

Assessment of the predatory capacity on mosquito larvae of *Jenynsia multidentata* (Anablepidae) in presence of vegetation under laboratory conditions

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ABSTRACT. Larvivorious fish have been studied as potential biocontrol agents of mosquito larvae and pupae through their trophic interactions. The use of native fish for mosquito control may have benefits for both aquatic biodiversity and human health. Evaluating the effect of vegetation on the predatory efficacy is fundamental to determine if this species can be used as a biocontrol agent. With the aim of evaluating *Jenynsia multidentata* (Jenyns, 1842) as a biocontrol agent of *Culex pipiens* Linnaeus, 1758, we tested its predatory capacity in the presence or absence of aquatic vegetation under laboratory conditions. Two independent experiments were conducted. Larval consumption at a density of 60 larvae (6 larvae/l) was significantly reduced with the vegetation increased. On the other hand, when the larval density was 120 (12 larvae/l), the predatory capacity of *J. multidentata* did not vary in the presence or absence of vegetation. This result indicated that vegetation effect on consumption could be related to prey density, since at the higher density of prey the probability of predator-prey encounter might be increased. *Jenynsia multidentata* is a good consumer in presence of vegetation and could be used as a potential biocontrol agent of mosquito larvae in natural environments with similar characteristics to the ones tested in these experiments.

KEYWORDS. Biocontrol agents, *Culex* larvae, larvae density, consumption rate.

RESUMEN. Evaluación de la capacidad depredadora de *Jenynsia multidentata* (Anablepidae) sobre larvas de mosquitos en presencia de vegetación en condiciones de laboratorio. Los peces larvívoros han sido estudiados como posibles agentes de control biológico de larvas y pupas de mosquitos a través de sus interacciones tróficas. El uso de peces nativos para el control de mosquitos puede tener beneficios tanto para la biodiversidad acuática como para la salud humana. La evaluación del efecto de la vegetación sobre la eficacia depredadora es fundamental para determinar si esta especie puede usarse como un agente de control biológico. Con el objetivo de evaluar a *Jenynsia multidentata* (Jenyns, 1842) como agente de biocontrol de *Culex pipiens* Linnaeus, 1758, probamos su capacidad depredadora en presencia o ausencia de vegetación acuática en condiciones de laboratorio. Se llevaron a cabo dos experimentos independientes. El consumo de larvas a una densidad de 60 larvas (6 larvas / l) se redujo significativamente con el aumento de la vegetación. Por otro lado, cuando la densidad larval fue de 120 (12 larvas / l), la capacidad depredadora de *J. multidentata* no varía en la presencia o ausencia de vegetación. Este resultado indicó que el efecto de la vegetación en el consumo podría estar relacionado con la densidad de presas, ya que a mayor densidad de presas se podría incrementar la probabilidad de encuentro entre depredador y presa. *Jenynsia multidentata* es un buen consumidor en presencia de vegetación y podría ser usado como un potencial agente de control biológico de larvas de mosquitos en ambientes naturales con características similares a las probadas en estos experimentos.

PALABRAS CLAVE. Agentes de biocontrol, larva de *Culex*, densidad larval, tasa de consumo.

The control of Culicidae based on chemical strategies causes a major impact on the environment and insecticides resistance in target species despite their efficiency (RAULINS & WAN, 1995). Since the 1980s, the anti-vectorial fight includes predation, parasitism and competition in a context of integrated mosquito management (WHO, 2004; KARUNAMOORTHY, 2011). Exotic fish species have been introduced to many areas worldwide because of their alleged ability to control mosquito larvae, but have become one of the main threats to biodiversity (GARCÍA-BERTHO, 2007;

