

Using *Gambusia* to control mosquito larvae in abandoned swimming pools: The New Orleans experience

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Introduction – The aftermath of Katrina

On August 29, 2005, Hurricane Katrina brought New Orleans a flood of unexpected and devastating consequences. Among them was the sudden creation of several thousand unattended swimming pools, littered with debris from the hurricane and receding flood waters. The mucky water in the pools provided ideal breeding habitat for mosquito vectors of West Nile virus, eastern equine encephalitis, and St. Louis encephalitis.

The city was in disarray when Mosquito Control staff returned to New Orleans to assess the situation about a month after the hurricane. Because the organization's headquarters had been under 8 feet of water during the flood, the buildings and equipment there were unusable. Most of the city was without water, electricity, and telephone service. Mosquito Control worked out of a cruise ship docked in New Orleans to provide offices and lodging for government workers.

The New Orleans landscape was a distressing quagmire of mud, filthy puddles, foul-smelling houses, hopelessly damaged automobiles, and every imaginable kind of trash. Swimming pools were littered with fallen trees, dead animals, furniture, and rotting mattresses. Although there are

no quantitative data for that time, it seemed more than half the pools contained mosquito larvae. *Culiseta inornata* was particularly numerous, along with *Culex quinquefasciatus* and *Cx. salinarius*. The larvae were sometimes so numerous that they appeared to form a carpet across a pool. Though the usual season for mosquito-borne diseases was ending, no one knew for sure whether the unprecedented mosquito production from swimming pools could set in motion a serious outbreak of disease at any time.

Everyone agreed that something should be done about the swimming pools as quickly as possible. Covering them was not a viable alternative because covers collected rainwater that continued to provide mosquito breeding habitat. It was not feasible to get rid of the pools because most homeowners presumably wanted to keep them. Besides, the whereabouts of many owners of the unattended pools was unknown. And it was logistically impossible to fill in a large number of pools in a city that was still in shock and preoccupied with an overwhelming roster of cleanup and reconstruction activities.

Thus began a program of emergency mosquito control conducted in an atmosphere of chaos and dysfunction that was at first overwhelming and continued to be extremely challenging for several years. At every step of the way, it was often necessary to move forward making do with less information than was really needed for confident decisions.

By November 2005, Mosquito Control staff were walking through backyards and listing addresses of houses with swimming pools. They applied a larvicide –usually Vectolex (*Bacillus sphaericus*), which the manufacturer had donated – to all pools observed to have mosquito larvae. However, larvicides could provide only temporary benefits, and the number of pools that could be inspected and treated during the months following Katrina was a small fraction of all those in the city. Large numbers of mosquito larvae continued to be seen in the swimming pools through the early months of 2006.

There were six cases of West Nile Virus diagnosed as Neural-Invasive Disease (NID) in Orleans Parish over the period of August-September 2005, though there had been only one or two cases per year during 2003 and 2004 (Figure 1). There were two cases of Saint Louis Encephalitis (NID), which had not occurred in Orleans Parish for years. Fortunately for disease transmission, there were very few people in New Orleans during the months immediately following Katrina. By the time many people were back, the disease transmission season was apparently over.

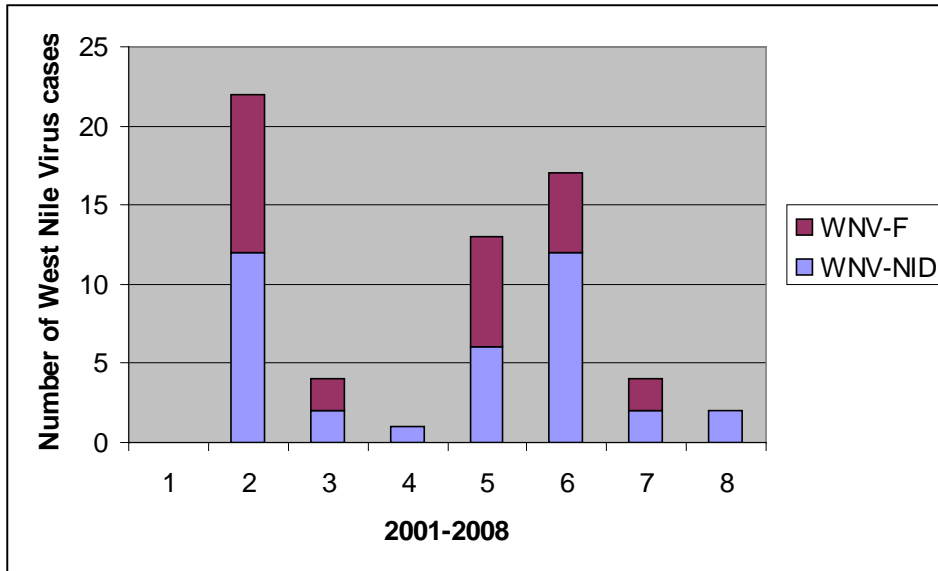


Figure 1. Number of West Nile Virus cases during 2002-2008. 2002 was the first year of West Nile Virus in New Orleans. WNV-NID = Cases diagnosed as “Neural Invasive Disease.” The total number of cases includes cases diagnosed as fever only (WNV-F). NID cases are considered a more reliable indicator of the incidence of the disease than “fever” cases.

Introducing *Gambusia* to swimming pools

In January 2006, a decision was made to introduce fish to the swimming pools. In theory, methoprene was another possibility for long-term suppression of mosquito production. However, Altocid briquets tested in swimming pools before Hurricane Katrina were ineffective, presumably because the methoprene was diluted by the large volume of water in a pool. The effectiveness of fish was apparent in pools that contained fish from the Katrina flood – a natural stocking of approximately 750 pools that was most prominent near Lake Pontchartrain and the numerous canals running through the city. Most of these fish were mosquito fish (*Gambusia affinis*), though sailfin mollies (*Poecilia latipinnia*), least killifish (*Heterandria formosa*), and sheepshead minnows (*Cyprinodon variegatus*) were also present in significant numbers (Caillouët 2007). Pools with these fish sometimes contained a few *Anopheles* larvae but seldom *Culex* or *Culiseta*.

A supply of fish was the most immediate concern for an introduction program. New Orleans Mosquito Control routinely introduced locally-trapped fish, primarily killifish, into unattended swimming pools during the years preceding Katrina. However, this source could not provide enough fish for so many pools. Fortunately, some of the catfish ponds in the region contained large *Gambusia* populations. A catfish farm in Georgia could provide plenty of *Gambusia*, but that source was ruled out because the fish were a mixture of *G. affinis* and *G. holbrooki*. Bringing *G. holbrooki* into Louisiana presented legal obstacles because that species was not known to be in the state. *G. affinis* was common in Louisiana.

In the end, *G. affinis* was transported by truck from a catfish farm in Yazoo, Mississippi. Twenty thousand fish at a time were deposited for holding in two circular, above-ground, aerated

swimming pools located in Slidell, Louisiana, on the outskirts of New Orleans. One of the pools was 18 feet in diameter and the other 15 feet in diameter. The cost for purchase and transport was approximately ten cents per fish. The fish sometimes suffered die-offs in the holding pools due to overcrowding and tail rot, but the supply from Mississippi was sufficient to compensate for setbacks.

The introduction of *Gambusia* to swimming pools began in April 2006 (Figure 2). Distributing the fish to so many swimming pools was a horrendous, labor-intensive job. Fortunately, the non-profit organization Operation Blessing, which provided volunteers from outside New Orleans to help with the recovery, assigned some of its volunteers to the swimming pool project (www.ob.org/projects/hurricane_relief/blog/tara_smith.asp). Operation Blessing's participation was critical for completing the fish introductions in a timely manner. In addition to providing volunteers, they covered expenses such as purchase and transport of mosquito fish, holding pools, plastic bags for transporting fish to swimming pools, ice chests, oxygen, signs, fish food, fungicide, and "Bug Buster" tee shirts used by the volunteers. This financial assistance was truly a blessing because it enabled procurement to proceed with flexibility and without the paperwork and delays associated with funding from FEMA or the city government.

Fish distribution started at 8 am each day with an assembly line that netted fish from the holding pools, counted out approximately fifty fish by pouring them into a calibrated cylinder and pouring those fish into a plastic bag, put oxygen into the bags, tied them, and placed the bags in a 50-120 gallon cooler without ice in the back of a pickup truck. About eight trucks were used each day.

The crew for each truck typically consisted of a Mosquito Control employee and two volunteers. Additional volunteers sometimes followed the truck in a van. It would have been an enormous job to check every house in the city for a swimming pool. The crews were able to work with swimming pool address lists running sequentially along the streets. The address lists were based on information from the Real Estate Association Multiple Listing Services and addresses recorded by Mosquito Control staff during the three thousand backyard inspections they conducted from November 2005 to April 2006.

One bag of fish was poured into each pool. The crews did not require permission to enter yards when no one was there, but they never climbed the fences of vacant houses if the gates were locked. When they were unable to enter a yard, they attempted to get fish into the swimming pool by throwing a "fish bomb" – a plastic bag arranged to open on impact – over the fence and into the pool. Vectolex was applied to suppress mosquito production until the fish established a larger population.

Gambusia introductions proceeded intensively through the spring and summer of 2006 (Figure 2). Because of the urgency and scale of the task, virtually all of the effort went into introducing fish. Aside from a few checks to confirm fish survival in pools when the introductions began, the field crews did not return to swimming pools during April-September 2006 to see whether the fish were still there.

Fish were introduced to a total of 1,278 unattended pools by September 2006 (Figure 2), and fish introductions proceeded at a less frantic pace during the autumn and winter. Although there were still plenty of swimming pools that had not yet been checked to see whether they needed fish, the swimming pool address lists were seriously incomplete. It was a welcome break in January 2007



Figure 3. Areal photos showing the same swimming pools before Hurricane Katrina (top) and after Katrina (bottom). The clean swimming pools before Katrina are turquoise. The unmaintained swimming pools after Katrina are brown.

Return to the pools

In October 2006 the field teams began returning to houses where fish had been introduced during the previous spring and summer. The purpose was to:

- Check whether fish were in the pools. Assessment of fish presence was based on actually seeing the fish or seeing characteristic ripples they made when breaking the surface of the water.
- Confirm that pools containing fish did not have mosquito larvae.
- Assess the magnitude of mosquito breeding in pools that did not have fish.

Autumn 2006 and winter 2007 were a time of uncertainty about how well the fish were working. In an alarming number of inspections, no fish were seen in pools to which they had previously been introduced. The implications were not entirely clear because the failure to see fish, particularly on cold, cloudy days, became more frequent as the winter advanced. Pool temperatures were as low as 11° C. It seemed that fish introductions had actually failed in some pools, but were fish really missing from so many pools? Or were we failing to see them because they were inactive? Were the fish reliably suppressing mosquito production when they were present in a pool? We were not sure. No *Culex* or *Culiseta* larvae were seen in the pools where fish also were seen, but there were substantial numbers of larvae in some of the pools where fish were *not* seen. Some of those pools may have contained fish.

We needed some way besides visual inspection to assess whether fish were in a pool. We tried seining pools that we knew to have fish in low or moderate numbers and sometimes did not catch any. We tried the same with commercial minnow traps and got the same result. As it turned out, we never found a reliable method for assessing fish during the winter. We had to wait for warmer temperatures in the spring, when we could rely upon visual assessment.

In May 2007, quality control tests were run with each inspection crew to assess the presence or absence of fish in a pool that another inspector had confirmed a few days earlier to contain fish. The same was done with pools known definitely *not* to contain fish. The results confirmed visual assessment to be completely reliable at that time. As the summer progressed, field crews returned to pools to which fish had previously been introduced. By the end of the summer nearly six hundred pools had been checked to see if the fish were still there, creating a solid data set for assessing the effectiveness of fish introductions. The crews introduced fish to every pool in which they did not see them. Unattended pools continued to turn up, and by the end of summer 2007, nearly 1,500 swimming pools had received one or more *Gambusia* introductions since the introductions began in April 2006.

Chlorine was of particular concern because empty bleach bottles were so commonly seen at unattended swimming pools. People sometimes dumped bleach into an unmaintained pool – even a neighbor’s pool – to “clean it up.” The bleach could kill the fish in that pool, thereby converting the pool into mosquito breeding habitat.

