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Field Studies of Caligus parasitic Infections among Cultured Seabass (*Dicentrarchus labrax*) and Mullet *(Mugil cephalus*) in Marine Fish Farms with Emphasis on Treatment Trials

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Abstract: Caligus parasites are the most infested crustacean parasites affecting marine water fish. This study carried out the more prevalence crustacean parasites infested cultured seabass (*Dicentrarchus labrax*) and mullet *(Mugil cephalus*). The clinical pictures as severe erosion and haemorrhaging of body surface, fins and buccal cavity of affected fish were recorded. The crustacean parasites were collected from skin of mullet fish and buccal cavity of seabass that identified as copepods of *Caligus minimus* (*C. minimus*) and *Caligus elongates* (*C. elongates*) from seabass and mullet respectively. Prevalence of infestation was apparent on adult fish in spring and early summer seasons. Histopathological examinations of skin, gills and buccal cavity revealed that severe inflammatory reactions to advanced degenerative and hyperplastic changes. Available pharmaceutical products for the treatment of Copepoda were Metriphonate, Potassium permanganate and Hydrogen peroxide, as well as problems related to their therapy is discussed. In conclusion; this study revealed that sea lice infestations existed on *Dicentrarchus labrax* and *Mugil cephalus* in Damietta, Egypt. Also, freshwater considered an effective in elimination *C. minimus* and *elongates*.

Key words: *Caligus parasitse* · Seabass · *Mugil cephalus* · Trichlorfon · Potassium permanganate · Freshwater

INTRODUCTION

In Egypt, *Dicentrarchus labrax* and *Mugil cephalus* fish products have reached to 19.027 and137.543 tons respectively [1]. The gradual increase of this production of fish resulted in serious pathological problems in all countries including Egypt where intensive aquaculture is practiced. In recent years, crustacean parasitic diseases are becoming more frequent in the aquaculture which was associated with morbidity and mortality causing substantial economic losses [2] and has been reported in cultured marine fish in Mediterranean region [3,4]. In Egypt, such parasites were isolated from skin and fins of fish as well as in their buccal and branchial cavities of seabass and mullet, the infested fish were appeared of food and lesions were observed on the head region, the buccal cavity, palate, tongue and base of the gill arch of *Dicentrarchus labrax* and skin of *Mugil cephalus* [5, 6]. The intensity of *C. elongates* and *minimus* infested *Mugil cephalus* and *Dicentrarchus labrax* respectively, increased in winter and decline at late spring [6]. In addition, Caligid infestations indicate were amongst the most notorious pests affecting farmed marine fishes [7] and cages cultured fishes [8]. There infestations don't actually kill seabass and mullet fish, unless the parasites occur in very large numbers, but the growth rate and market value may be reduced [9]. The family members bear certain characteristics are included a flattened body, external surfaces and adaptation to being surrounded by blood and mucus on the epithelial cells of their hosts [10]. Losses associated with disease were the result of retard of direct mortality or mortality due to secondary infections [10]. In many cases, increased fibroplasia and spongiosis is noticed within dermal collagenous connective tissue [5]. Metriphonate has been used as standard treatments for mobile stages of sea lice (*Caligus* sp.) by the

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immersion or bath method in European seabass and mullet fish in a concentration 300 ppm for 20 minutes [11, 12]. Also, malathion was widely use in infested farms which showed either poor efficacy or unsuitable therapeutic margins [13] and [14]. While, hydrogen peroxide (H_2O_2) was used as bath 1500 ppm for 20 minutes for the sea louse Caligus rogercresseyi eradication in Chile [15]. Freshwater was used as bath for 12hr was complete eradicates *Caligus* sp. infected *Mugil cephalus* [6]. This study aimed to describe *Caligus* sp. infested cultured seabass and mullet fish in marine farms as well as a simple and effective treatment against this pathogenic parasite.

