Indian J. Fish., 67(4): 154-159, 2020
DOI: 10.21077/ijf.2020.67.4.88605-19

Note

Amyloodinium sp. (Brown, 1931) (Dinoflagellida) infestation in captive stock of silver moony *Monodactylus argenteus* (Linnaeus, 1758)

DANI THOMAS, A. NAVANEETH KRISHNAN, P. EZHIL PRAVEENA, RAYMOND JANI ANGEL, M. KAILASAM AND K. P. JITHENDRAN

ICAR-Central Institute of Brackishwater Aquaculture, 75, Santhome High Road, R. A. Puram, Chennai - 600 028 Tamil Nadu, India
e-mail:danithomass@gmail.com

ABSTRACT

This study was undertaken to investigate the cause of mortality in the captive stock of silver moony *Monodactylus argenteus*. The fishes showed severe infection of dinoflagellate protozoan *Amyloodinium* sp. (Blastodinida, Oodiniaceae) on gills and skin with complete mortality of the stock within a week. Histopathological changes were evident in the gill tissues with severe lamellar epithelial cell hyperplasia and lamellar fusions with the presence of trophonts of *Amyloodinium* sp. Scanning electron microscopy (SEM) also revealed trophonts of *Amyloodinium* sp. of varying sizes in groups consisting of 3 to 5 trophonts tightly attached to gill lamellae. Source water contaminated with the tiny infectious form of the parasite (dinospores) favoured by higher salinity and low water temperature in the rearing tank could be the triggering factor for the spurt of infections. Proper quarantine and biosecurity protocols to prevent the potential sources of water-borne infection sources are likely to be far more effective than treatment.

Keywords: *Amyloodiniosis*, *Amyloodinium* sp., Histopathology, *Monodactylus argenteus,* Scanning electron microscopy, Silver moony, Trophonts

Amyloodinium sp. is one of the most severe parasitic infection affecting marine and brackishwater fishes in warm and temperate waters. The disease caused by this organism has been referred to as 'velvet' or 'rust' due to a powdery or velvety appearance on the infected fish and is commonly referred to as 'marine velvet' or amyloodiniosis (Reed and Francis-Floyd, 1994). *Amyloodinium* sp. infects the gills and skin of several fish species leading to severe infection and often complete mortality in wild and cultured fishes (Reed and Francis-Floyd 1994; Noga and Levy 2006; Kizhakudan *et al*., 2015; Nozzi *et al*., 2016). Life cycle of the parasite is direct and consists of three intermittent stages. First, a parasitic stage called trophont, which is attached *via* anchor-like roots and are actively feeding on infested gills, fins and body of the fish host. When trophont matures, it falls free of the host to become encysted reproductive phase in the sediments. The tomonts subdivide internally to produce an actively swimming infective dinospore, releasing in water column searching for a new host. The duration of this life cycle is temperature and salinity dependent and can range from seven to as many as twenty days in 10 to 60 ppt salinities (Landsberg *et al*., 1994; Kuperman and Matey 1999; Schwarz and Smith 2009). *Amyloodinium* sp. has a broad host and geographic range, causing fish mortalities in tropical and temperate environments. Rapid multiplication and spread of the parasite leading to high mortality are common in cultured fish, if the organism is not recognised and treated early in the course of the outbreak (Paperna 1980; Montgomery-Brock *et al.,* 2000). The conditions are more likely to occur in confinement or high fish densities, such as those that can arise in aquaculture. Therefore, health management measures are a vital part of intensified culture practices, lack of which results in severe loss due to mortality.

Captive breeding and seed production of silver moony *Monodactylus argenteus* as a brackishwater ornamental fish is being attempted at the Muttukadu Experimental Station (MES) of ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA) to augment breeding and seed production to commercial scale. The fish is highly priced and can adapt to a wide range of salinity (Kuiter and Tonozuka, 2001). However, diseases are an imminent part of this venture as in the case of any aquaculture activities. The present study was undertaken to investigate the causes of mass mortality recorded in the captive stock of *M. argenteus* at MES of ICAR-CIBA during September 2017.