PYKE, 2008). Larvivorious fishes such as *Gambusia affinis* (Baird & Girard, 1853) and *Gambusia holbrooki* Girard, 1859, both native to freshwater basins of the east coast of the USA, were introduced in different countries for the biological control of mosquitoes (MENON & RAJAGOPALAN, 1978; BENCE & MURDOCH, 1986; CHATTERJEE & CHANDRA, 1997; CABRAL *et al.*, 1998). At present, their negative impact on native species is being evaluated (HURLBERT *et al.*, 1972; CARMONA-CATOT *et al.*, 2013; WHO, 2016); nevertheless, governments continue to spend considerable funds on the

introduction of exotic fish species without knowledge of their biology, possible ecological risk and efficacy in biological control (EL-SABAAWI *et al.*, 2016; SERVIC, 2016). Recently, CABRERA *et al.* (2017) confirmed the presence of two species of the non-native mosquitofish *G. affinis* and *G. holbrooki* in Argentina, 70 years after its introduction, and highlighted that some health officials had recently released fishes to fight the transmission of dengue fever. This would suggest an indiscriminate introduction of these fish in this region.

Either way, some studies suggest that native fish can be equally or even more effective than exotic species as biocontrol agents (NELSON & LEWIS, 1992; HOMSKI *et al.*, 1994). *Jenynsia multidentata* (Jennyns, 1842) could be a biocontrol agent of mosquito species from Argentina. This fish is distributed in South America, in the River Plate, from Río de Janeiro in Brazil to central Argentina and Uruguay (GHEDOTTI & WEITZMAN, 1996). It is an omnivorous species (RINGUELET, 1967), strongly euryhaline (BAIGÚN *et al.*, 2002) and associated with aquatic vegetation (ESCALANTE, 1983; MENNI, 2004; QUEZADA-ROMEGIALLI *et al.*, 2009). The predation capacity of *J. multidentata* was evaluated under laboratory and field conditions with different results (MARTI *et al.*, 2006, BONIFACIO *et al.*, 2014), vegetation effect on predation efficiency is an important factor to consider if larvivorous fish are used as biocontrol agents in the environment, and such study has not been carried out in *J. multidentata* until now.

In coastal areas, the aquatic vegetation is an important biological component due to the diverse and abundant communities it supports, often much larger than unvegetated areas (CROWDER *et al.*, 1998). The diversity and abundance of macroinvertebrates generally increase with expanding vegetation biomass or density (CROWDER & COOPER, 1982; STONER & LEWIS, 1985). It is well known that some kinds of emergent vegetation favor mosquito's reproduction (JIANNINO & WALTON, 2004). For instance, *Culex pipiens* Linnaeus 1758, vector mosquito of St. Louis encephalitis virus (SLEV), West Nile virus (WNV), Rift Valley Fever virus (RVFV), filarial worms and some other wildlife pathogens like bird malaria (FARAJOLLAHI *et al.*, 2011), uses marsh habitats as natural breeding where *Typha dominguensis* Persson spreads along the perimeter (MUFARREGE *et al.*, 2011). In emergent vegetation, Culicidae larvae and pupae obtain food and protection from predators (ALMIRÓN & BREWER, 1996; THULLEN *et al.*, 2002).

The aquatic vegetation is used by macroinvertebrates as shelter (DIEHL, 1988), reducing predator-prey interactions (WERNER *et al.*, 1983; SIH, 1986; SHARMA, 1987; SAVINO & STEIN, 1989; ROJAS *et al.*, 2004; WILLEMS *et al.*, 2005). ABDEL-TAWWAB (2005) suggested that fish foraging success generally decreases as plant density increases, but this has not been determined with *J. multidentata*. There are several characteristics that a larvivorous fish must possess to be an efficient biocontroller and one of them is to be able to predate through vegetation (AHMED *et al.*, 1986). The aim

of this study was to determine the predatory capacity of *J. multidentata* on *C. pipiens* larvae in relation with the presence or absence of aquatic vegetation under laboratory conditions.

