

A Comparative Study Of The Health Status Of Tilapia Fish Through Various Environmental Changes

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ABSTRACT

A number of 360 apparently healthy and naturally infected Nile tilapia with body weight range (40-80) grams were obtained from Abassa fish farms, Sharkia Governorate, Egypt, random collected from pond with uncovered cement ponds (A) and greenhouse cement ponds (B) (180 from each) during winter season of 2013 /2014 (December and January). Water parameters were recorded at the collection time namely temperature, ammonia and dissolved oxygen. At the present work tilapia fish were subjected to clinical and post mortem examination. All Fish were examined for isolation and identification of bacterial, mycotic and parasitic pathogens and histopathological examinations were performed on infected fish tissue.

The isolated bacteria were *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *proteus vulgaris*, *Escherichia coli*, *Enterobacter intermedius*, *Enterobacter aerogenes*, *Enterobacter cloacae* and *Aeromonas hydrophila*. The bacterial prevalence was higher in pond B (57.8%) than in pond A (33.3%).

Mycological examination revealed that fungal isolation of four genera were isolated from pond (A) (*Penicillium* spp, *Aspergillus niger* , *Blastomyces* spp and *Scopulariopsis brevicaulis*) but in pond (B) were positive to five genera (*Penicillium* spp, *Aspergillus niger* , *Blastomyces* spp and *Scopulariopsis brevicaulis*). The fungal isolates were higher in pond (B)77.8% than in pond (A) 55.6%.

The examination of tilapia fish revealed the presence of four different parasitic agents were identified, as *Myxobolus tilapiae*, *Trichodina heterodontata* and two encysted metacercariae (EMC) *Centrocestus formosanus* in gills and *Heterophyes* sp in muscles .The parasitic agents was higher in pond (B) 77.8% than in pond 55.6%(A).

The results of the histopathological examination of naturally infected fish were also discussed.

It was concluded that it be good to using greenhouse in fish culture to avoid the decrease in water temperature during winter season but other factors must be taken in consideration like water sources to avoid spread of diseases.

INTRODUCTION

The tilapia fish is one of the most important economic fish in the world. Tilapia fish are primarily of the popular fish, easy to breeding, fast growth, cooler resistant, easy localization on various new environments.

Bacterial pathogens are the most serious disease problem in fish production

causing 80% of mortalities (1). The majority of bacterial infections are caused by gram-negative organisms and gram positive genus has been shown to cause disease in fishes. Bacterial organisms may be the primary cause of disease, or they may be secondary invaders, taking advantage of a breach in the fish's integument or compromise of its immune system.

Outbreaks of water born fungal infections of fish are common problems especially in fish farms and hatcheries. Along with zoospores' fungi, there are many conidial fungi found associated with fish diseases. Some of these genera involved are *Achlya* spp (2).(3) isolated *Penicillium* spp., *Aspergillus* spp., *Alternaria* s pp., from infected fishes. (4) Also reported conidial fungi from carp's fish and (5) isolated *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp. and *Saprolegnia* sp was observed in fish.

Fish may harbor many pathogens especially parasites which could interfere with the aquaculture industry.

The present work aimed to identify some bacterial, mycotic and parasitic agents in Nile tilapia fish during the influences of climatic changes (temperature fluctuation, dissolved oxygen concentration and unionized ammonia level). Histopathological changes were also recorded.

MATERIALS AND METHODS

Characters of two ponds

Data were collected from two fish ponds (referred to as pond A and B) located in Abassa- Sharkia Governorate, Egypt during the late winter season of 2013(December – January) Table (1).

Table 1.The characters of the two ponds under study

Items	Pond (A)	Pond (B)
Type of pond	Cement pond	Cement pond
Water source	Well	Ismailia canal (agriculture drainage + waste water)
Fish source	CLAR*	CLAR
Special management	----	Greenhouse

Central Laboratory for Aquaculture Research (CLAR)

Sample collection

A total number of 360 Nile tilapia fish were obtained from Abassa fish farms, Sharkia Governorate, Egypt. Fish samples were collected from green house ponds and uncovered cement ponds (pond A & B) 180 from each during the winter season of 2013/2014(December – January). Temperature, dissolved oxygen level and un-ionized ammonia were measured at the collection time using a thermometer, an oxygen meter and colorimeter Jenway Kits (Jenway Scientific Limited Company, UK). The fish samples weighing range (40-80 g) were transported to the laboratory in aerated farm water according to (6). Fish were subjected to clinical and post mortem examination according to (7). Bacteriological, mycological, parasitological and pathological examinations were performed.

Bacterial examination

Isolation and identification

Samples were taken under aseptic conditions from skin, gills, fins and the different organs of each fish and inoculated on nutrient broth, peptone water and selenite F broth and incubated at 25°C for 24 h. Sub culturing was carried out onto nutrient agar, blood agar, XLD and MacConkey's agar media and incubated aerobically at 25° C for 48 hrs The cultivated separate colonies were subjected to biochemical identification using standard bacteriological and biochemical procedures as described by (8).

Mycotic examination

Isolation of fungi was carried out from fish showing skin lesions, eye, fins, gills, mouth, spleen, liver and kidney. Swabs were aseptically taken from these organs and cultured in Potato Dextrose Agar (PDA) and Sabourauds Dextrose Agar (SDA) medium which incubated at 22C° for 3-7 days and examined daily. Preliminary identification depends on the color and morphology of colony and the confirmatory test of identification was done by examination of a portion of colony was added on slide to a drop of lactophenol cotton

blue, (L.P.C.B) then examined Microscopically (9).

Identification of the isolates

All positive cultures were examined for colonial growth, morphological features and microscopically characteristics. The morphological features include appearance of the cultures, rate of growth, texture of the surface colonies, colonies color. Microscopically examination was done for wet preparations of the skin lesions and mycelia cultured on (SDA) to detect septation of hyphae according to (10).

Parasitological examination

Fish were examined externally and internally for gross signs of parasitism and any abnormalities. Wet mounts were prepared from any noticed suspected lesions containing parasites or nodules. Scraped skin, fins and gills were examined with ordinary microscope at 10 – 40X magnification. Microscopically examination of fish tissues was carried out using compression technique as described by (11) for detection of encysted metacercaria which were lodged in/or attached to different organs and tissues (gills, muscles, liver, spleen and kidney). Collected parasites were fixed and stained for further identification according to (12). The prevalence and mean intensity of metacercarial infection were estimated according to (13).

Histopathological examination:

Tissue specimens taken from the organs (gills, skin, skeletal muscle and liver) that have lesions were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (HE) stains and then examined microscopically (14).

