#### **5.2 Biological Parameters**

#### 5.2.1 Mosquito Measures

Mosquito breeding on the marsh was measured in four separate ways, although they are all assumed to be closely linked and therefore to co-vary. One was through the larval sampling along the transects. A second means was through one-time targeted sampling in Area 1 and Area 2 that sought to precisely locate all breeding locations in mid-July 2004. Thirdly, the one-time July 2004 breeding surveys in Area and Area 2 were continued through 2004-2007 to determine the extent of breeding in all four areas. Finally, SCVC and USFWS continued to conduct routine surveillance every two weeks at set stations to determine the parts of the marsh that exceeded the 0.2 mosquito/dip threshold for aerial larviciding.

Almost all mosquito sampling data were analyzed using Kolmogorov-Smirnov tests initially, except for tests of presence-absence. No data sets were found to need analysis by Student's t-tests (either they were significantly different under Kolmogrov-Smirnov testing, or the data sets were not normal or log-normal distributions). Where significance was not determined under Kolmogorov-Smirnov tests, Mann-Whitney rank-sum tests were used. Tests of binomial distribution similarity were made when the variable returned presence-absence results. Significance for all tests was at p<0.05. Test data are provided in the Addendum, pp. 225-226, 235, 246, and 252-253. More details regarding the statistical tests are provided in Section 5.1, above.

The Before (pre-treatment) data for Area 1 (an Impact or Treatment area) were 2003-2004 for transect data, and 2004 data for targeted sampling. The control Before (pre-treatment) data Area 1 controls were Area 3 and Area 4 2003-2004 data for the transect sampling, and 2004 for targeted sampling. Post-treatment (After) data for Area 1 was 2005-2007 data, and its Control post-treatment (After) data were Area 3 and Area 4 data for 2005-2007. The Before (pre-treatment) data for Area 2 (also an Impact or Treatment area) were 2003-2005 for transect data, and 2004-2005 data for targeted sampling. The control Before (pre-treatment) data Area 2 controls were Area 3 and Area 4 2003-2005 data for the transect sampling, and 2004-2005 for targeted sampling. The control Before (pre-treatment) data Area 2 controls were Area 3 and Area 4 2003-2005 data for the transect sampling, and 2004-2005 for targeted sampling. Post-treatment (After) data for Area 2 was 2006-2007 data, and its Control post-treatment (After) data were Area 3 and Area 4 data for 2006-2007.

# 5.2.1.1 Larval Sampling at Transect Stations

This sampling methodology is not generally used by mosquito control professionals. Typically, mosquito surveillance is made to determine if marshes are breeding mosquitoes, and, if so, the extent of the breeding. Using set stations established without regard to breeding potential strikes many mosquito control professionals as an inappropriate means of determining the need for action. However, this technique was used in the Region 5 OMWM study at some of its sites (although not the Wertheim areas).

The data sets generated by this sampling effort contained many results showing no breeding. The sampling routine also resulted in many results of "dry" conditions (45.5 percent of all samples). Table 15 lists the percentage of dry stations, by area by year. Table 16 lists the comparison of treatment to post-treatment dry conditions for Area 1 and Area 2, along with the respective controls.

Year	Areas	Percent Dry
2003	1	42.6
	2	22.2
	2 3 4	39.8
		38.0
	All	35.5
2004	1	40.3
	2 3	64.6
	3	57.1
	4	63.2
	All	55.1
2005	1	50.0
	2	50.4
	3	55.5
	4	44.3
	All	49.4
2006	1	51.9
	2 3 4	31.0
	3	55.9
		37.4
	All	42.3
2007	1	62.6
	2 3	35.6
	3	54.3
	4	41.0
	All	47.6
All	1	49.0
	2	39.1
	3	51.3
	4	43.6
	All	45.5

 Table 15.
 Percentage Sampling Stations that were "Dry" for Transect Mosquito Sampling Efforts

Table 16. Comparison of Percentages of Pre-Treatment and Post-Treatment Dry Stations

Area	<b>Pre-Treatment</b>	<b>Post-Treatment</b>
Area 1	41.6	57.0
Area 1 controls	47.6	47.3
Area 2	42.9	33.6
Areas 2 controls	47.9	46.7

The number of dry stations recorded for Area 1 post-treatment was significantly greater; for Area 2, the number of dry stations decreased significantly. This implies that Area 1 may have become drier overall, while Area 2 became wetter. Areas 3 and 4, considered as controls for Area 1 and Area 2, did not have a significant change in the number of dry stations post-treatment. This suggests that any overall change in weather (and other environmental conditions, such as stream flow and upland water tables) did not significantly impact the percentage of dry stations. The 2006 USFWS study data report (James-Pirri et al., 2006), lacking direct measures of mosquito

breeding for all the study sites, interpreted wetter conditions as being more conducive to mosquito breeding. Therefore, the report used measures of drier conditions at treatment sites as a proxy for effective mosquito control. By this test, the changes to Area 1 would be judged as effective, and Area 2 treatment would be thought to have been ineffective.

This sampling effort directly measured mosquito breeding. One method was through the absence or presence of larvae at the transect sites, both including the dry stations (Table 17) and excluding the dry stations (Table 18). Area 1 showed significant declines in the sites where larvae were present after treatment. Area 2 had a significant relative decline (in comparison to its control site). The reductions in Area 1 from pre-treatment to post-treatment are most notable, as there was very little breeding post-treatment. The change in Area 2 is not as notable, although the percentage of sites where larvae were present decreased post-treatment in Area 2 controls, but increased post-treatment for its control sites.

