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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2019.349.355

Research Article
Field Studies on Amyloodiniosis in Red Sea Cultured Asian Seabass (*Lates calcarifer*) and Hamour (*Epinephelus polyphekadion*)

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Abstract

Background and Objective: *Amyloodinium ocellatum* infects the gills and skin of both marine and brackish water fishes. The aim of the present study was to examine pathogenesis, prevalence, trials for treatment and histopathological alterations of Amyloodinosis in naturally infested Asian Seabass Barramundi *Lates calcarifer* and Hamour *Epinephelus polyphekadion* in Ismailia Governorate, Egypt. **Materials and Methods:** A total number of 1447 Red Sea cultured Seabass (*Lates calcarifer*) broadstock and a total number of 53 Red Sea cultured Hamour, *Epinephelus polyphekadion* broadstock were collected and subjected for the study. Fishes showed symptoms of sudden death and respiratory distress besides Amyloodiniosis on gills and skin. All fishes were treated with various treatment protocols while gills of naturally infected fishes were examined histopathologically. **Results:** The clinical signs of infested fishes were flashing, surfacing, off food and respiratory distress. The intensity of infestation of Amyloodiniosis was more sever in Asian Seabass than *Epinephelus polyphekadion* while treatment of choice was copper sulphate (prolonged bath), freshwater bath and formalin consequently. **Conclusion:** Treatment of choice for *Amyloodinium ocellatum* infestation in Asian Seabass was copper sulphate (prolonged bath) followed by freshwater bath then formalin.

Key words: Amyloodiniosis, *Lates calcarifer*, *Epinephelus polyphekadion*, histopathological changes, seabass

Citation: Hussien Abd El-Fattah Mohamed Osman, Khairi Abd El-Fattah El-Battawy, Alaa El-Deen Zakaria Abu Brayka, Ahmed Ismael Noor El-Deen, Mona Saad Zaki, Nagwa Saad Rabie and Amany Mohmed Kenawy, 2019. Field studies on amyloodiniosis in red sea cultured asian seabass (*Lates calcarifer*) and hamour (*Epinephelus polyphekadion*). Pak. J. Biol. Sci., 22: 349-355.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Amyloodinium ocellatum is a dinoflagellate that infects the gills and skin surface of both marine and brackish water fishes. The disease caused by these organisms has been presented to as "velvet," "rust" and "gold dust disease" because of the shiny luster that the parasite give to heavily infected fish[1,2].

Amyloodiniosis is one of the important diseases of warm water marine fishes[3-5] infesting both food fish and aquarium fish worldwide[6]. *Amyloodinium ocellatum* is one of the fish parasites that can infest both elasmobranchs and teleosts[7,8] and most of the fish that live within its ecological range are susceptible to infections. Even freshwater fish, such as tilapia, is susceptible to infestation when they are in brackish water[7]. Species, most resistant to infestations can produce thick mucus or lay off low oxygen levels[6].

Gills are the primary site of infestation of Amyloodiniosis. Infestations may also involve the eyes and skin. Tomonts that may see in the intestinal tract were probably swallowed by the host. A single trophont can damage and kill several host cells[9,10], which probably produce severe injury on the host by trophonts. Mild infestations (1-2 trophonts per gill filament) cause little pathology. However, heavy infestations can cause severe or advanced gill hyperplasia, inflammation, hemorrhage and necrosis. Death may be occurred[7] within 12 h. Some acute mortalities are accompanied with apparently mild infections, suggesting that hypoxia may not always be the cause of death in all gill infestations. Osmoregulatory effects and secondary infections caused by epithelial damage may also be affected[2].

Amyloodinium protozoa can cause serious losses of aquarium fish or fish in high-density culture systems and has caused great problems in aquaria and mariculture systems. Thus the aim of the present study was to determine pathogenesis, prevalence, trials for treatment and histopathological alterations of Amyloodinosis in naturally infested Red Sea cultured Asian Seabass Barramundi *Lates calcarifer* and Hamour *Epinephelus polyphekadion* in Ismailia province, Egypt.

MATERIALS AND METHODS

Infected fish

Naturally infected Asian seabass: Study was carried out from May, 2016-April, 2017. A total number of 1447 Red Sea cultured Seabass Barramundi (*Lates calcarifer*) broadstock were collected from cement and earthen ponds from private fish farm at Ismailia Governorate, Egypt. Fish suffered from sudden death and respiratory distress, fish transferred to the laboratory of Hydrobiology, National Research Centre, Egypt. Clinical picture and postmortem lesions were recorded [11]. Fish body weight range was recorded (300-2500 g).

