum protein determination:

From fishes' serum, the total protein was estimated by Biuret method, (Wotton & Freeman, 1982).

Statistical Analysis:

The obtained data were analysed according to Petrie & Watsno (1999) and computerized using SPSS (1999).

RESULTS

1. Prevalence of infection with *I. hoferi:*

The prevalence of infection with *I.hoferi* in females of *O.niloticus* and *C.carpio* were 40% and 30% respectively. All examined fishes showed no external lesions while internal examination of apparent healthy *O. niloticus* showed microscopic and macroscopic white nodules in ovarian tissue (Fig.1) and greyish coloured ova. On the other hand, *C. carpio* showed only microscopic lesions in ovarian tissue, with the presence of white macroscopic nodules in liver, kidney and spleen in all infected fishes. Squash preparation from the nodules revealed the presence of thick-walled multinucleated spherical resting stages of variable sizes. The fungal growth in cultivated media (MEM-10) showed abundant hyphal growth characterized by evacuated hyphal wall with the cytoplasm migrating to the apex and no septation.

2. Prevalence of infection with *Myxobolus* spp:-

About 70% of *O.niloticus* and 50% of *C.carpio* were found to harbour *Myxobolus* spp. in their ovary. External Lesions were absent while internal examination of apparently healthy fish showed round white small cysts (< 0.5mm) in *C.carpio* and large size cysts (over 1.5mm diameter) scattered in ovary between ova. Squash preparations from cysts isolated from both species revealed numerous spores of Myxosporean parasites belonging to the Genus *Myxobolous* as two different species, having the same general morphological characters but differ in morphometeric features. Stained smear showed ovoid spores with two polar capsules of equal size situated in

anterior end of spore which were relatively large in spores isolated from *C.carpio*. The iodinophilous vacuole was not clearly detected. (Fig. 2).

Fecundity Estimation:

The specific relations (Table 1) revealed that fecundity of *O. niloticus* was highly affected by both types of infection. The B.Wt., B.L., B.D. and W_G (with *I. hoferi* only) were highly significantly increased, while the decrease in T. Egg No. , I_G , and I_H were highly significant for the first two and moderately for the third. With *C.corpio,* the infection showed moderately to highly significant increase in B.Wt. and B.L. only. The B.D., W_G, I_H , I_G and T. Egg No. decreased highly and moderately significant.

Total protein determination:

Serum total protein concentration was highly significant dropped in both species influenced by the parasite and the fungus. Also, comparison between the 2 species proved highly significance within *Myxobolus* and *I. hoferi* in B.Wt. B.L. and T. Egg No. Significance in B.D. was reported with *Myxobolus* only, while W_G with *I. hoferi* only.

Tables (2&3) expressed the correlations (+ve or –ve), through the discussed fecundity parameters in each fish influenced by the parasite and fungus.

Histopathological findings:

I. hoferi in *O. niloticus* revealed atresia and fibrosis that were seen clearly in ovarian architecture . Large number of melanomacrophages within the stroma were frequently accompanied with these changes. Inflammatory oedema was clearly prominant in the ovarian parenchyma of all examined specimens (Fig. 5).

I. hoferi colonies were seen in *C. carpio* within the parenchyma of ova in the form of large capsulated cysts or separate colonies (Fig. 3) which caused pressure atrophy of the adjacent uninfected ova.

Myxobolus of *O. niloticus* were seen in the parenchyma of the ovary with moderate number of melanomacrophages as well as excessive fibrosis being present (Fig. 4).

DISCUSSION

In this study, the prevalence of infection in *O. niloticus* was higher (40% & 70 %) than that of *C. carpio* (30 % & 50 %) with *I. hoferi* and *Myxobolus* spp. respectively. Myxosporean parasites are

very common in teleost fishes. Lom & Dykova (1995) recorded that gonads are infected by very few species. In this work, the spores detected in ovaries of both species were morphologically similar. The 2 isolates of Myxosporean parasites were (Myxozoa: Myxobolidae) Genus *Myxobolus.*

Two terms are applied for fecundity estimation: Absolute and Relative. The first term is defined as the total number of mature eggs in the ovary, while the second is concerned with the number of eggs related to several fish parameters as : B.L., B.Wt, B.D., and W_G.