The apparent absence of fish from pools to which they had been introduced set us to thinking why introductions would fail. We sometimes saw swimming pools with only one or two feet of water at

the bottom. They had apparently been drained by their owners and collected rain water after that. We also saw empty bleach or pool-chlorine bottles lying around unattended pools, reflecting a desperate effort of homeowners or neighbors to do something about the alarming state of the pools, sometimes during brief visits. However, draining a pool or dumping chlorine could exterminate the fish, thereby converting a pool into ideal mosquito breeding habitat if it was left unmaintained after that. Because well-intentioned but counterproductive interventions seemed to be a serious problem, a sign with the following message was left at every inspected swimming pool starting in July 2007:

Attention

Mosquito Control has been regularly treating this pool.
 Mosquito-eating fish have been released into this pool
 to control mosquitoes in the area.
 To avoid killing the fish, please DO NOT add chemicals
 to pool before the pool is in complete working order.
 Please inform Mosquito Control if you:

- restrict access to your pool
- drain, fill-in or remove the pool
- have a mosquito problem at your home.

Many of the swimming pools that needed emergency fish introductions in 2006 are now under owner maintenance or have been filled in (Table 1). Fish still provide mosquito control in approximately a thousand unattended pools, and approximately a hundred unattended pools have never had fish introduced because of inability to access the property or other reasons. During the past three years, a new crop of unattended swimming pools has emerged, primarily because people have abandoned their homes after defaulting mortgages.

Table 1. Status of swimming pools when first visited after Katrina and when last visited (or checked with the Pictometry aerial photo system)

Status at last visit	Status at first visit				Total
	Owner maintained	Natural fish ¹	No fish ²	Drained ³	
Owner maintained	2370	261	507	11	3149
Natural fish ¹	0	199	0	0	199
<i>Gambusia</i> ⁴	38	53	489	0	580
Drained ³	0	4	11	0	15
Removed ⁵	94	153	307	13	567
Don't know ⁶	1673	83	137	49	1942
Total	4175	753	1451	73	6452

1. Fish populations introduced to swimming pools by the Katrina flood.
2. *Gambusia* were introduced to unmaintained pools that did not already contain fish (unless the pool was completely drained).

3. Although it was not possible to introduce fish to drained pools, such pools were considered potential mosquito breeding sites because they might later collect rainwater.
4. Pools to which *Gambusia* were introduced previously and there were still *Gambusia* in the pool at the last visit.
5. Pools filled in or otherwise destroyed.
6. Pools not checked after the first visit.

The research program

In May 2007, systematic research was initiated in support of the swimming pool introductions. The research started with the following questions:

- How successful were the *Gambusia* introductions? In what percentage of the pools did *Gambusia* introduction lead to a subsequent fish population in the pool?
- Where introduction did not lead to a fish population, why did it fail? Were conditions in some of the swimming pools so adverse that the fish were unable to survive?
- What could be done to make fish introductions more reliable in the future?
- How effectively were the fish controlling mosquito production? How did this depend on ecological conditions in the swimming pools?
- How effective was Vectolex treatment at the time of fish introductions?

A broad selection of swimming pools was identified for monthly monitoring of physical and chemical factors, trash, leaves and aquatic vegetation, aquatic insect predators, mosquito larvae, and fish. Table 2 lists the information recorded at each inspection. The monitoring began in May 2007 and ended in December 2008 with a total of 433 pool inspections. Included were:

- pools where *Gambusia* introduction had failed;
- pools containing fish after successful *Gambusia* introduction;
- pools with natural fish populations from the Katrina flood;
- “control” pools to which fish had not been introduced.

Control pools provided information on the seasonal pattern of different species of mosquito larvae.

Experiments were conducted in a few other swimming pools to assess the impact of particular pool treatments such as Vectolex or bleach application upon fish and mosquito larvae.

The pool inspection data for the research program are shown in Appendix 1. Spearman rank correlation coefficients were calculated for every pair of variables (37 variables and 666 correlations coefficients). Spearman correlations are non-parametric and therefore capture any association between two variables – not just the linear component of association reflected by conventional correlation coefficients. Spearman correlations of all measured variables with mosquito larvae, pupae, and fish are shown in Table 2. Correlations among all the other variables are shown in Appendix 2.

Table 2. Information recorded during each swimming pool inspection in the research program

1. Month (MON)
2. Day (DAY)
3. Year (YR)
4. Pine leaves (PIN) – None=0, Low=1, Medium=2, High=3
5. Oak leaves (OAK) – None=0, Low=1, Medium=2, High=3
6. Miscellaneous leaves, not oak or pine (MLE) – None=0, Low=1, Medium=2, High=3
7. Sticks floating on the water (STI) – None=0, Low=1, Medium=2, High=3
8. Tree branches (TB) – None=0, Low=1, Medium=2, High=3
9. Wood such as boards (WOO) – None=0, Low=1, Medium=2, High=3
10. Grass growing into the water at the edge of the pool (GRA) – None=0, Low=1, Medium=2, High=3
11. Filamentous algae mats (ALG) – None=0, Low=1, Medium=2, High=3
12. Plastic bags (PLB) – None=0, Low=1, Medium=2, High=3
13. Plastic objects such as toys (PLO) – None=0, Low=1, Medium=2, High=3
14. Large objects such as furniture and appliances (LO) – None=0, Low=1, Medium=2, High=3
15. Glass bottles (GLB) – None=0, Low=1, Medium=2, High=3
16. Empty bleach bottles around the pool (BLB) – None=0, Low=1, Medium=2, High=3
17. Brown water color (BRW) – None=0, Low=1, Medium=2, High=3
18. Green water color (GRW) – None=0, Low=1, Medium=2, High=3
19. Water smell (WSM) – None=0, Low=1, Medium=2, High=3
20. pH (PH)
21. Salinity (SAL) – Parts per thousand
22. Chlorine (CHL) – Parts per million
23. Nitrate (NO₃) – Parts per million
24. Nitrite (NO₂) – Parts per million
25. Oxygen (OXY) – Parts per million
26. Ammonia (NH₃) – Parts per million
27. Temperature (TEM) – Degrees centigrade
28. Secchi disk (SEC) – Water clarity (meter depth of disk visibility)
29. Backswimmers (BSW) – None=0, Low=1, Medium=2, High=3
30. Water boatmen (WBO) – None=0, Low=1, Medium=2, High=3
31. Diving beetles (DBE) – None=0, Low=1, Medium=2, High=3
32. Dragonfly/mayfly nymphs (DRA) – None=0, Low=1, Medium=2, High=3
33. Water striders (WST) – None=0, Low=1, Medium=2, High=3
34. Mosquito pupae (PUP) – Number in eight dips (all species combined)
35. *Culex quinquefasciatus* larvae (CXQ) – Number in eight dips
36. *Culex salinarius* larvae (CXS) – Number in eight dips
37. *Anopheles* larvae (AN) – Number in eight dips (all species)
38. *Culex coronator* larvae (CXC) – Number in eight dips
39. Fish abundance (FISH) – None=0, Low=1, Medium=2, High=3

Table 3. Spearman rank correlations between fish abundance, abundance of mosquito larvae and pupae, and the physical, chemical, and biological variables¹

	Fish abundance	Culex quinquefasciatus	Culex salinarius	Culex coronator	Anopheles	Pupae
Fish abundance	1.000	-.223	-.072	-.122	-.222	-.180
	.	.000	.134	.011	.000	.000
Culex quinquefasciatus	-.223	1.000	.149	.223	.370	.339
	.000	.	.002	.000	.000	.000
Culex salinarius	-.072	.149	1.000	-.013	.203	.353
	.134	.002	.	.783	.000	.000
Culex coronator	-.122	.223	-.013	1.000	.175	.167
	.011	.000	.783	.	.000	.000
Anopheles	-.222	.370	.203	.175	1.000	.370
	.000	.000	.000	.000	.	.000
Pupae	-.180	.339	.353	.167	.370	1.000
	.000	.000	.000	.000	.000	.
Time	.192	-.193	-.029	-.107	-.115	-.038
	.000	.000	.546	.026	.017	.426
Leaves	-.014	-.029	-.040	-.023	-.055	-.088
	.769	.552	.405	.632	.254	.067
Sticks	-.112	.003	.006	.007	.095	.004
	.020	.951	.905	.877	.050	.936
Tree branches	.022	.047	-.044	.070	.091	-.034
	.647	.332	.359	.145	.060	.486
Wood	-.021	-.079	-.036	-.051	-.088	-.075
	.669	.101	.459	.293	.068	.120
Grass	.139	-.090	.038	-.053	.023	-.021
	.004	.062	.426	.273	.632	.663
Pine leaves	.033	.101	.051	.013	.212	.061
	.496	.036	.294	.785	.000	.205
Oak leaves	.021	.210	.137	.017	.051	.238
	.662	.000	.004	.724	.291	.000
Algae	.039	-.122	-.084	-.092	-.146	-.142
	.415	.011	.082	.057	.002	.003
Plastic bags	-.083	-.024	.007	.025	.034	.035
	.086	.619	.882	.610	.479	.475
Plastic	.148	-.070	-.001	-.047	-.013	-.028
	.002	.146	.977	.336	.790	.566
Large items	-.012	.034	-.039	.049	-.032	-.009
	.798	.478	.417	.307	.511	.845
Glass bottles	-.009	-.041	-.027	-.038	-.039	-.002
	.854	.391	.583	.436	.422	.961
Bleach bottle	.077	-.053	-.018	-.025	-.064	-.049
	.112	.274	.713	.602	.189	.308
Brown	-.071	.072	-.020	-.033	.006	.059
	.139	.137	.679	.492	.894	.222
Green	.112	-.073	.026	.000	.007	-.054

	.020	.130	.583	.998	.887	.264
Smell	-.113	.197	.118	-.025	-.018	.059
	.018	.000	.014	.602	.713	.219
pH	.001	.045	-.059	.074	.019	-.079
	.982	.364	.231	.134	.699	.110
Salinity	.079	.013	.063	-.009	.110	-.004
	.109	.791	.199	.856	.026	.943
T- Chlorine	.021	.037	.098	-.030	-.029	.044
	.677	.456	.046	.542	.553	.375
Nitrate	-.093	-.001	.117	-.027	.080	.009
	.061	.988	.018	.592	.106	.856
Nitrite	-.093	.011	.136	-.023	.053	.022
	.059	.817	.006	.636	.284	.661
Oxygen	.060	-.120	.043	-.083	-.022	.009
	.226	.014	.389	.091	.658	.853
Ammonia	.088	.143	.035	.006	.000	-.002
	.081	.005	.494	.903	.999	.962
Temperature	-.049	-.060	-.095	.021	-.061	-.172
	.325	.224	.054	.670	.219	.000
Secchi	-.122	-.164	.002	-.057	-.009	.006
	.023	.002	.976	.284	.864	.910
Back swimmer	-.204	.067	.082	-.095	-.033	.093
	.000	.162	.089	.048	.500	.053
Water boatman	-.187	.224	.145	.096	.240	.171
	.000	.000	.002	.046	.000	.000
Diving beetle	-.006	.030	-.035	.002	-.036	-.015
	.904	.532	.468	.961	.453	.761
Dragonfly nymphs	.027	-.024	.095	.085	.186	.115
	.577	.615	.049	.076	.000	.017
Water strider	.038	-.031	-.023	-.032	.131	.060
	.431	.525	.641	.508	.007	.213

1. Based on the data in Appendix 1. N = 433. The top number in each cell of the table is the Spearman correlation coefficient. The number beneath the correlation coefficient is the level of significance (two-tailed test). Asterisks indicate $P < .05$. Appendix 2 provides the Spearman correlation coefficients between all physical, chemical, and biological variables listed in Table 2.

Mosquito larvae in the swimming pools

How many of the swimming pools contained mosquito larvae? What kind of larvae? How many? How was this affected by whether there were fish in the pools? The answers to these questions have come from two sources of information:

- The records for every pool inspection in the research program and fish introduction program during 2006-2008 (summarized in Table 4). Research program records specified what kind of mosquito larvae and how many, but records in the fish introduction program only noted whether fish or mosquito larvae were in the pool without specifying what kind of larvae or

how many. Fish introduction program records were far from perfect, particularly in 2006 when inexperienced volunteers were involved, but their broad quantitative patterns should be valid.

- Samples of mosquito larvae from a smaller number of pools at the time of inspection. The larvae were identified and counted in the laboratory (Appendix 3 and Appendix 4).

Figure 4 presents the month-to-month percentage of inspected *pools without fish* that contained mosquito larvae. Month-to-month fluctuations in the percentage of pools containing mosquito larvae were large with conspicuous peaks. However, the peaks were not in the same months each year, though they were never in the summer.

