BOLETIM DO INSTITUTO DE PESCA

ISSN 1678-2305 online version

Scientific Article

Neoechinorhynchus buttnerae PARASITIC INFECTION IN TAMBAQUI (*Colossoma macropomum*) ON FISH FARMS IN THE STATE OF AMAZONAS*

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*Financial support: Embrapa (MP2 - 02.13.09.003.00.00) and Fapeam (PAPAC – 015/2014).

Received: January 04, 2018
Approved: February 18, 2019

ABSTRACT

The record of occurrence of acanthocephalan parasites on tambaqui farms in Northern Brazil has increased in recent years along with reports of associated economic losses, justifying further epidemiological studies of this parasite. As such, this study evaluated the occurrence of the acanthocephalan *Neoechinorhynchus buttnerae* and the parasite-host relationship in tambaqui (*Colossoma macropomum*) from three fish farms in the Amazonian town of Rio Preto da Eva, Brazil. Fish from the farms (N = 18 per farm) were collected for parasitological examination, determination of the relative condition factor and correlation tests between the parameters assessed. Of the fish examined, 37 were parasitized with *N. buttnerae* (68.5% prevalence), with intensity of infection variation ranging from 1 to 1,219 specimens per fish (12,279 specimens counted). In two farms was detected the high mean intensity of infection, 230.8 and 451.3 parasites per fish, with a total count of 4,155 and 8,123 parasites, respectively, whereas the third farm had a single fish infected with only one specimen. The weight-length ratio equation indicated that the fish were growing at the same rate in weight and length. It was observed significant correlation between parasite intensity and fish length, as well as significant negative correlation with parasite intensity and condition factor. The gastrointestinal tract contained ostracod invertebrates, which are intermediate *N. buttnerae* hosts. The high *N. buttnerae* intensity affect their physiological condition as suggested by the negative correlation between parasitic intensity and condition factor, emphasizing the need to implement suitable prophylactic measures to prevent infection by the parasite.

Key words: acantocephalan; black pacu; fish culture; fish parasite.

PARASITISMO POR *Neoechinorhynchus buttnerae* EM TAMBAQUI (*Colossoma macropomum*) DE PISCICULTURAS DO ESTADO DO AMAZONAS

RESUMO

Nos últimos anos tem crescido o registro de ocorrências de parasitos acantocéfalos em pisciculturas de tambaqui da região Norte do Brasil, com relatos de perdas econômicas, justificando assim a necessidade de aprofundar dados epidemiológicos desta doença parasitária. Assim, o objetivo deste estudo foi avaliar a ocorrência do acantocéfalo *Neoechinorhynchus buttnerae* e a relação parasito-hospedeiro em tambaqui (*Colossoma macropomum*) de três pisciculturas do município de Rio Preto da Eva (AM), Brasil. Foram amostradas três pisciculturas no município alvo, coletando 18 peixes em cada uma, para análises parasitológicas e cálculo do fator de condição relativo e correlações associadas. Foram quantificados 12.279 espécimes de *N. buttnerae* em 37 peixes parasitados (prevalência 68,5%), com intensidade de infecção variando de 1 a 1.219 parasitos por peixe. Duas propriedades apresentaram as maiores intensidades médias de infecção, 230,8 e 451,3 parasitas por peixe; com 4.155 e 8.123 parasitos quantificados, respectivamente, enquanto na terceira foi encontrado apenas 1 parasito em 1 peixe infectado. A equação da relação peso-comprimento indicou que os peixes estavam crescendo na mesma proporção em peso e comprimento. Foi observada correlação significativa entre intensidade parasitária e comprimento dos peixes, assim como correlação negativa significativa entre intensidade parasitária e fator de condição. No trato gastrointestinal dos tambaquis foi observada a presença de invertebrados ostracodas; hospedeiros intermediários desta espécie de acantocéfalo. Portanto, a alta carga parasitária de *N. buttnerae* pode ter comprometido o desempenho dos peixes e afetado a sua condição fisiológica como sugerido pela correlação negativa entre intensidade parasitária e fator de condição, e enfatiza-se a importância do emprego de medidas adequadas de profilaxia para prevenção de infecções por este parasito.

