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Use of the neotropical fish *Cnesterodon decemmaculatus* for long-term control of *Culex pipiens* L. in Argentina

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ABSTRACT

We released the native mosquito fish, *Cnesterodon decemmaculatus*, into suburban drainage ditches to evaluate its potential as a long-acting agent for the control of *Culex pipiens* larvae in natural breeding sites. The inoculation of predatory fish was conducted in nine ditches at three densities: 1, 7, and 13 fish/m^2 during a 2-year period (2006–2008). The number of immature stages of *Cx. pipiens* was recorded before and after release. On a monthly basis, the digestive-tract contents of some fish and the average number of offspring from the females was recorded. Fifteen weeks after release, a 99% reduction in the number of immature mosquito stages was recorded in the drainage ditches containing 13 fish/m^2, while at 22 weeks, a 99% reduction was also observed in those with 7 fish/m^2. The ditches with 1 fish/m^2 had lower densities of immature mosquito stages relative to the controls, but over the entire experiment these observations did not prove statistically significant. The number of offspring per adult *C. decemmaculatus* female ranged from 4 ± 1.4 (mean ± SD) to 7.4 ± 0.9. Larval remains were detected in the fish collected in January, February, March, June, and September of 2006.

1. Introduction

Worldwide mosquito problems are still a major human-health related issue. Efforts in mosquito control have involved chemical pesticides, but this method has become harmful to the environment and has increased the likelihood of pest resistance in the target insects. An interest in using larvivorous fishes for the control of mosquitoes has existed for several decades since larvivorous freshwater fishes have been shown to be effective natural enemies of mosquito larvae. Consistent with present-day knowledge, such fishes possess a number of attributes that would make them good candidates for biological control because of their innate predatory characteristics. They are capable of significantly reducing natural populations of mosquito larvae and have a broad host range as well as a great potential for the long-term control of mosquitoes in the field since they survive and reproduce naturally in the fresh water (Bay, 1985; Torrente et al., 1993; Lee, 2000; Martinez-Ibarra et al., 2002; van Dam and Walton, 2007). Nevertheless, this great interest in mosquito fish as insect control agents is tempered by the concerns of ichthyologists and ecologists about the possible negative aspects on non-target organisms within natural ecosystems (Gratz et al., 1996). For example, the release of non-native fishes into ecosystems can have significant consequences on the fauna of specific aquatic habitats (Goodman, 1991; Adams et al., 2003), mainly in places where fishes had been previously lacking (Wellborn et al., 1996; Hamer et al., 2002).

The most common mosquito fish, *Gambusia affinis* (Baird and Girard) (Cyprinodontiforme: Poecilidae), has been introduced from its native habitat in the southern United States to more than 60 countries within the continents of Europe, Africa, and Asia for the purpose of malaria control (Gerberich and Laird, 1985; Sala et al., 1985; Komak and Crossland, 2000; Ayala et al., 2007). Members of the Poecilidae are present in tropical and subtropical zones of the American continents and include indigenous fishes in those areas. These fishes are small freshwater or brackish-water, ovoviviparous teleosts, originally adapted for breeding in small pools without vegetation and with a low level of dissolved oxygen.

In Argentina, *Culex pipiens* L. has become a major nuisance in many regions. This mosquito also constitutes a significant vector for the West Nile virus in North America, from which region that invasive arbovirus may be carried southward by migratory birds (Hayes et al., 2005). Drainage ditches in the suburban areas of many cities in Argentina, being human-made and widely distributed, are among the most prolific breeding sites for *Cx. pipiens* larvae and allow vast numbers of mosquitoes to develop and emerge throughout the year (Campos et al., 1993). Marti et al. (2006), investigating drainage ditches in the suburbs of the city of La Plata, Argentina identified *Cnesterodon decemmaculatus*

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(Jenyns) (Cyprinodontiforme: Poecilidae) along with *Jenynsia multidentata* (Jenyns) (Cyprinodontiforme: Anablepidae) as the most common natural fish species present. These fishes have been cited previously by Ringuelet et al. (1967) as being larvivorous and are found exclusively in the southern part of the Neotropical region. Marti et al. (2006) concluded that the inoculation of fish species into such drainage ditches and the clearing away of obstacles impeding the flow of water within them could improve the natural control of *Cx. pipiens* by these two native species within the relevant sites in Argentina.

