Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(7): 363 – 381 (2020)
www.ejabf.journals.ekb.eg

The impact of co-infection of sea lice and its concurrent some bacterial diseases with field treatment trials in some marine cultured fishes

Soad S. A. Salama[1*] and Nesreen S.I. Yousef[2]
[1]Fish Microbiological Unit, Fish Diseases Department, Animal Health Research Institute, Egypt.
[2]Fish Parasitological Unit[2], Fish Diseases Department, Animal Health Research Institute, Egypt.
[*]Corresponding Author: Goodweza2009@yahoo.com

ARTICLE INFO

Article History:
Received: Aug. 7, 2020
Accepted: Oct. 25, 2020
Online: Oct. 27, 2020

Keywords:
Caligus minimus
sea bass
Dicentrarchus labrax
Malathion
V. alginolyticus.

ABSTRACT

Co-infection is frequently found between sea lice and other pathogens, with high prevalence in the field and is highly detrimental to fish health. In this study, a total of 100 sea bass (*Dicentrarchus labrax*) were collected from a private marine farm that suffered from severe co-infection. All fish were examined for bacteriological and parasitological infections with trials for treatment. The detected pathogens among the examined *D. labrax* were *V. alginolyticus* (80%) and sea lice, *Caligus minimus* (100%). The clinical pictures showing dark skin coloration, excess mucus secretion, and detached scales with sever hemorrhage at fins and the operculum. The internal organs showed congestion and hemorrhages with small grossly ascetic fluid in the abdominal cavity. Water samples were analyzed for confirmation of normal condition and all physicochemical parameters were within the normal ranges in the marine fish farm. The antibiotic susceptibility against 11 antibiotics was tested and *V. alginolyticus* sensitive to ciprofloxacin and Trimethoprim /Sulfamethoxazole. The naturally infected fish could be treated successfully by Malathion in a single dose (0.1 mg/L for 30 minutes) as a short duration bath and then fed a ration containing Ciprofloxacin (150 mg / Kg body wt.) for ten days. The mortality rate declined, the clinical signs disappeared and the fish returned to a normal state of health. Co-infections have a fundamental effect and can alter the course and the severity of different fish diseases. Co-infection (bacterial and parasitic diseases) need further studies by conducting more experiments in the fish farm and using modern and fast diagnostic methods to control these diseases.

INTRODUCTION

The subject of co-infections of aquatic animals by different pathogens has received little attention even though such infections are common in nature. Co-infections are defined as infection of the host by two or more genetically different pathogens where each pathogen has pathogenic effects and causes harm to the host in coincidence with other pathogens **(Mohamed *et al.*, 2016).** Bacterial infection considered the main cause of high mortalities and economic losses among fish diseases (**Eissa *et al*., 2010**). The European sea bass **(***Dicentrachus labrax*) is the most important commercial fish widely cultured in

Mediterranean areas and Egypt are the biggest producers (**FAO, 2013)** and is found to be susceptible to various pathogens of parasitic, bacterial and viral origin (**Azad *et al*., 2006**). **Salwany *et al.* (2019)** studied growth in aquaculture production is parallel with the increasing number of disease outbreaks, which could be affect the production, gains, and continuity of the global aquaculture industry. Vibrios, one of the most challenging pathogens in mariculture development causing high mortalities between fish farms (**Fadel, 2014**). *V. alginolyticus* is a halophilic vibrio and is considered the most frequent species living freely in water and sediment. It can survive in seawater even under starvation conditions while maintaining its virulence (**Mustapha, *et al*., 2012).** It causes many epizootic outbreaks among the Gilthead sea bream and European sea bass populations, which possess high economic value at marine aquarium **(Zorrilla, *et al.*, 2003).** In spite of that, antimicrobial chemotherapy has side effects, but it remains vitally important for treating bacterial diseases through appropriate diagnosis, antibiotic selection at suitable administration route and dose (**Mahmoud *et al.*, 2018**).

Crustacean parasites are considered an important limiting factor in the development of intensified fish culture **(Osman *et al.*, 2014)**. Among crustaceans, copepods are dominant. Sea lice (copepods) have been reported as one of the most critical problems in marine fish culture, causing severe negative effects on stock survival, growth and susceptibility to disease **(Maran *et al.*, 2009).** The ensuing losses from fish deaths as a direct or indirect consequence of parasitic copepod infestation may be huge **(Lafferty *et al.*, 2015).** Sea lice moreover, reproduce rapidly and survive in large populations **(Tully et al., 1993)**, which may affect wild fish populations **(Costello, 2009)**. The Caligidae is the most species rich family of the copepod order Siphonostomatoida. *Caligus* Müller, 1759 is the largest genus of the family, currently containing approximately 250 valid species **(Boxshall, 2015).** Sea lice can affect the growth, fecundity, and survival of their hosts because their feeding may cause skin lesions leading to osmotic problems and secondary infections and, if untreated, they can reach a level that is highly detrimental to the fish **(Bayoumy *et al.*, 2013).**

The control of parasitic organisms is a major concern in marine aquaculture. In particular, sea lice cause substantial economic losses on fish farms **(Costello, 2009)**. Due to their economic importance, control of sea lice on fish farms has been named one of the top priorities in aquaculture research by both scientists and aquaculture practitioners **(Jones *et al.*, 2014).**

Hence, the present study aimed to obtain the effectiveness of different treatment regimens of co-infection of sea lice with bacterial pathogens in the marine fish farm. Firstly, adequate control of sea lice is predicated on the ability to predict future lice levels from current farm and secondly, uses of different regimens to treatment bacterial infection.

MATERIALS AND METHODS

Case history

A private marine fish farm in Ezbt El-borg, Damietta Governorate (Egypt), rearing the sea bass (*Dicentrarchus labrax*) in earthen ponds was suffered from co-infection with cumulative mortality. The naturally infected *D. labrax* showed excess mucus secretion, sluggishness, surface swimming and collected on the surface of water as groups at the water inlet with gulping of air. Moreover, *D. labrax* were rubbing the body against hard objects and sides to get rid of the irritation induced by the parasites.

