Juvenile Turtles for Mosquito Control in Water Storage Tanks

GERARDO BORJAS,[1] GERALD G. MARTEN,[2] EDUARDO FERNANDEZ, AND HECTOR PORTILLO

División de Enfermedades de Transmisión Vectorial, Ministerio de Salud Pública de Honduras, Tegucigalpa, Honduras

J. Med. Entomol. 30(5): 943–946 (1993)

ABSTRACT Juvenile turtles, *Trachemys scripta,* provided highly effective control of mosquito larvae in cement tanks (pilas) where water was stored for household cleaning. When single turtles were introduced to tanks with histories of high mosquito production, nearly all turtles remained in good health and no mosquito larvae survived to the pupal stage. Families welcome turtles in their water storage containers in Honduras. Humane conditions for turtles can be assured by providing small quantities of table scraps to supplement their diet and by placing a small floating platform in the tank for basking. Although turtles can serve as alternate hosts for *Salmonella,* available evidence suggests that turtles in tanks should not be a source of human infection. Further confirmation that there is no *Salmonella* hazard should precede routine use of turtles for mosquito control.

KEY WORDS turtles, mosquito control, biological control

IN THE COURSE of the Integrated Dengue Control Project in El Progreso, Honduras (Fernández et al. 1992), the need arose for a method to provide long-term control of *Aedes aegypti* (L.) larvae in cement laundry tanks (pilas). More *Ae. aegypti* larvae and pupae are found in these tanks than in any other kind of container in the city.

Most homes have one of these tanks adjacent to the house to store water for washing clothes and dishes. Drinking water is not stored in the tanks, nor are clothes or dishes washed in the tanks themselves; water is dipped out of a tank for use.

No fully satisfactory method exists for controlling mosquito larvae in these tanks. Larvicides require repeated application. We tried cyclopoid copepods and small fish, but sooner or later the copepods disappeared down the drain (Marten et al. 1993), and the fish died, apparently because of stress resulting from fright when people used the tanks. Aquatic predators such as copepods and fish were also killed by household chemicals (e.g., bleach) that sometimes got into the tanks.

We decided to try juvenile turtles because they are predators of aquatic invertebrates such as mosquito larvae, they are too large to be lost down a drain, and they should be relatively resistant to small quantities of household chemicals. The sides of the tanks are too steep and smooth for turtles to climb. If people regard the turtles as pets, they might make an effort to maintain them in their tanks.

[1] Departamento de Biología, Universidad Autónoma de Honduras, Tegucigalpa, Honduras.

[2] New Orleans Mosquito Control Board, 6601 Lakeshore Drive, New Orleans, LA 70126 and Department of Tropical Medicine, Tulane University, New Orleans, LA 70112–2824.

Materials and Methods

Trachemys scripta (Schoepff) (formally *Pseudemys scripta*) was used for the trials. The biology of *T. scripta,* which is common throughout Central America and eastern North America, has been thoroughly described by Gibbons (1990).

Trachemys scripta is the main species of turtle in the international pet trade. We purchased our first three turtles from a pet shop in Honduras; they presumably were imported from a turtle farm in southeastern United States. We obtained the rest of our turtles directly from a farm in Louisiana.

Predation Experiments. Single turtles (≈6 mo old) were placed in plastic tubs (40 cm diameter) partially filled with water containing several hundred *Ae. aegypti* pupae and larvae (all instars) to observe which stages were eaten by the turtles.

Another series of experiments was conducted in three cement laundry tanks next to our laboratory. The tanks (55 cm long, 45 cm wide, 40 cm deep), smaller than most residential tanks, were half filled with water. A 6-mo-old turtle was introduced to each tank along with 600 third- and fourth-instar *Ae. aegypti.* In the next experiment, we placed 500 third- and fourth-instar *Ae. aegypti* into each of three tanks containing a 1-yr-old *T. scripta.* Finally, we placed 500 fourth instars in a tank with an adult *T. scripta* (18 cm carapace length) from a turtle farm. In all experiments, time for the turtles to eat larvae was observed.

Another experiment was conducted in the same three tanks after the first experiments were completed. The purpose of the second experiment was to see how the turtles responded to *Ae. aegypti* larvae that hatched from eggs as they would in a real laundry tank. A single 6-mo-old turtle was placed in each of three tanks along with 500 *Ae. aegypti* eggs. Each tank was nearly full of water (but not full enough to allow the turtles to escape), and each tank contained some leaves to provide food for the larvae. The number of larvae in each tank was counted 7 d after introducing the eggs, a time when most of the larvae would reach fourth instar. The same procedure was followed simultaneously with three control tanks without turtles.

