EFFICIENCY OF BRAZILIAN NATIVE ORNAMENTAL FISHES AS MOSQUITO LARVAE PREDATORS

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ABSTRACT

Fish are an alternative in mosquito biological control. Some species are proven to be effective in many countries, but are Brazilian native. Brazilian species may be larvivorous but this lacks information. Therefore, the widely used exotic species, *Poecilia reticulata* and *Betta splendens*, were compared with the Brazilian species *Hyphessobrycon eques* and *Pterophyllum scalare* for their efficiency as *Aedes aegypti* mosquito larvae feeders. Fifty mosquito larvae were offered to the fishes (n = 20) and after five minutes the remaining larvae were counted. *Betta splendens* ingested the largest larvae amount but when this value was divided by fish weights, *P. reticulata* was the species with the highest intake per gram. Brazilian native species did not differ from *B. splendens* in the ingestion per weight rate. Thus, it is concluded that the most efficient larvivorous fish was *P. reticulata*, but the Brazilian species showed a larvivorous potential, with fewer impacts on biodiversity.

Keywords: *Aedes aegypti*; angelfish; jewel tetra; larvivorous fish; mosquito control

EFICIÊNCIA DE PEIXES NATIVOS BRASILEIROS COMO PREDADORES DE LARVAS DE MOSQUITOS

RESUMO

Peixes são uma alternativa no controle biológico de mosquitos. Algumas espécies são comprovadamente eficientes em muitos países, porém não são nativas brasileiras. Espécies brasileiras podem ser larvófagas, porém há carência de informações. Portanto, foram comparadas as espécies exóticas geralmente utilizadas, *Poecilia reticulata* e *Betta splendens*, com as espécies brasileiras, *Hyphessobrycon eques* e *Pterophyllum scalare*, por sua eficiência como predadoras de larvas do mosquito *Aedes aegypti*. Foram oferecidas 50 larvas do mosquito para os peixes (n = 20) e, após cinco minutos, as larvas restantes foram contabilizadas. *Betta splendens* ingeriu a maior quantidade de larvas, mas quando esse valor foi dividido pelo peso dos peixes, *P. reticulata* foi a espécie com maior ingestão por grama. As espécies nativas não diferiram de *B. splendens* na taxa de ingestão por peso. Conclui-se que o peixe mais eficiente foi *P. reticulata*, mas as espécies brasileiras apresentaram potencial como larvófagas, com menores impactos na biodiversidade.

Palavras-chave: *Aedes aegypti*; acará-bandeira; mato-grosso; peixes larvófagos; controle de mosquitos

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INTRODUCTION

An efficient and responsible method to control *Aedes aegypti* is needed. The mosquito transmits zika, chikungunya and dengue, and its distribution is alarmingly increasing throughout the urbanized areas in the world (BHATT *et al.*, 2013; MESSINA *et al.*, 2015; AI *et al.*, 2016; NDEFFO-MBAH *et al.*, 2016). This is happening as a consequence of the increase of suitable places for laying their eggs (BENTLEY and DAY, 1989), its rapid growth and higher population densities (EISENSTEIN, 2016). The chemical control, despite being efficient (VAN DEN BERG *et al.*, 2012), is a weapon against the environment, because it pollutes the air and the water while suppressing non-target species and causing diseases in humans (HEMINGWAY and RANSON, 2000). This requires the search for alternative and efficient control means which has fewer impacts on the environment.

Biological control by fishes is not a new proposal, however it was forgotten during time even though its efficiency has been proven in other opportunities in several places (WU *et al.*, 1987; FLETCHER *et al.*, 1993; NAM *et al.*, 2000; LARDEUX *et al.*, 2002; MARTÍNEZ-IBARRA *et al.*, 2002). For the success of the mosquito control, the fishes used must be larvivorous, which are defined as small fish capable to easily move between thick weeds to reach the suitable places where mosquitoes lay their eggs (JOB, 1940). Other characteristics are defined, such as toleration to rough handling and transportation, short and prolific life cycle, surface carnivorous feeding habits with preference for mosquito larvae and non-impactant for the local fish community (CHANDRA *et al.*, 2008).

