**Macrocyclops albidus** (Copepoda: cyclopidae) for the Biocontrol of *Aedes albopictus* and *Culex pipiens* in Italy

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MACROCYCLOPS ALBIDUS (COPEPODA: CYCLOPIDAE) FOR THE BIOCONTROL OF AEDES ALBOPICTUS AND CULEX PIPIENS IN ITALY

RODOLFO VERONESI,1 MARCO CARRIERI,1 BETTINA MACCAGNANI,2 STEFANO MAINI1 AND ROMEO BELLINI1

ABSTRACT. The aim of our study was to assess the potential of Macrocyclops albidus as a biological control agent against the 1st and 2nd instars of Culex pipiens and Aedes albopictus. Under laboratory conditions of prey saturation (50 1st instars/copepod), an average of 58.98% of Cx. pipiens and 54.99% of Ae. albopictus larvae were killed by 1 copepod in 24 h. Trials run in big drums containing 200 liters of water showed that the M. albidus population, inoculated in April, efficiently controlled the mosquito population for the entire season. The predator was particularly effective against Ae. albopictus, as only 2 larvae of this species were found in the treated drums, compared to 814 larvae in untreated control drums throughout the study period. No difference was observed in the control efficacy between the 2 initial densities of copepods used. The reduction in Ae. albopictus density in the drums with 100 and 500 M. albidus with respect to the control drums was 99.90 ± 0.35% and 100.0 ± 0.0%, respectively. For Cx. pipiens, the reduction in density was 88.69 ± 13.51% and 84.65% in drums inoculated with 100 and 500 copepods, respectively. Macrocyclops albidus populations survived through the winter and continued to keep the mosquito population under control during the 2008 season. The M. albidus population developed very well both in drums placed in sunny and shaded areas and proved to be tolerant to both high (summer) and low (winter) temperatures. Trials performed on M. albidus survival in catch basins showed that after a few weeks, the copepod population dramatically decreased and subsequently disappeared. The main problem for copepod survival in catch basins seemed to be the low oxygen tension and accumulation of toxic substances, rather than copepods being flushed out in heavy rainfall episodes. During the period when copepods were present, they maintained the mosquito population under control; their partial disappearance from the catch basins, however, would require more re-colonization intervention to maintain mosquito control during the season.

KEY WORDS Macrocyclops albidus, Aedes albopictus, Culex pipiens, drums, catch basins, biocontrol

INTRODUCTION

It is well known that mosquito larvae are an important component of the trophic chain in most aquatic environments at all latitudes. The list of vertebrate and invertebrate predators is very long, provided that the ecological constraints are good enough to allow the predator’s survival, and that ecological management actions are adopted to enhance their predatory effectiveness (Collins and Washino 1985, Service 1993, Legner 1995, Kumar and Hwang 2006, Mogi 2007). In artificial habitats (i.e., cans, vats, tanks, catch basins), predators are usually absent, and for this reason, mosquito larval density can be very high. The possibility of using predators as a means of control is limited for many different reasons: the lack of mass rearing and storage facilities, problems related to their distribution, difficulties in maintaining a stable habitat colonization, and possible decrease of effectiveness in the presence of alternative prey. However, great interest has arisen around cyclopoid copepods (Copepoda: Cyclopoidea) as a mean of biological control of mosquitoes (Marten and Reid 2007), and in recent decades, several studies have been performed in the framework of integrated pest management programs. Apart from fish species (Walton 2007), copepods are so far the only predator group involved in such kind of studies in all continents, except Europe and Africa (Soto et al. 1999; Marten et al. 2000a, 2000b; Dieng et al. 2002; Kay et al. 2002a; Marti et al. 2004; Panagadí-Reyes et al. 2004; Rey et al. 2004; Kay and Nam 2005; Gionar et al. 2006; Pons et al. 2008).

Of cyclopoid copepods, Macrocyclops albidus (Jurine) is a cosmopolitan species that lives in temperate climatic regions, is highly adaptable to a very wide water temperature range (from 0 to 40 °C; Marten et al. 1994a), tolerates a wide range of pH levels (from 4.4 to 9.8), and resists high concentrations of pollutants like nitrogen and potassium (Dussart 1969). These ecological features make M. albidus suitable as a means of biological control, and several field studies have already been carried out on the use of copepods against Aedes albopictus (Skuse, 1894) in Florida and Louisiana (Marten et al. 1994a, Rey et al. 2004), and against Culex pipiens L. in Uruguay (Pons et al. 2008).

Copepods feed mainly on young 1st and 2nd instars, and if the larval mosquito density is very high, they consume only part of the prey’s body and then attack another prey, being able to lead to the death of up to 30–40 larvae per day.