Additional predation experiments were conducted in three laundry tanks at people's homes. The size of these tanks (≈150–190 cm long, 90 cm wide, 85 cm deep) was typical of most homes in the city. The purpose was to see how the turtles would perform in tanks that were used on a regular basis. We selected tanks with a history of large numbers of *Ae. aegypti* larvae.

Immediately before the experiments at people's homes, we removed naturally occurring larvae or pupae from the tanks, counted, and discarded them. Then a 6-mo-old turtle and a known number of first-instar *Ae. aegypti* (250–400 larvae) were introduced to each tank. The larvae were monitored on a daily basis until all of them disappeared or emerged as adult mosquitoes. The same procedure was followed with a control tank that did not have a turtle.

Field Trials. We selected six residential tanks for our first set of field trials. Each of the tanks had a history of large numbers of mosquito larvae, mostly *Ae. aegypti* but also some *Culex* spp. We started the trials by counting the number of naturally occurring mosquito larvae and pupae in each tank and introducing a 6-mo-old turtle, leaving the larvae and pupae in the tank with the turtle. The housewives then used the tanks in a normal fashion. No special care was provided for the turtles except to ensure they were not lost when a tank was cleaned.

The numbers of larvae and pupae in each tank were counted on a weekly basis for ≈2 mo. At each inspection, the turtle was examined to see if it appeared to be in good health. Two tanks without turtles in the same neighborhood were monitored at the same time as controls.

In the second set of field trials, we introduced single 8-mo-old turtles to 30 residential laundry tanks and subsequently inspected the tanks for mosquito larvae on a monthly basis. We placed a small floating platform in each tank so the turtles could climb out of the water. Housewives reported that the platforms were used frequently. Each platform was a piece of Masonite fiberboard (10 by 15 cm) held near the edge of a tank by a vertical wire hooked over the top of the tank and extending to the bottom of the tank through a hole in the middle of the platform.

The weight and shell length of the turtles was measured once every 3 mo. At the same time, each turtle was held in a plastic bowl with 50 ml of water for 3 d. This water containing turtle feces was analyzed for the presence of *Salmonella* by a specialist laboratory.

***Salmonella* Experiment.** The purpose of this experiment was to observe the quantity of *Salmonella* in a tank that contained a *Salmonella*-infected turtle. Several 4-mo-old *T. scripta* (produced on a farm from eggs not treated to eliminate *Salmonella*) were held in plastic bowls with 50 ml of water for 3 d to collect their feces for *Salmonella* analysis. A turtle, identified by this procedure to be strongly positive for *Salmonella*, was placed in a tank (190 by 85 by 85 cm) at the laboratory. The tank was filled with fresh tap water (without chlorine), and the turtle was fed by placing about five grains of rice in the tank each day and several thousand fourth-instar *Ae. aegypti* each week. The water in the tank was not changed.

One-liter water samples were taken from the tank 2 and 4 wk after introducing the turtle and analyzed for *Salmonella*. The turtle was removed from the tank at the end of the fourth week, and its feces were collected in 50 ml of water for 3 d and analyzed for *Salmonella*. One week later, feces were again collected and analyzed.

Results

Predation Experiments. When observed in the plastic tubs, 6-mo-old turtles ignored first-instar *Ae. aegypti*. They ate second instars and fed particularly vigorously upon third and fourth instars and pupae.

When third and fourth instars were placed in the three small tanks with 6-mo-old turtles, every turtle ate the 600 larvae in <20 min. The 1-yr-old turtles consumed their 500 larvae within 30 min. The adult turtle also ate all the larvae but less quickly than the juvenile turtles; several hours were needed for it to eat the 500 larvae.

When 500 eggs were placed in the small tanks at the laboratory with 6-mo-old turtles, 85% of the eggs hatched within 1 d. No larvae survived in the tanks with the turtles. An average of 285 ± 56 (mean ± SE) larvae was present in the three control tanks at the end of 7 d.

Similarly, none of the larvae introduced to the three residential tanks with turtles survived to the fourth instar; 16% of the larvae in the control tank completed their development to the pupal stage.

