Received: 19 September 2018 | Revised: 7 January 2019 | Accepted: 8 January 2019

DOI: 10.1111/jfd.12980

SHORT COMMUNICATION

WILEY Journal of Fish Diseases

Avermectins, praziquantel and levamisole have in vitro efficacy against *Neoechinorhynchus buttnerae* (Neoechinorhynchidae) in *Colossoma macropomum*: A Serrasalmidae from the Amazon

Lígia C. D. Oliveira[1] | Cláudia Majolo[2] | Franmir R. Brandão[3] | Caio Francisco S. Farias[4] | Maria Inês B. Oliveira[3] | Welliton B. Santos[5] | Patrícia C. Monteiro[3] | Maria Juliete S. Rocha[3] | Edsandra C. Chagas[2,3] | Marcos Tavares-Dias[1,6]

[1]Postgraduate Program on Tropical Biodiversity (PPGBio), Universidade Federal do Amapá (UNIFAP), Macapá, Brazil

[2]Embrapa Amazônia Ocidental, Manaus, Brazil

[3]Postgraduate Program on Fishery Sciences in the Tropics, Universidade Federal do Amazonas (UFAM), Manaus, Brazil

[4]Uninorte Laureate International Universities, Manaus, Brazil

[5]Universidade Nilton Lins, Manaus, Brazil

[6]Embrapa Amapá, Macapá, Brazil

Correspondence
Marcos Tavares-Dias, Embrapa Amapá, Macapáp, Brazil.
Email: marcos.tavares@embrapa.br

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 303013/2015-0

KEYWORDS: acanthocephalan, anthelminthics, infection, treatment

1 | INTRODUCTION

Neoechinorhynchus buttnerae Golvan, 1956 is an acanthocephalan of the family Neoechinorhynchidae for which the ostracod *Cypridopsis vidua* (Müller, 1776) Brady, 1867 is the intermediate host (Lourenço, Morey, Pereira, & Malta, 2018). This endoparasite causes great problems in aquaculture production of *Colossoma macropomum* Cuvier, 1818 from the Brazilian Amazon, where it infects 100% of the fish (Jerônimo et al., 2017; Lourenço, Morey, Pereira, & Malta, 2017; Matos, Oliveira, Gomes, & Silva, 2017), affecting the intestinal tissue of hosts and causing impairments of nutrient uptake (Matos et al., 2017) and leading to great economic losses of 100% of production (Silva-Gomes et al., 2017). However, this helminth endoparasite has been often neglected as causative agents of *C. macropomum* diseases. Therefore, as this acanthocephalan may have detrimental consequences for health of this farmed fish (Jerônimo et al., 2017; Lourenço et al., 2017; Matos et al., 2017), it is important to implement strategies to control them.

Anthelminthic drugs such as avermectins (ivermectin and emamectin benzoate) and benzimidazoles (albendazole, mebendazole and fenbendazole) have been used to treat parasitic infections by monogeneans, nematodes, digeneans and cestodes in different fish species (Collymore et al., 2014; Hardy-Smith et al., 2012; Shinn & Bron, 2012). Despite continuing need to expand the use of strategies for integrated management of parasite control among farmed fish (Shinn & Bron, 2012), treatment with anthelminthic drugs is a tool that has been little tested for controlling infections by acanthocephalan species. Levamisole, loperamide and praziquantel have been recommended for in vitro control of the acanthocephalans *Neoechinorhynchus rutili* Müller, 1780 and *Echinorhynchus truttae* Schrank, 1788 in *Oncorhynchus mykiss* Walbaum, 1792 (Taraschewski, Mehlhorn, & Raether, 1990). Praziquantel has also shown potential as an oral treatment against the acanthocephalan *Pomphorhynchus laevis* Müller, 1776 in *Barbus barbus* Linnaeus, 1758 (Zuskova et al., 2018). The number of antiparasitic drugs that are efficacious, consistent and safe and which can be used across a wide range of environmental conditions is very limited. Since no other suitable chemotherapeutic treatments for acanthocephalan infections in fish are available, the aim of this study was to investigate the efficacy, in vitro, of albendazole, levamisole, fenbendazole, mebendazole, praziquantel, ivermectin, loperamide and emamectin benzoate against *N. buttnerae* in *C. macropomum*.