 Table 17. Percentage of Stations Positive for Larvae

Area	<b>Pre-Treatment</b>	<b>Post-Treatment</b>
Area 1	7.3	0.2
Area 1 controls	6.6	7.8
Area 2	4.9	4.6
Area 2 controls	6.0	8.2

 Table 18.
 Percentage of Stations Positive for Larvae (Dry Stations Removed)

Area	<b>Pre-Treatment</b>	<b>Post-Treatment</b>
Area 1	12.5	0.6
Area 1 controls	12.7	14.9
Area 2	8.5	6.9
Area 2 controls	11.6	15.4

The number of larvae per dip was determined. Table 19 shows the results with all samples (including dry stations) considered (a dry station was considered to have no larvae in a hypothetical dip). Table 20 shows only the results from wet sampling points. The declines in larvae counts following the treatments for both Area 1 and Area 2 were statistically significant; however, the decreases in larvae for the control areas were also significant, which reduces the impact of the finding.

Table 17. Transeet Stations, Weath Larvae per				
Area	<b>Pre-Treatment</b>	<b>Post-Treatment</b>		
Area 1	0.58	0.02		
Area 1 controls	0.74	0.24		
Area 2	0.52	0.22		
Area 2 controls	0.64	0.25		

Table 19. Transect Stations, Mean Larvae per Dip

 Table 20.
 Transect Stations, Mean Larvae per Dip (Dry Stations Removed)

Area	<b>Pre-Treatment</b>	<b>Post-Treatment</b>
Area 1	1.00	0.04
Area 1 controls	1.42	0.45
Area 2	0.92	0.33
Area 2 controls	1.23	0.46

The data for Area 2 should be discussed in more detail (see Table 21). In 2006, immediately after treatment, mosquito breeding across Area 2 was very low, as measured at the transect stations. The data were comparable to results found in Area 1. However, many more larvae were detected, in more samples at more stations, in 2007. In some ways, there could be said to be more breeding than had occurred pre-treatment. Slightly fewer stations had "dry" results in 2006 than 2007, suggesting that the marsh was not especially wetter (at least by this measure). If the larvae per dip data are considered, it seems reasonable to conclude that mosquito control in Area 2, when compared to the full year sampling efforts in 2004 and 2005, were moderately successful (reductions of 25 to 65 percent), but not as good as were achieved in 2006, and not especially good in comparison to 2003. The positive larval samples were not clustered at a single station or part of the Area, so one physical problem (a persistent panne or slow-draining area of the marsh) was unlikely to have caused the problem. The two years considered together suggest that breeding has decreased, in a statistically significant fashion, but it is clear that there was great effectiveness in mosquito control in 2006 (the first year post-treatment) and less success in controlling mosquito breeding in 2007, as measured by sampling at the transect stations.

Year	Samples	Positive	Percent	Percent with	Larvae	Larvae/Dip	Larvae/Dip (wet
		Samples	Positive	Water Positive			stations only)
2003	252	9	3.6	4.6	94	0.37	0.48
2004	195	13	6.7	18.8	102	0.52	1.48
2005	127	6	4.7	9.5	105	0.83	1.67
2006	171	1	0.6	0.8	1	0.01	0.01
2007	219	17	7.8	12.1	85	0.39	0.60

Table 21. Area 2 Transect Mosquito Dip Data

# 5.2.1.2 One-time Targeted Sampling in Area 1 and Area 2

This sampling was undertaken in mid-July, 2004. All breeding locations across Areas 1 and 2 were identified and located by GPS following a high spring tide the first week in July (see the inundation studies, Section 4.3.3, below). These data were used to guide the construction plans for the marsh management project. Figures 26 and 27 show the results of these surveys. They demonstrate that a large number of locations in Area 1 and Area 2 were capable of breeding mosquitoes prior to the water management project. Ponds and back-blading areas were preferentially located in the areas where the most breeding occurred.

## 5.2.1.3 Repeated Targeted Sampling (Marsh-wide)

Surveys across each area were made in conjunction with the monthly transect sampling, beginning in 2004 (and including the sampling effort reported on just above). This effort was made with the aim of determining mosquito breeding across the extent of the marsh. Most mosquito professionals believe this is a more accurate measure to determine mosquito breeding occurring in a given area than use of transects. Dip samples were taken to quantify the amount of breeding associated with the determination that breeding was occurring. Some data points were recorded as "dry" when an attempt to dip was made, but insufficient water was present. Other times, sites previously determined to be breeding points were revisited to determine if they still supported breeding; if those sites were now dry, the point was sometimes recorded as a "dry" sample. On some occasions, the entire marsh area was too dry to support mosquito breeding. Some select measurements were therefore labeled "dry" to demonstrate an effort had been made to determine breeding. Finally, some obvious areas exist that could support mosquito breeding (such as pannes and pools). If these were encountered but they contained no water, they were sometimes labeled as a "dry" sample. Dry samples have been included in the data sets that follow, but it should be understood they constitute a source of sampler judgment that may influence the overall results.

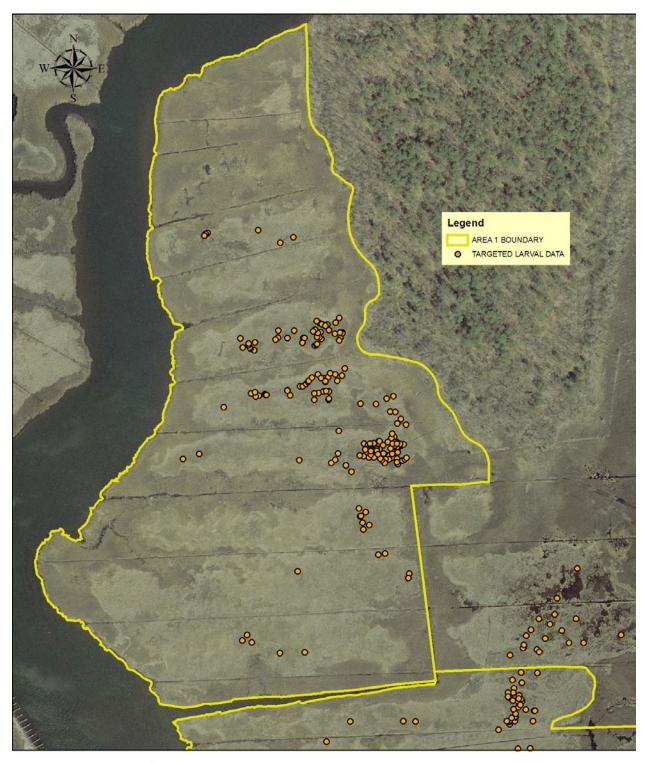




FIGURE 26 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 1 BREEDING LOCATIONS 2004