Fish sampling

A total of 100 moribund, freshly dead and alive adult *D. labrax* (150 $\pm$ 10 gm) were collected in ice box. Only alive fish were put in plastic bags pumped with full amount of oxygen and transported under safety condition to the Fish Disease Department Lab in Animal Health Research Institute.

Clinical and Post-mortem examination of fish

Fish samples under investigation were grossly examined for determination of clinical signs and any external parasite. The specimens were examined externally according to **Noga (2010).** The samples were dissected for detection of any abnormality internal lesions.

Water sampling

Water samples were collected from marine fish farm, according to standard **Canadian Council on Animal Care (2005).**Water parameters pH, temperature, dissolved oxygen, salinity, Un-ionized ammonia, Nitrate (NO3), Nitrite (NO2) and sulphate were measured.

Bacteriological examination

Loopfuls from (liver, spleen, kidney, gills, ascetic fluid and gall bladder) from examined fish under complete aseptic conditions and streaked onto trypticase soy agar(Oxoid) supplemented with 2% (w/v) sodium chloride, marine agar (Oxoid) and thiosulphate citrate bile salt agar (TCBS) plates then incubated at 25° C for 24-48hr. (**Whitman (2004).** Pure colonies are examined microscopically and biochemically according to Bergey's Manual of Systematic Bacteriology (**Garrity *et al*. 2001**). Furthermore, identity the bacterial strain using API*20 NE (BIO-Merieux, France). Then, sensitivity to 2, 4-diamino-6,7-diisopropypteridin (O/129) disc (150 μg) and the motility of the strain were examined in soft agar. Finally, pure colonies were transferred to glycerol broth 20% at -80°C (**Pujalte *et al.*, 2003**).

Antibiogram sensitivity discs:

Sensitivity was determined by the agar diffusion method (**Quinn *et al.*, 2002**) using 6 mm diameter commercial discs (Oxoid) included the following antibiotics,: Amoxicillin 25 µg (AX25) Ciprofloxacin 5 µg (CIP 5), Lincomycin 2 µg (L2), Oxolinic acid 2 µg (OA 2), Norfloxacin 30 µg (NOR30), Trimethoprime /Sulfamethoxazole 25µg (SXT25), Oxytetracycline 30 µg (TE30),Tetracycline 30 µg (T 30), Erythromycin 15 µg (E15), Nalidixic Acid 30 µg (NA30) and Gentamycin 10 µg (GN 10). Antibiotic sensitivity was tested on Mueller-Hinton agar supplemented with 1.5% NaCl (for *V. alginolyticus* isolate), inhibition zones diameters were interpreted as sensitive, intermediate and resistant according to **CLSI (2010**).

Parasitological preparations:

Gills, skin, fins and branchial chambers were investigated macroscopically using magnified lens to detect crustaceans. The isolated crustaceans were fixed by passage in alcohol-glycerin (30.0%, 50.0% and 70.0%) then the specimens were cleared in pure glycerin and mounted in glycerin-gelatin according to **Lucky (1977).** Identification of the detected copepod crustaceans were based on morphological features according to **Yamaguti (1963).**

Trials for treatment:

Experimental fish

The experiment was performed in the private marine fish farm, a number of 250 infected *D. labrax* with an average body of 150 ± 10 gm. were holding in 4 small earth ponds, it contains almost 200 liter of water from same source as fish farm with 60 fish each. Water quality was the same in all ponds.

Experimental Design:

The experiment was conducted to compare the effect of antibiotic (Ciprofloxacin) and antiparasitic (malathion) by exposure of infected sea bass to either single or combined treatment of two drugs. The total of 250 infected sea bass were divided into 4 groups and randomly screened of 10 fish for the presence of co-infection parasitic (*C. minimus*) and bacterial (*V. alginolyticus*), also free from any other pathogens. Treated fish feeding were 3% from fish weight divided twice /day. Group (1), was fed on ration containing Ciprofloxacin (150mg / Kg Body wt.) for 10 days (**Chatterjee and Chatterjee, 1992)**. Group (2), was exposed to a single dose of malathion (organophosphate insecticide) in the concentration 0.1 mg/L used as a short duration bath for 30 min. with possible repeating of treatment after one week from the first dose according to the condition of the infection, according to **Burka *et al*. (1997).** Group (3), was exposed to a single dose of Malathion in the concentration 0.1 mg/L for 30 min. and then followed by Ciprofloxacin (150 mg / Kg Body wt.) for ten days. Group (4), was kept as a control group and fed on non-treated ration (Table 4). During the experiment, fish was examined immediately after exposure to treatment. The morbidity and mortalities were recorded and at end of experiment, *D. labrax* were examined for the presence of pathogens to evaluate the efficiency of treatment methods.

RESULTS

Clinical and post-mortem changes:

Grossly, the naturally infected *D. labrax* showed dark skin coloration, excess mucus secretion, detached scales with sever hemorrhage at fins and the operculum, congested gills and slight abdominal distention. The results showed focal hemorrhage, abrasions on the skin, buccal cavity. Other fishes showed sluggish movement and uni or bilateral corneal opacity (Plate (1) A, B and Plate (2) A, B and C).

The most common post-mortem lesions were congestion and hemorrhages of the internal organs with small grossly ascetic fluid in the abdominal cavity. In addition to, paleness of liver and kidney were observed in some cases and discoloration of liver with flabby skeletal muscles (Plate (2) D).