RESULTS

Clinical signs

The clinical signs of natural mixed infected examined fish (parasitic, mycotic and Bacteria) in pond (A) were hemorrhage at the base of the fins, ulceration and emaciation, while in addition to these symptoms, mixed infected fish in pond (B) showed abdominal distention, eyes opacity, scales detachment and congested gills. In case of single parasitic infested fish in pond (A) showed abundant secretion of mucous, hemorrhagic spots and easily detached scales with ulcerative areas. However, in pond (B) showed no external signs with single parasitic infestation.

The main postmortem lesions in single infected fish (bacterial or mycotic) in pond (A&B) were sanguineous fluids in abdominal cavity, spleen and kidneys were congested and enlarged. Small grayish white foci on the liver surface was seen, distended gall bladder with bile, the surface of the liver has some hemorrhagic spots and enlarged.

The prevalence of infected Tilapia fish with bacterial, fungal and parasitic agents showed in Table (2) and the prevalence of single and mixed bacterial, fungal and parasitic agents in examined Tilapia fish in Table (3).

Bacteriological examination

The result in Table (2) revealed that out of 360 collected fish, 164 were positive for various bacterial agents. The bacterial prevalence was higher in pond B (57.8%) than in pond A (33.3%). The isolates were *Pseudomonas aeruginosa* (40 isolates), *Pseudomonas fluorescens* (26 isolates), *proteus vulgaris* (22 isolates), *Escherichia coli* (34 isolates), *Enterobacter intermedium* (14 isolates), *Enterobacter aerogenes* (14 isolates), *Enterobacter cloacae* (8 isolates) and *Aeromonas hydrophila* (6 isolates). The predominant microorganisms isolated were *Ps. aeruginosa* followed by *E. coli* while *E. colacea* and *A. hydrophila* were the least prevalent bacteria in the examined fish Table (4).

Mycological examination

Mycological examination revealed that 150 samples out of 180 fish were positive to fungal isolation of the five genera were isolated from green house highly incidence 83.3% (*Achlya* spp 73.33% , *Alternaria* spp 66.65%, *Rhizopus* spp 53.33%, *Mucor* spp 10.66% and *Penicillium* spp .9.33%) but in cement pond 90 samples out of 180 fish were positive to four genera were isolated (50%) (*Penicillium* spp 50%, *Aspergillus niger* 44.4%, *Blastomyces* spp 30% and *Scopulariopsis brevicaulis* 16.6%).

The morphological criteria of isolated fungi

Achlya spp: which among oomycete (order Saprolegniales) colony grow rapidly on SDA with dense aerial white mycelium in concentric rings, abundant, floccose, somewhat powdery in aged cultures which reverse to brownish colour, showing average growth rate per day at 20C°. *Achlya* spp. under microscope it is non septate hyphae, thin filaments and terminal hyphae swelling that will differentiate into the primary zoospores.

Alternaria spp : macroscopically rapidly growing downy or cottony colonies maturing within 5 days, grey to olive brown on the surface with short aerial hyphae brown-black on reverse due to pigment production. Microscopically *Alternaria* produces the pigment melanin therefore structures can appear brown to black in colour, dark septate hyphae large conidiophores are septate (transverse & longitudinal septations), simple or branched and occasionally exhibit a zigzag appearance, conidia (poroconidia) are brown, muriform, ovoid or obclavate, with an elongated 'beak-like' apical cell, solitary or acropetal chaining.

Rhizopus spp: Macroscopically, *Rhizopus* is a rapidly growing fungus that can fill a petrie dish with fluffy, cotton-candy like growth in under 5 days. Growth is generally whitish in colour which can turn brown with age as a result of the maturation of the sporangiospores within the sporangium. *Rhizopus* is grouped with other fungi of the Zygomycota phylum which cause similar infections commonly referred to as zygomycosis.

Mucor spp: macroscopically is a rapidly growing fungus which will fill a culture plate in a matter of a few days with a woolly growth resembling cotton candy. New growth is white in colour but turns a greyish-brown with aging. The reverse remains a pale white.

Penicillium spp : macroscopically rapid growth and become fully mature in about 5 days. The surface appearance is usually described as velvety to powdery. The colony colour is usually a green, blue-green or grey-green, often with a white edge.

Aspergillus niger : macroscopically Rapidly growing on Saboraud-Dextrose Agar starting with a white to yellowish felt-like mat of mycelia, quickly turning black as conidia develop the pigment aspergillin during maturation. Reverse remains white to pale in colour.

Blastomyces spp: macroscopically filamentous fungus form moderately slow growth, usually maturing in about 2 weeks, exhibits a cottony or downy texture. Colonies produce white areal hyphae on the surface which may be turn a yellowish to tan colour as the colony ages. The reverse is typically a light tan to brown.

Scopulariopsis brevicaulis: macroscopically colonies moderately rapid growth, reaching maturity in about one week. Colonies are velvety to powdery in texture. Colour may start off as white, quickly becoming a pinkish-brown or buff to cinnamon. Reverse is a honey to golden-brown in colour.

Parasitological examination

The prevalence of parasitic agents in examined fish in pond (A) were mixed ectoparasites; *Trichodina heterodontata* and the *Myxobolus tilapiae* 55.6%. The intensity with *T. heterodontata* ranged from moderate infestations of >30 parasites per field (100×) to heavy >150 parasites per field (100×). The parasites in pond (B) were two types of encysted metacercariae (EMC) with prevalence of 77.8% namely *Centrocestus formosanus* in gills and *Heterophyes* sp in muscles.

The Morphological identification of *Trichodina heterodontata* was ciliated protozoan characterized by robust, strongly sickle-shaped dentacle blades with pointed ends, the evenly tapering rays with pointed tips, the absence of any central inclusion and the prominence of the radial pins Fig(1)A.

Giemsa stained impressions smears made from skin, gills, and the plasmodial protozoan cysts revealed the presence of a myxosporean spores of *Myxobolus tilapiae*. The spore body was oblong to oval with anterior and posterior ends bluntly rounded. Characteristic two spherical to pyriform polar capsules of equal size located in anterior portion of the spore that contain polar filaments Fig (1) B.

The EMC *Heterophyes sp* were encysted between the muscle fibers and them spherical in shape and surrounded by yellow cysts and found in groups. The mean intensity of infection was 180 metacercariae/one gram fish muscles Fig (1) C.

The *Centrocestus formosanus* were found encysted in the gills of the fish, and were oval-shaped; the larvae presented an X-shaped excretory vesicle with dark granules inside, and a mean intensity of the infection of 10 parasites per gill lamellae Fig (1) D.

Physicochemical analysis of water

Analyses of variance for average physicochemical parameters of water from ponds under study are shown in Table (5). Water temperature among the ponds (B) 23 ± 2 °C with greenhouse management and ponds (A) uncovered 15 ± 2 °C.