TABLE 1 Efficacy of different concentrations of the anthelminthics in *Neoechinorhynchus buttnerae* de *Colossoma macropomum* in different time of exposure

Time	Control of DMSO (ml)	Live parasites	Dead parasites	EC_{50} (mg/L)
6 hr	0.45	30	0	–
24 hr	0.45	29	0.3 ± 0.5	
Time	**Control of Tween 80 (ml)**	**Live parasites**	**Dead parasites**	
6 hr	1.0	30	0	–
24 hr	1.0	30	0	
Time	**Control of distillated water (ml)**	**Live parasites**	**Dead parasites**	
6 hr	1.0	30	0	–
24 hr	1.0	28	0.7 ± 1.1	
Time	**Emamectin benzoate (mg/L)**	**Live parasites**	**Dead parasites**	**EC_{50} (mg/L)**
6 hr	12.5	30	0	–
	25.0	30	0	
	50.0	30	0	
	100	30	0	
	200	26	1.3 ± 0.6	
	300	30	0	
	400	30	0	
	500	30	0	
	600	29	0.3 ± 0.6	
24 hr	12.5	30	0	61.8
	25.0	25	1.7 ± 0.6	
	50.0	26	1.3 ± 1.1	
	100	1	9.7 ± 0.6	
	200	0	10.0 ± 0	
	300	1	9.7 ± 0.6	
	400	1	9.7 ± 0.6	
	500	4	8.7 ± 1.5	
	600	1	9.7 ± 0.6	
Time	**Ivermectin (mg/L)**	**Live parasites**	**Dead parasites**	**EC_{50}**
6 hr	1,000	30	0	–
	1,500	30	0	
	2,000	30	0	
	2,500	30	0	
	3,000	30	0	
	3,500	30	0	
	4,000	30	0	
	4,500	30	0	
	5,000	30	0	

(*Continues*)

TABLE 1 (Continued)

Time	Ivermectin (mg/L)	Live parasites	Dead parasites	EC_{50}
24 hr	1,000	0	0	2,900
	1,500	28	0.7 ± 1.1	
	2,000	26	1.3 ± 0.6	
	2,500	27	1.0 ± 1.0	
	3,000	13	5.7 ± 0.6	
	3,500	8	7.3 ± 0.6	
	4,000	7	7.7 ± 4.0	
	4,500	0	10.0 ± 0	
	5,000	0	10.0 ± 0	
Time	**Levamisole (mg/L)**	**Live parasites**	**Dead parasites**	**EC_{50}**
6 hr	50	30	0	–
	100	19	3.7 ± 1.1	
	150	25	1.7 ± 0.6	
	200	19	3.7 ± 1.5	
	250	20	3.3 ± 1.5	
	300	18	4.0 ± 1.0	
	350	11	6.3 ± 1.5	
	400	8	7.3 ± 0.6	
	450	7	7.7 ± 2.1	
24 hr	50	20	3.3 ± 1.5	73.3
	100	9	7.0 ± 1.0	
	150	11	6.3 ± 1.1	
	200	11	6.3 ± 1.1	
	250	9	7.0 ± 1.0	
	300	0	10.0 ± 0	
	350	0	10.0 ± 0	
	400	0	10.0 ± 0	
	450	0	10.0 ± 0	
Time	**Praziquantel (mg/L)**	**Live parasites**	**Dead parasites**	**EC_{50}**
6 hr	750	30	0	–
	1,000	30	0	
	1,250	30	0	
	1,500	30	0	
	1,750	30	0	
	2,000	30	0	
	2,250	29	0.3 ± 0.6	
	2,500	14	5.3 ± 0.6	
	2,750	0	10.0 ± 0	