Appendix 3 shows the counts of each mosquito species in larval samples taken from pools without fish. Every species had less than one larva/dip in most pools but much larger numbers in some pools. *Cx. quinquefasciatus* sometimes numbered hundreds of larvae/dip. Figure 5 (top) shows the month-to-month *number of pools without fish* that contained larvae of each mosquito species. Figure 5 (bottom) shows the month-to-month *total number of larvae* of each mosquito species in the samples. *Cx. quinquefasciatus* was the most abundant species – present throughout the year though numbers dropped in July and August. *Anopheles* larvae (*An. crucians* and *An. quadrimaculatus*) were also in pools throughout the year, but their numbers were generally much lower than *Cx. quinquefasciatus*. The number of *Anopheles* larvae varied noticeably from month to month but without any clear seasonal pattern. *Cs. inornata*, *Cx. salinarius*, and *Cx. restuans* were conspicuous from November to March. (A few *Cx. salinarius* were observed in August 2006.) *Cx. coronator* and *Cx. erraticus* extended from July to October with large numbers of *Cx. coronator* also seen in January.

The downward trend in Figure 4 is of particular practical interest because larval populations in swimming pools without fish reflect adult mosquito populations in the vicinity of the pools. Whereas 53% of inspected pools without fish contained larvae during March-August 2006, only 8% contained larvae during the same months in 2007 and 6% in 2008. This difference suggests a dramatic decline in adult mosquito populations from 2006 to 2007-2008, and a corresponding decline in the potential for disease transmission. We cannot be certain what was responsible for the decline, but we can speculate that the *Gambusia* introductions that proceeded through summer 2006 were a contributing factor because they reduced swimming-pool breeding habitat for mosquitoes after that.

How effective were the fish at preventing mosquito breeding in pools? While it was unusual to see mosquito larvae in *pools with fish*, it did happen. There were larvae in 36.0% of the 2,697 inspections of pools without fish, but there were larvae in only 2.2% of the 3,186 inspections of pools that contained fish (Table 4). Figure 6 shows substantial variation in the month-to-month percentage of inspected pools that contained both fish and mosquito larvae. Some months no larvae were seen in pools with fish, but during a few months as many as 30% - 40% of inspected pools with fish also had mosquito larvae.

What kinds of mosquito larvae were in the pools with fish? Appendix 4 shows the counts from larval samples. Almost all the larvae in pools with fish were *Cx. quinquefasciatus* or *Anopheles*. (Exceptions: Two *Cs. inornata* larvae were collected from one pool with fish; a single *Cs. inornata* larva was collected from another pool; and a single *Cx. salinarius* larva was collected from another pool.) The larvae most frequently seen in pools with fish were *Anopheles*, but *Cx.*

quinquefasciatus larvae were sometimes seen, and when present, they were typically much more numerous than *Anopheles*.

In our research inspections, the abundance of mosquito larvae in a pool was correlated negatively with the number of fish in that pool (Table 3). This was so for every species of mosquito larvae as well as mosquito pupae (all species combined). The negative correlations of *Cx. quinquefasciatus* and *Anopheles* larvae with fish abundance were particularly strong. *Cx. quinquefasciatus* larvae were only in pools where fish numbers were very low, and there were large numbers of *Cx. quinquefasciatus* larvae only in the unusual circumstance of fish looking visibly sick. Small numbers of *Anopheles* larvae were sometimes seen in pools with abundant and apparently healthy fish.

Table 4. Number of inspections in which mosquito larvae were observed in swimming pools containing fish and pools not containing fish.¹

Month	Fish present			Fish absent			Total inspections
	Larvae present	Larvae absent	Total	Larvae present	Larvae absent	Total	
Jan-06	0	5	5	0	6	6	11
Feb-06	0	6	6	0	8	8	14
Mar-06	9	14	23	8	12	20	43
Apr-06	0	617	617	255	346	601	1218
May-06	2	319	321	121	69	190	511
Jun-06	15	295	310	231	21	252	562
Jul-06	6	86	92	134	191	325	417
Aug-09	1	96	97	46	79	125	222
Sep-06	1	42	43	18	77	95	138
Oct-06	0	74	74	23	57	80	154
Nov-06	0	126	126	24	142	166	292
Dec-06	1	106	107	10	64	74	181
Total-06	35	1786	1821	870	1072	1942	3763
Jan-07	0	30	30	12	58	70	100
Feb-07	3	55	58	10	2	12	70
Mar-07	3	186	189	8	70	78	267
Apr-07	2	101	103	12	108	120	223
May-07	12	77	89	4	70	74	163
Jun-07	3	119	122	10	45	55	177
Jul-07	0	133	133	3	75	78	211
Aug-07	0	89	89	0	42	42	131
Sep-07	0	225	225	14	37	51	276
Oct-07	7	18	25	4	6	10	35
Nov-07	0	14	14	0	15	15	29
7-Dec	2	14	16	10	5	15	31
Total-07	32	1061	1093	87	533	620	1713
Jan-08	0	75	75	5	11	16	91
Feb-08	0	27	27	1	13	14	41
Mar-08	1	52	53	4	27	31	84
Apr-08	1	58	59	3	20	23	82
May-08	1	20	21	1	13	14	35
Jun-08	0	21	21	1	29	30	51
Jul-08	0	12	12	0	6	6	18
Aug-08	0	4	4	0	1	1	5
Total-08	3	269	272	15	120	135	407
Total Inspections	70	3116	3186	972	1725	2697	5883

¹This table is based on all inspections in the fish introduction program plus inspections in the research program.

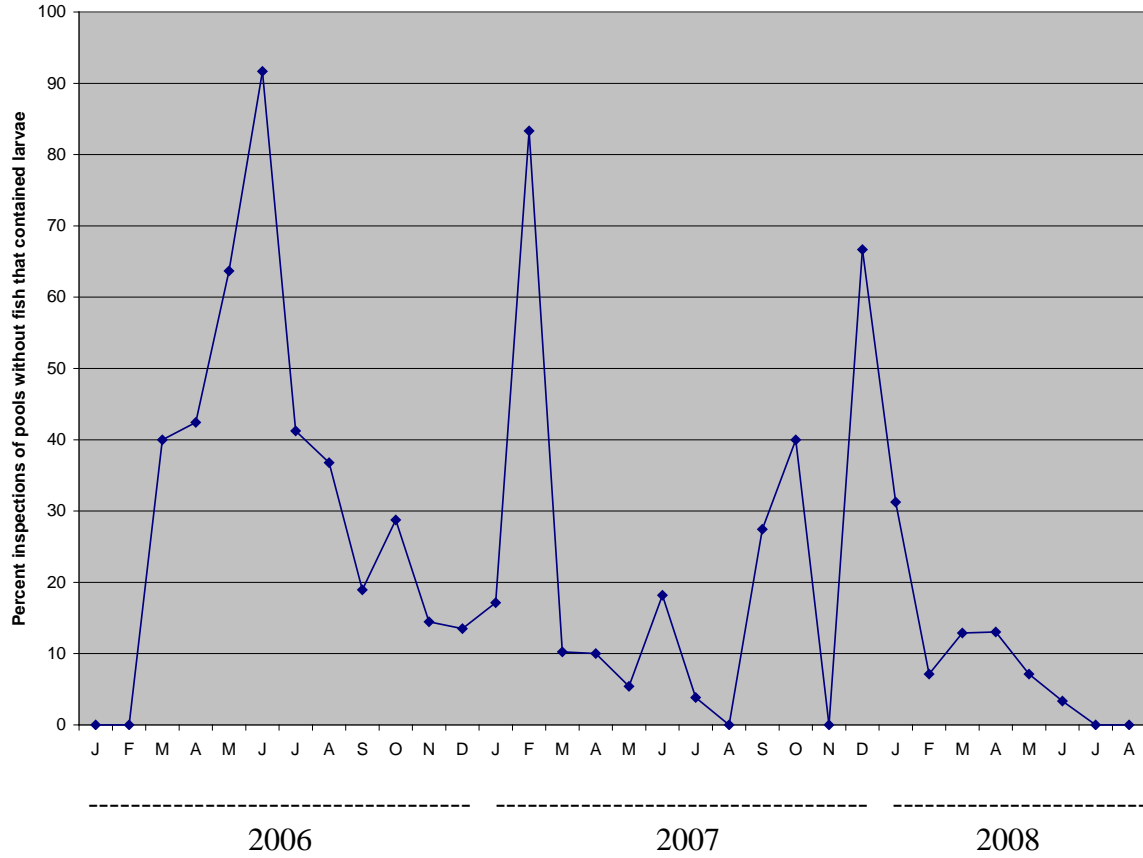


Figure 4. Percentage of inspections for which mosquito larvae were present in pools that *did not contain fish* (based on Table 4)

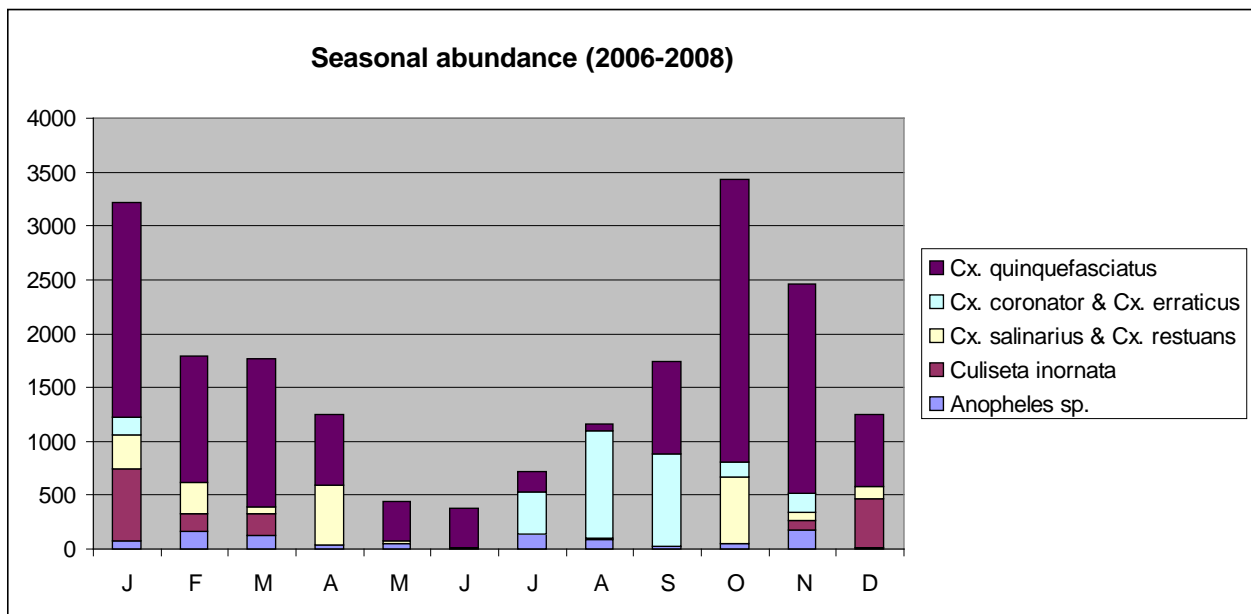
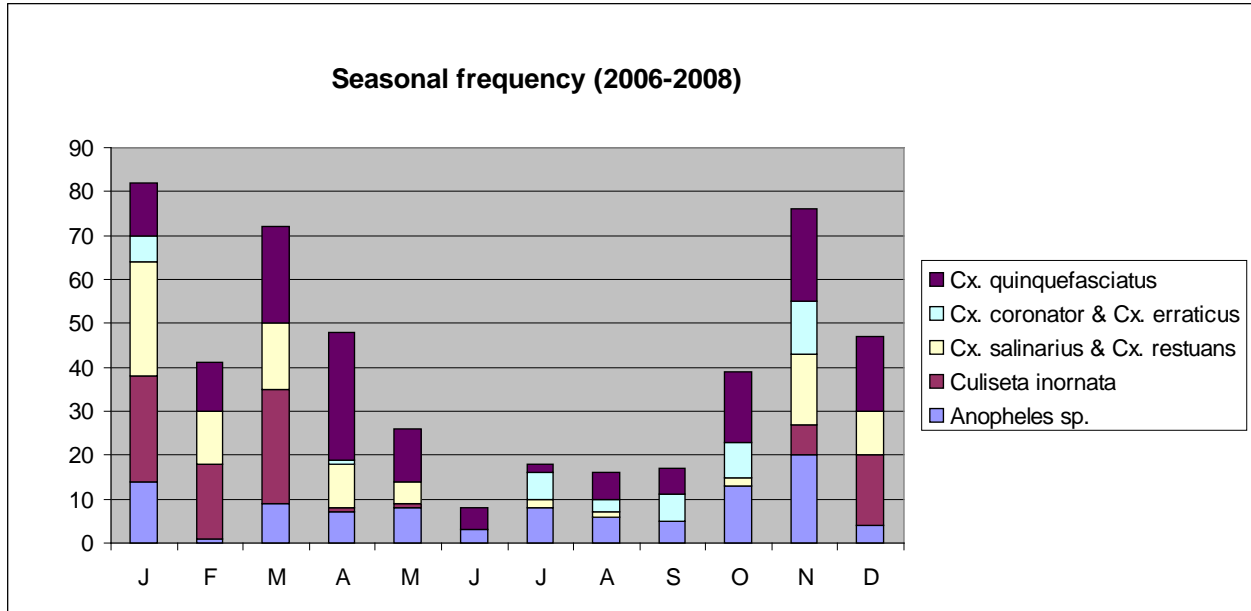


Figure 5. Representation of various species of mosquito larvae in swimming pools without fish (based on Appendix 3). The top graph shows the number of pools in which each species was found. The bottom graph shows the total number of larvae counted.

