The significance of aquatic habitat types when using *Gambusia* to control mosquito larvae in abandoned swimming pools in New Orleans after Hurricane Katrina

Gerald G. Marten, Cynthia Harrison, Mieu Nguyen

New Orleans Mosquito, Termite and Rodent Control Board, 2100 Leon C. Simon Drive, New Orleans, LA 70122. Email: gerrymarten@hawaii.rr.com. 2014.

ABSTRACT *Gambusia affinis* was introduced to approximately 1500 unmaintained swimming pools that provided breeding habitat for *Culex* and *Anopheles* mosquitoes. Because subsequent inspections revealed that fish were missing from 14% of those pools, an ecological study was conducted to clarify what was responsible. The study revealed four habitats of particular significance for fish and mosquito control. The "organic pollution" habitat, created by decomposing organic debris, was ideal for *Cx. quinquefasciatus* larvae. *Gambusia* appeared to tolerate low oxygen associated with severe pollution, but high ammonia concentrations were detrimental. If pollution was light, fish thrived and provided effective larval control; however, if pollution was severe, fish populations were depressed and mosquito control was incomplete. The "saline lake water" habitat was particularly suitable for *Anopheles* and *Cx. salinarius* larvae. Fish thrived, but *Anopheles* larvae sometimes survived with fish in a pool because floating debris such as pine needles provided refuge from fish predation. The "oak leaves" habitat was suitable for both *Culex* and *Anopheles* larvae but poor for fish, sometimes containing mosquito larvae even when fish were in a pool. The "floating algal mats" habitat was poor for mosquito larvae and excellent for fish. There was a natural reduction in mosquito production from 2006 to 2008 as ecological succession transformed pools from "organic pollution" and "saline lake water" to "floating algal mats."

Keywords mosquito larvae; Culex; Anopheles; mosquito control; mosquito fish; Gambusia; biological control; swimming pool; Hurricane Katrina; water quality

Introduction

Hurricane Katrina and the flooding of New Orleans led to miles of abandoned residential neighborhoods containing several thousand unattended swimming pools, many of the pools without maintenance for years (Moise et al., 2013) (Figure 1). The water in the pools – littered with rotting trees, household goods, and other debris from the hurricane and receding flood waters – provided breeding habitat for *Culex quinquefasciatus* Say, *Culex salinarius* Coquillett, *Culex restuans* Theobald, *Culex coronator* Dyar & Knab, *Culiseta inornata* Theobald, *Anopheles crucians* Wiedemann, *Anopheles quadrimaculatus* Say, and to a lesser extent *Cx. erraticus* Dyar & Knab and *Cx. nigripalpus* Theobald. Fortunately for disease control, there were very few people in New Orleans during the months immediately following Katrina, but as people returned, there was serious concern about what might happen with West Nile virus and other mosquito-borne diseases (Caillouët et al., 2008a; Reisen et al., 2008). Thus began a program of emergency mosquito control for a city in disarray, a challenge that moved beyond previous experience and continued with less than adequate information for several years (Marten et al., 2012).

Approximately 750 pools contained fish from the Katrina flood – mainly mosquito fish (*Gambusia affinis* (Baird and Girard)), but also sailfin mollies (*Poecillia latipinnia* (Lesueur)), least killifish (*Heterandria formosa* Girard), and sheepshead minnows (*Cyprinodon variagatus* Lacepède) (Caillouët et al., 2008b). In April 2006, the New Orleans Mosquito, Termite and

Rodent Control Board began to introduce *Gambusia affinis* from a catfish farm in Mississippi into the approximately 1,500 unmaintained swimming pools that did not already contain fish (Marten et al., 2012). Fish were held temporarily in a swimming pool that served as a holding tank and were transported to unmaintained swimming pools in plastic bags filled with oxygen – 50 fish in each bag. During transport the bags were stacked in large coolers (without ice) in the back of pickup trucks. All the fish in a single bag were poured into each unmaintained pool. To suppress mosquito production until the fish established full populations, VectoLex (*Bacillus sphaericus*, Lacey, 2007) was applied to each pool at the same time fish were introduced.

Gambusia was introduced to a total of 1,278 unattended pools by September 2006. In October, field crews began returning to the pools to see how the fish were doing, and fish were missing from 14% of the pools to which they had previously been introduced. Fish were reintroduced to swimming pools that did not contain them. The reintroduced fish survived in the great majority of those pools, but not all of them.

Although damage to fish during transport from holding tanks to swimming pools during the difficult circumstances of post-Katrina New Orleans was responsible for many of the failed fish introductions in 2006 (Marten et al., 2012), there were concerns that ecological conditions in some of the pools were interfering with the health and survival of the fish. Pools with large quantities of rotting trash and putrid water were particularly alarming. Even when there were fish in a pool, their numbers were sometimes small, and some pools with fish contained mosquito larvae.

A field research program was initiated to examine the hypothesis that ecological conditions in the pools were responsible for (a) failed fish introductions and (b) depressed fish populations in some pools where introduced fish survived. The ultimate objective was not only to understand problems with the fish introductions but also to identify measures that would make the introductions more effective for mosquito control.

Materials and methods

Quantitative assessment of ecological conditions in a broad selection of swimming pools began in May 2007 and ended two years later. Each inspection included the variables listed in Table 1. Salinity, oxygen, and temperature were measured with a YSI meter (Model 85-10 FT). Chlorine, nitrate, nitrite, and ammonia were measured with WaterWorks water quality test strips. Water odor was assessed by smelling water dipped from each pool. Mosquito larvae and pupae were assessed with eight dips around the edge of each pool, directed to spots with the most larvae or appearing particularly suitable for larvae (e.g., at the edge of floating plants or other floating objects). All collected larvae were taken to the laboratory for identification. *Culex* larvae were identified to species. *Anopheles* larvae were identified to genus but known from prior experience in New Orleans to be *An. crucians* or *An. quadrimaculatus*. All other variables in Table 1 were assessed visually.

Fifty-five pools were monitored with a total of 433 inspections, covering a broad range of ecological histories and conditions, including the source of water that flooded each pool, trash introduced to the pool by the hurricane and flood, and vegetation in the vicinity of the pool. Monitored pools also embraced a range of histories with regard to fish introductions, including:

- 11 pools containing Gambusia introduced by the Katrina flood;
- 11 pools containing successfully introduced *Gambusia*;
- 11 pools where *Gambusia* introduction had failed;
- 11 pools that were treated with Vectolex whenever mosquito larvae were observed during an inspection, but fish were never introduced;
- 11 “control” pools that never received fish introductions or insecticidal treatment.