MATERIALS AND METHODS

Sampling and Examination of Fish: During the year 2011-2012, 240 seabass (ranged from 300-350 g total body weight and 30-35 cm total length) and 240 mullet fish (150-200 g and 15-20 cm) infested with a parasitic copepod *Caligus* sp. were collected from cultured fish in Ezbat El Borg fish farms, Damietta Governorate in corresponding to Mediterranean Sea and examined for clinical signs, postmortem and isolation of parasites copepods in examined farms and left in refrigerator at 4°C for overnight then preserved in glycerin alcohol till examined.

Experimental Treatments: A total number of 140 *Dicentrarchus labrax* fingerlings (ranged from 8-10 cm total length) and 140 *Mugil cephalus* fingerlings (ranged from 5-7 cm total length) infested with a parasitic copepod *Caligus* sp. were transported in tanks partially filled, with seawater pumped from the same earthen ponds were aerated and transported to Lab. of hydrobiology Dept. National Research Centre (NRC). Twenty eight aquaria (40 x 60 x 30 cm) were filled with 100 liter of water and provided with aeration; 26 were filled with water from the same earthen ponds had a pH of 8.2 and salinity of 30 ‰ and 2 were filled with freshwater had a pH of 7.2 obtained from the storage tanks. Freshly prepared Potassium permanganate (*Akmavet*), Hydrogen peroxide (H_2O_2) (*Cure Chem, India*) and Metriphonate (trichlorfon 97% active ingredient; (*Adwia*) were added to the tanks according to the experimental protocol (Table 1 and 2). There were ten treatment tanks, one control tank for 10 fish of each species. Seabass and mullet fish individually were examined and exposed to the various treatments for either 5-30 min (short-duration bath) or 12 h (long-duration bath). *Dicentrarchus labrax* and *Mugil cephalus* exposed to different average time of treatments (including control) were examined immediately after completion of exposure.

Clinical Examination: The collected fish were examined clinically according to the methods described by Noga [16].

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, then fixed in 70% alcohol glycerin, passed through ascending grades of alcohol (70, 80, 90, 95% and absolute) cleared in xylol, mounted in Canada balsam or by clearing in lactophenol and mounted in glycerin gelatin [17]. The isolated *Caligus* species were identified according to Yamagute [18] and Markemitch [19].

Table 1: Prevalence and intensity of Caligidae parasites in *Dicentrarchus labrax* and *Mugil cephalus*

	Prevalence (%)				Intensity		
Parasite species	Season	No.of examined fish	No.of infested fish	%	Season	Average S.D	Sn-1
Caligus minimus	Spring	60	36	51	Spring	10.20±0.6	8.8
	Summer	60	55	91.6	Summer	23.15±0.3	22.6
	Autumn	60	0	0	Autumn	0	0
	Winter	60	0	0	Winter	0	0
Caligus elongatus	Spring	60	54	90.0	Spring	22.55±0.55	21.5
	Summer	60	43	71.3	Summer	16.37±0.49	15.5
	Autumn	60	0	0	Autumn	0	0
	Winter	60	0	0	Winter	0	0

$Chi^2 = (16.77)^{**}$ ** = Significant at ($P < 0.01$)

**Means within the same column of different letters are significantly different at ($P < 0.05$).

Table 2: Showing condition of the fish and copepods after different treatments and time exposures to remove *Caligus minmus* from Seabass

