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SHORT PAPER

First Report of *Amyloodinium ocellatum* (E. Brown) E. Brown & Hovasse, 1946 (Dinoflagellate, Blastodiniales, Ooodiniaceae) from Sobaity Seabream, *Sparidentex hasta* (Valenciennes, 1830) Cultured in Persian Gulf of Iran

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Abstract

A study was conducted to determine the cause of mortalities in juvenile sobaity seabream, *Sparidentex hasta* (Valenciennes, 1830) cultured in a commercial fish farm hatchery located at Suza, southeast of Qeshm (Iran). Anorexia, anoxia, and excess mucous secretion were the main sympotoms observed in moribund fishes. Microscopic examination of the gill filaments revealed the presence of the trophonts of the causative agent, *Amyloodinium ocellatum* (E. Brown) E. Brown & Hovasse, 1946. This is the first report of *A. ocellatum* infestation in sobaity seabream cultured at marine fish hatcheries in Iran.

Introduction

Aquaculture has the world's fastest growing rate among the food production sector and mariculture has the steadily developing over the last few decades. The increasing demand for seafood has intensified fry and fingerling production, but diseases are one of the crucial restricting factors in marine fish hatcheries and representing severe costs to producers (Murray & Peeler, 2005; Shinnet al., 2015). Generally, the open design of aquaculture facilities allows the transmission of infectious pathogens, where they achieve optimum conditions to cause a disease occurrence (Mladineo et al., 2010).

Dinoflagellates are primary producers in the marine ecosystem, and numerous species are also known as parasites of a wide variety of hosts from protists to metazoans (Gómez, 2012). Amyloodiniosis (marine velvet), caused by the dinoflagellate ectoparasite *Amyloodinium ocellatum* (E. Brown) E. Brown & Hovasse, 1946, is one of the most important diseases that represents a major threat for marine organisms (fishes, crustaceans and bivalves) in warm and temperate waters (Noga & Levy, 2006). According to a cosmopolitan distribution, this pathogen is capable of living and reproducing in different ecological habitats (temperature ranges: 16 to 30°C and salinity ranges: 10 to 60) and infesting both feral and cultured fish (Paperna, 1984). *A. ocellatum* causing severe morbidity and mortality in various aquaculture facilities, with attaching to gill and skin of cultured fishes and usually with rapid spread within a few days (Masson, Blaylock, & Lotz, 2011).

The parasite has a triphasic life cycle: (i) Trophont (parasitic or feeding phase), the causative agents attach to the epithelium of gill and skin of host through rhizoids

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and feeds on the host through the stomopode. (ii) Tomont (encapsulated or reproductive phase), the trophonts separate from the host after feeding stage and fall to the pond bottom where they form a cyst membrane to become the tomont. (iii) Dinospore (free living or infective phase), the infective agents hatch from tomonts and freely search a new host using flagella. After the attachment to the host, dinospores quickly transform into trophonts (Figure 1) (Brown, 1931).

A. ocellatum is a non-specific parasite in host selection, hence it has been recorded from different cultured fish in several parts of the world (Colorni, 1994). The only reference of amyloodiniosis in sparids from Persian Gulf was that one reported by Tareen (1986) during a parasitology study on *Acanthopagrus cuvieri*. Occurrence of amyloodiniosis was recorded by Masson *et al.* (2011) in red snapper (*Lutjanus campechanus*) in USA and by Ramesh Kumar *et al.* (2015) in silver pompano (*Trachinotus blochii*) in India. Saraiva *et al.* (2011) reported massive infestation of *A. ocellatum* in Portuguese marine fish farms causing high mortalities in seabass, seabream and turbot. Also, marine velvet was responsible for the mass mortality of milkfish fry in a commercial fish hatchery in Philippines (Cruz-Lacierda, Maeno, Pineda, & Matey, 2004). Soares *et al.* (2012) have reported mortality of farmed meagre (*Argyrosomus regius)* due to *A. ocellatum* infestation. Recently, it was isolated from the gills of cultured cobia (*Rachycentron canadum*) in Brazil (Gómez & Gast, 2018). However, information about marine fish parasites in Persian Gulf water is still scarce in the literature.

Sobaity seabream, *Sparidentex hasta* (Valenciennes 1830) is a popular marine food fish and a desirable candidate species for marine fish farming in the Persian Gulf and the Oman Sea regions. Nowadays, Sobaity is cultured in floating cages and at land-based farms in southern part of Iran, especially in Hormozgan and Bushehr provinces. One definitive factor to the sustainable development of this economically species is the increase of the knowledge about most important diseases affecting sobaity culture. This study describes the first case of amyloodiniosis in the Iranian commercial hatcheries of marine fish that can be used as basic information for the future studies.

