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Outbreak of mortality among cage-reared cobia (*Rachycentron canadum*) associated with parasitism

Surto de mortalidade em bijupirá (*Rachycentron canadum*) criado em tanque-rede associado ao parasitismo

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Abstract

This study reports a disease outbreak among juvenile cobia (*Rachycentron canadum*) farmed in cages in the state of Rio de Janeiro, Brazil, caused by the dinoflagellate *Amyloodinium ocellatum* and the monogenean *Neobenedenia melleni.* Two thousand five hundred fish were stocked at 0.4 kg/m³ in a set of 12 m³ tanks, in autumn (mean weight 15.0 ± 7.3 g) and in winter (mean weight 43.0 ± 5.6 g). Freshwater baths were administered as a routine treatment, as the symptoms were detected followed by two collection samples. Firstly in May 2011 (n = 5) and secondly in September 2011 (n = 10). In the first sample, the prevalence of *N. melleni* on the body surface was 100% and the mean intensity was 42.0 ± 1.7, while in the second sample the prevalence was 60% with a mean intensity 3.0 ± 0.2 and mean abundance 1.8 ± 0.4. *Amyloodinium ocellatum* was only found in the second sample, at a prevalence 100% and mean intensity 46.8 ± 3.4. The cause of fish mortality was possibly associated with a decrease in fish resistance after the first contact with monogenean parasites, allied with respiratory difficulty caused by the presence of *A. ocellatum* in the gills.

Keywords: Marine fish, parasitology, disease, culture, Brazil.

Resumo

Este estudo relata a mortalidade em massa de juvenis de bijupirá (*Rachycentron canadum*) criados em tanques-rede no estado do Rio de Janeiro, Brasil, causada pelo dinoflagelado *Amyloodinium ocellatum* e o monogenea *Neobenedenia melleni.* Dois mil e quinhentos peixes estavam estocados à densidade de 0,4 kg/m³ em tanques-rede de 12 m³, no período do outono (15,0 ± 7,3 g; peso médio ± DP) e inverno (peso médio de 43,0 ± 5,6 g). Banhos de água doce foram realizados rotineiramente à medida que os sintomas eram detectados, quando então, foram efetuadas duas colheitas, sendo a primeira em maio de 2011 (n = 5) e a segunda em setembro de 2011 (n = 10). Na primeira colheita, a prevalência de *N. melleni* foi de 100% e a intensidade média de 42,0 ± 1,7, e na segunda, a prevalência foi de 60% e intensidade média de 3,0 ± 0,2 e abundância média de 1,8 + 0,4. Na segunda colheita, observou-se a presença de *A. ocellatum* (prevalência de 100%, intensidade média de 46,8 ± 3,4). Possivelmente, a causa da mortalidade estava relacionada à redução na resistência dos peixes após o primeiro contato com o parasito monogenea, juntamente com a dificuldade respiratória causada pelo dinoflagelado nas brânquias.

Palavras-chave: Peixe marinho, parasitologia, doença, cultivo, Brasil.

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Cobia (*Rachycentron canadum* Linnaeus, 1766; known as *bijupirá* in Brazil) have aroused great interest in the aquaculture sector and scientific community, and are an emerging species for marine aquaculture (CAVALLI; HAMILTON, 2007). Cobia production started in Asia and has spread rapidly to other continents because of their positive characteristics such as rapid weight gain (around 4.5 kg per year), easy spawning in captivity (ARNOLD et al., 2002; SOUZA-FILHO; TOSTA, 2008) and availability of technology for fingerling production (HOLT et al., 2007). On the other hand, it presents some problems relating to diseases caused by the monogenean helminth *Neobenedenia melleni* MacCallum, 1927 (Monogenea: Capsalidae), which is one of the main constraining factors on cobia aquaculture development (LIAO et al., 2004; OGAWA et al., 2006; CAVALLI et al., 2011). The dinoflagellate protozoan *Amyloodinium ocellatum* Brown, 1931, has been reported in larval stages of these fish (LIAO et al., 2004; BENETTI et al., 2008). Nevertheless, no information on dinoflagellate infestation in juvenile cobia in Brazil has been registered. This study reports an outbreak of mortality among juvenile cobia caused by the monogenean *N. melleni* and the dinoflagellate *A. ocellatum.*

In order to ascertain the cause of symptoms, two collections were made. In the first, in May 2011 (autumn), the fish (n = 5) weighed 15.0 ± 7.3 g and had a total length of 16.0 ± 2.3 cm. The second sample was taken soon after the first observations of mortality, in September 2011 (winter), when the fish (n = 10) presented weight and total length of 43.0 ± 5.6 g and 22.0 ± 2.5 cm, respectively. The water temperature, dissolved oxygen and pH were measured using a Hanna HI 9828 multiparameter meter and the salinity using an Atago HHR-2N refractometer.

Fish presenting erratic swimming and gasping at the water surface were randomly caught with a net, anesthetized in eugenol solution (75 $mg.L^{-1}$), weighed and measured. After that, gills and mucus from the body surface were collected and fixed in a 5% formalin solution for parasitological analysis. The dinoflagellates were quantified using a Sedgewick Rafter chamber, following the methodology developed by Jerônimo et al. (2011), and were identified in accordance with Eiras et al. (2006) and Martins et al. (2001). The monogeneans were counted under a stereomicroscope, mounted in Hoyer's medium and identified in accordance with Whittington and Horton (1996).

