Abbassa Int. J. Aqua., V0l.12 No.(1) 2019. *85-110*

CAUSES OF ULCERATIVE SYNDROME IN MARINE FISHES AND TRIAL TO CONTROL

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Received 4/ 8/ 2019 *Accepted 8/ 9/ 2019*

Abstract

The present study was carried out on one hundred and forty five naturally diseased marine fishes represented two species; seventy European seabass (*Dicentrarchus labrax*) and seventy five Gilthead sea bream (*Sparus aurata*) were collected from private fish farms in Damietta governorate. The collected fish submitted to full clinical, postmortem, bacteriological, mycotic, parasitological and histopathological examination. In addition, the physicochemical analysis of water pond holding fish was measured. The common clinical signs and the postmortem finding of examined fish were observed and recorded. The result of bacteriological examination revealed the isolation of *V. alginolyticus* from *D. labrax* and *S. aurata* with total prevalence rates 53.57% and 40.084%, respectively. Also, isolation of *A.hydrophila* from *S. aurata* with prevalence rate 33.75%. The antibiogram sensitivity of isolated strains were done and showed that of *V. alginolyticus* and *A. hydrophila* revealed that it was highly sensitive to Ciprofloxacin and Nalidixic acid. From the macroscopic and microscopic characters of isolated fungi from *S. aurata*, it cleared that the isolated fungi was *Aphanomyces sp.* with prevalence 26.16%. Parasitic examination of fish revealed infection of *D. labrax* with crustacean parasites identified as copepods of *Caligus minimus* with total prevalence rate 100%. The histopathological finding were studied. The trial of treatment of Copepoda by using Bafry D - 50/500 in a dose 0.75ml/L (375ppm) 10 minutes for successive 3 days was sufficient to eliminate all copepods without effect on fish health.

INTRODUCTION

In Egypt, fish considered a main factor of food security, social and economic development as it contributes in providing animal protein and one of the main sources of the national income. Sea bass (*Dicentrarchus labrax*) and

Gilthead sea bream (*Sparus aurata*) are the main cultured fish species in the Mediterranean area Abdel-Aziz *et al*. (2013).

The aquaculture industry has been plagued with disease problems caused by bacterial, fungal and parasitic pathogens. The most common diseases of fish are those that affect the skin which is a primary defense mechanism, the symptoms are mainly ulcerative and hemorrhagic skin patches Hassan *et al*. (2010).

Bacterial diseases mount the most influential sector of disease problems that has direct colossal impacts on Egyptian mariculture Grisez and Ollevier (1995). Bacterial ulcers are a common fish disease problem; they are one of the most difficult problems to deal with, especially if large numbers of fish are affected.

Vibriosis come on the top list of pathogens with direct jeopardy to mariculture development due to high mortalities associated with their invasion to fishes. *Vibrio alginolyticus* was considered to be predominant fish pathogens in both wild and cultured marine fish, responsible for high mortality rate and characterized by septicemia, dermal ulceration, ascitis and haematopiotic necrosis Mahmoud *et al*. (2018).

Aeromonas is considered the most important fish pathogens. These bacteria are responsible for ulcer type diseases and causing outbreaks with a mortality rate of 80-100% in a short period of time Kusdarwati *et al*. (2017).

Many of the fungi that affect fishes are considered opportunists, attacking the fishes when they are stressed or immune-compromised because of unfavorable environmental conditions, or secondary to bacterial or viral infections, or when they have lost their mucus protection because of trauma or excessive handling causing deep mycoses, ocular and skin lesions, fatal ulceration and necrohemorrhagic dermatitis on different parts of skin Cutuli *et al*. (2015).

Deleterious tissue damage is considered the main indicator of parasitic infestation among fish population. Crustacean ectoparasites are more frequently encountered in the aquaculture industry, it was found that about 25% of parasites infesting fish considered being crustaceans Tansel and Fatih, (2012).

Bafry D - 50/500 is a mixture of several disinfectants (H2O2 and silver ions). Hydrogen peroxide has been used in aquaculture as immersion (Bath) treatment against many different disease-causing organisms, including external parasites, bacteria and fungi, on different species and life-stages of fish Shehab El-Din *et al*. (2017).

The aim of the present study was to through light on infectious diseases affecting cultured marine fish causing ulcerative lesions under Egyptian conditions. Furthermore, trials for effective treatment and control ectoparasitic copepodes.

MATERIALS AND METHODS

Naturally infected fish:

A total of 145 moribund naturally diseased marine fishes represented two species, 70 European seabass (*Dicentrarchus labrax*) with an average body weight 80±5 gm and an average length 18± 2 cm and 75 Gilthead sea bream (*Sparus aurata*) with an average body weight 140±5 gm and an average length 18± 2 cm were collected from private fish farms in Deeba triangle in Port Said - Damietta way, Egypt. The collected fish were transported in large tanks filled with water of the same sources and supplied with battery air pumps to Fish Health and Management Department Lab., at Central Laboratory for Aquaculture Research, Abbassa, Abou Hammad Sharkia, Egypt (CLAR) and were subjected immediately to full clinical examination and postmortem finding also, bacteriological, mycological and parasitological investigation .