Subadults of *M. argenteus* (n = 100, mean length 11.3±0.4 cm and mean weight 41±20 g) were collected from Vennangupattu backwater of Kancheepuram District, off Chennai. They were transported in open tank with oxygen

supply to MES of ICAR-CIBA, located at Muttukadu near Chennai, India, as part of the captive broodstock development programme. The fishes were acclimatised and transferred to three circular one-ton capacity (FRP) quarantine tanks filled with filtered seawater and provided with continuous aeration. As a prophylactic measure, before transferring to the rearing tank, fishes were given freshwater treatment and 200 ppm formalin bath (30 min) and released into 10 t capacity FRP tank with continuous aeration and flow-through. Fishes were fed @ 3 to 5% body weight with formulated broodstock feed. Uneaten feed and faecal matter were syphoned out daily.

Forty-five days after stocking, during September 2017, these sub-adult fishes in the broodstock tank exhibited abnormal behaviour and clinical symptoms. Within 24 h after onset of clinical symptoms, mortality started and 100% loss of stock occurred within a span of one week. The case was suspected for parasitic infections and detailed investigations were carried out. Physicochemical parameters, such as temperature, salinity, pH, total ammonia nitrogen (TAN), dissolved oxygen, nitrate and nitrite of the rearing water were measured at the time of infection.

Five live fishes with advanced stage of clinical signs were examined for gross lesions and comprehensive necropsy. The fishes were sacrificed and subjected to microscopic and histopathological examinations. The body surface, fins and gills were closely examined for the presence of ectoparasite under a stereozoom microscope (Nikon SMZ25, Japan). Similarly, the visceral organs were also dissected out and examined for any internal parasites. Gills and other visceral organs were fixed in 10% neutral buffered formalin and processed by standard histological analysis using an automatic tissue processor (Microme, Germany) and histoembedder (Histostar, Thermo Scientific, USA). Tissue sections were cut (4-5 µm) using a rotary microtome (Leica RM 2245, Germany) and stained with haematoxylin and eosin (H&E). The histological changes were analysed by light microscopy (Nikon Eclipse E200, Japan) and digital images were taken using a Nikon DS-Fi2 digital camera using Nikon NIS-Elements Imaging software suite (Version F 4.30.01). The gill tissues were processed for scanning electron microscopy (SEM). A small portion of infected gills were fixed in 0.1 M cacodylate buffer (pH 7.5) containing 2.5% glutaraldehyde (Karnovsky 1965) for 2 h at 4^0C. Afterwards, samples were washed in cacodylate buffer for 1 h and then post-fixed in 1% cacodylate-buffered osmium tetroxide for 1 h at 4^0C. After double fixation, the samples were dehydrated through an ascending series of ethanol and the dehydrated samples were critical point dried, mounted on specimen holders and sputter coated with 20 nm gold-palladium. Tissue preparations were examined under a scanning electron microscope (SEM) (JSM-IT300, Jeol Ltd, Japan).

The silver moony fish stock exhibited abnormal behaviour like rapid breath (perceptible by the fast opercular movement) and rubbing or scratching on the tank wall. The affected fish which were kept apart from the stock, showed sluggish movement, change in colour and reduced appetite. Fishes came to the surface very frequently and swam near the surface; later the gills became very pale. The fish showed decreased appetite or off-feed, anorectic and changed skin colour from silver-yellow to yellow-brown with velvet or rust like appearance on the skin surface, swimming near the surface, rubbing against objects in the tank or on the bottom, moving upside down and respiratory distress. Clinical signs observed were indicative of sporadic infestation by parasites in sub-adults of silver moony in the rearing tank. Within a week, an outbreak of hyper infestation occurred resulting in mass mortality. Closer examination of the skin revealed scale loss, brown sheen and patchy accumulation of mucus as gross pathological symptoms (Fig. 1).