Fifteen days after the second collection, a total of 2,500 juvenile cobia died. In May 2011, the water temperature was 24.0 ± 0.8 °C, pH 7.9 ± 0.3, dissolved oxygen 6.0 ± 0.7 $mg.L^{-1}$ and salinity 30.0 ± 1.2 $g.L^{-1}$. At the first sampling, the prevalence of *N. melleni* was 100%, with mean intensity of 42.0 ± 8.7 and abundance of 42.0 ± 8.7 was found in the body surface mucus. In September 2011, the water temperature was 20.8 ± 1.1 °C, pH 7.9 ± 0.4, dissolved oxygen 6.8 ± 0.7 $mg.L^{-1}$ and salinity 30.0 ± 0.9 $g.L^{-1}$. At the second sampling, the prevalence of *N. melleni* was 60%, with mean intensity of 3.0 ± 0.2 and abundance of 1.8 ± 0.4. In the gills, the observed prevalence of *A. ocellatum* was 100%, with mean intensity of 46.8 ± 3.4 and abundance of 46.8 ± 3.4.

The mean intensity of *N. melleni* observed in this study is considered to be high and harmful for farmed fish (JITHENDRAN et al., 2005). Monogenean infestations are common in cultivated marine fish and almost always are responsible for high mortality rates (DEVENEY et al, 2001; CRUZ-LACIERDA et al., 2004). The main factors responsible for parasite dissemination are directly associated with water quality and temperature (MORAES; MARTINS, 2004). Increased water temperature and high organic matter content in floating cages can favor monogenean reproduction (PAN, 2005). Water temperature may influence occurrences of fish parasites such as dinoflagellates (MARTINS et al., 2001; PEREIRA et al., 2011) and *Neobenedenia* sp. (OGAWA et al., 2006). Monitoring water quality and fish health is important as a prophylactic measure in order to avoid parasite reproduction (JERÔNIMO et al., 2011). In the present study, from the first to the second sampling, the reduction in the water temperature of 3.5 °C may have been responsible for decreased fish resistance and greater parasite occurrence. These data are in agreement with the hypothesis proposed by Pereira et al. (2011), which highlights the importance of sanitary surveillance in the summer season in order to avoid fish mortality caused by parasite reproduction.

Although cobia are considered to be disease-resistant fish, they can harbor a great variety of pathogenic agents over their life cycle, including viral diseases (e.g. lymphocystis), bacterial diseases (vibriosis) and the parasites *Neobenedenia* sp. and *A. ocellatum* (LIAO et al., 2004). Infestations by *Amyloodinium* sp. and *Neobenedenia* sp. have been reported in other fish species (NOWAK, 2007; SANCHES, 2008; KERBER et al., 2011; HIRAYAMA et al., 2009), and monogeneans are one of the most important parasites affecting cobia cultivation (KERBER et al., 2011). During the growth phase of *Lates calcarifer*, this ectoparasite was responsible for the mortality of 200,000 fish in Australia (DEVENEY et al., 2001). Recently, the first occurrence of *N. melleni* among cage-reared cobia in southeastern Brazil was registered (KERBER et al., 2011). Parasite proliferation is associated with a monoxenic life cycle and high stocking densities (THONEY; HARGIS JUNIOR, 1991; TAKEMOTO et al., 2004).

Fish parasitized by *N. melleni* showed skin lesions, apathy, anorexia, pale eyes, increased mucus production and hemorrhages, with death due to secondary infections (OGAWA et al., 1995; HIRAYAMA et al., 2009). Like in the sample of the present report, Kerber et al. (2011) also observed lesions on the skin, head and fins, as well as respiratory difficulty and corneal opacity.

The infestation by *N. melleni* may have been reduced from the first to the second sampling through treatment for minimizing fish mortality, which consisted of immersion baths in freshwater for 5 min, at 96-hour intervals.

This, along with the gasping behavior of the fish in order to increase gill ventilation, suggests that gas exchange had become impaired, as a consequence of high infection by *A. ocellatum* in the gills, since no monogenean was found in this organ.

In cobia, occurrences of *Amyloodinium* sp. have generally been reported in larval stages (LIAO et al., 2004; BENETTI et al., 2008). Although low numbers of *N. melleni* were found in the second sampling, it can be inferred that monogeneans may have caused a decrease in fish resistance, thus leading to dinoflagellate attachment. This may be explained by a reduction in fish resistance after the first contact with monogeneans.

According to Noga and Levy (2006), *A. ocellatum* is one of the most important parasites in marine fish cultivation. The fish tend to search for better oxygenated sites in tanks (REED; FRANCIS-FLOYD, 1994). This ectoparasite has been reported in both estuarine and marine fish, especially when reared at high stocking densities (REED; FRANCIS-FLOYD, 1994). However, in the present study, the cobia were stocked at low density (0.4 kg/m^3), throughout the observation period.

The pathogenicity of dinoflagellates is associated with attachment of rhizocysts to host cells, performed by trophonts, thereby causing necrosis (PAPERNA, 1980; MARTINS et al., 2001). More than 200 trophonts per gill filament can cause hyperplasia, inflammation, hemorrhage and necrosis (PAVANELLI et al., 2008).

In this study, the events that caused deleterious actions on the organism and the presence of *N. melleni* may have enhanced the possibility of secondary infections. Control over fish parasites among marine cage-reared fish is the most important challenge for marine aquaculture.

Our report suggests that fish mortality was caused by the first infestation by the monogeneans followed by the dinoflagellates. Prophylactic measures need to be adopted in fish cultivation, so as to avoid parasite reproduction (REED; FRANCIS-FLOYD, 1994).

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