The results revealed that the average of dissolved oxygen concentration 6.5 ± 0.5 in pond (B) and 5.7 ± 0.8 in pond (A). The unionized ammonia (mg/l) showed that the pond (B) had higher value (0.05 ± 0.01) than pond (A) (0.002 ± 0.001).

Histopathological Findings:

Uncovered cement pond samples (Pond A):

The gills revealed congestion brachial blood vessels and secondary lamellar capillaries, hemorrhage and edema (Fig. 2). The tips of the

gill filaments showed focal proliferation of the epithelial covering of the secondary lamellae and fusion of the filaments which focally necrotic and were replaced with lymphocytes and macrophages. Few round eosinophilic bodies were scattered on the affected gill arch (Fig 3). The gill rakers showed edema, congestion, hemorrhages and few leukocytic infiltrations mainly with lymphocytes with unilateral sloughing of the secondary lamellae and desquamation of the covering epithelium. Several trophozoites of *Trichodina* were visualized on gill arch, in mucous plug of pale bluish substance containing desquamated epithelial cells and few leukocytes and between the secondary lamellae (Figs 4 and 5). Few leukocytes infiltrations with hyperplasia of the mucus-secreting cells were also noted.

The skin of fish infected with *Myxobolus* revealed areas of necrosis or erosions in the epidermis and extended into the dermis. The contagious epidermis showed spongiosis, increased and hypertrophied mucous secreting cells and spongiosis besides few leukocytes infiltrations (Fig. 6). Meanwhile, the dermis showed edema, hemorrhage and extensive aggregation of round cells.

The muscles appeared homogenous and eosinophilic (hyalinized) or necrotic besides widely separated bundles (Fig 7).

The liver showed severe congestion in the hepatportal blood vessels and hepatic sinusoids besides few extravasated erythrocytes (Fig 8). In addition to coagulative necrosis with eosinophilic granular cells in individual hepatocytes represented by pyknosis and disappearance of the nuclei, and round cells infiltrations mostly lymphocytes in the portal areas were detected (Fig. 9). Interstitial aggregations of lymphocytes were seen. These cells were invaded and replaced the interstitial tissue (Fig 10). Meanwhile, the mycotic lesions represented by granulomatous reaction and extensive coagulative necrosis with irregular elongated eosinophilic mycotic elements (Fig 11).

Green house Samples (Pond B)

The gills of fish infected with mixed (parasitic, mycotic and bacterial) showed severe congestion, hemorrhage, proliferation of the secondary lamellar epithelium and lymphocytes infiltrations. Some gill-filaments showed several encysted metacercaria with fibrous capsule (Figs 12 & 13).

The skin of fish infected with Single Mycotic infection revealed areas of ulceration with complete disappearance of the epidermis. Such areas were invaded with numerous basophilic hyphae (Fig 14). The other areas showed spongiosis and severe hydropic degeneration.

The dermal skeletal muscles showed numerous encysted metacercaria which encapsulated with thin fibrous tissue capsule. The affected muscles were hyalinized (Zenker's

degeneration) and appeared more eosinophilic (Fig15). Erosions, spongiosis, increased mucus-secreting cells and hydropic degeneration were also found besides numerous encysted metacercaria in muscles.

The liver of examined fish explicated the three mixed infections showed severe congestion in hepatoportal blood vessels and leukocytes infiltration were represented the septicemic lesions. Necrosis in the pancreatic acini and intense aggregations of lymphocytes with bacterial colonies were observed (Fig 16). Coagulative necrosis with thin light hyphae and sporocysts or sporoblasts was confirmed the presence of mycotic infection (Fig 17). Only one case showed viable encysted metacercaria with thick fibrous capsule with no evidence of cellular infiltrations or hepatocyte degeneration (Fig 18) in addition to severe congestion, hemorrhage and necrosis in the liver (Fig 19).

Table 2. The prevalence of bacterial, fungal and parasitic agents in examined tilapia fish

Items	Fish No.	Bacterial infection		Fungal infection		Parasitic infection	
		No. of diseased fish	%	No. of diseased fish	%	No. of diseased fish	%
Pond (A)	180	60	33.3	90	50	100	55.6
Pond (B)	180	104	57.8	150	83.33	140	77.8

Table 3. The prevalence of single and mixed bacterial, fungal and parasitic agents in examined tilapia fish .No. of examined fish / pond = 180

Items	App. Healthy Fish		Single Bacterial infection		Single Mycotic infection		Single Parasitic infection		Mixed parasitic +bacterial		Mixed parasitic +mycotic		Mixed parasitic +mycotic +Bacterial	
	Fish Number	%	No. of diseased fish	%	No. of diseased fish	%	No. of diseased fish	%	No. of diseased fish	%	No. of diseased fish	%	No. of diseased fish	%
Pond (A)	34	18.88	14	7.77	32	17.77	18	10	24	13.33	36	20	22	12.22
Pond (B)	-	-	4	2.22	36	20	26	14.44	-	-	14	7.77	100	55.55

Table 4. Type, number and percentage of the isolated bacterial *spp.* from examined tilapia

Pond	Pond A(180 fish)		Pond B(180 fish)	
	No	%	No.	%
<i>Ps. aeruginosa</i>	10	18	30	16.6
<i>Ps. fluorescen</i>	2	1.1	24	13.3
<i>p. vulgaris</i>	8	4.4	14	7.8
<i>E. coli</i>	16	8.9	18	10
<i>E. intermedius</i>	12	6.7	2	1.1
<i>En.aerogenes</i>	4	2.2	1	18
<i>E.colacea</i>	8	4.4	0	0
<i>A.hydrophila</i>	0	0	6	3.3
Total	60	33.3	104	57.8

Table 5. The physicochemical parameters of water samples from the ponds

Items	Water temp (°C)	DO (mg/L)	Unionized ammonia (mg/l)
Pond (A)	15±2	5.7±0.8	0.002± 0.001
Pond (B)	23±2	6.5±0.5	0.05 ± 0.01