Palavras-chave: acantocéfalos; tambaqui; piscicultura; parasitos de peixes.

INTRODUCTION

Brazilian aquaculture has grown significantly in recent years, with production reaching an annual value of 1.2 billion dollars in 2016, 77% corresponding to the 507,120 tons obtained from fish farming. The state of Rondônia was the top producer in 2016, with a yield of 90,640 tons, nearly 18% of total national production. The state of Amazonas ranked 8th, producing 21,080 tons of fish, but the Amazonian town of Rio Preto da Eva was considered the main national fish producing center, with 13,380 tons in 2016 (IBGE, 2016). Tambaqui (*Colossoma macropomum*) was the predominant native species reared in Rondônia and Amazonas, as well as in a number of other Brazilian states, with total production of 136,990 tons, accounting for 27% of national aquiculture production (IBGE, 2016).

In the context of aquacultural growth, Tavares-Dias and Martins (2017) predict yearly losses of 84 million dollars with direct and indirect costs caused by disease on Brazilian fish farms. Many pathogens can compromise tambaqui farming such as the acanthocephalan, whose records of occurrence of this parasite has increased in Northern Brazil as well as the description of their damage to fish (Malta et al., 2001; Silva et al., 2013; Chagas et al., 2015; Dias et al., 2015; Jerônimo et al., 2017; Lourenço et al., 2017; Matos et al., 2017; Pereira and Morey, 2018; Oliveira et al., 2019). According to Silva-Gomes et al. (2017), this endoparasite causes significant economic losses in tambaqui farming. Assessment of production indexes such as weight gain, final biomass and production per area revealed a more than 200% decline in the growth of acanthocephalan-infected fish, which directly affected gross production income.

Acanthocephalans are the smallest group of parasites, with nearly 1,100 species (Bush et al., 2001). More than the half of these are worms in wild and captive fish (Nickol, 2006). The life cycle of acanthocephalans is indirect and based on the food chain, requiring an arthropod as intermediate host and a vertebrate as definitive host, with the occasional use of a paratenic host (Santos et al., 2013). One aspect to underscore is that to date, *Neoechinorhynchus buttnerae* has been the only acantocephalan species detected infecting pure tambaqui (Malta et al., 2001; Thatcher, 2006; Eiras et al., 2010; Dias et al., 2015; Jerônimo et al., 2017; Lourenço et al., 2017; Matos et al., 2017; Pereira and Morey, 2018).

The pathogeny caused by acanthocephalans is characterized by stiffening and thickening of the host intestinal wall, a consequence of mechanical damage from the parasite's proboscis, triggering an intense inflammatory process primarily involving macrophages, Langerhans cells and lymphocytes (Dezfuli et al., 2008; Sanil et al., 2011; Jerônimo et al., 2017). More severe cell responses, such as leukocyte cell infiltration, muscle tissue metaplasia and necrosis across the intestinal layers are observed in sites with proboscis penetration. Also, histochemical techniques revealed that the host increases acid mucosubstance production in response to infection (Matos et al., 2017).

Considering the impact of acanthocephalan infection in fish farming and the need for further and more extensive epidemiological studies on the issue, the present study aimed at evaluating the occurrence of *Neoechinorhynchus buttnerae* in tambaqui farming (*Colossoma macropomum*) and the parasite-host relationship on three fish farms in the town of Rio Preto da Eva, Amazonas (AM) state, Brazil.