Naturally infected grouper fish: A total number of 53 Red Sea cultured Hamour *Epinephelus polyphekadion* broadstock were collected from cement ponds. Fish suffered from sudden death, respiratory distress. Fishes were transferred to the laboratory. Hydrobiology Department, National Research Center, Egypt. Clinical signs and postmortem lesions were recorded[11]. Fish body weight range was also recorded (2000-4000 g).

Clinical and postmortem signs: Infested fishes were clinically observed in the ponds for any external abnormalities, swimming behavior, respiration, feeding and escape reflex. Eyes, ulcers, skin and fins after dissecting all internal organs: gills, swim bladder, intestine, gonads also vascular organs heart, liver, spleen and kidneys were observed Noga[2].

Parasitological examination: Parasitological examination was carried out by scraping of skin, fins, eyes and mouth also scraping of gills with covered mucous with drop of filtered sea water and examined under light microscope according to Lucky[12].

Prevalence and intensity of infestation: Prevalence of infection and intensity of infestation were calculated, intensity of infestation was calculated (Number of trophonts per microscopic field, X40) according to Lucky[12].

Identification of the isolated parasite: Identification of the isolated parasite was identified by microscopical examination of wet mount preparations and stained with iodine as described earlier[12]. From the morphological features and measurement of dimensions of the parasite identification was carried out according to previous report[2].

Treatment trial for naturally infected fish: Treatment was performed using copper sulphate and formalin (Table 1).

Experimental design for treatment trials: A total number of 120 Red Sea cultured Asian Seabass broad stock were divided into four groups (30 fish each); 1st group (30 fish) was considered as a negative control (without treatment). The 2nd one (30 fish) was treated with copper sulphate prolonged bath for 12 days (3 day treatment then rest one day then continued) and repeated after 7 days[4].

Table 1: Design of treatment for *Amyloodinium* in Asian Seabass

Drug of treatment	Type of treatment	Dose (ppm)	Duration of treatment	Repetition
Control	Without	Without	-	-
Copper sulphate	Prolonged bath	0.03 mg L^{-1}	For 12 days, one day rest after 3 days treatment and continued	After 1 week
Formalin	Prolonged bath	100-150 mg L^{-1}	For 6 h	After 3 days
Freshwater	Dipping	-	5 min	After 3 days

The 3rd group was treated with prolonged bath formalin (37% formaldehyde) for 6 h and repeated after 3 days Paperna[13], while the 4th group was treated with freshwater bath for 5 min treatment repeated after 3 days[6]. All treated fish were examined for parasitological examination during treatment time.

Histopathological examination: Naturally infected fish with Amyloodiniosis were examined histopathologically. Samples were taken from gills of naturally infested fishes and preserved in 10% neutral buffered formalin, processing and examination was carried out accordingly[14].

Statistical analysis: Data were analyzed for significant differences using the ANOVA test at $p<0.05$. Correlation and regression (linear and quadratic) at confidence level of 95 and 99% was performed using SPSS program version 9.0.

RESULTS

Clinical signs and postmortem lesions: Lack of feeding, surfacing, flashing and rubbing against hard objects in the ponds or on the bottom and coughing back flushing water across the gills leading to skin scale sloughing and spotted accumulated mucus (Fig. 1). Gill primary lamellae sloughing (Fig. 2) may occur. The skin of heavily infected fish may be appeared dark and have a gold or brown sheen (Fig. 3a). Respiratory distress with gulping the atmospheric air may occur due to accumulation of mucous on gills (Fig. 3b).

Parasitological examination

Identification of the isolated parasite: Identification of the isolated parasite was identified by microscopical examination X400 of wet mount preparation. From the morphological and measurement of parasite dimensions (Trophonts dimension range from 50-350 µm) (Fig. 4). Identification revealed that the isolated parasite was identified as *Amyloodinium ocellatum* (Fig. 4).

The parasite attached to fish tissue is called trophont. The trophont is nearly pear-shaped to spherical with a dark brown to golden is easily seen with magnification (400X). The trophont attached to the fish by means of an attachment plate, which was visible with a light compound microscope.

Prevalence and intensity of infection of Amyloodiniosis in infected fish: Out of 1447 Asian Seabass Barramundi, 1022 fish infected with Amyloodiniosis with percentage 67.8% while out of 53 Hamour, *Epinephelus polyphekadion* 31 fish infected with Amyloodiniosis with percentage 58.4%, (Table 2). Intensity of infestation was more sever in Seabass (97-134 trophont/microscopic field (40X) (Fig. 5a) than grouper, *Epinephelus polyphekadion* (17-33 trophont/ microscopic field 40X) (Fig. 5b, Table 3).

