National Fish and Wildlife Foundation

Final Programmatic Report

Project Name and Number: Oyster Restoration in St. Mary's River (MD) (2006-0100-009

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1) Summary

Project sought to define pathways toward increasing oyster biomass and commercial harvests in the St. Mary's River by means of strategic placement and management of farmed and selectively-bred oysters, and measure the results of these efforts. By forming an important cadre of new oyster farmers, we also intended to generate greater citizen demand for stable or improving water quality and more official concern for environmental degradation within a bellwether watershed. More specifically, we assessed the ability of farmed, selectively bred native oysters raised in bags on floats to achieve progress toward Maryland oyster restoration goals relative to wild oyster performance employing traditional oyster-reef methods. We also planned to assess the extent to which impacts of farmed oyster floats on water quality and aquatic biodiversity in the river can be measured. Analysis of data carefully gathered during the year-long duration of the project, and public dissemination of this information, largely achieved these goals.

2) Introduction

The St. Mary's River is a part of the lower Potomac River drainage system and is one of Maryland's significant waterways. As the site of the first English settlement and capital of Maryland, its legacy goes beyond that of an important commercial and recreational river to one of vital historic importance. Wholly contained within St. Mary's County, the St. Mary's River historically was one of the most pristine watersheds on the western side of the Chesapeake Bay and supported a commercially viable oyster fishery.

Though still among the cleanest waterbodies on the western side of the Chesapeake Bay, the St. Mary's River watershed is beset by the consequences of rapid development in recent years: increasing pollution and erosion, and declines in water quality and biological diversity. With impervious surfaces in the watershed increasing

toward a threatening level, impairment classification is more than a distant threat. But this is also an area where, despite the intensive development that is in progress, the application of holistic thinking and sound management practices can do much to preserve traditional values and environmental quality.

One important casualty in the St. Mary's River is the native oyster (*Cassostrea virginica).* In the mid-twentieth century the species remained so abundant in the river that the oysters "did not grow to large size, presumably because of overcrowding," according to one report.[i] But by the mid-1980s the local mortality rate reached 80% for a combination of reasons including principally the arrival of the parasitic diseases MSX and Dermo. Commercial landings dropped from 439,700 pounds in 1973 to 305,788 pounds in 1986, 161,298 pounds in 1998 and a mere 9,418 pounds in 2001.[ii]

Spat settlement on wild oyster reefs in the river has been poor in recent years, far from sufficient for the river to make a proportional contribution to Maryland's ambitious goal to increase oyster biomass tenfold by 2010 from a 1994 baseline. For this reason, wrote Dr. Donald W. Meritt, program director at the Horn Point Lab at the University of Maryland Center for Environmental Sciences, that "plans are to greatly expand efforts using hatcheries as tools for oyster rehabilitation in Maryland."

In 2003, several of those who later founded the St. Mary's River Watershed Association (SMWRA) developed a detailed management plan for Hilton Run, one of the watershed's 16 subwatersheds. This plan was designed as a prototype for the watershed as a whole. Oyster regeneration is listed in the plan's working documents as a key means of achieving major ecological benefits for the watershed and creating new sources of income for local people. Maryland authorities have long believed that fortifying old oyster reefs with extra shell, and planting seed oysters raised by volunteer amateur farmers, could revive the beds. But the diseases MSX and Dermo attack young fingernail-sized oysters planted on the old beds, and almost all of them die before reaching the three-inch size that makes them legal to harvest. Richard Pelz, founder and proprietor of the Circle C Oyster Ranch on St. Jerome Creek in St. Mary's County, takes an alternative approach. He places seed oysters in plastic nets and attaches these to floats where they grow not on the seafloor but close to the surface, somewhat protected from blue crabs by polyurethane mesh..

Over 15 years of oyster farming in St. Jerome Creek, though no scientific analysis confirms this, Pelz alleges biodiversity increases near Circle C's cluster of floats. Water quality has also improved, he claims. The extent to which oysters can "clean up" water is often exaggerated: though their filtration ability is prodigious, they can filter only what comes to them. Nonetheless, Pelz says that improvements in St. Jerome Creek's water quality vouch for their impressive capabilities. The SMRWA-led oyster restoration project, described below, served the broad need to test these assertions and the efficacy of using this method to improve water quality and oyster production in the St. Mary's River.