Statistical analysis followed procedures employed by Marten et al. (1996). To start, Spearman rank correlation coefficients (Gibbons, 1992) were calculated for every pair of variables in Table

1. 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.

Swimming pool "habitat types" were identified by means of factor analysis – principle components analysis with varimax rotation (Tabachnick, 2007) – for all the variables in Table 1, applied separately to data from all pools containing fish and all pools without fish. Each "factor group" was a set of intercorrelated variables representing a distinct ecological situation reflected in the data. The "factor loading" for a particular variable with regard to a particular factor group indicated the strength of that variable's association with that factor group. "Factor scores" for each inspection represented the strength of each factor group at a particular swimming pool and inspection time.

Multiple regression analysis was applied with the abundance of fish and the abundance of particular species of mosquito larvae as dependent variables and all other variables in Table 1 as independent variables. Log (X+1) transformation was applied to counts of mosquito larvae and ammonia concentration before including them in statistical analyses. Partial regression coefficients from the regression analysis helped to suggest direct causal relationships between fish, mosquito larvae, and ecological conditions in the swimming pools.

Empty bleach bottles were frequently seen at unmaintained swimming pools because homeowners who visited their unoccupied houses were alarmed at the condition of the pool and dumped bleach to "clean it up." The bleach could kill the fish, converting the pool into mosquito breeding habitat if left unmaintained after that. We conducted field experiments in July 2007 to assess the impact of bleach dumping on fish. Sixteen liters of bleach were poured into each of three pools that contained thriving *Gambusia* populations and no detectable chlorine in the water (Figure 2). Chlorine concentrations and fish numbers were monitored after that.

Results

Figure 3 shows the month-to-month number of pools without fish that were observed to contain larvae of each mosquito species. *Culex quinquefasciatus* was the most abundant species – present throughout the year and sometimes numbering hundreds of larvae/dip, though declining in numbers during the summer. *Anopheles* larvae (*An. crucians* and *An. quadrimaculatus*) were also in pools throughout the year, but their numbers were generally much lower than *Cx. quinquefasciatus*. *Cx. salinarius* and *Cx. restuans* were most conspicuous from October to April, and *Culiseta inornata* was numerous from November to March. *Culex coronator* and *Cx. erraticus* extended from July to November with large numbers of *Cx. coronator* also seen in January.

Table 2 shows Spearman rank correlations between (a) fish numbers and numbers of mosquito larvae or pupae and (b) all the other variables in Table 1. The quantity of manufactured objects in a pool – such as bottles, plastic bags, toys and other plastic objects, furniture, and appliances – was not correlated with the presence or abundance of fish or mosquito larvae. A few elements of water quality such as pH and nitrate concentration were also uncorrelated with fish or mosquito larvae. Some aquatic insects had negative correlations with particular species of mosquito larvae. However, water boatmen had strong positive correlations with both *Culex* and *Anopheles* larvae, and dragon fly nymphs had a strong positive correlation with *Anopheles* larvae.

Impact of fish on mosquito larvae

How effective were the fish at preventing mosquito breeding in swimming pools? While it was not common to see mosquito larvae in pools with fish, it did happen. Based on all swimming pool inspections during the fish introduction program (2006-2008), larvae were seen in 2.2% of the inspections of pools that contained fish (N=3,186), compared to 36.0% of the inspections of pools without fish (N=2,697). The larvae seen most frequently in pools with fish were *Anopheles*. *Culex quinquefasciatus* larvae were seen less frequently, but when present, they were typically much more numerous than *Anopheles*. Larvae of the other mosquito species were virtually never seen in pools with fish.

While the impact of fish on mosquito larvae was mainly a consequence of whether or not there were fish in a pool, the number of fish also mattered. Mosquito larvae were most numerous in pools where fish numbers were low. Every species of mosquito larvae, as well as pupae, had a strongly negative Spearman rank correlation with fish numbers (Table 2), and the regression coefficients in Table 3 for the association of *Cx. quinquefasciatus* and *Anopheles* larvae with fish abundance were strongly negative. In fact, there were very large numbers of *Cx. quinquefasciatus* larvae only in the unusual circumstance of small fish numbers and fish that seemed sick because their movement was abnormal (e.g., not fleeing quickly when disturbed). Moderate numbers of *Anopheles* larvae were sometimes seen in pools with numerous and apparently healthy fish.

Water quality and fish survival

Inspections of hundreds of swimming pools during 2007-2008 showed that if there was an established fish population in a swimming pool during that period, the population almost always remained in the pool for as long as it contained water. Most of the observed instances of established fish populations disappearing from pools were when the pools were drained. Although homeowners sometimes demolished their pools or maintained them after draining, many drained pools were left unmaintained, leaving them to collect rainwater and produce mosquitoes because they no longer contained fish. However, pool draining was not the only problem. A small number of the fish introductions and reintroductions in 2007 did in fact fail; in addition, established fish populations disappeared from four of the 55 monitored pools during 2007-2008; and the fish in a few pools appeared unhealthy. Every pool where these problems occurred had conspicuous signs of organic pollution (e.g., foul odor).

The “*Gambusia* tolerance” column in Table 4 provides a summary of water-quality factors known to be harmful to *Gambusia* when extreme. Comparing the “extreme values” column with the “*Gambusia* tolerance” column in Table 4, observed values of temperature, pH, and salinity were always within the range of published tolerances for *Gambusia*.

The pools were observed to be clustered into two salinity ranges when quantitative data collection began in May, 2007:

- Pools with salinity in a range of 4-10 ppt. These pools had been flooded during the Katrina flood with brackish water from Lake Pontchartrain, whose salinity can be as high as 16 ppt.
- Pools with salinity in a range of 0.1-0.3 ppt. These pools had not been flooded with brackish water.

The salinity of pools flooded with brackish water declined steadily over time. Most of those pools were in a salinity range of 1.5-5.0 ppt by the middle of 2008, and almost all were less than 2.0 ppt by the middle of 2009. The salinity of pools not flooded with brackish water remained in a range of 0.1-0.3 throughout the entire period.