MATERIAL AND METHODS

Jenynsia multidentata (total length 5-7 cm) were captured with hand nets (1 mm mesh size) from the artificial lagoons of the Institute of Hydrobiological Development (31°31'50.37"S, 68°32'15.15"W), San Juan province, Argentina. The specimens of *J. multidentata* (10♀, 20.IV.2014) used in the experiment became part of the Scientific Collection of Vertebrates of the Institute and Museum of Natural Sciences of the National University of San Juan, Argentina. Mosquito larvae were collected with a 300 ml dipper from urban irrigation ditches, transported to the laboratory in a 250 ml plastic container and reared to fourth-instar. *Culex pipiens* larvae identification was based on taxonomic key from MITCHELL & DARSIE (1985) and ROSSI *et al.* (2002).

Laboratory experiment. Habitats with different stem densities were simulated in an indoor laboratory to investigate the influence of emergent vegetation on the predatory capacity of *J. multidentata*. Laboratory experiments were conducted in 20 l containers with 1 kg of sand as substrate, 10 l of dechlorinated water and one *J. multidentata* female. The area of the container and water depth were 616 cm² and 20 cm respectively. The emergent vegetation of mosquito breeding habitats was simulated with dry *Arundo donax* Linnaeus, 1753 stems of 2 cm ± 0.23 in diameter and 30 cm in length. The experimental containers were kept at room temperature and water temperature was recorded by means of a data logger. The water temperature was 17.84 ± 1 °C.

The first experiment was conducted in 30 containers, 10 of which included 20 dry stems at 5 cm (20 stems / 616 cm²) that were distributed across the surface of the container (T1), 10 containers had 10 stems at 5 cm (10 stems / 308 cm²) in half of the container (T2) and the remaining 10 were left free of vegetation as control treatment (TC). After a 24-hour fast to standardize hunger levels, *J. multidentata* females were fed daily with 60 fourth-instar *C. pipiens* larvae for 7 days. The surviving larvae were counted and removed after 24 h. The second experiment was like the first one only that in this case T2 was eliminated and *J. multidentata* females were fed daily with 120 fourth-instar *C. pipiens* larvae. To maintain adequate oxygenation levels in the experimental containers in both experiments, a partial water refill corresponding to 25% of the total volume was carried out daily.

Data analysis. Normality and homogeneity of variance were analyzed using the Levene test and Shapiro Wilk tests respectively. To compare the consumption between treatments, Kruskal-Wallis tests and Tukey test (p = 0.05) were carried out in experiment 1 and Mann-Whitney U test (SOKAL & ROHLF, 1969) in experiment 2. Finally, the Spearman correlation coefficient was used to analyze the

relationship between consumption and days. The R Studio Version 1.0.136 was employed for statistical analyses.

RESULTS

The average number of *C. pipiens* larvae consumed by *J. multidentata* at a larval density of 6 larvae/l ($n=60$) (Fig. 1) was $50 \pm 15.8/24h$ in T1 and $60/24h$ in T2 and TC, with significant differences observed ($H=70$, $p=5.062e-16$). In T1, *J. multidentata* consumption was significantly reduced compared to T2 and TC, and it was an equally effective

consumer in the treatment with area free of vegetation (T2) and TC (Fig. 1). Days were not a statistically significant variable ($r=-0.05$, $p\text{-value}=0.5$) in this experiment (Fig. 2).

The average number of *C. pipiens* larvae consumed by *J. multidentata* at a density of 12 larvae/l ($n=120$) larvae was 84 ± 27.5 in T1 and 78 ± 28 in TC. *J. multidentata* was equally effective as a mosquito larvae consumer in the treatment with stems (T1) and TC ($W=1404$, $p=0.28$) (Fig. 3). In this experiment, there was a significant effect of days on larval consumption with a negative correlation ($r=-0.55$, $p\text{-value}=2e-09$) (Fig. 4).

