2008 Sanctuary Assessment

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## **Introduction**

Patent tong surveys were used to sample eight oyster sanctuaries. The surveys were undertaken with the objectives outlined below.

### **Objectives**

The objectives of the sanctuary sampling were:

- 1) Determine the distribution of oysters, oyster density, mortality, and size distribution;
- 2) Compare oyster abundances, distributions and sizes of oysters sampled to those sampled from the same sanctuaries in 2007;
- 3) Determine Perkinsus marinus (Dermo) infection levels in oysters;
- 4) Estimate oyster clumping percent single/, double/ or more than three/shell;
- 5) Estimate shell present as retrievable by patent tongs;
- 6) Evaluate bottom quality using a standardized, transect-based evaluation method; and
- 7) Evaluate how well past locations have served as sanctuaries based on growth rates, disease prevalences, mortality and unpredicted oyster losses.

### **Background**

In 2007, patent tong surveys were undertaken at five sanctuaries in an effort to estimate the density and spatial distribution of oysters planted at those sites over the last few years. In 2008, the surveys were repeated to the greatest extent possible to critically focus on objective #2 above, as well as to assess the repeatability of the survey technique, test the accuracy/applicability of the ArcGIS software being used, and determine the detectability of annual variation potentially caused by planting, mortality or poaching.

This report presents the results of that effort including measurements of the distribution of oysters, oyster density, mortality and size distribution, *Perkinsus marinus* (Dermo) diagnoses, shell scores as a measure of bottom quality and the beginnings of an in-depth analysis of the patent tong survey technique. All data was collected and entered into a Microsoft Access database.

## **Methods**

### Patent Tong Surveys

For the 2008 sanctuary assessment, eight oyster sanctuaries were sampled as shown in Figure 1. The sanctuaries investigated included Dobbin Point and Ulmstead Point in the Magothy River; Lake Ogleton in the Severn River; Hickory Thicket and Strong Bay (seven separate sites) in the Chester River; and Oxford, State's Bank, and Shoal Creek II in the Choptank River. Of these eight sanctuaries, five sanctuaries were also sampled in 2007. The 2007 surveys included Strong Bay (seven separate sites), Shoal Creek II, State's Bank, Lake Ogleton, and Ulmstead Point. For the Strong Bay sanctuary, the seven separate sites within the sanctuary are shown in Figure 2.



Figure 1. Locations of sanctuaries surveyed in 2008.

## Figure 2. Locations of surveyed sites within Strong Bay sanctuary.

The dark blue polygon in the center of the figure represents the historic Strong Bay oyster bar as defined by Yates. The multicolored polygons in the figure (from northwest to southeast: dark green, light green, beige, pink, blue, orange and green) represent spat-on-shell plantings from different years ranging from 2001-2008. Each individual square within the color scheme represents a fixed sampling area, 25-meter by 25-meter, where one patent tong grab was taken during the survey.



Patent tong surveys were conducted to determine the quantity and spatial extent of oysters on each site. This survey was initiated by using ArcGIS to overlay a grid of 25-meter by 25-meter cells on each of the bars. Patent tong samples were then collected within each identified cell as the wind and tide allowed. Within each cell, at least one patent tong grab was collected. Within every other cell, two patent tong grabs were collected. The differential grab pattern was undertaken to assess the variability between replicate grabs and between separate surveys. For all sites sampled, the location of each grab was recorded using a global positioning system (GPS). The shell score (amount of shell brought up in each grab), the number of oysters, and the lengths of all oysters were recorded from each grab. The shell score was a relative value where 0 = no shell; 5 = tongs full of shell; and 1, 2, 3, and 4 = intermediate amounts of shell. A shell score of >2 shows significant amounts of shell were present on the bottom at that location.

Once the patent tong surveys were completed, the data were analyzed spatially using ArcGIS. Natural neighbor interpolation was used to extrapolate the patent tong abundance data over the entire bar. Natural neighbor interpolation is one of the simplest interpolation methods. A neighborhood about the interpolated point is identified and a weighted moving average is taken of the observation values within this neighborhood. The oyster population calculated by this method is referred to as the "GIS" estimate in later tables and in the equation below.

Based on data from Allen and Paynter (2007), the natural neighbor interpolation was thought to be a significant underestimate of the population, so the oyster population estimates were also calculated using the DeLury correction. This correction used an equation derived from the patent tong efficiency estimated by Allen and Paynter (2007). The DeLury correction equation is as follows:

L = 389 + 3.88 GIS Where: L = Corrected estimate of initial population in bushels GIS = GIS estimate of initial population in bushels Note a bushel is assumed to be 300 oysters

Using the DeLury corrected population values, maps of oyster density and shell score were created for each sanctuary site using ArcGIS; these maps are included in Appendix 1. Extensive analysis was conducted comparing the DeLury estimates to other methods of population estimation and testing the ability of the DeLury method to detect changes in the population.

### **Groundtruthing**

As part of the sanctuary assessment, the bottom was visually surveyed by divers by swimming bottom transects within the sanctuary. At least 10 random points were generated within each sanctuary site; these random points marked the starting points of bottom transect lines. Depending on the location, a 50-, 100-, or 200-meter line was deployed from each of the starting points in a manner that maximized the area covered. Endpoint locations were recorded using a tablet PC (personal computer) equipped with a GPS receiver. Divers equipped with underwater communication gear were then sent down to make observations on bottom type. The divers stopped at numbered tags, attached to the line at 2-meter intervals. Tag number and bottom conditions were radioed back to the data logger onboard the boat. The two major bottom characteristics reported were the presence (E) or absence (N) of exposed shell and substrate composition (H = shell hash, S = sand, and M = mud). If mud or sand was reported, a rough depth of sediment was also reported. This process was repeated until all of the points had been assessed.