Treatment	No. fish	Time	Treatment Concentration	Condition *Mugil cephalus*	Condition Copepods	No. of free copepods	No. of attached copepods
Fresh water	10		12 h	Good	Dead	34	0
Freshly prepared Pot. permanganate	10	20 min	3 mg/L	Good	Active	31	6
Freshly prepared Pot. permanganate	10	10 min	5 mg/L	Good	Dead	21	5
Freshly prepared Pot. Permanganate	10	5 min	10 mg/L	Stressed	Dead	16	2
Freshly prepared Hydrogen peroxide	10	30 min	10 mg/L	Good	Active	30	5
Freshly prepared Hydrogen peroxide	10	20 min	15 mg/L	Good	Dead	20	1
Freshly prepared Hydrogen peroxide	10	10 min	20 mg/L	Stressed	Dead	15	2
Metriphonate	10	30 min	1 mg/L	Good	Active	9	5
Metriphonate	10	20 min	2 mg/L	Good	Dead	45	2
Metriphonate	10	10 min	3 mg/L	Stressed	Dead	40	1
Malathion	10	30 min	0.1 mg/L	Good	Active	9	5
Malathion	10	20 min	0.15 mg/L	Good	Dead	45	1
Malathion	10	10 min	0.2 mg/L	Stressed	Dead	40	1
Seawater control	10	12 h	40 ‰	Good	Inactive	2	12

Chi^2 = (20.44)** ** = Significant at ($P < 0.01$)

Histopathological Examination: The affected skin, gills and buccal cavity of seabass and mullet fish 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 left 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 and E) stain according to Carleton *et al.* [20].

Treatment Trials:

- Freshwater (H_2O) obtained from the storage tanks). It was used as abath for 20-min freshwater treatment according to Landsberg *et al.* [21].
- Malathion (DiazinonTC 95%; o,o.diethylo-2-isopropyl-6-methyl-4-pyrimidinyt-phosphorothioate) obtained from *ADWIA* Company. It 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 according to Burka *et al.* [12].
- Metriphonate (Trichlorofon 97%) obtained from *ADWIA* Company. It 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 according to Pike [11].
- Potassium permanganate ($KMnO_4$) obtained from *Akma Vet* Company. It was used as freshly prepared in concentration 5mg/l for (10-min) immersion treatment according to Abd El-Alim *et al.* [22].

Hydrogen peroxide (H_2O_2) obtained from *Cure Chem. India* Company. It was used as freshly prepared in concentration 1500 ppm 20 min treatment according to McAndrew *et al.* [23].

Statistical Analysis: The statistical analysis was made according to SAS [24] in two steps using analysis of variance (ANOVA): 1st step to show the significance differences in intensity of different parasites among different seasons. 2nd steps using the Chi^2 test to examine the differences in incidences of different parasites among different seasons, different treatments and different weights and lengths.

RESULTS

Clinical Examination: The main clinical signs in naturally infested seabass and mullet fish showed in the form of motion slow swimming at the surface of the water (lethargic) and debilitated with extensive mucus as well as they become off food and rubbing against hard object. In addition, hemorrhagic areas on gill cover and in late stages, external ulcers located in the head region were appeared on infested seabass fish and the gills of mullet

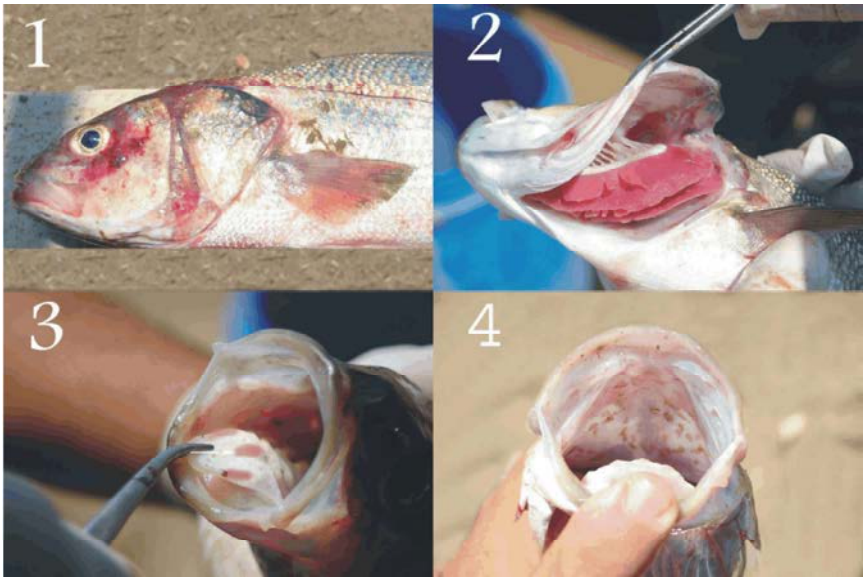

Plate A: Showing in infested seabass, poetical hemorrhage on skin (1), congestion of gill filament (2), poetical hemorrhage on tongue (Arrow) (3) and heavy infestation of *caligus* sp. in buccal cavity (4).