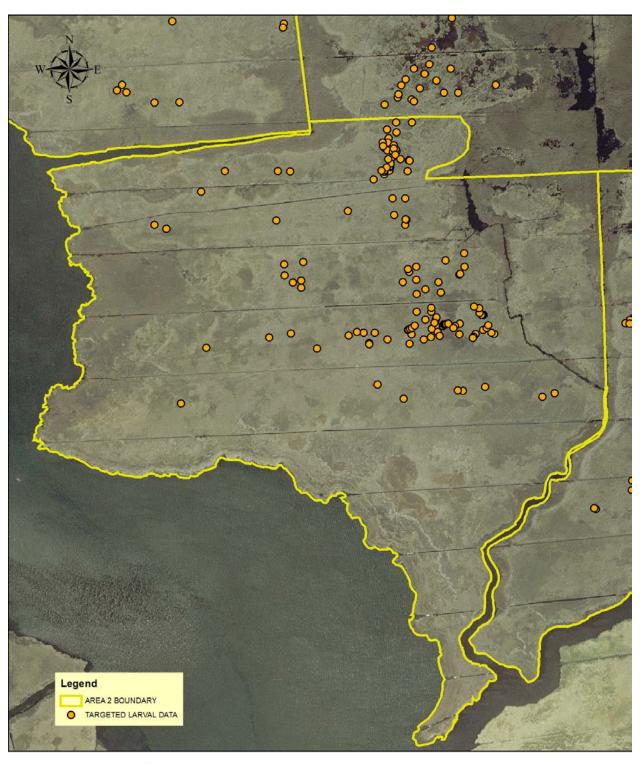




FIGURE 27 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 2 BREEDING LOCATIONS 2004 The data collected here supports two analyses of mosquito breeding: one, it identifies the number of locations across each season where breeding occurred; secondly, since samples were taken at every breeding site that could be located, the number of larvae should represent some approximation of overall breeding across the marsh (however, different kinds of breeding locations support difference intensity of sampling with dippers; a sample from a small pothole is more likely to capture a considerable percentage of larvae compared to a sample from a larger panne or puddle). Thus, the data allow measures of breeding site changes and intensity to be determined, pre- and post-project, and in comparison to the control locations.

The intensity of the sampling effort effects the determination of breeding sites. Many more locations were sampled across all four areas in 2006 and 2007, and also in Area 1 (and, to a lesser degree, Area 2) in 2004. This needs to be understood in terms of the data presentation, below.

Figures 28-30 (Area 1), Figures 31-33 (Area 2), Figures 34-37 (Area 3), and Figures 38-41 (Area 4) illustrate the number of sites where mosquitoes were detected from 2005-2007 for Areas 1 and 2 and from 2004-2007 for Areas 3 and 4. These figures show that post-treatment, the extent of mosquito breeding decreased in Area 1. In 2007, approximately one-tenth the number of locations were found to have larvae compared to 2004, although the sampling intensity was greater post-project in 2007. In 2006, the number of positive locations was approximately one-third of the number found in 2004 (although the number of samples had increased). The parts of the marsh where breeding occurred in Area 2 also decreased, although not as dramatically. The number of locations positive for larvae across the seasons post-treatment was approximately the same for Area 2, compared to pre-treatment sampling results. However, nearly twice as many samples were made post-treatment, and so the reduction in breeding was actually considerable.

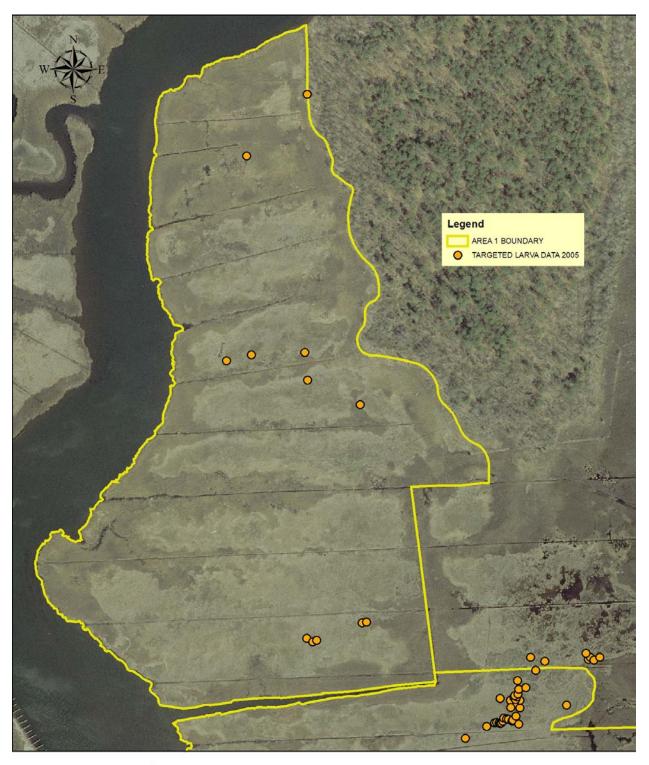




FIGURE 28 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 1 BREEDING LOCATIONS 2005