Some ornamental fish species are used as larvivorous species (CHAPMAN, 1974; KUMAR and HWANG, 2006; CHANDRA *et al.*, 2008). Despite their efficiency, in Brazil they are not native species, and this is considered a misguided strategy because it negatively affects native populations (AZEVEDO-SANTOS *et al.*, 2016). Thus, the main objective was to evaluate the efficiency of Brazilian native ornamental fishes as predators of *A. aegypti* larvae, comparing with two of the most used species for mosquito control.

MATERIAL AND METHODS

Animals and stock conditions

The species tested were guppy (*Poecilia reticulata*), betta (*Betta splendens*), jewel tetra (*Hyphessobrycon eques*) and angelfish (*Pterophyllum scalare*). All the species, except for the guppies, were obtained in a commercial establishment. In pilot surveys conducted in a coastal city of the State of São Paulo, in Brazil, was verified that the wild guppies had a larger size than the fish bred in captivity, which is why the wild animals were chosen for this study. They were collected in a water channel of São Vicente, São Paulo, Brazil, using baited traps to avoid physical injury to the animals. The *A. aegypti* larvae were achieved through partnership with the São Vicente Regional Service of the Endemies Control Superintendence (SUCEN SR-2). The larvae were born in laboratory conditions from an *A. aegypti* population with no contact with the natural environment. The adults laid their eggs in traps placed inside the cages, and these eggs were hatched until the larvae reached the IV instar.

The animals were stocked in three 45 L tanks (one for each species) with controlled temperature (25.0 ± 1.0 ºC), pH (6.8 ± 0.3) and dissolved oxygen (>5.00 mg L^{-1}) and fed *ad libitum* with commercial feed. The bettas, due to their aggressiveness with conspecifics (JOHNSON and JOHNSON, 1973), were stocked in individual 6 L aquaria under the same conditions as the others. All animals remained at least two weeks in stock conditions prior to the tests to exclude effects of capture or transport. The larvae were collected and immediately used in tests.

This study was approved by the UNESP IB/CLP Animal Use Ethics Committee (CEUA-UNESP/CLP) under the protocol number 24/2016.

Experimental design and procedures

Were used 20 fish of each species, randomly chosen from the stock populations, weighed them and placed individually in 6 L aquaria without visual contact with the outside, totaling 20 samples per species. The temperature was maintained constant at 25.0 ± 1.0 °C. The acclimation period occurred during 24 hours in which individuals were not fed, so that when testing started, the animals were in fasting conditions.

The test consisted in offering the animals, at once, 50 stage IV larvae of *A. aegypti* and, five minutes later, the fish were removed from the aquaria and all the remaining larvae were accounted. This period was chosen based on a pilot experiment in which the predation occurred mostly during the first five minutes after the food presentation.

Statistical analysis

After testing normality with Shapiro-Wilk test and homoscedasticity with Levene's test and having both confirmed, the amount of ingested larvae by each fish species was compared using analysis of variance (ANOVA), followed by Tukey's HSD, as well as the number of ingested larvae per gram of fish. Statistical differences were set at $p<0.05$.

RESULTS AND DISCUSSION

Siamese fighting fish ate more larvae (29.50 ± 11.24; number of larvae ± standard deviation) than all other species (Figure 1; $p<0.05$). Angelfish ingested 13.84 ± 13.02 larvae; guppies ate 5.78 ± 4.63 larvae and jewel tetras consumed 2.39 ± 3.18 larvae.