We observed apparently normal *Gambusia* populations in pools with as little as 0.1 ppm oxygen. The fish breathed at the surface when oxygen was low, presumably mixing air into water passing over their gills. There was only one monitored pool in which *Gambusia* introductions failed repeatedly (Figure 4). That pool was littered with trash and contained approximately 0.3 meters depth of foul-smelling water that was never observed to contain measurable oxygen. The ammonia concentration at different times was in a range of 6-10 ppm.

High concentrations of ammonia are known to be toxic to fish (Table 4). Among the 55 monitored pools we saw seven with ammonia concentrations greater than 10 ppm at one time or another, including a pool with 40 ppm for an extended period. There were fish in all of these pools. However, even though fish managed to survive high ammonia concentrations, the negative regression coefficient for ammonia in Table 5 shows that fish numbers were distinctly reduced where ammonia concentrations were high.

While high concentrations of nitrite are also toxic, there was no measurable nitrite in the vast majority of sampled pools (Table 4). The nitrite concentration at one pool was 6 ppm on one occasion, and 2 ppm at another pool, but the fish appeared normal. Although we had no evidence that nitrite was completely excluding fish from swimming pools, the negative regression coefficient for nitrite in Table 5 indicates there were smaller fish populations in pools with higher nitrite.

When we poured bleach into three swimming pools to assess its impact on the fish, the chlorine concentration 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 dropped to less than 0.1 ppm. Nonetheless, every pool once again had a thriving fish population within a month, despite the fact that no fish were reintroduced to those pools. Bleach dumping remained a concern, however, because the negative regression coefficient in Table 5 for empty bleach bottles indicates that fish populations were smaller at pools where bleach dumping had occurred recently. The positive regression coefficient for chlorine (dependent variable: *Cx. quinquefasciatus*) suggests that depression of fish by bleach was translating into larger numbers of *Cx. quinquefasciatus* larvae.

Swimming pool habitat types

The factor analysis revealed four factor groups in pools that did not contain fish (Table 6), each factor group representing a prominent habitat type. Most pools fell clearly within a single habitat type, though some pools at some times displayed the characteristics of two habitat types. Occasionally none of the four habitat types was clearly represented.

Organic pollution. Factor Group 1 in Table 6 represents pools cluttered with tree branches and other rotting debris, leading to low oxygen concentrations, a characteristic foul odor, high ammonia concentration, high pH, and large numbers of *Cx. quinquefasciatus* larvae (Figure 4). The positive factor loading for water temperature indicates that this habitat type was stronger during the summer, when decomposition was most intense. The negative factor loading for “time” indicates that this habitat type was most prominent at the beginning of the data collection period (May 2007) and diminished during the subsequent two years. The positive regression coefficient for foul odor and the negative coefficient for oxygen in Table 3 suggest that they were the elements of this habitat type that most directly connected *Cx. quinquefasciatus* larvae to it.

Saline lake water. Factor Group 2 in Table 6 represents pools with a high salinity (4-8 ppt) because they were flooded during the hurricane with brackish water from Lake Pontchartrain. The water surface in the “saline lake water” habitat type typically contained floating pine needles, twigs, or other small plant debris, which presumably did not sink to the bottom because of the buoyancy and strong surface tension of saline water (Figure 5). This habitat type was particularly favorable for *Anopheles* and *Culex salinarius* larvae as well as other aquatic insects such as dragonfly nymphs, water striders, and water boatmen. The negative factor loading of “foul odor” for Factor Group 2 shows that the water in this habitat type lacked the putrid quality of water in the “organic pollution” habitat type. While *Cx. quinquefasciatus* larvae were sometimes seen in the “saline lake water” habitat type, it was not ideal for that species. The positive regression coefficients for pine needles and grass in Table 3 indicate that these factors were particularly significant for connecting *Anopheles* larvae to this habitat type. It was common to see *Anopheles* larvae clinging to floating pine needles clustered around the edge of a pool or grass growing into the water from the edge. The *aufwuchs* film of microalgae and bacteria on the surface of floating leaves, twigs, and grass provided a concentration of food for the larvae.

Oak leaves. Factor Group 3 in Table 6 represents pools with oak leaves, distinctively brown water (from tannins in oak leaves), and an abundance of water boatmen (Figure 6). This habitat type was favorable for both *Cx. quinquefasciatus* and *Anopheles* larvae. The high regression coefficient for oak leaves in Table 3 suggests that oak leaves were the most direct connection between *Cx. quinquefasciatus* larvae and this habitat type.

Floating algal mats. Factor Group 4 in Table 6 represents pools with floating mats of filamentous algae, backswimmers, diving beetles, and water striders (Figure 7). Water clarity was high in part because shading from algal mats suppressed phytoplankton production. Mosquito larvae of all species were generally absent from these pools. The positive factor loading for water temperature in Factor Group 4 indicates that this habitat type was stronger during the summer, when there was more light to support algal growth. The negative regression coefficient for algal mats in Table 3 indicates algal mats were particularly significant for the negative association of *Cx. quinquefasciatus* larvae with this habitat type. Because algal mats covered so much of the water surface, they not only suppressed production of phytoplankton food for mosquito larvae but may also have interfered with oviposition. The negative regression coefficients for water striders (dependent variable: *Cx. quinquefasciatus*) and backswimmers and diving beetles (dependent variable: *Anopheles*) in Table 3 suggest that predation by these aquatic insects may have contributed to low numbers of mosquito larvae in this habitat.

Table 7 shows the four groups of intercorrelated variables identified by the factor analysis for pools that contained fish. There are striking similarities between the factor groups in Table 6 and Table 7, but there are also conspicuous differences between Table 6 and Table 7 that reflect the impact of fish predation.

Organic pollution. Factor Group 1 in Table 7 represents pools littered with tree branches and other trash and characterized by low oxygen, high ammonia, low water clarity, foul odor, and the full range of aquatic insect predators. The physical/chemical characteristics of this ecological situation are very similar to Factor Group 1 in Table 6. However, *Cx. quinquefasciatus* larvae were not associated with this habitat type when fish were in the pool (Table 7), despite the fact that *Cx. quinquefasciatus* larvae thrived in this habitat type when fish were absent (Table 6). The explanation for the difference seems to be predation by fish, an interpretation supported by the highly significant negative regression coefficient for fish abundance (dependent variable: *Cx. quinquefasciatus*) in Table 3. Fish appeared normal in this habitat as long as the pollution was not too severe.