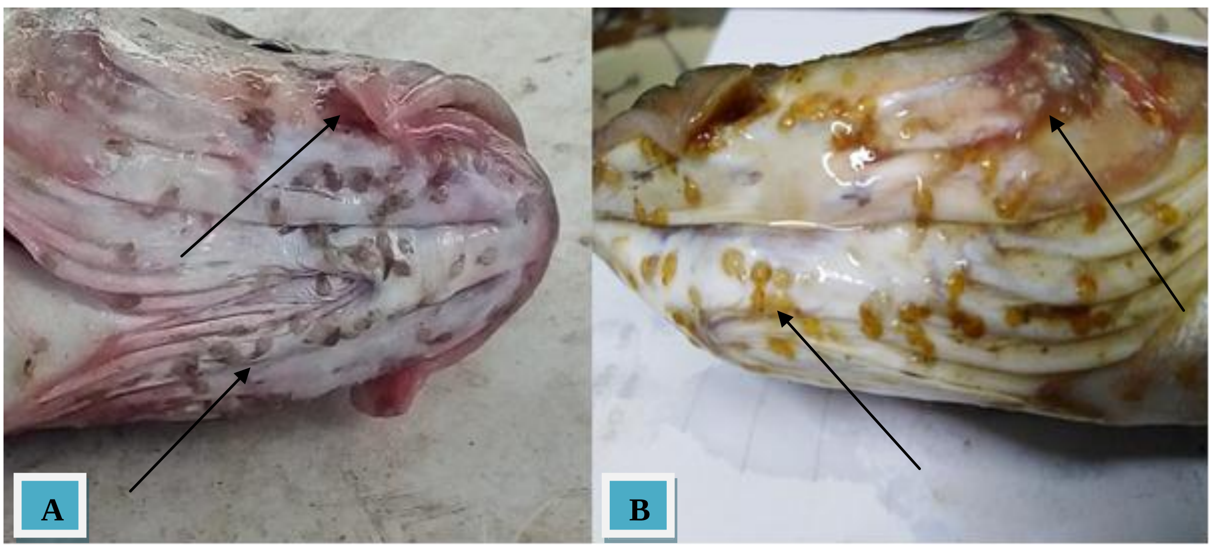

Plate (1): Naturally infected *D. labrax* showing a large number of sea lice (*Caligus minimus*) on head region with excessive mucus and hemorrhage at operculum cover.

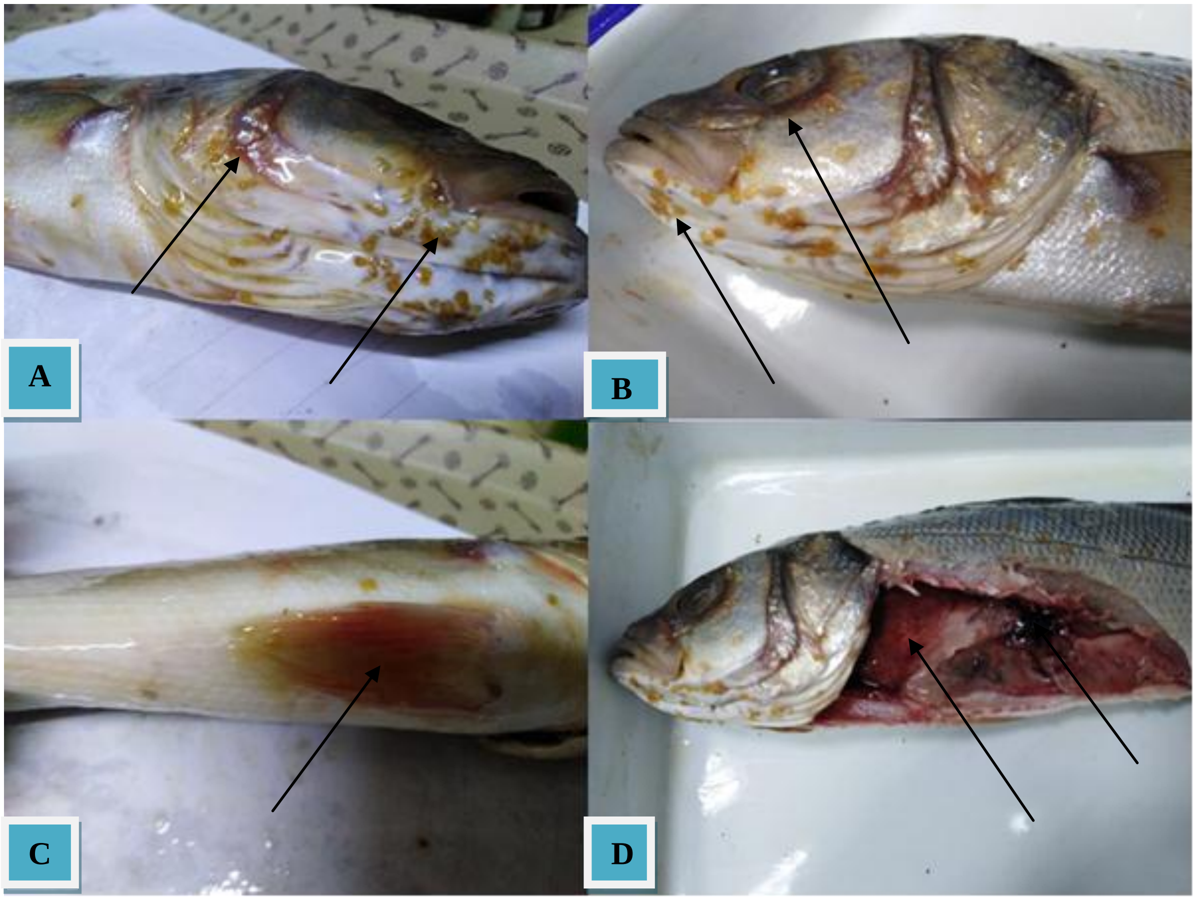

Plate (2): (A) Naturally infected *D. labrax* showing a large number of sea lice all over the body especially on head region**. (B)** D. labrax showing corneal opacity. **(C)** showing focal hemorrhage at abdominal region especially at fins and **(D)** *D. labrax* showing discoloration of liver with small grossly ascetic fluid in the abdominal cavity.

Water analysis

The physiochemical parameters of water were recorded in Table (1) revealed that all parameters within permissible limit and within the normal ranges for sea bass culture.

Table (1): Water quality of the marine farm containing infected fish.