This report provides detailed data on findings, and indicates the future role of farmed, selectively bred, and float-raised oysters in the overall restoration effort on the St. Mary's River.

[1] Kennedy, Victor S. and Linda L. Breisch, "Maryland's Oysters: Research and Management." Maryland Sea Grant College, 1981
[1] Tarnowski, Mitchell. "A Brief History of Oyster Population Surveys in Maryland." Maryland Department of Natural Resources, 2002

3) Procedures

OYSTER FILTRATION STUDIES- Field Microcosm Study

A set of mesocosm (containers filled with ambient river water) studies were conducted at Chesapeake Bay Field Laboratory on St. George Island (St. Mary's County). This island separates the lower Potomac River from the St. Mar's River and the laboratory itself is located on St. George Creek, a tributary of the St. Mary's River. We wished to begin trials of oyster filtration in our tanks in the fall of 2006, but delays in NFWF funding and construction prevented data acquisition before cold weather (<42 ^{o}F) set in and the oysters reduced their filtration rates.

Two large (950 gallon polyethylene) tanks- (mesocosms) were used for initial experiments in the spring 2007 once water temperatures exceeded 42 ^{o}F (Figure 1). Tanks were filled with St. George Creek water, then pumps (1080 gph) were used to pull water from the bottom of the tank and returned it to the tank's surface. Water was returned horizontally a few centimeters beneath the water's surface. Two Circle C oyster floats were used, one containing about 600 three-inch oysters and the other containing dead shell of similar size to control for other filtering organisms and shell structure. During experimentation each oyster float was placed in its own closed system tank, and water circulated throughout the experiment. This design mimicked the natural flow of an estuary and reduced gravitational settling of suspended particles. Prior to each trial the tanks were rinsed to remove all sediment and debris. The tanks were then filled with approximately 700 gallons of water directly from St. George Island Creek and circulated. Tank circulation current was measured using a Marsh McBirney flow meter were lowered into the tanks and tied so they would remain stationary in the circulating current.

Figure 1. Large mesocosm tanks with floats containing 3 bags of oysters (left) and 3 bags of non-living shell (right) at Chesapeake Bay Field Laboratory (CBFL). Kevin Boyle (center) was our student research assistant.

Following float placement in the two mesocosm tanks, 1 L water samples were collected every hour, for five hours. Baseline samples were collected at time zero. Sample bottles were rinsed with mesocosm water three times prior to each collection. During this time the temperature (˚C), salinity (ppt), and dissolved oxygen (% and ppt) were recorded using a YSI 600-QS Multi-parameter Water Quality Monitor (Yellow Springs Instrument Company, Yellow Springs, OH).

Total Suspended Solids (TSS) analyses were conducted following Chesapeake Biological Laboratory (CBL) Standard Operating Procedures (SOP's). At each sampling period, between 300 ml and 500 ml of water were drawn from each 1 L sample bottle using a volumetric pipette. These aliquots were filtered using a Millipore vacuum filtration system and pre-combusted and weighed (to 0.1 mg) Whatman 0.7µm glass fiber filters. Filter pads containing suspended solids were dried on glass plates in a drying oven at 105^{o}C for 24 hours. The filters were then re-weighed to the nearest 0.1mg using a Mettler AT261 analytical balance to calculate TSS.

Because of structural difficulties in supporting the large tanks, their instability, and the weight of the floats containing 600 oysters; we abandoned the large tanks and the large floats (each with 3 bags of oysters) in favor of a smaller system. The new (smaller) system, built and first used in the late spring and summer of 2007, had six rectangular (2'w x 3' l x 2'd) tanks. Each tank had the same circulation system described above for the large tanks, but contained a single bag of 200 oysters. Three experimental tanks contained bags with live oysters and 3 control tanks contained bags with non-living shell. Because the overall volume of the system was reduced substantially, the time between TSS sampling was reduced from 1 hour to 30 minutes. A total of 11 trials were conducted between June and September of 2007. The initial trial was used to calibrate sampling times and to trouble shoot the new system, so the data collected at that time was

not used in the final analysis. At the conclusion of the experiment, all 6 bags were opened, live oysters and dead shells were counted, and surface colonization was assessed.