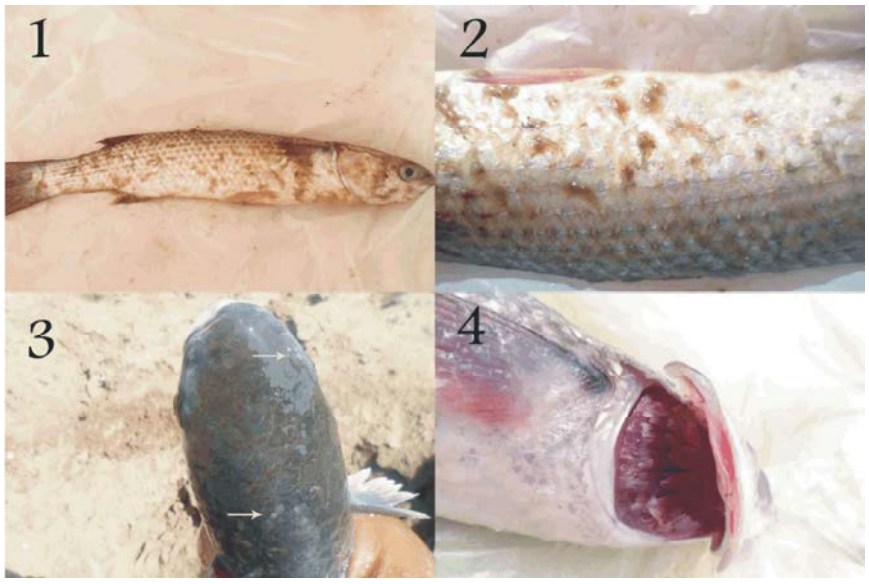

Plate B: Showing on *Mugil cephalus* heavy infestation with Caligus sp. on skin (1 and 2), head (arrow) (3) and hyperplasia in the lamellae of the filaments (4).

appeared as mosuic in shape. The tongue of seabass was also severely ulcerated and extensive focal brown spotted dotes were observed on the skin of head region, buccal cavity, palate and tongue was obvious even macroscopically (Plates A and B).

Parasitological Examination: Crustacean parasite 8-15 parasitic copepods were collected from the buccal cavity of European seabass. The body length of the female measures 2.8 mm, the male parasite is 1.2 mm in length with 4 legs. The cephalothorax, the cephalic zone, lateral zones and thoracic zone are clearly identified. The abdomen has posterior tagma which includes an abdomen and caudal rami which is greater than the thoracic zone.The thorax is segmented tofourth leg-bearing. The genital segment, the oviduct channel, intestine and immature eggs are also definable. Female characterized by long bar-shaped mature and immature eggspouches while

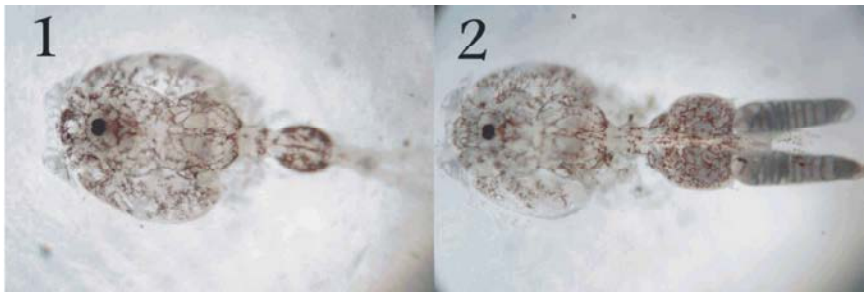

Plate C: General view of male dorsal surface, *Caligus minimus* (1) and female (2).