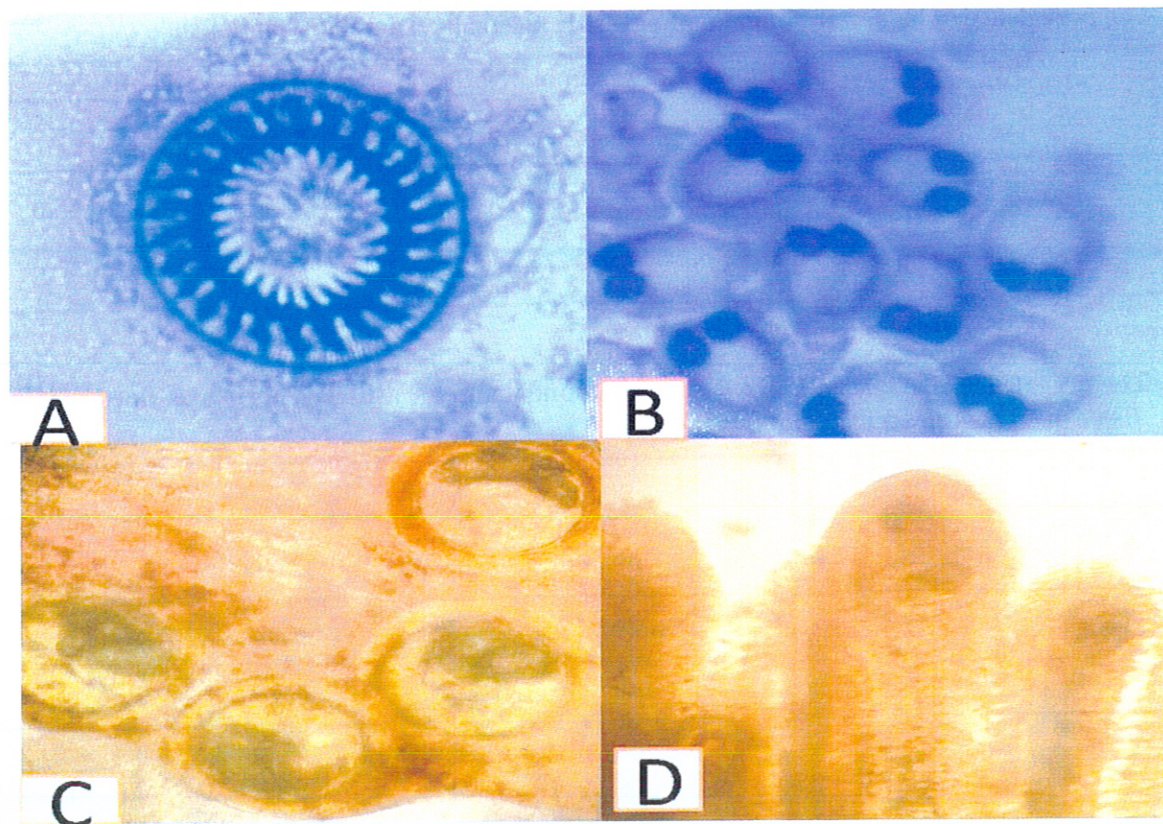
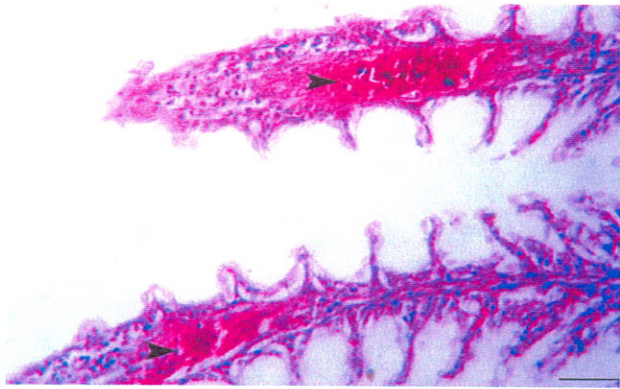
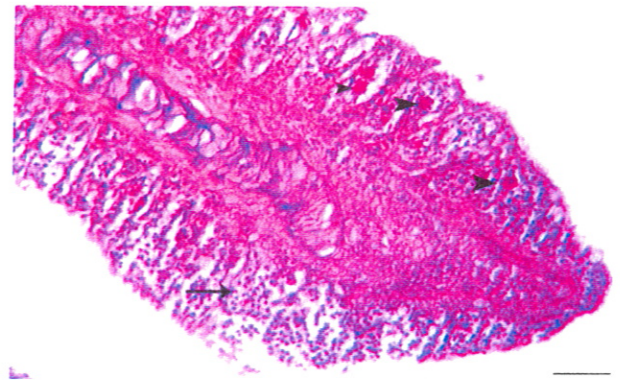


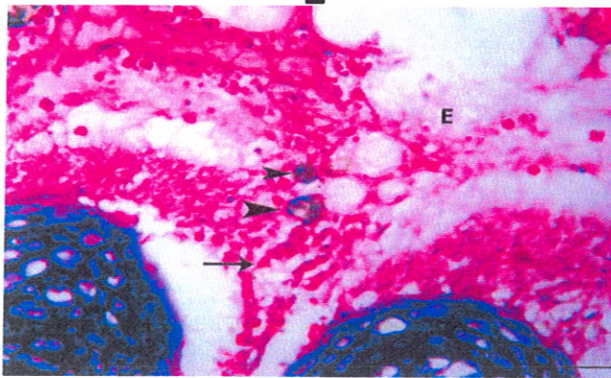
Fig. 1. (A) *Trichodina heterodontata* (B) *Myxobolus spp* spores (Giemsa stain) (C) Group of Encysted metacercariae of *Heterophyes sp* between muscles fibers. (D) Encysted metacercariae of *C. formosanus* in gill filaments fresh smear.



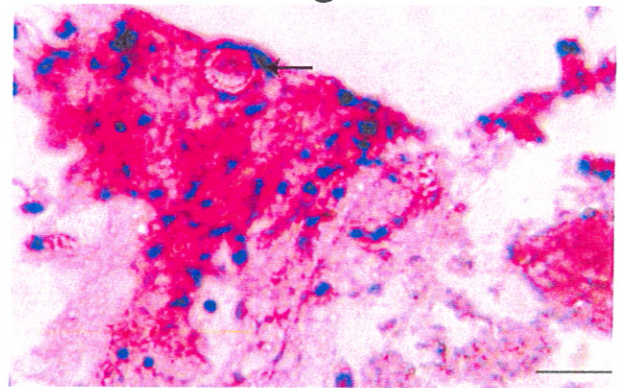
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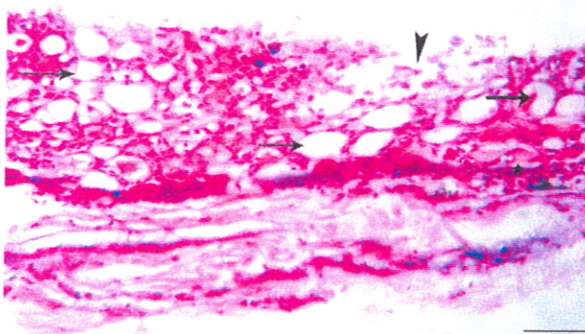


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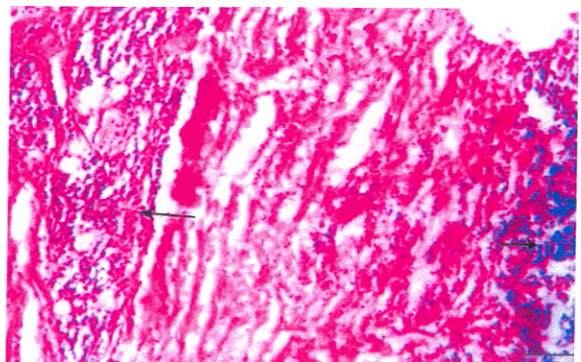
Fig. 2. Gills of farm (A) showing congestion in brachial blood vessels H, E X 300 .