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The mass rearing of copepods appears to be feasible and cheap (Suarez et al. 1992, Marten et al. 1997), and this aspect makes it possible to hypothesize their use in control programs against *Culex pipiens molestus* Forskal and *Aedes albopictus* (Skuse), the 2 main species that colonize Italian urban areas, sharing very often the same larval habitats (Celli et al. 1994, Carrié et al. 2008, Bellini et al. 2009). *Aedes albopictus* is actually the most dangerous species in Italy, both for its diurnal biting activity and for the sanitary concern related to its competence in transmitting several arboviruses causing severe human diseases, like chikungunya and dengue (Rezza et al. 2007, Carrié et al. 2011). This species is extending its geographic influence in Europe (ECDC 2009), and from 1990 to date, it has now colonized all urban and peri-urban areas of plains and low hills of Italy. In this context, cyclopoids could find use in water collections that cannot be eliminated, such as recycled tanks with capacities up to several hundreds of liters used to store rainwater, groundwater, or water supply (drums, cans, tubes, barrels, etc.) and manholes for drainage of rainwater.

The complete weekly emptying of tanks, or the use of tight covers can be effective in preventing egg laying by female mosquitoes. Citizens are often obligated to take these actions by specific mayoral ordinance, but very often recommendations/obligations are not respected, and closures usually appear to be inappropriate. In catch basins, the control of mosquito populations is conducted by periodic larvicidal treatments from May to October, with high costs for public administrations. They can retain water volumes up to 50–60 liters, usually characterized by high concentration of organic substances in suspension and/or deposited at the bottom.

Our study focused on the effectiveness of *M. albidus* against *Cx. pipiens* and *Ae. albopictus* larvae and on its ability to permanently colonize artificial breeding habitats.

**MATERIALS AND METHODS**

*Macrocyclops albidus*

An *M. albidus* strain collected in a ditch in the countryside of Crevole (44°43′15″N, 11°08′56″E), Emilia–Romagna region, Italy, has been reproduced at the CAA Laboratory using 15–30-liter capacity containers filled with tap water. The diet consisted of a mix of various live microorganisms, with a predominance of ciliated protozoa and rotifers (*Blepharisma* spp., *Tetrahymena* spp., *Paramecium* spp., *Trichocerca* spp.). Organic substances necessary for microorganism development were provided by adding dry cat food, shredded beef, liver, and vegetables (Friskies®, Nestlé S. A. Vevey, Switzerland) to the water.

**Predation capacity under laboratory conditions**

The predation capacity was assessed by placing 1 *M. albidus* adult in a cylindrical plastic jar with 40 ml of dechlorinated tap water and a small amount of dry cat food. Each individual was offered 50 1st-stage larvae of *Ae. albopictus* (N = 25) or *Cx. pipiens* (N = 10). Identical jars containing only 50 larvae of *Ae. albopictus* (N = 15) or *Cx. pipiens* (N = 15) acted as controls. Surviving larvae were counted at 24 and 48 h. Both mosquito species were reared at the CAA Medical and Veterinary Laboratory, where an *Ae. albopictus* strain (collected in Rimini, northern Italy) and an autogenous *Cx. pipiens* strain have been maintained for many years. The trials were conducted in an air-conditioned room at 26°C, 75% relative humidity, with a light:dark photoperiod of 14:10 h.

**Survival, population growth, and predatory efficacy in drums**

The trial was run in semifield conditions from April to October 2007. Large plastic containers, filled with 200 liters of tap water, were placed in an open air area close to the CAA Laboratory in Ronchi di Crevole (Bologna, Italy; 44°45′22.28″N, 11°08′20.10″E). On April, 18, 2007, in 10 drums placed in the shade, 50 g of lyophilized hay were added to the water to increase the attractiveness toward gravid mosquitoes. After 7 days, *M. albidus* adults were introduced: 100 copepods in 3 drums (COP100), and 500 copepods in another 3 (COP500), while 4 drums without copepods acted as controls. Beginning May 10, 2007, biweekly sampling was performed to assess the presence of mosquito larvae, pupae, and copepods, by using a mosquito net (13 × 15 cm, mesh of 0.1 mm). The net was swept gently only once with spiral movements from the surface of the water towards the bottom of the drum, to a depth of 30–40 cm. The net content was put in a white plastic tray (40 × 20 cm) with some water. The abundance of *M. albidus* was estimated by averaging the number of adults and immature specimens counted in 10 squares of a grid that had been drawn on the bottom of the tray (square size of 2.5 × 2.5 mm). All the 3rd- and 4th-stage mosquito larvae and pupae were classified and counted.