Water quality analysis:

The physico-chemical analysis of water holding fish were done in the field immediately for measuring the water parameters as (temperature, salinity,

pH, dissolved oxygen, saturation of the oxygen, electric conductivity (E.C), nitrate, nitrite, phosphate and total solid). The physicochemical analysis of water samples were carried out in accordance to standard analytical methods APHA (2005).

Clinical investigation and Post-mortem examination of fish:

Moribund and clinically diseased fish were grossly examined for determination of any clinical abnormalities and any external parasite. The clinical abnormalities and post-mortem changes were recorded according to Noga (2010).

Bacteriological examination :

Samples for bacteriological examination were collected under aseptic precaution from gills, liver, spleen, skin ulcers, blood, kidney and fins, inoculated into tryptic soy broth (TSB) and streaked on tryptic soy agar (TSA) with 1% sodium chloride (NaCl), the suspected purified colonies were picked up and streaked over specific media as Thiosulphate Citrate Bile Salt sucrose Agar (TCBS) (Biolife, Milan, Italy). The inoculated media were incubated at 25°c for 24-48 hrs; the isolated bacteria were subjected to identification according to Bergey's Manual of Determinative Bacteriology (1994).

Antibiogram sensitivity:

The antibiogram sensitivity test was done according to the limits given by Schäperclaus *et al*., (1992) using disc diffusion method on Muller's Hinton agar medium and interpretations of zones of inhibitions were recorded. The antibiotic disces used were ciprofloxacin, colistin sulphate, rifampine, florefenicol, lincomycin, nalidixic acid.

Mycological examination:

The isolation of fungi were carried out from moribund and diseased Fish. The sample were taken From eyes, skin ulcer, fins gills, mouth and inoculated into Sabaroud's Dextrose Agar (SDA) medium plates and incubated at 20 ± 2oc

For 3 - 4 days and subculture on the Same medium (SDA) for purification, all positive culture were examined for colonial growth, morphological feature and microscopic characteristic. The microscopical examination was done from wet preparation from skin ulcer, eyes, gills, mouth and fins and also from growth on SAD to detect septation of hyphae according to Dvarak and Atanoesk (1969).

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 Paperna (1980) and Özak (2006).

Histopathological examination :

Samples for histopathological examination were freshly taken from skin and gills of infested fish. Histopathological techniques were carried out according to Roberts (2001).

Treatment trials:

The trial for treatment beginning with remove the causes by rising salinity and adjust pH by water exchange or partial change of pond water, addition of Ciprofloxacin in a dose 5mg/10kg body weight for bacterial infection. For controlling fungal infection using lime and salt for 3 successive days at a ratio 1:1 in a concentration of 0.5% gave a good results and using sea water supply after partial removal of pond water. For control copepods infestation, forty infested *Dicentrarchus labrax* with copepod kept in glass aquaria 40×40×80 cm at the same environment specially salinity and provided with aeration, divided into four groups each group contain 10 infested fish and treatment trials applied using Bafry D-50/500 (H_2O_2 and silver ions) FMT:106 Makram Obeid St., Nasr City, Cairo, Egypt. First group treated with fresh water.2nd group treated with Bafry D-50/500 in a dose 0.5ml/L (250ppm) for 10 minutes, 3rd and 4th groups treated with dose 0.75ml/L (375ppm) and 1ml/L (500ppm) for 10 minutes respectively for successive 3 days.

RESULTS AND DISCUSSION

Physico-chemical analysis of water:

The water parameters during the study for water holding Seabass (*D. labrax*) site (1) and Seabream (*S. aurata*) site (2) were recorded in Table (1). The physico-chemical analysis of site (1) were in normal range with exception slight decreasing in dissolved oxygen. Whereas site (2) showed decreasing of water temperature, decreasing of water salinity, slight decreasing in dissolved oxygen and slight increasing in pH. These results in agreement with (Zaki *et al*., 2011; Abou El-Atta, 2013 and Moustafa *et al*., 2014). Actually, there is a close relationship between environmental stress and emergence of outbreaks of fish diseases. Deterioration in water quality stresses cultured fishes with consequent increase in the chance of opportunistic pathogens to invade fish causing disease condition Roberts (2012). Numerous epizootics of bacterial and mycological infection have been erupted after long exposure to low water temperatures, whereas cold temperatures completely halt the activity of immune system, subsequently eliminate fish defense against invading pathogens Zorrilla *et al*. (2003). The virulence of the causative pathogens is exacerbated by exposure to higher pH values and reduced dissolved oxygen levels Moustafa *et al*. (2014).

Table 1. Water quality parameters measures of sites holding examined fish.

Water parameters										
Sampling sites	**Temperature**	**Salinity**	**pH**	**DO**	**Saturation**	**EC**	**NO2**	**NO3**	**PO4**	**TS**
Site 1	29°c	28‰	7.8	4 mg/L	52.5%	10000	0.004 mg/L	0.2 mg/L	0.2 mg/L	140 mg/L
Site 2	19.8°c	4.5‰	8.8	3.75 mg/I	45%	9000	0.019 mg/L	0.23 mg/L	0.18 mg/L	90 mg/L

DO = Dissolved oxygen, NO2 = Nitrite, NO3 = Nitrate, PO4 = Phosphate, EC = Electric conductivity, TS = Total solid.