The transect data were then analyzed in the lab. Each point was assigned a number value from 1 (best) to 5 (worst) depending on the bottom characteristics reported. The point assignation is detailed below:

Number Value	Bottom Characteristics
1	Shell present with hard substrate
2	Shell present with shell hash, still fairly hard bottom
3	Shell present with sand or mud
4	No shell present with shallow sand or mud (<2
	centimeters)
5	No shell present with deep sand or mud soft-bottom ( $\geq 2$
	centimeters)

An average of all of the points along each transect was then calculated. This number was assigned to its respective transect line and compared to shell score distributions. Transect values were remarkably similar to shell scores.

Video footage was collected at some sites when visibility allowed, and is available upon request.

## Water Quality

The temperature (°C), salinity and dissolved oxygen (mg/L) concentration at 0.5 meter below the surface of the water and 0.5 meter above the bottom were collected at each site at the time of sampling using a YSI 6600 (YSI Incorporated, Yellow Springs, Ohio) sonde.

## **Results**

### Patent Tong Surveys

As noted previously, the maps of oyster and shell distributions are included in Appendix 1 of this report. Separate maps for each surveyed sanctuary site illustrate (1) the oyster density in animals per square meter ( $m^2$ ), and (2) the recorded shell score, which reflects a measure of the amount of shell present at the point of sampling.

Using the oyster density data, oyster population estimates for each of the sanctuary sites were calculated; these computations are presented in Table 1. Two estimates are presented. The first estimate, "GIS Estimate," is the number generated by the nearest neighbor ArcGIS analysis. The second estimate, "DeLury Estimate," is the population estimate when corrected using the equation generated by the bar cleaning experiments conducted previously (Allen, et al. 2006).

Although there is a very high degree of uncertainty until further calibration of patent tong surveys are conducted on <u>planted</u> oysters, observations suggest that the DeLury estimate is likely a much closer estimation to the real number due to possible undersampling of high density areas using the GIS method.

Bar Name	River	Planting Year	Oysters Planted	GIS Estimate (Oysters)	DeLury Estimate (Oysters)
Strong Bay	Chester	2005e/2008b	9,490,000	51,800	318,000
Strong Bay	Chester	2005d	11,520,000	36,170	257,000
Strong Bay	Chester	2005c	15,040,000	28,910	229,000
Strong Bay	Chester	2004/2005b	1,480,000	105,000	524,000
Strong Bay	Chester	2005a	11,350,000	62,870	361,000
Strong Bay	Chester	2003b/2007	6,400,000	70,400	390,000
Strong Bay	Chester	2003a	12,200,000	50,500	313,000
Strong Bay	Chester	2008	8,400,000	78,300	421,000
Hickory	Chester	2006-2008	6,200,000	38,100	265,000
Oxford	Choptank	N/A		57,100	338,000
Shoal Creek	Choptank	2003-2008	60,690,000	43,740	286,000
State's Bank	Choptank	2005-2008	24,230,000	102,900	516,000
Lake Ogleton	Severn	2006-2007	14,280,000	78,960	423,000
Ulmstead	Magothy	2006	1,670,000	74,700	406,000
Dobbin Point	Magothy	1999	3,100,000	73,300	401,000

Table 1. Population estimates of sanctuaries surveyed in 2008.

Notes: (1) The population estimates were based on data collected during the patent tong surveys of planted areas.

(2) The GIS estimate was generated by the nearest neighbor ArcGIS analysis.

(3) The DeLury estimate is a refinement of the GIS estimate, using the DeLury correction equation noted in the text.

Box counts are a representative of recent mortality. Given the box count numbers shown in Table 2, natural mortality is low on most sanctuaries. Not unexpectedly, Dobbin Point in the Magothy River, which is composed of old oysters planted in 1999, shows the highest apparent mortality of 35 percent. The apparent mortality is likely representative of many years of cumulative mortality since the bar is not disturbed by harvest with minimal likelihood of poaching since the Magothy River is entirely closed to shellfishing. All other box counts are remarkably low, indicating low natural mortality and low infection by *P. marinus*, which causes Dermo disease.

The box count data are important because they suggest disease or other sources of natural mortality have not had a significant impact on these populations in recent years. Had a disease or other natural mortality event occurred after the oysters were of moderate size (>25 millimeters), a much higher percentage of boxes would have been expected. The box counts would be expected to be approximately equal to the number of missing live oysters (i.e., 2007 live oysters = 2008 live oysters + 2008 boxes). However, that was not the case, leading us to conclude that disease did not kill a large number of oysters. Thus, either early spat mortality occurred, which would leave little or no scar/box, or substantial poaching of adults has occurred, again leaving no boxes. As mentioned earlier, it is suspected that significant mortality of spat has occurred/is occurring, but as reported by several sources including Larry Simns, president of the Maryland Watermen's Association, poaching is widespread. In fact, the survey team discovered harvest divers on Strong Bay on one sampling outing, and reported them to the Natural Resource Police (NRP). However, when the police arrived, they allowed the harvesters to continue collecting oysters because they claimed they could not enforce the closure area due to a missing buoy(s).

Bar Name	Planting Plot	Live Count (Total)	Box Count (Total)	Apparent Mortality (%)
Dobbin Point	1999	81	28	35
Hickory Thicket	2007	1,259	25	2
Lake Ogleton	2008	3,869	167	4
Oxford	1999, 2000	54	5	9
Shoal Creek II	2003-2008	2,610	94	4
State's Bank	2003, 2007	879	19	2
Strong Bay	2003a	292	4	1
Strong Bay	2003b	102	1	1
Strong Bay	2005a	403	4	1
Strong Bay	2005b	41	3	7
Strong Bay	2005c	83	0	0
Strong Bay	2005d	227	8	4
Strong Bay	2005e	315	14	4
Strong Bay	2008	1067	22	2
Ulmstead Point	2006	481	19	4

Table 2. Number of live and dead oysters (boxes) counted on each bar.