Water parameters	Results	Permissible limits
pH	**8.2**	**7.5 - 8.5**
Tempreature 0C	**20±3 0C**	**---**
Dissolved oxgyen	**5.5**	**5 – 6 mg/L**
Salinity PPT	**28**	**28 – 35 ppt for marine farm**
Un ionized ammonia	**0.01**	**0.0 – 0.0125 mg/L**
Nitrate(No3)	**8**	**10 mg/L**
Nitrite(NO2)	**0.3**	**0.0 - 0.3 mg/L**
Sulphate	**170**	**<3000 mg/L**

Bacteriological examination

After using both Phenotypic and Biochemical characteristics of isolated bacteria by conventional and API®20 NE, the isolates was confirmed to be *V. alginolyticus* (80%). *V. alginolyticus* isolates were circular colony with regular edges, slightly convex on TSA. The bacterial isolates were gram–negative, motile, oxidas, Indole production, gelatine hydrolysis, large yellow coloring (sucrose-fermenting) colonies on TCBS agar (Plate 4) and catalase positive, urease but Simon citrate were negative and finally were sensitive to O/129 (150 µg). *V. alginolyticus* characteristics were summarized in table (2).

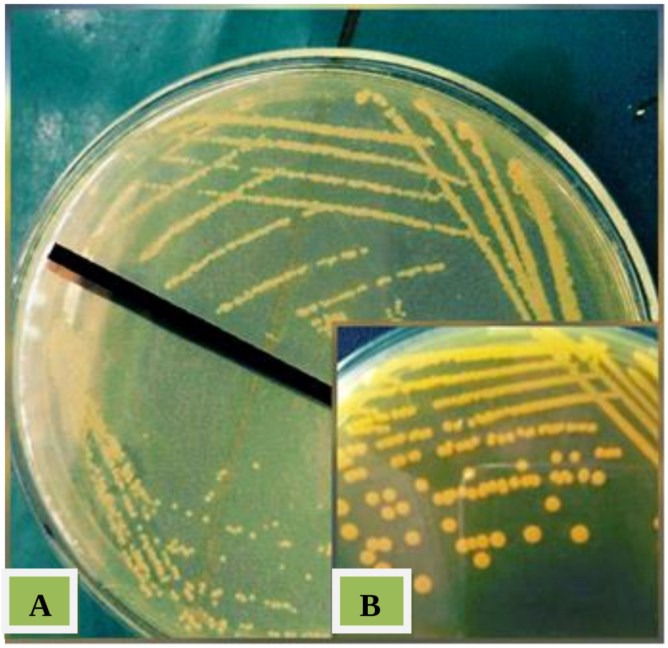

Plate (4): (A) *V. alginolyticus* showing circular colony with regular edges, slightly convex on TSA. (B) *V. alginolyticus* showing large yellow coloring (sucrose-fermenting) colonies on TCBS agar.

Table (2): Biochemical characteristics of *V.alginolyticus* by API*20 NE

Biochemical tests		*V.alginolyticus*	Biochemical tests		*V.alginolyticus*
NO_3	Potassium nitrate	+	MAN	Mannitol assimilation	+
TRP	Tryptophan production	+	NAG	N acetyl Glucosamine assimilation	-
GLU	Glucose fermentation	+	MAL	Maltose assimilation	+
ADH	Arginine Dihydrolase	-	GNT	Potassium Gluconate assimilation	+
URE	Urease	-	CAP	Capric acid assimilation	-
ESC	Esculin	+	LDI	Adipic acid assimilation	-
GEL	Gelatin	+	MLT	Malate assimilation	+
PNG	Para Nitrophenyl D Galactopyranosidase B Glucosidase	-	CIT	Tri sodium Citrate assimilation	-
GLU	Glucose assimilation	V	PAC	Phenyl acetic acid assimilation	-
ARA	Arabinose assimilation	+	OX	Oxidase	+
MNE	Mannose assimilation	-			

Antibiogam

The results of sensitivity to antibiotic revealed that *V. alginolyticus* was sensitive to Ciprofloxacin, Trimethoprime /Sulfamethoxazole while resistance to Lincomycin, Gentamycin, Norfloxacin, Oxytetracycline. Moderate sensitive to Nalidixic Acid, Erythromycin, Tetracycline, Oxolinic acid were recoeded in Table (3).

Table (3) Antibiotic sensitivities of *V.alginolyticus*

Antibiotics discs	*V. alginolyticus*
AX(25 µg)	R
CTP (5(µg)	S
E (15µg)	I
L(2 µg)	R
GN (10 µg)	R
NOR(30 µg)	R
NA(30µg)	I
OA(2 µg)	I
TE(30µg)	R
SXA(25µg)	S
T(30 µg)	I
O/129(150 µg)	S

Parasitological examination

Parasitological examination in the present study revealed sever infection of sea lice *Caligus minimus* from the sea bass with infestation rates 100%. Identification of the parasites was carried out according to **Yamaguti, (1963)** and their morphometric features as follows:

Order : *Siphonostomatoida*

Genus : *Caligus Müller*, 1785

Species : *Caligus minimus* **Otto, 1821**

Description: 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 to fourth leg bearing. 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. (Plate3).

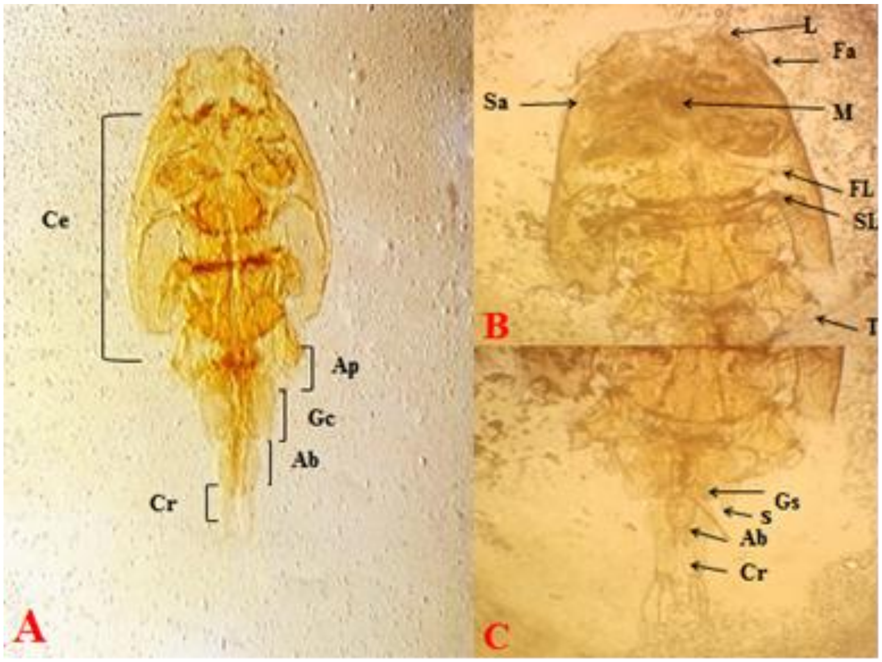

Plate (3): A- male *Caligus minimus* whole parasite (X 50), B- cephalothorax of *Caligus minimus* (X 400) and C- posterior part of *Caligus minimus* (X 400).