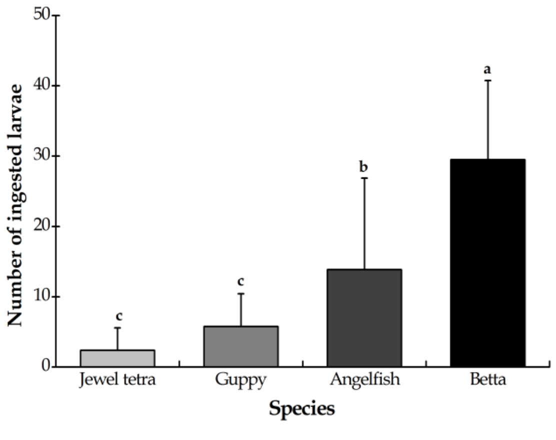

Figure 1. Number of *Aedes aegypti* larvae ingested by Brazilian native (jewel tetra – *Hyphessobrycon eques* and angelfish – *Pterophyllum scalare*) and non-native (Guppy – *Poecilia reticulata* and Betta – *Betta splendens*) ornamental fish species. Different letters indicate statistical differences between species ($p<0.05$).

However, when the values are considered proportionally to the fish weights, the most efficient species in larvae ingestion was the guppy (Figure 2; $p<0.05$), ingesting 2.05 ± 1.10 larvae per gram. The other species did not show statistical differences; betta ingested 1.94 ± 0.53 larvae per gram; angelfish, 1.25 ± 0.86; and jewel tetra, 0.85 ± 0.95 larvae per gram.

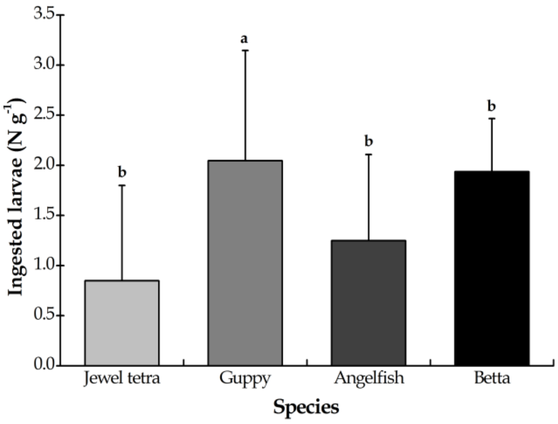

Figure 2. Amount of *Aedes aegypti* larvae ingested by the weights of Brazilian native (jewel tetra – *Hyphessobrycon eques* and angelfish – *Pterophyllum scalare*) and non-native (Guppy – *Poecilia reticulata* and Betta – *Betta splendens*) ornamental fish species (N g^{-1}). Different letters indicate statistical differences ($p<0.05$).

The Brazilian native ornamental fish species are suited to be used in mosquito control programs. Both *H. eques* and *P. scalare* were efficient, and their use would not cause great damages to local fish populations. Even though they do not consume the highest amounts (Figure 1), the proportions of larvae ingested by their weights are similar of *B. splendens* (Figure 2). In this case, the native species have an advantage over the Siamese fighting fish, because they group in shoals (CARVALHO and DEL-CLARO, 2004; GÓMEZ-LAPLAZA and GERLAI, 2011) while *B. splendens* tends to be aggressive with other fishes (BRADDOCK and BRADDOCK, 1955; JOHNSON and JOHNSON, 1973). Consequently, a large group can eat a higher amount of larvae compared with a single individual.

The species with the greatest efficiency to capture mosquito larvae is *P. reticulata* because it showed the highest larvae ingestion relatively to the body weight and also groups in shoals (SEGHERS, 1974). The guppy is less effective than the most widely used species for mosquito control, *Gambusia affinis* (CHANDRA *et al*., 2008; SARWAR, 2015), but the latter does not breed well in tropical environments (SARWAR, 2015). Nevertheless, individual guppies are able to consume up to 102 larvae per day, and females are more efficient than males (SENG *et al*., 2008). The use of non-native species to control another invasive species such as *A. aegypti* is not recommended by the World Health Organization (WORLD HEALTH ORGANIZATION, 2003). This is considered to be a threat to native biodiversity by reducing or negatively affecting local populations (AZEVEDO-SANTOS *et al*., 2016), even when they are efficient mosquito larvae predators (EL-SABAAWI *et al*., 2016). In some places native fishes are already proven to be more effective than introduced species , as in Australia (RUSSELL *et al*., 2001; LAWRENCE *et al*., 2016), India (RAO *et al*., 2015), Iran (SHAHI *et al*., 2015) and Mexico (MARTÍNEZ-IBARRA *et al*., 2002).