Twelve samplings were performed, with the last one taken on October 22, 2007. The minimum and maximum water temperatures at 15–30-cm depth were recorded in the early morning, using a thermometer submerged in the water.

**Influence of the sun on *M. albidus* survival and predatory activity**

Starting June 5, 2007, more drums were placed in sunny areas: 3 of them had 100 *M. albidus,*
while 2 without copepods acted as controls. The influence of sun exposure on *M. albidus* survival and on its predatory activity was assessed by means of paired comparisons between the number of *M. albidus*, *Ae. albopictus*, and *Cx. pipiens* (3rd and 4th instars and pupae) in the drums placed in sunny or in shady areas.

**Winter survival and predatory activity after one year from inoculation**

During the winter of 2007–8, the 10 drums placed in the shady areas were maintained without adding either water or organic material. To evaluate the copepod population development, 14 samplings were performed from April 25, 2008, until October 28, 2008. The minimum and maximum water temperatures at 15–30-cm depth were registered.

**Predation capacity in catch basins**

In 2007, a preliminary trial was run in Marano di Castenaso (BO; 44°31′33″N, 11°27′16.97″E). Twenty catch basins (Fig. 1) were chosen along roads with and without trees at the borders. On July 9, 2007, 100 copepods were introduced in each of the 10 catch basins randomly arranged, while 10 catch basins without copepods acted as control. Biweekly samplings were performed from June 26 (before the beginning of the trial) to October 22, 2007. Two samples were taken from each catch basin, waiting 3 min after the opening of the grid. By using a 0.5-liter dipper, 2 subsequent samplings were performed, waiting 3 min between the 1st and 2nd. The total number of copepods, the number of larvae (categorized by species and instar), and number of pupae were registered. The minimum and maximum air temperature and the oxygen concentration in the water were recorded (using oxygen meter by HANNA Instruments®, Villa-franca Padovana (PD), Italy, model HI 9146-04) after every sampling session. Total rainfall during the trial was also measured.

**Factors affecting *M. albidus* survival in the catch basins**

In 2008, the impact of high concentrations of organic substances was assessed by comparing *M. albidus* survival in dirty and cleaned catch basins (from which all the sediments had been removed during the previous 6 months). The trial was run in San Carlo (FE; 44°48′11.55″N, 11°24′35.34″E) in 145 catch basins displaced along 7 roads, each one marked with a permanent color and identified by a code. Fifty *M. albidus* adults per catch basin were introduced on April 26, 2008, followed by 50 more individuals on June 28, 2008. During the 2nd release, catch basins were treated with Vectobac® DT (*Bacillus thuringiensis* israelensis de Barjac, H-14, 3400 ITU/mg), Bleu Line s.r.l., Forlì, Italy.

![Diagram of a typical catch basin in Italy](image)

**Fig. 1.** Section of a typical catch basin in Italy. Common side dimensions are about 40 × 40 cm, with a number of exceptions.

From April 26 to August 5, 2008, 30 catch basins randomly chosen were sampled according to the protocol described above. Immature mosquitoes were categorized as follows: 1st–2nd instars, 3rd–4th instars, and pupae. The presence/absence of copepods in the water sampled and the total rainfall were also recorded.

**Descriptive statistics and data analysis**

The predatory activity of *M. albidus* against *Ae. albopictus* and *Cx. pipiens* larvae (1st plus 2nd instars) after 24 and 48 h is presented as mean ± standard deviation. The predatory activity of *M. albidus* against both species over time was assessed by means of the Wilcoxon rank sum test with continuity correction. The sum of the number of 3rd and 4th instars and pupae in drums with 100 and 500 copepods was compared to those found in drums without copepods (only drums placed in shady areas were considered).

The Wilcoxon rank sum test with continuity correction was used also to assess the effect of drum exposure to direct sunlight on *M. albidus* and mosquito populations. The numbers of *M. albidus* and of 3rd instars and pupae of *Ae. albopictus* and *Cx. pipiens* in drums exposed to the sun were compared with those counted in drums placed in shady areas.

Paired comparisons were performed between the number of immature mosquitoes of the 2 species in drums with and without copepods exposed or unexposed to direct sunlight.

The survival and predatory capacity of *M. albidus* in catch basins was also evaluated by means of the Wilcoxon rank sum test.

**RESULTS**

**Predatory capacity of *M. albidus***

Data on the predatory capacity of *M. albidus* under conditions of prey saturation with 50 1st
Macrocyclops albidus to control mosquitoes

and 2nd instars of *Ae. albopictus* and *Cx. pipiens* are the following: 28.36 ± 3.56 *Ae. albopictus* larvae predated within 24 h, and 37.92 ± 4.47 within 48 h, while on average 1.9 ± 2.9 and 3.2 ± 3.0 larvae died at the same times in the controls; 29.90 ± 4.23 *Cx. pipiens* larvae predated at 24 h and 39.60 ± 3.27 at 48 h, while the average numbers of larvae that died in the controls were 1.0 ± 1.73 at 24 h, and 1.60 ± 2.10 at 48 h.