Plate D: General view of male dorsal surface, *Caligus elongates* (1) and female (2).

male parasite characterized by the first and second antennae of the parasites, can be clearly noticed and separated in frontal plates identified at the mid-dorsal line, where the lunules are large and the length of the tagma is greater than the thoracic zoneas well as the semen glands in both sides of the genital segment (Plate, C). Based on the morphological characters, these crustaceans are related to Calgidae, *Caligus minimus* [7]. Other crustacean parasite 2-3 parasitic copepods were collected from the skin of infested *Mugil cephalus*. The body length of the female measures 3.2 mm, the male parasite is 2.1 mm in length with 4 legs. The body was flattened, elongated or spherical with brown spotted coloration and has two characterized long bar-shaped egg pouches or strings (Plate, D). Based on the morphological characters, these crustaceans are related to Calgidae, *Caligus elongates* [25].

Prevalence of Infestation: As shown in Table (1), the infestation rate of *C. minimus* with a high prevalence of infested marine seabass and mullet in summer season was 91.6 and 71.3% respectively, followed by spring season was 51 and 90% respectively and the infestation was absent in autumn and winter season. While, the mean intensity per seabass and mullet fish in summer season was 22.6 and 15.5 respectively, followed by spring season were 8.8 and 21.5% respectively. The prevalence and intensity of *C. minimus* and were high throughout the year with a distinct peak of intensity in summer and decline in spring season. While, the highest prevalence and intensity of *C. elongates* infested *Mugil cephalus* showed higher values in spring and decline in summer season.

Histopathological Examinations: Skin showed the parasite caused traumatic lesions with complete loss of epithelium, congestion of dermal blood vessels associated with dermal oedema (Plate, E1). As well as presence of the developmental stage of parasite attached to the necrotic buccal cavity and skin lesion (Plate, E2).

Musculature showed intense infiltration with inflammatory cells mainly EGCs mixed with mononuclear cells and necrosis of the underlying musculature (Plate, E3).

Gills revealed large number of lamellar capillaries with necrotized pilaster cells and lamellar epithelium with hemorrhage, lamellar aneurysm with mucous cell hyperplasia (Plate, E4), multilayered lamellar epithelium and necrotic gill filaments with many lymphocytes infiltrate the gill lamellae.

Kidney revealed necrotic lesion in the renal tubules with nuclear pyknosis and cellular fragmentation as well as presence of cellular debris (Plate, E5). Intense mononuclear cell aggregation infiltrating the necrotized renal tubules, as well as interstitial tissues were demonstrated (Plate, E.6).