Materials and Methods

This study was conducted in a marine fish hatchery located at Suza, southeast of Qeshm (Iran) (26º 45′ 21.38″ N, 56º 1′ 45.81″ E), during the summer of 2017. Sobaity juveniles (mean length: 14±3 cm; mean weight: 23±4 g) were reared for mariculture activity in 8 t capacity concrete ponds, holding seawater (salinity: 30-33, dissolved oxygen: >5 mg l^{-1}, pH: 7.2 to 8.3, temperature: 28–32°C) with continuous aeration and flow-through seawater system.

On May 18th 2017, the fish showed loss of appetite and lethargic swimming behavior with low mortality (around 20%). One to two days laterof the occurrence of these clinical signs, the fish mortality rate was around 50%. Newly dead and moribund fishes were subjected to microscopic observations. The gills were dissected and the gill filaments were collected for detection of pathogenic/parasitic infection. The dissected gill parts were placed in a Petri dish containing normal saline water and checked under binocular microscope at total magnification of 40x or 100x. Parasites attached on the gills were examined live and were sensitively removed using fine forceps and preserved in 70% ethanol. For the identification, Brown (1931) and Brown & Hovasse (1946) were followed.

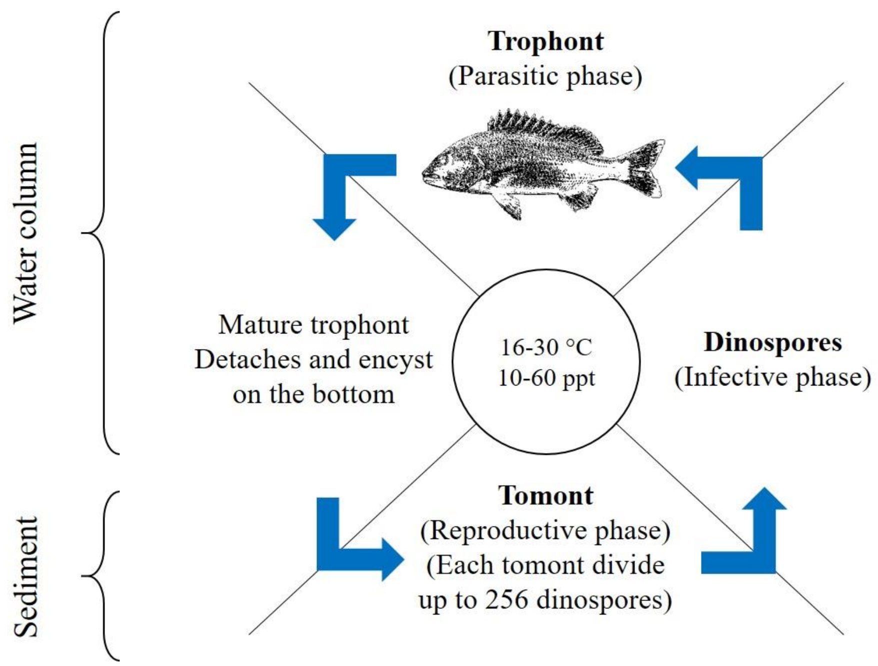

Figure 1. Life cycle of Amyloodiniosis agent *Amyloodinium ocellatum* (E. Brown) E. Brown & Hovasse, 1946.

Results

The primary symptoms of moribund fishes were darkening of the skin color. Infested fishes showed abnormal swimming behavior like jerky movement, swimming in circles and moving upside down with severe respiratory distress. Heavily infested fish showed signs of depression, anorexia, anoxia, gaping of mouth and sudden collapse. In the advanced stages, the gills became very pale and the affected fishes secreted excess mucous. Mortality was observed within 12–16 hours after onset of these clinical symptoms.

Through the parasitology observations, *A. ocellatum* was identified from the gill of *S. hasta* juveniles. There is no observation in skin infestation, even in heavily infested ponds. Under light microscopy, infected gills revealed several spherical brownish trophonts attached to the epithelium and measuring 80–230 µm in diameter. Detached trophonts were also presented between the gill lamellas (Figure 2). Necrosis of the lamellae and eventual erosion of gill epithelium were apparent at the trophont attachment sites.

Discussion

The dinoflagellate *Amyloodinium ocellatum* is one of the most dangerous ectoparasites that infests marine fish either in nature or aquaculture facilities. Therefore, amyloodiniosis is a major risk for land-based farming especially in semi-intensive and intensive systems, where, without a rapid detection, it can cause fatal epizooties and high mortality.

Entry routes of epizootic infestations in rearing systems probably include infected fish, infected live feed and contaminated water. Additionally, dinospores of *A. ocellatum* could transfer in aerosol droplets (Roberts-Thomson *et al*, 2006).