(*Continues*)

TABLE 1 (Continued)

Time	Praziquantel (mg/L)	Live parasites	Dead parasites	EC_{50}
24 hr	750	30	0	1,900
	1,000	30	0	
	1,250	30	0	
	1,500	29	0.3 ± 0.6	
	1,750	19	3.7 ± 2.5	
	2,000	16	4.7 ± 1.1	
	2,250	5	8.3 ± 1.5	
	2,500	0	10.0 ± 0	
	2,750	0	10.0 ± 0	
Time	**Albendazole (mg/L)**	**Live parasites**	**Dead parasites**	**EC_{50}**
6 hr	250	30	0	
	500	30	0	
	750	30	0	
	1,000	30	0	
	1,250	30	0	
	1,500	30	0	
	1,750	30	0	
	2,000	30	0	
	2,250	30	0	
24 hr	250	30	0	–
	500	30	0	
	750	30	0	
	1,000	29	0.3 ± 0.6	
	1,250	29	0.3 ± 0.6	
	1,500	28	0.7 ± 0.6	
	1,750	29	0.3 ± 0.6	
	2,000	30	0	
	2,250	27	1.0 ± 1.0	
Time	**Mebendazole (mg/L)**	**Live parasites**	**Dead parasites**	**EC_{50}**
6 hr	100	30	0	–
	150	30	0	
	200	30	0	
	250	30	0	
	300	30	0	
	350	30	0	
	400	30	0	
	450	30	0	
	500	30	0	

(*Continues*)

TABLE 1 (Continued)

Time	Mebendazole (mg/L)	Live parasites	Dead parasites	EC_{50}
24 hr	100	30	0	–
	150	29	0.3 ± 0.6	
	200	30	0	
	250	29	0.3 ± 0.6	
	300	28	0.7 ± 1.1	
	350	30	0	
	400	30	0	
	450	29	0.3 ± 0.6	
	500	29	0.3 ± 0.6	
Time	**Fenbendazole (mg/L)**	**Live parasites**	**Dead parasites**	
6 hr	0.2	30	0	
	0.3	30	0	
	0.4	30	0	
	0.5	30	0	
	0.6	30	0	
	0.7	30	0	
	0.8	30	0	
	0.9	30	0	
	1.0	30	0	
24 hr	0.2	30	0	
	0.3	30	0	
	0.4	30	0	
	0.5	30	0	
	0.6	30	0	
	0.7	30	0	
	0.8	30	0	
	0.9	30	0	
	1.0	30	0	
Time	**Loperamide (mg/L)**	**Live parasites**	**Dead parasites**	
6 hr	20	30	0	
	40	30	0	
	60	30	0	
	80	30	0	
	100	30	0	
	120	30	0	
	140	30	0	
	160	30	0	
	180	30	0	
24 hr	20	29	0.3 ± 0.6	
	40	26	1.3 ± 1.1	
	60	29	0.3 ± 0.6	
	80	30	0	
	100	30	0	
	120	29	0.3 ± 0.6	
	140	29	0.3 ± 0.6	
	160	28	0.7 ± 1.1	
	180	26	1.3 ± 1.5	