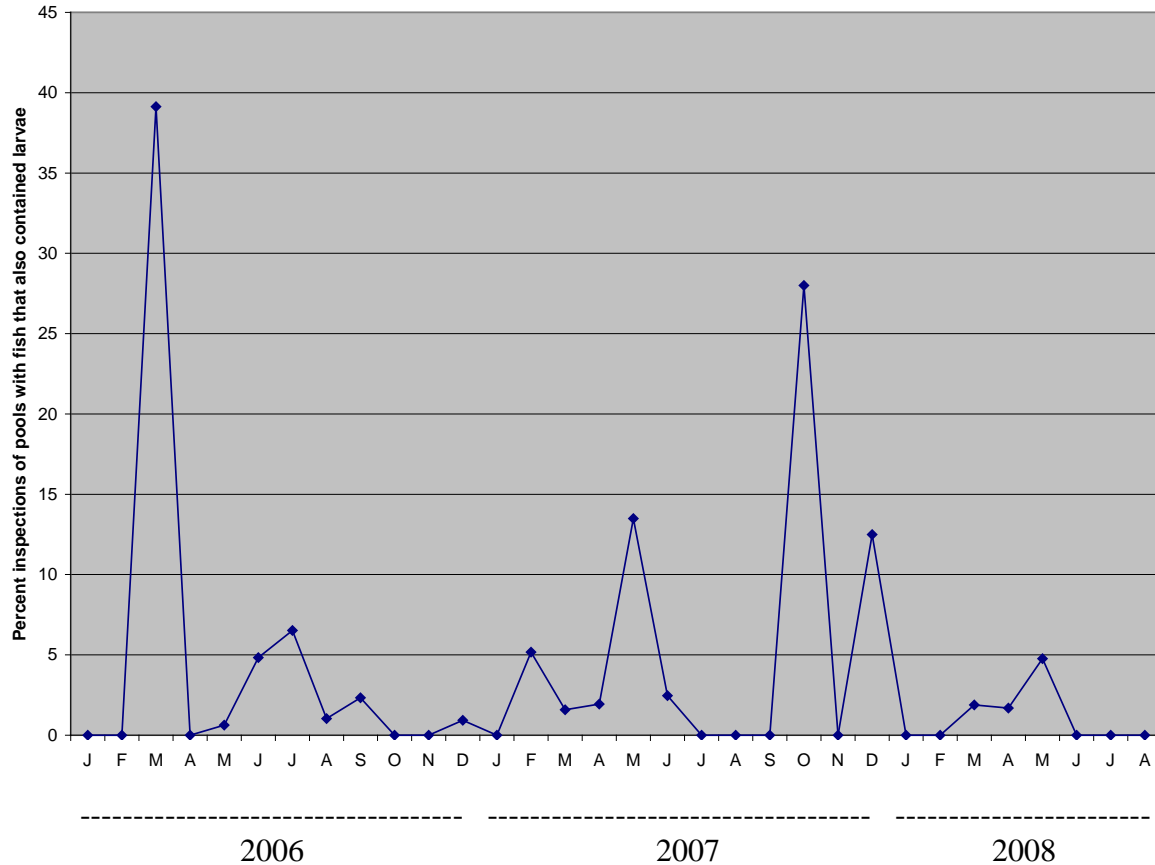


Figure 6. Percentage of inspections for which mosquito larvae were present in pools that contained fish (based on Table 4). The highest peaks are not precise because of relatively small sample sizes for those months.

Physical and chemical conditions in the swimming pools

Appendix 1 presents the original data for physical and chemical conditions in the swimming pools. Table 5 provides a summary of physical/chemical factors known to be harmful to *Gambusia* at extreme values. A key question was “Were extremes of chemical or physical factors in unmaintained swimming pools killing fish or suppressing their populations?”

We found only one swimming pool in which *Gambusia* introductions failed repeatedly. The pool was less than completely full, it was littered with trash (including empty bleach bottles), and the dark-brown water smelled strongly similar to sewage and rotten eggs. There was never measurable oxygen in the water, and the ammonia level (40 ppm) was by far the highest that we observed in any pool – well above the generally accepted tolerance of 1 ppm for *Gambusia* (Walton 2007). Although Walton’s assessment of *Gambusia*’s ammonia tolerance was based on numerous laboratory studies, Hubbs (2000) reported that *Gambusia* survived in heavily-polluted field conditions with an ammonia concentration of 10 ppm. In our research program, we saw fish in 16 swimming pools with ammonia concentrations greater than 10 ppm.

The nitrite concentration at one pool was 2.0 ppm on one occasion, slightly greater than *Gambusia*'s published tolerance of 1.5 ppm (Lewis & Morris 1986), but the fish were fine. No nitrite was detected at the same pool on other occasions.

For all pools, the values for temperature, pH, and salinity were comfortably within the range of published tolerances for *Gambusia* (Table 5). The *Gambusia* thrived in many pools with no measurable oxygen in the water. It was rare to encounter detectable concentrations of chlorine, though toxic concentrations were observed on occasion.

In July 2007, to assess the impact of bleach dumping on fish survival, we poured four gallons of bleach into each of three pools that had thriving *Gambusia* populations and no detectable chlorine in the water (Figure 7). The bleach was introduced by walking around each pool and pouring the bleach at the edge. The chlorine concentration in the pools immediately increased to 0.1-0.5 ppm, and massive fish deaths followed within a few hours. No fish were seen in any of the pools the next day, when chlorine levels had dropped to less than 0.1 ppm. Nonetheless, every pool once again had a thriving fish population within a month, despite the fact no fish were reintroduced to these pools. We therefore conclude it is conceivable to exterminate all the fish in a pool with bleach, but it appears difficult, particularly since our experiments employed more bleach than people told us they had dumped. Recovery of fish populations after bleach dumping could be slower at winter temperatures.

Table 5. Summary of physical/chemical conditions recorded during 433 swimming pool inspections during the research program.

	<i>Gambusia</i> tolerance ¹	Common values ²	Extreme values	% extreme ³
Oxygen (ppm)⁴	0.2–35	2–11	0–0.3	7%
Ammonia (ppm)	0–1	0–0.4	4–40	4%
Temperature (°C.)⁵	0.5–42	25–35	-----	----
pH⁵	4.7–10.2	6.0–9.0	-----	----
Salinity (ppt)⁶	0–58	0.1–5	8–10	3%
Chlorine (ppm)	0–0.8	0	0.5–1.5	<1%
Nitrite (ppm)	0–1.5	0–0.15	0.5–2.0	<1%

1. Source: Walton (2007). Source for nitrite tolerance: Lewis & Morris (1986). Interpretation of published tolerances is difficult because they are typically based on laboratory tests reported as LC50 values for various periods of exposure, a testing procedure that does not have clear implications for *population viability*.
2. Approximately 90% of the inspected pools in Appendix 1 were within this range.
3. Percentage of inspected pools in the extreme range.
4. For oxygen, the extreme values of interest (i.e., possibly harmful to fish) are *less than* the “common values.”
5. For temperature and pH, all observed values were within the “common values” range indicated in the table.
6. Pools in the 3-10 ppt salinity range appeared to have been flooded with water from Lake Pontchartrain.



Figure 7. One of the swimming pools treated experimentally with bleach to see its impact on the fish population. Fish normally thrived in this pool. Pouring four gallons of bleach into the pool led to high fish mortality, but the fish population recovered within a month.

Fish survival in the swimming pools

Due to logistical limitations during the 2006 crash program for introducing *Gambusia* into swimming pools, many of the pools were not checked to see whether they in fact contained fish until many months after the introductions. In the end, only 86% of those pools contained fish the first time they were checked (Table 6). This raised a question about what had happened in the pools that did not have fish.

- *Did the fish fail to establish a population in 14% of the pools to which they were introduced?*
- *Or did the fish successfully establish populations in some or all of those pools, but for some reason the populations were lost before the pools were checked?*

We had the impression that virtually all the fish populations, once established in a pool, continued to inhabit the pool for as long as there was water. The main times that we observed fish to disappear from pools were when pools were completely drained. Instead of being demolished or maintained after the draining, some of those pools were subsequently neglected, leaving them to collect rainwater and converting them into mosquito breeding habitat because they no longer contained fish. However, this kind of pool draining was not common enough to account for the 14% of pools that were missing fish after the introductions in 2006.

Our swimming pool inspections provided quantitative information to assess whether established fish populations were, for whatever reason, disappearing from swimming pools on a significant scale. We tabulated the presence or absence of fish with regard to the number of months that had passed between introducing the fish to a pool and the first inspection of that pool after the fish introduction (Table 7). Because some inspections happened only a few months after introducing the fish, and others were more than a year later, it was possible to see whether the percentage of pools positive for fish declined with the passage of time after the fish were introduced. A decline would indicate that some of the pools were losing their fish populations each month. However, the Spearman rank correlation between the percentage of inspected pools containing fish and the passage of time was positive ($r=0.61$; $p=0.0025$), not negative, strongly indicating there was no decline. It therefore appears that a loss of established fish populations before the pools were checked is not an explanation for the 14% of pools without fish.

Continuous monitoring of a selection of pools during 2007-2008 reinforced this conclusion. In June-July 2007, we introduced *Gambusia* (35 fish per pool) to 47 pools known not to have fish at that time. For some of the pools, it was the first time fish were introduced. For others, it was the second time, after the first introduction had failed. After introducing the fish, the pools were checked monthly for fish and mosquito larvae. Every one of those pools had a thriving fish population within a month and sustained the population for the duration of the monitoring. The same was true for other pools that we knew to have fish and revisited on later occasions during 2007-2008.

We are left with the conclusion that most instances of fish missing from pools after their introduction in 2006 were because the fish failed to establish a population in the first place. The failures seem to be due to damage the fish experienced during transport to the pools. Transport during this period was in oxygenated plastic bags in large ice chests (without ice), where the fish were sometimes held from early morning until late afternoon. We observed that some fish appeared unwell when poured from the bag into a pool, particularly if it was late in the day. During 2007-2008, fish were transported quickly to swimming pools in large coolers without plastic bags, and that worked much better.

The percentages for April-November 2006 at the bottom of Table 6, which show how many pools subsequently had fish after each month of *Gambusia* introductions, reveal a time pattern for the success of introductions during that period. The percentages were lower for April and May, when the introductions began, and increased during succeeding months as the program gained experience and apparently delivered fish to the pools in better condition.

Table 6. Number of first inspections after *Gambusia* introduction at which fish were observed in the swimming pool

<i>First Inspection</i> ¹	Month of <i>Gambusia</i> introduction							
	<i>Apr-06</i>	<i>May-06</i>	<i>Jun-06</i>	<i>Jul-06</i>	<i>Aug-06</i>	<i>Sep-06</i>	<i>Oct-06</i>	<i>Nov-06</i>
Sep-06	12/13 ²	1/2	2/2	5/8	2/2	1/1	---	---
Oct-06	6/7	0/3	3/4	0/0	1/1	0/0	0/0	---
Nov-06	0/0	1/2	0/1	1/1	3/4	0/0	0/0	0/0
Dec-06	7/10	4/5	2/3	3/3	1/1	1/1	1/1	1/1
Jan-07	8/11	4/5	8/10	9/13	6/7	0/1	3/4	0/0
Feb-07	2/3	1/2	0/0	0/1	0/0	0/0	0/0	0/0
Mar-07	10/16	2/5	5/5	4/5	6/8	0/0	0/0	0/0
Apr-07	4/6	0/0	7/7	7/7	7/7	0/0	0/0	1/1
May-07	29/29	13/13	27/29	10/11	10/11	1/1	1/1	0/0
Jun-07	10/11	3/3	9/9	3/4	3/4	2/2	2/2	1/1
Jul-07	17/22	18/20	8/9	6/6	6/6	1/1	1/1	2/2
Aug-07	15/16	14/15	8/10	9/9	9/9	2/2	2/2	1/1
Sep-07	25/28	14/15	10/12	4/4	4/4	0/0	2/2	1/1
Oct-07	8/8	0/0	2/2	2/2	2/2	0/0	0/0	0/0
Total	150/180	75/90	91/105	104/117	60/66	8/9	12/13	7/7
Percentage ³	85%	83%	87%	88%	91%	90%	92%	100%

¹ Month of first inspection after *Gambusia* introduction.