(a) (b)

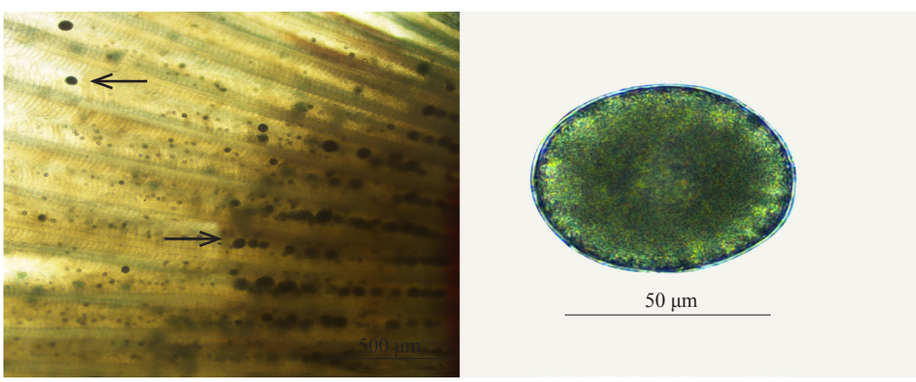
Fig. 1. *M. argenteus.* (a) Uninfected healthy fish; (b) Affected fish showing discoloration, descaling and mucous secretion

Based on necropsy, the cause of mass mortality was identified as *Amyloodinium* sp. infestation. Affected fish may die suddenly, exhibiting few clinical signs, but behavioural and physical changes were seen before death in most cases. The most common site of infection observed were the gill; hence respiratory difficulties seem to be one of the most common signs as evident in this case. Gills of infected fish showed excessive mucus and pale discolouration followed by mortality observed within 24 h after the onset of these clinical symptoms. Light microscopy of gill revealed a large number of round shaped and dark brownish parasitic stage or feeding stage of *Amyloodinium* sp. known as trophonts. Gill filaments showed distended appearance with the presence of vegetative stage, mechanically lodged between them (Fig. 2), which attaches to the skin and gill tissues by means of root-like structure called rhizoids.

Brown (1931) described *Amyloodinium* sp. as one of the most important pathogenic parasite affecting the cultured marine and brackishwater fishes. The parasite is reported to cause devastating disease and mortality due to the rapid reproduction of the organism when fishes are crowded, especially in closed aquaculture systems (Francis-Floyd and Floyd 2011). Severe outbreaks have been reported in cultured European seabass (Nozzi *et al*., 2016), Indian halibut (Kizhakudan *et al*., 2015) and silver pompano (Ramesh Kumar *et al*., 2015). In the present study, *Amyloodinium* sp. infestation in silver moony was observed in both gill and skin tissues. Within 24 h after the onset of these clinical symptoms, low-level mortality @ 2-3 nos. per day continued for three to four days, which was followed by a phase of rapid mortality (25 to 30 fish per day) leading to 100% loss of stock within a weeks time.

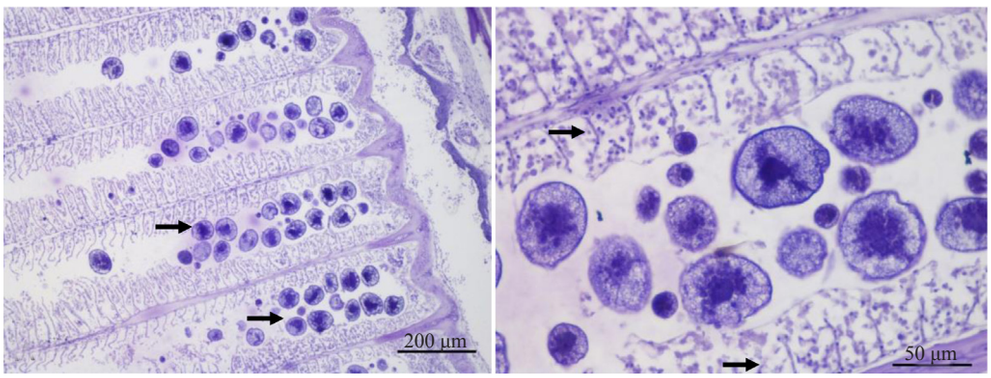
Water quality parameters recorded in the rearing tank during the infection were as follows: temperature 27±2^{0}C, salinity 31±1‰, pH 8±0.1, dissolved oxygen 4.9±0.1 ppm, total ammonia nitrogen 0.02±0.003 ppm, nitrate 0.01±0.002 ppm and nitrite 0.002±0.001 ppm. Environmental factors can vigorously promote the infestation of fish by ectoparasite *Amyloodinium* sp. Dinospore production, infectivity and life-cycle are temperature and salinity dependent and it can occur at a wide range (temperature 16 to 30^{0}C and salinities 10 to 60‰). Duration of the life cycle can range from 7 to as many as 20 days depending on the temperature and salinity (Landsberg *et al*., 1994; Kuperman and Matey 1999; Schwarz and Smith 2009). Salinity is the only factor positively related to the trophont of *Amyloodinium* sp. Dissolved oxygen, water temperature and pH, have a significant negative relationship with trophonts. In the present study, it was evident that water quality parameters *viz*., low water temperature and higher salinity were favourable for the proliferation of *Amyloodinium* sp. in the rearing system, leading to hyper infestation and the mass mortality of silver moony stock.