Saline lake water. Factor Group 2 in Table 7 represents conditions that were unpolluted (i.e., a negative factor loading for foul odor) and particularly favorable for *Anopheles* larvae and other aquatic insects such as dragonfly nymphs, water striders, and water boatmen. This is very similar to Factor Group 2 in Table 6. Many of the fish in this habitat were introduced to the swimming pools by the Katrina flood. “Saline lake water” was the best habitat type for *Anopheles* larvae if fish were present. *Anopheles* were able to conceal themselves from fish predation by clinging to pine needles, twigs, and grass. *Cx. salinarius* larvae were prominent in the “saline lake water” habitat when fish were absent (Table 6) but not associated with this habitat when fish were present (Table 7).

Oak leaves. Factor loadings for Factor Group 3 in Table 7 represent pools with a large quantity of oak leaves, a low number of fish, and positive factor loadings for *Cx. quinquefasciatus*, *Cx. salinarius*, and *Anopheles* larvae. This habitat type is very similar to Factor Group 3 in Table 6. Although fish reduced the number of mosquito larvae in all habitat types, “oak leaves” was the best habitat for *Cx. quinquefasciatus* if fish were in the pool. The explanation is presumably that fish numbers were depressed in this habitat, and fish predation on mosquito larvae was correspondingly diminished.

Floating algal mats. Negative factor loadings for Factor Group 3 in Table 7 indicate an additional ecological situation, which is the opposite of that represented by the positive factor loadings for Factor Group 3 in Table 7. With algal mats, clear water, high fish abundance, and high temperatures, this habitat type is similar to Factor Group 4 (the floating algal mats habitat type) in Table 6. Backswimmers, diving beetles, and water striders were prominent in this habitat when fish were absent (Table 6) but not when fish were present (Table 7). Mosquito larvae were almost never seen in this habitat type when fish were in the pool, apparently due to a paucity of food in the clear water and high predation by robust fish populations. The negative association of this habitat type with the “oak leaves” habitat type is probably because shading from oak trees was unfavorable for algae growth.

Table 8 shows the frequencies of the four habitat types in 2007 and 2008. The percentages in Table 8 are not precise estimates for each habitat type in the city’s entire unmaintained swimming pool population because monitored pools were not a random sample of all pools. Nonetheless, the percentages reflect real changes from 2007 to 2008. The “organic pollution” and “saline lake water” habitat types were common in 2007, but were no longer significant in 2008. The number of pools with the “floating algal mats” habitat type increased from 2007 to 2008, in part because many “organic pollution” and “saline lake water” pools changed to “floating algal mats” by 2008. All “oak leaves” swimming pools in 2007 continued with the “oak leaves” habitat type in 2008.

Tables 6-8 are based on quantitative data starting in May 2007. What are the implications for the time before data collection began? Because some of the key features of each habitat type can be discerned by just looking at a swimming pool, we know from visual observations during 2006 that all four habitat types were already conspicuous at that time. In fact, the “organic pollution” and “saline lake water” habitat types were even more common and more pronounced during 2006 than they were during 2007.

Discussion and conclusions

Water quality and fish

The statistical analyses in this study confirmed our hypothesis that ecological conditions in the pools were responsible for failed fish introductions and depressed fish populations. Ammonia, which is one of the most conspicuous products of decomposing organic matter, emerged as the prime candidate for explaining failed or struggling fish populations, though the fish were more resilient to ammonia than published laboratory studies would suggest. Walton (2007) concluded from laboratory studies that *Gambusia*’s ammonia tolerance is one ppm. Liang and Wong (2000) observed 2%-4% *Gambusia* mortality with three days’ exposure to ammonia in the range of 1-5 ppm and 10%-70% mortality with ammonia in the range of 5-25 ppm. However, we encountered live fish in swimming pools with months of exposure to ammonia concentrations in a range of 10-40 ppm, an observation in accord with Hubbs’ (2000) report that *Gambusia affinis* survived in a heavily-polluted pond with an ammonia concentration of 10 ppm. The discrepancy between field observations and laboratory studies may be because laboratory studies evaluated tolerances with bioassay analysis estimating LD50s. What happened in swimming pools was a population response, in which a pool contained fish if any of the fish were able to survive and reproduce under long-term exposure to ammonia. However, high ammonia concentrations were not without negative consequences. Pools with high ammonia were the ones where fish had problems.

Ammonia combines with oxygen to form nitrite, which can be toxic to fish at concentrations exceeding 1.5 ppm (Lewis & Morris, 1986). High nitrite concentrations in the swimming pools were associated with smaller fish populations, but high nitrite was not common enough, or continuous enough, to impact many swimming pools. Chlorine from bleach dumping had a devastating short-term impact on fish populations, but we did not see evidence that bleach dumping eradicated fish from the pools. While oxygen was low in severely polluted pools, low oxygen was not noticeably detrimental to *Gambusia* except possibly when there was no measurable oxygen at all.

When we began the study, we expected to find a connection between severe organic pollution and struggling fish populations, and that connection was confirmed. We also discovered a connection that we had not anticipated. Fish populations were depressed in pools with a large quantity of oak leaves in the water, an effect supposedly due to chemicals leaching from the leaves, though the mechanism is not known (Brandenberg, n.d.).

Effectiveness of *Gambusia* predation

This study confirmed the effectiveness of *Gambusia* predation on mosquito larvae in the great majority of swimming pools to which fish were introduced, though predation was incomplete where fish populations were depressed by severe organic pollution or large numbers of oak leaves. Where fish populations were not depressed, predation shortfalls were observed mainly with *Anopheles* larvae protected from predation by vegetation or other objects at the water surface, a result in accord with long-standing experience using fish for mosquito control (Hildebrand, 1919; Meisch, 1985; Malhotra & Prakesh; 1992, Swanson et al., 1996).

Aquatic insect predators

In addition to eating mosquito larvae, fish eat aquatic insects that prey on mosquito larvae. While water boatman numbers were depressed in pools with fish (Table 5), and backswimmers, diving beetles, and water striders were less numerous in the "floating algal mats" habitat when fish were present, insect predators were prominent in the other habitats even when fish were present (Tables 6 and 7).