The objective of our work was therefore to release the native mosquito fish, *C. decemmaculatus*, into drainage ditches to evaluate the potential of this species as a long-term agent for controlling *Cx. pipiens* immature stages within the mosquito's natural breeding sites. Considering that *Cx. pipiens* is the only species of mosquito breeding in drainage ditches, we hypothesized that *C. decemmaculatus* have the potential to suppress natural populations of *Cx. pipiens* in suburban area of La Plata city.

2. Materials and methods

2.1. Study area and selection of test sites

From September through December of 2005, we surveyed 600 drainage ditches (average dimensions of these ditches were $25 \times 0.60 \times 0.25$ m with respect to length, width, and water depth) located in the suburban area of La Plata (Argentina) and selected 12 for this study on the basis of size and the presence or absence of mosquito larvae, larvivorous fish, water, and vegetation and/or algae. In these 12, we also measured the temperature, pH, and conductivity (μS/cm) of the water.

We inoculated fish in nine ditches, but left three sites without fish and containing immature stages of mosquitoes to serve as a control. *Cx. pipiens* is the only mosquito species identified in the drainage ditches. The numbers of immature stages in the 12 ditches during the pretreatment period were compared by the one-way ANOVA test. The abundance was transformed through logarithmic function ($\log x + 1$).

2.2. Survey of the population of mosquito immature stages and release of fish

Cnesterodon decemmaculatus specimens were collected from the field sites with a 100-μm-mesh net and transported in plastic containers containing 3 l of field water. At the laboratory, the fish were separated into male–female pairs. Twenty-four hours after collection, the pairs of fish were released at total numbers of 10, 50, and 100 pairs corresponding to 1, 7, and 13 fish/m^2, respectively. Each level of fish inoculation was performed in triplicate, with the three remaining ditches serving as a control group without fish. The size of the male fish in length ranged from 1.5 to 2.5 cm, whereas that of the females was from 2 to 3.5 cm.

During the pretreatment, the number of mosquito larvae and their instars were recorded in the 12 ditches on four sampling dates. A 250-ml dip was used as the sampling unit (three samples per ditch). The fish were released in January 2006.

After the treatment period, the mosquito larvae at each of the sites were monitored twice a week during the first year (January, 2006 through May, 2007) and every 2 weeks in the second year (June, 2007 through January, 2008). The immature mosquito stages obtained were transported in plastic containers (3-l), and at the laboratory, the number and density of larvae and pupae were recorded for each site along with the sampling date.

2.3. Statistical analysis

In order to detect statistically significant differences among the different treatment groups and the controls, we employed a model for repeated measurements over time. The variable to be analyzed was the average number of larvae per sample. The covariance structure for these repeated non-equidistant measurements was the Spatial-Power-Law covariance (*SP POW*), whose mathematical expression is $\sigma^2\rho^{|ti - tj|}$ where σ^2 is the variance of one observation, ρ is the correlation between observations within the same experimental unit, and the exponent is a measurement of the distance between two times. This statistical structure was detected as the best in all analyses according to the criteria of Akaike (AIC) and Schwarz's Bayesian (BIC). We considered only the first sampling period (from release date to June 2006) since during this stage two of the three treatment methods (50 and 100 pairs per site) eliminated all larvae. The best covariance structure for the first period is the autoregressive of the first order AR(1), which structure is equivalent to the previous one for the particular case of equidistant data.