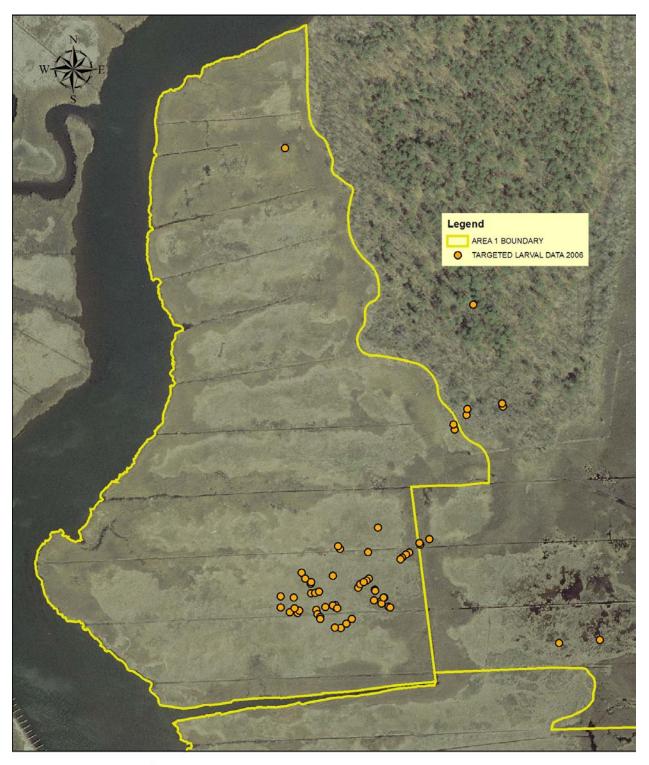




FIGURE 29 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 1 BREEDING LOCATIONS 2006

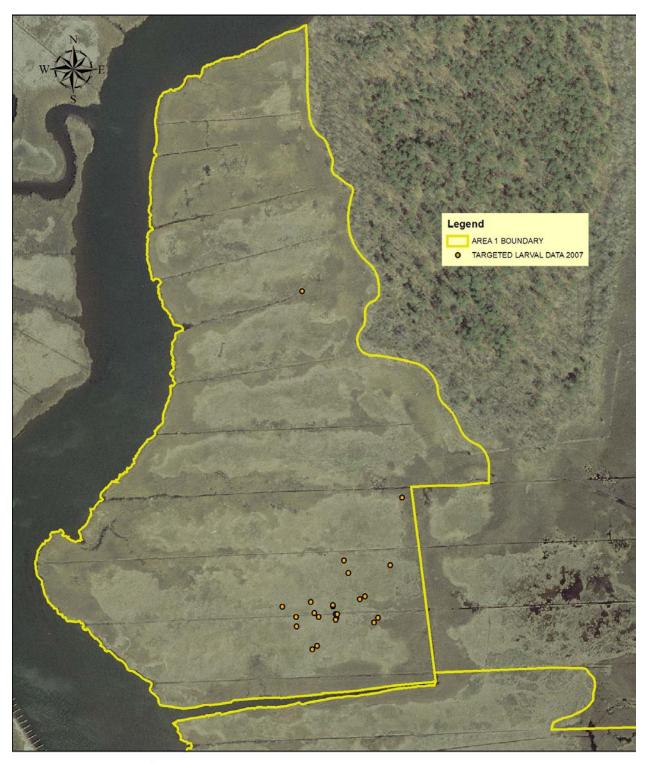




FIGURE 30 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 1 BREEDING LOCATIONS 2007

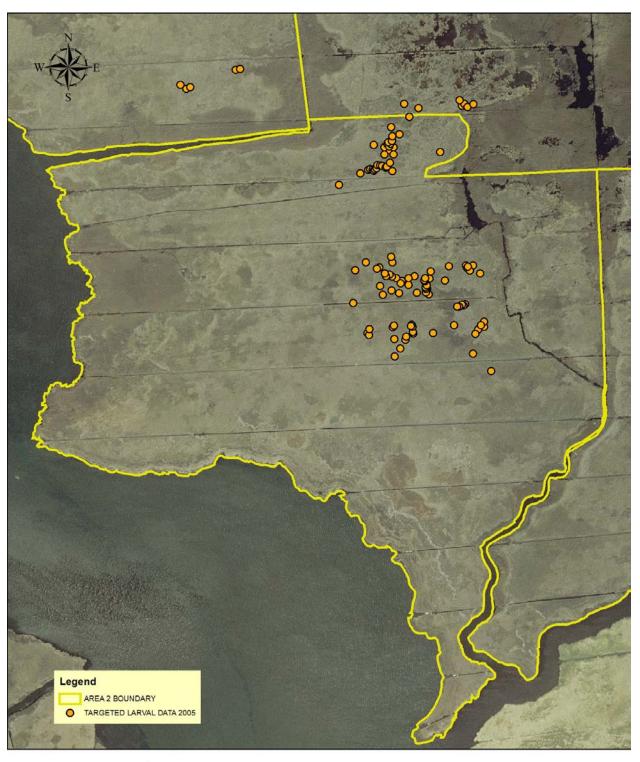




FIGURE 31 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 2 BREEDING LOCATIONS 2005