How effective were the aquatic insect predators for mosquito control? While *Cx. quinquefasciatus* larvae were negatively associated with the abundance of water striders, and *Anopheles* larvae were negatively associated with the abundance of diving beetles and backswimmers (Table 3), the statistical analysis revealed no other negative impacts of aquatic insects on larval numbers. Carlson et al. (2004) frequently observed *Culex* and *Anopheles* larvae coexisting with aquatic insect predators in unattended swimming pools in Kenya. In New Orleans, *Anopheles* larvae coexisted with predators such as dragon fly nymphs, water striders, and water boatmen, particularly in the "saline lake water" habitat (Tables 6 and 7). Ecological conditions favorable for one seemed favorable for all. Similarly, *Culex* larvae, *Anopheles* larvae, and water boatmen thrived together in the "oak leaves" habitat. Water boatmen emerged from the data as a particularly strong indicator of favorable conditions for both *Culex* and *Anopheles* larvae (Table 3).

Management insights from the habitat types

The fact that the factor analysis in this study revealed the same habitat types in two separate data sets – swimming pools without fish (Table 6) and pools with fish (Table 7) – reinforces our confidence in the reality of those habitats. Differences between the habitats can help to explain why fish, and particular species of mosquito larvae, flourished in some pools and not others. The habitat differences can also provide a basis for tailoring the management of unmaintained swimming pools to the ecological conditions in each pool.

The "organic pollution" habitat type, characterized by foul odor, high turbidity (reflecting an abundance of bacterial food for mosquito larvae), low oxygen, and high ammonia concentration was a consequence of rotting materials cast into pools by the hurricane and flood. This habitat was particularly suitable for *Cx. quinquefasciatus* larvae. Fish provided effective control except when pollution was so severe that it sickened fish and depressed their populations. Severe organic pollution was common enough during 2006 to be responsible for (a) a substantial number of the failed fish introductions that year and (b) fish populations that survived but were not healthy enough to provide effective larval control. The "organic pollution" habitat type persisted for as long as two years after Katrina in pools that were never cleaned out, gradually declining as the organic debris decomposed. Draining and cleaning a pool with this habitat type, and then refilling it with water and stocking it with fish, should eliminate the pollution and ensure effective mosquito control even if the pool continues without further maintenance. If thorough cleaning is not feasible, simply removing trash should help to wind down the organic pollution more quickly.

The "saline lake water" habitat type was a consequence of flooding with brackish, nutrient-rich lake water. It was particularly favorable for *Anopheles* and *Cx. salinarius* larvae, whose main natural habitat in the vicinity of New Orleans is brackish marshes. Fish thrived in this habitat and effectively eliminated *Cx. salinarius* larvae. High salinity had no negative impact on *Gambusia* populations, but it enabled an accumulation of pine needles or twigs on the surface of the water, providing refuge for *Anopheles* larvae and reducing the effectiveness of fish predation. This observation is in accord with the report of Duryea et al. (1996) that *Gambusia* predation on *Anopheles* larvae was incomplete in unmaintained swimming pools in New Jersey when they contained floating materials. The "saline lake water" habitat diminished in strength over a period of three years because the salinity gradually declined during that time. The most important management is to replace the water to reduce the salinity. Otherwise, it is necessary to continually remove pine needles, twigs, and other floating materials.

The "oak leaves" habitat type was favorable for all species of mosquito larvae and had particularly high levels of mosquito production (indicated by the high correlation between oak leaves and pupae numbers in Table 2). The decomposing leaves appeared to provide an abundant food supply for the larvae. Fish populations were low, and larvae often survived even with fish in the pool. This habitat was location specific, associated with overhanging oak trees, and the number of pools with this habitat did not decline with the passage of time. Since pools with a continuous influx of oak leaves can continue to produce mosquitoes even with fish in the pool, such pools should be priority candidates for demolition or conversion to full maintenance.

The "floating algal mats" habitat type, which was similar to a healthy pond ecosystem, supported thriving fish populations. The filamentous algae in this habitat can give it a "messy, stagnant water" appearance (Figure 7), which at first glance might seem favorable for mosquito breeding. However, mosquito production was in fact very low, even in the absence of fish, presumably because the clear water, shaded by algal mats, provided little food for mosquito larvae and possibly because of predation by backswimmers and diving beetles. Despite past reports associating algal mats with *Anopheles* production in aquatic habitats not involving swimming pools (Hildebrand, 1919; Hess & Hal,l 1943), mosquito production from our New Orleans swimming pools was virtually nil with fish in "floating algal mats" habitat. This habitat was in some swimming pools during the first year after Katrina but was particularly common by the third year, when the "organic pollution" and "saline lake water" habitat types faded away – a natural ecological succession from more favorable to less favorable conditions for mosquito larvae. It appears to be sound mosquito management to allow unattended pools with *Gambusia* in "floating algal mats" habitat to continue that way if they cannot be put into proper pool maintenance.

Acknowledgments

We wish to thank New Orleans Mosquito, Termite & Rodent Control Board staff for helping to make the collection of quality data a reality under difficult circumstances. Greg Thompson, who was in charge of locating unmaintained swimming pools and managing the records of fish introductions and follow-up pool inspections, was helpful in many ways, including provision of useful support information for this study. Michael Carroll commented on manuscript drafts.