² X/Y: X = Number of inspections for which fish were observed at the pool.
Y = Total number of inspections.

³Percentage of inspections positive for fish. The overall percentage for introductions in 2006 was 507/587 = 86.4%

Table 7. *Gambusia* survival in swimming pools as a function of time since introduction¹

Number of months since fish introduction²	Number of inspections with fish present	Total number of inspections	Percentage of inspections with fish present
1	5	6	87%
2	10	14	71%
3	10	12	83%
4	7	11	64%
5	22	28	79%
6	19	26	73%
7	21	27	78%
8	27	32	84%
9	26	31	84%
10	29	35	83%
11	55	63	87%
12	50	53	94%
13	60	62	97%
14	59	65	91%
15	45	53	85%
16	31	33	94%
17	25	28	89%

¹ Based on Table 6.

² First inspection since fish introduction.

Spearman rank correlation coefficient between (a) the number of months after introduction and (b) the percentage of pools with fish = 0.68 (two-sided P = 0.0025).

Ecological conditions of significance for mosquito larvae and fish

The impact of fish on mosquito larvae can be better understood in the context of ecological conditions in the swimming pools. Pool inspection records in the research program (Appendix 1) were designed to clarify:

- different ecological situations that commonly occurred in the swimming pools;
- whether fish populations, particular species of mosquito larva, and the effectiveness of fish for reducing the larvae were associated positively or negatively with each of those ecological situations.

The following statistical methods were used for data analysis:

- **Factor analysis**, which provides *factor loadings* (Tables 8 and 9) that identify groups of intercorrelated variables. Each factor group – a subset of all the variables – corresponds to a different major ecological situation represented in the data. A factor loading for a particular variable in a particular group indicates the strength of the association of that variable with the

ecological situation represented by that group. Most important for the swimming pool program, factor analysis can provide insights into:

- which ecological situations were favorable or unfavorable for each species of mosquito larvae;
 - which ecological situations were favorable or unfavorable for fish;
 - how the number of mosquito larvae was associated with the absence, presence, or abundance of fish in each ecological situation.
- **Multiple regression analysis** (Tables 10, 11, and 12), which provides *regression coefficients* pointing to variables that had the strongest association with a designated “dependent variable” (e.g., the abundance of fish or a particular species of mosquito larvae) after removing the “effect” of other correlated variables in the same data set. Multiple regression coefficients are the closest we can come to identifying direct causal relationships.

The factor analysis was conducted separately for data coming from pools containing fish and data coming from pools without fish. Table 8 lists the four most prominent ecological situations encountered in *pools without fish*.

- **Group 1** in Table 8 represents pools that were cluttered with debris, including rotting plant material that led to low oxygen concentrations, a characteristic foul odor, ammonia (a decomposition by-product toxic to fish), and higher pH (Figure 8). The positive factor loading for water temperature indicates that this ecological situation predominated during the summer. The negative factor loading of “time” in Group 1 indicates that this situation diminished during the two years covered by the data (May 2007 – May 2009). In other words, Hurricane Katrina cast large quantities of debris into many pools, creating “polluted” conditions that gradually diminished over the following years. The positive regression coefficients for smell and oxygen in Table 10, where the abundance of *Cx. quinquefasciatus* larvae is the dependent variable, suggest that strong odor and low oxygen were factors in Group 1 most directly connecting *Cx. quinquefasciatus* to the group.
- **Group 2** in Table 8 represents conditions that were particularly favorable for *Anopheles* and *Culex salinarius* larvae as well as other aquatic insects such as dragonfly/mayfly nymphs, water striders, and water boatmen (Figure 9). Salinity was high but declining with the passage of time, suggesting flooding with saline water from Lake Pontchartrain or canals within the city at the time of Hurricane Katrina. While these pools could support an abundant aquatic fauna, the negative factor loading for “smell” shows that their organic richness did not take the polluted form so characteristic of Group 1. *Cx. quinquefasciatus* larvae were not part of this group, presumably because the water was too clean to attract oviposition. The positive regression coefficients for pine leaves and grass in Table 11, where the abundance of *Anopheles* larvae is the dependent variable, indicate that these factors were particularly significant for connecting *Anopheles* larvae to this situation. It was common to see *Anopheles* larvae clinging to floating pine leaves clustered around the edge of a pool (Figure 10) or grass growing into the water from the edge (Figure 11). The positive regression coefficients for water boatmen and dragonfly nymphs in Table 11 suggest that coexistence of mosquito larvae with these predators was due to an abundance of food that allowed all to thrive together.
- **Group 3** in Table 8 represents pools with oak leaves, distinctively brown water (from tannins in oak leaves), and an abundance of water boatmen (Figure 12). This group represents a

particularly favorable situation for *Cx. quinquefasciatus* larvae and also favorable for *Anopheles* larvae. The high regression coefficient for oak leaves in Table 10 suggests they were the most direct connection between *Cx. quinquefasciatus* larvae and this ecological situation. The positive regression coefficients for water boatmen in Tables 10 and 11 suggest that water boatmen connected *Cx. quinquefasciatus* and *Anopheles* larvae to this group because the abundance of water boatmen acted as an indicator of favorable food conditions for both boatmen and mosquito larvae.

- **Group 4** in Table 8 represents pools with floating algal mats, clear water (i.e., high Secchi disk readings), backswimmers, diving beetles, and water striders (Figure 13). Mosquito larvae – both *Culex* and *Anopheles* – were generally absent from these pools. The positive factor loading for water temperature indicates that this ecological situation predominated during the summer. The negative regression coefficient for algal mats in Table 10 indicates they were particularly significant for the negative association of *Cx. quinquefasciatus* larvae with this situation. Because algal mats covered so much of the water surface, they suppressed production of phytoplankton food for mosquito larvae and may have interfered with oviposition. The water clarity in this situation was the opposite of the turbid conditions so attractive to *Cx. quinquefasciatus* larvae and reflected a dearth of bacterial and protozoan food for all kinds of mosquito larvae. The negative regression coefficient for backswimmers and diving beetles in Table 11 suggests that predation by these aquatic insects may have contributed to the low numbers of *Anopheles* larvae in this ecological situation.

Table 9 shows ecological situations identified for *pools that contained fish*. The ecological situations in Table 9 bear a striking resemblance to those already seen in Table 8 for pools without fish. It is reassuring that the factor analysis found the same ecological situations (i.e., similar variable groups) in the two independent data sets – with and without fish. This result reflects not only the reality of these ecological situations but also the quality of the data that revealed them.

However, in addition to the similarities between Tables 8 and 9, there are some significant differences between the two tables. While it seems fish did not significantly affect physical/chemical conditions in the pools, fish dramatically impacted the abundance of aquatic insects (including mosquito larvae) in a way that was different in the different ecological situations represented by the factor groups in Table 9.

- **Group 1** in Table 9 represents pools littered with trash, including rotting branches and sticks, and characterized by turbid water (low Secchi disk reading), low oxygen, foul odor, and high ammonia. The physical/chemical characteristics of this ecological situation are very similar to Group 1 in Table 8, but the aquatic insect fauna is different. This group includes a diversity of predatory insects, presumably because of an abundant food supply, but *Cx. quinquefasciatus* larvae were not associated with Group 1 in Table 9, despite the fact that *Cx. quinquefasciatus* larvae thrived in Group 1 pools where fish were absent (Table 8). The explanation for the difference seems to be predation by fish, an interpretation supported by the highly significant negative regression coefficient for fish abundance in Table 10.
- **Group 2** in Table 9 represents conditions that were unpolluted (i.e., a negative factor loading for smell) and particularly favorable for *Anopheles* larvae and other aquatic insects such as dragonfly/mayfly nymphs, water striders, and water boatmen. This is very similar to Group 2

in Table 8. Whether fish were present or absent, this was the best ecological situation for *Anopheles* larvae, presumably because there was an abundant food supply and *Anopheles* could avoid fish predation by clinging to pine needles, sticks, and grass in this habitat. *Cx. salinarius* larvae were prominent in Group 2 when fish were absent (Table 8) but not associated with this group when fish were present (Table 9). This was presumably because of effective fish predation on *Cx. salinarius* larvae.

- **Positive factor loadings for Group 3** in Table 9 represent pools with a large quantity of oak leaves and a low number of fish. This ecological situation is similar to Group 3 in Table 8. Although mosquito larvae were generally suppressed by the presence of fish in swimming pools, the positive factor loadings of *Cx. quinquefasciatus*, *Cx. salinarius*, and *Anopheles* larvae for Group 3 in Table 9 point to Group 3 as the best ecological situation for mosquito larvae when fish were present. The positive regression coefficient for oak in Table 10, and the negative coefficients for fish and water temperature in Tables 10 and 11, suggest the following explanations for why this ecological situation is so favorable for mosquito larvae:
 - Decomposing oak leaves provided an abundance of food for mosquito larvae.
 - Lower numbers of fish resulted in less fish predation on mosquito larvae.
 - Group 3 was most common in the winter, when fish were not so active, further diminishing fish predation on mosquito larvae.
- **Negative factor loadings for Group 3** in Table 9 indicate an additional ecological situation, which is the opposite of that represented by the positive factor loadings for Group 3 in the same table. With algal mats, clear water, high fish abundance, high temperatures (indicating summer), and the absence of *Culex* and *Anopheles* larvae, this ecological situation is similar to Group 4 in Table 8. The paucity of mosquito larvae in this situation seems due to a combination of low food supply for the larvae (indicated by clear water) and high predation by fish.

The regression coefficients in Table 12, where fish abundance is the dependent variable, point to additional connections between fish populations and ecological conditions in a pool. The positive coefficient for tree branches indicates that not only algal mats, but also tree branches in the water, provided particularly favorable conditions for fish. The positive coefficient for pH shows that fish were more abundant under more alkaline conditions, an observation in accord with published information that a pH of 7.2-8.2 is optimal for *Gambusia* (Walton 2007).

The negative coefficients in Table 12 for ammonia, nitrite, and empty bleach bottles (which reflect chlorine dumping) suggest that even though it is rare for the concentration of toxic chemicals such as ammonia, nitrite, and chlorine to be high enough to wipe out a fish population, lesser concentrations had a depressing effect on fish abundance and possibly their vitality as predators. This is significant for the effectiveness of fish predation on mosquito larvae because we never saw *Cx. quinquefasciatus* larvae in pools with thriving fish populations. We saw *Cx. quinquefasciatus* larvae only in association with low fish numbers. *Anopheles* larvae appeared less vulnerable to fish predation, sometimes coexisting in small numbers with healthy fish populations if a pool contained grass, leaves, or other objects that provided refuge.

Table 8. Intercorrelated variables for swimming pools not containing fish¹**GROUP 1****Positive factor loadings**

Tree branches (.64)
 Large items (.54)
 pH (.52)
 Water temperature (.51)
 Smell (.45)
 Ammonia (.36)
Cx. quinquefasciatus larvae (.30)
 Temperature (.17)

Negative factor loadings

Oxygen (-.57)
 Time (-.56)

GROUP 2**Positive factor loadings**

Dragonfly/mayfly nymphs (.68)
 Pine (.57)
Anopheles larvae (.56)
 Water strider (.51)
 Water boatmen (.45)
Cx. salinarius larvae (.43)
 Salinity (.40)
 Grass (.31)

Negative factor loadings

Time (-.30)
 Algal mats (-.21)
 Smell (-.11)

GROUP 3**Positive factor loadings**

Oak leaves (.60)
 Plastic bags (.47)
Cx. quinquefasciatus larvae (.43)
 Water boatmen (.36)
 Nitrate (.38)
 Brown water (.27)
 Smell (.26)
Anopheles larvae (.21)

Negative factor loadings

Large items (-.40)
 Tree branches (-.36)
 Wood (-.33)

GROUP 4**Positive factor loadings**

Secchi disk (.61)
 Backswimmers (.56)
 Diving beetles (.53)
 Algae mats (.47)
 Water striders (.38)
 Sticks (.33)
 Temperature (.28)

Negative factor loadings

Grass (-.34)
Anopheles larvae (-.29)
Cx. quinquefasciatus larvae (-.29)
Cx. coronator larvae (-.26)
Cx. salinarius larvae (-.20)

1. Factor loadings based on principle components analysis with varimax rotation.

Table 9. Intercorrelated variables for swimming pools containing fish.¹**GROUP 1****Positive factor loadings**

Tree branches (.72)
 Water boatmen (.71)
 Backswimmers (.64)
 Water striders (.55)
 Diving beetles (.54)
 Dragonfly/mayfly nymphs (.34)
 Ammonia (.37)
 Large items (.35)
 Sticks (.30)
 Smell (.24)