Fig. 1: Asian Seabass infected with *Amyloodinium* on the skin and fins with emaciation, excessive secreted mucous on the body surface eroded fins with sloughing of some scales

Fig. 2: Complete sloughing of primary gill lamellae of Asian Seabass infected with *Amyloodinium ocellatum* (arrows)

Table 2: Prevalence of *Amyloodinium ocellatum* infection of investigated species

Fish species	Latin name	No. of examined fish	No. of infected fish	Infection (%)
Asian seabass	*L. calcarifer*	1447	1022	67.8
Hamour	*E. polyphekadion*	53	31	58.4

Table 3: Intensity *Amyloodinium ocellatum* trophonts/microscopic field (10X)

Fish species	No. of examined fish	No. of trophont/microscopic field
Asian seabass, *L. calcarifer*	1507	97-134 trophonts
Hamour, *E. polyphekadion*	53	17-33 trophonts

Table 4: Treatment efficacy of amyloodiniosis in seabass and repetition of treatment

Drug of treatment	Type of treatment	Dose (ppm)	Duration of treatment	References	Repetition	Efficacy of treatment (%)
Control	Without	-	-	-	-	0
Copper sulphate	Prolonged bath	0.03 mg L^{-1}	For 12 days	Noga and Levy[4]	After 1 week	100**
Formalin	Prolonged bath	100 mg L^{-1}	For 7 h	Paperna[13]	After 3 days	90*
Freshwater	Immersion (dipping)	-	For 5 min	Lawler[6]	After 3 days	95**

*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level, n = 30

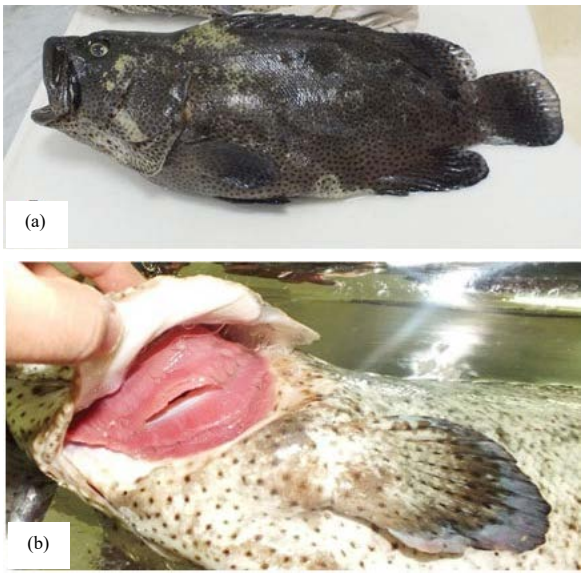

Fig. 3(a-b): Hamour *Epinephelus polyphekadion* (a) Opening mouth due to respiratory distress with darkening of skin accompanied with shining appearance and (b) Gills suffered from paleness with excessive mucous secretion

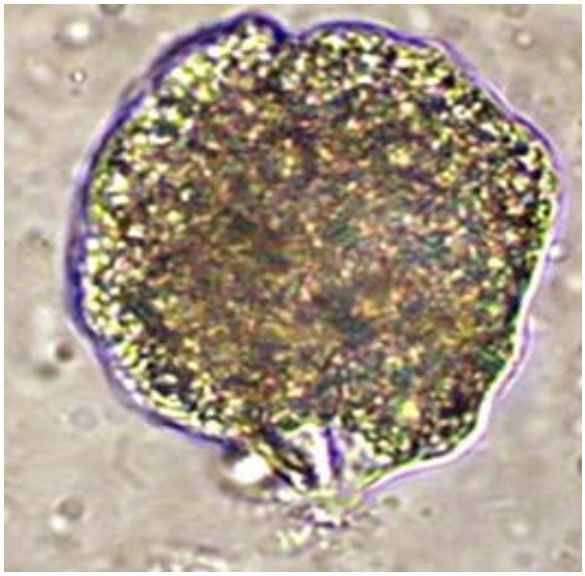

Fig. 4: Wet mount of *Amyloodinium* trophont (400X)

Treatment trials of Amyloodiniosis: Treatment was carried out using copper sulphate, formalin and freshwater bath. Treatment of choice was copper sulphate that achieved efficacy of treatment 100% from the first time followed by freshwater bath 95% then formalin 90% consequently. After repetition of formalin and freshwater bath, the treatment efficacy reached 100%. After treatment, fishes were removed to another tank free from tomonts and trophonts (Table 4).