References

Brandenberg, D., n.d. Toxic pond plants. eHow. Retrieved May 14, 2014, from www.ehow.com/list_6182906_toxic-pond-plants.html.
Caillouët, K. A., S. R. Michaels, X. Xiong, I. Foppa & D. M. Wesson, 2008a. Increase in West Nile neuroinvasive disease after Hurricane Katrina. Emerging Infectious Diseases 14, No. 5. Available from http://wwwnc.cdc.gov/eid/article/14/5/07-1066.htm.
Caillouët, K. A., J. C. Carlson, D. M. Wesson & F. Jordan, 2008b. Colonization of abandoned swimming pools by larval mosquitoes and their predators following Hurricane Katrina. Journal of Vector Ecology 33: 166-172.
Carlson, J., J. Keating, C. M. Mbogo, S. Kahindi, & J. C. Beier, 2004. Ecological limitations on aquatic mosquito predator colonization in the urban environment. Journal of Vector Ecology 29: 331–339.
Duryea, R., J. Donnelly, D. Guthrie, C. O'Malley, M. Romanowski & R. Schmidt, 1996. *Gambusia affinis* effectiveness in New Jersey mosquito control. Proceedings of the 83rd Annual Meeting of the New Jersey Mosquito Control Association: 95-102.
Gibbons, J. D., 1992. Nonparametric statistics: an introduction. Sage Publications, Newbury Park, Calif.
Hess, A. D. & T. F. Hall, 1943. The intersection line as a factor in anopheline ecology. Journal of the National Malaria Society 2: 93-98.
Hildebrand, S.F., 1919. Fishes in relation to mosquito control in ponds. U.S. Public Health Reports, No. 21: 1113-1128.
Hubbs, C., 2000. Survival of *Gambusia affinis* in a hostile environment. Southwestern Naturalist 45: 521-522.
Lacey, L. A., 2007. *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. In: Floore, T. G. (ed) Biorational Control of Mosquitoes, American Mosquito Control Association Bulletin No. 7: 133-163.
Lewis, M. L. & D. P. Morris, 1986. Toxicity of nitrite to fish: A review. Transactions of the American Fisheries Society 115: 183-195.
Liang, Y. & M. H. Wong, 2000. Reclamation of wastewater for polyculture of freshwater fish: Bioassays using Chlorella and Gambusia. Archives of Environmental Contamination and Toxicology 39: 506-14.
Marten G. G., M. F. Suárez & R. Astaeza, 1996. An ecological survey of *Anopheles albimanus* larval habitats in Colombia. Journal of Vector Ecology 21: 122-131.
Marten G. G., C. Harrison, M. Nguyen, S. Sackett, G. Thompson, M. Carroll & C. Riegel, 2010. The use of *Gambusia* to control mosquito larvae in abandoned swimming pools: The New Orleans experience. New Orleans Mosquito, Termite, and Rodent Control Board report. Available from http://gerrymarten.com/publicatons/pdfs/GM_new-orleans-swimming-pools.pdf.
Marten G., C. Harrison, M. Nguyen, S. Sackett, G. Thompson, M. Carroll & C. Riegel, 2012. The New Orleans experience: Using *Gambusia* to control mosquito larvae in abandoned swimming pools. Wing Beats 23(1): 27-35.
Malhotra, M. S. & A. Prakesh, 1992. Enhancing the efficacy of *Gambusia affinis* to control mosquito breeding in ponds. Indian *Journal of* Malariology *29*: 65-68.
Meisch, M. V., 1985. *Gambusia affinis affinis*. In: Chapman, H. C. (ed) Biological control of mosquitoes, American Mosquito Control Association Bulletin, No. 6: 3-17.
Moise, I. K., K. S. Brown, C. Riegel, E. Kalipeni & M. O. Ruiz, 2013. Geographic assessment of unattended swimming pools in post-Katrina New Orleans, 2006–2008. Annals of the Association of American Geographers 103: 1160-1175.
Reisen, W. K., R. M. Takahashi, B. D. Carroll, & R. Quiring, 2008. Delinquent mortgages, neglected swimming pools, and West Nile virus, California. Emerging Infectious Diseases 14: 1747-1749.

Swanson, C., J. J. Czech & R. H. Piedrahita, 1996. Mosquito fish: Biology, culture, and use in mosquito control. California Mosquito and Vector Control Association and University of California Mosquito Research Program.
Tabachnick, B. G., 2007. Using Multivariate Statistics. Pearson/Allyn & Bacon, Boston.
Walton, W. E. 2007. Larvivorous fish including *Gambusia*. In: Floore, T. G. (ed) Biorational Control of Mosquitoes, American Mosquito Control Association Bulletin, No. 7: 184-220.

Table 1 Information recorded during each swimming pool inspection[1]

---

1. Address and date
2. Pine needles floating on the water (None, Low, Medium, High)
3. Oak leaves (None, Low, Medium, High)
4. Miscellaneous leaves, not oak or pine (None, Low, Medium, High)
5. Twigs floating on the water (None, Low, Medium, High)
6. Tree branches (None, Low, Medium, High)
7. Wood floating at water surface (None, Low, Medium, High)
8. Grass growing into the water at the edge of the pool (None, Low, Medium, High)
9. Filamentous algae mats at or near the surface (None, Low, Medium, High)
10. Plastic bags (None, Low, Medium, High)
11. Plastic objects such as toys ((None, Low, Medium, High)
12. Large objects such as furniture and appliances (None, Low, Medium, High)
13. Glass bottles (None, Low, Medium, High)
14. Empty bleach bottles around the pool (None, Low, Medium, High)
15. Brown water color (None, Low, Medium, High)
16. Green water color (None, Low, Medium, High)
17. Foul water odor (None, Low, Medium, High)
18. pH
19. Salinity (parts per thousand)
20. Chlorine (parts per million)
21. Nitrate (parts per million)
22. Nitrite (parts per million)
23. Oxygen (parts per million)
24. Ammonia (parts per million)
25. Temperature (degrees centigrade)
26. Water clarity (meter depth of Secchi disk visibility)
27. Backswimmers – Notonectidae (None, Low, Medium, High)
28. Water boatmen - Corixidae (None, Low, Medium, High)
29. Diving beetles – Dytiscidae (None, Low, Medium, High)
30. Dragonfly/mayfly nymphs – Odonata (None, Low, Medium, High)
31. Water striders – Gerridae (None, Low, Medium, High)
32. Mosquito pupae (number in 8 dips, all species combined)
33. *Culex quinquefasciatus* larvae (number in 8 dips)
34. *Culex salinarius* larvae (number in 8 dips)
35. *Anopheles* larvae (number in 8 dips)
36. *Culex coronator* larvae (number in 8 dips)
37. Fish abundance (None, Low, Medium, High)

[1]The complete data set can be accessed online at Marten et al. (2012, Appendix 1).

Table 2 Spearman rank correlations between (a) fish numbers and numbers of mosquito larvae or pupae and (b) all the other variables in Table 1. The top number in each cell of the table is the Spearman correlation coefficient; the bottom number (in italics) is the two-tailed level of significance. N = 433. This table contains only variables with at least one correlation having P<.05