Negative factor loadings

Oxygen (-.38)
 Secchi disk (-.32)
 Grass (-.30)
 Time (-.28)

GROUP 2**Positive factor loadings**

Salinity (.74)
 Dragonfly/mayfly nymphs (.64)
 Water striders (.58)
 Pine leaves (.53)
 Plastic items (.50)
 Sticks (.46)
Anopheles larvae (.39)
 Grass (.38)
 Water boatmen (.28)

Negative factor loadings

Time (-.63)
 Large items (-.50)
 Smell (-.34)
 Algal mats (-.25)

GROUP 3**Positive factor loadings**

Cx. quinquefasciatus larvae (.55)
 Brown water (.53)
 Oak leaves (.52)
Cx. salinarius larvae (.43)
 Water boatmen (.33)
Anopheles larvae (.19)
 Smell (.19)

Negative factor loadings

Algae mats (-.52)
 Fish abundance (-.50)
 Water temperature (-.46)
 Time (-.33)

1. Factor loadings based on principle components analysis with varimax rotation.

Table 10. Normalized multiple regression coefficients (Dependent variable: number of *Cx. quinquefasciatus* larvae)¹

	<u>Positive coefficients</u>		<u>Negative coefficients</u>	
	<i>Coefficient</i>	<i>Significance</i>²	<i>Coefficient</i>	<i>Significance</i>²
Smell	.21	<.001	Fish abundance	-.16 .001
Chlorine	.19	<.001	Water striders	-.11 .11
Oak leaves	.16	.003	Oxygen	-.10 .06

Water boatmen	.10	.10	Water temperature	-.09	.08
			Algae mats	-.07	.14

1. Based on all swimming pools.
2. Two-tailed test.

Table 11. Normalized multiple regression coefficients (Dependent variable: number of *Anopheles* larvae)¹

	<u>Positive coefficients</u>			<u>Negative coefficients</u>	
	<i>Coefficient</i>	<i>Significance²</i>		<i>Coefficient</i>	<i>Significance²</i>
Water boatmen	.19	.001	Fish abundance	-.22	<.001
Nitrate	.17	<.001	Diving beetles	-.11	.04
Pine leaves	.14	.01	Water temperature	-.10	.05
Dragonfly nymphs	.11	.09	Backswimmers	-.10	.06
Wood	.10	.04			
Grass	.07	.18			

1. Based on all swimming pools.
2. Two-tailed test.

Table 12. Normalized multiple regression coefficients (Dependent variable: Fish abundance)¹

	<u>Positive coefficients</u>			<u>Negative coefficients</u>	
	<i>Coefficient</i>	<i>Significance²</i>		<i>Coefficient</i>	<i>Significance²</i>
Algae mats	.18	.015	Water boatmen	-.30	.001
Tree branches	.15	.067	Ammonia	-.18	.006
pH	.15	.026	Pine leaves	-.16	.05
			Nitrite	-.11	.08
			Bleach bottles	-.09	.21

1. Based on swimming pools that contained fish.
2. Two-tailed test.



Figure 8. Examples of Group 1 in Tables 8 and 9: Heavy organic loading, foul odor, turbid water, and ammonia. Particularly favorable for *Cx. quinquefasciatus* larvae. A small amount of water (as in the bottom photo) can concentrate the pollution, providing habitat for exceptionally high densities of *Cx. quinquefasciatus* larvae.



Figure 9. Examples of Group 2 in Tables 8 and 9: A high level of biological productivity and large populations of aquatic invertebrates. Favorable habitat for *Anopheles* larvae.



Figure 10. Floating pine needles (the brown strip around the edge of the pool) provide refuge for *Anopheles* larvae.



Figure 11. Grass at the edge of a pool and growing into the water provides refuge for *Anopheles* larvae.



Figure 12. An example of Group 3 in Tables 8 and 9: Heavy influx of oak leaves and typically dark brown water due to tannins from the oak leaves. Favorable habitat for *Culex* and *Anopheles* larvae but inhibiting for fish.



Figure 13. An example of Group 4 in Table 8 and negative Group 3 loadings in Table 9: A large cover of floating algal mats and clear water. Unfavorable habitat for mosquito larvae but favorable habitat for fish.

Vectolex performance

It was standard procedure in the swimming pool program to treat all pools with Vectolex (*Bacillus sphaericus*) at the same time *Gambusia* was introduced, even if no mosquito larvae were seen in the pool. In addition, pools that contained larvae but no fish were treated with Vectolex whenever no *Gambusia* were on hand to introduce. The purpose of Vectolex treatment was temporary suppression of mosquito production, presumably extending beyond the treatment day because *B. sphaericus* can “recycle”. This could bridge the gap until introduced *Gambusia* built up a larger population or someone returned to a pool to introduce *Gambusia*.

Was Vectolex really performing as expected? To check, in June 2007 three swimming pools were monitored for mosquito larvae after treating them with Vectolex. At the same time, water samples were taken from the pools approximately twice a week to assay the survival of newly hatched *Cx. quinquefasciatus* larvae in the laboratory.

Two of the pools contained moderately turbid, brown water (Secchi disk visible to 1.3 meters below the surface of the pool), a common condition for unattended pools. One of those pools averaged 60 *Cx. quinquefasciatus* larvae/dip at the time of Vectolex treatment, and the other averaged 8 larvae/dip. The Vectolex killed all the larvae within a day after treatment, and no *Culex* larvae were seen in these two pools during the two weeks of observation after that. Water samples from these pools a day after treatment killed all *Cx. quinquefasciatus* larvae in the laboratory assay, but water samples collected from the pools during the 6th to 14th day after treatment killed only 15% - 65% of the larvae. One of the pools averaged approximately two *Anopheles* larvae/dip at the time of Vectolex treatment, and the number of *Anopheles* larvae in that pool remained about the same after the treatment.

The third pool had a strong organic loading. Its water was very turbid (Secchi disk visible to only 0.8 meters) and distinctly foul-smelling. There were approximately 500 *Cx. quinquefasciatus* larvae/dip and 200 pupae/dip at the time of Vectolex treatment. All larvae were apparently killed within a day after treatment, but there were 1-2 pupae/dip during the following 10 days. New *Cx. quinquefasciatus* larvae started appearing in the pool 10 days after treatment. Water samples up to 10 days after treatment killed all *Cx. quinquefasciatus* larvae in the laboratory assay, but only 30% of larvae died in water samples collected 14 days after treatment.

Hot tubs attached to two pools were subjected to the same procedure of Vectolex treatment and monitoring. One contained 20 *Cx. quinquefasciatus* larvae/dip and the other 50 larvae/dip at the time of treatment. No larvae were observed in the hot tubs until three weeks after the treatment. Water samples from the tubs a day after treatment killed all *Cx. quinquefasciatus* larvae in the laboratory assay. Water samples from one of the hot tubs killed all larvae during the following two weeks that samples were collected, while water samples from the other tub during that period killed 87% - 97% of the larvae.

In conclusion, Vectolex killed all the larvae in a swimming pool at the time of application, but it killed only a fraction of the larvae after that. As expected for *B. sphaericus*, persistence (presumably due to “recycling”) was greater in the swimming pool with higher organic loading,

but even there the kill was only partial after the first day and distinctly ineffective by the 14th day. For practical purposes, Vectolex treatment seems no better than applying oil to the surface of a swimming pool.

The discrepancy between observed Vectolex performance in swimming pools and what might have been expected with “recycling” can be attributed to the fact that information on Vectolex performance comes primarily from application in shallow water. The incomplete kill in swimming pools after the first day is not surprising, since *B. sphaericus* can be expected to sink to the bottom, beyond the reach of *Cx. quinquefasciatus* larvae. Vectolex in hot tubs probably performed better, for at least a few weeks after treatment, because the larval feeding zone was replenished with *B. sphaericus* “recycling” from the bottom of the shallow container. The failure of Vectolex to suppress *Anopheles* production is no surprise as *B. sphaericus* is known to be ineffective against *Anopheles* and *Aedes* larvae (Lacey 2007).

Summary and conclusions

New Orleans Mosquito Control checked nearly every swimming pool in the city to ascertain whether it was properly maintained. “Pictometry” aerial photographs were a big help for locating swimming pools and preparing address lists of houses to be visited for possible *Gambusia* introduction. Pictometry reduced the number of houses to check in the field from thousands that had no swimming pools to a number that was manageable with available manpower.

Cx. quinquefasciatus and *Anopheles* (*An. crucians* and *An. quadrimaculatus*) were the mosquito larvae most frequently seen in swimming pools. *Cx. quinquefasciatus* larvae were present throughout the year. They were sometimes very numerous, though less common during the summer. *Anopheles* larvae were also seen throughout the year, but their numbers were seldom large. *Cs. inornata*, *Cx. salinarius*, and *Cx. restuans* larvae could be very numerous during November to March, and *Cx. coronator* during July to October.

Approximately 750 pools contained fish introduced into them by the Katrina flood (mainly *Gambusia affinis*). The fish introduction program began in April 2006. By the end of 2006, *Gambusia affinis* was introduced to 1,335 unattended swimming pools that did not already contain fish. *Gambusia* was introduced to 136 pools during 2007, 82 pools during 2008, and 40 pools during 2009.

When pools were checked later, fish were missing from 14% of the pools to which they were introduced in the crash program during 2006. It was important for the management of the program to know what happened to those fish. Were they unable to establish populations in “problem pools” because those pools contained polluted water or were in some other way unsuitable for *Gambusia* survival? Did the fish successfully establish populations that disappeared from the pools later? The fact that people sometimes dumped bleach into unmaintained swimming pools to “clean them up” was of particular concern because bleach could kill the fish, thereby converting that pool into mosquito breeding habitat. Experimental dumping of bleach into swimming pools confirmed that bleach caused massive fish deaths immediately after application, but in every instance some of the fish survived and their populations were quickly back to normal. It appears the main way that fish were lost from pools was when they were drained. Such pools could subsequently become mosquito breeding habitat if left to collect rainwater.

Although bleach dumping and pool draining were destructive to fish, it was unusual for swimming pools to lose fish populations once they were established. It appears that most of the pools without fish after the introductions in 2006 were missing the fish because of damage to the fish during transport to the pools. This conclusion is supported by our observation that fish sometimes appeared unwell when they were poured into a pool, particularly if this happened late in the day when they had been in plastic bags since early in the morning.

With more leisurely circumstances during 2007-2008, we were able to transport fish to swimming pools quickly, and the introductions were virtually always successful, including reintroductions to pools where fish failed to establish a population the first time. The only exceptions occurred with less than one percent of the pools, where fish failed to establish a population, or managed to establish only a small and sickly population, because a pool was severely polluted and contained toxic concentrations of ammonia.

Mosquito larvae were seen in only 2.2% of inspected pools containing fish, compared to 36% of inspected pools without fish. The larvae in pools with fish were most frequently *Anopheles*. *Culex* larvae were only occasionally seen in pools with fish, but *Cx. quinquefasciatus* numbers could sometimes be high if fish numbers were particularly low.

Our quantitative documentation of habitat conditions in the swimming pools (data in Appendix 1) started in May 2007 (20 months after Hurricane Katrina), and ended two years later. The following habitat conditions appeared most significant for mosquito production (Table 8 and Table 9):

- **Organic pollution.** Turbid and foul-smelling water, associated with a large quantity of rotting materials, provided particularly favorable habitat for *Cx. quinquefasciatus* larvae when there were no fish in a pool. Although we do not have quantitative data for ecological conditions in the swimming pools during the first year after Hurricane Katrina, we observed during numerous pool visits that this ecological situation was very common and pronounced during the first year and could be traced back to plant debris or dead animals cast into pools by the hurricane and flood. In our quantitative documentation, this condition weakened conspicuously as pools proceeded into the third year after Katrina, presumably because the debris had gradually decomposed. The low oxygen concentrations characteristic of this habitat did not seem to bother the fish, though ammonia concentrations were high enough in a very small percentage of pools to suppress fish populations. Fish generally provided effective mosquito control in this habitat.
- **Ecologically productive without pollution.** The ecological conditions in these pools favored *Anopheles* larvae and often supported large numbers of aquatic insect predators such as dragonfly/mayfly nymphs, water boatmen, and water striders. These pools did not have the putrid conditions and numerous *Cx. quinquefasciatus* larvae so characteristic of pools with organic pollution. *Anopheles* larvae frequently survived even when fish were present, apparently because the larvae were able to avoid predation by clinging to floating pine needles, grass, or small sticks around the edge of a pool.

