P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Research Article

DOI: 10.37422/IJVS/20.075

Studies on Prevailing Parasitic Fish Diseases in Pre-mature Cultured *Sea bass, Dicentrarchus labrax, Sea bream* and *Mugil cephalus* at Ismailia, Province with Special References to Control

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Article History: 20-058 Received: March 08, 2020 Revised: July 03, 2020 Accepted: July 20, 2020

ABSTRACT

This study concentrated on the assessment of the prevailing parasitic fish diseases in some marine fishes at Ismailia province and how to control the infestation using microalgae. This study was carried out on 1080 pre-mature fish (360 *Dicentrarchus labrax* (225±25g) and 360 *Saprus aurata* (150±25g) and 360 *Mugil cephalus* (125±25g) collected from similar ponds of studies to be examined at the end of treatment. In addition to that we followed non-treated fish (1080 premature). The infested fish showed dark color and respiratory signs. Post-mortem lesions were a presence of congestion or paleness and destruction of gill filaments. The total prevalence of infestation was the total prevalence of parasitic infection of non-treated fishes was 45.83%. The highest percentage was in *D. labrax* 56.94% followed by *S. aurata* 47.22%, the lowest percentage in *M. cephalus* 33.33%. The total prevalence of parasitic infection in premature treated with 2g algae was 28.79%, followed by 3g algae was 23.60%, while the lowest percentage with 5g algae was 20.37%, respectively. The detected species of parasites were protozoal parasites, *Amyloodinium ocellatum* and *Riboscyphidia* in additions of marine monogenea, *Lamellodiscus diplodicus* isolated from *D. Labrex*, *M. Cephalus* and *S. aurata*. The present study concluded that, the use of microalgae instead of fish meal decreased parasitic infestation in marine fish. The histopathological alteration of natural infested examined fishes was also recorded.

Key words: *D. labrax, S. aurata, M. cephalus,* Parasites, Protozoa, Trematodes, Histopathology.

INTRODUCTION

Marine aquaculture represents one of the fastest growing food producing sectors (Muthenna *et al.*, 2012). The clinical signs of most investigated fishes displayed no characteristic lesions on some of the infested fishes except showing congestion, excessive mucous secretion, sticky or pale gills and grayish coloration with hemorrhages all over the body surface especially at the base of fins and the abdomen (Eissa *et al.*, 2010; Osman *et al.*, 2019). The skin and gills showed focal erosions, the parasites produced inflammatory reactions, hyperplastic changes in the skin (Noor El Deen *et al.*, 2013).

Parasitic diseases can be affected on wild and cultured fishes directly (mortality rate) or indirectly (morbidity rate) causing increase economic cost (Lagrue and Pouline, 2015; Shaheen *et al.*, 2017). Parasitic infections cause morbidity in the signs of absence of reflex, off food, rates of conversion become more less and prolonging the period of growth that causes more expenses (Noor El Deen *et al.*, 2010; Trujillo-Gonzalez *et al.*, 2015; Osman *et al.*, 2019).

Gills of naturally infected fish revealed that hyperplasia of the secondary gill lamellae with edema and infiltration of inflammatory cells (Engi, 2013). El-Lamie (2007) showed severe vacuolar degeneration along with few leucocytic infiltration. Algae would play a positive role in enhancement of disease resistance in fishes. (El-Feky *et al.*, 2017). So that the present study aimed to understand the effects of supplementation of microalgae in the diets of some marine fish species, Gilthead Sea bream *(Saprus aurata*), Seabass (*Dicentrarchus labrax*) and mullet (*Mugil cephalus)* to control parasitic infestation at Ismailia province, Egypt.

Cite This Article as: Ahmed IENED, IAM Eissa, HAM Osman, AAA Zaid, OM Darwish, 2020. Studies on prevailing parasitic fish diseases in pre-mature cultured *Sea bass*, *Dicentrarchus labrax*, *Sea bream* and *Mugil cephalus* at ismailia, province with special references to control. Int J Vet Sci, x(x): xxxx. www.ijvets.com

MATERIALS AND METHODS

Fish sampling

A total of 1080 premature fish (360 *D. labrax* (225±25g) and 360 *S. aurata* (150±25g) and 360 *M. cephalus* (125±25g). Fish were collected from cement ponds at Fish Research Center of Faculty of Aquaculture at Ismailia governorate, fishes were collected from assimilatory ponds of studies for examining at the end of treatment. In addition to examined non treated fish (1080 premature) of Agriculture, Suez Canal Univ. in Ismailia province. The collected specimens were carefully examined for signs of diseases specially gills, eyes, abdominal, skin blisters, ulcers, hemorrhages, fishes were transported in battery aerated tanks to the wet Lab. of National Research Center, Giza for isolation and identification of microbial agents.