Fig. 3. Necrotic secondary lamella, lymphocytes and macrophages infiltrations with hyperplasia of the secondary lamellar epithelium H, E X 300 .

Figs. 4,5. Several trophozoites of trichodina in the gill arch, mucous plug of pole bluish substance containing desquamated epithelial cells and few leucocytes between the secondary lamellae. H, E X1200



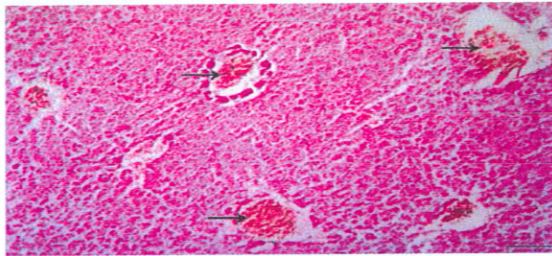
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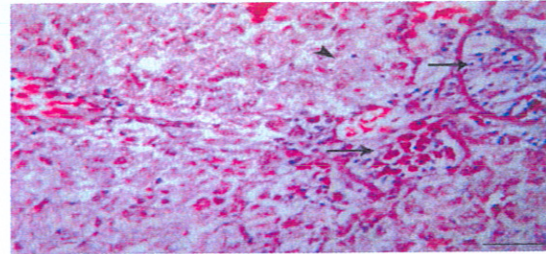
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Fig. 6 The skin of fish infected with myxobolus show areas of necrosis in the epidermis, spongiosis and hypertrophied mucous cells with few leukocytic infiltration H, E X 1200.

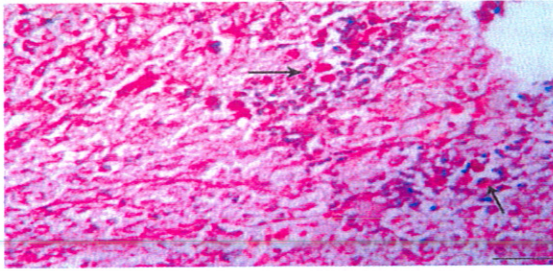
Fig. 7. The muscle is homogenous and hyalinized besides widely separated bundles H, E X 300'



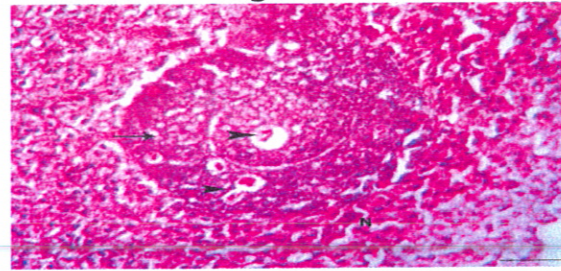
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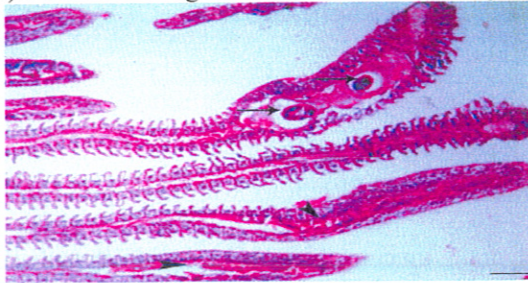
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Fig. 8. Liver of farm A showing congestion the hepatic portal blood vessels H, EX300.

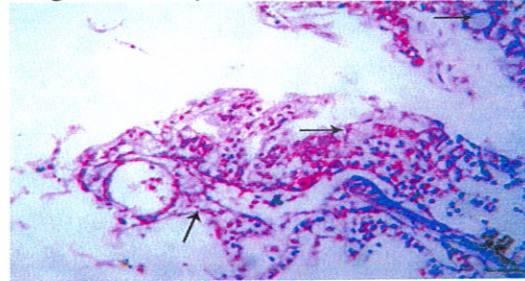
Fig. 9. Coagulative necrosis and eosinophilic granular cells and round cells H, E X200.

Fig (10) Granulocytes invading and replaced the interstitial cells H, E X 1200

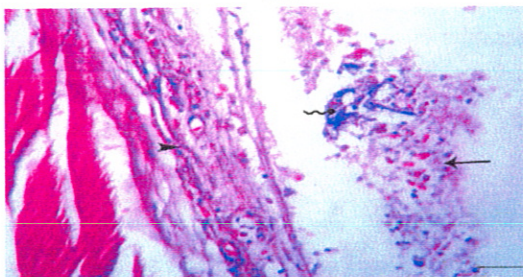
Fig (11) Extensive coagulative necrosis with irregular elongated eosinophilic mycotic elements H, E X 1200



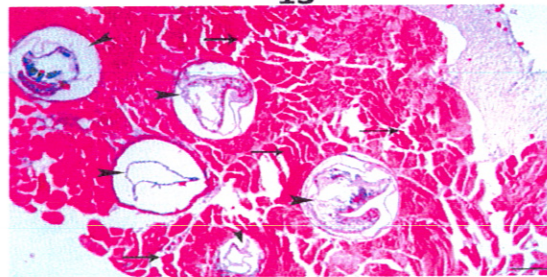
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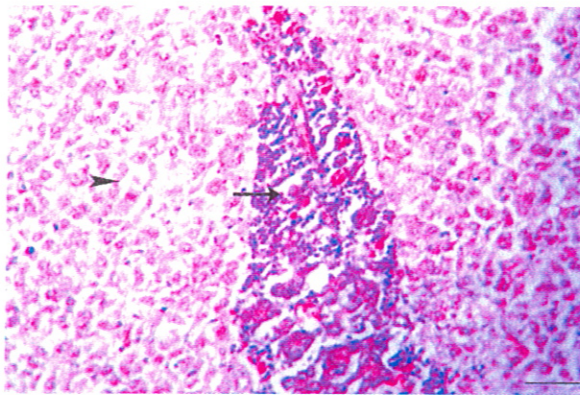
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Fig. 12. Gills of farm (B) showing sever congestion, hemorrhage, proliferation of the secondary lamellae and encysted metacercaria capsules H, E X 300

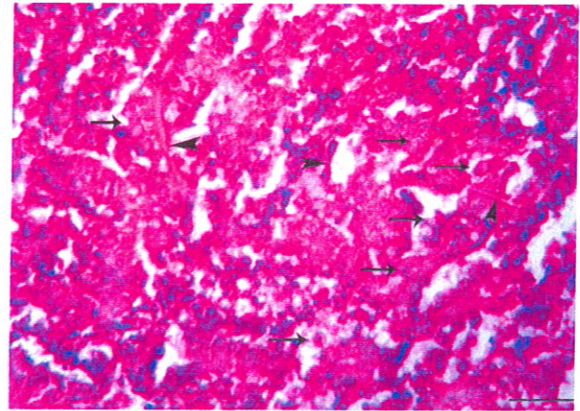
Fig. 13 Showing degeneration of the secondary lamellae, lymphocytes and fibrous capsular of EMC H, E X 1200

Fig. 14. Skin of tilapia farm (B) showing ulceration and numerous basophilic hyphae of mycotic elements H, E X 1200 .