Table 3 shows the mean shell heights in millimeters (mm) of the oysters measured in the surveys, as well as the maximum density in oysters per square meter (m<sup>2</sup>). Maximum density is the highest density of oysters sampled in a single patent tong grab. Oyster sizes were fairly well correlated with planting year or age although recently overplanted populations such as Shoal Creek II and State's Bank showed reduced mean sizes due to the increased number of smaller oysters in the population.

Bar Name	Year Planted	Mean Shell Height (mm)	Maximum Density (oysters/m <sup>2</sup> )	
Dobbin Point	1999	128.4	16.6	
Hickory Thicket	2007	50.2	201.7	
Lake Ogleton	2008	34.9	127.6	
Oxford	1999, 2000	99.2	11.8	
Shoal Creek II	2003-2008	50.5	175.2	
State's Bank	2003	46.7	88.2	
Strong Bay	2003a	105.9	17.7	
Strong Bay	2003b	93.5	5.5	
Strong Bay	2005a	92.2	36.5	
Strong Bay	2005b	106.5	9.4	
Strong Bay	2005c	93.0	23.8	
Strong Bay	2005d	100.7	40.9	
Strong Bay	2005e	57.0	89.5	
Strong Bay	2008	53.4	202.8	
Ulmstead Point	2006	75.0	50.3	

Table 3. Mean shell height and maximum density of oysters on surveyed bars.

Oyster distributions were patchy in all areas surveyed. Several sanctuaries showed maximum densities above 100 oysters/m<sup>2</sup> including Hickory Thicket, Lake Ogleton, Shoal Creek II, and Strong Bay 2008. However, many showed maximal densities far below the goal including Dobbin Point, Oxford, and many sites within Strong Bay (2003a, 2003b, 2005a, 2005b, 2005c and 2005d). To achieve the desired population density, it is recommended that these sites be remediated by planting additional oysters except where disease is threatening (such as the Oxford site, as shown in Table 4).

Shell score showed high correlations with oyster distribution (see Appendix 1). Areas of restored bars with high shell scores were typically the areas where oysters were found. Few oysters were found in areas with low shell score at any site. Some areas within sites showed high shell scores without oysters, indicating that the planting may have missed that area or the oyster may have been illegally removed from that area.

Dermo is highest in oysters from the Oxford oyster bar in Tred Avon River at 93-percent infected with a weighted prevalence of 2.1 (Table 4; weighted prevalence is a measure of intensity calculated as the mean of 25 to 30 individual infections in a sample scored by microscopic evaluation from 0 (negative) to 5 (heavily infected)). It would be expected to see mortality due to Dermo in this population soon unless meteorological events create an environment unfavorable to the parasite (cold wet winter followed by cool wet spring and summer). All other populations appear to be unthreatened by parasitic infections at the time of the sampling (Fall 2008).

	Date	Prevalence	Weighted
Bar Name	Planted	(%)	Prevalence
Dobbin Point	1999	23	0.8
Hickory Thicket	2007	23	0.15
Lake Ogleton	1999, 2006,	44	0.9
Strong Bay	2005a	10	0.05
Strong Bay	2005b	3	0.03
Strong Bay	2005d	13	0.07
Strong Bay	2005e	32	0.29
State's Bank	2008	0	0
Shoal Creek II	2008	0	0
Oxford	1999, 2000	93	2.13
Ulmstead Point	2006	22	0.5

## Table 4. Perkinsus marinus infections (Dermo) from sampled sites.

### Comparative Analysis of 2007 and 2008 Sanctuary Assessments

The following summarizes an analysis of data collected in 2007 and 2008 from repetitive patent tong grabs within 11 different oyster sanctuaries throughout northern Chesapeake Bay. The metrics collected were used to produce a population estimate for each bar within the oyster sanctuaries as well as to validate systematic patent tonging as a sampling methodology. The methodology employed to generate the population estimates for each bar utilizes a stratified cell design previously unexplored in Maryland prior to the 2007 assessment.

## Stratified Cell Population Estimate

Each bar targeted for population estimation within the oyster sanctuaries was overlaid with a sampling grid of 25-meter by 25-meter square cells. In 2007, a single patent grab was taken near the center of each sampling grid cell and the number of live oysters caught in the grab was recorded. In 2008, each point taken in 2007 was revisited and sampled again with either one or two patent tong grabs. The points in 2008 with two patent tong grabs were used to analyze the precision of repetitive patent tong grabs.

The number of live oysters caught in each patent tong grab in 2007 and 2008 were standardized to the sampling area of the patent tongs employed. Choptank River sites were sampled with 1.61-square meter ( $m^2$ ) patent tongs, while all other sites were sampled with 1.81- $m^2$  patent tongs. Those oyster densities, which are based on the total number of live oysters in each patent tong grab, were classified as one of four strata based on the scale outlined in Table 5.

Oyster Density (Live Oysters /m <sup>2</sup> )	Strata
0-2	Low
3-6	Moderate
7-15	High
16+	Very High

### Table 5. Oyster density classifications.

An average oyster density was calculated for each strata within all sanctuary bars and multiplied by the area of one sampling grid cell ( $625 \text{ m}^2$ ) and the number of grabs classified as the corresponding strata to produce a strata population estimate within each bar. All individual strata population estimates within each bar were added together to produce a population estimate for the entire bar (see the example in Table 6). These calculations were made for all bars surveyed (Table 7).

Lake Ogleton 2007 Strata	Area Grid Cell (m <sup>2</sup> )	Mean Oyster Density (m <sup>2</sup> )	Number of Cells Per Strata	Strata Population Estimate
Low	625	0.76	62	29,500
Medium	625	3.22	27	54,400
High	625	8.81	8	44,000
Very High	625	46.32	3	86,800
			Total Estimate:	214,700

Table 6. Stratified cell population estimation example.

Table 7. Corrected-GIS and stratified cell population estimates.