Physical parameters were analyzed in Excel to determine if the environmental conditions in each tank were the same. TSS concentrations were also plotted in Excel over time to determine if time and treatment and had an effect on the concentration of suspended material. One-way analysis of variance statistical tests were used to compare the effects of time within each treatment for TSS. An analysis of covariance was used to determine if there were differences due to live and dead shell treatments.

OYSTER FILTRATION STUDIES - Controlled Laboratory Studies of Individual Oysters

Because field mesocosm studies were temperature dependent and assessed a large number of oysters filtering at one time, we conducted a small scale study in the winter of 2006-2007 that compared the filtering capability of individual native St. Mary's River oysters to individual selectively-bred Circle C oysters. These studies were conducted in the flow-through estuarine system at St. Mary's College of Maryland (Figure 2).

The study's design was quite similar to that conducted at CBFL, except that raw river water pumped from the St. Mary's River was used as a source of TSS for this series of experiments. Six oysters, 3 selectively-bred oysters from Circle C and 3 native oysters from the St. Mary's River oysters were placed in individual aquaria. The aquaria were filled with river water that was then circulate in much the same way as done at CBFL but on a much smaller scale.

A small pilot study was conducted to determine if TSS assessed by a Hach DR/2400 portable spectrophotometer (Loveland, CO) was equivalent to gravimetric assessment using pre-weighed filters. We wanted to do this to reduce the cost and time of filtration, but there is a controversy over the accuracy of spectrophotometric (Hach) methods in measuring TSS (Sadar, 2002). The published, manufacturers methods for the Hach greatly over-estimated suspended solids. But we were able to slightly modify the Hach methods and the results from our pilot experiment demonstrated with high significance (tested by t-Test) that the Hach method was equivalent to the filtration method. Therefore, we used the Hach spectrophotometer because it was a quick and reliable.

The six individual aquaria were sampled for TSS every 15 minutes during 2-hour trials. Six separate trials were conducted between January and March 2007. We tested to see if trial had a significant effect on filtration rate, and it did not. Therefore, we collapsed all data and conducted a final analysis of TSS removal by a two-way analysis of variance where type of oyster and time were the two independent variables.

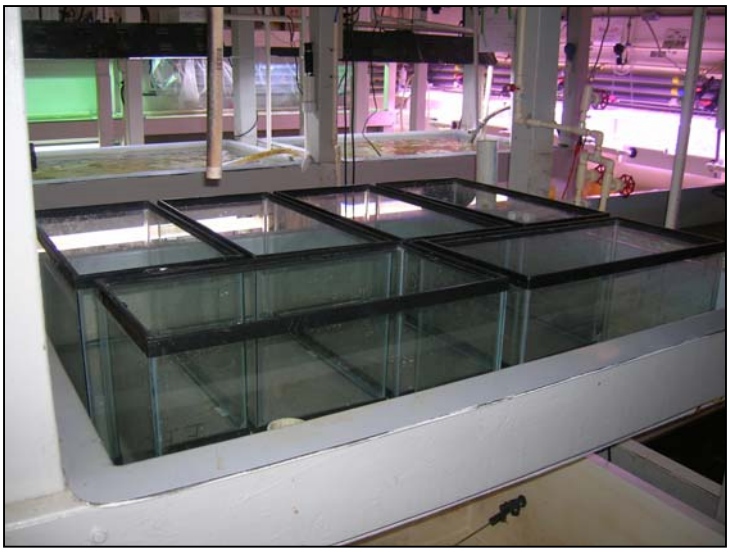


Figure 2. SMCM wet laboratory with circulating St. Mary's River water and experimental aquaria.