Histopathological studies showed varying degrees of epithelial proliferation in gill tissue with various developing stages of the parasite seen between the gill filaments. Severe epithelial hyperplasia was extensive throughout the length of the gill filament and consequent partial fusion of the secondary lamellae resulted in complete obliteration and distortion of the lamellar structure of the gill (Fig. 3). In many areas, the trophonts were surrounded by the gill filament as a cap. Severe infiltrations by the inflammatory cells were seen in both primary and secondary gill lamellae. Discrete areas of necrotic and sloughed gill tissue were observed (Fig. 4). The trophonts are encysted in gill tissue with massive proliferation of brachial epithelium surrounding the encysted parasitic trophonts. It might be due to an adaptive mechanism developed by the affected fishes to withstand the irritants due to the heavy load of protozoans (Meissner and Diamandopoulos, 1977). Erythrocytes heavily infiltrated



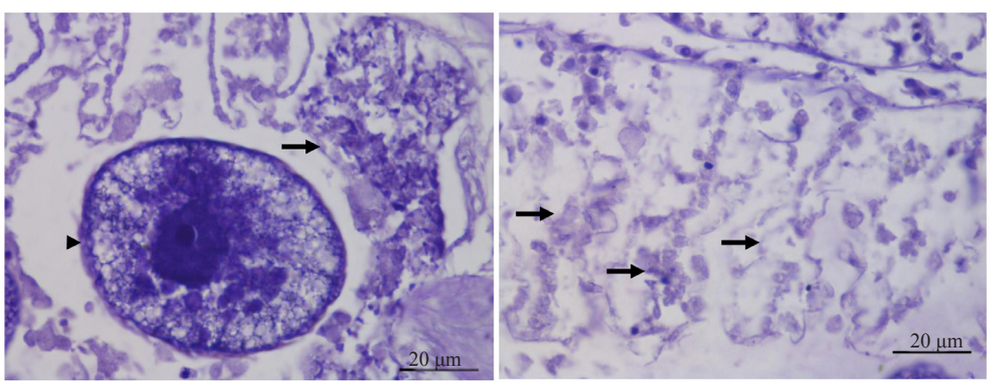
(a) (b)

Fig. 2. (a) Wet mount of the gills showing trophonts of *Amyloodinium* sp. between gill lamellae; (b) Detached trophont of *Amyloodinium* sp.



(a) (b)

Fig. 3. (a) Section of gill arch showing trophonts of *Amyloodinium* sp. (arrow) in clumps (H&E; 10x); (b) Section of gills showing necrosis (arrow) with inflammatory cell infiltration in the secondary lamella around the trophonts (H&E; 40x)