Drums’ carrying capacity for *M. albidus* over time

Stable drum colonization in the drums inoculated with 100 and 500 individuals was observed, with a constant increase of the number of individuals, which reached the maximum density in the period August–September (Fig. 2A). In October, the copepod population decreased to the initial density measured during the 1st sampling after inoculation. In the control drums, where *M. albidus* had not been inoculated, no accidental colonization occurred. The Wilcoxon’s test showed statistically significant differences in the copepod density between the control and the treated drums, but not between the drums inoculated with the 2 copepod densities (Table 1).

Macrocyclops albidus predation against *Ae. albopictus* and *Cx. pipiens* in drums: The presence of immature individuals of both mosquito species was detected contemporarily for the 2 species for the first time on May 25 in the control drums. In total, in the control drums, the number of 3rd and 4th instars and pupae was 814 for *Ae. albopictus*, and 10,464 for *Cx. pipiens*. Only 2 *Ae. albopictus* mature larvae were found in one single drum containing 100 copepods, on August 23. The Wilcoxon test showed a statistically significant difference between the density of *Ae. albopictus* larvae and pupae in the drums with and without copepods (Table 1). As shown in Fig. 2B, this difference occurred during the period July–October; as during the preceding weeks, *Ae. albopictus* was detected at very low density level both in treated and control drums. On the contrary, no statistically significant difference was found between the density of *Ae. albopictus* in drums inoculated with the 2 initial densities of *M. albidus* (Table 1 and Fig. 2B).

Both the copepod densities (COP100 and COP500) were effective in reducing the number of *Cx. pipiens* larvae with respect to the control drums (Table 1 and Fig. 2C), while no statistically significant difference was observed between the 2 treatments (Table 1 and Fig. 2C). In the drums with *M. albidus*, an infestation by *Cx. pipiens* occurred on July 9, when more than 10 egg batches were counted.

The percentage of reduction of *Ae. albopictus* density in the drums with 100 and 500 *M. albidus* individuals with respect to the control drums was 99.90 ± 0.35% and 100.0 ± 0.0%, respectively.
For *Cx. pipiens*, the percentage of reduction was 88.69 ± 13.51% in the drums inoculated with 100 copepods and 84.65 ± 18.48% in those inoculated with 500 copepods.

### Influence of drum exposure to direct sunlight on *M. albidus* and mosquito densities (2007)

The average minimum temperatures of the water were 17.5 ± 3.9°C in the drums placed in shady areas and 16.8 ± 5.1°C in those placed in sunny areas, while the maximum average temperatures were 23.0 ± 3.0°C and 29.9 ± 7.1°C, respectively. The average numbers of *M. albidus*, *Ae. albopictus*, and *Cx. pipiens* specimens in the drums placed in sunny or shady areas are reported in Table 2.

In the drums inoculated with *M. albidus*, no statistically significant difference was found in the copepod densities between drums placed in sunny or shady areas, even though the absolute average number of *M. albidus* was higher in the drums placed in the shade compared to those placed in sunny areas (Tables 2 and 3). The average densities of 3rd and 4th instars and pupae of both species were not statistically different between the drums placed in the sun or in the shade (Tables 2 and 3). In the control drums, no accidental inoculation occurred, and copepods were never found. The average numbers of mosquito larvae of the 2 species were lower in the drums exposed to the sun than in those placed in shady areas, even though the differences were not statistically significant (Tables 2 and 3).

Concerning the impact of drum exposure to the sun, under sunny conditions the average densities of *Ae. albopictus* and *Cx. pipiens* in the drums with *M. albidus* were statistically lower with respect to the control drums (Tables 2 and 3): in the drums placed in the shade, the average densities of the 2 mosquito species were low, and the differences between copepod-treated drums and controls were not statistically significant (Tables 2 and 3).

### Macrocyclops albidus’ density in drums 1 year after inoculation (2008)

On April 25, 2008, 1 year after the introduction of *M. albidus* in the drums, copepods were still present in all of them, with no difference in their density between drums inoculated in 2007 with 100 or 500 copepods (Table 4). An increase of *M. albidus* density was observed from the last 10 days of July to the end of September, with no difference between the drums inoculated in 2007 with 100 or 500 copepods. The 2008 mean seasonal *M. albidus* density was 91.48 ± 96.73 copepods per net (Fig. 3A), similar to the mean seasonal density registered in 2007 (89.10 ± 85.66 copepods per net).