OYSTER FLOAT BIODIVERSITY- Field Procedures

In the St. Mary's River Watershed Association (SMRWA) project, ten Circle C kits (each including a 3' by 6' float, three bags containing a total of about 600 seed oysters, securing devices) were deployed in the St. Mary's River near St. Mary's College of Maryland (SMCM) by October 30, 2006. Each of 10 waterfront homeowners who have docks along the St. Mary's River north of St. Inigoes Creek and south of the freshwater St. Mary's River (Tippity Witchity Island) agreed to "host" a Circle C float as well as an adjacent second float containing shells but no live oysters. Owners and locations (Figure 3) of these properties are as follows:

1. Peter and Susan Messitte
2. Jim and Karen Nutter.
3. Captain Russell Crenshaw, Jr. and Mrs. Flavienne Crenshaw
4. Doug and Cynthia Gardiner
5. Gary and Linda Williams
6. Doug and Robin Cook
7. Mr. & Mrs. Richard Timbie / Dr. & Mrs. John Harmon
8. Tom Schmidt
9. Mr. & Mrs. Robert Maddox
10. Elmer Brown

During the winter of 2007, while the live oysters in the floats remained dormant because of 40 degree F or lower temperature water the floats were given regular checks to assure moorings and floats were weathering the winter. However, during the winter several problems occurred. At site 8 - Glen Mary Farm – Tom Schmidt, owner-because of a communications error the live oysters placed there were prematurely harvested and consumed in January 2007 by people not working on the project. Replacement live oysters could not be attached to the float because the time difference would invalidate

the**.** In addition, two bags on the live oyster float at site 6 – Doug and Robin Cook, owners - were vandalized so only one live bag remained at that site. And thanks to the combination of very cold weather, ice formation, and very low tides during February 2007, most of our original oysters literally froze to death. At three docks we experienced 100% mortality. At only two of the docks did high percentages of oysters survive. We are not alone with these kinds of misfortunes. We replaced the dead oysters in the early spring of 2007, but because of this extensive replacement at just about every site, and because of the late placement of oyster floats in the River (October 30, 2006), it was impossible for us to assess the growth of the oysters during this study. A slight budget modification was required since we needed to purchase more oysters than anticipated.

We measured the diversity of organisms colonizing all oyster bags at each site on five different cruises. On June 27th we sampled Site 1 (Williams), on June 29th we sampled Site 10 (Brown), on August 1st we sampled sites 7 (Harmon) and 9 (Maddox), on August 30th we sampled sites 2 (Nutter) and 3 (Crenshaw), and we finished on September 21st by sampling sites 1 (Messitte), 4 (Gardiner), and 6 (Cook). On each sampling date we left the college dock by 9 a.m. and used the SMCM's 25 foot C-Hawk vessel to pull floats from the water (Figure 4). Prior to sampling we recorded water and air temperatures, dissolved oxygen concentration, salinity, and pH taken with a YSI model XLM600 water quality sonde (Yellow Springs, Ohio).

The procedures were the same for each float at each dock on all sampling dates. Floats were first detached from the dock and brought gently along side the research vessel. We slipped a 2' x 3' fiberglass window screen tray mounted on a ¾" diameter PVC pipe frame underneath the bag to be sampled. The plastic bands holding the bag to the float were clipped with diagonal cutters and the bag and its support screen lifted on board the boat. This procedure assured that organisms in the bag would not wash out when the bag was taken out of the water. The bag was taken from the screen tray and placed on a 2' x 3' x 1.5' (d) plastic box and rinsed with ambient river water for 1 minute. The bag was turned over and rinsed again for one minute. Organisms were washed out of the box and retained on a fine mesh sieve. The bag was placed on the deck and examined for blue crabs inside the bag, and then the bags were opened on the June 27th (Williams dock) and on the August 1st (Maddox dock), and five oysters removed for surface analysis. Organisms collected on sieves were then counted or preserved for later identification.