(Ce = cephalothorax , Ap = apron , Gc = genital complex , Ab = abdomin ,Cr = caudal rami , L = lunules , Fa = first antenna ,M= mouth ,Sa = second antenna , Fl = first leg ,Sl = second leg ,Tl = third leg , Gs = genital segment ,S = spines)

Treatment Experiment

In the current study, the trials to treat the infected *Dicentrarchus labrax* by used one or combined drugs were met with success recorded in Table (4). The effective treatment considered when it reduces parasitic infection with decrease the mortality rate. After treatment, the treated groups (1 & 3) recorded less level of re- isolation of *V. alginolyticus* than control group (4). Sea lice (*Caligus minimus*) recorded high decrease in lice infection in group (2 & 3) relatively to the control group (4) but sea lice slightly increase in group (1) and control group (4) while nearly disappear in group (3). In the experiment, used of oral treatment of bacterial infection of sea bass recorded that the best treatment result in case of combined two drugs in group (3). In addition, the mortality rate was 15% in group (3) but increase in other groups and very high in control group. Also, the treated fish group (3) recorded the clinical signs were disappeared and the fish returned to normal state of health.

Table (4): Efficacy of trials treatment of infected *D. labrax***.**

Fish group	No. of infected *D. labrax*	Protocol of Treatment with Ciprofloxacin and Malathion	Mortality	
			No. of fish	%
Group (1)	60	fed on ration containing Ciprofloxacin (150 mg / Kg Body wt.) for ten days	24	40%
Group (2)	60	was exposed to a single dose of Malathion (0.1 mg/L for 30 minutes) as bath treatment.	12	20%
Group (3)	60	was exposed to a single dose of Malathion (0.1 mg/L for 30 minutes) as bath treatment and then fed ration containing Ciprofloxacin (150 mg / Kg Body wt.) for ten days	9	15%
Group (4)	60	non-treated	48	80%

DISCUSSION

In the present study, the main clinical signs observed in infested sea bass fish with sea lice were excessive mucus production, sluggishness, collected on the surface of water as groups at the water inlet with gulping of air, 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 (**Eissa *et al.*, 2012**; **Smit *et al.*, 2014** and **Maather & Heba, 2015).** 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 microorganisms, reported by **Noor *et al.* (2012).**

Regarding the postmortem examination, it was revealed congestion and hemorrhages of the internal organs with small grossly ascetic fluid in the abdominal cavity. In addition to, paleness of liver and kidney were observed in some cases and discoloration of liver with flabby skeletal muscles. In the present study, postmortem lesions recorded among infected *D. labrax* were supported by **Nahla *et al.* (2014)** and **Maather and Heba (2015).** In this marine farm we found most of physiochemical parameters of water within the normal ranges as reported by **Nahla *et al.* (2014)** except water temperature was variable due to sea bass are eurythermic (5-28 °C). In this study found that temperature play a very important role to occur the fish diseases.

Infectious diseases are one of the main bottlenecks for future development of marine aquaculture in Egypt. Knowledge about the epidemiology, etiology, diagnosis and treatment of the main bacterial diseases of marine farmed fish are crucial to prevent severe economic losses. All various bacterial fish pathogens, such as Vibrio, streptococci, photobacteria, tenacibaculosis and viruses with un- control environmental condition are the causes of high rate of morbidity and mortalities in fish farms (**Jehan *et al*., 2019** ; **Nahla *et al*., 2014).** In the present study, affected fish was confirmed to be infected by *V. alginoloiyticus* which a more serious bacterial diseases, as like recorded by **Moustafa *et al.* (2010), Abou Okaada (2013)** and **Dalia, (2017)** who isolated *V. alginolyticus* from sea bream, sea bass and solea fish with the same phenotypic characters. In our study, Ciprofloxacin is more effective to treatment of *V. alginolyticus* infection. This finding is agreed with **Quinn *et al.* (2002), Ali (2007)** and **Jehan *et al.* (2019**). *Vibrio* spp. were able to develop resistance mechanism to resist antibiotics (**Abualreesh, 2017**) which could be related to highly use of these antibiotics reaching to aquaculture and increase its concentration in water agriculture /municipal wastes (**Abdel-Aziz *et al*., 2013**).

Parasitic infections increase the risk of secondary bacterial diseases and can act as a vehicle to transmit bacterial pathogens (**Holzer *et al*., 2006**). This synergistic interaction was demonstrated by many experimental studies (**Busch *et al*., 2003**), which showed increased mortality rates in parasitized/ bacteria co-infected fish. This synergistic effect caused by parasites reducing the resistance of fish to other secondary bacterial infections (**Bowers *et al*., 2000**). In some instances, the parasites harbor the bacteria and deliver it to their host while feeding (**Bowers *et al*., 2000**).

Concerning the description and systematic part of sea lice *C. minimus* this agrees with **Tansel *et al*. (2012**) and **Noor El-Deen *et al.* (2013**). Regarding the infection of sea lice *C.minimus* in the sea bass (*D. labrax*) in the present study agree with **Khoa *et al.* (2018) and Noor El-Deen *et al*. (2013)** who reported that Copepod infestation, especially by *C. minimus* has been reported in Mediterranean sea bass (*D. labrax*). The parasites will damage the host's skin and epidermal layer, leading to stress, osmotic problems and secondary infections (**Saraiva *et al*., 2015 and Khoa *et al*., 2018**). Also, agree with **Shinn *et al.* (2015**) who reported that 80% of sea bass farms worldwide have been affected by this parasite, which resulted in a 30 to 50% loss of fish stock. However, the impact of parasitism was limited only to economic and environmental aspects (**Heuch *et al*., 2005**). The co-infection of *C. minimus* with bacteria supported by **Xu *et al.* (2007**) who assumed that ectoparasite provides a portal of entry for invasive bacteria through mechanical damage of the fish epithelium.

