Field Studies on *Caligus Disease* among Cultured *Mugil Cephalus* in Brackish Water Fish Farms

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Abstract: This study deals with clinical, post-mortem examination and parasitological identification of *Caligus sp* affecting infested *Mugil cephalus*, at Kafr El Sheikh Governorate fish farms. Histopathological examination of gills was investigated and recorded. Some treatment trials with freshwater, Metriphonate and freshly prepared Potassium permanganate were done. Freshwater with a 20-min immersion was successfully killed all copepods. While, short-term treatment for 30-min and 50-min immersion treatments with Metriphonate (20mg/l) and freshly prepared Potassium permanganate (10 mg/l) respectively, against *Caligus sp* infested *Mugil cephalus* were less effective than fresh water. These results concluded that, fresh water considered an effective in elimination of *Caligus sp* in *Mugil cephalus*.
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1. Introduction

Parasites have recently been highlight at serious pathogenic problems in cultured mullet fish in marine and brackish water **(1)**. Among the parasites, Copepode family is commonly found on fishes cultured in brackish water **(2)**. Most of the Caligus species are exclusively marine and some species attack brackish water fishes **(1).** In Egypt, such parasites were isolated from mullet fish *Mugil cephalus* (3). Attachment sites of these copepods were the body surface and gill cavities of the fish **(4)**. However, don't actually kill *Mugil cephalus*, unless the parasites occur in very large numbers, but the growth rate and market value of the *Mugil cephalus* may be reduced (**5** and **6)**. Fish with large numbers of parasites were emaciated; the body colour was darkened, Extensive abrasions with loosing and sloughing of the skin. The respiratory disturbance is accompanying with massive mucous secretions and severe destruction of the gills as well as causing severe losses and mortalities up to 50% (**7** and **3).** Losses associated with disease are the result of direct mortality due to secondary infections **(8** and **9).** *Caligus sp* respond differently to treatment. Fresh water controls *Caligus sp* (**10**) perhaps because of their osmoregulatory tolerances, which appears to be restricted to marine and brackish conditions **(11).** This study describes a simple and effective treatment against this pathogenic parasite.

2. Material and Methods

Fish:

A total number of 56 *Mugil cephalus* (ranged from 25-30 cm total length) infested with a parasitic copepod *Caligus* sp were collected from brackish water fish farms corresponding to Brullus lake and transported in tanks partially filled with brackish water pumped from the same ponds, were aerated and transported to Lab. of hydrobiology Dept. NRC. Before testing, two storage tanks were filled with water, the first with the same water of ponds and the second with freshwater. Eight aquaria (40 x 60 x 30 cm) were filled with 100 liter of water and aerated; seven aquaria were filled with brackish water had a pH of 8.2 and a salinity of 10 %o, and one was filled with freshwater had a pH of 7.2 obtained from the storage tanks. Freshly prepared Potassium permanganate (Akmavet) and Metriphonate (trichlorfon 97% active ingredient); (Adwia) were added to the tanks according to the experimental protocol (Table 1). There were seven treatment aquaria and one control. All *Mugil cephalus*, individually examined to record infestations and observed for clinical pictures. *Mugil cephalus* were exposed to the various treatments for either 5 -30 min (short-duration bath) or 12 h (long-duration bath) and immediately examined after exposure times.

Water:

Seven water samples collected from the same ponds *Mugil cephalus* and one from storage fresh water tank; in July at summer season 2011 for estimation of pH and salinity. A clean water sampling bottles each of about 1litre volume, equipped with stoppers. The water samples were taken a hand's breadth below the pond water surface. The bottles were labeled with locality, date and time of collection. The water samples were collected from 10 pounds of

Mugil cephalus and the average of their analysis were taken.

Clinical examination:

The collected *Mugil cephalus* were examined clinically according to the methods described by **(12)** paying an attention to the *Mugil cephalus* behaviors in the ponds, changes in colour, respiratory manifestations with a special attention to the gills.

Parasitological examination:

The microscopic parasites were collected by a fine brush, special needle or eye dropper, washed for several times in fresh water until the specimens had died and left in refrigerator at 4°C to completely relaxed. The crustaceans examined directly under light microscope. The isolated Copepods species were identified according to **(13,14,15 and 16).**

Physico – chemical analysis of water:

A total nine water samples, were collected from the different fish ponds; and one from stored freshwater tank. Determination of pH value of water samples collected from examined ponds and samples of storage freshwater tanks was measured by means of a digital PH meter (Ph. CP. HANNA instruments. Italy) and salinity (ppt) was estimated by DR 2010 (at wave length 530, programs 88).