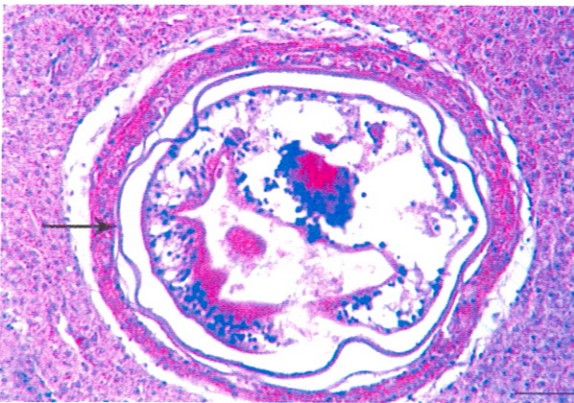
Fig. 15. The dermal skeletal muscles showing numerous encysted metacercaria with thin fibrous capsule and zinger's necrosis H, E X 300



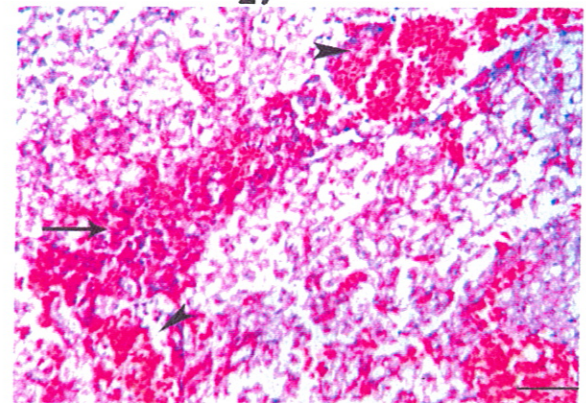
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Fig. 16. Liver of farm (B) show coagulative necrosis in the hepatocytes and pancreatic acini with intensive aggregations of lymphocytes H, E X 1200 .

Fig (17) Coagulative necrosis with thin light hyaline H, E X 1200 .

Fig (18) One encysted metacercaria with fibrous capsule with no evidence of cellular reaction H, E X 300 .

Fig (19) Severe congestion, hemorrhaged and necrosis in the liver H, E X 1200

DISCUSSION

At the present work clinical and post mortem examination of Tilapia fish revealed different lesions with different causative agents.

Regarding the single parasitic infected fish in Pond (A), the present study revealed that the clinical signs of infected fish were agree with that obtained by (15) who studied external parasites in cultured *O. niloticus*. On the other hand, in Pond (B) were agreed with that results met by (16,17).

The clinical signs and postmortem examinations of bacterial infected fish, were nearly agree with that obtained by (18) who studied the major bacterial diseases affecting Tilapia *Oreochromis niloticus* and other spp. in Port Said and agree with (19) who isolated *pseudomonas spp.* from 30.83% of examined *O. niloticus* and the clinical signs were redness all over the body, abdominal swelling, eyes cloudiness, scales detachment and congested gills.

The finding of clinical signs and postmortem examinations of mycotic infected fish were supported those recorded by (20,21).

Data obtained in table (2) revealed that out of 360 collected fish, 164 were positive for various bacterial isolates. The bacterial prevalence was higher in green house Pond (B) than in uncovered cement pond (A). Data obtained in table (4) revealed that, eight genera of bacteria were isolated from examined fish. The isolated strains were *Ps. aeruginosa* (40 isolates), *E. coli* (34 isolates), *Ps. fluorescens* (26 isolates), *p.vulgaris* (22 isolates), *E. intermedius* (14 isolates), *E. aerogenes* (14 isolates), *E. cloacae* (8 isolates) and *A. hydrophila* (6 isolates). This result was going hand with (19,22) also (18) who suggest that pollution with sewage and heavy metals make the immune response of fish suppressed leading to more bacterial infection for fish.

In Considering the Water temperature among ponds (A) $15\pm 2^{\circ}\text{C}$ and the ponds (B) $23\pm 2^{\circ}\text{C}$ maybe explain the large numbers of isolated bacteria. These results agree with (23) who concluded that water temperature ranging from $27\text{-}32^{\circ}\text{C}$ seemed to be the most effective for rearing of Nile tilapia juveniles and fries.

The predominant microorganisms isolated were *Ps. aeruginosa* followed by *E. coli* while *En. colacea* and *A. hydrophila* were the least prevalent bacteria in the examined fish. This result was agree with that obtained by (24) who isolated *A. hydrophila* by 43.77% and *Ps. fluorescence* by 29.63 % from skin ulcers affection in *Tilapia nilotica*. Meanwhile (19) found *pseudomonas spp.* in 30.83% of *Oreochromis niloticus* the prevalence of infection revealed significant difference among four batches, it was 43.33% (April 2008), 24.44% (August 2008), 21.11% (November 2008) and 17.77% (January 2009); and the organisms were mainly isolated from liver and kidney (35 and 30%, respectively). In another study (25) conducted bacterial examinations of *Oreochromis niloticus* cultured at Abassa Fish farms. *E. coli*, *Enterobacter colacea*, *C. frunidii* and *Y. intermediate* were isolated. It was found in this work that, a great relationship occurs between the bacterial flora of the alimentary

tract and that of the environment in which the fish lives. Also, (26) isolated *A. hydrophila*, *A. veronii*, *E. coli* and other bacterial spp. from semi-intensively cultured tilapia, *O. niloticus*. (27) Recovered 8 isolates of *Proteus vulgaris* from 6 fish out of 50 Nile tilapia collected from fish pond in Assiut Governorate.