Babiker & Ibrahim (1979) suggested that *O.niloticus* fecundity increased with length increase. At length (11- 13cm) it ranged from 300 to 550 oocytes while in length (29-32cm), it reached 2800 oocytes. This +ve relation was linked by Gerking (1978) and Morita & Takashima (1998) with Salmonids to a certain stage of maturation. The variability between fecundity and either B.L. and B.Wt in catfishes was stated by Rinne and Wanjala (1983) to be partially explainable by the fish's continuous or multiple or fractional spawning nature. Moreover, in their research with *T. zillii* Danzie & Wangila (1980) had attributed high fecundity in relation to B.Wt in pond – raised fish to " Runting " where the food requirements per fish is small thus the energy derived from food is used in egg production to maintain high fecundity. However, in this study such idea was not detected, where the parasitic spores and nodules replacing the ovarian tissue played an obstacle towards egg production. Besides, the high mortality caused by infection has led to increase the amount of food per fish. Thus, the food was then directed to fattening in both fish species presenting a high significant series of increases in B.Wt and B.L. Brummer *et al.* (1991) proved that the prevalence of *Myxobolus* spores increased with pollution in roach. Their study presented a tendency for decrease myxosporean infection with increasing age, which may be due to increasing the immune response with age. This is corroborative to the +ve correlation between B. Wt. , B.L. and B.D. in the *Myxobolus* infected groups of *O. niloticus* and *C. carpio.*

In annual spawners (as *C.carpio*), the mature ovary forms 20% of the total body weight while in *O. niloticus* that spawns several times, the gonads are about 4% (Gerking, 1978). Tacon *et al.* (1996) added an idea that the rhythm of *O. niloticus* ovarian development is related to the mouthbrooding nature. Gartner & Zwerner, (1988) found that livers infected with *I. hoferi* appeared to contain less liver tissue. With the functional tissue had been destroyed as the fungus had overcome host defences. This replies the question of –ve

correlation presented by *I. hoferi* in *C. carpio* between I_H and each of B.Wt. and B.D. In this research, the atresia and fibrosis of the ovary, the less intensity of infection referred to species susceptibility difference (McVicar, 1999) and finally the presence of only microscopic resting spores might present 3 different explanations for the highly significant decrease in B.D. and W_G of *C. carpio* group infected with *I. hoferi*. The opposite happened with *O. niloticus* where the highly significant increase in B.D. and W_G (W_G expressed a –ve correlation with each of B.Wt. , B.L. and B.D.) which is explained by the severe inflammatory oedema in the ovary caused by the fungus.

On the contrary, *Myxobolus* infection caused excessive fibrosis in both species which stands as an explanation for the highly significant decrease of W_G (with a –ve correlation in *O. niloticus* between W_G and each of B.Wt. , B.L. and B.D.).

Absolute fecundity (T. Egg No.) presented a highly significant decreasing picture among the 2 species influenced by the 2 infections. This is caused by the atrophy of the atretic follicles on the uninfected adjacent ova.

The mean value of I_G in noninfected *O. niloticus* is 2.8 ± 0.8, which is similar to that of EL Ashram (1997) (2.75 ± 1.57). The I_G of parental females was highly correlated with ovarian growth reflected by W_G (Tacon *et al.* 1996). I_G also greatly underestimates reproductive investments in *T.zilli* by Coward & Bromage (1998). In infected fish of both species, the changes occurring in the ovaries resulted in decrease of I_G (significant with *Myxobolus* as the decrease in W_G was prominent).