Clinical signs and postmortem examination

The sampled fishes were examined for clinical picture. Examination was done on live fishes. Fish specimens under investigation were examined for observation of any external infections or visible lesions according to Noga (2010). For examination of the internal abnormalities, the postmortem investigation was carried out according to Woo (2006).

Parasitological examination

Under the dissecting microscope (Optika), gill biopsies were prepared by dissection of the gill arc, placed on a slide, covered with coverslips, submerged in water and examined freshly under a light microscope (Optika). The isolated monogeneans were collected, fixed in 5% buffered formol saline, preserved in an equal amount of 70% alcohol and 5% glycerin. The monogenean including different isolated developmental stages was identified and classified following the diagnostic keys outlined by Oliver (1968), Lucky (1977), Gonzfilez-Lanza *et al.* (1991) and Whittington (2004). For Ryboscyphidia infection, protozoa gill and skin scraping, and fixed with methanol, dried with air and covered with cover slide for examination.

Isolation and identification

Identification of the isolated protozoal and monogentic trematodes was performed according to Noga (2010) and Paperna (1996).

Diet

A basal diet was formulated to contain (32.5% crude protein, 3.50 crude fibers, 3.91% ash, and 4420 Kcal.kg-1 gross energy). Microalgae feed additive with equal amounts (*Dunula salina*, *Amphora coffeaeformis* and *nanochloropsis*) containing ferrus sulphate according to Rebort (2012) was added to the diet at the levels of 0.0 (control), 2, 3 and 5g/kg-1 diet. Premature fishes were fed their respective diets twice a day (at 8 am and 1 pm) for 8 weeks.

Treatment trial for naturally infected fishes

A total of 1080 fish from a total transported fish, 360 premature each from *D. labrax, S. aurata* and *M. cephalus* were obtained from private marine fish farms at Ismailia province. Fishes were randomly distributed in triplicate into three treatment groups and non-treated groups for examined fish infections in the Fish Nutrition Lab, Research Center, Ismailia. Treatments were carried out in 12 cement ponds, at a rate of 90 fish/pond. The collected marine fishes for examined work were acclimated to the system for 2 weeks. Initially, premature fishes were randomly collected after two months from each pond, weighted and the average initial weights of each species were recorded then examined for parasitological infections. Water temperature, dissolved oxygen, salinity, and pH were adjusted and observed daily.

Histopathological Examination

Tissue samples from infected examined organs were fixed in 10% formalin, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin (H&E) according to Roberts (1992).

Statistical analysis

Statistical analysis Data were subjected to one-way analysis of variance (ANOVA) at a 95% confidence limit, according to Duncan (1955).

Ethical considerations

The list of committees of ethics of scientific research at National Research Centre, Egypt does not include fish.

RESULTS AND DISCUSSION

In Egypt, fish diseases affected fish production especially parasitic infections which are about 80% of fish diseases (Eissa, 2002). This record attributed to the longtime of warm atmosphere and weather which preferable to reproduction of the intermediate hosts for parasitic life cycle (Younes *et al*., 2016). Regarding the clinical signs in the naturally infected fishes (*D. labrax*, *S aurata* and *M. cephalus*) revealed no pathognomonic clinical abnormalities. Some infected fish showed dark colour and respiratory signs (Fig. 1: A, B and C). These results nearly agree with that recorded by Badawy (1994). These results may be attributed the lower respired oxygen of gill epithelium which causes by attached of parasites causing mass destruction of gill epithelium cells.

Post-mortem examination displayed, hemorrhagic spots on gill cover, abdomen and on the bases of fins, abrasions or ulcers on the surface (Fig. 2A and 2B). These results nearly agree with that recorded with Noga (2010). Excessive mucus secretion with marbling appearance on gills. Gill tips were sticking with greyish colors in *D. Labrax* and *S. aurata* and in *M. Cephelus* (Fig. 3A). These results agreed with the findings of El Lamie (2007) who recorded that areas of congestion and paler as mosaic in *M. labrex*. These results may be caused due to destruction of the efferent vessels by protozoal or monogenean parasites were the blood pressure is low and extensive hemorrhages are causes very hard clotted blood brings about rapid occlusion of the infested vessels, thrombus is formed resulting in ischemia which in turn leads to necrosis in some areas occurred due to the inflammation and congestion of some areas with progressive degeneration of the other parts of the gill filaments causing the appearance of such phenomena (Eissa, 2002).