### Efficacy in mosquito larvae control during the 2nd year after inoculation

Apart from some *Culiseta annulata* (Schrank) larvae found in the last 2 samplings in 2008, only

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### Table 1. Wilcoxon rank sum test with continuity correction, comparing the number of *Aedes albopictus*, *Culex pipiens*, and *Macrocyclops albidus* in drums inoculated with 100 copepods (COP100) and 500 copepods (COP500) between them and with the control drums.

<table>
<thead>
<tr>
<th>M. albidus treatment</th>
<th>Ae. albopictus</th>
<th>Cx. pipiens</th>
<th>M. albidus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COP500 Control</td>
<td>COP500 Control</td>
<td>COP500 Control</td>
</tr>
<tr>
<td>COP100</td>
<td>W 78 6.5</td>
<td>70.0 21.0</td>
<td>78 138</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.00001</td>
<td>&lt; 0.004</td>
<td>&lt; 0.00001</td>
</tr>
<tr>
<td>COP500</td>
<td>W</td>
<td>6.0</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.00001</td>
<td>&lt; 0.005</td>
<td>&lt; 0.00001</td>
</tr>
</tbody>
</table>

### Table 2. Average number of *Macrocyclops albidus*, *Aedes albopictus*, and *Culex pipiens* in the drums placed in sunny or shady conditions (mean ± standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>M. albidus</th>
<th>Ae. albopictus</th>
<th>Cx. pipiens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.00 ± 0.00</td>
<td>25.62 ± 25.70</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>M. albidus</td>
<td>11.92 ± 10.69</td>
<td>15.41 ± 21.33</td>
<td>11.92 ± 10.69</td>
</tr>
<tr>
<td>Shade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.00 ± 0.00</td>
<td>110.42 ± 87.17</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>M. albidus</td>
<td>124.79 ± 80.29</td>
<td>30.75 ± 42.34</td>
<td>124.79 ± 80.29</td>
</tr>
</tbody>
</table>
Aedes albopictus (1,342 4th instars and pupae) and Culex pipiens (5,586 4th instars and pupae) specimens were found in the drums during the 2nd year. In control drums, the detection of Aedes albopictus and Culex pipiens larvae occurred for the first time on May 23. In the drums with copepods, Aedes albopictus larvae were never recorded, while the presence of Culex pipiens larvae was detected for the first time on July, 5, when more than 10 egg batches were counted on the water surface. The Wilcoxon rank sum test showed a statistically significant difference in Aedes albopictus density between control and treated drums (Table 4 and Fig. 3B), and the percent reduction was 100%, as mosquito larvae were never found in the drums with Macrocyclops albidus.

Culex pipiens density was statistically lower in the treated drums than in the control ones, particularly from July 19 to August 15. Data collected on the other sampling dates showed lower numbers of Culex pipiens immature mosquitoes (Fig. 3C), and the percent reduction observed in the drums with copepods was on average 68.78 ± 31.62%.

Survival and predation capacity in catch basins (2007)

During the trial, the rainfall was scarce; the total rainfall measured 64 mm, and only one heavy rain occurred on August 31. After 103 days from the inoculation of Macrocyclops albidus, 60.0% of the catch basins were still colonized by copepods, but their density showed a high variability (Table 5). The trend of the copepod and mosquito population in control and copepod-treated catch basins is depicted in Fig. 4A–C. In the catch basins in which Macrocyclops albidus survived, their mean density decreased from the date of release to the beginning of August, and then it started to increase again until the end of the trial on October 20. Culex pipiens and Aedes albopictus were

Table 3. Wilcoxon rank sum test with continuity correction, comparing the number of Aedes albopictus (Ae), Culex pipiens (Cx), and Macrocyclops albidus (Ma) in drums with and without copepods placed in sunny and shady positions. The comparison between control drums without copepods in sunny and shady conditions was not performed.