			Live	Amount	DeLury	Stratified Cell
	Planted	Year	Oyster	Planted	Population	Population
Bar Name	in 2008?	Sampled	Count	(millions)	Estimate	Estimate
		2007	1,074	0	408,000	215,000
LAKE OGLETON	Yes	2008	3,869	14.65	477,000	1,449,000
		2007	889	18.89	305,000	256,000
SHOAL CREEK	Yes	2008	2,610	25.33	307,000	1,073,000
		2007	314	7.9	338,000	80,000
STATES BANK	Yes	2008	879	19.37	595,000	389,000
		2007	523	0	294,000	83,000
STRONG BAY 2003a	No	2008	292	0	340,000	121,000
		2007	254	10.1	354,000	54,000
STRONG BAY 2003b	Yes	2008	102	15.12	437,000	44,000
		2007	1,033	0	355,000	265,000
STRONG BAY 2005a	No	2008	403	0	400,000	150,000
		2007	58	0	481,000	14,000
STRONG BAY 2005b	No	2008	41	0	605,000	16,000
		2007	254	0	239,000	72,000
STRONG BAY 2005c	No	2008	83	0	235,000	28,000
		2007	460	0	255,000	108,000
STRONG BAY 2005d	No	2008	227	0	270,000	74,000
		2007	178	0	310,000	56,000
STRONG BAY 2005e	No	2008	315	0	346,000	158,000
		2007	1,956	0	393,000	273,000
ULMSTEAD	No	2008	481	0	406,000	175,000
		2007	6,993	36.89	3,731,000	1,475,000
TOTAL		2008	9,302	74.47	4,418,000	3,677,000

For those bars that received a planting between the 2007 and 2008 sampling events, the stratified cell technique appears more sensitive to the large addition of oysters than the DeLury-corrected technique with the exception of Strong Bay 2003b, which is believed to have been heavily poached between sampling events. For the remaining sanctuary bars that were not planted, the stratified cell technique shows more variation in population estimation than the DeLury-corrected technique, which may indicate that the stratified cell technique is less sensitive to small changes in population.

Figure 3 illustrates that potential limitation of the stratified cell technique by comparing the amount of oysters planted (after expected mortality in the first year) on six different sanctuary bars with the population difference detected by the stratified cell population estimation between the 2007 and 2008 sampling events. The graph shows very little population difference detected below an expected population of 2 million oysters.



Figure 3. Population difference detected vs. expected population on six individual oyster bars.

Repetitive Patent Tong Sampling Precision

In 2008, patent tong sampling was repeated at all 2007 sanctuary sampling points whereby one or two patent tong grabs were captured for each 2007 sampling point. The double patent tong grabs were executed in immediate succession and in close proximity to one another. The shell score, primary substrate, and density of oysters were recorded for all grabs. Shell score is a measure of the amount of shell material captured in each patent tong grab on an increasing scale from 0 to 5. Oyster densities from each patent tong grab were stratified two ways, based on narrow or relaxed abundance ranges as shown in Table 8. The range selection may influence the strata population estimates based on abundance distributions.

Table 8. Oyster density category limits.

Oyster Density Categories – Narrow Limits	<b>Oyster Density Categories – Relaxed Limits</b>
Low - Live Counts Between 0-2	Low - Live Counts Between 0-10
Medium - Live Counts Between 3-6	Medium - Live Counts Between 11-20
High - Live Counts Between 7-15	High - Live Counts Between 21-30
Very High - Live Counts Between 16+	Very High - Live Counts Between 31+

Two classification schemes were explored because the narrow category limits of oyster density, defined by the statistical distribution of oyster densities, appeared biologically insignificant and were relaxed for comparison. All double patent tong grabs from 2008 were compared for disagreement between their category values of shell score, primary substrate, and oyster density. Additionally, oyster densities from the narrow and relaxed limit categories were analyzed with and without double grabs that caught zero oysters. The oyster density, shell score, and substrate instances of disagreement between double patent tong grabs are reported as percentages in Tables 9 and 10.

Table 9. Patent tong dou	ıble grab disagreement su	ımmary – narrow limit oyster (	densities.
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Patent Tong Double Grab Disagreement Summary	Shell Score	Shell Score +/- 1	Primary Substrate	Oyster Density with Zeros	Oyster Density without Zeros
Total Points Sampled	2720	2720	2720	2720	2720
Total Double Grabs	1045	1045	1045	1045	544
Instances of Disagreement at Double Grabs	422	107	244	195	200
Percent Disagreement of Double Grabs	40%	10%	23%	19%	37%

Patent Tong Double Grab Disagreement Summary	Shell Score	Shell Score +/- 1	Primary Substrate	Oyster Density with Zeros	Oyster Density without Zeros
Total Points Sampled	2720	2720	2720	2720	2720
Total Double Grabs	1045	1045	1045	1045	544
Instances of Disagreement at Double Grabs	422	107	244	92	81
Percent Disagreement of Double Grabs	40%	10%	23%	9%	15%

Table 10. Patent tong double grab disagreement summary - relaxed limit oyster densities.

Shell score differed 40 percent of the time between the 2008 double patent tong grabs; however, when one degree of variation around shell score is allowed (Shell Score +/- 1), the amount of disagreement between the double grabs drops to only 10 percent. Because this metric is assigned subjectively, the most accurate disagreement measurement comes from the allowance of one degree of variation around shell score. Primary substrate differed 23 percent of the time between double grabs, which suggests that the bottom type within oyster sanctuaries is heterogeneous on a small scale. Oyster density disagreement between patent tong grabs within the narrow limit category increases from 19 to 37 percent with the exclusion of doubles grabs that had zero oysters. However, when the oyster density categories are relaxed, the disagreement between patent tong grabs drops over 50 percent, to 9-percent disagreement with zeros and 15-percent disagreement without zeros. The relaxed oyster density category limits reflect a more biologically meaningful disagreement value and analyzing oyster densities without zeros theoretically eliminates sampling grid cells that are positioned on the margins of a bar, which allows for conclusions about the more dense portions of the sanctuary bars.