To date, most of the information about *A. ocellatum* infestation come from fish farming in tropical water (Noga & Levy, 2006). Amyloodiniosis has been reported on *S. senegalensis* from Portuguese fish farms causing mass mortalities (Soares et al., 2011). Currently, similar mortality recorded from two seabass farms in Egypt (Bessat & Fadel, 2018). In addition, marine velvet has been frequently diagnosed in the Mediterranean mariculture including *Dicentrarchus labrax* and *Sparus aurata* (from all the Mediterranean countries), *Diplodus puntazzo* (Greece) and mugilids (Italy) (Alvarez-Pellitero, 2004). This study reportsthe first occurrence of *A. ocellatum* infection in reared *S. hasta* in Persian Gulf region.

The primary preferred site of infection of *A. ocellatum* is mostly the gill epithelium of the host fish (Seoud et al., 2017). Damage to infested cells is related to the insertion of the rhizoids of the trophonts into the host cells causing focal erosion of the epithelium (Paperna, 1980). In advanced stages of infection, the parasites lead to gill hyperplasia and epithelial destruction, with subsequent respiratory impairment (Alvarez-Pellitero, 2004). Severe damage to gill epithelium leading to osmoregulatory impairment and secondary microbial infections (Noga & Levy, 2006; Noga, 2012).

It is described in numerous articles that the presence of *A. ocellatum* stimulates secretion of mucous in affected fish (Moreira *et al.* 2017; Ramesh Kumar *et al.* 2015). Similar pattern of mucous secretion were detected in infested sobaity juveniles. Moreover, changes in mucous production can be interpreted as a strategy to decrease attachment of the dinospores when fish are exposed to pathogen agents.

In conclusion, *S. hasta* reports as a new host of *A. ocellatum* in Persian Gulf water. Microscopic examination represents a useful method to diagnosis of Amyloodiniosis in fish farm, which could help to the detection and control of *A. ocellatum* infections. However, further study must be performed to deeper understand the physiological responses of fish to *A. ocellatum* outbreaks, which could help for management of the Amyloodiniosis in aquaculture systems.

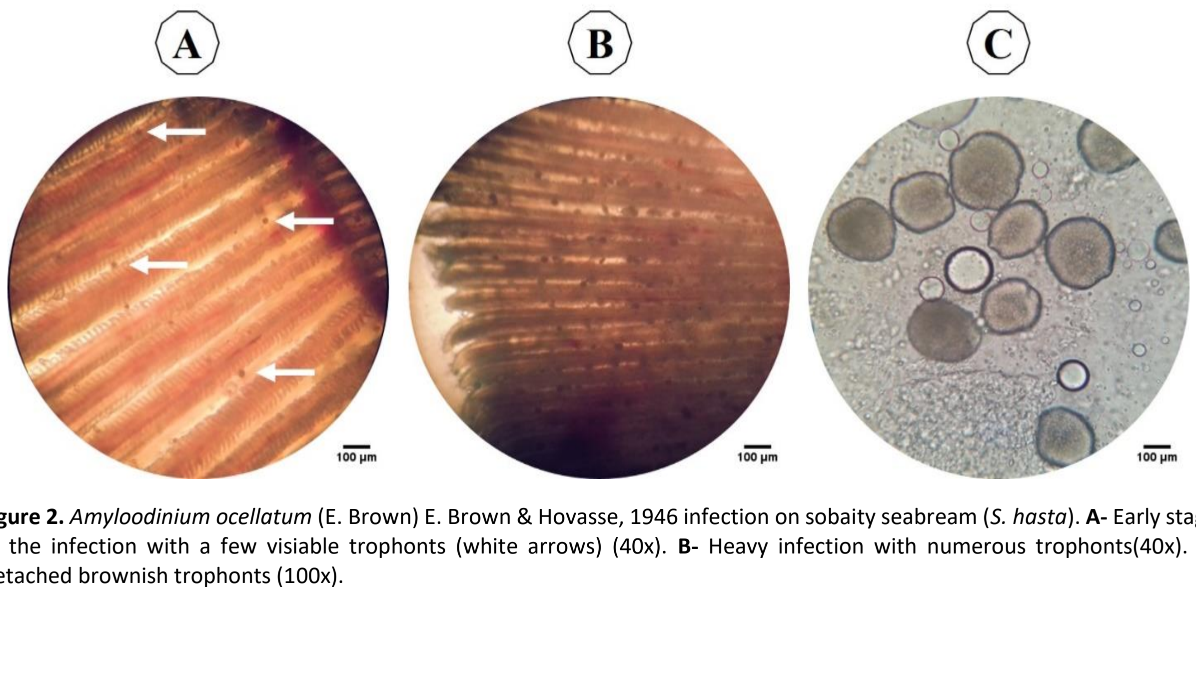

Figure 2. *Amyloodinium ocellatum* (E. Brown) E. Brown & Hovasse, 1946 infection on sobaity seabream (*S. hasta*). **A-** Early stage of the infection with a few visiable trophonts (white arrows) (40x). **B-** Heavy infection with numerous trophonts(40x). **C-** Detached brownish trophonts (100x).

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