Susca *et al.* (2001) had defined vitellogenin as a glycolipophosphoprotein synthesized in the liver and used as precursor of the yolk proteins. The decrease in I_G & I_H obtained by both infections was explained by the slow down of egg production (T. Egg No.) thus inhibiting the pronounced influx of protein yolk from the liver to the ovary (Shackley *et al.*, 1981).

Since hepatic vitellogenin production is mastered by estradiol hormone secreated from the ovary (Wallace *et al.*, 1987), the disturbances resulting in the infected fish ovaries stands for the highly significant decrease of serum TP.

The authors throw light on the parasitic and fungal deceptive nature affecting good conditioned fish (Paperna, 1996), with the missing of external lesions (Ogawa *et al.* 1992), their easy quick

transmission which ends with complete organ failure and above all their uncurability. Finally, they insist on the necessity of broodstock gonadal examinations and exclusion of infected individuals specially *O. niloticus* being highly susceptible, severely affectable and hardly detectable.

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REFERENCES

Babiker, M. M. and Ibrahim, H. (1979). Studies on the biology of reproduction in the cichild *Tilapia nilotica* (L): gonadal maturation and fecundity. J. Fish. Biol., *14*:437-448.

Brummer, K. H. ; Tellervo, V. E. and Pugachev, O. N. (1991). Myxosporea parasites in roach, *Rutilus rutilus* (Linnaeus), from four lakes in central Finland. J. Fish. Biol., *38*:573-586.

Coward, K. and Bromage, N. R. (1998). Histological classification of *zillii*. J. Fish Biol., *53*:285-302.

Danzie, S. and Wangila, B. C. C. (1980). Reproductive biology, length-weight relationship and relative condition of pond raised *Tilapia zillii* (Gervais). J. Fish.Biol., *17*:243-253.

EL- Ashram, A. M. (1997). Some reproductive studies on some freshwater fish with regard to some pathological and toxicological factors Ph. D.

Gartner, J. V. and Zwerner, D. E. (1988). An Icthyophonus type fungus in the deep sea pelagic fish *Scopelogadus beanii* (Gunther) Pisces : Melamphaidae : pathology, geographical distribution and ecological implications. J. Fish Biol., *32*:459- 470.

Gerking, S. D. (1978). Ecology of freshwater fish production. Blackwell scientific publications, Oxford London Edinburgh.

Landsberg, J. H. and Lom, J. (1991). Taxonomy of the genus *Myxobolus* (Myxobolidae : Myxosporea) current listing of species and revision of Synonyms. Sys. Parasit., *18*:165-186.

Lom, J. and Dykova, I. (1995). Myxosporea (Phyulum Myxozoa) in Fish Diseases & Disorders. Vol.I by Woo, P.T.K.

Macintosh, D. J. (1985). Tilapia Culture: Hatchery Methods for *Oreochromis mosambicus* and *Oreochromis niloticus* with special reference to all male fry production. Inst. of Aquaculture, Univ. of Sterling, Sterling.

McVicar, A. H. (1999). *Icthyophonus* and related organisms in Fish Diseases & Disorders. Vol. III by Woo, P.T.K. and Bruno, D.W.

Morita, K. and Takashima, Y. (1998): Effect of female size on fecundity and egg size in white- spotted charr: Comparison between sea- run and resident forms. J. Fish Biol., *53*:1140-1142.

Histological studies on the host response to *Myxobolus artus* Akhmerov, 1960 (Myxozoa : Myxobolidae) infection in the skeletal muscle of carp, *Cyprinus carpio*. J. fish boil. *41*:363 – 371.

Paperna, I. (1996). Parasites infections and disease of fishes in Africa FAO/ CIFA technical paper 563pp.

Petrie, A. and Watson, P. (1999). Statistics for veterinary and animal Science 1st ed., pp. 90-99. The Blackwell Science Ltd. UK.