### Sensitivity Analysis of Patent Tong Method

An analysis of live count data was conducted on 11 individual oyster bar sites (4 planted, 7 not planted) where patent tong grabs were taken at identical locations in both 2007 and 2008. The difference in live count was calculated by subtracting the live count in 2007 from the live count in 2008. Positive differences in live counts between years indicate the addition of oysters at that point (natural recruitment or planting), no difference indicates the population at that point was unchanged and negative differences indicate the removal of oysters at that point (natural mortality, harvest or poaching).

The mean difference in live count between 2007 and 2008 on all planted bars was  $10.17\pm2.63$  oysters while the mean difference in live count on bars that were not planted was  $-1.09\pm1.54$  oysters. These results indicate there is a large, positive difference in the amount of oysters at identical points when a planting occurred between sampling events while there is zero or a slightly negative difference in live count when bars were not planted (see Figure 4; additional details are in Appendix 2).

**Figure 4. Mean differences in live count between unplanted and planted populations.** Note the significant number of positive differences in the planted populations compared to the unplanted.



Appendix 2 shows the individual histograms of the difference in live count in 2007 and 2008 for all planted and not planted bars as well as by bar sampled. Planted bars are shown in green while not planted bars are shown in red. The trends of a large, positive difference on planted bars in contrast to little or no difference on not planted bars are also present when bars are examined individually. For example, the mean difference in live count on Lake Ogleton (planted, Appendix 2, Figure 2.3) was 17.80 $\pm$ 7.30 oysters while the mean difference in live count on Strong Bay 2005d (not planted, Appendix 2, Figure 2.11) was 2.19 $\pm$ 1.35 oysters. Although the meaning of the magnitude of these differences is not finite, the increase in the live count of oysters on planted bars was significantly higher than the increase in live count of oysters on unplanted bars, indicating that patent tongs can detect planting events.

#### **Oyster Clumping**

Another method to determine the sensitivity of the patent tong method is to examine trends in the grouping of oysters on a bar. A high percentage of single oysters paired with a low percentage of 2+ clumps (defined here as two or more oysters) could indicate poaching, while the opposite (low percentage of singles paired with a high percentage of 2+ clumps) could indicate natural settlement or population addition due to planting since hatchery shell contains multiple spat per shell. The amount of singles and clumps as a percentage of the total live count was compared on the 11 oyster bars where identical points were sampled in both 2007 and 2008.

The distributions of the grouping of oysters (single or 2+ clumps) in 2007 and 2008 for all planted and not planted sanctuaries as well as separately by bar are shown in Appendix 3. The 2007 percentages are shown in blue while 2008 percentages are shown in red. In general, the percentage of singles decreased while the percentage of 2+ clumps increased from 2007 to 2008 on planted bars. This trend is evident in the distribution of all planted bars combined (Appendix 3, Figure 3.1) as well as for individual bars, with the strongest trend seen at Lake Ogleton (Appendix 3, Figure 3.3). In contrast, the percentage of singles and 2+ clumps was relatively unchanged from 2007 to 2008 on bars that were not planted. The percentage of singles increased while the percentage of 2+ clumps declined on all bars that were not planted (Appendix 3, Figure 3.2), which may be reflective of natural mortality or

poaching. Strong Bay 2005d is a good example of a bar that was not planted that also had relatively little change in the percentage of singles and clumps (Appendix 3, Figure 3.11). One interesting example of a possible detection of poaching is Strong Bay 2003b, where although the bar was planted the number of singles increased while the number of 2+ clumps decreased from 2007 to 2008 (Appendix 3, Figure 3.7).

## **Groundtruthing**

Groundtruthing verified that most restored bars had hard bottom with shell present (Table 11). Notable exceptions were Dobbin Point, Oxford, Strong Bay 2003a and 2005c all of which had mean values above 3.0. Otherwise, bottom quality of the surveyed reefs was considered good.

Bar Name	Year Planted	Mean Bottom Characteristic Score
Dobbin Point	1999	3.24
Hickory Thicket	2007	1.51
Lake Ogleton	2008	2.22
Oxford	1999, 2000	4.13
Shoal Creek II	2003-2008	1.87
State's Bank	2003	1.93
Strong Bay	2003a	3.31
Strong Bay	2003b	1.47
Strong Bay	2005a	2.68
Strong Bay	2005b	1.87
Strong Bay	2005c	3.05
Strong Bay	2005d	2.71
Strong Bay	2005e	1.93
Strong Bay	2008	2.22
Ulmstead Point	2006	1.75

### Table 11. Groundtruthing mean bottom characteristic score.

Key

Number Value	Bottom Characteristics	
1	Shell present with hard substrate	
2	Shell present with shell hash, still fairly hard bottom	
3	Shell present with sand or mud	
4	No shell present with shallow sand or mud (<2	
	centimeters)	
5	No shell present with deep sand or mud soft-bottom ( $\geq 2$	
	centimeters)	

### Water Quality

The surface temperature throughout the survey period ranged from a low of 2.65°C to a high of 16.16°C, with an average surface temperature of 9.63°C. The bottom temperature throughout the survey period ranged from a low of 2.78°C to a high of 16.11°C, with an average bottom temperature of 9.61°C. The surface salinity throughout the survey period ranged from a low of 6.9 to a high of 10.67, with an average surface salinity of 9.00. The bottom salinity throughout the survey period ranged from a low of 7.63 to a high of 11.54, with an average bottom salinity of 9.42. The surface dissolved oxygen throughout the survey period ranged from a low of 7.5 mg/L to a high of 14.58 mg/L, with an average surface dissolved oxygen of 10.50 mg/L. The bottom dissolved oxygen throughout the survey period ranged from a low of 6.90 mg/L to a high of 13.95 mg/L, with an average bottom dissolved oxygen of 8.56 mg/L.