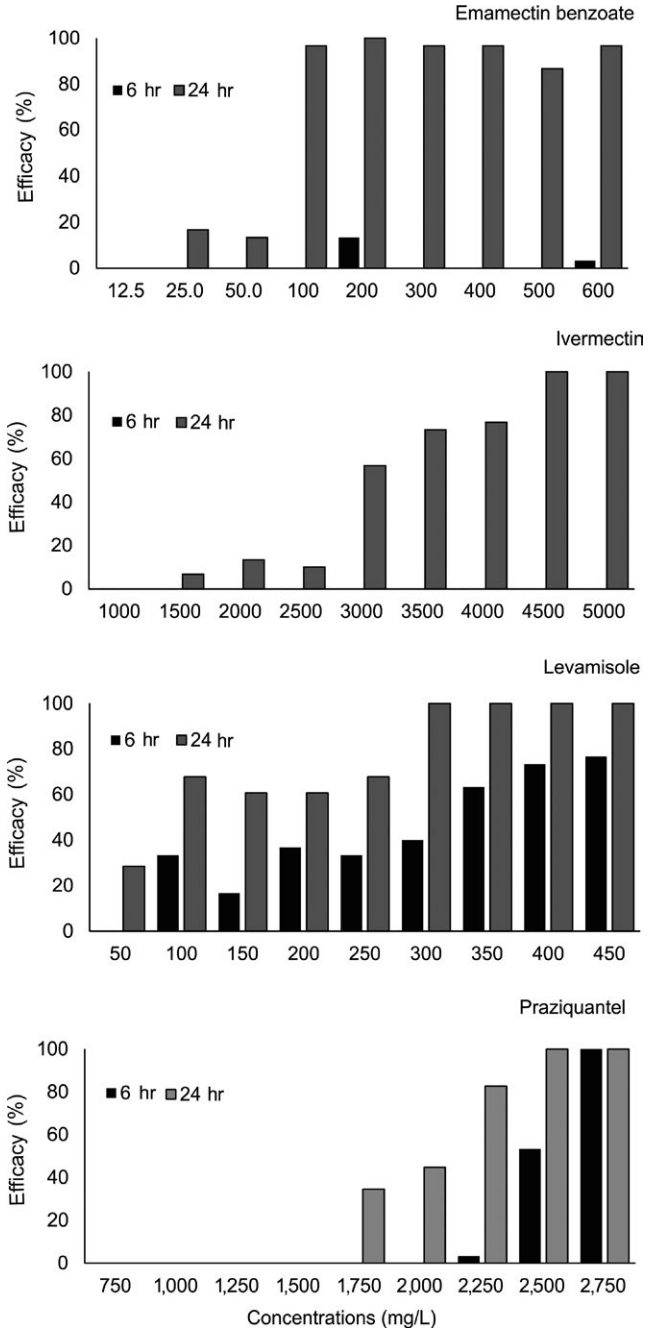

FIGURE 1 Efficacy of the anthelminthics against *Neoechinorhynchus buttnerae* of *Colossoma macropomum* in 6 and 24 hr of exposure

For these trials in vitro, we used eight anthelmintic drugs and forty juveniles of *C. macropomum* (100.0 ± 20.0 g) obtained from a commercial fish farm in Manaus, Amazonas state (Brazil). All the fish had the intestine naturally infected by *N. buttnerae*. Parasites obtained were stored in Eagle's minimal essential at room temperature (25.0 ± 1.0°C) in combination with different concentrations of albendazole, levamisole, mebendazole, fenbendazole, emamectin benzoate, ivermectin, loperamide and praziquantel. The efficacy of concentrations of each anthelminthic drug was tested using three replicates per treatment and 10 adults of *N. buttnerae* in each replicate. The endoparasites were considered dead when had totally lost mobility. The effective concentration ($EC_{50\text{-}24\ hr}$) and 95% confidence interval were determined for each anthelminthic drug, using the software Trimmed Spearman–Karber method (Hamilton, Russo, & Thurston, 1977).