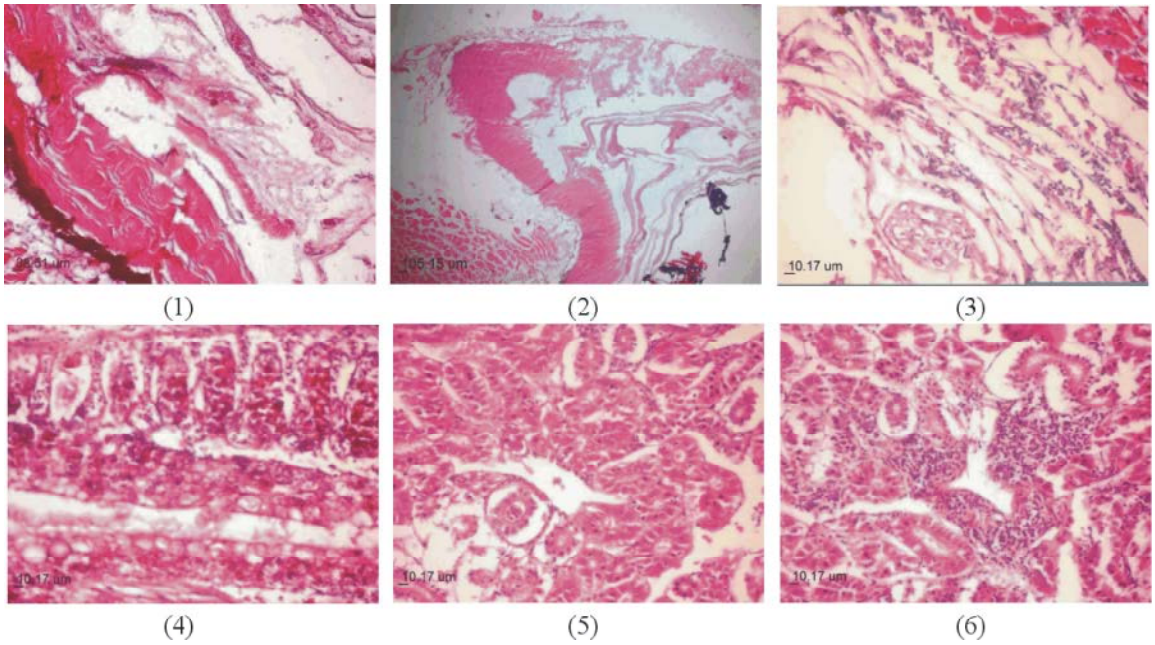

Plate E: Showed traumatic lesions, congestion of dermal blood vessels (1), necrosis of the underlying musculature (3) in infested skin of *Mugil cephalus*, necrotic lesion in buccal cavity of infested seabass (2), necrotized pilaster cells of lamellar capillaries and lamellar epithelium with hemorrhage, lamellar aneurysm with mucous cell hyperplasia (4) in infested gills of *Mugil cephalus*, necrotic lesion in the renal tubules with nuclear pyknosis, cellular fragmentation and cellular debris (5) in infested *Mugil cephalus*, intense mononuclear cell aggregation infiltrating the necrotized renal tubules and interstitial tissues in infested seabass (6).

Table 3: Showing condition of the fish and copepods after different treatments and time exposures to remove *Caligus elongatus* from *Mugil cephalus*

Treatment	No. fish	Time	Treatment Concentration	Condition of *Mugil cephalus*	Condition Copepods	No. of free copepods	No. of attach copepods
Fresh water	10		12 h	Good	Dead	8	0
Freshly prepared Pot. permanganate	10	20 min	3 mg/L	Good	Active	7	0
Freshly prepared Pot. permanganate	10	10 min	5 mg/L	Good	Dead	5	4
Freshly prepared Pot. permanganate	10	5 min	10 mg/L	Stressed	Dead	4	2
Freshly prepared Hydrogen peroxide	10	30 min	10 mg/L	Good	Active	8	0
Freshly prepared Hydrogen peroxide	10	20 min	15 mg/L	Good	Dead	6	0
Freshly prepared Hydrogen peroxide	10	10 min	20 mg/L	Stressed	Dead	5	2
Metriphonate	10	30 min	10 mg/L	Good	Active	3	7
Metriphonate	10	20 min	20 mg/L	Good	Dead	12	0
Metriphonate	10	10 min	30 mg/L	Stressed	Dead	10	6
Malathion	10	30 min	0.1 mg/L	Good	Active	9	5
Malathion	10	20 min	0.15 mg/L	Good	Dead	45	1
Malathion	10	10 min	0.2 mg/L	Stressed	Dead	40	1
Seawater control	10	12 h	40‰	Good	Inactive	7	0

$Chi^2 = (18.33)^{**}$ ** = Significant at ($P < 0.01$)

Treatment Trials: As shown in tables (2 and 3); treatment trials of naturally infested seabass and mullet with *Calgius* sp. were applied using fresh water, freshly prepared Potassium permanganate, Hydrogen peroxide, Malathion and Metriphonate and revealed that, the best suitable and effective fresh water followed by the Metriphonate in the concentration of 25 mg/liter for 20 minutes and Malathion in the concentration 0.15 mg/L for 20 minutes which cause a great damage to the parasite. Also, freshly prepared Potassium permanganate in a concentration of 10 mg/liter for 5 minutes and Hydrogen peroxide (H_2O_2) bath 1500 ppm for 30 minutes was partially effective.