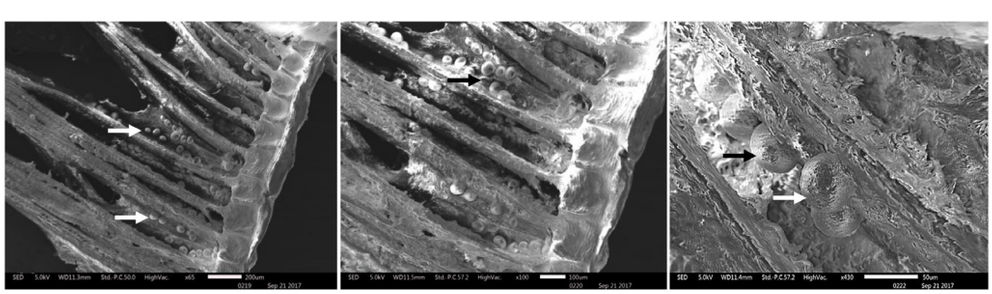


(a) (b)

Fig. 4. (a) Section of gill arch showing trophont of *Amyloodinium* sp.; (b) Necrosed gill tissue with inflammatory cells (H&E; 100x)

both primary and secondary gill lamellae. Lamellar fusion with disorganisation was evident in almost all gill tissues. Gill tissue impairment due to the presence of parasites causes asphyxiation and finally death of fishes.

Under the SEM, trophonts of *Amyloodinium* appeared round or spherical in shape (Fig. 5). Trophonts of varying sizes (40 to 60 µm dia) were located along gill filaments and between lamellae. Their basal portion was narrow and formed a very short stalk or peduncle inserted into the cells for more robust anchoring of the parasite. Groups consisting of 3 to 5 trophonts were found tightly attached to the gill lamellae. Altered gill structures, with gill filaments enlarged and swollen and partial or full fusion of lamellae transformed into the club-like structure were visible. Epithelial cells at the point of attachment were partially destroyed and concentrated around the peduncle.



(a) (b) (c)

Fig. 5. Scanning electron micrographs of the gills showing *Amyloodinium* sp. trophonts lodged in infected gills of silver moony (Scale bar, a: 200 µm; b: 100 µm; c: 50 µm)

The organism has a simple (direct) life cycle that allows the parasite to reproduce rapidly if unnoticed in aquaculture systems. Each tomont may release as many as 256 dinospores, capable of infecting susceptible fish (Reed and Francis-Floyd 1994). The attached, feeding stage may be difficult to treat and the encysted reproductive phase may be completely resistant to drug treatment and can remain ineffective for at least two weeks in the absence of a fish host. Freshwater dips of 3-5 min cause trophonts to detach from infected fish. Repeated treatments for 2-3 weeks are usually required to control an *Amyloodinium* outbreak. Formalin and hydrogen peroxide has been used as an effective therapeutic compound against *Amyloodinium* infestation in aquaculture (Ramesh Kumar *et al*., 2015). The most probable routes through which parasites may be introduced are infected fish or infected food or contaminated water. In the present case, the probable root for the introduction of parasites could be the contaminated source water, since it started after 45 days of stocking and the feeding was done using formulated feed. Preventing the introduction of the parasite in the first place is extremely important in any aquaculture operation. Quarantine and biosecurity are essential to prevent recurring problems with *Amyloodinium* infestation in aquaculture facilities and is likely to be more effective than treatment. Avoiding the potential sources of infection and setting up of disinfection protocols to prevent the accidental introduction or movement of infectious materials around the facility is vital.

We found that the reason for mass mortality of silver moony fish stock was due to high infestation by *Amyloodinium* sp. The organism›s life cycle allows rapid reproduction accompanied by wide range of environmental tolerance and resistance of the tomont stage to chemotherapeutants makes the organism difficult to control in an aquaculture system. As there is yet no safe and effective treatment for *Amyloodinium*, it is extremely important to avoid entry of the parasite into an aquaculture facility.

Acknowledgements

The authors are thankful to the Director, ICAR-CIBA, Chennai for providing necessary facilities for the study. The authors also thank Mr. N. Jagan Mohan Raj, Senior Technical Assistant for technical assistance.

References

Brown, E. M. 1931. Note on a new species of dinoflagellate from the gills and epidermis of marine fish. *Proceedings of the Zoological Society of London*. Oxford, UK, p. 345-346. https://doi.org/10.1111/j.1469-7998.1931.tb06196.x.