Site 1: Water holding Seabass (D. labrax) fish. Site 2: Water holding Seabream (S. aurata) fish.

Clinical and post-mortem examinations:

Clinically, moribund diseased fish showed opaque of the skin, loss of balance, respiratory distress, off-food, swim near to water surface, excessive mucus secretion, loss of scales, hemorrhage on gill cover, behind the pectoral fin, anal fin and caudal peduncle, tail and fin rots and erosion extended to reach epidermal layer causing ulceration (Plate1 A and B) and (Plate2 A, B, C and D). *D. labrax* rubbed themselves on solid substrate due to attachment of crustacean with fish, ulceration was a result of penetration of skin with crustacea for feeding which leads to physiological hemeostasis including osmotic stress anemia, hypoproteinanemia, immune state decreasing and suppressed which facilitate the invasion with secondary bacterial infection specially Vibrio species which it occur ready in water environment, these result agree with Lester and Hayward (2006). The excessive mucus secretion, may be to dilute the irritation and act as a defense mechanism against the infestation and showed emaciation may be due to crustacean infestation which reduce fish appetite and became off food, this agreed with Nagasawa (2004). In addition *S. aurata* showed appearance of grey-white cotton wool tufts growth on lateral aspect of the body when removed showed irregular margin ulceration, also, cotton like growth on mouth, eye causing blindness uni or bilateral (Plate2 A, B, C and D). Unilateral or bilateral corneal opacity of the eyes maybe attributed to inflammatory local odema due to in cease permeability of the capillary endothelium leading to escape of plasma protein under the effect of exotoxin or cytotoxin produced by infected microorganisms. These results were identical to those obtained by (Taghrid, 2011 and Abou El-Atta, 2013). The lesions focal or diffused hemorrhage on the different parts of the body in both fishes may be occurred due to various factors such as potent bacterial proteases (proteolytic enzymes) these results were similar to that mentioned Abou El-Atta (2013). The main postmortem lesions were congested gills with excessive mucous (in some cases the gills were pale), contact of parasites on gill filaments of seabass causing gill damage, destruction of gill tissue and secondary bacterial infection were occur resulted in death of infected fish, these result agree with (Noor El

Deen *et al*., 2012 and Marzouk *et al*., 2018), accumulation of fluid in abdominal cavity, these result were due to septicemia which caused by secondary bacterial infection specially Vibrio species which secret it's toxins and protedytic enzymes these result agree with Enany *et al*. (2011), congested kidney, congested enlarged liver, distended gall bladder with bile secretion and the intestine free of any food (Plate1 C and Plate 2 E).

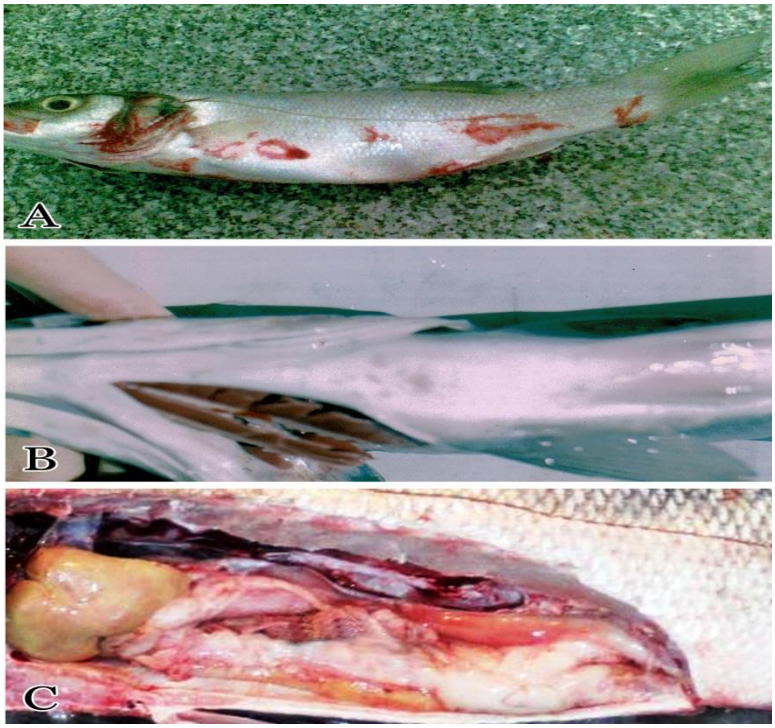

Plate 1. A- *D. labrax.* showing haemorrhagic patches on the external body surface. B- *D. labrax.* showing marbilling like appearance on gills, Caligus attached to skin. C- *D. labrax.* showing pale liver.