MATERIAL AND METHODS

The tambaqui (*Colossoma macropomum*) were obtained in October 2016 from three commercial fish farms (F1, F2 and F3) in the town of Rio Preto da Eva (AM). Feeding regime and water quality data were also collected. In the growth stage, the three farms used extruded commercial feed containing 28% crude protein (CP) for omnivorous fish, provided twice a day. During fish collection we measured water temperature (ºC) and dissolved oxygen (mg L^{-1}) using an YSI Pro20 oxygen meter (Ohio, USA), pH using a YSI Environmental pH100 pH meter (Ohio, USA) and total ammonia (mg L^{-1}) by the indophenol method (APHA, 1998).

We collected 18 fish on each farm, using a trawl net. The fish were immediately anesthetized with 100 mg L^{-1} of benzocaine before being measured for weight and standard length and then killed for autopsy and parasitological examination purposes. The protocols used complied with ethical principles established by the Animal Ethics Committee of Embrapa Pesca e Aquicultura (certificate no. 24, protocol 10/2016). The gastrointestinal tract of the fish was collected and examined under stereomicroscope for the presence of acanthocephalan parasites, which were collected, counted and identified as recommended by Eiras et al. (2006) and Thatcher (2006).

The data collected were used to calculate the parasitological indexes of prevalence, mean intensity and mean abundance, as described by Bush et al. (1997). Hosts weight and length measurements were used to calculate weight to length ratio by the equation $Wt=aL^b$, where Wt is total weight in grams, L the standard length in centimeters and a and b the constants. The constants were estimated by linear regression of the transformed equation: W=log a + b x log. The significance level of the coefficient of correlation r was estimated and the b value tested using the t-test to check if b=3 (Santos, 1978). The relative condition factor was calculated according to Le Cren (1951), and analyzed using the Student's t test ($p < 0.05$).

Spearman's correlation coefficient was applied to determine the possible weight to standard length correlations of the hosts and the intensity of infection, in addition to verifying parasite intensity and the relative condition factor (Zar, 1999).

RESULTS

The water quality parameters on the three fish farms were similar, except for the lower dissolved oxygen levels (2.52 mg L^{-1}) and higher ammonia levels (0.79 mg L^{-1}) on farm 1, where eutrophication was observed (Figure 1).

We examined 54 tambaqui with mean weight of 561.0 ± 333.9 g (206.6-2,200.0g) and mean length of 25.2± 4.4 cm (18.4-43.0 cm) (Table 1). The condition factor ranged from 0.98-1.04 (Table 1).

A total of 12,279 *N. buttnerae* specimens were counted in the 37 infected fish, indicating a 68.5% of prevalence in three farms. The intensity of infection ranged from 1 to 1,219 parasites per fish. All the fish sampled on farms 1 and 2 were infected by the parasites, totaling 4,155 and 8,123 specimens, respectively, whereas only one fish, infected by a single parasite, was detected on farm 3. Accordingly, mean intensity and mean abundance were higher on farms 1 and 2 (Table 1). Ostracods were detected in the gastrointestinal tract of some fish in two fish farms (1 and 2) (Figure 2).

Considering that the fish farm 3 has only one fish infected, we decided to exclude this farm from the following analyzes. The weight-length ratio equation for the fish was $W_t=0.1362*L^{2.5572}$ and $r^2=0.8899$. The value of *b* did not differ statistically from 3, and the growth was considered isometric ($t_c < t_{0.05;52}$) (Figure 3). This result indicates that the fish were growing proportionally by