Rinne, J. N. and Wanjala, B. (1983). Maturity, fecundity and breeding seasons of the major catfishes (Suborder: Siluroidea) in Lake Victoria, East Africa. J. Fish Biol., *23*: 357- 363.

Shackley, S. E. ; King, P. E. and Gordon, S. M. (1981). Vitellogenesis and trace metals in a marine teleosts. J. Fish Biol., *18*:349- 352.

Shulman, S. S. (1984). Class Myxosporidia. Opredelitel Parasitov Presnovodnych Ryb Fauny SSSR1, 88-251. Leningrad: Nauka (In Russian).

Spanggaard, B. ; Gram, L. ; Okamoto, N. and Huss, H. H. (1994). Growth of the fish pathogenic fungus, *Icthyophonus hoferi* measured by conductivity and microscopy. J. Fish Diseases, *17*:145- 153.

Spanggaard, B. ; Huss, H. H. and Bresciani, J. (1995). Morphology of *Icthyophonus hoferi* assessed by light and electron microscopy. J. Fish Diseases, *18*:567- 577.

SPSS (1999). SPSS for windows . Release 10.0.1.27 Oct. 1999. Standard Version. Copyright SPSS Inc., 1989- 1999.

Susca, V. ; Corriero, A. ; Bridges, C. R. and DeMetrio, G. (2001). Study of the sexual maturity of female bluefin tuna purification and partial characterization of vitellogenin and its use in an enzyme- linked immunosorbent assay. J.Fish Biol., *58*:815- 831.

Tacon, P. ; Ndiaye, P. ; Cauty, C. ; LeMenn, F. & Jalabert, B. (1996). ovarian development in the mouthbrooding cichlid fish *Oreochromis niloticus*. Aquacult., *146*:261- 275.

Wotton, I. D. and Freeman, H. (1982). Microanalysis in Medical Biochemistry. Churchill, Livingstone, Edinburght, London, Melbourne and NewYork.

Wallace, R. A. ; Selman, K. ; Greeley, M. S. ; Jr, Begovac, P. C. ; Lin, Y. - W. P. & McPherson, R. (1987). Current status of oocyte growth. Proceedings of the Third International Symposium on the Reproductive Physiology of Fish. (Idler, D.R., Crium, L.W.& Walash, J. M., eds), pp. 167-177. St John's, Canada: Memorial University of Newfoundland.

Table (1): Fecundity estimation for females of *O.niloticus* & *C.carpio*

	BFM	O.niloticus			C.carpio			
		Free	Mysob.	I. bolleri	Free	Mysob.	I. bolleri
Relative fecundity	B.Wt	1295 ±96	2226 ±158 ****	1620 ±[illegible] ****	1217 ±26	1316 ±53 *** ####	1393 ±37 ** ####
	B.L.	1697 ±87	2613 ±176 ****	1923 ±17 ****	1628 ±52	1769 ±42 **** ####	1720.43 **** ####
	B.D.	6316 ±83	7087 ±220 ****	6701 ±23 ****	6894 ±83	6701 ±126 **** ####	6701 ±48 ****
	W.G	1128 ±99	889 ±150 ****	1400 ±20 ****	2089 ±20	1418 ±153 ****	1691±145**** ###
Relative parameters	k_0	2.8 ±0.3	1.1 ±0.2 ****	1.4 ±0.3	3.7 ±0.6	1.1 ±0.04 ***	1.7 ±0.3
	k_1	4.5 ±0.04	0.8 ±0.2 ***	1.1 ±0.1 ***	2.1 ±0.4	0.9 ±0.1 ***	1.2 ±0.2 **
	K	1.9 ±0.04 —	1.6 ±0.1	1.6 ±0.04	1.4 ±0.1 —	1.2 ±0.04 —	1.2 ±0.04
Absolute fecundity	T. Egg No.	2376 ±121	199 ±37 ****	5[illegible] ****	4549 ±91	1638 ±23 **** ####	3583±8 **** ####
	T.P	7.2 ±0.7	4.4 ±0.7 ****	5.3 ±0.6 ****	5.7±0.6	3.7±0.8 ****	4 ±0.7 ***

* P < 0.05 ** P < 0.01

*** P < 0.005 **** P < 0.0001

* Sig. between each parasite with the free group

Sig. between each parasite within both fish species

Table (2): Correlation matrix of *O. niloticus* fecundity among the discussed parameters influenced by the parasite & fungus.