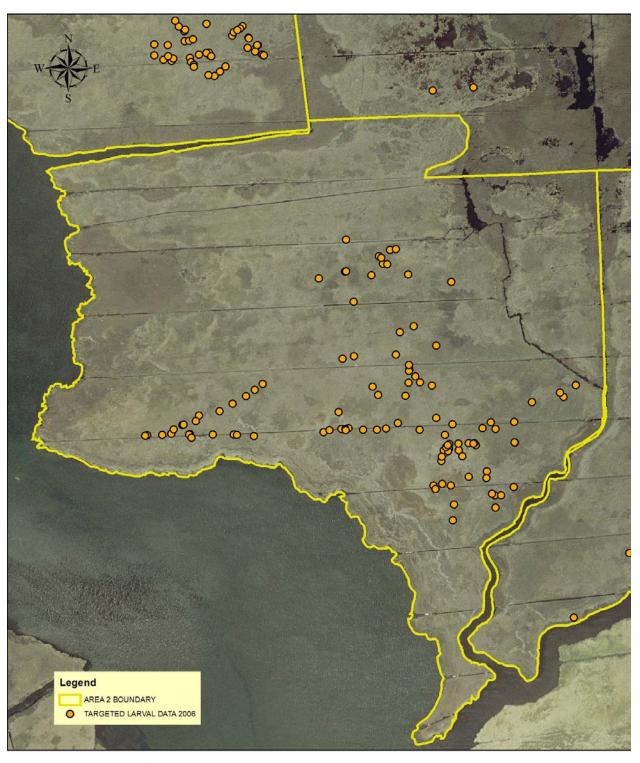




FIGURE 32 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 2 BREEDING LOCATIONS 2006

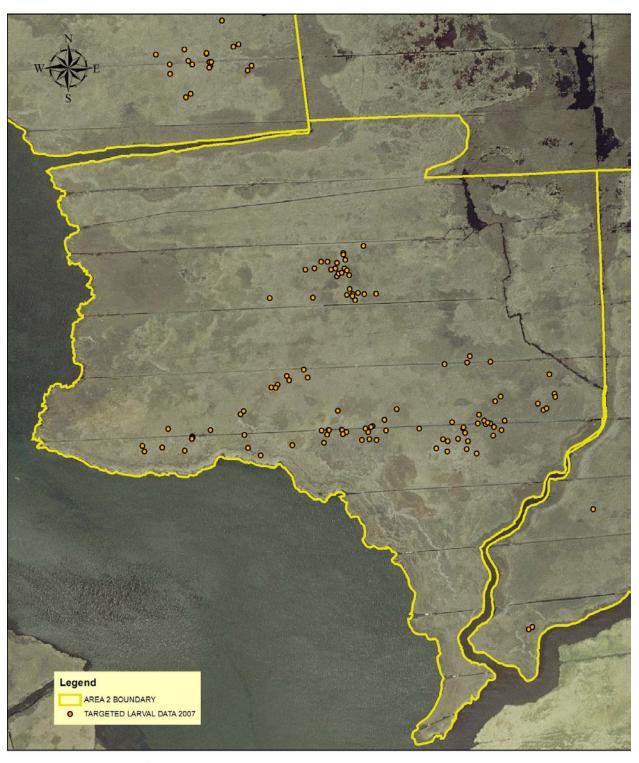




FIGURE 33 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 2 BREEDING LOCATIONS 2007

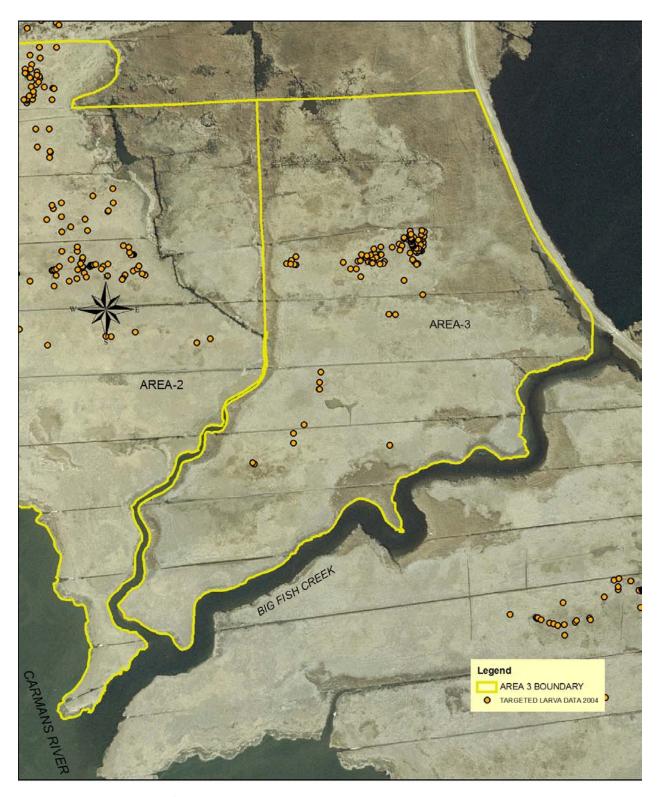


FIGURE 34 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 3 BREEDING LOCATIONS 2004

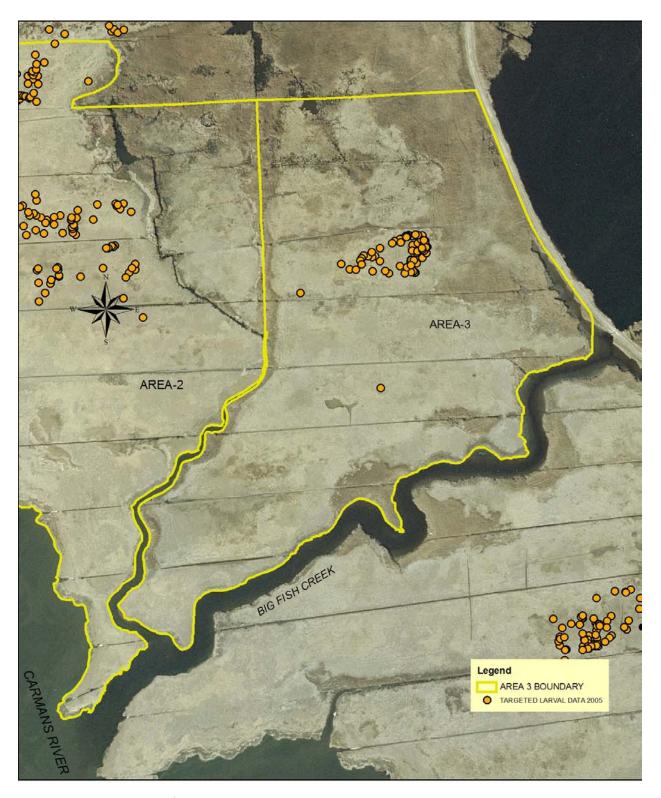




FIGURE 35 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 3 BREEDING LOCATIONS 2005