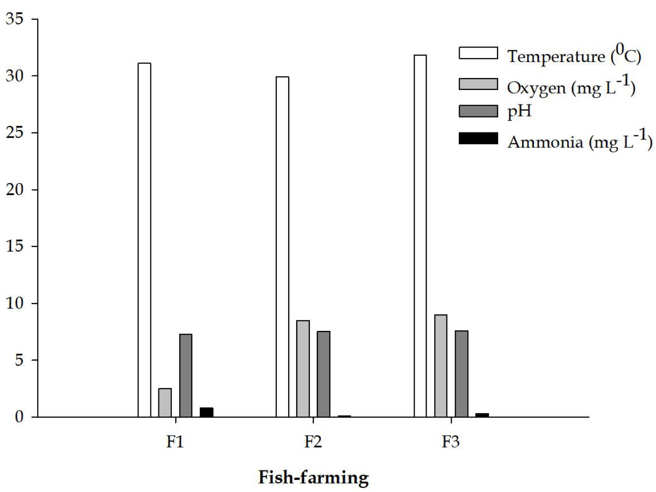

Figure 1. Water quality parameters on the three tambaqui (*Colossoma macropomum*) farms evaluated in Rio Preto da Eva, AM.

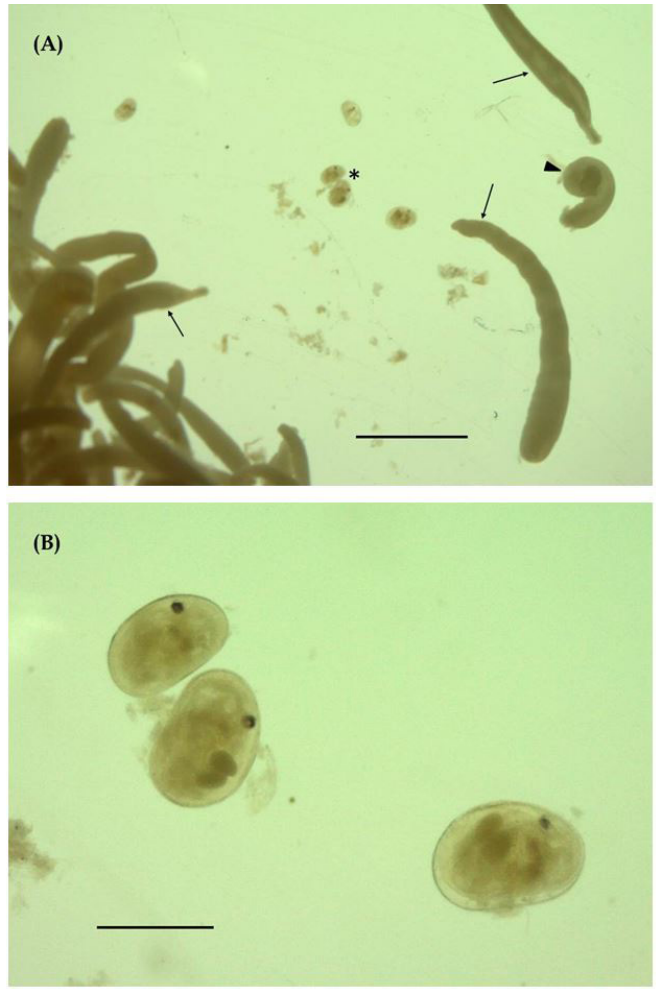

Figure 2. (A) Adults (arrows) and juvenile (arrowhead) of acanthocephalan *Neoechinorhynchus buttnerae* and exemplars of ostracods (*), in detail in (B), detected in the gastrointestinal tract of tambaqui (*Colossoma macropomum*) in fish farms in the state of Amazonas. Scale bars: A = 150 µm; B = 50 µm.

Table 1. Body weight, total length, condition factor and parasitological indexes for tambaqui (*Colossoma macropomum*) parasitized by acanthocephalan (*Neoechinorhynchus buttnerae*) from three fish farms in Rio Preto da Eva, AM.