Histopathological examination: Advanced hyperplasia of primary gill lamellae with edema in the core of the primary gill filaments (Fig. 6a), hyperplasia of the secondary gill filaments with Telangiectasia with sever infiltration of inflammatory cells (Fig. 6b), with hyperplasia of mucous cells accompanied with epithelial cells and gill necrosis (Fig. 6c), deformity and *Amyloodinium* trophonts with some particles of monogenea were present in between the secondary gill lamellae (Fig. 6d).

DISCUSSION

Amyloodinium ocellatum was first described by Brown[15] and it is one of the most important pathogenic parasitic diseases affecting cultured marine and brackish water fish[4]. The parasite produced a powdery or velvety appearance on infected fish and the resulting disease was commonly known

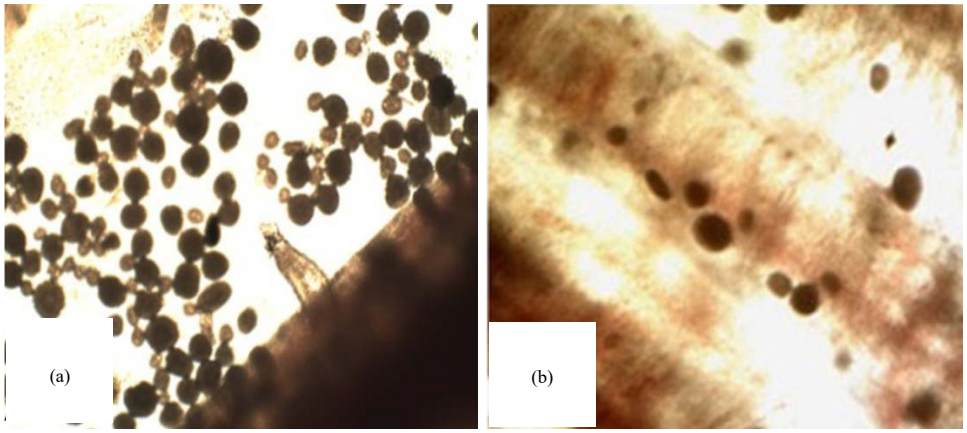

Fig. 5(a-b): (a) Mixed infection of heavy infestation with *Amyloodinium ocellatum* trophonts (arrows) and *Benedenia* sp. (arrow) on gills of Asian Seabass and (b) Wet mount of *Amyloodinium ocellatum* trophonts on gills of *Epinephlus polyphekadion*

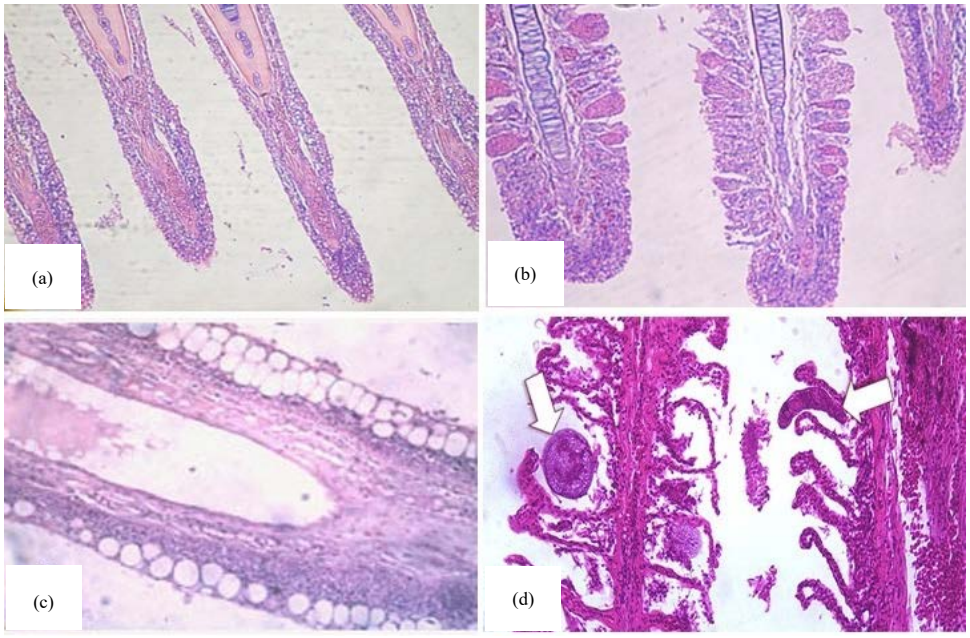

Fig. 6(a-d): (a) Advanced hyperplasia of primary gill lamellae with edema of the core of the primary gill filaments (arrows), (b) Hyperplasia of the secondary gill filaments with telangiectasia with infiltration of inflammatory cells (arrows), (c) Hyperplasia of goblet epithelial cells (arrows) and (d) Gill necrosis, deformity of primary gill lamellae, hyperplasia of secondary gill lamellae and *Amyloodinium* trophonts with some particles of monogenea were present in between the secondary gill lamellae (white arrows) (H and E, X200)