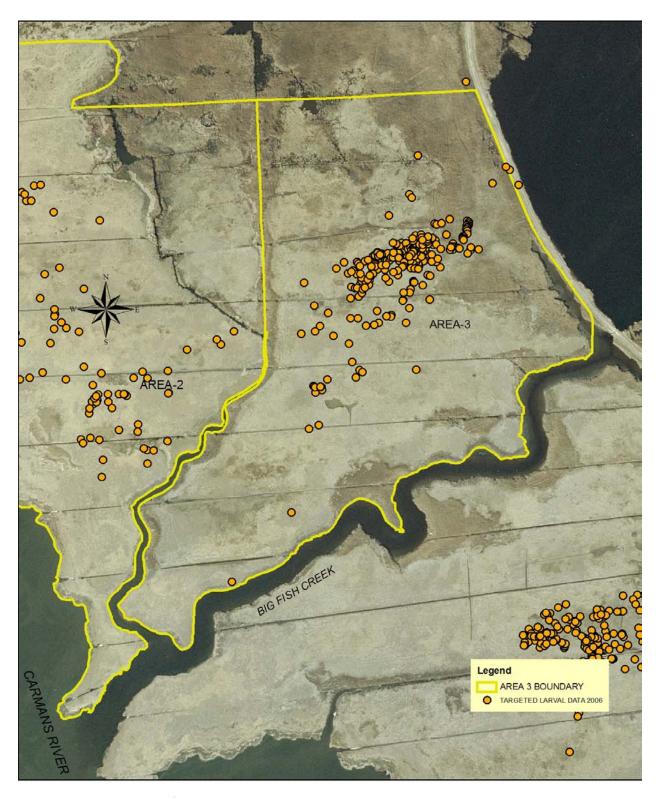




FIGURE 36 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 3 BREEDING LOCATIONS 2006





FIGURE 37 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 3 BREEDING LOCATIONS 2007



FIGURE 38 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 4 BREEDING LOCATIONS 2004



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FIGURE 39 WERTHEIM NATIONAL WLDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 4 BREEDING LOCATIONS 2005



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FIGURE 40 WERTHEIM NATIONAL WLDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 4 BREEDING LOCATIONS 2006

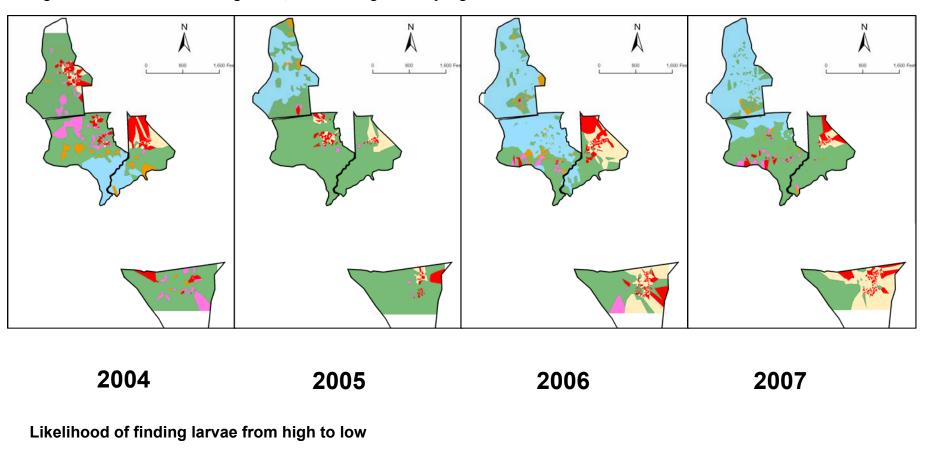


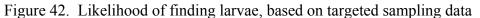
Cashin Associates, P.C.

FIGURE 41 WERTHEIM NATIONAL WILDLIFE REFUGE WATER MANAGEMENT DEMONSTRATION PROJECT AREA 4 BREEDING LOCATIONS 2007 Figure 42 is a statistical analysis of the geographical extent of breeding, created by SCVC. All sampling data points in the targeted data set were assigned either 1 (larvae found) or 0 (no larvae) (a presence/absence classification. Moran's I spatial statistic with associated Z-scores was generated for each point (Chen et al., 2005; Kitron et al., 1996; Ryan et al., 2004). They were grouped into three different categories:

- a. Z>2.0 "clustering of similar values"
- b. -2.0<Z<-2.0 "values as expected"
- c. Z<-2.0 "exceptional values dissimilar to surrounding values"

The two values considered were larvae found and no larvae, creating six different categories. Thiessen polygons (Glass et al., 1994; Cohen et al., 2006) were created around each point, using simple interpolation to determine edges. Polygons with from the same categories that neighbored each other were combined to create area-wide polygons.





Larvae found, clusters 🗾 Larvae found as expected Larvae found, exception 🦳 No larvae, exception 📰 No larvae as expected 📰 No larvae, clusters

The data from the targeted surveys have been further analyzed. Table 22 presents the relationship between the samples that contained larvae over time and across the areas. Table 23 aggregates the results into pre-treatment and post-treatment groups. The percentage of samples that were positive for larvae post-treatment was statistically significantly lower in both Area 1 and Area 2.