	Fish farms		
	1	2	3
Weight (g)	461.85±91.30	644.53±177.5	576.59±538.20
Total length (cm)	23.66±1.68	27.60±2.47	24.24±6.49
Condition fator	1.04±0.09	0.98±0.17	1.00±0.00
Examined fish	18	18	18
Parasitized fish	18	18	1
Prevalence (%)	100	100	5.6
Mean intensity	230.8	451.3	1.0
Mean abundance	230.8	451.3	0.05
Range of Intensity	54-931	81 – 1,219	1
Total number of parasites	4,155	8,123	1

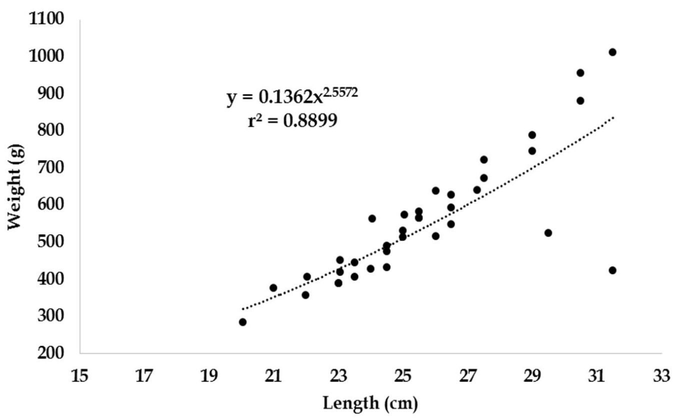

Figure 3. Scatter plot of standard length versus total weight of tambaqui (*Colossoma macropomum*) (n = 36) from two fish farms in Rio Preto da Eva, AM.

weight and length. The correlation between parasite intensity and fish weight was not significant, however the correlation between parasite intensity and length was significant (Figure 4). Another significant correlation was obtained between parasite intensity and condition factor (Kn) (Figure 5).

DISCUSSION

The acanthocephalan *N. buttnerae* was identified in this study in tambaqui from three fish farmers in the municipality of Rio Preto da Eva, state of Amazonas. The area of occurrence of this parasite has increased in the last years, mainly in the northern region of Brazil, infecting the tambaqui (Malta et al., 2001; Dias et al., 2015; Jerônimo et al., 2017; Lourenço et al., 2017; Matos et al., 2017; Pereira and Morey, 2018). In part, this is due to the presence in ponds of ostracod invertebrates, which are intermediate *N. buttnerae* hosts, as registered in two evaluated fish farms. The acanthocephalans collected in the present study infected 100% of the fish collected on farms 1 and 2, with intensity ranging of 54 to 931 on farm 1 and 81 to 1,219 on farm 2. In fish farm 3 only one parasite was found in the tambaqui evaluated, a fact that may be related to the application of chemical treatment in the water and subsequent drying of the tank, after the recording of acanthocephalans in the fish of the previous harvest. Other records of acanthocephalan infection in tambaqui reared in the state of Amazonas describe infection intensity (parasite per fish) of 31 to 406 (Malta et al., 2001), 81 to 708 (Matos et al., 2017), 15 to 720 (Silva-Gomes et al., 2017), 107 to 921 (Lourenço et al., 2017). Similarly, a study in the state of Roraima recorded acanthocephalan intensity of 188 to 388 parasites per tambaqui (Pereira and Morey, 2018). This variation in parasite indices may be due to intensifying production systems without concurrently using good hygienic practices, contributing to the dissemination of the intermediate host and parasite to other fish farms in the north region. The mean intensity of tambaqui infection by *N. buttnerae* (230.8 on farm 1 and 451.3 on farm 2) is above that reported