Histopathological examination:

The affected skin , gills and gill arches of *Mugil cephalus* were fixed in 10% phosphate buffered formalin, then dehydrated in ascending grades of alcohol and cleaned in xylol, then embedded in paraffin wax and cut into thin sections (5um) and floated on warm water (just below the melting point of the paraffin) the sections are lefted from the water bath on microscope slides, coated with a minimal amount of Myer's albumin then allowed to dry thoroughly and then stained with (H&E) stain according to **(17).**

Treatment trials:

1- Freshwater. It was used with a 20-min freshwater dip treatment.
2- Potassium permanganate (KMnO4). It was used freshly prepared in a concentration of 5 mg/l for (10-min) immersion treatment.
3-Metriphonate (Trichlorfon) is an organophosphate insecticide and can be used at 0.25 to 20 mg/l, as a continuous bath for seven to ten days for (20-min) immersion treatment.

3. Results

Clinical examination:

The clinical signs of affected *Mugil cephalus* were loss of appetite, become debilitated with extensive mucous, rubbing against ponds plastic silk, nervous and respiratory manifestations. In addition, extensive focal brown spotted dotes on the skin, fins and red appearance on mouth can be seen (Fig.1 and 2).

Parasitological examination:

The microscopical examination revealed large numbers of copepods which was attached firmly to skin, fins and gill archer. The length of copepods were ranges from 5 to 6 mm long, the body was flattened, elongated or spherical with brown spotted colouration and have two characterized long bar-shaped egg pouches or strings. The crustacean parasite which was collected from some infected *Mugil cephalus* were identified as *Caligus sp* (Fig. 3).

Histopathological examination:

The parasites induced many branchial lesions showed severe inflammatory reactions to advanced degenerative and hyperplastic changes. The inflammatory changes were mainly observed in gill arch and congestion of lamellar blood vessels and adhesion of most gill filaments and lamellae (Fig. 4 and 5).

Treatment trials:

Treatment trials of naturally infested *Mugil cephalus* with *Calgius sp*, were applied using freshwater, freshly prepared Potassium permanganate and Metriphonate. It was noted that, the best suitable and effective treatments were freshwater followed by freshly prepared Potassium permanganate as it cause a great damage to the parasite at concentration of 10 mg/liter for 5 min and Metriphonate at a concentration of 20 mg /liter for 20 min.(Table, 1).

Table 1: Showing condition of the fish and copepods after different treatments and time exposures to remove *Caligus sp* from *Mugil cephalus* .

Treatment	Time	Treatment Concentration	Condition of *Mugil cephalus*	Condition of Copepods	Number of free copepods	Number of attached copepods
Fresh water		12 h	Good	Dead	10	0
Freshly prepared Pot.permanganate	20 min	3 mg/L	Good	Inactive	3	5
Freshly prepared Pot.permanganate	10 min	5 mg/L	Good	Dead	8	0
Freshly prepared Pot.permanganate	5 min	10 mg/L	Stressed	Dead	7	0
Metriphonate	30 min	10 mg/L	Good	Inactive	5	2
Metriphonate	20 min	20 mg/L	Good	Dead	9	0
Metriphonate	10 min	30 mg/L	Stressed	Dead	8	0
Brackish water control	12 h	10 %o	Good	Active	28	20

Figure (1): Showing *Mugil cephalus* red mouth X 40.

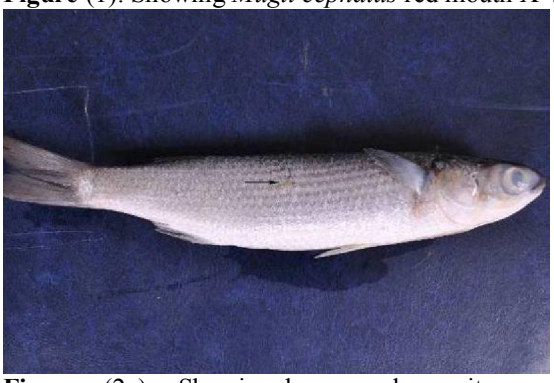

Figure (2) : Showing brown red parasite on skin (arrow) of *Mugil cephalus* .x 40

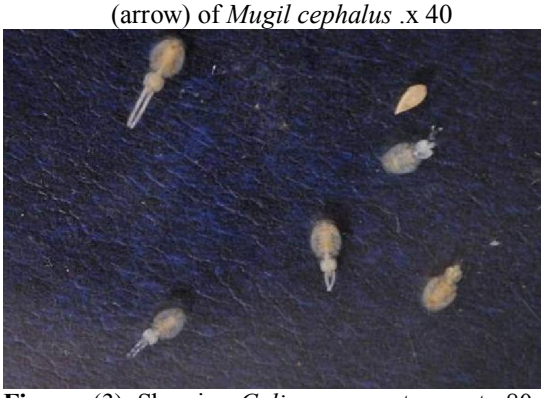

Figure (3): Showing *Caligus sp*. wet mount.x80

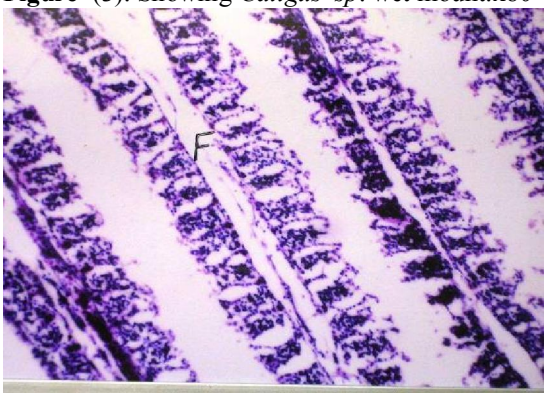

Figure (4): Gill of *Mugil cephalus* showing hyperplasia in the lamellae of the filaments (F) . H&E X 40.