Table 22. Ferentage Targeted Samples that Contained Larvae				
Year	Areas	<b>Percent Positive</b>	Percent Positive (excluding dry samples)	
2004	1	39.4	43.6	
	2	34.9	37.6	
	3	47.7	49.7	
	4	35.0	36.6	
2005	1	4.2	5.2	
	2	28.7	36.0	
	3	26.8	35.6	
	4	25.1	34.5	
2006	1	10.8	11.6	
	2	13.5	14.3	
	3	46.9	48.5	
	4	34.3	35.7	
2007	1	3.6	4.5	
	2	15.1	17.8	
	3	24.4	30.6	
	4	33.2	39.4	

 Table 22. Percentage Targeted Samples that Contained Larvae

Table 23. Comparison of Percentages of Pre-Treatment and Post-Treatment Targeted Samples
that Contained Larvae (dry samples excluded)

Area	<b>Pre-Treatment</b>	<b>Post-Treatment</b>
Area 1	43.6	7.5
Area 1 Controls	42.2	38.2
Area 2	36.9	15.7
Area 2 Controls	38.3	38.9

The number of larvae in each positive sample were counted. These data have been considered in two ways. One is the number of larvae per dip, which is a measure of the intensity of breeding across the areas. These data are presented in Tables 24 and 25. The larvae per dip declined by an order of magnitude for Area 1 post-treatment, while in the control areas for Area 1 the decline was on the order of 50 percent. These differences were significant, although the difference in larvae for the control sites between pre- and post-treatment was also significant. In Area 2, the larvae per dip declined by approximately two-thirds pre-treatment to post-treatment, which was significant, while in the Area 2 controls the decline was approximately 10 percent, and was not significant. Pre-treatment, approximately 15 percent more larvae were found per dip in Area 2 compared to its control sites, which was not found to be significant, but after treatment, there

were less than half the number of larvae per dip in Area 2 compared to the controls, which was a

significant difference. These data all suggest that the treatment decreased mosquito breeding.

Year	Areas	Larvae/Dip
2004	1	5.5
	2	5.3
	2 3 4	6.4
	4	6.3
2005	1	0.4
	2 3	3.1
	3	2.7
	4	1.4
2006	1	0.6
	2 3	1.4
		4.9
	4	2.8
2007	1	0.6
	2	1.7
	3	1.3
	4	2.9

Table 24. Number of Larvae per Dip, Targeted Sampling (dry sites not included)

Table 25.	Comparison of Pre-Treatment and Post-Treatment Larvae per Dip, for Targeted
Sampling	

Area	<b>Pre-Treatment</b>	<b>Post-Treatment</b>
Area 1	5.5	0.5
Area 1 Controls	5.9	3.0
Area 2	4.3	1.5
Area 2 Controls	3.7	3.3

Tables 26 and 27 compare the number of larvae found in each Area for each year. They are presented as averages per sampling event. Comparing these data, the decline from Area 1 pre-treatment to post-treatment was more than an order of magnitude. This change was statistically significant. The number of larvae found in each sampling event across all of Area 2 declined by approximately 50 percent post-treatment; the large variability and high number of "unusual" (outlier) data points meant this was not a statistically significant change. However, at the same time, the number of larvae found in the Area 2 control areas increased by approximately 50 percent post-treatment, and a the number of larvae per event for Area 2 post-treatment was significantly lower than the number of larvae found in the control areas post-treatment.

Year	Areas	Larvae/Event
2004	1	191
	2	147
	3	104
	4	157
2005	1	4
	2	48
	3	24
	4	22
2006	1	16
	2	47
	3	120
	4	96
2007	1	15
	2	41
	3	34
	4	100

Table 26. Number of Larvae per Targeted Sampling Event

Table 27. Comparison of Pre-Treatment and Post-Treatment Larvae per Targeted Sampling Event

Area	<b>Pre-Treatment</b>	<b>Post-Treatment</b>
Area 1	191	11
Area 1 Controls	129	68
Area 2	91	44
Area 2 Controls	61	88

Similarly, the number of sites in each one of the targeted sampling events where larvae were found was compared (Table 28). The data show that mosquito breeding can be affected by annual conditions (many fewer breeding sites were found in 2005, for instance) and other factors (in the control areas, the number of breeding sites was much more in Area 4 than in Area 3 in 2007, for instance). These data were aggregated as pre-treatment and post-treatment data for each area (Table 29). The number of breeding places that could be detected across Area 1 each event (on average) declined by an order of magnitude post-treatment (which was statistically significant). The decline for Area 2 post-treatment was smaller (approximately 40 percent), and was not found to be significant. However, the control areas for Area 2 had a 60 percent increase post-treatment, and the comparison of post-treatment breeding sites found there were a significantly lower number of breeding sites in Area 2 post-treatment compared to the controls.

Year	Areas	Larvae/Event		
2004	1	15.2		
	2	10.5		
	3	8.6		
	4	9.6		
2005	1	0.5		
	2	5.6		
	3	3.9		
	4	5.5		
2006	1	3.3		
	2	4.7		
	3	12.5		
	4	12.4		
2007	1	1.2		
	2	4.3		
	3	5.1		
	4	10.6		

Table 28. Number of Sites with Larvae per Targeted Sampling Event

Table 29. Comparison of Pre-Treatment and Post-Treatment Sites with Larvae per Targeted Sampling Event

Area	<b>Pre-Treatment</b>	<b>Post-Treatment</b>
Area 1	15.2	1.6
Area 1 Controls	9.1	8.5
Area 2	7.7	4.5
Area 2 Controls	6.3	10.2

Therefore, by several measures, the targeted sampling found very effective larval control in Area 1. The control achieved in Area 2 was not to the same degree; however, the project reduced mosquito larvae numbers and breeding locations significantly.