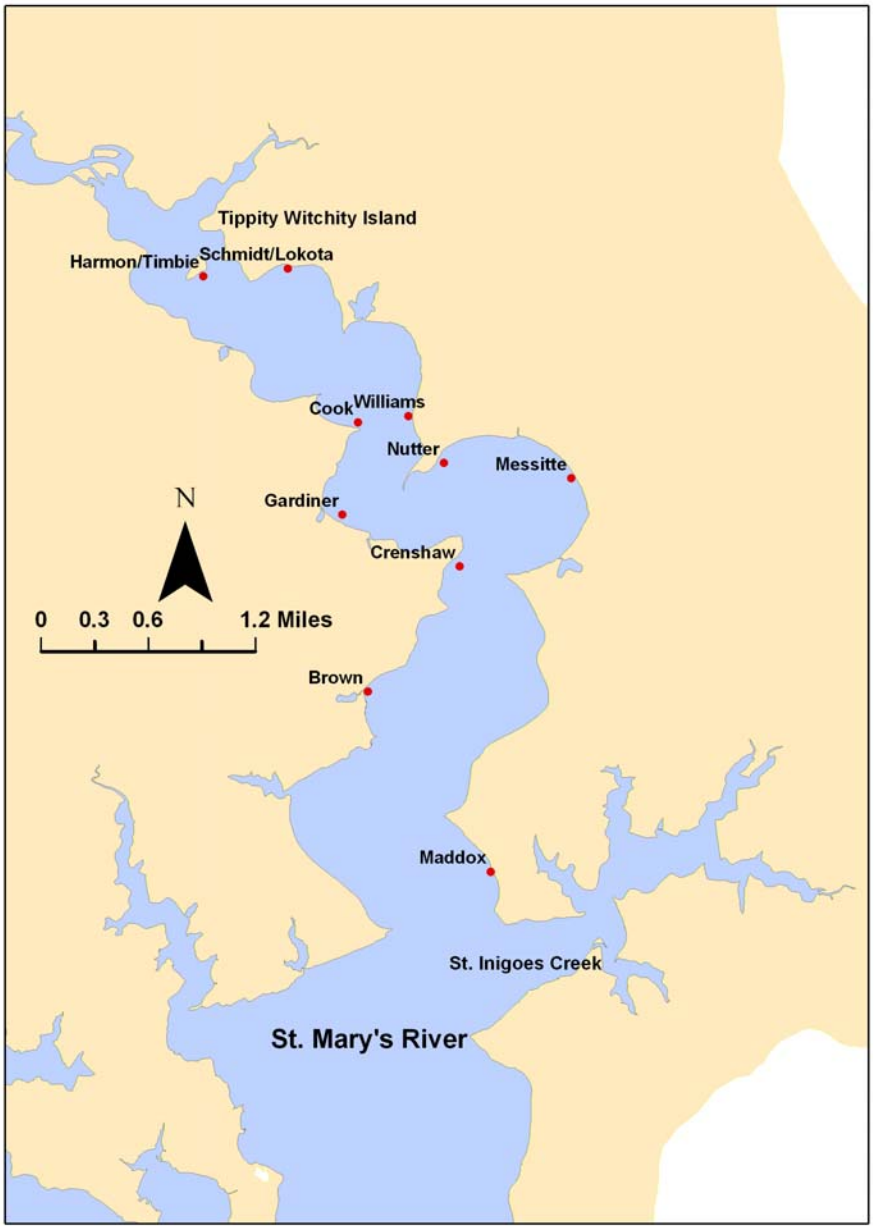


Figure 3. Locations of SMRWA sites where Circle C oyster floats were placed.

All fish and most of the grass shrimp (*Paleometes pugio*) were field identified, counted, and then released. The remaining invertebrates were placed into one quart mason Jars with 70% ethanol. These invertebrates were later processed, identified and counted in the laboratory. Once all 6 bags on live and dead floats had been sampled, the bags were reaffixed to the floats and again tied to the docks.

Figure 4. Research coordinator, Henry Bush, pulls a float from the St. Mary's River while student volunteers record data.