## **Conclusions**

Sanctuary surveys were undertaken with many objectives in mind (location of relevant data in parentheses):

- 1) Determine the distribution of oysters, oyster density, mortality, and size distribution (Tables 1, 2 and 3);
- 2) Determine Perkinsus marinus (Dermo) levels in oysters (Table 4);
- Compare oyster abundances, distributions and sizes of oysters sampled to those sampled from the same sanctuaries in 2007 (Table 5, Maps, Appendix 2, Comparative Analysis section);
- 4) Estimate oyster clumping percent single/, double/ or more than three/shell (Appendix 3);
- 5) Estimate shell present as retrievable by patent tongs (Appendix 1);
- 6) Evaluate bottom quality using a standardized, transect-based evaluation method (Appendix 4); and
- 7) Evaluate how well past locations have served as sanctuaries based on growth rates, disease prevalences, mortality and unpredicted oyster losses (below).

Healthy populations of oysters were found at all sites but oyster densities were low. A refined statistical approach for estimating oyster abundance and density will be developed over the next few years. Mortalities of adult oysters are low as estimated by box counts, which are not always reliable representatives of death, but in the case of sanctuaries boxes may be good proxies. The sizes of the oysters are as expected – they get larger as they get older. A separate report has analyzed growth rates over 10 years (Paynter, et al., 2010) and shows the same trends.

Sanctuary surveys in 2008 were conducted as identically to the previous 2007 surveys as possible for several reasons. First, the monitoring team wanted to assess the repeatability of patent tong surveys, separated in time by a year, with regards to their representation of oyster distribution and populations estimates. The second reason was to determine whether significant changes in population distribution caused by unknown impacts, including poaching, could be detected.

Oyster clump frequency analysis suggested that many of the restored plots in Strong Bay may have been illegally harvested. The frequency of singles increased dramatically between 2007 and 2008 at

Strong Bay 2003b, 2005a, 2005b, and 2005c. It is not expected that these clumps would be disrupted spontaneously or by natural events such as cownose rays. Rather, these data indicate that the affected bars have been significantly impacted by harvest gear; however it is not possible to definitively prove that to be the case.

The 2008 double patent tong grabs show good precision across shell score, substrate, and oyster density, especially with expanded category limits. This suggests that meaningful bottom variation on sanctuary oyster bars occurs at scales larger than the 25-meter by 25-meter sampling grid cells and that patent tonging at that scale is an appropriate survey approach. The relaxed oyster density category limits reflect a more biologically meaningful disagreement value and analyzing oyster densities without zeros theoretically eliminates sampling grid cells that are positioned on the margins of a bar, which allows for conclusions about the more dense portions of the sanctuary bars.

Estimates were remarkably comparable between years except for State's Bank and Strong Bay 2005b both of which were seeded in 2008. Patent tong surveys, conducted using a systematic, GIS-based grid protocol, appear to be a repeatable method to estimate population abundance and distribution. However, oyster abundance predictions by the software (off the shelf produced by ESRI Inc, Redlands, CA) appeared insensitive to large changes in oysters sampled between years. Thus, development of a new method for estimating oyster populations based on a stratified cell analysis has begun.

### **Recommendations for Future Population Assessments**

Overall, systematic patent tonging is precise at small scales and using the stratified cell technique can produce population estimates that are more sensitive to large population changes than the DeLurycorrected estimates that have been used in the past. Although the stratified cell population estimation technique is insensitive to small population changes, the scale of oyster planting and harvest in northern Chesapeake Bay may not require a population estimation technique that is sensitive to population changes of less than an order of magnitude. Not all bars analyzed responded as predicted and this is likely due to the large environmental variation present in the Bay between years. However, the data for all planted and all not planted bars combined (Appendices 2 and 3, Figures 2.1, 2.2, 3.1, 3.2) are compelling and indicate the sensitivity of the patent tong technique to the addition of oysters to the sanctuaries. The point comparison and grouping analyses indicate the patent tong method detects the addition of oysters through a positive increase in the difference in live count between years and an increase in the percentage of 2+ clumps on the bar after planting while sanctuaries that were not planted show little to no difference in both live count and grouping. It is interesting to note the results of these analyses indicate possible poaching on the one sanctuary where poaching was observed. Future analysis may show that ovster density estimates should be based only on those grabs that contain oysters (i.e., do not count zeroes) and that oyster coverage within any given plot can be reasonably estimated as the percentage of grabs containing oysters. These statistics will be refined over the next few years.

In summary, the oysters appeared to be growing well, but they were distributed in highly patchy networks throughout the sanctuary areas. The patchiness is probably the result of a combination of planting technique, bottom quality and high spat mortality occurring shortly after planting (estimated at 85 percent within 2 months). Natural mortality was low after this initial early spat mortality. Dermo was detected in all pre-2008 plantings and at low levels at all sites but Oxford. Poaching appears to be a growing problem and analytical techniques are currently being developed to quantify the impacts of poaching on sanctuaries and managed reserves. Evidence of natural recruitment on any of the sanctuaries surveyed was minimal (i.e., few, if any, natural spat have been found).

### **Literature Cited**

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- Paynter, K.P., V. Politano, H.A. Lane, S.M. Allen, and D. Meritt. 2010. Growth rates and *Perkinsus marinus* prevalence in restored oyster populations in Maryland. J. Shellfish Res. 29: 309-317.





Figure 5. Clumps of large oysters from Shoal Creek, Choptank River.

## Appendix 1



























































## Appendix 2

### **Distributions of Differences in Live Count at Identical Points**

The following histograms show the distribution of the differences in live counts in patent tong grabs taken at identical points in both 2007 and 2008. Red bars are sanctuaries that were not planted between 2007 and 2008 sampling events, and green bars are planted sites. Figure 2.1 is the distribution for all sites that were not planted. Figure 2.2 is the distribution for all planted sites. Figures 2.3 through 2.13 are the distributions for the individual sites, shown in alphabetical order.