Malathion, was used for treatment of naturally infested sea bass with sea lice (*Caligus sp*.) by using a single dose of 0.1 mg/L Malathion as immersion bath for 30 min (short duration bath) with possible repeating of treatment after 1 week from the first treatment according to the condition of infestation to obtain complete control of sea lice. These findings nearly agree with **Eissa *et al.* (2020**) who used Malathion to control the infestation of sea lice (*Caligus sp*.) among the reared sea bream fish at the pond level by using a single dose of Malathion solution as a bath treatment. The above results agree with **Noor El-Deen *et al.* (2013**) who used Malathion in treatment trials of naturally infested sea bass by *Calgius sp*. in different concentration including 0.1 mg/L Malathion as immersion bath for 30 min. The effectiveness of Malathion was due to its inhibition of many enzymes, especially acetylcholinesterase (AChE) activity in cholinergic nervous systems of the parasite where the function of AChE is to mediate the hydrolysis of the nurotransmitter acetylcholine, which subsequently terminates transmission through the synaptic membrane, resulting in spastic paralysis. These findings agree with **Noor El-Deen *et al.* (2013)** and **Josip Barisic *et al.* (2019)**. In the present study, the effective treatment of the infected *D. labrax* by used of combined treatment from both drugs (Malathion and Ciprofloxacin) reduces parasitic and bacterial infection with decrease the mortality rate. This findings is agree with many field studies like **Shoemaker *et al.* (2008), El- Hady and El-Khatib (2000)** and **Nahla *et al.* (2014).** The authors also advised farmers to use hydrogen peroxide as a disinfectant for ponds and oxygen donor and dose should be carefully calculated based on type of aquaculture and fish species.

CONCLUSION

This study resolved field outbreaks problem and dealing with this outbreaks depends on early intervention in the progression of disease. The exact diagnosis of causative agents and not checking any causative agent with best treatment to control all agents causing disease. Co-infections are very common in nature and occur when hosts are infected by two or more different pathogens either by simultaneous or secondary infections. Co-infection (bacterial and parasitic diseases) need further studies to explained this combination and better to clarify co-infection by conducting more experiments in the fish farm and using modern and fast diagnostic methods to control this diseases.

REFERENCES

Abdel-Aziz,M.; Eissa, A.E.; Hanna, M. and Abou Okada, M.(2013). Identification some pathogenic Vibrio/ Photobactrium species during mass mortalities of cultured Gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentratus labrax*) from some Egyptian coastal provinces. International Journal of Veterinary Medicine and Science, 1:87-95.

Abou Okada, M.M.O. (2013). Some studies on causes of mortalities among sea bream and sea bass. M.V.Sc, Thesis, Faculty of Vet. Med. Cairo University.

Abualreesh, M.H. (2017). Development of Standard Operational Procedures for Bacterial Management in Marine Fish Hatcheries, Master Thesis, Faculty of the University of Miami.

Ali, M. N. (2007). Clinical and bacteriological characteristic flavobacteriosis in semi-intensive cultured sea bass (*Dicentrarchus labraz*). Egypt J. of Aquatic biol. And fisheries, 11(3): 112-127.

Azad, I.S.; Jithendran, K.p.; Shekhar, M.S.; Thirunavukkarasu, A.R. and Pena, L.D. (2006). Immunolocalisation of nervous necrosis virus indicates vertical transmission in hatchery produced Asian sea bass (*Lates calcarifer Bloch*) – A case study. Aquaculture, 255(1-4):39-47.

Bayoumy, E.M. ; Baghdadi, H.B. and Hassanain, M.A. (2013). New Record of Parasitic Praniza Larva of *Gnathia pantherina*; Smit and Basson, 2002; from Arabian Gulf Greasy Grouper *Epinephelus tauvina* Caught from Saudi Coastal Water of Dammam. Global Veterinaria. 11(4): 414-419.

Bowers, J.M.; Mustafa, A.; Speare, D.J.; Conboy, G.A.; Brimacombe, M.; Sims, D.E. and Burka, J.F. (2000). The physiological response of Atlantic salmon, *Salmo salar L.*, to a single experimental challenge with sea lice *Lepeophtheirus salmonis.* J. Fish Dis. 23:165–172

Boxshall, G.A. (2015). Copepoda (copepods). In: Rohde K, editor. Marine parasitology. p. 123–138.

Burka, J.F.; Hammell, K.L.; Horsberg, T.E.; Johnson,G.R. ; Rainnie, D.J. and Speare, R. (1997). Drugs in salmonid aquaculture-A review. J. Vet. Pharm. Therap., 20: 333-349.

Canadian Council on Animal Care (2005). Guidelines on: the care and use of fish in research, teaching and testing. Appendix D.

CLSI (2010). Performance Standards for Antimicrobial Susceptibility Testing, 20th Informational Supplement. Clinical and Laboratory Standards Institute, M100-S20 & M100-S-20-U

Chatterjee, M. and Chatterjee, M. (1992). Control of fish disease by cifran ciprofloxacin caused by *Pseudomonas aeruginosa*. Enviro. And Ecolo. Kalyani, 10(3): 574-575.

Costello, M.J. (2009). The global economic cost of sea lice to the salmonid farming industry. J. Fish Dis. 32, 115–118.

Dalia, A.A.A. (2017). Comparative studies on clinical and molecular profiles of *vibrio spp*. in Solea fish. PhD, Thesis, Faculty of Vet. Med. Cairo University.

El-Hady, M.A. and El-Khatib, N.R.(2009): Mixed aetological agents of nodular from disease in Seabastes Marinus. Zagazic Vet. J., 37(3): 147-154.

Eissa, N.M.E.; Abou El-Ghiet, E.N.; Shaheen, A. and Abbass, A. (2010). Characterization of Pseudomonas Species Isolated from Tilapia "*Oreochromis niloticus*" in Qaroun and Wadi-El-Rayan Lakes, Egypt. Glob. Vet., 5: 116-121.