Table 1: Showing total parasitic infestation in non-treated premature examined fish

Fish species	No of examined fish	No of infected of non-treated fish	No- treated %	Treated %
D. labrax	360	230	63.88	36.12
S. aurata	360	193	53.61	46.39
M. cephalus	360	133	36.94	63.06
Total	1080	556	51.48	48.52

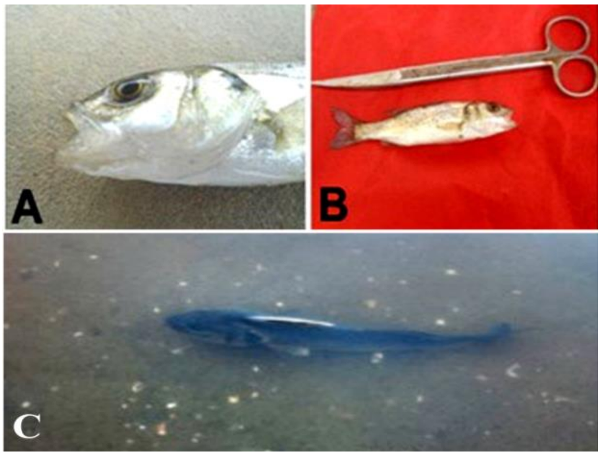

Fig. 1: Showing open mouth respiratory distress sign in (A) premature and (B) fingerlings of *D. labrax* (C) a dark color of premature *D. labrax*.

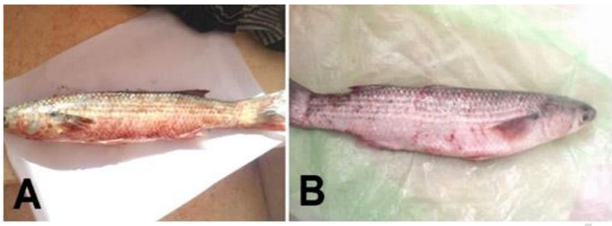

Fig. 2: Showing (A and B) haemorrhages all-over the body surface of *mugil cephalus.*

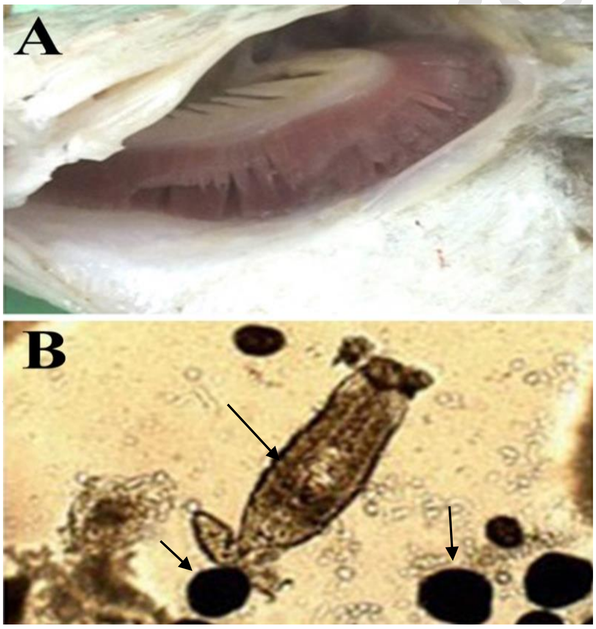

Fig. 3: Showing (A) gills of *D. labrax* suffered from sloughing of primary gill lamellae infected with *Amyloodinum ocellatum*, (B) Scraping from gills showing mixed infection of Amyloodinum and monogenea sp. (arrows) in *S aurata.*

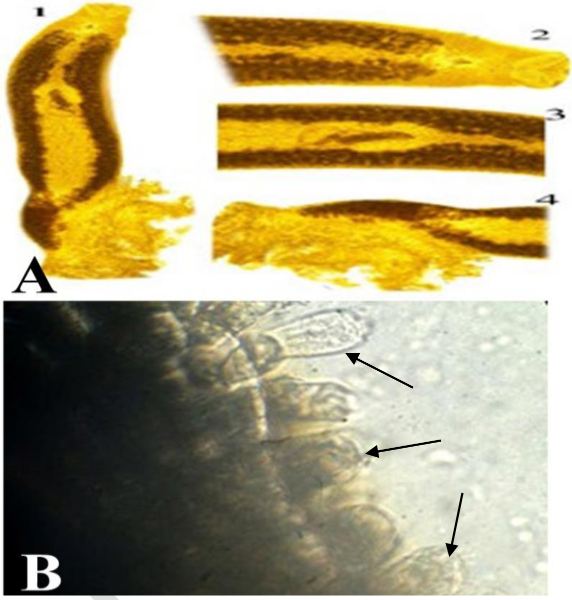

Fig. 4: Showing (A) marine monogenea (*Lamellodiscus dipolicus)* (1) whole worm with (2) anterior end and (4) posterior end isolated from *D. labrax*, (B) Magnified view showing heavy infestation of Riboscyphidia sp. attached to gills of *D. labrax* (arrows).