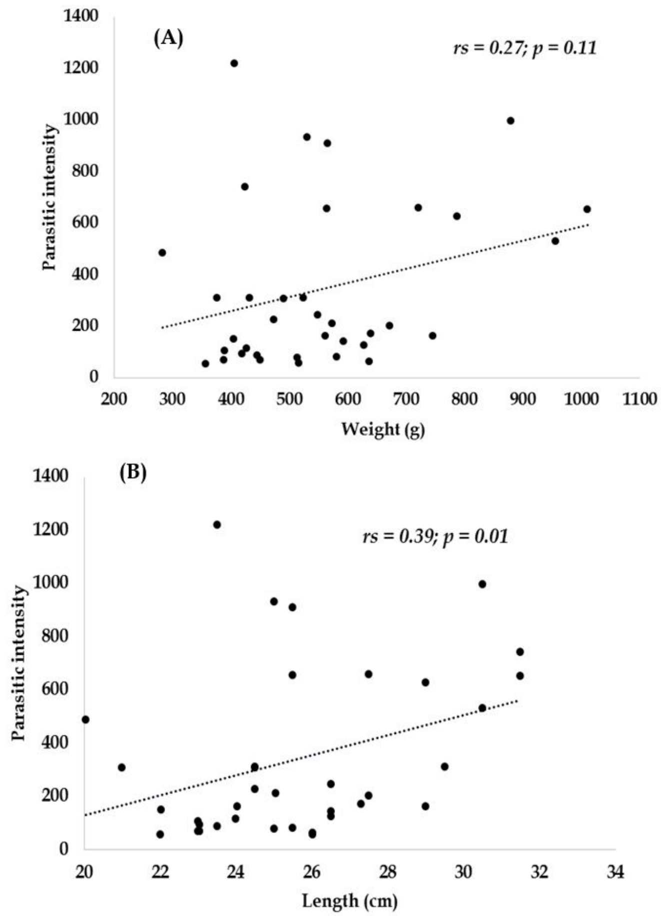

Figure 4. Correlation between acanthocephalan parasite (*Neoechinorhynchus buttnerae*) intensity and fish weight (g) (A) and standard lenght (cm) (B). Data obtained from 36 tambaqui (*Colossoma macropomum*) on two fish farms in Rio Preto da Eva, AM.

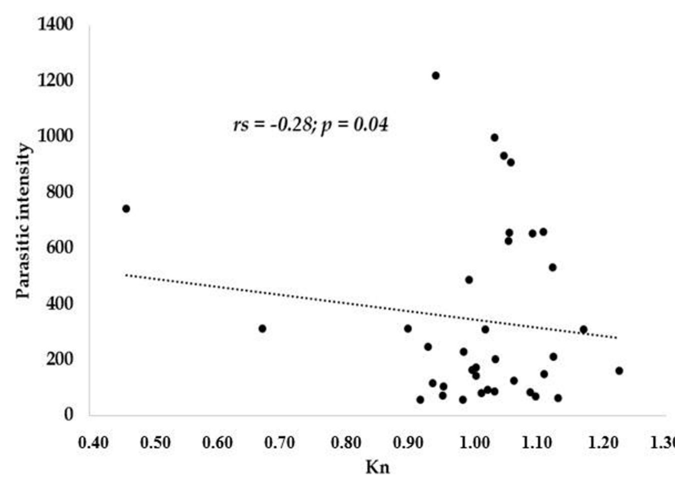

Figure 5. Correlation between acanthocephalan parasite (*Neoechinorhynchus buttnerae*) intensity and relative condition factor of tambaqui (*Colossoma macropomum*). Data obtained from 36 fish on two fish farms in Rio Preto da Eva, AM.

in other studies (Malta et al., 2001; Silva-Gomes et al., 2017; Lourenço et al., 2017; Pereira and Morey, 2018) and similar to that found by Jerônimo et al. (2017). With a mean intensity of 125.26, Malta et al. (2001) recorded the death of tambaqui weighing 162.68 to 299.64 g from *N. buttnerae* infection, which occurred by partial or total occlusion of the intestinal tract, which hindered the absorption capacity of the fish. No recent infected tambaqui deaths have been reported although the infection is known to damage their physiological function (Jerônimo et al., 2017). Tambaqui weighing between 30 g and 2 kg, with mean intensity of 476.8 in Amazonas and 262.7 in Rondônia, showed physiological changes such as cachexia and thickening of the intestinal wall, associated with intestinal secretion of yellowish catarrhal-like mucous (Jerônimo et al., 2017). Matos et al. (2017) observed marked morphological and immunological changes in tambaqui with *N. buttnerae* such as hypertrophy and hyperplasia of goblet cells, leukocyte infiltration in the intestinal submucosa, thickening of the muscle layer and increased production of cellular acidic glycoconjugates. Silva-Gomes et al. (2017) observed a more than 200% decline in tambaqui growth with acanthocephalan mean intensity between 103.25 and 152.5, which, despite being lower than that reported here, reduced gross production income. As such, despite the differences in host size, historical records indicate a long period without fish death from *N. buttnerae* infection. No fish deaths were observed in the present study, but the high parasitic intensity likely affected fish performance, as reported by Silva-Gomes et al. (2017).