Oyster Shell Surface Processing Procedures

The five oysters taken randomly from each bag in the field were placed in plastic bags and put on ice in a cooler. In the SMCM wet laboratory (Figure 2), the oysters were placed in plastic trays and water from the St. Mary's River was constantly pumped through the tank. When the surface analyses were conducted (within 5 days of sampling) the individual oysters were were gently agitated to remove sediments. Each individual oyster was placed in a culture dish containing river water and was then viewed through a Nikon SMZ1500 stereoscope (Model C-DSS115, Japan) at 7.5x magnification. A color video camera (Sony Hyper HAD, CCD-IRIS/RGB) was attached to the stereoscope so that the view-field of the scope could be seen on a monitor. A transparent plastic sheet with a grid of 336 evenly-dispersed black dots was attached to the monitor, so that a 0.42 square inch area of the view-field was covered. Oysters were allowed to acclimate for

one minute under the scope before they were examined and also allowed for sediment to settle. For each oyster, 3 random view-fields were examined for 30 seconds each. All motile organisms (tube worms, mud crabs, and amphipods) were identified and counted and fixed barnacles were also counted. Other fouling organisms (bryozoans, algae) were evaluated by counting the number of dots on the transparency where the fouling organism occupied and converting to a percentage of area covered.

4) Results

OYSTER FILTRATION STUDIES- Field Microcosm Study

At the conclusion of this set of experiments, we opened all 6 bags used in all 10 trials. Our objective was to determine if there were significant differences between the bags with live oysters and between the bags with non-living shell. These results are shown in Table 1. All the living bags contained at least 88 living oysters and equivalent numbers of mussels and barnacles. Likewise, non-living bags had equivalent amounts of shell and shell colonization by barnacles. We therefore felt that comparisons across bags of the same type were legitimate and that the consistency of results was meaningful.

Table 1. Contents of bags after completion the field microcosm study (following Trial 10.) for oyster filtration at CBFL.

Bags	Live oysters	Shell (empty)	mussels	barnacles	spat
Live 1	99	92	20	12	1
Live 2	88	116	7	24	0
Live 3	92	31	2	2	0
Shell 1	0	161	0	75%	0
Shell 2	0	155	5	80%	0
Shell 3	0	168	2	85%	1

The results of the modified field microcosm study at Chesapeake Bay Field Laboratory (CBFL) were interesting and conclusive (Figures 3 and 4). All 10 trials are shown in Figure 3 for each bag, and while there is no real significance in presenting the results by bag, this method does allow for comparisons between trials and between living oysters in bags to dead shell in bags. It is important to note that in all tanks regardless of whether they contained living or non-living shell, the amount of TSS suspended in the tanks declined, and declined at a relatively constant rate. This indicates that both oysters and non-living shell with filter-feeding colonists removed suspended material over all time increments. The fact that non-living shell removed so much TSS surprised us, but

these shells (especially non-living shell) were heavily colonized by filter-feeding barnacles in particular (Table 1). It is also interesting to note from Figure 3 that there is surprising consistency between the different bags of the same trial. For example, the bags in Trial 3 (the yellow lines in Figure 3) were always the least effective in removing TSS because initial TSS concentrations on that date were low. Conversely, Trial 2 (the bright pink line of Figure 3) started with the highest TSS concentrations and ended with relatively high TSS after 2.5 hours. The consistency of the slopes of these individual lines (trials) relative to one another confirmed that all live bags and all dead bags were very similar to one another, and that they only differed slightly between trials. From these results we conclude that bags with living shell were not different from each other and bags containing dead shell were not significantly different from one another. We can also conclude from these results that the only differences between all the live bags were due to the initial concentrations of TSS on an individual trial date. The same was true for all bags containing non-living shell.

The purpose of this experiment was to determine if living Circle C oysters filtered water and removed more TSS than the equivalent amount of non-living shell. When we compared all the TSS removal rate data by living shell to the TSS removal rate data for non-living shell, Figure 4 was the result. This figure also shows the lines of best fit, their equations, and their correlation coefficients (r-squared values) produced by simple linear regression. The slope of the living shell in removing TSS was -7.1219 (yellow line of Figure 4), considerably greater than the slope of the non-living shell in removing TSS, -4.6267 (blue line of Figure 4). We compared these two lines by analysis of covariance and found that the Circle C oyster filtration rate was significantly ($P<0.01$) greater than the non-living shell filtration rate. This is the important finding from this study and shows the combined and separate filtering capabilities of living oysters, and the organisms that inhabit their shells.