Only levamisole, praziquantel, emamectin benzoate and ivermectin caused mortality of *N. buttnerae*. The EC_{50} was also determined for the anthelminthic drugs (Table 1). For levamisole, the 95% confidence interval was 49.3–108.8 mg/L; for praziquantel, 1,800–2,000 mg/L; for emamectin benzoate, 52.7–72.4 mg/L and for ivermectin, 2,700–3,100 mg/L. However, the effects of emamectin benzoate and levamisole were not dependent on concentration. Praziquantel also presented high efficacy in vitro against *N. rutili* and *E. truttae* in *O. mykiss* (Taraschewski et al., 1990). Low concentrations of ivermectin in the diet of *Danio rerio* Hamilton, 1822 had little efficacy (24%) against the nematodes *Pseudocapillaria tomentosa* Dujardin, 1843, while emamectin benzoate had 90% efficacy (Collymore et al., 2014). Despite this positive effect of emamectin benzoate, its use is restricted for agriculture in most of the states in Brazil, thus limiting its use in aquaculture, which has not legal approbation.

The concentrations of emamectin benzoate and ivermectin that were used showed high in vitro efficacy against *N. buttnerae* of *C. macropomum* after 24 hr of exposure, but the efficacy of praziquantel and levamisole started at 6 hr of in vitro exposure (Figure 1). Levamisole showed low efficacy against *N. buttnerae* at 6 hr of exposure. Taraschewski et al. (1990) reported that levamisole presented efficacy against *N. rutili* and *E. truttae* after 1 hr of exposure. No concentration of albendazole, fenbendazole, mebendazole or loperamide showed in vitro efficacy against *N. buttnerae* of *C. macropomum*, since they are poorly soluble in water, resulting therefore in variable and incomplete bioavailability (Ghanbarzadeh et al., 2016; Ibrahim & Al-Anazi, 2013; Pacheco et al., 2018; Romero, Navarro, Sanchez, & Valero, 2014; Taraschewski et al., 1990). Similar inefficacy of albendazole, fenbendazole and mebendazole has also been reported in relation to species of acanthocephalans, nematodes, monogeneans and trematodes parasitizing different fish species (Reimschuessel, Gieseker, & Poynton, 2011; Romero et al., 2014; Taraschewski et al., 1990; Zhang et al., 2014).

Loperamide did not show efficacy against *N. buttnerae* in *C. macropomum* in the present study, contrary to our expectations. However, loperamide was effective against *N. rutili* and *E. truttae*, because it causes contractions and necrosis in acanthocephalans, along with mitochondrial swelling that may result in expulsion of the cytoplasm out of the tegument pores (Taraschewski et al., 1990).

The acanthocephalan *N. buttnerae* of *C. macropomum* presented slow movements only after 6 hr of exposure to the highest concentrations of emamectin benzoate, ivermectin and praziquantel. Reimschuessel et al. (2011) also reported that there was a tardy response after in vitro exposure of *Acolpenteron ureteroecetes* Fischthal & Allison, 1940 to emamectin benzoate and ivermectin. However, Zhang et al. (2014) reported an immediate change in the movement

of *Dactylogyrus vastator* Nybelin, 1924 after exposure to praziquantel. Praziquantel has a mechanism of action based on depolarization of the tegument of helminth parasites, thus leading to entry of calcium into the cells, hence causing immobilization, spasmodic contractions and paralysis (Hardy-Smith et al., 2012; Sitja-Bobadilla, Felipe, & Alvarez-Pellitero, 2006; Thomas & Timson, 2018; Williams, Ernst, Chambers, & Whittington, 2007).

To conclude, our results showed that 100 mg/L of emamectin benzoate, 300 mg/L of levamisole, 4,500 mg/L of ivermectin and 2,500 mg/L of praziquantel had the highest efficacy against *N. buttnerae*. These concentrations can be recommended for use in the diet of *C macropomum* after previous evaluation of the toxicity. Therefore, these first results represent a great step forward regarding integrated management for controlling *N. buttnerae* in farmed *C. macropomum*.

ACKNOWLEDGEMENTS

The authors also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for the productivity research grant awarded to M. Tavares-Dias (# 303013/2015-0) and Acanto project from Embrapa.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL DISCLOSURES

This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA), and authorization from Ethics Committee in the Use of Animal of the Embrapa Amazônia Ocidental (N° 02/2017) was carried out.