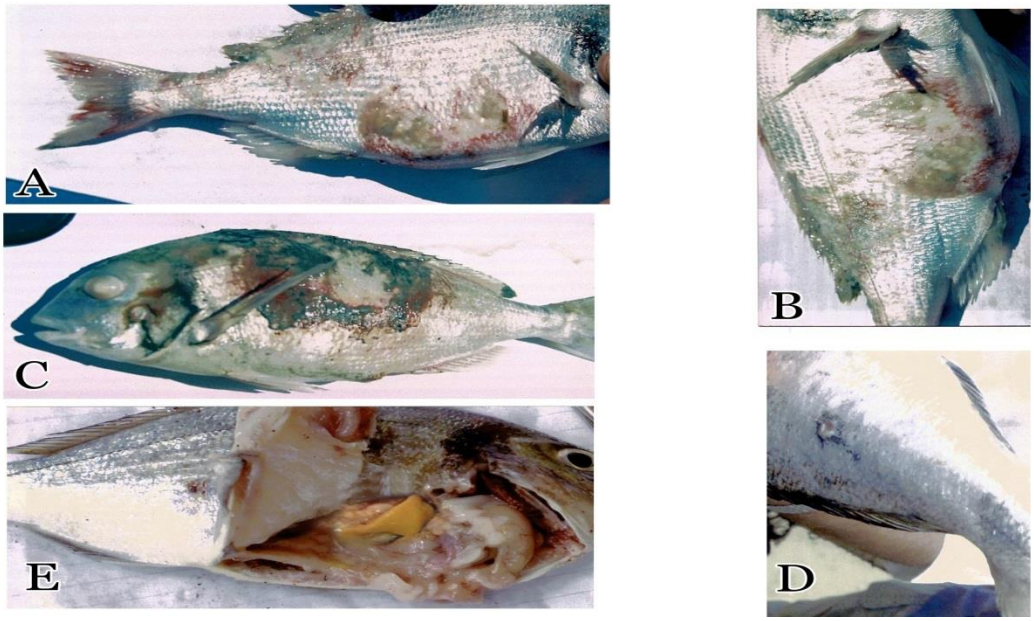

Plate 2. A&B *S. aurata* showing abdominal ascites, detached scales, fin rot of pectoral fin, diffused hemorrhage of abdominal cavity, redness and hyperanemia of anal opening, erosion, irregular ulceration and cotton wool like tufts growth on the fin and abdomen. C- *S. aurata* showing blindness of the eyes with cotton wool like tufts growth. D- *S. aurata* showing focal necrosis, erosion and irregular ulceration. E- *S. aurata* showing pale gills and liver.

Bacteriological examination:

Morphological and biochemical characterization of bacterial isolates which were isolated from diseased *D. labrax* and *S. aurata* are shown in (Table 2). In this study the result of bacteriological examination were recorded in (Table 3). The total prevalence of *V. alginolyticus* was 53.57% and 40.084% from seabass and seabream, respectively which were lower than those reported by Abdel-Aziz. *et al*. (2013) who recorded *V. alginolyticus* in 87.28% and 82.19% respectively. This might be attributed to the difference in seasons, water quality, pollution of water by heavy metals, locality and types of pisciculture used. Also, the main factors influencing the occurrence and distribution of Vibrio in aquatic environments were water temperature, nutrient availability and the association with marine organisms. While it

was higher than that recorded by Tanekhy (2013) 16.66 % and 17.14%, respectively and Saad and Atallah (2014) 13.8% and 10.3%, respectively. Such increase could be accredited to the difference in site and time of sampling, immune status of fish and disease resistance Grisez *et al*. (1997). *V. alginolyticus* act as primary and secondary infected pathogen, it isolated from seabass with high prevalence from skin 21 strains with percentage (28%) followed by liver with percentage (25.33%) as shown in (Table 4).This may be due to infestation with *Caligus minimus* which leads to erosion and ulceration of skin and excessive mucus secretion and decrease immune state and suppressed immune system these accelerate and enhance the bacterial infection, these result nearly accepted with those mentioned with Enany *et al*. (2011) and Abou El-Atta (2013). The *V. algindyticus* was obtained from seabream with high prevalence from liver 21 strains with percentage (23.15%) followed by skin with percentage (20.0%) as shown in (Table 4), preference of liver tissue in seabream could be related to some of the virulence determinants owned by the pathogens which augment their septicemic nature with final predisposition into the toxin neutralizing vessel this was in agreement with Abdel-Aziz. *et al*. (2013).

In regards to the Incidence of *A. hydrabhila* among naturally infected seabream; results revealed that it was 33.75%. this rate was higher than that obtained by Zorrilla *et al*. (2003) who isolated *Aeromonas spp*. from seabream with a frequency lower than 10%. *A. hydrophila* can persist in the salinity environment reach 6-7% so, it cause Aeromonas septicemia in fresh, brackish and marine environment, these results were in agreement with Abou El-Atta (2013). *A. hydrophila* is considered as one of the most important pathogen responsible for haemorrhagic septicemia in a wide variety of marine water fish. The highest intensities isolated from seabream were in skin with percentage (23.75%) as shown in (Table 4). These results

were supported by Vethaak (1992) who isolated *A. hydrophila* from ulcers and lesions of ulcerated European flounder.

Table 2. The biochemical and morphological characters of isolated bacteria.