The model employed was the following:

$$Y_{ijk} = \mu + \alpha_i + \beta_j \cdot t + \epsilon_{ijk}$$

where, Y_{ijk}, the response variable: the number of larvae per sample; μ, general mean; α_i, effect of the treatment (0–10-50–100 fish pairs); β_j, coefficient for the effect of time; t, time; ε_{ijk}, random error.

The analysis was done by means of the INFOSTAT 2001 program.

The percent reduction in mosquito larvae was compared between each of the different treatments and the control by means of the following formula (Kim et al., 2002):

$$\% \text{ reduction} = \frac{(A \times b/a - B)}{(A \times b/a)} * 100$$

where, *A*, number of mosquito larvae recorded in the controls after the treatment; *a*, number of mosquito larvae recorded in the controls before the treatment; *B*, number of mosquito larvae recorded in each treatment group after the treatment; *b*, number of mosquito larvae recorded in each treatment group before the treatment.

2.4. Persistence of fish within the natural environment after release

After introduction of the fish into the drainage ditches, 10 specimens of *C. decemmaculatus* were removed at random monthly from some of the ditches and dissected in the laboratory to determine the digestive contents under natural feeding conditions. The number of brood in each female was recorded as an indication of the establishment and reproduction of the fish population within these breeding sites.

2.5. Laboratory trial of predatory capacity of C. decemmaculatus on mosquito rafts

Plastic containers with 250 ml of water were used to evaluate whether the fish would consume egg rafts. A single *C. decemmaculatus* and one *Cx. pipiens* egg raft were added to each container. The presence of mosquito-egg rafts in the containers was then recorded 24 h after the exposure. Three replicate experiments were carried out with both sexes of fish involving a total of 18 containers.

3. Results

3.1. Study area and selection of the test sites

Upon surveying 600 drainage ditches within the study area, we detected about 32% with *C. decemmaculatus*, 11% without water, 5% with a marked development of the alga, *Oscillatoria brevis* (Kütz), along with the absence of culicine larvae, and 52% contained immature stages of *Cx. pipiens* mosquito. In all the ditches, we noted the accumulation of different types of refuse; such as cans, shards of glass, pieces of paper and cardboard, soap, detergent, and bleach.

Of these 600 ditches, the 12 that were selected for the study were among those where the immature stages of the culicines had been recorded. We collected the fish for the test from drainage ditches located in the same area. At this site the pH values were between 8.1 and 8.5, the conductivity between 580 and 750 (μS/cm), and the water temperature at 20.0 ± 1.3 °C. In the ditches where these fish were released, the pH values varied between 7.9 and 8.4, the conductivity between 530 and 690 (μS/cm), and the water temperature at 20.4 ± 1.0 °C.

3.2. Survey of the population of mosquito immature stages and release of fish

No significant differences ($F = 1.76$, df = 11, 37, $p = 0.09$) were observed in the mean number of larvae and pupae in the 12 ditches before the release of *C. decemmaculatus*, with the values remaining at average of 344 ± 64 (Mean ± SD) stages per sample.

In April 2006, 15 weeks after the introduction the predatory fish, no immature stages of *Cx. pipiens* were collected from the ditches in which we had introduced 100 male and female pairs of *C. decemmaculatus* (13 fish/m^2), giving a 99.3% reduction in those stages (Fig. 1). In the ditches with either 50 such pairs (7 fish/m^2) or 10 (1 fish/m^2), a reduction in *Cx. pipiens* immature stages of 72.7% and 47.1% was observed. By June (22 weeks after predator introduction) in the ditches where 50 pairs of fish had been released, the degree of reduction was 99.2%. Moreover, in the ditches that had received only 10 pairs of fish, we obtained a 40.6% reduction. During the winter and spring months (July through October), only the ditches inoculated with 10 pairs of *C. decemmaculatus* contained *Cx. pipiens* immature stages, which was similar in number to the control ditches. By mid-October, the *Cx. pipiens* larval counts in the control ditches had begun to rise and thereafter continued to do so. By the beginning of January 2007, a figure similar to the mean counts recorded a year earlier in January 2006 (the pretreatment value) occurred. Finally, in the ditches originally planted with 10 fish pairs, we recorded a rise in the number of larvae from the end of October onward, but except at the beginning of this sampling period, that increase never attained the mean value of the controls. In the ditches having received 50 and 100 fish pairs, no further mosquito larvae were detected throughout the rest of the sampling period.