- **Oak leaves.** Pools with a large number of oak leaves provided favorable habitat for both *Culex* and *Anopheles* larvae. Fish populations were generally low in this habitat. Mosquito larvae occasionally survived in this habitat even when fish were in the pool.
- **Algal mats.** Pools with a dense cover of algal mats and clear, shaded water contained few mosquito larvae, even in the absence of fish. Fish thrived in this habitat, and mosquito control was most absolute when fish were present.

Most kinds of predatory aquatic insects seemed to have little negative impact on mosquito larvae. Except for a negative association of backswimmers and diving beetles with *Anopheles*, mosquito larvae tended to be most abundant where aquatic insect predators, particularly water boatmen, were also abundant. Apparently, conditions favorable for mosquito larvae were favorable for all.

Bacillus sphaericus killed all *Culex* larvae when applied to swimming pools. Typically, there was partial mortality of *Culex* larvae hatching into a pool for about two weeks after the treatment, and no impact after that. *B. sphaericus* should therefore be regarded as an effective one-time treatment for *Culex* larvae in swimming pools. It apparently failed to provide longer-term control because it sank to the bottom of the pool, beyond the reach of *Culex* larvae feeding at the surface. *B. sphaericus* did not kill *Anopheles* larvae.

Because swimming pools without fish are similar in function to gravid traps used to monitor *Culex* populations, the abundance of mosquito larvae in pools without fish reflects the abundance of adult mosquito populations in the immediate vicinity. The percentage of pools containing larvae was high during 2006 and much lower during 2007-2008, suggesting that adult mosquito populations were also lower at that time. The number of West Nile Virus cases in New Orleans followed the same pattern, dropping from a high in 2006 to a low in 2007-2008, similar to 2003-2004 (Figure 1). It seems this decline in mosquitoes and West Nile Virus from 2006 to 2007-2008 can be attributed, at least in part, to the fact that the vast majority of unattended swimming pools in New Orleans contained fish by the end of 2006. It is possible that a decline in the organic enrichment of many pools during the years following Katrina also contributed to a lower number of mosquitoes by 2007-2008.

While nearly all the swimming pools left unattended because of Hurricane Katrina are now under effective mosquito control, other pools are being abandoned as people default on home mortgages.

Recommendations

The following recommendations come from our experience with fish introductions after the Katrina disaster. Many of the recommendations should be relevant not only to hurricanes and floods but also to other disasters that interfere with swimming pool maintenance, such as severe earthquakes. The recommendations apply as well to controlling mosquito production from abandoned swimming pools under less traumatic circumstances, such as the large number of abandoned pools now appearing around the country due to home mortgage defaults.

Identifying a reliable source of fish before they are needed. The beginning of our fish introduction program was delayed several months while tracking down an adequate supply of fish.

Identifying houses with swimming pools. The *Pictometry aerial photo system* proved critical for listing the addresses of swimming pools without walking up and down streets to find them. Up-to-date Pictometry data not only provide addresses but also show whether a pool is maintained or not. Google Earth could serve a similar function. *Past building permits and real estate listings* are another source of information for swimming pool addresses. It is best to arrange access to this information ahead-of-time because government permitting offices and real estate associations may not be functioning in the aftermath of disaster. *Neighborhood associations* can be a source of information about swimming pool locations and provide manpower for reconnaissance after a disaster.

Larvicides. It may be necessary to use larvicides on an unprecedented scale until mosquito production in swimming pools is controlled by fish introductions. It is best to work out a large-scale larviciding strategy ahead-of-time: what larvicides will be used; how large quantities can be obtained quickly; how a large number of pools can be treated quickly; and whether particular larvicide manufacturers might donate their products.

Introducing healthy fish. Fish in holding tanks should be free of disease and other stresses such as crowding, low oxygen, or extreme temperatures. In New Orleans, the most serious stress occurred during transport from holding tanks to swimming pools, the stress being particularly severe during high summer temperatures. Fish for introduction in the afternoon should be collected from holding tanks at mid-day, so transport time from storage tank to swimming pool is no more than a few hours, and fish should be protected from heat stress during that time. Staff should be alert to telltale signs of unhealthy fish (e.g., drooping fins and dark color), so only healthy fish are placed in swimming pools. During the early months of fish introduction, we put the fish in oxygenated plastic bags because we considered it the safest way to transport the fish. It also had the advantage that inexperienced field workers could introduce fish by simply pouring the contents of a bag into a pool. However, we later switched to transporting fish in large coolers without bags. It saved the work of putting fish in bags, and fish seemed to arrive at the pools in better condition.

Communicating with residents. There should be a clear policy concerning what the public should know about the fish introduction program and what is expected from the public to help the program succeed. Public service announcements through the media proved particularly helpful at paving the way for inspection or fish-introduction teams. In addition, if a resident is at home when fish are introduced, that person can receive appropriate information first hand from the people who introduce the fish. If no one is home, a notice or sign should be posted to provide the information. Expectations for resident cooperation might include:

- Putting no chemicals harmful to fish in the pool.
- Draining the pool only if it will be filled in immediately, kept completely dry, or put into proper maintenance.
- Informing the mosquito control agency if the fish disappear (so fish can be reintroduced).
- Informing the mosquito control agency if an unattended pool is converted to proper maintenance (so it is no longer necessary for the agency to monitor the pool).

Systematic records. Systematic data about the condition of inspected pools are essential for a well organized program. A simple form completed during every swimming pool inspection should include:

- Whether or not the pool is properly maintained.
- Whether fish are already in the pool.
- Whether fish were introduced at the time of inspection.
- Whether there are mosquito larvae in the pool. (If so, at all such pools or a systematic subsample of them, there should be a standard number of dips to estimate larval numbers and a sample of the larvae preserved in alcohol for species identification).
- Whether the pool was treated with a larvicide at the time of inspection.

All pools, or a subsample of them, should be checked approximately six weeks after fish introduction to confirm that they contain viable fish populations. It is particularly important during the early months of a fish introduction program to confirm that the introductions are working as expected.

Access to properties. Access can be particularly difficult when residents are not at home and gates are locked. It can be dangerous to enter a backyard without explicit permission from the resident. The legal basis for access to properties should be clear ahead-of-time and updated according to circumstances after the disaster. The public should be thoroughly informed about access policies and procedures.

Supervision and training of volunteers. Operation Blessing's volunteers were critical for the success of the fish introduction program in New Orleans. However, volunteers require careful supervision because they often lack experience appropriate for the work at hand. Moreover, even people who volunteer with the best of intentions can be distracted from the focus that the work requires, particularly under extraordinary conditions such as the devastation after Hurricane Katrina. Volunteer roles should be clearly defined, and they should be told what is expected of them down to procedural details. Maintaining a high standard in this regard can be a challenge if volunteer turnover is high.

Cleaning trash out of pools. Organic materials rotting in the water support high numbers of bacteria, protozoa, and other small organisms that serve as food for mosquito larvae. Putrid conditions are particularly favorable for *Cx. quinquefasciatus*, and trash of any kind can provide *Anopheles* larvae refuge from predators. The sooner trash is removed from a pool, the sooner the organic loading will dissipate and the carrying capacity for mosquito larvae decline.

Organizational self-sufficiency. While coordination with other government agencies can offer numerous advantages, we were impressed with the importance of being able to function without depending on other agencies in the aftermath of disaster. For example, measures to compel property owners to maintain their swimming pools – which may draw on authority beyond a mosquito control agency's domain – may be a part of mosquito control under normal circumstances. However, during the chaos that prevailed for more than a year after Katrina, coordinating with enforcement agencies to compel property owners to maintain their pools or eliminate the pools was not a viable option at a time when many homeowners were out of the city and other agencies were overburdened with their own priorities. We found it most effective to assume sole responsibility for getting fish into as many pools as possible as rapidly as possible, leaving organizationally more complex tasks for later.

Many of the above considerations will function at a higher standard after disaster if they are part of mosquito control operations before the disaster. In some instances, they can be built into routine

mosquito control operations for unattended pools in a way that can be scaled up when necessary. In other instances, they may not be appropriate for routine operations, but there can be some kind of preparation beforehand, such as a “dry run” or review of preparedness.

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Appendix 2. Spearman rank correlations among variables measured in the research program. N = 433. The number on top is the Spearman correlation coefficient. The number beneath the correlation coefficient is the level of significance (two-tailed test). Asterisks indicate $P < .05$. (Correlations involving mosquito larvae and fish are shown in Table 3.)

	Time	Leaves	Sticks	Tree branches	Wood	Grass	Pine leaves	Oak leaves	Algal mats	Plastic bags	Plastic objects
Time	1.000	.126	-.090	-.321	.039	-.052	-.214	-.205	.195	-.201	.151
	.	.009	.061	.000	.416	.281	.000	.000	.000	.000	.002
Leaves	.126	1.000	.012	-.188	.119	-.103	-.159	-.286	-.063	-.103	.060
	.009	.	.801	.000	.013	.032	.001	.000	.192	.032	.217
Sticks	-.090	.012	1.000	.126	-.082	-.128	.010	-.054	.001	.157	-.013
	.061	.801	.	.009	.090	.008	.828	.259	.990	.001	.785
Tree branches	-.321	-.188	.126	1.000	-.124	-.143	.119	.006	-.030	.086	-.007
	.000	.000	.009	.	.010	.003	.013	.905	.532	.074	.888
Wood	.039	.119	-.082	-.124	1.000	.137	-.121	-.116	-.052	-.051	-.017
	.416	.013	.090	.010	.	.004	.012	.016	.283	.292	.721
Grass	-.052	-.103	-.128	-.143	.137	1.000	.017	-.121	-.113	-.077	.010
	.281	.032	.008	.003	.004	.	.725	.012	.019	.112	.841
Pine leaves	-.214	-.159	.010	.119	-.121	.017	1.000	.060	-.137	.177	.125
	.000	.001	.828	.013	.012	.725	.	.212	.004	.000	.009
Oak leaves	-.205	-.286	-.054	.006	-.116	-.121	.060	1.000	-.175	.132	-.116
	.000	.000	.259	.905	.016	.012	.212	.	.000	.006	.016
Algal mats	.195	-.063	.001	-.030	-.052	-.113	-.137	-.175	1.000	.006	.051
	.000	.192	.990	.532	.283	.019	.004	.000	.	.905	.292
Plastic bags	-.201	-.103	.157	.086	-.051	-.077	.177	.132	.006	1.000	-.196
	.000	.032	.001	.074	.292	.112	.000	.006	.905	.	.000
Plastic objects	.151	.060	-.013	-.007	-.017	.010	.125	-.116	.051	-.196	1.000
	.002	.217	.785	.888	.721	.841	.009	.016	.292	.000	.
Large items	-.099	-.028	-.059	.305	-.020	-.043	-.112	-.064	.094	-.256	-.072
	.039	.561	.222	.000	.679	.378	.020	.184	.051	.000	.138
Glass bottles	-.163	.081	-.039	.024	.149	.117	-.059	-.055	-.109	-.061	-.124
	.001	.093	.420	.618	.002	.015	.219	.255	.024	.203	.010
Bleach bottles	-.154	-.140	-.005	-.009	-.023	.163	.294	-.058	-.021	-.014	-.013
	.001	.004	.921	.852	.628	.001	.000	.231	.663	.774	.781
Brown water	-.036	.017	.002	.041	.005	-.048	.084	.164	-.230	.035	-.009
	.460	.720	.973	.401	.911	.318	.083	.001	.000	.471	.848
Green water	.089	-.025	.055	-.006	.005	.041	-.050	-.120	.208	-.044	.041
	.066	.610	.252	.902	.916	.393	.296	.013	.000	.358	.395
Foul smell	-.165	-.076	-.005	.132	.016	-.034	-.017	.174	.004	-.001	-.005
	.001	.114	.920	.006	.735	.484	.726	.000	.940	.976	.912
pH	-.315	-.136	.004	.231	.029	.018	.145	.073	-.087	.098	-.004
	.000	.006	.938	.000	.559	.718	.003	.137	.076	.046	.928
Salinity	-.404	-.088	.053	.129	.032	.151	.180	.070	-.053	.054	.131
	.000	.075	.286	.009	.516	.002	.000	.155	.286	.273	.008
Total chlorine	-.141	-.033	.029	-.062	-.008	.098	.141	-.023	-.032	.045	-.071
	.004	.498	.551	.211	.874	.046	.004	.639	.512	.368	.151
Nitrate	-.110	-.016	-.010	-.047	-.025	-.072	-.059	.139	.044	.039	-.068
	.026	.750	.832	.339	.610	.145	.232	.005	.376	.426	.170