Therefore, although the use of non-native fishes in mosquito control programs in Brazil is succeeded (LIMA *et al*., 2010), the use of jewel tetra and angelfish as mosquito larvae controllers should and must be encouraged, since they can be effective mosquito larvae predators and are Brazilian native species. Another alternative to reduce the number of introduced fishes into the environment is the integrated use of different methods along with the fishes (ARAÚJO *et al*., 2015; SARWAR, 2015; CAIXETA *et al*., 2016). Some chemicals already used for controlling *A. aegypti* in low and environmentally safe concentrations tend to reduce the mosquito infestation, such as pyriproxyfen (CAIXETA *et al*., 2016) and spinosad (PEREIRA *et al*., 2016). Moreover, reduced doses of silver nanoparticles synthesized with *Sonneratia alba* extracts can boost the larvae predation by *P. reticulata* (MURUGAN *et al*., 2017). Future studies are needed to assess the Brazilian native species sensitivity to chemical larvicides and support their use in integrated programs.

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REFERENCES

AI, J.; ZHANG, Y.; ZHANG, W. 2016 Zika virus outbreak: "a perfect storm". *Emerging Microbes and Infections*, *5*(21): 1-3.

ARAÚJO, H.; CARVALHO, D.; IOSHINO, R.; COSTA-DA-SILVA, A.; CAPURRO, M. 2015 *Aedes aegypti* control strategies in Brazil: Incorporation of new technologies to overcome the persistence of dengue epidemics. *Insects*, *6*(2): 576-594.

AZEVEDO-SANTOS, V.M.; VITULE, J.R.S.; GARCIA-BERTHOU, E.; PELICICE, F.M.; SIMBERLOFF, D. 2016 Misguided strategy for mosquito control. *Science*, *351*(6274): 675-675.

BENTLEY, M.D.; DAY, J.F. 1989 Chemical ecology and behavioral aspects of mosquito oviposition. *Annual Reviews of Entomology*, *34:* 401–421.

BHATT, S.; GETHING, P.W.; BRADY, O.J.; MESSINA, J.P.; FARLOW, A.W.; MOYES, C.L.; DRAKE, J.M.; BROWNSTEIN, J.S.; HOEN, A.G.; SANKOH, O.; MYERS, M.F.; GEORGE, D.B.; JAENISCH, T.; WINT, G.R.W.; SIMMONS, C.P.; SCOTT, T.W.; FARRAR, J.J.; HAY, S.I. 2013 The global distribution and burden of dengue. *Nature*, *496*(7446): 504-507.

BRADDOCK, J.C.; BRADDOCK, Z.I. 1955 Aggressive behavior among females of the Siamese Fighting fish, *Betta splendens*. *Physiological Zoology*, *28*(2): 152-172.

CAIXETA, E.S.; SILVA, C.F.; SANTOS, V.S.V.; OLEGÁRIO DE CAMPOS JÚNIOR, E.; PEREIRA, B.B. 2016 Ecotoxicological assessment of pyriproxyfen under environmentally realistic exposure conditions of integrated vector management for *Aedes aegypti* control in Brazil. *Journal of Toxicology and Environmental Health, Part A*, *79*(18): 799-803.

CARVALHO, L.N.; DEL-CLARO, K. 2004 Effects of predation pressure on the feeding behaviour of the serpa tetra *Hyphessobrycon eques* (Ostariophysi, Characidae). *Acta Ethologica, 7*(2): 89-93.

CHANDRA, G.; BHATTACHARJEE, I.; CHATTERJEE, S.N.; GHOSH, A. 2008 Mosquito control by larvivorous fish. *The Indian journal of medical research*, *127*(1): 13-27.

CHAPMAN, H.C. 1974 Biological control of mosquito larvae. *Annual review of entomology*, *19*(172): 33-59.

EISENSTEIN, M. 2016 Disease: poverty and pathogens. *Nature*, *531*(7594): S61-S63.