Although the density of the fish population was not recorded in the nine ditches where the fish were inoculated, we verified the continuing presence of *C. decemmaculatus* specimens through samplings made from the time of their release in January 2006 up to the end of the experiment in January 2008.

By means of the Repeated Measurements analysis, we were able to confirm that the larval counts found in the control ditches were significantly different from values recorded for the ditches receiving 10 ($F = 3.49$, df = 1, 8, $p = 0.0986$), 50 ($F = 17.93$, df = 1, 8, $p = 0.0029$), and 100 fish pairs ($F = 30.95$, df = 1, 8, $p = 0.0005$). The number of immature larval stages recorded in the ditches planted with 10 fish pairs, in turn, differed significantly from the

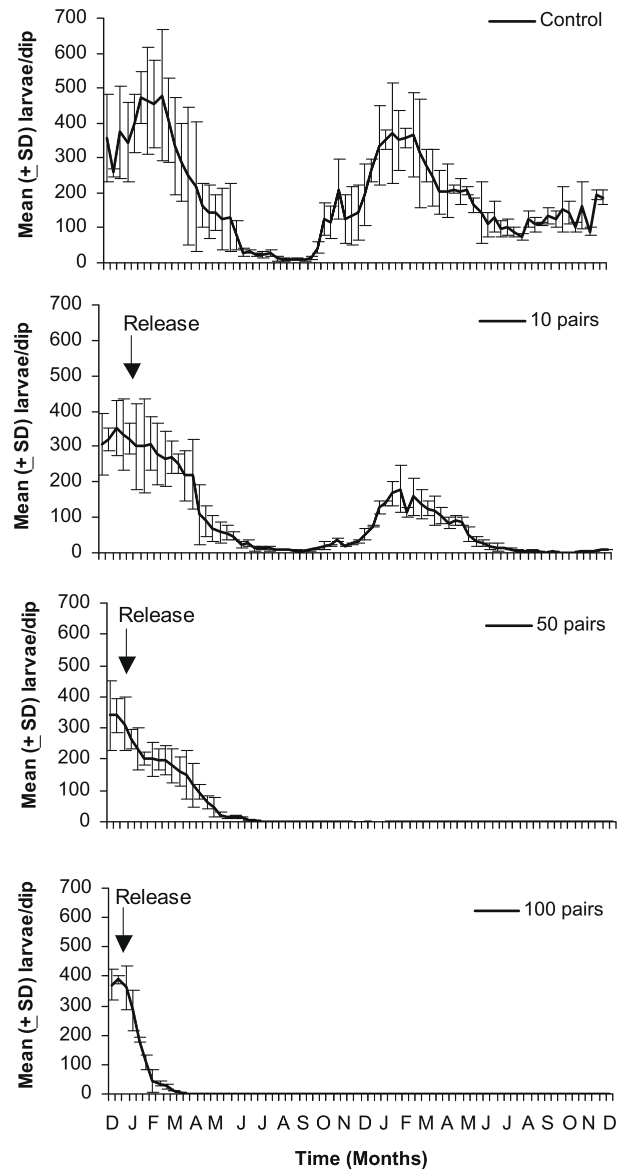

Fig. 1. Average number of larvae per sample during a 2-year study in the ditches where 100 (13 fish/m^2), 50 (7 fish/m^2), and 10 pairs (1 fish/m^2) of *Cnesterodon decemmaculatus* were released and in the control ditches lacking fish. An arrow indicates the time of release.

data for the ditches inoculated with either 50 ($F = 5.60$, df = 1, 8, $p = 0.0456$) or 100 pairs ($F = 13.65$, df = 1, 8, $p = 0.0061$); whereas the larval counts from these latter two experimental groups were not significantly different ($F = 1.77$, df = 1, 8, $p = 0.221$).