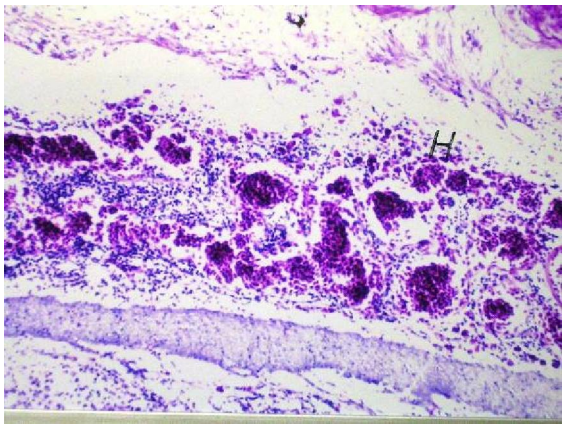

Figure (5): Gill of *Mugil cephalus* showing haemorrhage in the arch (H). H&E X 40.

4. Discussion

The present study deals with *Caligus disease* among cultured *Mugil cephalus* in brackish water at Kafr El Sheikh Governorate fish farms, Egypt. *Mugil cephalus* was in the aggregation as groups at the water inlet with severe respiratory distress and swam rapidly in circles, gulping of air at the water surface. These signs may be attributed to massive mucous secretions due to the irritation from contact of parasites and egg strings with the gill filaments & gill damage. Such results were nearly similar to that found by (**3, 18 and 19**). The results showed focal hemorrhage, abrasions on the skin and mortality was observed. These may be attributed to the parasites penetrate skin for fed and facilitate the invasion of the opportunistic micro-organisms. The present results agree with **(20, 5, 21 and 19).** The gross external examination of *Mugil cephalus* showed paleness of gills with numerous focal brown dots on the gill arches mainly unilateral and in some cases was bilateral. Alternative dark congested pale ischemic areas, marbling appearance of gills. These results are nearly similar to that reported by **(22)** and it may be attributed to destruction of the efferent vessels. The mechanism by which the destruction of the efferent vessels may happen by copepod crustaceans, where the blood pressure is low and no extensive haemorrhages are caused and the very short clotting time of blood brings about rapid occlusions of the vessel then thrombus is formed resulting in ischemia, which in turn leads to necrosis. Such explanation was recorded by **(23).**

In this study, histopathological examinations of the infested gills revealed congestion of lamellar and bronchial blood vessels with severe inflammatory cell infiltration. In advanced stages, adhesion of most secondary lamellae in some gills was observed. These results may be attributed to the mechanical injury

induced by the parasite that ingest infiltrated cells and epithelial cells that proliferate due to stimulation of attachment leads to slow haemorrhage, rapid blood clot, thrombus, ischemia and finally necrosis which manifests the picture of marbling appearance. The present findings nearly agree with results reported by **(24** and **5)**.

Regarding the prevalence of *Caligus disease* in relation to different some water parameters. The results revealed that, *Caligus* infestation found in *Mugil cephalus* cultured in brackish water , it may be attributed to the parasite cannot tolerate substantial osmatic changes in fresh water. The present findings nearly agree with the results found by **(25 and 26).**

Concerning the treatment using fresh water, it was revealed that the effectiveness of the fresh water treatment may be attributed to osmotic concentration. The present findings nearly agree with the results found by **(11).** Freshly prepared Potassium permanganate 5 mg per liter treated *Caligus sp* without harming in aquaria water. This may be attributed to strong oxidizing properties of Potassium permanganate, the present findings nearly agree with results found by **(27** and **28)** who recorded that Potassium permanganate kill skin and gill pathogens via its strong oxidizing properties. Moreover, the present study revealed that the effective concentration of Metriphonate was 20 mg per liter for 20 min. It was suggested that the effectiveness Metriphonate was due to its acetylcholine inhibiting effect on Caligus sp. These findings nearly agree with (**29,30, 31** and **32).** They reported that Metriphonate cause asphyxia and toxicity of the parasite. The results with (**33)** recorded that the standard *Caligus sp* treatment *in Salmon* appears to be Trichlorfon (300 mg/L, 15-60 min, 3-12°C).The results with pike may be due to the difference of the species , land host and /or they may be also due to difference of environmental conditions and /or breeding system.

In the present study, these results concluded that, fresh water considered the best and effective to eliminate *Caligus sp.* which affect *Mugil cephalus* in brackish water fish farms.

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