		Icthyophonus								
Myxobolus		B.Wt.	B.L.	B.D.	W_G	I_G	I_H	K	T.Egg No.	T.P.
	B.Wt.			# +ve		## +ve				
	B.L.									
	B.D.	## +ve	# +ve		# +ve	# +ve		# - ve		
	W_G	# -ve	# -ve	## - ve		# +ve				
	I_G				# -ve					
	I_H									
	K									
	T.Egg No.									
	T.P.									

Correlation is significant at the 0.05 level (2- tailed).
Correlation is significant at the 0.01level (2- tailed).

Table (3): Correlation matrix of *C. corpio* fecundity among the discussed parameters influenced by the parasite & fungus.

Myxobolus \ *Icthyophonus*	B.Wt.	B.L.	B.D.	W_G	I_G	I_H	K	T.Egg No.	T.P.
B.Wt.		## +ve				# - ve			
B.L.	## +ve								
B.D.						# - ve			
W_G							## +ve		
I_G			## +ve			# +ve		# +ve	
I_H								## +ve	
K			# +ve	# - ve	# +ve				
T. Egg No.									
T.P.				# +ve					

Correlation is significant at the 0.05 level (2- tailed).
Correlation is significant at the 0.01level (2- tailed).

EXPLANATION OF FIGURES

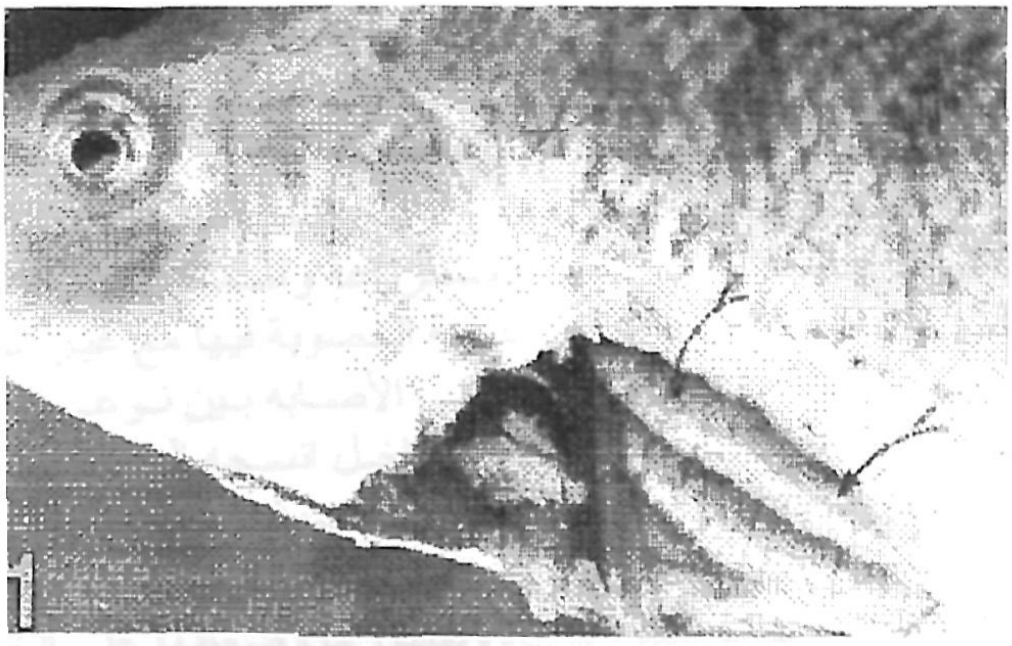
Fig. (1): *O.niloticus* showing various size nodules of *I.hofri*

Fig. (2): *Myxobolus* spp. Isolated from *O. niloticus* ovary. Giemsa's stain. (x 1000).