ORCID

Marcos Tavares-Dias https://orcid.org/0000-0002-8376-1846

REFERENCES

Collymore, C., Watral, V., White, J. R., Colvin, M. E., Rasmussen, S., Tolwani, R. J., & Kent, M. L. (2014). Tolerance and efficacy of emamectin benzoate and ivermectin for the treatment of *Pseudocapillaria tomentosa* in laboratory Zebrafish (*Danio rerio*). *Zebrafish*, *11*, 490–497. https://doi.org/10.1089/zeb.2014.1021

Ghanbarzadeh, S., Khalili, A., Jouyban, A., Emami, S., Javadzadeh, Y., Solhi, M., & Hamishehkar, H. (2016). Dramatic improvement in dissolution rate of albendazole by a simple, one-step, industrially scalable technique. *Research in Pharmaceutical Sciences*, *11*(6), 435–444. https://doi.org/10.4103/1735-5362.194868

Hamilton, M. A., Russo, R. C., & Thurston, R. V. (1977). Trimmed spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science & Technology*, *11*, 714–719. Correction. 12: 417. https://doi.org/10.1021/es60130a004

Hardy-Smith, P., Ellis, D., Humphrey, J., Evans, M., Evans, D., Rough, K., … Nowak, B. (2012). In vitro and in vivo efficacy of anthelmintic compounds against blood fluke (*Cardicola forsteri*). *Aquaculture*, *334–337*, 39–44. https://doi.org/10.1016/j.aquaculture.2011.12.037

Ibrahim, M. A., & Al-Anazi, F. K. (2013). Enhancement of the dissolution of albendazole from pellets using MTR technique. *Saudi Pharmaceutical Journal*, *21*, 215–223. https://doi.org/10.1016/j.jsps.2012.03.001

Jerônimo, G. T., Pádua, S. B., Belo, M. A. A., Chagas, E. C., Taboga, S. R., Maciel, P. O., & Martins, M. L. (2017). *Neoechinorhynchus buttnerae* (Acanthocephala) infection in farmed *Colossoma macropomum*: a pathological approach. *Aquaculture*, *469*, 124–127. https://doi.org/10.1016/j.aquaculture.2016.11.027

Lourenço, F. S., Morey, G. A. M., Pereira, J. N., & Malta, J. C. O. (2017). Ocorrência de *Neoechinorhynchus* (*Neoechinorhynchus*) *buttnerae* Golvan, 1956 (Acanthocephala: Neoechinorhynchidae) em *Colossoma macropomum* (Cuvier, 1818) Characiformes: Serrasalmidae) provenientes de uma piscicultura da Amazônia brasileira. *Folia Amazónica*, *26*(1), 1–8. https://doi.org/10.24841/fa.v26i1.41

Lourenço, F. S., Morey, G. A. M., Pereira, J. N., & Malta, J. C. O. (2018). The development of *Neoechinorhynchus buttnerae* (Eoacanthocephala: Neoechinorhynchidae) in its intermediate host *Cypridopsis vidua* in Brazil. *Acta Parasitologica*, *63*(2), 354–359. https://doi.org/10.1515/ap-2018-0040

Matos, L. V., Oliveira, M. I. B., Gomes, A. L. S., & Silva, G. S. (2017). Morphological and histochemical changes associated with massive infection by *Neoechinorhynchus buttnerae* (Acanthocephala: Neoechinorhynchidae) in the farmed freshwater fish *Colossoma macropomum* Cuvier, 1818 from the Amazon State, Brazil. *Parasitology Research*, *116*, 1029–1037. https://doi.org/10.1007/s00436-017-5384-3

Pacheco, P. A., Rodrigues, L. N. C., Ferreira, J. F. S., Gomes, A. C. P., Veríssimo, C. J., Louvandini, H., … Katiki, L. M. (2018). Inclusion complex and nanoclusters of cyclodextrin to increase the solubility and efficacy of albendazole. *Parasitology Research*, *117*(3), 705–712. https://doi.org/10.1007/s00436-017-5740-3