Field Trials. The tanks used for the first field trials contained large numbers of *Ae. aegypti* lar-

Table 1. Mosquito larvae and pupae observed in laundry tanks (pilas) after introducing turtles

Parameter	With turtle	Without turtle
No. tanks	6	2
Initial no. larvae[a]	108 ± 50	80 ± 57
Initial no. pupae[a]	12 ± 4	4 ± 4
Total no. inspections	33	7
No. 3rd and 4th instar larvae[b]	0.2 ± 0.1	67 ± 30
No. pupae[b]	0	36 ± 35

[a] Number (mean ± SE) of larvae or pupae naturally present in tanks immediately before turtles were introduced.
[b] Number (mean ± SE) of larvae or pupae present in tanks during monitoring period.

vae and pupae before the turtles were introduced (Table 1). Third and fourth instars in tanks with turtles were reduced by more than 99% compared with larvae in control tanks. We never observed pupae in a tank with a turtle. After the turtles were removed at the end of 2 mo, the tanks once again contained large numbers of larvae and pupae.

The turtles were well received by the people. Many gave the turtles names, expressed affection for them, and took precautions to prevent their loss. Most of the turtles received nutritional supplementation from bits of food that fell into the water when dishes were washed, or from table scraps (e.g., a piece of tortilla or a few grains of rice) that were thrown into the tank for the turtle from time to time. The turtles in the first field trials, which did not have platforms for basking, appeared to remain in normal health throughout the 2 mo of observation.

The turtles in the second field trials were also effective in controlling mosquitoes. Thousands of *Ae. aegypti* hatched in the tanks with these turtles, and no pupae were observed during 6 mo of monitoring. (Results from the second field trials are not reported in detail because they are still in progress.)

Forty-seven percent of the turtles in the second field trials disappeared from their tanks during the 6 mo following their introduction. Presumably, most of them escaped, although some may have been stolen. Only one turtle died during the 6-mo period; a box of detergent (with bleach) was accidentally spilled into its tank. With one exception (a turtle that grew little and appeared sick), the turtles appeared to be in normal health. The growth in length of different turtles ranged from 1.6 to 5.3% per month (averaged over the 6-mo period). Weight growth ranged from 8 to 20% per month. None of the turtles tested positive for *Salmonella*.

***Salmonella* Experiment.** No *Salmonella* were detected in the tank water sampled 2 and 4 wk after introducing a *Salmonella*-infected turtle. No *Salmonella* were detected in the turtle feces collected at the end of the experiment. None of the thousands of *Ae. aegypti* larvae placed in the tank as food for the turtle survived to the adult stage.

Discussion

The use of juvenile turtles appears to be a convenient, effective, and long-lasting method of biological control for mosquito larvae in laundry tanks. Our turtles ate large numbers of second to fourth instars in the laboratory experiments, and no larvae were known to survive to the pupal stage in field trials. Because our adult turtle also ate mosquito larvae, we can expect turtles to continue providing effective mosquito control as they become older.

Escape is the most significant limiting factor when using turtles for mosquito control. A tank cannot be filled to the top or a turtle will quickly escape. It should be possible to reduce the escape rate substantially by drilling a small hole a few inches below the top of each tank to prevent it from filling completely.

Tanks are a humane habitat for turtles if they have a small floating platform for basking. The turtles could develop shell or skin problems if they never leave the water. It is also important that the turtles get enough food. Families should be encouraged to provide table scraps on a regular basis, although foods (e.g., chicken) that might be contaminated with *Salmonella* should be avoided. Food supplementation does not interfere with turtle predation on mosquito larvae.

It should not be difficult to establish a supply of turtles for mosquito control. Commercial turtle farms in the southeastern United States produce several million *T. scripta* each year. Any country that wants turtles for mosquito control should be able to produce adequate numbers of a native variety at low cost.

The importation of turtles for mosquito control is an ecologically sensitive matter. We imported a small number of *T. scripta* for our field trials because *T. scripta* is native to Honduras, and large numbers of imported *T. scripta* are present because of the pet trade. Non-native species should not be imported for mosquito control because they could damage native fauna if they escape. Wild-caught turtles should not be used because natural populations could be depleted, and turtles from nature might be infected with *Salmonella*.

Turtles can serve as alternate hosts for *Salmonella* that cause human gastroenteritis (Lamm et al. 1972, D'Aoust et al. 1990), but they cannot serve as hosts for *Salmonella* that cause typhoid. The turtles in our field trials came from turtle farms where the eggs were treated with an antibiotic to eliminate *Salmonella*. The treatment is simple, inexpensive, 100% effective, and applied by commercial turtle producers to millions of eggs every year (Michael-Marler et al. 1983, Siebeling et al. 1984).