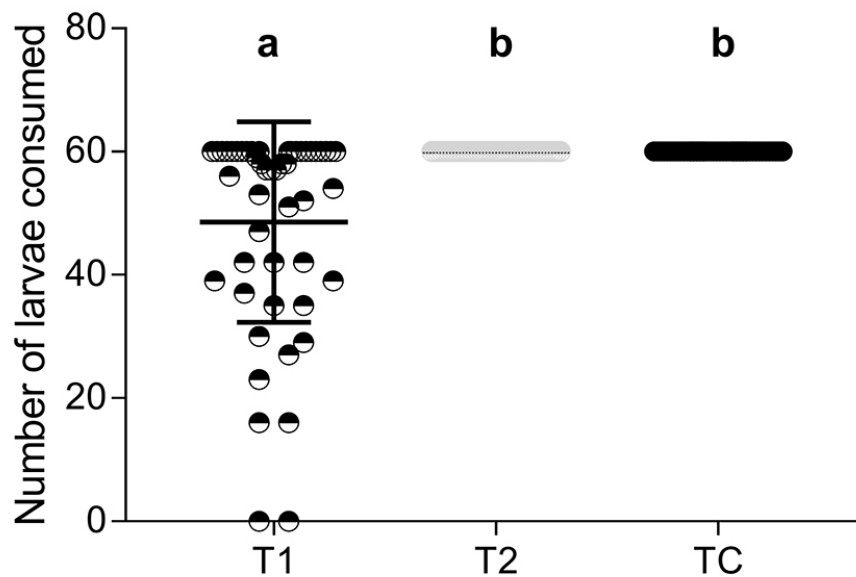


Fig. 1. *Jenynsia multidentata* (Jenyns, 1842) consumption of 60 *Culex pipiens* Linnaeus, 1758 fourth-stage larvae at different vegetation densities: (T1) 20 stems, (T2) 10 stems and (TC) without stems. Each circle represents the daily consumption of a female specimen and the horizontal bars indicate the average consumption. The letters (a, b) at the top of the figure indicate significant differences between treatments ($p < 0.05$).

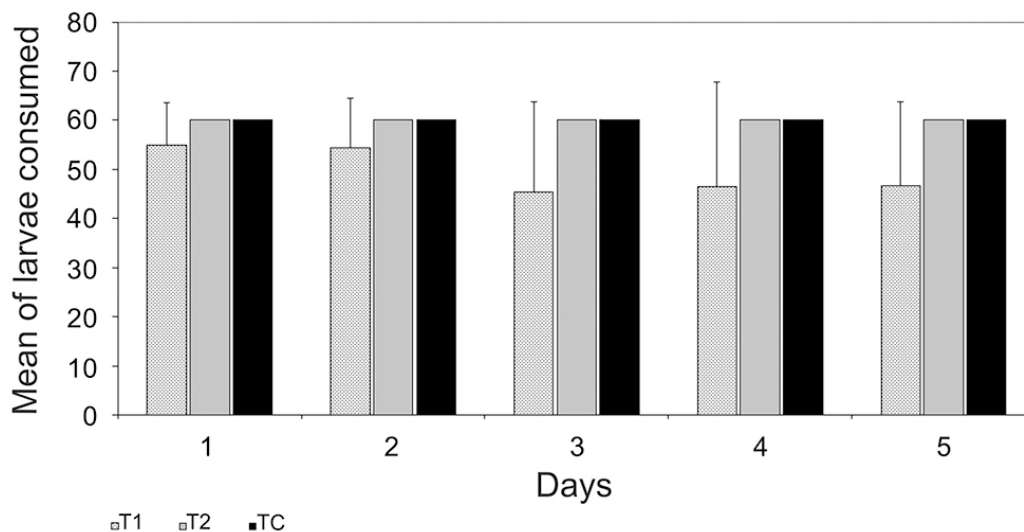


Fig. 2. Daily consumption (mean \pm SD) of *Jenynsia multidentata* (Jenyns, 1842) on 60 *Culex pipiens* Linnaeus, 1758 fourth-stage larvae at different densities of vegetation: (T1) 20 stems, (T2) 10 stems and (TC) without stems, for 5 days.