## **5.2.1.4 Larvicide Applications**

A goal of the project was to decrease larvicide applications (because mosquito breeding had been decreased due to alterations made to the marsh). The number of applications from 1999 to 2006 was obtained. The area that larvicides were allowed to be applied across Wertheim by USFWS was determined through a sampling program similar to the targeted sampling described above. On a weekly basis, at least 25 samples were taken in each of the zones that were used to define Wertheim treatment areas (prior to 2004, Areas 1 2 and 3 were in one treatment area, and Area 4 was part of another). At least 5 larvae would need to be detected in the samples (a mean larvae incidence of 0.2/dip) in order for a treatment to occur. This same standard was applied in 2004 through 2007, but each area was considered to be its own spray block, and in order for a

treatment to be considered, the same 0.2 larvae/dip standard needed to be met. The change in boundaries means that it is not entirely accurate to compare pre-2004 data to the 2004 and later data sets. However, relative changes in the larvicide frequencies can be discussed, and changes from the area averages can be determined.

Thus, the applications from 1999-2007 and pre-project and post-project mean numbers of applications were determined, by area, and with reference to the control areas (see Table 30 and Table31). It is clear that post-project the number of applications decreased in both areas. The difference is not so great in Area 2, but comparisons between the number of applications made in Area 2 in 2006 and 2007 to the number made in Areas 3 and 4 for 2006 and 2007 show that the number of applications in Area 2 were less than in the control sites. Relatively, the number of applications in the control areas increased post-project, but the number of applications in Area 2 was less than the average made pre-treatment. The number of applications has decreased across Area 1 by approximately 90 percent, comparing both to pre-project conditions and with respect to the control areas. The decrease in Area 2 was more than 50 percent in comparison to preproject means and in comparison to post-project controls. It should be understood that the change in methodologies for determining when applications should be made makes this analysis somewhat less valid. In addition, in 2007 in Area 2, reports by samplers that the water on the marsh in breeding areas was low and decreasing (the marsh was "drying down") meant that certain applications were not made although the larval criteria had been exceeded. This has been done in other areas at other times; this decision-making, reached on the basis of sampler judgments, helps explain why the transect sampling showed increases in mosquito larvae for 2007 compared to 2006, but no additional larvicide applications were made.

Area	1999	2000	2001	2002	2003	2004	2005	2006	2007
1	8	15	12	8	10	9	1	2	1
2	8	15	12	8	10	9	5	4	4
3	8	15	12	8	10	9	5	14	6
4	8	13	14	7	16	9	7	12	9

 Table 30.
 Larvicide Applications

Area	Pre-Project	Post-Project
Area 1	10.3	1.3
Area 1 Controls	10.9	8.8
Area 2	9.6	4.0
Area 2 Controls	9.7	10.3

In addition to the change in the number of applications, there were major differences in the areas of the different marsh segments that were treated, too. The project allowed SCVC to refine treatment areas. At times, only 5 acres were treated in Area 1, and a smaller treatment block for Area 2 (~8 acres) was also identified. Thus, instead of making treatments across the entire areas when needed, often only parts of the areas were treated after the project. However, it is not a good measure of the effectiveness of the mosquito control that has been achieved to compare the acreage treated after the project, since it is conceivable refinements in the treatment blocks might have been identified pre-project, too.

It is accurate to note the declines in the number of larvicide applications are in line with other measures of decreased mosquito breeding post-treatment, reported above.

#### **5.2.1.5 Mosquito Measures Conclusions**

The data presented here show that, usually in statistically significant ways, the treatments made at Wertheim decreased the amount of mosquito breeding occurring in the treatment areas. This was found in terms of the number of larvae produced, and the number of sites where they breed. The data show the effect was much greater in Area 1 than Area 2. Area 2, by some measures, had some breeding problems in 2007. Overall, especially in the first year post-treatment, the level of control achieved in Area 2 was also significant. There are some indications in the data that Area 1 became drier post-treatment, and Area 2 became wetter; however, these were indirect measures and may not be entirely accurate.

There was a very limited potential for confounding of the impact of treatment, due to continued larvicide applications across the site during the project. The number of larvicide applications was much greater in the control areas than the post-project treatment areas. Larvicide applications are intended to reduce adult mosquito emergence, and would not directly reduce larvae presence as measured here, because larvicides are applied only after larvae have been sampled and the marsh is found to meet the criteria for treatment. Therefore, it seems unlikely that one or two areas were affected unequally by the continued larvicide applications, or that their continued use affected the data materially.

The Long-Term Plan has a goal to reduce larvicide use County-wide by 75 percent. The primary means to reach this goal was to be the installation of various kinds of water management projects in marshes that currently receive aerial larvicide applications. The data presented above indicates that this project led to reduced mosquito breeding. The reduction in breeding appears to be of the scope that would allow the County to achieve its mosquito reduction goals, if similar results were found for other water management projects (Table 30).

Year	1999	2000	2001	2002	2003	2004	2005	2006	2007
Area 1*	128.0	240.0	192.0	128.0	160.0	144.0	16.0	32.0	16.0
Refined									2.0
(Actual)									
Area 1									
Treatments									
Area 2*	151.2	283.5	226.8	151.2	189.0	170.1	94.5	75.6	75.6
Refined									25.9
(Actual)									
Area 2									
Treatments									
Total*	279.2	523.5	418.8	279.2	349.0	314.1	110.5	107.6	91.6
Refined									27.9
Total									
Theoretical							69	70	75
Percent									
Reduction**									
Actual									92
Percent									
Reduction**									

Table 32. Area Larvicided, Areas 1 and 2 (ha)

\*2007 data based on applications treating entire Area, not refined blocks

\*\* compared to the 1999-2004 baseline annual larvicide application acreage of 360.6 ha