The condition factor and the weight to length ratio of fish are quantitative fish health indicators (Marinho et al., 2013; Silva et al., 2013). In the present study, the weight to length ratio indicated the isometric growth of the fish. However, it was observed that the increase in parasite intensity likely affected negatively the condition factor of tambaquis. Similarly, Silva-Gomes et al. (2017) comparing the production indexes of infected and non-infected tambaqui farms, found that *N. buttnerae* infection decreased the condition factor. Jerônimo et al. (2017) found that infected fish developed cachexy, that is, impairment of the condition factor. In the present study, the parasite intensity had correlation with fish length, result that corroborates the Lourenço et al. (2017) findings with similar mean intensity.

Ostracod occurrence in the gastrointestinal tract of fish corroborates Lourenço et al. (2018), who found that the ostracod *Cypridopsis vidua* was an intermediate host in the *N. buttnerae* life cycle. After ingesting *N. buttnerae* eggs, *C. vidua* hosts the parasite for 29 days, until it develops to the cysticum stage, which is infectious to tambaqui (Lourenço et al., 2018). Ostracods were also recorded as intermediate hosts of *Neoechinorhynchus* gender such as *Cypridopsis helvetica*, *C. vidua* e *Cypria turneri* to acanthocephalan *N. cristatus, N. saginatus* and *N. rutili*, respectively (Merritt and Pratt, 1964; Uglem and Larson, 1969; Uglem, 1972). Ostracods also are intermediate hosts of *Anguillicola crassus*, a nematode of the freshwater eel *Anguilla spp.*, although only 14.2% of the ostracod *Physocypria nipponica* were found with third stage *A. crassus* larvae (Moravec et al., 2005). Crustaceans in general are listed as intermediate hosts of acanthocephalans (Santos et al., 2013).

Adopting adequate prophylaxis measures to prevent *N. buttnerae* dissemination is important given the increasing occurrence of acanthocephalan parasites on fish farms in Northern Brazil, particularly in the states of Amazonas, Rondônia and Amapá (Malta et al., 2001; Silva et al., 2013; Chagas et al., 2015; Dias et al., 2015; Jerônimo et al., 2017; Lourenço et al., 2017; Matos et al., 2017), and more recently Roraima (Pereira and Morey, 2018). These measures include controlling the water supply, disinfecting ponds and equipment to reduce or eliminate potential intermediate hosts, and instituting quarantine protocols to avoid contamination of parasite-free areas (Chagas et al., 2015).

CONCLUSIONS

The occurrence of *Neoechinorhynchus buttnerae* in tambaqui fish farms was recorded. The prevalence of acanthocephalan in tambaqui from the three fish farms in the state of Amazonas was 68.5%, with intensity of 1 to 1,219 parasites per fish and total count of 12,279 specimens. The high *N. buttnerae* intensity in the two fish farms likely affects the physiological condition of tambaqui, as suggested by the negative correlation between parasitic intensity and condition factor. In conclusion, adequate prophylaxis measures are recommended to prevent *N. buttnerae* infection on fish farms.

ACKNOWLEDGEMENTS

The authors thank Pollyana Alves de Araújo for recording the images of the ostracods.

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