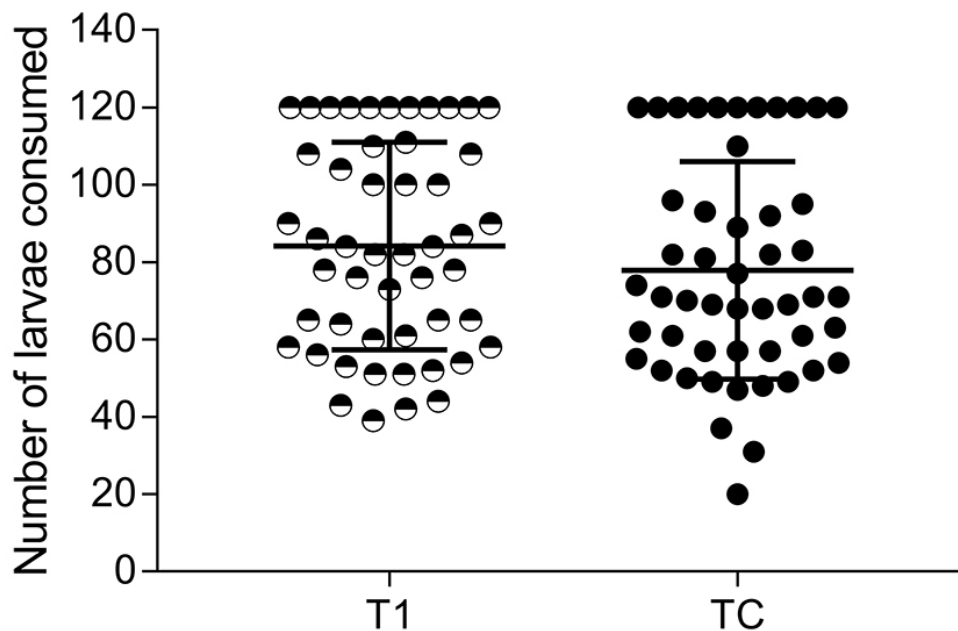


Fig. 3. *Jenynsia multidentata* (Jenyns, 1842) consumption of 120 *Culex pipiens* Linnaeus, 1758 fourth-stage larvae at different vegetation densities: (T1) 20 stems and (TC) without stems. Each circle represents the daily consumption of a female specimen and the horizontal bars indicate the average consumption.

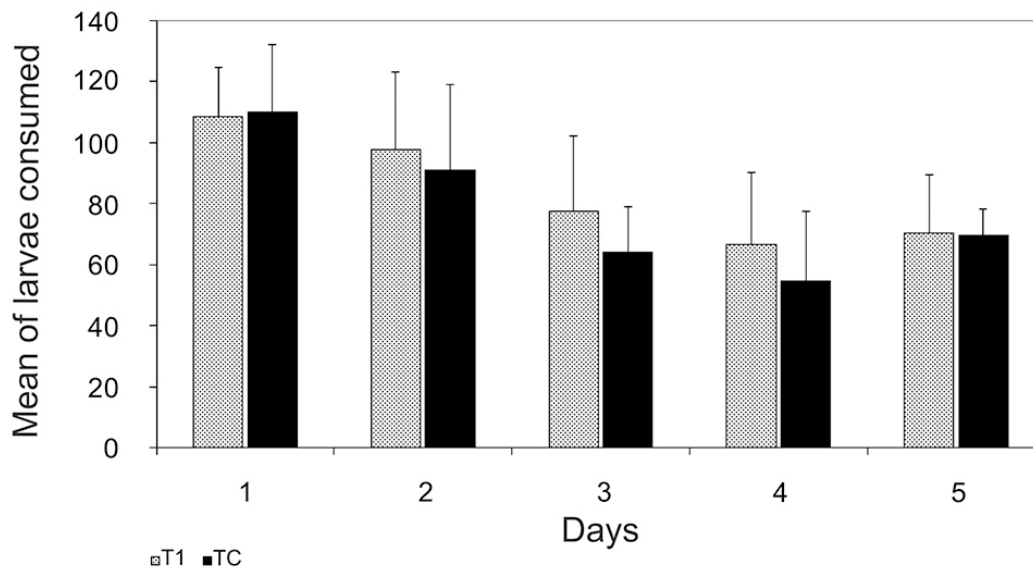


Fig. 4. Daily consumption (mean \pm SD) of *Jenynsia multidentata* (Jenyns, 1842) on 120 *Culex pipiens* Linnaeus, 1758 fourth-stage larvae. (T1) 20 stems, (TC) without stems, for 5 days.