Characters	***V. alginolyticus***	***A. hydrophila***
Shape	Slightly curved rod	Rod
Gram staining	-ve	-ve
Motility	Motile (swarming)	Motile
Growth on TCBS	+ve yellow colonies	-ve
Growth on Aeromonas base agar	-ve	+ve green colonies
Growth on Pseudomonas base agar	-ve	-ve
Growth on NaC%		
0%	-ve	+ve
3%	+ve	+ve
5%	+ve	+ve
7%	+ve	-ve
10%	+ve	-ve
Oxidase test	+ve	+ve
O/F (oxidation-fermentation)	F (fermentative)	F (fermentative)
Growth on 5%Nacl	+ve	+ve
Indole	+ve	+ve
Voges Proskaur	+ve	+ve
Methyl red	+ve	+ve
Arginine	-ve	+ve
H2s production	-ve	-ve
Catalase	+ve	+ve
Citrate utilization	+ve	+ve

Table 3. The prevalence of each isolates among naturally infected fish.

Fish species	Seabream (*S. aurata*)		Seabass (*D. labrax*)	
Isolets	No.	%	No.	%
V. alginolyticus	95	40.084	75	53.57
A.hydrophila	80	33.75	-	-
Aphanomyces sp.	62	26.16	-	-

Table 4. Distribution of isolated strains in different organs and tissues of examined fish.

Organ		Gill	Skin	Fin	Eye	Liver	Spleen	Kidney	Total
No.of isolates of *V. alginolyticus* from Seabass, *D. labrax.*	NO.	9	21	-	-	19	11	15	75
	%	12	28	-	-	25.33	14.66	20	
No.of isolates from Seabream, *S. aurata*.	NO.	25	63	46	11	37	22	33	237
	%	10.5	26.6	19.4	4.64	15.6	9.3	13.9	
V. alginolyticus	NO.	9	19	15	-	22	12	18	95
	%	9.47	20.0	15.78	-	23.15	12.63	18.94	
A. hydrophila	NO.	8	19	13	-	15	10	15	80
	%	10.00	23.75	16.25	-	18.75	12.50	18.75	
Aphanomyces sp.	NO.	8	25	18	11	-	-	-	62
	%	12.90	40.32	29.03	17.74	-	-	-	

Antibiogram sensitivity :

As shown in Table (5), the susceptibility patterns of the isolated bacterial strain agents revealed that all isolates were sensitive to Ciprofloxacin while vary in susceptibility to the others chemotherapeutic agents. These results confirmed with the previous findings by Quinn *et al*. (2002); Abdel-Aziz *et al*. (2013) and El-khatib (2014) who mentioned that Ciprofloxacin was one of the effective choices for treatment of most gram negative bacterial fish diseases.

Table 5. Antibiogram of sensitivity of isolated strains.

Antbiotic disc	Code symbol	Concentration (µg)	*V. alginolyticus*	*A.hydrophila*
Ciprofloxacin	Cip5	5 µg	+++	+++
Amoxicillin	AMX25	25 µg	R	+
Tetracycline	T30	30 µg	±	+++
Nalidixic Acid	NA30	30 µg	+++	+++

R=Resistant

Mycological examination:

The wet mount preparation from cotton like tufts from naturally infected *Seabream, (S. aurata*) was identified as *Aphanomyces sp*. It was isolated in a

total number 62 strain with prevalence 26.16% as shown in Table (3). This rate was higher than obtained by Abou El-Atta (2013) recorded *Aphanomyces sp.* from the same fish with rate 11.66% and very higher from Abdel-Latif *et al*. (2016) who isolated the same species from Seabream with rate 0.88%. The highly percentage of isolation from skin 25 isolates (40.32 %) as shown in Table (4). This was agree with Abdel-Latif *et al*. (2016) who isolated the same species from skin of Seabream and similar to Abou El-Atta (2013) who isolated it with high rate from skin (30.61%).

Parasitological examination:

The parasitological examination revealed severe infestation by large numbers of crustacean species which was attached firmly to gills, skin and fins and buccal cavity of Seabass, (*D. labrax*). Based on the morphological characters, these crustaceans are related to the Copepoda, family Calgidae and were identified as *Caligus minimus* (Plate 3). These results agreed with Özak (2006) who recorded the same species from the same host in Turkey, Sterud (2002) isolated *C. minimus* from bucccal cavity of seabass in Norway, Noor El-Deen (2013) recorded it from seabass in Egypt and Khoa *et al*. (2018) from Malaysia. While Tansel and Fatih (2012) isolated the same species from the gill and external surfaces of the brawn wrasse marine fish.

The morphological description of *Caligus minimus*:

The body length of the female measures 3.1 mm, the male parasite is 1.6 mm in length with 4 legs. Lunules, 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. The cephalothorax, the cephalic zone, lateral zones and thoracic zone are clearly identified. The last part is the abdomen has posterior tagma which includes an abdomen and caudal rami which is greater than the thoracic zone. The thorax is segmented to fourth leg-bearing. The genital segment, the oviduct channel, intestine and immature eggs are also definable. Female characterized by long bar-shaped mature and immature eggs pouches while 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 (Plate 2). The morphological characters and measurements of *C. minimus* were similar to that obtained by (Paperna, 1980; Ragias *et al*., 2004 and Noor El-Deen, 2013).