Reimschuessel, R., Gieseker, C., & Poynton, S. (2011). *In vitro* effect of seven antiparasitic on *Acolpenteron ureteroecetes* (Dactylogyridae) from largemouth bass *Micropterus salmoides* (Centrarchidae). *Diseases of Aquatic Organisms*, *94*, 59–72. https://doi.org/10.3354/dao02303

Romero, M. C., Navarro, M. C., Sanchez, J. M., & Valero, A. (2014). Peppermint (*Mentha piperita*) and albendazole against anisakiasis in an animal model. *Tropical Medicine and International Health*, *19*, 1430–1436. https://doi.org/10.1111/tmi.12399

Shinn, A. P., & Bron, J. E. (2012). Considerations in the use of antiparasitic drugs in aquaculture. In B. A. Austin (Ed.), *Infectious disease in aquaculture prevention and control* (pp. 190–217). Cambridge, UK: Woodhead Publishing Limited. https://doi.org/10.1533/9780857095732.2.190

Silva-Gomes, A. L., Coelho-Filho, J. G., Viana-Silva, W., Braga-Oliveira, M. I., Bernardino, G., & Costa, J. I. (2017). The impact of *Neoechinorhynchus buttnerae* (Golvan, 1956) (Eoacanthocephala: Neochinorhynchidae) outbreaks on productive and economic performance of the tambaqui *Colossoma macropomum* (Cuvier, 1818), reared in ponds. *Latin American Journal of Aquatic Research*, *45*(2), 496–500. https://doi.org/10.3856/vol45-issue2-fulltext-25

Sitja-Bobadilla, A., Felipe, M. C., & Alvarez-Pellitero, P. (2006). In vivo and in vitro treatments against *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) parasitizing the gills of gilthead sea bream (*Sparus aurata* L.). *Aquaculture*, *261*(3), 856–864. https://doi.org/10.1016/j.aquaculture.2006.09.012

Taraschewski, H., Mehlhorn, H., & Raether, W. (1990). Loperamid, an efficacious drug against fish-pathogenic acanthocephalans. *Parasitology Research*, *76*, 619–623. https://doi.org/10.1007/BF00932573

Thomas, C. M., & Timson, D. J. (2018). The mechanism of action of praziquantel: six hypotheses. *Current Topics in Medicinal Chemistry*, *18*, 1575–1584. https://doi.org/10.2174/1568026618666181029143214

Williams, R. E., Ernst, I., Chambers, C. B., & Whittington, I. D. (2007). Efficacy of orally administered praziquantel against *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in yellowtail kingfish *Seriola lalandi*. *Diseases of Aquatic Organisms*, *77*, 199–205. https://doi.org/10.3354/dao01824

Zhang, X. P., Li, W. X., Ai, T. S., Zou, H., Wu, S. G., & Wang, G. T. (2014). The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control *Dactylogyrus vastator* (Monogenea). *Aquaculture*, *420–421*, 302–307. https://doi.org/10.1016/j.aquaculture.2013.09.022

Zuskova, E., Piackova, V., Machova, J., Chupani, L., Steinbach, C., Stara, A., & Velisek, J. (2018). Efficacy and toxicity of praziquantel in helminth-infected barbel (*Barbus barbus* L.). *Journal of Fish Diseases*, *41*(4), 643–649. https://doi.org/10.1111/jfd.12764

How to cite this article: Oliveira LCD, Majolo C, Brandão FR, et al. Avermectins, praziquantel and levamisole have in vitro efficacy against *Neoechinorhynchus buttnerae* (Neoechinorhynchidae) in *Colossoma macropomum*: A Serrasalmidae from the Amazon. *J Fish Dis*. 2019;42:765–772. https://doi.org/10.1111/jfd.12980