as "marine velvet", velvet disease or Amyloodiniosis. The organism is a dinoflagellate ectoparasite and has been reported in a wide range of marine and estuarine fish. It is one of a very few organisms that can infect both teleosts and elasmobranchs[2,16].

Regarding the clinical signs and postmortem lesions, the present study revealed that the first indication of an *Amyloodinium* infection was dying fish. *Amyloodinium* should always be considered as a possible cause of mortality when a disease outbreak involving marine or brackish water

fish took place. Behavioral signs might include anorexia or complete lack of feeding activity, flashing (rubbing against hard objects in the tanks or on the bottom substrate) and coughing (back flushing water across the gills). The skin of heavily infected fish could have a dull gold or brown sheen. Examination of the skin might revealed scale loss and patchy accumulation of mucus. Respiratory distress, gulping the atmospheric air could occur or even jumping outside water of the pond. The result nearly agreed with that obtained in previous research[2-4].

The gills are usually the primary site of infestation. Heavy infestations could also involve the skin, fins and eyes. Tomonts seen in the gastrointestinal tract[17] were probably swallowed by the host. So, the most characteristic sign was respiratory distress because of accumulation of mucous on gill filaments or sloughing of the primary gill filaments leading to respiratory failure and suffocation of the infected fishes and death[2].

Regarding prevalence and intensity of Amyloodiniosis, the current investigation revealed that from out of 1447 Asian Seabass Barramundi fish 1022 fish infected with Amyloodiniosis with percentage 67.8% while out of 53 Hamour *Epinephelus polyphekadion* 31 fish infected with Amyloodiniosis with percentage 58.4%. Intensity of infestation was higher in Seabass (97-134 trophont/microscopic field (40X) than grouper *Epinephelus polyphekadion* (17-33 trophont/microscopic field), the results nearly agreed with that obtained in earlier research[2,5].

Concerning treatment trials for Amyloodiniosis, the present investigation revealed that treatment of choice was copper sulphate that achieved efficacy of treatment 100% from the first time followed by freshwater bath 95% then formalin 90% consequently. Treatment could be prolonged because Tomonts and trophonts are resistant for treatment dinospores only susceptible for treatment. The results nearly agreed with the results of Noga[2], Noga and Levy[4], Lawler[6], Soares *et al*.[8] and Paperna[13].

Regarding the histopathological changes produced from infection of Amyloodiniosis in naturally infected Asian Seabass and Hamour broadstock present study revealed that advanced hyperplasia of primary gill lamellae with edema of the core of the primary gill filaments, hyperplasia of the secondary gill filaments with telangiectasia with sever infiltration of inflammatory cells, with hyperplasia of mucous cells accompanied with epithelial cells and gill necrosis, deformity and *Amyloodinium* trophonts and some particles of monogenea were present in between the secondary gill lamellae, the results nearly coincided with the previous results[2].

CONCLUSION

It was concluded that Amyloodiniosis in fish is an epidemic disease recorded for the first time in Egypt causing mortality in Asian Seabass and Hamour broad stock. The current investigation revealed the best treatment of Amyloodiniosis infection which included prolonged bath of copper sulphate, formalin and freshwater bath (immersion) consequently. Treatment of choice for Amyloodiniosis was prolonged bath of copper sulphate with a dose 0.03 mg L^{-1} for 12 days repeated one week for 2 times followed by freshwater bath for 5 min (immersion).

SIGNIFICANCE STATEMENT

This study discovered and compared the pathogenesis of Amyloodiniosis in Asian Seabass, *Lates calcarifer* and Hamour, *Epinephelus polyphekadion* and discovered also applicable treatment for Amyloodiniosis in Asian Seabass. Treatment was prolonged bath of copper sulphate followed by formalin then freshwater bath (immersion), but the treatment of choice for Amyloodiniosis was prolonged bath of copper sulphate with a dose 0.03 mg L^{-1} for 12 days repeated one week for 2 times. This study will help the researcher to uncover the critical areas of treatment parasitic protozoal diseases affect Asian Seabass fish that many researchers were not able to explore.

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