Concerning the prevalence of *C. minimus* infested seabass it was 100% this findings similar to results reported by Paperna (1980); Yeler (1988) and Abou El Atta (2013) while it was higher than recorded with Noor El-Deen *et al*. (2013). This difference may be attributed to the locality from which fish samples obtained. In the current study ectoparasites infection increased the susceptibility of fish for secondary bacterial infection; where the most fish samples infested with crusteacean are infected with *V. algynolyticus*. This result supported by Abo El- Atta (2013) who isolated *V. algynolyticus* from sea bass infested by crusteacean parasites, Raja *et al*. (2014) who said that attachment of crustacean parasites on marine fishes open the way for secondary microbial infection, El-khatib (2014) who suggested that possible role of parasites in enhancing infections of fish with bacterial pathogens.

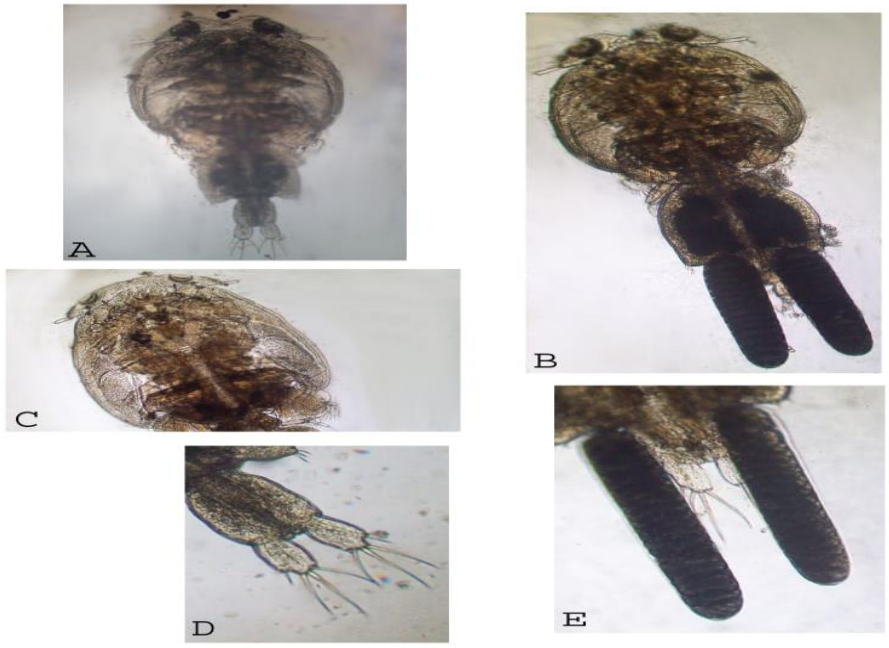

Plate 3. Light photomicrograph of *Caligus miminus* showing A-Adult male (X 40); B-Adult female (X 40);C- Cephalic region; D-Caudal ramai (X 100); E-Egg strings (X 100).

Histopathological findings:

The results of histopathological examination in *D. labrax*; skin showing epithelial desquamation in the dermis and degeneration in the epidermal cells with focal superficial sloughing to deep ulceration these 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 Noor El-Deen *et al*. (2013). Gills showing sever hyperplasia of the epithelial covering, congestion and desquamation of secondary lamellae epithelium. Liver showing severe vacuolar degeneration in the hepatocytes. kidney showing collapse of the capillary tuft (Glomeruli) with accumulation of edematous fluids in the Bowman's capsule, with hyaline droplet degeneration (Plate 4). About *S. aurata*; gills showing sever hyperplasia, aneurysm, fusion, ballooning dilatation of secondary lamellae and marked sloughing in the primary and secondary lamellae. Liver showing perivascular edema odmixed with inflammatory cells with fibrous tissue proliferation around bile ducts. Kidney hyaline droplet degeneration with accumulation of edematous fluids in the Bowman's capsule (Plate 5). Generally, the tissues of infected fishes revealed various proliferative, degeneration and circulatory changes. Gills are the primary organ which directly reflects the water pollution, contamination and diseases causing factors. In the present work gills showing sever hyperplasia of the epithelial covering, congestion and desquamation of secondary lamellae epithelium. Hyperplasia of lamellar epithelium could be interpreted as defense responses of the fish, as these alterations increase the distance across which waterborne irritants must diffuse to reach the blood stream Pandey *et al*. (2008). The most common cause of cellular degeneration in gill filaments is oxygen deficiency as a result of gill toxicity Mohamed (2009). Alterations in the liver may be useful as markers that show former exposure to environmental stressors Velmurugan *et al*. (2007). In the present study, vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulation system Gingerich (1982). On the other hand,

Eder and Gedigk (1986) suggested that oxygen deficiency as a result of gill degeneration may be the most common cause of the cellular degeneration in the liver. These histopathological changes are in are in agreement with those obtained by Avci *et al*. (2014). kidney showing collapse of the capillary tuft (Glomeruli) with accumulation of edematous fluids in the Bowman's capsule, with hyaline droplet degeneration. This may be due to proliferation of endothelial and mesangial cells accompanied by inflow of leukocytes leading to adhesion of tufts as well as degeneration of renal tubules may be due to bacterial or parasitic diseases Jones *et al*. (1997). The edema of the Bowman's capsules resulted in hypoproteinamia, decrease the colloidal substances, breakdown the cement substance and the endothelial cells leads to the passage of fluids to the surrounding media. Hyaline droplets (accumulations of intracytoplasmic protein absorbed from the glomerular filtrate) arise from glomerulonephritis Kashgarian (1998). These histological alterations on kidney have been observed by several authors in fish (Mohamed, 2009 and Lidia *et al*. 2011).