Fig. (3): Cross section in the ovary of *C. corpio* showing rest stage cyst of *I.hoferi.* H &E stain (x 400)

Fig (4): Cross section in the ovary of *O. niloticus* showing many parts of *Myxobolus* spp. Spores. H &E stain (x 400).

Fig. (5): *O. niloticus* ovary infected with *I. hoferi* showing severe inflammatory oedema. H& E stain (x 400).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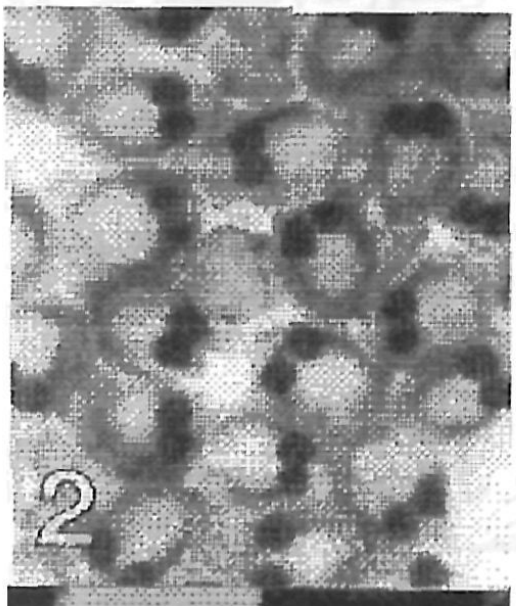
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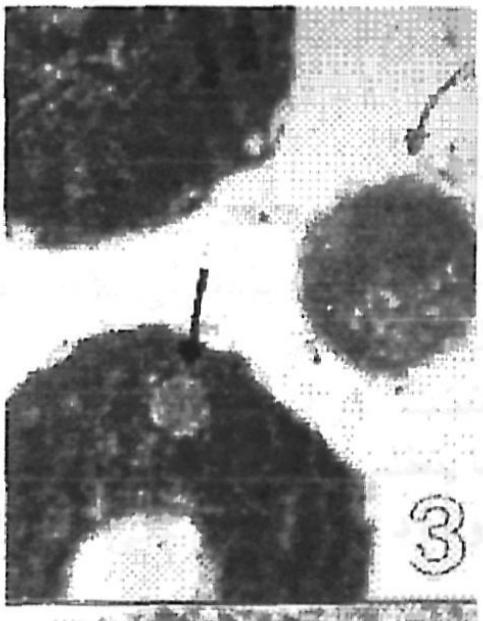
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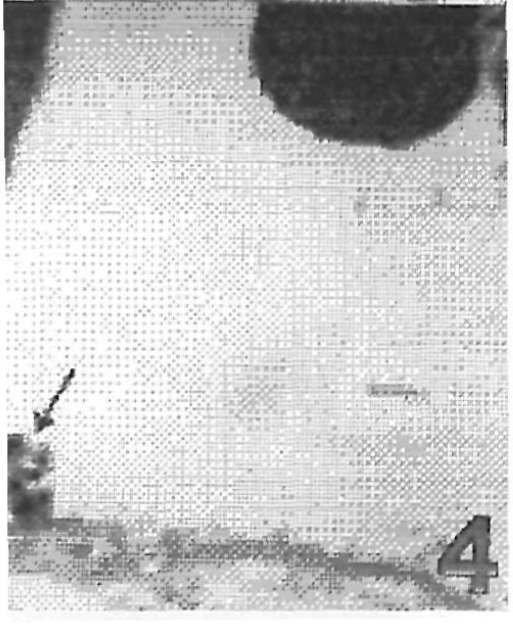
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