# Quantiles

Ν

100.0%	maximum	160.0
99.5%		137.6
97.5%		33.0
90.0%		6.0
75.0%	quartile	1.0
50.0%	median	0.0
25.0%	quartile	0.0
10.0%		-4.8
2.5%		-36.8
0.5%		-198.4
0.0%	minimum	-200.0
Moment	S	
Maan		1 001000
iviean		-1.091633
Std Dev	24.393515	
Std Err Mean		1.539705
upper 95% Mean		1.9408131
lower 95% Mean		-4.12408

-4.12408 251

3	6
2	U

## Figure 2.2 ALL PLANTED SITES Distributions Difference 2



100.0%	maximum	265.0
99.5%		249.1
97.5%		143.9
90.0%		44.0
75.0%	quartile	6.0
50.0%	median	0.0
25.0%	quartile	0.0
10.0%		-4.0
2.5%		-20.3
0.5%		-274.0
0.0%	minimum	-274.0
Moment	S	

Mean	10.168421
Std Dev	44.441365
Std Err Mean	2.6324793
upper 95% Mean	15.350067
lower 95% Mean	4.9867748
Ν	285

Figure 2.3 Site=Lake Ogleton Distributions 2008-2007 Difference



100.0%	maximum	177.0		
99.5%		177.0		
97.5%		168.2		
90.0%		87.7		
75.0%	quartile	39.0		
50.0%	median	6.0		
25.0%	quartile	-1.0		
10.0%		-10.2		
2.5%		-274.0		
0.5%		-274.0		
0.0%	minimum	-274.0		
Moments				

17.794872
64.453619
7.2979353
32.326918
3.262826
78

Figure 2.4 Site=Shoal Creek II Distributions 2008-2007 Difference



maximum	265.0	
	265.0	
	171.4	
	18.5	
quartile	1.0	
median	0.0	
quartile	0.0	
	-1.5	
	-16.0	
	-97.0	
minimum	-97.0	
Moments		
	maximum quartile median quartile minimum <b>s</b>	

8.0895522
38.887821
3.359396
14.734307
1.444797
134

Figure 2.5 Site=State's Bank Distributions 2008-2007 Difference



100.0%	maximum	132.0
99.5%		132.0
97.5%		131.5
90.0%		26.9
75.0%	quartile	13.8
50.0%	median	0.0
25.0%	quartile	-1.8
10.0%		-4.9
2.5%		-9.9
0.5%		-10.0
0.0%	minimum	-10.0
Moments		

Mean	10.525
Std Dev	28.014637
Std Err Mean	4.429503
upper 95% Mean	19.484515
lower 95% Mean	1.5654845
N	40





100.0%	maximum	8.000
99.5%		8.000
97.5%		7.850
90.0%		4.800
75.0%	quartile	2.500
50.0%	median	0.000
25.0%	quartile	-1.000
10.0%		-3.800
2.5%		-4.000
0.5%		-4.000
0.0%	minimum	-4.000
Moments		

Mean	0.6341463
Std Dev	2.8262705
Std Err Mean	0.4413893
upper 95% Mean	1.5262274
lower 95% Mean	-0.257935
N	41

## Figure 2.7 Site=Strong Bay 2003b Distributions 2008-2007 Difference



100.0%	maximum	9.00
99.5%		9.00
97.5%		9.00
90.0%		5.00
75.0%	quartile	2.00
50.0%	median	0.00
25.0%	quartile	0.00
10.0%		-4.00
2.5%		-21.00
0.5%		-21.00
0.0%	minimum	-21.00
Moments		

Mean	0.1515152
Std Dev	5.184841
Std Err Mean	0.902565
upper 95% Mean	1.9899798
lower 95% Mean	-1.68695
N	33

Figure 2.8 Site=Strong Bay 2005a Distributions 2008-2007 Difference



100.0%	maximum	33.0
99.5%		33.0
97.5%		25.3
90.0%		8.2
75.0%	quartile	2.0
50.0%	median	0.0
25.0%	quartile	-0.5
10.0%		-27.8
2.5%		-162.5
0.5%		-194.0
0.0%	minimum	-194.0
Moments		

-6.894737
32.519601
4.3073263
1.7338747
-15.52335
57

Figure 2.9 Site=Strong Bay 2005b Distributions 2008-2007 Difference



100.0%	maximum	9.00
99.5%		9.00
97.5%		9.00
90.0%		4.40
75.0%	quartile	0.00
50.0%	median	0.00
25.0%	quartile	0.00
10.0%		-2.00
2.5%		-16.00
0.5%		-16.00
0.0%	minimum	-16.00
Moments		

Mean	-0.28125
Std Dev	4.6920274
Std Err Mean	0.8294411
upper 95% Mean	1.4104063
lower 95% Mean	-1.972906
N	32

Figure 2.10 Site=Strong Bay 2005c Distributions 2008-2007 Difference



100.0%	maximum	29.00	
99.5%		29.00	
97.5%		29.00	
90.0%		1.00	
75.0%	quartile	0.00	
50.0%	median	0.00	
25.0%	quartile	-0.25	
10.0%		-5.20	
2.5%		-30.00	
0.5%		-30.00	
0.0%	minimum	-30.00	
Moments			

Mean	-0.684211
Std Dev	7.3635525
Std Err Mean	1.194526
upper 95% Mean	1.736129
lower 95% Mean	-3.10455
N	38

## Figure 2.11 Site=Strong Bay 2005d Distributions 2008-2007 Difference



100.0%	maximum	35.00	
99.5%		35.00	
97.5%		35.00	
90.0%		13.10	
75.0%	quartile	0.00	
50.0%	median	0.00	
25.0%	quartile	0.00	
10.0%		-0.70	
2.5%		-11.00	
0.5%		-11.00	
0.0%	minimum	-11.00	
Moments			