Nitrite	-.225 .000	-.006 .897	-.016 .744	-.025 .608	-.062 .213	-.018 .720	-.001 .992	.170 .001	.027 .583	.054 .274	-.006 .897
Oxygen	.243 .000	.124 .012	.007 .890	-.224 .000	.001 .978	.089 .069	-.149 .002	-.052 .294	.019 .698	-.013 .794	-.055 .269
Ammonia	-.060 .234	-.016 .753	.046 .359	.221 .000	-.082 .103	-.048 .342	.082 .105	.043 .398	-.065 .196	.027 .587	.081 .110
Temperature	-.197 .000	-.153 .002	.030 .547	.109 .026	-.036 .466	-.031 .526	.106 .032	-.065 .189	.199 .000	.039 .427	.012 .814
Secchi disk	.008 .878	.029 .589	.025 .647	-.222 .000	.056 .293	.003 .957	-.076 .154	-.068 .205	.141 .008	.122 .023	-.092 .086
Back swimmer	-.216 .000	.010 .842	.080 .095	.166 .001	-.072 .136	-.055 .254	-.092 .055	.065 .176	.110 .022	.046 .340	-.039 .420
Water boatman	-.286 .000	.023 .628	.221 .000	.244 .000	-.018 .713	-.186 .000	.124 .010	.188 .000	-.146 .002	.143 .003	-.085 .078
Diving beetle	-.175 .000	.038 .427	.164 .001	.197 .000	-.113 .019	-.076 .115	.023 .632	-.065 .180	.054 .264	.002 .962	.092 .056
Dragonfly nymphs	-.177 .000	-.105 .030	.065 .179	.123 .010	-.015 .758	.131 .006	.275 .000	.008 .867	-.031 .518	.098 .043	-.099 .041
Water strider	-.156 .001	-.109 .024	.206 .000	.211 .000	-.086 .075	.061 .205	.280 .000	.001 .978	-.055 .254	.015 .753	.043 .379

	Large items	Glass bottles	Bleach bottle	Brown water	Green water	Foul smell	pH	Salinity	Total chlorine	Nitrate	Nitrite
Time	-.099 .039	-.163 .001	-.154 .001	-.036 .460	.089 .066	-.165 .001	-.315 .000	-.404 .000	-.141 .004	-.110 .026	-.225 .000
Leaves	-.028 .561	.081 .093	-.140 .004	.017 .720	-.025 .610	-.076 .114	-.136 .006	-.088 .075	-.033 .498	-.016 .750	-.006 .897
Sticks	-.059 .222	-.039 .420	-.005 .921	.002 .973	.055 .252	-.005 .920	.004 .938	.053 .286	.029 .551	-.010 .832	-.016 .744
Tree branches	.305 .000	.024 .618	-.009 .852	.041 .401	-.006 .902	.132 .006	.231 .000	.129 .009	-.062 .211	-.047 .339	-.025 .608
Wood	-.020 .679	.149 .002	-.023 .628	.005 .911	.005 .916	.016 .735	.029 .559	.032 .516	-.008 .874	-.025 .610	-.062 .213
Grass	-.043 .378	.117 .015	.163 .001	-.048 .318	.041 .393	-.034 .484	.018 .718	.151 .002	.098 .046	-.072 .145	-.018 .720
Pine leaves	-.112 .020	-.059 .219	.294 .000	.084 .083	-.050 .296	-.017 .726	.145 .003	.180 .000	.141 .004	-.059 .232	-.001 .992
Oak leaves	-.064 .184	-.055 .255	-.058 .231	.164 .001	-.120 .013	.174 .000	.073 .137	.070 .155	-.023 .639	.139 .005	.170 .001
Algal mats	.094 .051	-.109 .024	-.021 .663	-.230 .000	.208 .000	.004 .940	-.087 .076	-.053 .286	-.032 .512	.044 .376	.027 .583
Plastic bags	-.256 .000	-.061 .203	-.014 .774	.035 .471	-.044 .358	-.001 .976	.098 .046	.054 .273	.045 .368	.039 .426	.054 .274
Plastic objects	-.072 .138	-.124 .010	-.013 .781	-.009 .848	.041 .395	-.005 .912	-.004 .928	.131 .008	-.071 .151	-.068 .170	-.006 .897
Large items	1.000 .	.040 .409	-.074 .124	.039 .416	-.083 .086	.189 .000	.118 .017	.062 .214	-.043 .385	-.030 .550	.034 .498
Glass bottles	.040 .409	1.000 .	.116 .016	-.035 .469	.008 .868	.002 .965	.070 .158	.059 .231	-.006 .906	-.049 .323	-.043 .382

Bleach bottle	-.074 .124	.116 .016	1.000 .	-.047 .330	.092 .056	-.034 .487	.118 .017	.166 .001	.174 .000	-.033 .509	.058 .245
Brown water	.039 .416	-.035 .469	-.047 .330	1.000 .	-.841 .000	.026 .596	.002 .973	-.033 .504	.011 .825	-.026 .601	-.053 .285
Green water	-.083 .086	.008 .868	.092 .056	-.841 .000	1.000 .	-.023 .637	-.008 .875	.014 .772	-.051 .304	.044 .375	.077 .119
Foul smell	.189 .000	.002 .965	-.034 .487	.026 .596	-.023 .637	1.000 .	.085 .085	.062 .208	.029 .562	.195 .000	.228 .000
pH	.118 .017	.070 .158	.118 .017	.002 .973	-.008 .875	.085 .085	1.000 .	.381 .000	.144 .003	-.012 .808	-.052 .292
Salinity	.062 .214	.059 .231	.166 .001	-.033 .504	.014 .772	.062 .208	.381 .000	1.000 .	.119 .016	-.012 .810	.071 .150
Total chlorine	-.043 .385	-.006 .906	.174 .000	.011 .825	-.051 .304	.029 .562	.144 .003	.119 .016	1.000 .	.089 .072	.187 .000
Nitrate	-.030 .550	-.049 .323	-.033 .509	-.026 .601	.044 .375	.195 .000	-.012 .808	-.012 .810	.089 .072	1.000 .	.549 .000
Nitrite	.034 .498	-.043 .382	.058 .245	-.053 .285	.077 .119	.228 .000	-.052 .292	.071 .150	.187 .000	.549 .000	1.000 .
Oxygen	-.156 .002	.029 .553	-.089 .072	-.056 .258	.062 .210	-.164 .001	-.270 .000	-.224 .000	-.082 .096	-.025 .613	-.116 .019
Ammonia	.001 .981	-.078 .121	.000 .999	.121 .016	-.038 .457	.153 .002	.086 .092	.179 .000	-.004 .941	.203 .000	.142 .005
Temperature	.047 .343	.005 .920	.105 .034	-.057 .252	.102 .037	.143 .004	.267 .000	.115 .019	.155 .002	.177 .000	.205 .000
Secchi disk	-.043 .419	-.021 .701	-.073 .175	-.078 .146	-.026 .630	-.121 .024	-.071 .187	.044 .414	.011 .834	-.063 .239	-.004 .934
Back swimmer	.040 .402	.001 .984	.114 .018	.026 .587	-.050 .302	.036 .458	.040 .420	.067 .177	-.018 .709	.044 .377	.078 .115
Water boatman	.034 .486	-.071 .144	-.115 .017	.156 .001	-.141 .003	.081 .093	.115 .020	.091 .065	.058 .240	.002 .966	.030 .546
Diving beetle	-.037 .441	-.072 .138	-.027 .575	-.135 .005	.079 .101	.015 .754	.052 .294	.139 .005	-.038 .444	-.024 .630	-.011 .823
Dragonfly nymphs	.029 .551	-.013 .796	.103 .033	-.056 .244	.081 .091	-.075 .121	.039 .432	.165 .001	.018 .720	-.076 .124	-.026 .604
Water strider	.001 .990	-.064 .187	.184 .000	-.004 .940	.038 .437	-.043 .378	.025 .616	.147 .003	.125 .011	-.040 .415	.107 .030

	Oxygen	Ammonia	Temperature	Secchi disk	Back swimmer	Water boatman	Diving beetle	Dragonfly nymphs	Water strider
Time	.243 .000	-.060 .234	-.197 .000	.008 .878	-.216 .000	-.286 .000	-.175 .000	-.177 .000	-.156 .001
Leaves	.124 .012	-.016 .753	-.153 .002	.029 .589	.010 .842	.023 .628	.038 .427	-.105 .030	-.109 .024
Sticks	.007 .890	.046 .359	.030 .547	.025 .647	.080 .095	.221 .000	.164 .001	.065 .179	.206 .000
Tree branches	-.224 .000	.221 .000	.109 .026	-.222 .000	.166 .001	.244 .000	.197 .000	.123 .010	.211 .000
Wood	.001 .978	-.082 .103	-.036 .466	.056 .293	-.072 .136	-.018 .713	-.113 .019	-.015 .758	-.086 .075

Grass	.089 .069	-.048 .342	-.031 .526	.003 .957	-.055 .254	-.186 .000	-.076 .115	.131 .006	.061 .205
Pine leaves	-.149 .002	.082 .105	.106 .032	-.076 .154	-.092 .055	.124 .010	.023 .632	.275 .000	.280 .000
Oak leaves	-.052 .294	.043 .398	-.065 .189	-.068 .205	.065 .176	.188 .000	-.065 .180	.008 .867	.001 .978
Algal mats	.019 .698	-.065 .196	.199 .000	.141 .008	.110 .022	-.146 .002	.054 .264	-.031 .518	-.055 .254
Plastic bags	-.013 .794	.027 .587	.039 .427	.122 .023	.046 .340	.143 .003	.002 .962	.098 .043	.015 .753
Plastic objects	-.055 .269	.081 .110	.012 .814	-.092 .086	-.039 .420	-.085 .078	.092 .056	-.099 .041	.043 .379
Large items	-.156 .002	.001 .981	.047 .343	-.043 .419	.040 .402	.034 .486	-.037 .441	.029 .551	.001 .990
Glass bottles	.029 .553	-.078 .121	.005 .920	-.021 .701	.001 .984	-.071 .144	-.072 .138	-.013 .796	-.064 .187
Bleach bottle	-.089 .072	.000 .999	.105 .034	-.073 .175	.114 .018	-.115 .017	-.027 .575	.103 .033	.184 .000
Brown water	-.056 .258	.121 .016	-.057 .252	-.078 .146	.026 .587	.156 .001	-.135 .005	-.056 .244	-.004 .940
Green water	.062 .210	-.038 .457	.102 .037	-.026 .630	-.050 .302	-.141 .003	.079 .101	.081 .091	.038 .437
Foul smell	-.164 .001	.153 .002	.143 .004	-.121 .024	.036 .458	.081 .093	.015 .754	-.075 .121	-.043 .378
pH	-.270 .000	.086 .092	.267 .000	-.071 .187	.040 .420	.115 .020	.052 .294	.039 .432	.025 .616
Salinity	-.224 .000	.179 .000	.115 .019	.044 .414	.067 .177	.091 .065	.139 .005	.165 .001	.147 .003
Total chlorine	-.082 .096	-.004 .941	.155 .002	.011 .834	-.018 .709	.058 .240	-.038 .444	.018 .720	.125 .011
Nitrate	-.025 .613	.203 .000	.177 .000	-.063 .239	.044 .377	.002 .966	-.024 .630	-.076 .124	-.040 .415
Nitrite	-.116 .019	.142 .005	.205 .000	-.004 .934	.078 .115	.030 .546	-.011 .823	-.026 .604	.107 .030
Oxygen	1.000 .	-.224 .000	-.391 .000	.134 .012	-.063 .204	-.170 .001	.014 .784	.010 .841	-.158 .001
Ammonia	-.224 .000	1.000 .	.157 .002	-.299 .000	.014 .780	.102 .044	.024 .633	-.072 .156	.087 .087
Temperature	-.391 .000	.157 .002	1.000 .	.044 .416	.058 .243	-.058 .239	.020 .685	-.058 .236	.105 .034
Secchi disk	.134 .012	-.299 .000	.044 .416	1.000 .	.030 .580	-.173 .001	-.057 .287	.092 .084	.065 .227
Back swimmer	-.063 .204	.014 .780	.058 .243	.030 .580	1.000 .	.272 .000	.262 .000	.010 .829	.167 .000
Water boatman	-.170 .001	.102 .044	-.058 .239	-.173 .001	.272 .000	1.000 .	.134 .005	.124 .010	.254 .000
Diving beetle	.014 .784	.024 .633	.020 .685	-.057 .287	.262 .000	.134 .005	1.000 .	.005 .912	.168 .000

Dragonfly nymphs	.010	-.072	-.058	.092	.010	.124	.005	1.000	.428
	.841	.156	.236	.084	.829	.010	.912	.	.000
Water strider	-.158	.087	.105	.065	.167	.254	.168	.428	1.000
	.001	.087	.034	.227	.000	.000	.000	.000	.