Salmonella might be introduced to a laundry tank through contaminated bird droppings, dirty hands, or scraps of contaminated food, but infection of *Salmonella*-free turtles by tank water does not seem likely. To be infected, a turtle normally must be exposed to a *Salmonella* concentration exceeding 1,000 bacteria per ml (Brown 1978). The water in laundry tanks is usually quite clean, and clean water should not be suitable for *Salmonella* propagation (Ronald Siebeling, personal communication). Moreover, the tanks contain >1,000 liters of water, which dilutes bacteria to very low concentrations. Although a turtle might be infected by eating contaminated food, none of our turtles in laundry tanks became positive for *Salmonella* during the field trials.

Even if a turtle were infected, it does not appear likely it would be a source of infection for people who use the tank. A person must ingest at least a million *Salmonella* cells to be infected (Ronald Siebeling, personal communication), but the concentration in tank water should be low. It appears contaminated tank water could be a source of human infection only if the bacteria are amplified on food contaminated by the water. This is not a likely scenario in Honduras because it is customary to consume perishable foods the same day they are prepared.

Our *Salmonella*-infected turtle stopped releasing *Salmonella* when we kept it in a tank. This result is compatible with the view that tank water is not a favorable medium for *Salmonella*, but it does not tell us what will happen if a turtle continues to release *Salmonella*. More experiments are needed to document conclusively whether turtles can be infected with *Salmonella* under tank conditions and, if so, whether they can serve as a source of human infection.

In conclusion, juvenile turtles offer impressive possibilities for mosquito control, but there are risks of misuse. Turtles should be used only under humane conditions and only in a way that does not endanger indigenous fauna. Although available evidence suggests that the risk of *Salmonella* infection is low or nil, the use of turtles for mosquito control should proceed with extreme caution until the *Salmonella* issue is resolved.

Acknowledgments

We wish to thank Mary Cush, Reyna Lozano, Consuelo Gutierrez, Catalina Sherman, and other members of the Integrated Dengue Control Project for their contributions to the experiments and field trials. Emmett Aucoin (Cajun Farm, Pierre Part, LA) provided turtles and information on turtle farming. Ronald Siebeling (Department of Microbiology, Louisiana State University) provided the *Salmonella*-infected turtle, analysis of samples for *Salmonella*, and information on *Salmonella* in turtles. The research was funded by The Rockefeller Foundation (Health Sciences Division) through contracts with the Pan American Health Organization, the International Center for Community-Based Health Research (Johns Hopkins University), and the Tulane School of Public Health and Tropical Medicine.

References Cited

Brown, M. L. 1978. Salmonellosis in the baby green turtle. M.S. thesis, Louisiana State University, Baton Rouge.

D'Aoust, J. Y., E. Daley, M. Crozier & A. M. Sewell. 1990. Pet turtles: a continuing international threat to public health. Am. J. Epidemiol. 132: 233–238.

Fernández, E., I. Lagos, H. Portillo & G. Borjas. 1992. Community-based *Aedes aegypti* control programme in Honduras, pp. 279–282. *In* S. B. Halstead & H. Gomez-Dantes [eds.], Dengue—a worldwide problem, a common strategy. International Conference on Dengue and *Aedes aegypti* Community-Based Control. Mexican Ministry of Health and Rockefeller Foundation, Mexico.

Gibbons, J. W. [ed.]. 1990. Life history and ecology of the Slider Turtle. Smithsonian Institution Washington, DC.

Lamm, S. H., A. Taylor, E. J. Gangarosa, H. W. Anderson, W. Young, M. H. Clark & A. R. Bruce. 1972. Turtle-associated salmonellosis. I. An estimation of the magnitude of the problem in the United States, 1970–1971. Am. J. Epidemiol. 95: 511–517.

Marten, G. G., G. Borjas, M. Cush, E. Fernández & J. W. Reid. 1993. Control of larval *Aedes aegypti* (Diptera: Culicidae) by cyclopoid copepods in peridomestic breeding containers. J. Med. Entomol. (in press).

Michael-Marler, S., M. L. Brown & R. J. Siebeling. 1983. Eradication of *Arizona hinshawii* from artificially infected turtle eggs. Appl. Environ. Microbiol. 45: 748–754.

Siebeling, R. J., D. Caruso & S. Neuman. 1984. Eradication of *Salmonella* and *Arizona* species from turtle hatchlings produced from eggs treated on commercial turtle farms. Appl. Environ. Microbiol. 47: 658–662.

Received for publication 28 July 1992; accepted 16 March 1993.