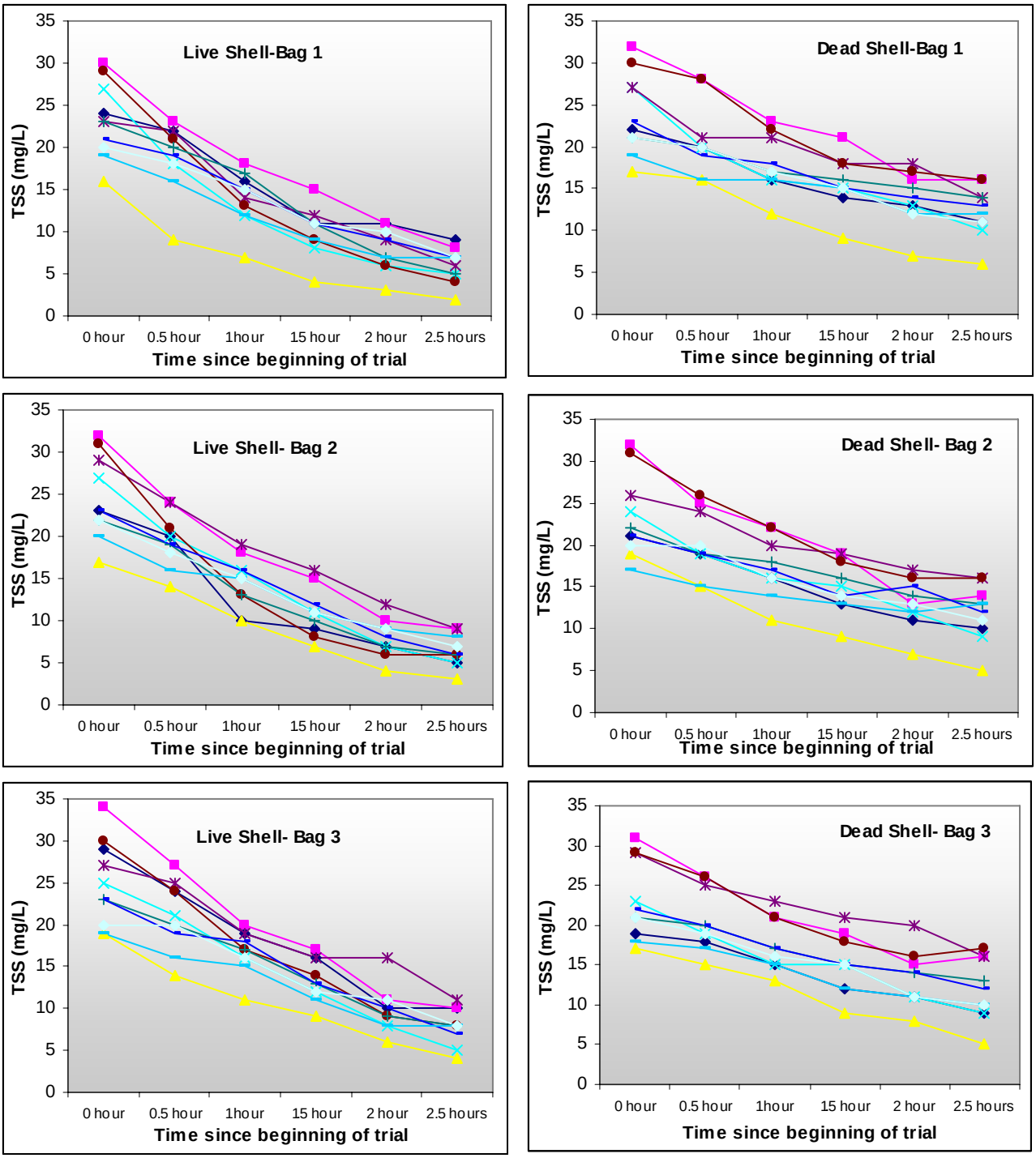


Figure 3. Filtration rates (removal of TSS) by filter-feeding organisms over the 2.5 hour trial period. Each colored line is an individual trial, and there were 10 separate trials during the course of this experiment. Each of the 6 bags was suspended in its own tank that was filled with the same water from St. George Creek. Therefore, the initial concentrations of TSS for each bag in a trial are nearly the same.