<table>
<thead>
<tr>
<th>Sun</th>
<th>Species</th>
<th>W</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>With M. albidus</td>
<td>Ma</td>
<td>64</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Ae</td>
<td>36</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Cx</td>
<td>34.5</td>
<td>0.83</td>
</tr>
<tr>
<td>Shade</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without M. albidus</td>
<td>Ma</td>
<td>64</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>Ae</td>
<td>0</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>Cx</td>
<td>8</td>
<td>&lt; 0.02</td>
</tr>
</tbody>
</table>

1 NA, nonapplicable; Idem, the cell contains the results of a comparison already presented in another cell.

Table 4. Wilcoxon rank sum test with continuity correction, comparing the number of Aedes albopictus, Culex pipiens, and Macrocyclops albidus in drums in which 100 or 500 Macrocyclops albidus had been introduced 1 year before.

| No. M. albidus | 500 | Control without copepods | 500 | Control without copepods | 500 | Control without copepods |
|               |     |                          |     |                          |     |                           |
| 100           |     |                           |     |                          |     |                           |
| W             | 98  | 14                        | 85  | 38                       | 107.5 | 192                       |
| P             | NA  | < 0.00001                 | 0.56| < 0.006                  | 0.68  | < 0.00001                 |
| 500           |     |                           |     |                          |     |                           |
| W             | 14  |                           | 46  |                          | 193  |                           |
| P             | < 0.00001 |                       | < 0.02 |                     | < 0.00001 |   |
the sole mosquito species detected during the course of the study (Figs. 4B, 4C). The predatory activity of *M. albidus* significantly reduced the average number of larvae of the former species (*Cx. pipiens*: $W = 7, P = 0.026$) but not of the latter (*Ae. albopictus*: $W = 13.5, P = 0.179$).

Factors affecting *M. albidus* survival conditions in catch basins (2008)

*Weather conditions and oxygen tension:* During the trial, the weather was particularly rainy (342 mm of rain from April 26 to August 5),
with some heavy rainfall events (on July 21, 80 mm of rain was measured; Fig. 5), but no difference was found in the number of colonized catch basins before and after the heavy rains. The average oxygen tension measured in the catch basins ranged between 0.0 and 1.03 mg/l.

**Macrocyclops albidus survival:** In 2008, after the 1st release of *M. albidus* (on April 26), the percentage of catch basins with copepods decreased abruptly (minimum value: 6.0%) on June 28 (Fig. 6A). One month after the 2nd release, the percentage of colonized catch basins was 54.8%, and the numbers decreased again to 6.67% at the 3rd sampling, on August 5 (Fig. 6B).

**Predation capacity of *M. albidus* in catch basins:** *Culex pipiens* larvae were found at the 3rd sampling on May 10 in 2 catch basins, while *Ae. albopictus* was found for the first time on June 6 in 2 catch basins. In the catch basins where *M. albidus* was present, the percentage of reduction after the 1st inoculation was 70.0% for *Cx. pipiens* and 76.2% for *Ae. albopictus*. The statistical analysis did not show significant differences in the mean number of 3rd and 4th instars and pupae between catch basins with or without copepods for either of the 2 species (*Cx. pipiens: W = 7, P = 0.8778; Ae. albopictus: W = 6, P = 0.6198; Fig. 7A, 7B). After the 2nd inoculation, the percent reduction of the mosquito population was 10.5% for *Cx. pipiens* and 68.9% for *Ae. albopictus*, but the statistical analyses showed no significant difference in the mosquito population of both species (*Cx. pipiens: W = 4, P = 1; Ae. albopictus: W = 0, P = 0.1; Fig. 7A, 7B).

**DISCUSSION**

If the catch basins from which the copepod population disappeared are excluded from the evaluation of the predatory efficacy of *M. albidus*, the average reduction between copepod-treated catch basins and controls is 77.71 ± 12.93% and in this case the difference was statistically significant. Under laboratory conditions, *M. albidus* showed a very high predatory capacity against 1st instar larvae. On average, 30 *Cx. pipiens* larvae were killed within 24 h, and 40 larvae were killed within 48 h. When *Ae. albopictus* was offered as the prey, in 24 h, on average 28 larvae were killed, and these data are similar to the findings by Rey et al. (2004), Calliari et al. (2003), and Tranchida et al. (2009), but lower than 44 larvae predated in 24 h found by Marten 1990b) and Marten et al. (1994a).

Marten and Reid (2007) proposed that 30–40 larvae killed per day should be considered as the minimum threshold for the predatory capacity above which the species could be recommended for the use in mosquito control programs. In our opinion, the evaluation of the predatory efficacy under laboratory conditions provides important information on the efficacy of *M. albidus* as a means of biological control, but its use under field conditions is still to be established.