Mycological examination revealed that 150 samples out of 180 fish were positive to fungal isolation of the five genera were isolated from green house highly incidence 83.3% (*Achlya spp* 73.33%, *Alternaria spp* 66.66%, *Rhizopus spp* 53.33%, *Mucor spp* 10.66% and *Penicillium spp* .9.33%) but in cement pond 90 samples out of 180 fish were positive to four genera were isolated (50%) (*Penicillium spp* 50%, *Aspergillus niger* 44.4%, *Blastomyces spp* 30% and *Scopulariopsis brevicaulis* 16.6%). These fungal species are infectious through contamination of water (28). Firouzbakhsh (29) reported *Aspergillus Niger* isolated from common carp and eggs. *Aspergillus niger* is the cause of the internal and external infection in fishes. *Saprolegnia sp.*, *Penicillium sp.* and *Mucor sp.* were reported in the eggs of *Aspencer percicus* (30). Refai (20) reported Eight different genera, *Aspergillus*, *Rhizopus*, *Mucor*, *Saprolegnia*, *Fusarium* and *Pencillium* from *Oreochromis spp.* and *Clarias gariepinas*. (31) isolated *Aspergillus sp.*, *Alternaria sp.*, *Blastomyces sp.* and *Rhizopus sp.* from silver carp and goldfish. According to (21), fungi have wide range of infection, depending on the management of farm and environment. However, many of the fungal genera have virulence factor which cause fish diseases under favorable predisposing environment. Role of ecology is important factor, which influence the diversity of fungus genera on the fish (32). According to (33), diversity of water molds depends upon the interaction of physicochemical factors. It may be stressed that poor pond management increases the chances of occurrence of fish diseases. Isolation of *Aspergillus spp.* from pond water has given an indication of pond contamination. Hence, attention must be paid to carry out; good pond and fish health management, through the use of good quality

inputs such as feed and water. Moreover, regular fish health monitoring may also be practiced.

The parasitic agents in examined Tilapia in pond (A) were mixed ectoparasites; *Trichodina heterodontata* and the *Myxobolus tilapiae* with prevalence rate 55.6%. This result was nearly similar with that recorded by (15, 34) who found *Trichodina spp.* infection rate in *O. niloticus* was 63.7% in winter and disagree with their results in case of *Myxobolus spp* which were not recorded in winter and recorded in summer with prevalence (38.5%).

The total prevalence of EMC in examined Tilapia from pond (B) was 77.8 % and all were infested with two types of EMC *Centrocestus formosanus* in gills and *Heterophyes sp* in muscles. The Heterophyid metacercariae *C. Formosa* were found infested gills of Cichlid fish in Brazil collected in winter with prevalence of infection 100 % (16). Our results agree with (17) who showed infestation rate of encysted metacercariae in tilapia species was 77.37% and disagree with (35) who showed infestation rate of encysted metacercariae (*Diplostomum tilapiae*, *Centrocestus formosanus* and *Heterophyes sp.* in tilapia species was 13.1%. These variations might be attributed to the factors affecting cercarial penetrations, site and time of sampling and the immunological status of the fishes. Also, the type of water seems to play an important role in the infection of fish by different types of heterophyid parasites. This role may be more influential to the first intermediate host (snails).

The Results of water parameters of selected water can be depicted from (Table.5). Water temperature, average DO concentration and the unionized ammonia increased in farm (B) with agriculture drainage water than farm (A) where source of water was well in winters supports the growth of aquatic microorganisms due to which concentration of spores have been increased mucus due to which skin is more prone to infections. Above findings have been supported by (36).

Regarding to the histopathological results of the present study, fish obtained from uncovered

cement pond (pond A) showed different lesions in gills, skin, muscles and liver.

Gills of fish infected with mixed bacterial, parasitic and fungal infections revealed congestion brachial blood vessels and capillaries, secondary lamellar together with hemorrhage and edema. Skin of fish infected with *Myxobolus* revealed necrosis and erosions beside different alterations occurred in dermis and epidermis. Meanwhile, the dermis showed edema, hemorrhage and extensive aggregation of round cells and *trichodina* -protozoa in epidermis. Liver of fish infected with bacterial infection showed severe congestion in the hepatportal blood vessels and hepatic sinusoids besides few extravasated erythrocytes. Interstitial aggregations of lymphocytes were seen. These cells were invaded and replaced the interstitial tissue. Meanwhile, the mycotic lesions represented by granulomatous reaction and extensive coagulative necrosis with irregular elongated eosinophilic mycotic elements. This result was similar to that obtained by (37) who found that skin of fish infected with *Trichodina* species showed, sloughing of the epidermal layer and the remained dermis was oedematous and infiltrated with leucocytes and melanine-carrying cells which aggregate between the muscle layers. He added that, the epithelial lining cells of the gill filaments showed, proliferative changes, including, hyperplasia and hypertrophy. In most cases, the epithelial proliferation of the lamellae started from their apices and extended towards the basal portations. Sometimes, those proliferative changes extend to other lamellae where one, two, or three lamellae appeared adherent to each other. In some fish, epithelial proliferation was extensive leading to fusion of secondary lamellae. Also (38,39) reported histological lesions of fishes infected experimentally by enterobacteriaceae including necrosis of liver. Roberts (40) Concluded that fish have catarrhal enteritis with eosinophilic granular cells indicate severe allergic or bacterial condition.

Teruo (41) Studied histopathological *Pseudomonas fluorescens* infection in Tilapia and revealed the presence of either focal

necrosis or granulomas in the livers with pale coloration and spotty lesions. In gills with spotty lesions, bacteria invaded connective tissue of gill filaments, causing focal necrosis, infiltration of inflammatory cells and fibrin precipitation. Some spotty lesions were packed by macrophages, in which bacterial cells were not observable. Those lesions in most cases accompanied epithelial hyperplasia.

On the other hand skin of fish obtained from Cement Pond samples (pond B) revealed areas of ulceration with complete disappearance of the epidermis. Such areas were invaded with numerous basophilic hyphae of mycotic element. The other areas showed spongiosis and severe hydropic degeneration of the epidermis. Gills showed severe congestion, hemorrhage, proliferation of the secondary lamellar epithelium and lymphocytes infiltrations. Some gill-filaments showed several encysted metacercaria with fibrous capsule. Liver of examined fish explicated the 3 infections; bacterial, parasitic and fungal infections. Severe congestion in hepatoportal blood vessels, and leukocytes infiltration were represented the septicemic lesions of bacterial infection. Coagulative necrosis with thin light hyphae and sporocysts or sporoblasts was confirmed the presence of mycotic infection. The dermal skeletal muscles showed numerous encysted metacercaria which encapsulated with thin fibrous tissue capsule. The affected muscles were hyalinized (Zenger's degeneration) or necrosis and appeared more eosinophilic erosions, spongiosis, increased mucus-secreting cells and hydropic degeneration were also found in the covering skin besides numerous encysted metacercaria in muscles.