DISCUSSION

The present study deals with Caligus disease among cultured seabass and mullet fish in marine water at Damietta Governorate fish farms, Egypt. The main clinical signs observed in infested seabass and mullet fish with crustacean infestations were excessive mucus production, sluggishness, collected on the surface of water as groups at the water inlet with gulping of air in earthen ponds and air pump in aquaria as well as, rubbing the body against hard objects and sides of to get rid the irritation induced by the parasites. These signs are as a result of the attachment by means of second pair of the antennae which were inserted into the host epidermal tissue which caused the low respired oxygen of destructed gill epithelium of the parasites. These results are in agreement with those reported by Eissa *et al.* [26]. The results showed focal hemorrhage, abrasions on the skin, buccal cavity and mortality was observed. These may be attributed to the parasites penetration of the skin for fed and facilitate the invasion of the opportunistic micro-organisms. These results are in agreement with that reported by Noor *et al.* [6] and Woo [27].

Regarding the postmortem examination, it was revealed paleness of gills alternative dark congested pale ischemic areas, marbling appearance of gillswith numerous focal brown dots on the gill arches mainly unilateral and in some cases was bilateral which 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 hemorrhages were 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 Noor El Deen [28]. The main lesions are observed on the skin of the head region, the buccal cavity, the palate, the tongue and the base of the gill arch which resulted the integument where parasites are located showed ulceration of the epidermis with marked inflammatory of the dermis as a result of the attachment and feeding activity of the parasites, these results agree with those reported by Ragias *et al*. [5].

Concerning the description and systematic part of the parasite is given *Caligus* male and female *elongatus* on *Mugil cephalus*, this agrees with Noor El- Deen [6] who isolated the same genus from the same host and site. The parasite under discussion isolated from skin of *Mugil cephalus*. These results may be due to the males die after copulation (38–54 days after hatching). However the host and locality varied from those mentioned before. The parasite measurements and morphological characters are nearly similar to that obtained by Williams [29] so it was identified as *Caligus elongatus* [30].

The second parasite under discussion isolated from buccal cavity and this is agreed with Tansel *et al.* [2] who collected female parasite from the buccal cavity of Brawn wrasse, *Labrus merula L.*, in Izmir Bay, Aegean Sea. Comparing the present data with the previous data, it is clear that the parasite has all morphological characters and measurements of *Caligus minimus* [31 and 32].

Regarding the prevalence of *C. minimus* infested seabass was high throughout the year in summer (91.6%), followed by spring (51%) and disappear in autumn and winter season. The mean intensity per seabass fish and the minimum-maximum parasite load per infested fish were 22.6 and 8.8 % in summer and spring respectively. These results may be attributed to many factors such as light and temperature as well as due to absence of parasite death. The results of this study showed that *C. minimus* can cause severe pathological changes in cultured seabass especially during summer and spring months. This may be explained by the fact that this fish species during hot months (when both parasite intensity and prevalence were high). In addition, the currents in these ponds are minimal, thus, facilitating the transfer of parasites from fish to fish. The present findings agree with previous results reported by Abd El-Alim *et al.* [22] and McAndrew *et al.* [23].