	Fish abundance	Culex quinquefasciatus	Culex salinarius	Culex coronator	Anopheles	Pupae
Fish abundance	1.000	-.223 *.000*	-.072 *.134*	-.122 *.011*	-.222 *.000*	-.180 *.000*
Culex quinquefasciatus	-.223 *.000*	1.000	.149 *.002*	.223 *.000*	.370 *.000*	.339 *.000*
Culex salinarius	-.072 *.134*	.149 *.002*	1.000	-.013 *.783*	.203 *.000*	.353 *.000*
Culex coronator	-.122 *.011*	.223 *.000*	-.013 *.783*	1.000	.175 *.000*	.167 *.000*
Anopheles	-.222 *.000*	.370 *.000*	.203 *.000*	.175 *.000*	1.000	.370 *.000*
Pupae (all species)	-.180 *.000*	.339 *.000*	.353 *.000*	.167 *.000*	.370 *.000*	1.000
Time[1]	.192 *.000*	-.193 *.000*	-.029 *.546*	-.107 *.026*	-.115 *.017*	-.038 *.426*
Grass	.139 *.004*	-.090 *.062*	.038 *.426*	-.053 *.273*	.023 *.632*	-.021 *.663*
Pine needles	.033 *.496*	.101 *.036*	.051 *.294*	.013 *.785*	.212 *.000*	.061 *.205*
Oak leaves	.021 *.662*	.210 *.000*	.137 *.004*	.017 *.724*	.051 *.291*	.238 *.000*
Algal mats	.039 *.415*	-.122 *.011*	-.084 *.082*	-.092 *.057*	-.146 *.002*	-.142 *.003*
Green water	.112 *.020*	-.073 *.130*	.026 *.583*	.000 *.998*	.007 *.887*	-.054 *.264*
Foul odor	-.113 *.018*	.197 *.000*	.118 *.014*	-.025 *.602*	-.018 *.713*	.059 *.219*
Salinity	.079 *.109*	.013 *.791*	.063 *.199*	-.009 *.856*	.110 *.026*	-.004 *.943*
Nitrite	-.093 *.059*	.011 *.817*	.136 *.006*	-.023 *.636*	.053 *.284*	.022 *.661*
Oxygen	.060 *.226*	-.120 *.014*	.043 *.389*	-.083 *.091*	-.022 *.658*	.009 *.853*
Ammonia	.088 *.081*	.143 *.005*	.035 *.494*	.006 *.903*	.000 *.999*	-.002 *.962*
Temperature	-.049 *.325*	-.060 *.224*	-.095 *.054*	.021 *.670*	-.061 *.219*	-.172 *.000*
Secchi disk	-.122 *.023*	-.164 *.002*	.002 *.976*	-.057 *.284*	-.009 *.864*	.006 *.910*
Back swimmers	-.204 *.000*	.067 *.162*	.082 *.089*	-.095 *.048*	-.033 *.500*	.093 *.053*
Water boatmen	-.187 *.000*	.224 *.000*	.145 *.002*	.096 *.046*	.240 *.000*	.171 *.000*
Dragonfly nymphs	.027 *.577*	-.024 *.615*	.095 *.049*	.085 *.076*	.186 *.000*	.115 *.017*

Water striders	.038	-.031	-.023	-.032	.131	.060
	.431	*.525*	*.641*	*.508*	*.007*	*.213*

[1]Time elapsed since data collection began in May 2007.

Table 3 Normalized multiple regression coefficients, based on all swimming pools (N=433 pool inspections)

Dependent variable: Number of *Cx. quinquefasciatus* larvae

	Positive coefficients			Negative coefficients	
	Coefficient	Significance[1]		Coefficient	Significance[1]
Foul odor	.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
			Algal mats	-.07	.14

Dependent variable: Number of *Anopheles* larvae

	Positive coefficients			Negative coefficients	
	Coefficient	Significance[1]		Coefficient	Significance[1]
Water boatmen	.19	.001	Fish abundance	-.22	<.001
Nitrate	.17	<.001	Diving beetles	-.11	.04
Pine needles	.14	.01	Water temperature	-.10	.05
Dragonfly nymphs	.11	.09	Backswimmers	-.10	.06
Wood	.10	.04			
Grass	.07	.18			

[1]Two-tailed test.

Table 4 Summary of key water-quality factors recorded during 433 swimming pool inspections

		Observed		
	Gambusia tolerance[1]	Common values[2]	Extreme values[3]	% extreme[4]
Oxygen (ppm)	0.2–35	0.8–12	0–0.3	4%
Ammonia (ppm)	0–1	0–0.2	4–40	7%
Temperature (ºC.)	0.5–42	10–35	-----[5]	-----
pH	4.7–10.2[6]	6–9	5.0–5.5	1%
Salinity (ppt)	0–58	0.1–6	8–10	3%
Chlorine (ppm)	0–0.8	0	0.2–1.5	1%
Nitrite (ppm)	0–1.5[7]	0	0.2–2.0	1%

[1]Based on laboratory studies summarized by Swanson et al. (1996) and Walton (2007).
[2] "Common values" is the range encompassing 90% of the pool inspections.
[3]For ammonia, temperature, salinity, chlorine, and nitrite, extreme values harmful to fish are greater than the "common values." For oxygen and pH, extreme values harmful to fish are less than the "common values."
[4]Percentage of pool inspections in the "extreme values" range.
[5]All observed values for temperature were within the "common values" range.
[6]Optimal pH for *Gambusia*: 7.2-8.2 (Walton 2007).
[7]Source for nitrite tolerance: Lewis & Morris (1986).

Table 5 Normalized multiple regression coefficients, based on swimming pools that contained fish (N=244 pool inspections)

Dependent variable: Fish abundance

Positive coefficients	Coefficient	Significance[1]	Negative coefficients	Coefficient	Significance[1]
Algal mats	.18	.015	Water boatmen	-.30	.001
Tree branches	.15	.067	Ammonia	-.18	.006
pH	.15	.026	Pine needles	-.16	.05
			Nitrite	-.11	.08
			Bleach bottles	-.09	.21

[1]Two-tailed test.

Table 6 Results of the factor analysis for swimming pools not containing fish. Each factor group is a set of intercorrelated variables (factor loadings in parentheses)

FACTOR GROUP 1 (Organic pollution)

Positive factor loadings	Negative factor loadings
Tree branches (.64)	Oxygen (-.57)
Large items (.54)	Time (-.56) [1]
pH (.52)	
Water temperature (.51)	
Foul odor (.45)	
Ammonia (.36)	
Cx. quinquefasciatus larvae (.30)	

FACTOR GROUP 2 (Saline lake water)

Positive factor loadings	Negative factor loadings
Dragonfly nymphs (.68)	Time (-.30) [1]
Pine needles (.57)	Algal mats (-.21)
Anopheles larvae (.56)	Foul odor (-.11)
Water striders (.51)	
Water boatmen (.45)	
Cx. salinarius larvae (.43)	
Salinity (.40)	
Grass (.31)	

FACTOR GROUP 3 (Oak leaves)

Positive factor loadings	Negative factor loadings
Oak leaves (.60)	Large items (-.40)
Plastic bags (.47)	Tree branches (-.36)
Cx. quinquefasciatus larvae (.43)	Wood (-.33)
Water boatmen (.36)	
Nitrate (.38)	
Brown water (.27)	
Foul odor (.26)	
Anopheles larvae (.21)	

FACTOR GROUP 4 (Floating algal mats)

Positive factor loadings	Negative factor loadings
Water clarity (.61)	Grass (-.34)
Backswimmers (.56)	*Anopheles* larvae (-.29)
Diving beetles (.53)	*Cx. quinquefasciatus* larvae (-.29)
Algal mats (.47)	*Cx. coronator* larvae (-.26)
Water striders (.38)	*Cx. salinarius* larvae (-.20)
Floating twigs (.33)	
Temperature (.28)	

[1]Time elapsed since data collection began in May 2007.