Francis-Floyd, R. and Floyd, M. R. 2011. *Amyloodinium ocellatum*, an important parasite of cultured marine fish. *Southern Regional Aquaculture Centre*. *Publication No. 4705*, p. 1-12.

Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell. Biol*., 17: 137-138.

Kizhakudan, J. K., Ezhil Praveena, E., Maran, B. A. V., Otta, S. K., Bhuvaneswari, T., Rajan, J. J. S., Krishnamoorthi, S. and Jithendran, K. P. 2015. Investigation on the mortality of juveniles in captive stock of the Indian halibut *Psettodes erumei*. *Indian J. Mar. Sci*., 44: 1217-1223.

Kuiter, R. H. and Tonozuka, T. 2001. Pictorial guide to Indonesian reef fishes. Part 2. Fusiliers - Drago nets, Caesionidae - Callionymidae. *Zoonetics*, Australia, p. 304-622.

Kuperman, B. I. and Matey, V. E. 1999. Massive infestation by *Amyloodinium ocellatum* (Dinoflagellida) of fish in a highly saline lake, Salton Sea, California, USA. *Dis. Aquat. Org*., 39: 65-73. DOI: 10.3354/dao039065.

Landsberg, J. H., Steidinger, K. A., Blakesley, B. A. and Zondervan, R. L. 1994. Scanning electron microscope study of dinospores of *Amyloodinium* cf. *ocellatum*, a pathogenic dinoflagellate parasite of marine fish and comments on its relationship to the Peridiniales. *Dis. Aquat. Org*., 20: 23-32.

Meissner, W. A. and Diamandopoulos, G. T. 1977. Neoplasia. In: Anderson, W. A. D. and Kissane, J. M. (Eds.), *Pathology.* The CV Mosby Co., St. Louis, USA, p. 640-691.

Montgomery-Brock, D., Sylvester, J. Y., Tamaru, C. S. and Brock, J. 2000. Hydrogen peroxide treatment for *Amyloodinium* sp. on mullet (*Mugil cephalus*) fry. *Centre for Tropical and Sub Tropical Aquaculture, Aqua Tips Regional Notes,* 11(4): 4-6.

Noga, E. J. and Levy, M. G. 2006. Phyllum Dinoflagellida. In: Woo, P. T. K. (Eds.), *Fish diseases and disorders*, *vol 1: Protozoan and metazoan infections*. CAB International: Oxford, UK, p. 16-45.

Nozzi, V., Strofaldi, S., Piquer, I. F., Di Crescenzo, D., Olivotto, I. and Carnevali, O. 2016 *Amyloodinum ocellatum* in *Dicentrarchus labrax*: Study of infection in salt water and freshwater aquaponics. *Fish Shellfish Immunol*., 57: 179-185. DOI:10.1016/j.fsi. 2016.07.036.

Paperna, I. 1980. *Amyloodinium ocellatum* (Brown, 1931), (Dinoflagellida) infestation in cultured marine fish at Eilat, Red Sea: epizootiology and pathology. *J. Fish Dis*., 3: 363-372. https://doi.org/10.1111/j.1365-2761.1980.tb00421.x.

Ramesh Kumar, P., Nazar, A. K. A., Jayakumar, R., Tamilmani, G., Sakthivel, M., Kalidas, C., Balamurugan, V., Sirajudeen, S., Thiagu, R. and Gopakumar, G. 2015. *Amyloodinium ocellatum* infestation in the broodstock of silver pompano *Trachinotus blochii* (Lacepede, 1801) and its therapeutic control. *Indian J. Fish*., 62: 131-134.

Reed, P. A. and Francis-Floyd, R. T. 1994. *Amyloodinium* infections of marine fish In: *Fact Sheet VM-90, a series of the College of Veterinary Medicine*, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, USA, 12 pp.

Schwarz, M. H. and Smith, S. A. 2009. *Getting acquainted with Amyloodinium ocellatum*. Commercial Fish and Shellfish Technology. Virginia Cooperative Extension. Fact Sheet, Pub. 600-200, Virgina, USA, 2 pp.

Date of Receipt : 05.04.2019
Date of Acceptance : 20.09.2020