Our results were in accordance with (42) who examined histopathological naturally infected tilapia fish with *Saprolegnia spp.* The skin showed epithelial desquamation in the epidermis which displayed either erosion to ulceration in the infected area. The other epidermal cells suffered vacuolar degeneration and focal necrosis. The underlying dermis was edematous and contained fragments from the fungal hyphae with focal aggregation of

melanomacrophages cells. The underlying muscles exhibited intramuscular edema, hyaline degeneration and Zinger's necrosis. The necrotic muscles infiltrated with numerous mononuclear leukocytes and some melanomacrophage. Similar result reported by (25) who stated that, the experimentally infected Nile tilapia, by *E. coli*, *Citrobacter freundii*, *Yersinia intermedia*, and *Enterobacter cloacae* showed focal hyperplasia, desquamation in the secondary lamellae and hemorrhages in the gill arch. The hepatopancreas revealed vacuolar degeneration and necrosis in some hepatocytes with nuclear pyknosis together with mucinous degeneration. On the other hand, the experimentally infected Nile tilapia, by *Enterobacter cloacae* revealed tubular degeneration in the kidney with depletion of hematopoietic tissue.

Banu et al., (43) Observed degenerative changes, cytoplasm fat vacuoles and lymphocyte infiltration in liver of Nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila* beside focal necrosis of hepatocyte. The hemorrhages and intensive lymphocyte infiltrations in liver both in macroscopic and microscopic levels revealed a visceral hemorrhagic septicemia.

Ibtsam (44) Concerning musculature oedematous and infested with encysted metacercariae was appear surrounded with serous fluid which contain a network of fibrin. It may be attributed to irritation of infective parasite and their product.

CONCLUSION

The present study emphasized the importance of favorable water quality parameters for successful rearing of fish as well as use of overwintering system (green house). Also this study substantiate the relationship between pond water source and water temperature as well as infection by different fish diseases

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المخلص العربي

دراسة مقارنة للحالة الصحية لأسماك البلطي خلال التغيرات البيئية المختلفة

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تم في هذه الدراسة تجميع ٣٦٠ سمكة من أسماك البلطي النيلى وزن (٤٠-٨٠) جراما جمعت بشكل عشوائى من أحواض اسمنتية مكشوفة مزرعة (A) احواض اسمنتية مغطاة (B) (١٨٠ من كل منهما) في أواخر فصل الشتاء من عام ٢٠١٣ (ديسمبر - يناير) من المعمل المركزى بالعباسة - محافظة الشرقية . في ذلك الوقت تم تسجيل درجات الحرارة، و نسبة الأمونيا و الأوكسجين الذائب. تم فحص جميع الأسماك فحص ظاهرى وفحص تشريحي وعمل فحوصات ميكروبيولوجية وطفيلية وبارتولوجية .

كانت العلامات المرضية على الأسماك المصابة فى الأحواض قبل الصيد تبدأ بفقدان فى الشهية، عتامة فى العين، ، زيادة فى كمية المخاط المغطى للجسم ، تعفن فى الزعنفه الظهرية والذيلية و وجود سوائل ارتشاحية دموية فى التجويف البطنى ثم النفوق. بعد النفوق كشفت دراسة الأحشاء فى الأسماك امتلاء التجويف البطنى بكميات كبيرة من السوائل المدممة، تليف فى الكبد وتضخم فى الطحال والمرارة حيث كانت ممتلئة بالعصارة الصفراوية.

أوضحت الفحوص الطفيلية والبكتيرية والفطرية أن الأسماك المصابة قد تم عزل منها مسببات مرضية ميكروبيولوجية وطفيلية كانت كالأتي : من ٣٦٠ عينة التي تم جمعها، ١٦٤ كانت إيجابية لمختلف المعزلات البكتيرية. كان انتشار البكتيريا العالي في المزارع الاسمنتية المغطاة (B) من الأسماك في البرك المكشوفة (A). كانت السلالات المعزولة سيدوموناس ارجينوزا (٤٠ عترة)، سيدوموناس فلوريسينس (٢٦ عترة)، بروتييس فلجارس (٢٢ عترة)، البكتيريا القولونية (٣٤ عترة)، انتروبيكتر انترميدنس (١٤ عترة)، انتروبيكتر اروجنس (١٤ عترة)، انتروبيكتر المذرقية (٨ عترات) والإيروموناس هيدروفيل (٦ عترات).

وكشف الفحص الفطري أن ١٥٠ عينة من ١٨٠ كانت إيجابية إلى العترات الفطرية. تم عزل خمسة أجناس من المزارع الاسمنتية المغطاة (B) بنسبة ٨٣,٣٪ (أكليا ٧٣,٣٪، الترنايا ٦٦,٦٦٪، الرايزوبس ٥٣,٣٣٪، الميكور ١٠,٦٦٪ والبنسليوم ٩,٣٣٪) ولكن ٩٠ عينة من ١٨٠ إيجابية إلى أربعة أجناس بنسبة ٥٠٪ (البنسليوم ٥٠٪، اسبرجيلس نيجر ٤٤,٤٪، بلاستومييسيس ٣٠٪ و اسكيبواوربسس ١٦,٦٪) على التوالي.

كشفت الفحص الطفيلي لأسماك البلطي وجود أربعة أنواع مختلفة، ميكوبولاس تلابي، تريكودينا هيتروندنتاتا و الميتاسركاريا المتحوصلة (EMC) سينتروسيتس في الخياشيم والهيتروفس في العضلات. وكانت الإصابة الطفيلية أعلى في المزارع المغطاة مزرعة (B) ٧٧,٨٪ من المزارع المكشوفة مزرعة (A) ٥٥,٦٪.

وكانت نتائج الهستوباثولوجي في الجلد والخياشيم والعضلات والكبد والتي زادت في شدتها مع المزارع المغطاة مزرعة (B) وقد أصبحت أقل حدة في مجموعة الأسماك من المزارع المكشوفة مزرعة (A).

أظهرت نتائج درجات حرارة الماء أن درجة الحرارة ارتفعت في مزرعة (B) 23 ± 2 درجة مئوية من مزرعة (A) $15 \pm$. كان تركيز الأكسجين الذائب في بركة مع مياه الصرف الزراعي في مزرعة (B) كان أعلى بنسبة $6,5 \pm 0,5$ بالمقارنة مع غيرها حيث كان مصدر مياه الآبار $5,7 \pm 0,8$. كانت نسبة الأمونيا في البركة مع مياه الصرف الزراعي (B) كان أعلى قيمة ($0,05 \pm 0,01$) من مزرعة (A) التي تزود المياه من بئر ($0,02 \pm 0,001$).

وتخلص الدراسة إلى أن نوعية المياه في الأسماك المستزرعة واحدة من أهم العوامل الرئيسية التي تؤثر على جودة الأسماك، لذلك تحتاج إلى العناية من أجل منع التأثيرات المحتملة الناجمة عن تجاهلها لتقييم صحة الأسماك.