3.3. Persistence of fish within the natural environment after release

Within the contents of the digestive tracts dissected from the *C. decemmaculatus* specimens sampled throughout the experimental period, there were algae, crustaceans, and the remains of larval exoskeletons (mainly siphons). These latter culicine-derived materials were, moreover, detected in fish specimens sampled during the months of January through March, June, and September of 2006.

The number of offspring per *C. decemmaculatus* adult female varied between 4.0 ± 1.4 and 7.4 ± 0.9 throughout the 2-year sampling period (Table 1).

Table 1
Monthly number of *Cnesterodon decemmaculatus* and mean (±SD) of broods from dissected female fish from each sampling period.

Date	Sex of the collected *C. decemmaculatus*	Means (±SD) of broods per female
2006		
January	6 ♀ - 4♂	5.4 + 1.1
February	5♀ - 5♂	6.8 + 0.4
March	2♀ - 8♂	6 + 0
April	7♀ - 3♂	7.4 + 0.9
May	4♀ - 6♂	4.8 + 1.3
June	9♀ - 1♂	5.4 + 1.7
July	3♀ - 7♂	7 + 1
August	5♀ - 5♂	6.4 + 1.5
September	8♀ - 2♂	6.1 + 1.3
October	1♀ - 9♂	6 + 0
November	7♀ - 3♂	5.7 + 2.2
December	0♀ - 10♂	0
2007		
January	4♀ - 6♂	6.3 + 0.6
February	7♀ - 3♂	6.1 + 1.5
March	8♀ - 2♂	6.3 + 0.8
April	5♀ - 5♂	5.4 + 1.1
May	9♀ - 1♂	5.6 + 1.8
June	7♀ - 3♂	6.6 + 1.34
July	3♀ - 7♂	4.7 + 0.57
August	2♀8♂	4 + 1.4
September	5♀ - 5♂	5.8 + 1.5
October	2♀ - 8♂	6.5 + 0.7
November	6♀ - 4♂	5.6 + 0.8
December	3♀ - 7♂	6.7 + 0.6

3.4. Laboratory trial of predatory capacity of C. decemmaculatus on mosquito rafts

Culex pipiens egg rafts were consumed by both male and female *C. decemmaculatus* within 24 h of exposure.

4. Discussion

Larvivorous fishes have been used as agents for controlling various species of culicine vectors that breed in different types of water, both natural (temporary and permanent ponds) and artificial (waste waters, storage tanks, canals, drainage ditches) (Swanson et al., 1996). Our results following the release of *C. decemmaculatus* in the field coincide with the findings from other experiments carried out under natural conditions in which the utilization of fish as biocontrol agents was successful (Meisch, 1985; Scott, 2006; Howard and Omlin, 2008).

The release of 13 *C. decemmaculatus*/m^2 resulted in a reduction in *Cx. pipiens* larval stages of 99% in residential drainage ditches within 15 weeks of the introduction of the fish. These results are in agreement with the findings of Howard et al. (2007) who introduced *Oreochromis niloticus* L. specimens into bodies of water colonized by *Anopheles gambiae* (Giles), *A. funestus* (Giles), and various species of the subfamily Culicinae. Fifteen weeks later the population density of the *Anopheles* species had been reduced by some 94% and those of the Culicinae subfamily by 75%. Likewise, in our study, the release of 50 pairs of fish into the *Cx. pipiens* breeding areas proved effective in eliminating the larvae 22 weeks later. Marti et al. (2006) were able to reduce the *Cx. pipiens* population in a drainage ditch situated within the same study site 17 days after the release of some 1700 *C. decemmaculatus* specimens, which introduction would be equivalent to a density of about 113 fish/m^2; this predator input resulted in a reduction in the mosquito population within a shorter period of time. In our study, the time required to eliminate the larvae in the mosquito breeding sites in La Plata at densities of 7 or 13 fish/m^2 was always earlier than the time within which the mosquito population within the control ditches underwent a reduced density as a result of the onset of winter. On the basis of these results together with the predatory capacity on *Cx. pipiens* egg rafts (observed in the laboratory), we conclude that, at least during the time interval documented in this present work, the introduction of new predatory fish specimens for the purpose of reducing the number of immature larval stages of this particular culicine is unnecessary. A similar finding was recorded in study by Howard et al. (2007) on *O. niloticus* in Kenya.