DISCUSSION

The use of larvorous fish as a method of biological control of mosquito larvae is dated; there are relatively few studies that evaluate the vegetation effect on mosquito larvae predation efficiency and none of *J. multidentata*. This parameter is fundamental to determine if a larvorous fish species can be used as biocontrol agent in marsh areas where macrophytes are predominant. Emerging vegetation obstructs the visual field and hinders the swimming behavior of forage fish (DIEHL, 1988; MANUTANGE *et al.*, 2000). Studies that were

carried out with other fish species obtained different results; for instance, WILLEMS *et al.* (2005) concluded that vegetation density has a negative correlation with larval consumption in *Pseudomugil signifier* Kner, 1866 and *G. holbrooki*, while CABRAL *et al.* (1998) determined that vegetation has no effect on prey consumption in field experiment with *G. holbrooki*.

Our results showed that *J. multidentata* is a good consumer through vegetation. However, we observed differences in the two experiments carried out with dissimilar number of larvae. Experiment 1 (60 mosquito larvae) showed that *J. multidentata* predatory capacity on *C. pipiens* larvae

was maximum in both treatments, with vegetation plus free area and control. Probably, the low larval density coupled with vegetation decreased the predator-prey encounter.

On the other hand, in experiment 2 (120 mosquito larvae), the predatory capacity of *J. multidentata* was not reduced in the presence of vegetation and the average consumption rate was higher than in experiment 1 in T1 and TC. This could be due to the increase in the number of larvae that were exposed, which could increase both the probability of predator-prey encounter and prey contrast with vegetation (CABRAL *et al.*, 1998), or because the time spent searching and identifying a prey depends on larval concentration (HOLLING & BUCKINGHAM, 1976). Larval consumption was considerably reduced over days, probably because of the rise in larval density that increased the survival rate of the prey in experiment 2. The reason for this could be the prey handling time that limits the number of prey the predator can process per unit of time (HOLLING & BUCKINGHAM, 1976).

The consumption rate of *J. multidentata* under the tested conditions was like the one found in other larvivorous fish that were evaluated and proposed as biocontrol agents, such as *Girardinus metallicus* Poey, 1854 (HERNÁNDEZ CONTRERAS *et al.*, 2004), *P. signifer*, *G. holbrooki* (WILLEMS *et al.*, 2005), *Poecilia reticulata* Peters, 1859, *Carassius auratus auratus* (Linnaeus, 1758) (VALERO *et al.*, 2006) and *Cnesterodon decemmaculatus* (Jenyns, 1842) (MARTI *et al.*, 2006; TRANCHIDA *et al.*, 2010). *Jenynsia multidentata* consumption results obtained by MARTI *et al.* (2006) at a density of 60 larvae were like those achieved in this experiment and inferior at a larval density of 120. The results showed that *J. multidentata* predatory capacity was greater, even in the presence of vegetation, than those obtained by other authors in experiments without vegetation (MARTI *et al.*, 2006, BONIFACIO *et al.*, 2014). This could be due to the differences in water volume used in both experiments. Lower predatory capacity was observed in *J. multidentata* females in experiments with small volumes of water (BONIFACIO *et al.*, 2014). The fish energy expenditure could decrease with a lower volume of water that, in turn, would reduce the consumption of larvae. There is probably a relationship between the consumption capacity of *J. multidentata* and the water volume available for the fish to swim.

The use of indigenous fish for mosquito control may have benefits for both aquatic biodiversity and human health (LAWRENCE *et al.*, 2016). Our results would indicate that since *J. multidentata* is a good consumer of larvae among vegetation, it could be used as a potential biocontrol agent of mosquito larvae in natural environments with similar characteristics to the ones tested in the present study. Further research will be necessary to determine whether the results observed in laboratory conditions are similar in natural breeding sites of mosquitoes.

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