Table 7 Results of the factor analysis for swimming pools containing fish. Each factor group is a set of intercorrelated variables (factor loadings in parentheses)

FACTOR GROUP 1 (Organic pollution)	
Positive factor loadings	Negative factor loadings
Tree branches (.72)	Oxygen (-.38)
Water boatmen (.71)	Water clarity (-.32)
Backswimmers (.64)	Grass (-.30)
Water striders (.55)	Time (-.28)[1]
Diving beetles (.54)	
Ammonia (.37)	
Large items (.35)	
Dragonfly nymphs (.34)	
Floating twigs (.30)	
Foul odor (.24)	
Water temperature (.17)	

FACTOR GROUP 2 (Saline lake water)	
Positive factor loadings	Negative factor loadings
Salinity (.74)	Time (-.63)[1]
Dragonfly nymphs (.64)	Large items (-.50)
Water striders (.58)	Foul odor (-.34)
Pine needles (.53)	Algal mats (-.25)
Plastic items (.50)	
Floating twigs (.46)	
Anopheles larvae (.39)	
Grass (.38)	
Water boatmen (.28)	

FACTOR GROUP 3 (Oak leaves)	FACTOR GROUP 3 (Floating algal mats)
Positive factor loadings	Negative factor loadings
Cx. quinquefasciatus larvae (.55)	Algal mats (-.52)
Brown water (.53)	Fish abundance (-.50)
Oak leaves (.52)	Water temperature (-.46)
Cx. salinarius larvae (.43)	
Water boatmen (.33)	
Anopheles larvae (.19)	
Foul odor (.19)	

[1]Time elapsed since data collection began in May 2007.

Table 8 Percentage of monitored swimming pools that were in each habitat type. Based on "factor scores" for each pool inspection with respect to each factor group in Tables 6 and 7. Number of swimming pools monitored each year = 43

	2007	2008
Organic pollution	35%	2%
Saline lake water	23%	0 %
Oak leaves	14%	14%
Floating algal mats	16%	51%

Figure 1. Aerial photos showing the same swimming pools before Hurricane Katrina (top) and after Katrina (bottom). Maintained swimming pools before Katrina are turquoise. The unmaintained swimming pools after Katrina are brown. Source: Pictometry

Figure 2. One of the swimming pools treated experimentally with bleach to see its impact on the fish population. Fish normally thrived in this pool. Pouring 16 liters of bleach into the pool led to high fish mortality, but the fish population recovered within a month

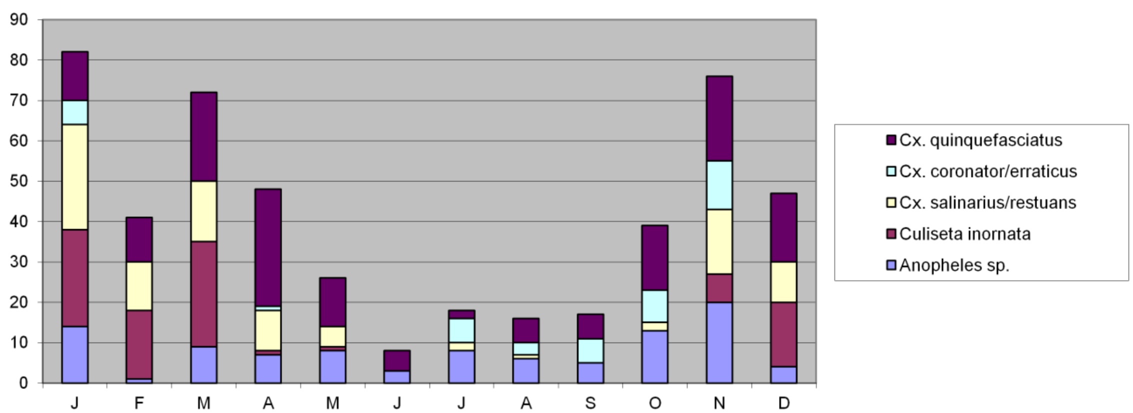

Figure 3. The number of inspections of swimming pools without fish during 2006-2008 in which each species of mosquito larvae was observed in the pool. Source: Marten et al. (2012)

Figure 4. An extreme example of the “organic pollution” habitat type (Factor Group 1 in Tables 6 and 7), characterized by rotting debris, foul odor, high ammonia concentration, and low oxygen. Particularly favorable for *Cx. quinquefasciatus* larvae. *Gambusia* maintained normal populations in this habitat type if pollution was not severe, but the fish were unhealthy and their populations depressed if pollution was severe. This particular pool had been drained and subsequently collected rainwater at the bottom. Pollution from the large quantity of trash was concentrated in a small amount of water. *Gambusia* never survived when introduced to this pool

Figure 5. An example of the “saline lake water” habitat type (Factor Group 2 in Tables 6 and 7). This habitat type supported large populations of aquatic insects. Floating pine needles (the brown strip around the edge of the pool) provided *Anopheles* larvae refuge from predation by fish

Figure 6. An example of the “oak leaves” habitat type (Factor Group 3 in Tables 6 and 7), characterized by a heavy influx of oak leaves and dark brown water due to tannins from oak leaves. Favorable habitat for *Culex* and *Anopheles* larvae but inhibiting for fish. A Mosquito Control sign with information for homeowners is next to the pool

Figure 7. An example of the “floating algal mats” habitat type (Factor Group 4 in Table 6 and negative Factor Group 3 loadings in Table 7), characterized by filamentous algae mats and clear water. Unfavorable habitat for mosquito larvae but particularly favorable for fish