EL-SABAAWI, R.W.; FRAUENDORF, T.C.; MARQUES, P.S.; MACKENZIE, R.A.; MANNA, L.R.; MAZZONI, R.; PHILLIP, D.A.T.; WARBANSKI, M.L.; ZANDONÀ, E. 2016 Biodiversity and ecosystem risks arising from using guppies to control mosquitoes. *Biology Letters*, *12*(10): 20160590.

FLETCHER, M.; TEKLCHAIMANOT, A.; YEMANE, G.; KASSAHUM, A.; KIDANE, G.; BEYENE, Y. 1993 Prospects for the use of larvivorous fish for malaria control in Ethiopia: search for indigenous species and evaluation of their feeding capacity for mosquito larvae. *Journal of Tropical Medicine and Hygiene*, *96*(1): 12-21.

GÓMEZ-LAPLAZA, L.M.; GERLAI, R. 2011 Can angelfish (*Pterophyllum scalare*) count? Discrimination between different shoal sizes follows Weber's law. *Animal Cognition*, *14*(1): 1-9.

HEMINGWAY, J.; RANSON, H. 2000 Insecticide Resistance in Insect Vectors of Human Disease. *Annual Review of Entomology*, *45*(1): 371-391.

JOB, T. 1940 An investigation on the nutrition of the perches of the Madras coast. *Records of the Indian Museum*, *42*: 289-364.

JOHNSON, R.N.; JOHNSON, L.D. 1973 Intra- and interspecific social and aggressive behaviour in the siamese fighting fish, *Betta splendens*. *Animal Behaviour*, *21*(4): 665-672.

KUMAR, R.; HWANG, J.-S. 2006 Larvicidal efficiency of aquatic predators: A perspective for mosquito biocontrol. *Zoological Studies*, *45*(4): 447-466.

LARDEUX, F.; SECHAN, Y.; LONCKE, S.; DEPARIS, X.; CHEFFORT, J.; FAARUIA, M. 2002 Integrated Control of Peridomestic Larval Habitats of *Aedes* and *Culex* Mosquitoes (Diptera: Culicidae) in Atoll Villages of French Polynesia. *Journal of Medical Entomology*, *39*(3): 493-498.

LAWRENCE, C.; RUTHERFORD, N.; HAMILTON, R.; MEREDITH, D. 2016 Experimental evidence indicates that native freshwater fish outperform introduced *Gambusia* in mosquito suppression when water temperature is below 25°C. *Hydrobiologia*, *766*(1): 357-364.

LIMA, J.W. de O.; CAVALCANTI, L.P. de G.; PONTES, R.J.S.; HEUKELBACH, J. 2010 Survival of *Betta splendens* fish (Regan, 1910) in domestic water containers and its effectiveness in controlling *Aedes aegypti* larvae (Linnaeus, 1762) in Northeast Brazil. *Tropical Medicine & International Health*, *15*(12): 1525-1532.

MARTÍNEZ-IBARRA, J.A.; GUILLÉN, Y.G.; ARREDONDO-JIMÉNEZ, J.; RODRÍGUEZ-LÓPEZ, M. 2002 Indigenous fish species for the control of *Aedes aegypti* in water storage tanks in Southern México. *BioControl*, *47*(4): 481-486.

MESSINA, J.P.; BRADY, O.J.; PIGOTT, D.M.; GOLDING, N.; KRAEMER, M.U.G.; SCOTT, T.W.; WINT, G.R.W.; SMITH, D.L.; HAY, S.I. 2015 The many projected futures of dengue. *Nature Reviews Microbiology*, *13*(4): 230-239.

MURUGAN, K.; DINESH, D.; PAULPANDI, M.; SUBRAMANIAM, J.; RAKESH, R.; AMUTHAVALLI, P.; PANNEERSELVAM, C.; SURESH, U.; VADIVALAGAN, C.; ALSALHI, M. S.; DEVANESAN, S.; WEI, H.; HIGUCHI, A.; NICOLETTI, M.; CANALE, A.; BENELLI, G. 2017 Mangrove helps: *Sonneratia alba*-Synthesized silver nanoparticles magnify guppy fish predation against *Aedes aegypti* young instars and down-regulate the expression of envelope (E) gene in dengue virus (Serotype DEN-2). *Journal of Cluster Science*, *28*(1): 437-461.