<table>
<thead>
<tr>
<th>Days from inoculation</th>
<th>N</th>
<th>% colonized catch basins</th>
<th>No. <em>Ma</em>2 dippers (mean ± SD, only colonized catch basins)</th>
<th>No. <em>Ma</em>2 dippers (mean ± SD, all catch basins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>100.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>80.00</td>
<td>8.71 ± 5.44</td>
<td>6.10 ± 6.12</td>
</tr>
<tr>
<td>28</td>
<td>10</td>
<td>80.00</td>
<td>3.67 ± 1.51</td>
<td>2.20 ± 2.20</td>
</tr>
<tr>
<td>49</td>
<td>10</td>
<td>80.00</td>
<td>8.20 ± 5.27</td>
<td>4.30 ± 5.40</td>
</tr>
<tr>
<td>70</td>
<td>10</td>
<td>70.00</td>
<td>8.20 ± 642</td>
<td>4.10 ± 6.08</td>
</tr>
<tr>
<td>84</td>
<td>10</td>
<td>70.00</td>
<td>14.14 ± 18.69</td>
<td>9.90 ± 16.72</td>
</tr>
<tr>
<td>103</td>
<td>10</td>
<td>60.00</td>
<td>4.67 ± 2.88</td>
<td>2.80 ± 3.22</td>
</tr>
</tbody>
</table>

1 SD, standard deviation.

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Fig. 5. Rainfall measured in San Carlo (FE) in the period April 26–August 5, 2008.
conditions is affected by many factors that need to be evaluated, such as the dimension of the water container, the water temperature, the nutritional state, and food preferences in the presence of different preys (Micieli et al. 2002, Rao Ramakrishna and Kumar 2002, Kumar and Ramakrishna Rao 2003, Calliari et al. 2003, Rey et al. 2004).

In our study, the good performances obtained by *M. albidus* under laboratory conditions were not fully confirmed by the trials performed in drums and catch basins. In the drums, the predatory activity against *Cx. pipiens* seemed to be lower than that of *Ae. albopictus*, thus confirming data reported by other authors (Marten and Reid 2007). The average percentage reduction of *Cx. pipiens* ranged from 86.7% in 2007 to 73.0% in 2008. Due to the accumulation of organic materials (mainly leaves) in the course of the season, the drums were quite attractive for *Cx. pipiens* females, and high numbers of egg batches and larvae were found. Under these conditions, the number of *Cx. pipiens* larvae overpassed the feeding capacity of the *M. albidus* population. On the contrary, the drum’s colonization by *Ae. albopictus* was much lower both in shady and in sunny positions, and *M. albidus* achieved a complete control against this species during the 2 year study, after one single inoculation.

According to Marten et al. (1994b), the population of *M. albidus* inoculated in water drums disappeared after 3 months, due to the high summer temperatures of the water, which reached 37–38°C. In our trials, in 2007 the temperature was higher than 37°C for 21 days, and it surpassed 40°C for 9 consecutive days, with a peak temperature of 42°C. It has been reported that many copepod species during the summer time may move towards the bottom, where the water temperature can be lower. According to our observations, the survival of the copepod population cannot be ascribed to this behavior, as only 50–70 liters of water remained in the drums, forming a water column of 20–30 cm along which the temperature was likely the same. The average

**Culex pipiens**

![Graph of Culex pipiens with and without copepods](image)

**Aedes albopictus**

![Graph of Aedes albopictus with and without copepods](image)

Fig. 6. Percentage of *Macrocyclops albidus* survival in catch basins at increasing time periods in the 2 trials. Trial 1: inoculation on April 26, 2008; trial 2: inoculation on June 26, 2008.

Fig. 7. Trends of the mean density of 3rd and 4th instars and pupae of *Culex pipiens* and *Aedes albopictus* in 30 catch basins with and without *Macrocyclops albidus*. Trial 1: from the 1st inoculation on April 26 to June 28, 2008; Trial 2: from the 2nd inoculation on June 28 to August 5, 2008.
temperatures measured in the early morning were higher in the drums placed in the shady positions than in the sunny ones. This apparently contradictory finding can be explained by the fact that the drums exposed to the sun contained less water due to evaporation, and this allowed a more rapid decrease of the temperature during the night. The *M. albidus* strain used in our study also showed high tolerance to the low winter temperatures. In fact, during the winter of 2007–08, the average minimum daily temperature was around 0°C, but 60 days of below-zero temperatures were registered. In the drums, the water froze at the surface, but below the ice, *M. albidus* could successfully overwinter. These ecological traits make *M. albidus* suitable as a means of biocontrol of the mosquito populations in drums instead of fishes, which usually do not survive the winter. In order to promote the use of *M. albidus* as a biocontrol agent, it is necessary to inform the users about good management practices to maintain a healthy and numerous copepod population. For example, it is necessary to replenish the water in the drums, and if a complete renewal of the water is needed to clean the drum, part of the copepod’s population should be collected before and reintroduced immediately after the cleaning operations. The normal use of the water does not lead to the extinction of the colony, because *M. albidus* tends to live near to the walls and at the bottom of the drums (Marten et al. 1994b).