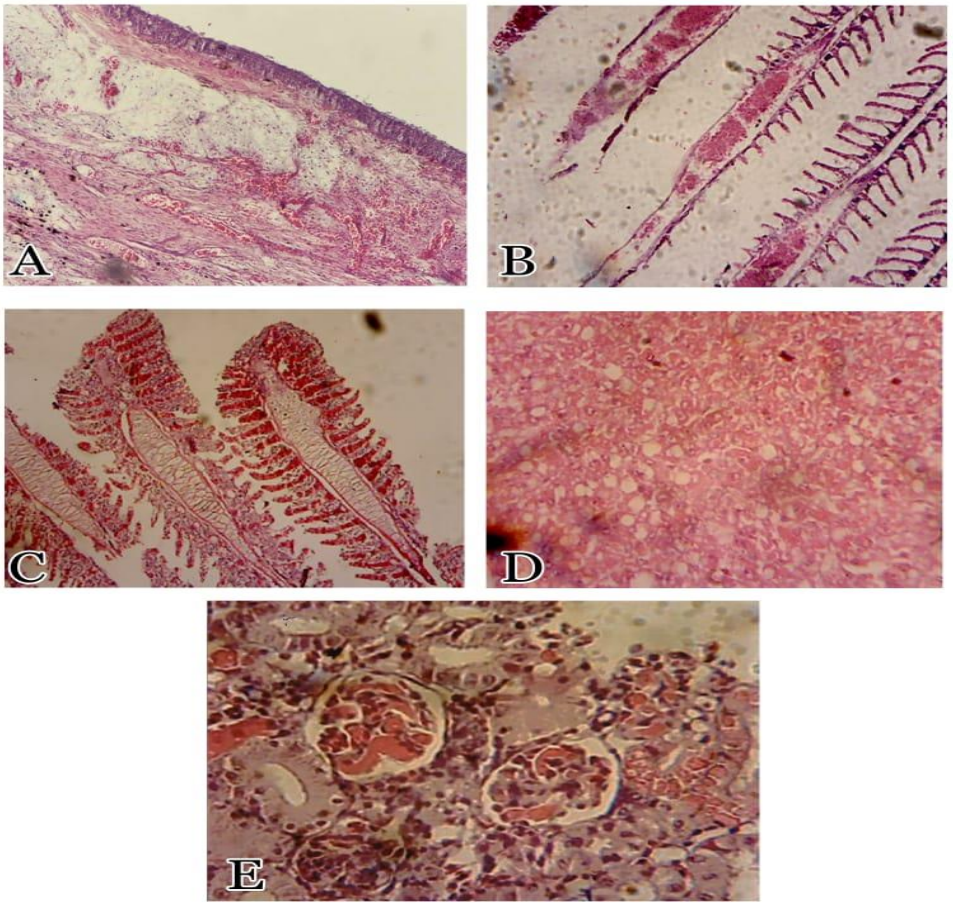

Plate 4. A- Photomicrograph of section in the skin of *D. labrax* showing epithelial desquamation in the dermis and degeneration in the epidermal cells with focal superficial sloughing to deep ulceration. B- Photomicrograph of section in the gills of *D. labrax*. showing hyperplasia of the epithelial covering, congestion and desquamation of secondary lamellae epithelium (H&E X 300).C-Photomicrograph of section in the gills of *D. labrax*. showing sever hyperplasia of secondary lamellae epithelium (H&E X 400). D-Photomicrograph of section in the liver of *D. labrax*. showing severe vacuolar degeneration in the hepatocytes (H&E X 400). E- Photomicrograph of section in the kidney of *D. labrax*. showing collapse of the capillary tuft (Glomeruli) with accumulation of edematous fluids in the Bowman's capsule, with hyaline droplet degeneration (H&E X 400).