Eissa, I.A.M.; Maather, E. and Mona, S.Z. (2012). Studies on Crusteacean Disease of sea bass, *Morone labrax*, in Suez Canal, Ismaillia Governorate. Life Science journal, 9(3): 512-518.

Eissa, A.; Abdelsalam, M.; Mahmoud, A.M.; Younis, N. A;. Abu Mhara, A. A. and El Zlitne R. A. (2020). Cutaneous fibropapilloma in Egyptian-farmed gilthead sea bream (*Sparus aurata*;*Linnaeus*, 1758). Aquaculture International 28:2081–2091. https://doi.org/10.1007/s10499-020-00579-0

Er, A. and Kayiş, Ş. (2015). Intensity and prevalence of some crustacean fish parasites in Turkey and their molecular identification. Turkish Journal of Zoology, 39(6), 1142–1150.

Evans, J.W. (1962). Records of The Australian Muesum (parasitic copepod from Austuralian waters by Poul Heegaard). XXXV (9): 149-234.

Fadel, A.H. (2014). Studies on Vibriosis in Sea bass (*Dicentrarchus labrax*). M.V.Sc, Thesis, Faculty of Vet. Med. Zagazig University.

FAO (2013). Fishers and Aquaculture Department. In: FAO Fishers and Aquaculture Department [online]. Italy.

Garrity, G.M.; Winters, M and Searles, D.B. (2001) Taxonomic outline of the prokaryotic genera. In Bergey's Manual of Systematic Bacteriology ed. Garrity, G.M. Springer-Verlag, New York, NY: Bergey's Manual Trust.

Heuch, P.A.; Bjørn, P.A.; Finstad, B.; Holst, J.C.;Asplin, L. and Nilsen F. (2005). A review of the Norwegian "National Action Plan against Salmon Lice on salmonids": the effect on wild salmonids. Aquaculture 246(1–4):79–92

Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T. (1994). Bergey's Manual of Determinative Bacteriology, 9th edn. Baltimore: Williams & Wilkins.

Horsberg, M.(2004). Evidence for occurrence of an organophoshate-resistant type of acetylcholinesterase in strains of sea lice (*Lepeophtheirus salmonis Kroyer*). Pest Management Science, 60(12): 1163-1170.

Holzer, A.S.; Sommerville, C. and Wootten, R. (2006). Molecular studies on the sea-sonal occurrence and development of five myxozoans in farmed *Salmo trutta L.* Parasitology 132:193–205

Jehan, I. A.; El-Gohary, M.S. and Marowa, M. M.(2019). Monitoring of bacterial mass mortalities in farmed pre-growing stage Gilthead sea bream (*Sparus aurata*) with control trail. Animal Health Research Journal Vol. 7, No. 4, November 2019.

Jones, A.C.;Mead, A.;Kaiser, M.J.;Austen, M.C.V.; Adrian, A.W.;Auchterlonie, N.A.;Black, K.D.;Blow, L.R.; Bury, C.;Brown, J.H.;Burnell, G.M.; Connolly, E.; Dingwall, A.; Derrick, S.; Eno, N.C.;Gautier, D.J.H.; Green, K.A.;Gubbins, M.; Hart, P.R.; Holmyard, J.M.; Immink, A.J.;Jarrad, D.L.; Katoh, E.; Langley, J.C.R.; Lee, D.O.;Le Vay, L.; Leftwich, C.P.;Mitchell, M.; Moore, A.;Murray, A.G.; McLaren, E.M.R.;Norbury, H.; Parker, D.;Parry, S.O.; Purchase, D.; Rahman, A., Sanver, F., Siggs, M., Simpson, S.D., Slaski, R.J., Smith, K., Syvret, M.L.Q., Tibbott, C.; Thomas, P.C.; Turnbull, J.; Whitely, R.; Whittles, M.; Wilcockson, M.J.; Wilson, J.;Dicks, L.V. and Sutherland, W.J. (2014). Prioritization of knowledge needs for sustainable aquaculture: a national and global perspective. Fish and Fisheries Volume 16, Issue 4

Josip, B. ;Cannon, S. and Quinn, B. (2019). Cumulative impact of anti-sea lice treatment (*azamethiphos*) on health status of Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) in aquaculture. Nature research journal. Scientific Reports 9:16217

Khoa, T. N. D; Suhairi M; Sabri M. and Faizah H. (2018). The life cycle of *Caligus minimus* on Seabass (*Lates calcarifer*) from floating cage culture. Thalassas: An International Journal of Marine Sciences, In press, 1–9.

Lafferty, K. D.;Harvell, C. D.;Conrad, J. M.;Friedman, C. S., Kent, M. L.;Kuris, A. M. and Saksida, S. M. (2015): Infectious Diseases Affect Marine Fisheries and Aquaculture Economics. Annual Review of Marine Science, 7(1), 471–496.

Langdon, J. and Jones, B. (2002). Design and implementation of health testing protocols for fish with special reference to sample size, test sensitivity and specificity, predictive value and risk, Australian Standard diagnostic techniques for fish diseases.

Lucky, Z. (1977). Methods for the diagnosis of fish diseases American Publishing Co., Pvt. Ltd., New Delhi, Bombay Calcutta and New York.

Maather, M.M.E. and Heba, I.A. (2015). Isopods infection in relation to vibriosis of some marine fishes. Egy. J. Aquac., Vol.5, No. (2): 13-30 (2015) ISSN: 2090-7877.

Mahmoud, S. A.; El-Bouhy. Z. M. ; Hassanin, M. E. and Fadel, A. H. (2018). *Vibrio alginolyticus* and *Photobacterium damselae subsp. Damselae*: Prevalence, Histopathology and Treatment in sea bass *Dicentrarchus labra*. J. Pharm. Chem Biol. Sci. , December 2017-February 2018; 5(4) : 354-364 x.

Maran, B.V.; Seng, L. T.; Ohtsuka, S.; Nagasawa, K. and Nagasawa, K. (2009). Records of *Caligus* (Crustacea: Copepoda: Caligidae) from Marine Fish Cultured in Floating Cages in Malaysia with a Redescription of the Male of *Caligus longipedis* Bassett-Smith, 1898. Zoological Studies, 48(6), 797–807.