According to our results, in the catch basins, both in 2007 and 2008, the predatory activity of *M. albidus* against *Ae. albopictus* led to a dramatic decrease of the mosquito population, but this very good result was not sufficient to promote the use of the copepods for biocontrol purposes in such kinds of breeding habitats because of the low percentage of survival in the catch basins after relatively short periods. In fact, the copepod population rapidly decreased: In 2007, 103 days after inoculation, *M. albidus* survived in 60% of the catch basins, while in 2008, we observed the disappearance of the copepods in more than 94% of the catch basins after 40 days. In a similar trial in Colombia, *Mesocyclops longisetus* Thiebaud was present in 49.2% of the catch basins after 8 months (Suárez-Rubio and Suárez 2004). In a study carried on in Australia, the catch basins’ colonization by *Mesocyclops* sp. remained stable for 1 year, resistant to flushing events during the rainy season, and it survived in the wet soil during the dry season (Kay et al. 2002). In our conditions, the nonsuitable water quality, and in particular the low oxygen tension measured in the catch basins (0.0–1.03 mg/l), might have been the main reason for the decrease until disappearance of *M. albidus*. The response of *M. albidus* and other benthic cyclopoid copepods to hypoxia and anoxia was investigated by Tinson and Laybourne-Parry (1985). This species died within 6 h when confronted by totally anoxic conditions and survived 4 days at oxygen saturation levels as low as 25%. It is likely that the fermentation processes of the abundant organic material might have caused the decrease of the oxygen tension and the production of potentially toxic substances, like hydrogen, methane, ammonia, hydrogen sulfide, and phosphine, the toxic effects of which against copepods were not investigated in our study. On the contrary, the heavy rains, which were expected to lead to leaching, did not affect the survival and density of the copepod population, as demonstrated by the samplings performed before and after the heavy rain episodes.

For their use in other artificial water containers, it has to be considered that *M. albidus* is not resistant to a complete absence of water, so it is not suitable for small artificial water containers, like those used in the cemetery for the flowers, or the plant pot saucers (Marten 1990a, Tranchida et al. 2009), unless something is placed in the bottom of the container to hold moisture when the rest of the container dries out.

The ability of *M. albidus* to reproduce in artificial water habitats, and to rapidly increase its population starting from an inoculative release make it possible to evaluate how many copepods are to be released according to the characteristics of the breeding habitat. The main aim should be to reach the right copepod density able to achieve the control over the mosquito population within the shortest time. Concerning how many individuals should be recommended for the initial inoculative release, our study found that 100 copepods per drum of 200-liter capacity inoculated during spring were sufficient to ensure mosquito control for the entire summer season. In fact, no difference was found in the level of control of the *Ae. albopictus* larval population between drums inoculated with 100 or 500 copepods per drum. With respect to *Cx. pipiens*, Fig. 2C shows that in 3 samplings performed in the period of highest mosquito density (August), a difference occurred in the number of immature mosquitoes between COP100 and COP500 drums, but not always in the same direction. We can conclude that copepods cannot keep the *Cx. pipiens* population in drums as low as they do with *Ae. albopictus*, but there is no statistically significant difference in this performance between the 2 copepod densities studied, as confirmed by the Wilcoxon test ($P = 0.93$).

As a conclusion we can say that *M. albidus* is a beneficial organism of great interest, and its well-known poliphagy represents an advantage, because it allows the species to be released early in the season when the target prey is not present, yet. *Macrocyclops albidus* proved to be very efficient against *Ae. albopictus* and suitable to be used in some kinds of water habitats, like
drums, ponds for animals, pots for aquatic plants, etc. In these types of breeding habitats, the predatory capacity of *M. albidus* during the period of maximum *Cx. pipiens* population development was not so efficient as with *Ae. albopictus*, maybe due to the higher density of the former species in comparison to the latter. Currently, only partially positive results do not allow us to recommend the use of *M. albidus* in catch basins under urban conditions in northern Italy as the sole means of biological control, as this would mean a very intense copepod release program in the course of the season, and its costs would not be lower than performing larvicide treatments. For an integrated control program, the use of copepods could be effective in all kinds of water containers in which chemical insecticides are not to be used, but also in catch basins together with long-lasting *Bti*-based products, if and when they will be available on the market. Marten and Bordes (1993) found that routine application of *Bti* when treating tires or ground-water habitats with cyclopoids provides immediate and long-term control of larvae belonging to species for which cyclopoids are effective. Copepods also showed low sensitivity to some chemical mosquito larvicides; therefore, it is possible to reintroduce *Macrocylops* to catch basins on a regular basis with *Bti* or selective chemical mosquito larvicides, and this could be worth further investigations.

**ACKNOWLEDGMENTS**

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**REFERENCES CITED**