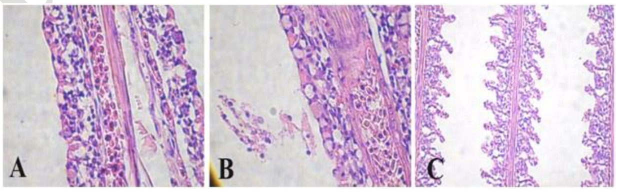

Fig. 5: Gills of *D. labrax* showing (A) congestion and mild to moderate hyperplasia of gill lamellae, (B) severe hyperplasia of gill lamellae and leucocytic infiltration mainly lymphocytes and few macrophages with hyperplasic mucous cells (C) Severe vacuolar degeneration and hyperplasia of secondary lamellae along with leucocytes infiltration.

Regarding to Parasitological examination of fish specimens present study revealed, that fish specimens were examined for parasites (macroscopically and microscopically) (Fig. 3B; Fig. 4 A and B). Identification of the parasites was carried out according to its morphometric measurements.

Amyloodinium ocellatum was first described by Brown (1931) and it is one of the most important pathogenic ecto-parasitic protozoal disease affecting cultured marine water fishes. The parasite induces a velvety appearance on skin of infected fish, and the resulting disease was commonly known as "marine velvet," velvet disease, or *Amyloodiniosis*. The organism is a dinoflagellate ectoparasite and has been reported in a wide range of marine hosts (Alvarez-Pellitero, 2008; Noga, 2010; 2012; Osman *et al*., 2019).

The main clinical signs in naturally infested *S. aurata* and *D. labrax* fishes showing congestion and mucus on gill filaments. Monogenea is a class of platyhelminthes, common parasites of the external surface gills and skin of all species of fishes (Eissa, 2002; Trujillo-González *et al*., 2018; Osman, 2005; Osman *et al.*, 2013). The examined parasites were collected from gills of *S. aurata*.

Concerning to the total prevalence of parasitic infestation of non-treated premature marine fishes, present study displayed that the total prevalence was 45.83%. The highest percentage in *D. labrax* was 56.94% followed by *S. aurata* 47.22%, the lowest percentage in *M. cephalus* 33.33%. The present investigation indicated that the total prevalence of parasitic infestations in examined fishes vary from species than other. These results nearly are similar to that recorded by Shaheen *et al.* (2017). These variations may be due to behavior and habitat of each fish.

The total prevalence of parasitic infection of treated premature fish was 21.48%. The highest percentage was in *D. labrax* 23.88% followed by *S. aurata* 23.61%, the lowest percentage in *M. cephalus* 16.94% These, results nearly similar to that obtained by Noor El Deen *et al.* (2013) and Shi *et al.* (2017) who recorded that feeding by β-carotene from *D. salina* by reason of enhancement β-carotene due to complement activity increased, and on the other hand, serum lysozyme activity increased, and the result indicated that body immune levels increased (Amar *et al*., 2004). In the other trial Japanese parrotfish (*Oplegnathus fasciatus*) and spotted parrot fish (*Oplegnathus punctatus*) larvae were fed with β–carotene supplement rotifers, resulted in survival rates of β–carotene supplemented groups of both Japanese and spotted parrotfish higher than the control one. Tachibana *et al*. (1997) also stated that survival, skin, flesh-color, and also antioxidant capacity factors in rainbow trout were increased due to Dunaliella supplementation.

These variations may be due to cultured ponds riches with microalgae that also, may be attributed to anti-parasitic effect of algae on parasitic infestation due to presence of polysaccharides that act as complement activity increased and on the other hand, serum lysozyme activity increased, and the result indicated that body immune levels increased.

Regarding the histopathological examination, present study revealed that gills showed hyperplasia of the secondary gill lamellae with edema and infiltration of inflammatory cells (Fig. 5 A, B and C), the obtained results were supported by Mai (2009). These results go with the findings of Darwish *et al*. (2000) and Zhou *et al*. (2014) who found increase in numbers of melano-macrophage centers, depletion of lymphocytes and congestion of blood vessels. Regarding gills of naturally infected fish revealed that hyperplasia of the secondary gill lamellae with edema and infiltration of inflammatory cells. These results nearly agree with that recorded by Engi (2013) and disagree with Woo and Bruno (2011) who found granulomas in examined fish. This may be attributed to different site and kind of study and species of fish and environmental conditions.

Conclusion

This study concluded that, the use of microalgae instead of fish meal decreased parasitic infestation in marine fish. The histopathological alteration of natural infested examined fishes was also recorded. In addition, the microalgae increased and enhanced the innate immune response in fishes that fed on it.

Acknowledgment

Great thanks to all my staff for hardworking in this study. We acknowledge STDF for their fund, support and trust in us to achieve this project (5715) under the supervision of P1 Professor Dr. Ahmed Noor El Deen. Thank you all.

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