Mohamed, H. K.; Simon, M.; Gokhlesh, K.;Mahmoud, A. and Mansour, E. (2016). The impact of co-infections on fish. Veterinary Research 47:98

Moustafa, M.; Mohamed, L.A.; Mahmoud, M. A.; El.gendy, M. Y. and Soliman, W.S. (2010). "Bacterial Infections Affecting Marine Fishes in Egypt", Journal of American Science, vol. 6, no.11, pp. 603-612, 2010.

Nahla, R.; Nadia, A.A. and Soad, S.A. (2014). Trials to control sea bass mortality due to concomitantly microbial and parasitic infection. 4th conference of central laboratory for Aquaculture Research (2014), 283-302.

Noga, E.J. (2010). Fish disease Diagnosis and Treatment. Mosby-yearbook, Inc. watsworth publishing Co., USA, pp: 366-369.

Noor El-Deen, A.E.; Abd El Hady, O.K.; Shalaby, S.I. and Zaki, S.M. (2012). Field studies on Caligus disease among cultured *Mugil cephalus* in brackish water fish. Life Science journal, 9(3): 733-737.

Noor El-Deen, A.E.; Mahmoud and Azza, H.M. Hassan (2013). Field Studies of Caligus parasitic Infections among Cultured Seabass (*Dicentrarchus labrax*) and Mullet (*Mugil cephalus*) in Marine Fish Farms with Emphasis on Treatment Trials . Global Veterinaria 11 (5): 511-520.

Osman, H.A.M.; Hassan, M.A. and El-Refaey, A.M.E. (2014). Studies on *Sarcotaces Sp.* Copepoda,Philichthyidae) Infestation (Black Bag Disease) among Some Marine Fish Species of Arabian Gulf, Saudi Arabia. World Applied Sciences Journal 32(9):1780-1788.

Otto, A. W. (1821). Conspectus animalium quorundam maritimorum nondum editorum. Pars prior. Quam patri dilectissimo ad cineres usque venerando Bernhardo Crristiano [sic] Otto. 1-20. Typis Universitatis, Vratislaviae.

Pike, A.W. (1989). Sea lice major pathogens of farmed Atlantic salmon Parasitology Today, 5: 291-297.

Pujalte, M.J.; Sitjà-Bobadilla, A.; Macián, M.C.; Belloch, C.; Álvarez-Pellitero, P.; Pérez-Sánchez, J.; Uruburu, F. and Garay, E. (2003). Virulence and Molecular Typing of *Vibrio harveyi* Strains Isolated from Cultured Dentex, Gilthead Sea bream and European Sea bass. Syst. Appl. Microbiol 2003; 26(2): 284-292.

Saraiva, A.; Costa, J.; Serrão, J.; Eiras, J. C., and Cruz, C. (2015). Study of the gill health status of farmed sea bass (*Dicentrarchus labrax* L., 1758) using different tools. Aquaculture, 441, 16–20.

Salwany, M.Y.; Al-saari, N.; ,Mohamad, A.; Mursidi, FA.; Mohd-Aris, A.; Amal, M.N.A.; Kasai, H.; Mino, S.; Sawabe,T. and Zamri-Saad, M. (2019). Vibriosis in Fish: A Review on Disease Development and Prevention. American Fisheries society Volume 31, issue 1:3-22 published: 24 September 2018 https://doi.org/10.1002/aah.10045

Shinn, A. P.; Pratoomyot, J.; Bron, J. E.;Paladini, G.; Brooker, E. E. and Brooker, A. J. (2015). Economic costs of protistan and metazoan parasites to global mariculture. Parasitology, 142(1), 196–270.

Shoemaker, C.A.; Klesius, P. H. and Evans, J. (2008). Concurrent infectins (parasitism and bacterial disease) in tilapia. 8th International Symposium on Tilapia in Aquaculture 2008.

Smit, N.J., Bruce, N.L. and Hadfield, K.A. (2014). Global diversity of fish parasitic isopod crustaceans of the family Cymothoidae. International Journal for parasitology: Paras. And Wilds, 3 (2014): 188-197.

Tansel, T. and Fatih, P. (2012) Ectoparasitic sea lice, *Caligus minimus* (Otto 1821, Copepoda: Caligidae) on Brawn wrasse, *Labrus merula* L., in Izmir Bay, Aegean Sea. Journal of Animal Science, 11(38): 208-2011.

Tookwinas, S.; Perngmark, P.; Sirimontaporn, P.; Tuaucharoen, S. and Sangsakul, P. (1986). Study on aquatic environment of sea bass cage culture at Songkhla Outer Lake: investigation on the cause of sudden fish mortality. Thai Fisheries Gazette, 39(3): 255-263.

Torrissen, O.; Jones, S.; Asche, F. and Guttormsen, A.(2013). Salmon lice—impact on wild salmonids and salmon aquaculture. J Fish Dis 36:171−194

Tully, O. and Whelan, K.F. (1993). Production of nauplii of *Lepeophtheirus salmonis* (Krøyer) (Copepoda: Caligidae) from farmed and wild salmon and its relation to the infestation of wild sea trout (*Salmo trutta L*.) off the west coast of Ireland in 1991. Fish Res 17:187−200

Whitman, A.K. (2004). Finfish, Shellfish Bacteriology Manual: Techniques and Procedures. UK: A Blackwell Publishing company/Iowa State Press; 2004, p 258..

Xu, D.H.; Shoemaker, C.A. and Klesius, P.H. (2007). Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia, *Oreochromis niloticus* (L.). J Fish Dis 30:233–238

Yamaguti, S. (1963). Parasitic Copepoda and Branchiura of Fishes. Interscience publishers, A Division of John Wiley & Sons,New York. pp.1104

Zorrilla, I.; Morin˜ igo, M.A.; Castro, D.; Balebona, M.C. and Borrego, JJ.(2003). Intraspecific characterization of *Vibrio alginolyticus* isolates recovered from cultured fish in Spain. J Appl Microbiol 2003;95:1106–1116.