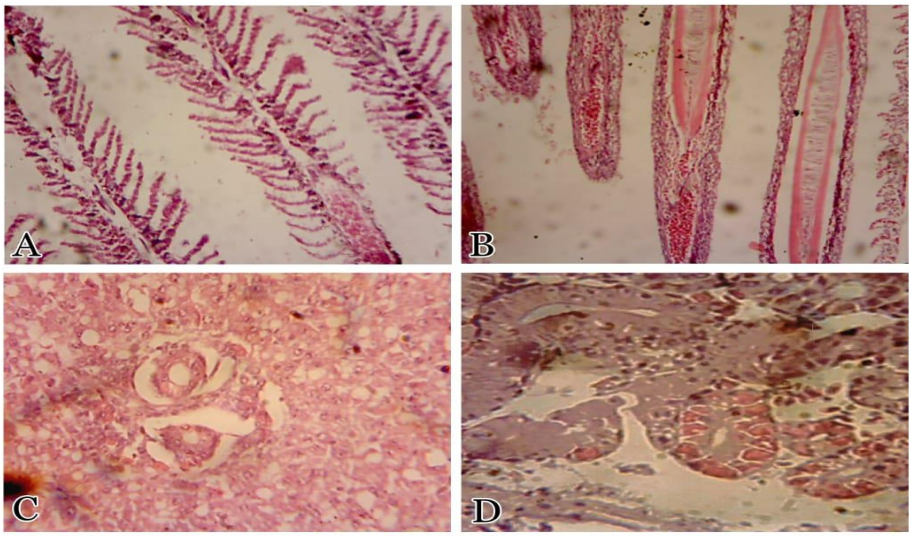

Plate 5. A-Photomicrograph of section in the gills of *S. aurata* showing aneurysm, fusion and ballooning dilatation of secondary lamellae (H&E X 300). B-Photomicrograph of section in the gills of *S. aurata* showing marked sloughing in the primary and secondary lamellae (H&E X 400). C-Photomicrograph of section in the liver of *S. aurata* showing perivascular edema odmixed with inflammatory cells with fibrous tissue proliferation around bile ducts (H&E X 400).D-Photomicrograph of section in the kidney of *S. aurata* hyaline droplet degeneration with accumulation of edematous fluids in the Bowman's capsule (H&E X 400).

Treatment trials:

Treatment trials of naturally infested Seabass (*D. labrax*) with *Caligus miminus* using freshwater for 12 hrs and Bafry D-50/500 in different concentration as bath for 10 minutes for successive 3 days. It was noted that, the freshwater treatment gave a good result in control of *Caligus miminus* attached to *D. labrax* as a bath for 12 hrs. these results agree with Noor El Deen *et al*., (2013). While the using of Bafry D-50/500 revealed that application of a dose 0.5ml/L (250ppm) 10 minutes for successive 3 days had no effect on copepods , but treated in a dose 0.75ml/L (375ppm) 10 minutes for successive 3 days was sufficient to eliminate all copepods by second day of treatment without effect on fish health, whereas treated in a dose 1ml/L (500ppm) for 10

minutes showed disappearing of copepods but fish die in the second day of treatment. So, the best dose was 0.75ml/L (375ppm) 10 minutes for successive 3days in treatment and control of all attached Caligus. This may be attributed to a 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 Noor El Deen *et al*. (2013). Hydrogen peroxide has no environmental implications, when added to water, it breaks down into oxygen and water over time and the formation of these by-products is one reason that hydrogen peroxide is considered to be relatively safe for the environment Hartmann (2010). Silver ions have an oligodynamic effect and react as catalysts and react with the protiens of the ectoparasites and this proteins will be inactivated or precipitated, they are added for long-term efficiency. Hydrogen peroxide and silver ions have a synergetic effect as a disinfectant.

CONCLUSION

In the present study, we concluded that treatment beginning with remove the causes by rising salinity and adjust pH by water exchange or partial change of pond water, addition of Ciprofloxacin in a dose 5mg/10kg body weight for bacterial infection. For controlling fungal infection using lime and salt for 3 successive days at a ratio 1:1 in a concentration of 0.5% gave a good results and using sea water supply after partial removal of pond water. Bafry D-50/500 in a dose 0.75ml/L (375ppm) 10 minutes for successive 3 days considered the best chemical and effective to eliminate *Caligus miminus* which infested and affect *D. labrax* in marine aquaculture and has no effect on healthy state of treated fish.

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دراسة مسببات ظاهرة التقرحات فى الأسماك البحرية ومحاولة السيطرة عليها

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الملخـــــــــص العربـــــــى

أجريت هذه الدراسة على مائة وخمسة وأربعين سمكة بحرية ٧٠ من اسماك القــاروص و٧٥ من اسماك الدنيس. تم جمعهما من مزارع الأسماك الخاصة في محافظة دمياط حيث خضعت للفحص الظاهرى، الداخلى، البكتريولوجى، الفطرى، الطفيلى والهستوباثولوجى. بالاضافة الى قياس معــايير جودة المياة. اظهر الفحص البكتريولوجي عن عزل ميكروب الفيبرو الجينولتكس مــن القــاروص و الدنيس حيث كانت نسبة الاصابة ٥٣.٥٧ ٪ و ٤٠.٠٨٤ ٪، على التوالي. أيضا، عــزل ميكــروب الإيروموناس هيدروفيلا من الدنيس مع معدل اصابة ٣٣.٧٥ ٪. تم إجراء حساسية المضادات الحيوية للسلالات المنعزلة وأظهرت حساسيتها للسيبروفلوكساسين وحمض الناليديكسيك. و لقد اظهر الفحص المورفولوجى و الميكروسكوبى لعينات الفطريات ان الفطر المعــزول مــن اســماك الــدنيس هــو افانوميسيس بمعدل انتشار ٢٦.١٦ ٪. كما اوضح الفحص الطفيلي لاسماك القاروص عزل نوع مــن القشريات الطفيلية كاليجس مينيمس وكانت النسبة الكلية للإصابة ١٠٠%. وقد تم دراســة التغيــرات الهستوباثولوجية . ولقد تبين من محاولات العلاج ان استخدام بافرى د ٥٠/٥٠٠ بتركيز ٠.٧٥ مل/ لتر (٣٧٥ جزء فى المليون) ١٠ دقائق لمدة ٣ أيام متتالية كافية للقضاء على جميع القشــريات دون تأثير على صحة الأسماك.