Mean	2.1875
Std Dev	7.6556431
Std Err Mean	1.3533393
upper 95% Mean	4.9476537
lower 95% Mean	-0.572654
Ν	32

Figure 2.12 Site=Strong Bay 2005e Distributions 2008-2007 Difference



100.0%	maximum	160.0	
99.5%		160.0	
97.5%		160.0	
90.0%		9.3	
75.0%	quartile	1.0	
50.0%	median	0.0	
25.0%	quartile	0.0	
10.0%		-10.5	
2.5%		-59.0	
0.5%		-59.0	
0.0%	minimum	-59.0	
Moments			

## Figure 2.13 Site=UmIstead Point Distributions 2008-2007 Difference



100.0%	maximum	74.0	
99.5%		74.0	
97.5%		74.0	
90.0%		56.6	
75.0%	quartile	33.0	
50.0%	median	-1.0	
25.0%	quartile	-1.0	
10.0%		-100.4	
2.5%		-200.0	
0.5%		-200.0	
0.0%	minimum	-200.0	
Moments			

Mean	-4.266667
Std Dev	60.325861
Std Err Mean	15.57607
upper 95% Mean	29.140682
lower 95% Mean	-37.67402
Ν	15

### Appendix 3

#### Comparison of Oyster Grouping between 2007 and 2008

The following figures show the distributions of oyster groupings (singles or 2+ clumps) as a percentage of the total live count between 2007 and 2008. The 2007 data is shown in blue, and 2008 data is shown in red. A high percentage of single oysters paired with a low percentage of 2+clumps (2 or more oysters) could indicate poaching, while the opposite (low percentage of singles paired with a high percentage of clumps) could indicate natural settlement or population addition due to planting since hatchery shell contains multiple spat per shell. Figure 3.1 shows the distribution for all planted bars. Figure 3.2 shows the distribution for all bars that were not planted. Figures 3.3 through 3.10 are the distributions for the individual sites, in alphabetical order.

Figure 3.1 All Planted Bars



Figure 3.2 All Not Planted Bars



Figure 3.3 Lake Ogleton



Figure 3.4 Shoal Creek



Figure 3.5 States Bank



Figure 3.6 Strong Bay 2003a



Figure 3.7 Strong Bay 2003b



Figure 3.8 Strong Bay 2005a



Figure 3.9 Strong Bay 2005b



Figure 3.10 Strong Bay 2005c



Figure 3.11 Strong Bay 2005d



Figure 3.12 Strong Bay 2005e



Figure 3.13 Ulmstead



# Appendix 4

## 2008 Water Quality Data

Sanctuary Bar Name	Sample Date	Sub Area	Water Column Location	Temperature (°C)	Salinity	Dissolved Oxygen (mg/L)
Strong Bay	11/4/08	2005e	Surface	2.65*	9.5*	14.58*
Strong Bay	11/4/08	2005e	Bottom	2.78*	9.7*	13.95*
Strong Bay	11/4/08	2005d	Surface	2.65*	9.5*	14.58*
Strong Bay	11/4/08	2005d	Bottom	2.78*	9.7*	13.95*
Strong Bay	2/9/09	2005c	Surface	2.65*	9.5*	14.58*
Strong Bay	2/9/09	2005c	Bottom	2.78*	9.7*	13.95*
Strong Bay	11/3/08	2005b	Surface	2.65*	9.5*	14.58*
Strong Bay	11/3/08	2005b	Bottom	2.78*	9.7*	13.95*
Strong Bay	11/3/08	2005a	Surface	2.65*	9.5*	14.58*
Strong Bay	11/3/08	2005a	Bottom	2.78*	9.7*	13.95*
Strong Bay	11/3/08	2003b	Surface	2.65*	9.5*	14.58*
Strong Bay	11/3/08	2003b	Bottom	2.78*	9.7*	13.95*
Strong Bay	11/3/08	2003a	Surface	2.65*	9.5*	14.58*
Strong Bay	11/3/08	2003a	Bottom	2.78*	9.7*	13.95*
Strong Bay	11/13/08	2008	Surface	2.65*	9.5*	14.58*
Strong Bay	11/13/08	2008	Bottom	2.78*	9.7*	13.95*
Hickory Thicket	11/17/08	2006- 2008	Surface	NC	NC	NC
Listen Thister	11/17/08	2006-	Detterre	NG	NG	NG
HICKORY THICKEE	12/8/08	2008	Bottom	NC	NC	NC
Oxford	12/8/08	N/A	Surface	5.76	MM	7.5
Oxford	12/0/00	N/A	Bottom	5.86	MM	6.9
Shoal Creek II	12/2/08	2003-2008	Surface	6.19	9.88	11.24
	12/2/08	2003-				
Shoal Creek II	12/9/09	2008	Bottom	6.2	10.6	8.5
State's Bank	12/0/00	2003-	Surface	4.21	9.87	11.19
	12/8/08	2005-				
State's Bank	. / . /	2008	Bottom	4.22	10.5	8.48
Lake Ogleton	4/9/09	2006- 2007	Surface	9 24	10.67	10 78
	4/9/09	2006-	Surface	5.21	10.07	10170
Lake Ogleton		2007	Bottom	9.32	11.54	10.04
Ulmstead Point	5/6/09	2006	Surface	16.23	7.56	11.5
Ulmstead Point	5/6/09	2006	Bottom	15.96	6.84	7.73
Dobbin Point	5/6/09	1999	Surface	16.16	6.9	10.79
Dobbin Point	5/6/09	1999	Bottom	16.11	7.63	9.72

Legend:\*- Strong Bay water quality was collected at one sub area and applied to neighboring sub areas because of their close proximity, NC – Data not collected due to lack of meter availability, MM – Data not recorded due to meter malfunction.