In the 12 drainage ditches that we monitored, the immature larval stages of *Cx. pipiens* were either virtually absent or at least at low levels during the winter. Nevertheless, during the following spring the larval counts in the control ditches increased progressively up to the population levels measured in previous investigations performed on the study area (Campos et al., 1993). The number of culicine immature larval stages was maintained below the value measured in the control ditches, we can conclude that even a density of only 1 *C. decemmaculatus*/m^2 is useful for maintaining this vector at low population densities, though such an input is still insufficient for its absolute control.

The observation that the digestive tracts of dissected predatory fish contained the remains of mosquito larvae as well as unicellular and filamentous algae, copepods, and chironomids has been reported previously by Marti et al. (2006). In our study, this finding serves to confirm the supposition that the reduction in *Cx. pipiens* larval stages seen in the experimental groups indeed resulted from the presence and action of the predatory fish. Unlike the results of this work, however, Marti et al. (2006) also found residues of fish skeleton and scales. The identification of larval remains in the digestive tracts of the predatory fish and the presence of their offspring in the ditches after planting of the male–female pairs allow us to conclude that *C. decemmaculatus* is capable of adapting itself and reproducing in the surrounding areas. Moreover, since this species is native to Argentina, its release into the breeding sites of *Cx. pipiens* would not prove harmful to the environment. The advantage in using native species for such a purpose is that they not only serve to eliminate the culicine immature larval stages but also remain for prolonged periods of time within the environment by their reproductive capabilities. Furthermore, with such native predators the non-target fauna are not compromised by their presence (Chandra et al., 2008). Within this context, the example of *G. affinis* illustrates the inconveniences that may result when a predator is released into an environment unlike its customary surroundings. When this species, a native of the United States, was released into the rice fields of Italy or into the drainage ditches and canals of Sudan (Gratz et al., 1996; Rupp, 1996) to suppress *Anopheles* mosquitoes, it was ineffective in controlling the various species of that genus.

Another important determinant of the success or failure of predatory fish introduced into an environment devoid of fish as controlling agents for mosquitoes is the chemical, physical, and biological characteristics of the body of water. The introduced fish must become adapted to the new surroundings in order to survive and reproduce. In our work the pH, conductivity, and temperature values measured at the moment of introducing the fish into the experimental ditches were comparable to the equivalent parameters in the ditches in which they were originally captured so that the transition between the two environments represented no challenge whatsoever to the predators. Moreover, a consideration of the household effluents released into all of those ditches would certainly suggest that *C. decemmaculatus* is capable of tolerating a level of water pollution that often limits the effectiveness of other species of predatory fish after their distribution into non-customary and ecologically compromised surroundings (Weiser, 1991; de la Torre et al., 1997).

In conclusion, *C. decemmaculatus* is a predatory fish species with innate attributes that are appropriate for its application for long-term *Cx. pipiens* control in human-made aquatic habitats. We demonstrated that this species has the reproductive capacity and the ability of self-sustaining themselves after initial releases in absence of immature stages of mosquito prey as was demonstrated by the presence of algae and crustaceans in the contents of their digestive tract throughout the seasons. The introduction and maintenance of these predators in household drainage ditches should constitute an appropriate system of reducing the *Cx. pipiens* populations within the suburban zones of La Plata and possibly elsewhere under comparable environmental conditions.

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