NAM, V.S.; YEN, N.T.; HOLYNSKA, M.; REID, J.W.; KAY, B.H. 2000 National progress in dengue vector control in Vietnam: survey for *Mesocyclops* (Copepoda), *Micronecta* (Corixidae), and fish as biological control agents. *The American Journal of Tropical Medicine and Hygiene*, *62*(1): 5-10.

NDEFFO-MBAH, M.L.; DURHAM, D.P.; SKRIP, L.A.; NSOESIE, E.O.; BROWNSTEIN, J.S.; FISH, D.; GALVANI, A.P. 2016 Evaluating the effectiveness of localized control strategies to curtail chikungunya. *Scientific Reports*, *6*(23997): 1-9.

PEREIRA, B.B.; CAIXETA, E.S.; FREITAS, P.C.; SANTOS, V.S.V.; LIMONGI, J.E.; DE CAMPOS JÚNIOR, E.O.; CAMPOS, C.F.; SOUTO, H.N.;

RODRIGUES, T.S.; MORELLI, S. 2016 Toxicological assessment of spinosad: Implications for integrated control of *Aedes aegypti* using larvicides and larvivorous fish. *Journal of Toxicology and Environmental Health, Part A*, *79*(12): 477-481.

RAO, J.C.S.; RAO, K.G.; RAJU, C.S.; SIMHACHALAM, G. 2015 Larvicidal efficacy of four indigenous ornamental fish species of lake kolleru, India. *Journal of Biodiversity and Environmental Sciences*, *7*(1): 164-172.

RUSSELL, B.M.; WANG, J.; WILLIAMS, Y.; HEARNDEN, M.N.; KAY, B.H. 2001 Laboratory evaluation of two native fishes from tropical North Queensland as biological control agents of subterranean *Aedes aegypti*. *Journal of the American Mosquito Control Association*, *17*(2): 124-126.

SARWAR, M. 2015 Control of Dengue Carrier *Aedes* Mosquitoes (Diptera : Culicidae) larvae by larvivorous fishes and putting it into practice within water bodies. *International Journal of Preventive Medicine Research*, *1*(4): 232-237.

SEGHERS, B. H. 1974 Schooling Behavior in the Guppy (*Poecilia reticulata*): an evolutionary response to predation. *Evolution*, *28*(3): 486-489.

SENG, C.M.; SETHA, T.; NEALON, J.; SOCHEAT, D.; CHANTHA, N.; NATHAN, M.B. 2008 Community-based use of the larvivorous fish *Poecilia reticulata* to control the dengue vector *Aedes aegypti* in domestic water storage containers in rural Cambodia. *Journal of Vector Ecology*, *33*(1): 139–144.

SHAHI, M.; KAMRANI, E.; SALEHI, M.; HABIBI, R.; HANAFI-BOJD, A.A. 2015 Native larvivorous fish in an endemic malarious area of Southern Iran, a biological alternative factor for chemical larvicides in malaria control program. *Iranian Journal of Public Health*, *44*(11): 1544-1549.

VAN DEN BERG, H.; ZAIM, M.; YADAV, R.S.; SOARES, A.; AMENESHEWA, B.; MNZAVA, A.; HII, J.; DASH, A.P.; EJOV, M. 2012 Global trends in the use of insecticides to control vector-borne diseases. *Environmental Health Perspectives*, *120*(4): 577-582.

WORLD HEALTH ORGANIZATION. 2003 *Use of fish for mosquito control.* Arab Mass Media, Cairo. 77p.

WU, N.; WANG, S.; HAN, G.; XU, R.; TANG, G.; QIAN, C. 1987 Control of *Aedes aegypti* larvae in household water containers by Chinese cat fish. *Bulletin of the World Health Organization*, *65*(4): 503-506.