On the other hand, the prevalence of *C. elongates* infested *Mugil cephalus* was high throughout the year in spring season (90%) and in summer season (71.3%) and disappear in autumn and winter season. The mean intensity per seabass fish and the minimum-maximum parasite load per infested fish were 21.5 and 15.5 % in summer and spring respectively due to the lack of recruitment and parasite death. The results of this study showed that *C. elongates* can cause very low intensity and without apparent mortality. This may be explained by the fact that this fish species during hot months fish. These results are similar to that of *C. elongatus* described by Jonsdottir *et al.* [33] and Boxshall and Halsey [34].

The present study showed histopathological lesions mainly in the skin, gills and muscle. Skin lesions were characterized by complete loss of epithelium and necrosis of the underlying musculature with mononuclear cell aggregation, lamellar epithelial hyperplasia especially mucous cell. Similar histopathological pictures were recorded by Kabata [35] and Costello [36]. These lesions

may be attributed to the copepods attachment and feeding activity of the parasites as the parasitic copepods have been reported to feed on host mucous, tissues and blood. These results agree with that recorded by Johnson *et al.* [37] and Cruz-Lacierda *et al.* [38]. The increase of phagocytic cells in response to fish parasitizes as a defense mechanism for elimination of parasites is clear in this study. Mucous cell hyperplasia demonstrated in this study confirmed that the mucosal reaction is involved in the response to the Caligus copepod as mucous contain substances with biostatic activities. These results agree with that recorded by Eissa [39].

Regarding Metriphonate was used as standard treatments for mobile stages of sea lice (*Caligus* sp.) by the immersion bath method in seabass and *Mugil cephalus*. It was used in different concentrations, the best dose 20 mg per liter for 20 min. The effectiveness of Metriphonate was due to its inhibit acetylcholinesterase activity in cholinergic nervous systems of *Caligus* sp. Metriphonatepose toxic hazards not only to fish stock but also to the environment and repeatedly exposed to organophosphates can develop persistence. These findings agree with Fallang *et al*. [40]. Also, Malathion was used in different concentration in a best dose 0.15 mg/L for 20 min. The effectiveness of Malathion was due to inhibit many enzymes, especially acetylcholinesterase and cause a blockage of cholinergic nerve transmission in the parasite, resulting in spastic paralysis [16]. Their therapeutic index is low and they affect cholinesterase both in the host and the parasite, as well as other organisms in the aquatic environment. They represent a safety risk for chemical handlers when they administer treatments and parasite resistance is well documented [12] and the effective concentration of freshly prepared Potassium permanganate 5 mg per liter for 10 min treated *Caligus* sp. without harming in aquaria water which may be attributed to strong oxidizing properties of Potassium permanganate. The present findings nearly agree with results found by Abd El-Alim *et al*. [22] who recorded that Potassium permanganate kill skin and gill pathogens via its strong oxidizing properties. Their therapeutic index is low in presence of organic matters [41]. While, hydrogen peroxide was used in different concentration; the best dose 1500 ppm for 30 min. This may be attributed toa strong oxidizing agent and induce mechanical paralysis caused by the formation of bubbles in the haemolymph which detaches the lice and they float to the water surface. These results were in agreement with that recorded by Thomassen [42] and Athanassopoulou *et al*. [43]. On the other hand, Hydrogen peroxide is corrosive and must therefore be handled with great care. Transport of hydrogen peroxide requires certain precautionary measures, as it is defined as hazardous goods in great quantities and has been used against sea lice in the treatment of chalimus and mobile stages. In addition to, it was damage the gills of fish in experimental treatments and some mortality have been reported when farmed fish are treated [12]. For all these disadvantages, the chemical is not likely to be used for Mediterranean fish.

Finally, freshwater is considered the best and the safe in addition to its effectiveness to eradication of Sea lice instead *Dicentrarchus labrax* and *Mugil cephalus* cultured in marine ponds. These results may be attributed to osmotic concentration and potential. The present findings agree with Noor El- Deen *et al.* [6].

The obtained results concluded that, freshwater is considered the best and effective application to eliminate C. *minimus* and *elongatus* which infested European *Dicentrarchus labrax* and *Mugil cephalus* respectively.

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