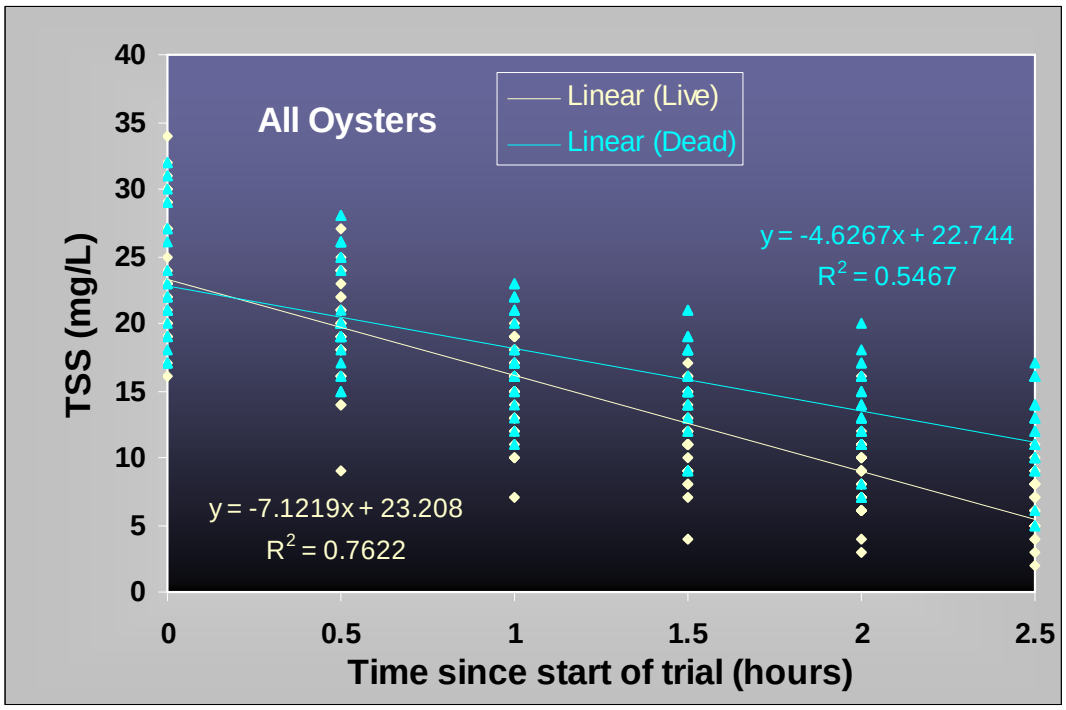


Figure 4. TSS concentrations in all tanks over all trials for live (light yellow) and dead (bright blue) oysters. Simple linear regression was used to construct lines of best fit, equations for these lines, and their correlations.

Controlled Laboratory Studies of Individual Oysters

Our small scale study in the winter of 2006-2007 that compared the filtering capability of individual native St. Mary's River oysters to individual selectively-bred Circle C oysters was not supported by NFWF funds, but because the results are important to this study we include the basic results here. These studies were quite similar to those conducted at Chesapeake Bay Field Laboratory (CBFL). However, instead of non-living shell, we used native oysters so the comparison was Circle C oyster to native St. Mary's River oyster filtration capacity. As in the CBFL study, TSS reduction was studied over time.

Our statistical tests showed that there was a significant reduction in TSS over the 15 minute time intervals when all 6 trials were combined. This reduction in TSS took place for both Circle C and native St. Mary's River oysters. The other comparison made in the two-way analysis of variance showed that the Circle C oyster filtration rates (reductions in TSS) were significantly ($P<0.01$) greater than the native oyster filtration rates.

OYSTER FLOAT BIODIVERSITY
Results from floats placed at home owner's docks

The organisms collected from bags with living oyster shell (Table 2) and from bags with non-living oyster shell (Table 3) were surprisingly similar in both the kinds of different organisms

Table 2. All organisms found in all float bags with living oyster shell at all homeowner sites during the study.

		Skillet Fish	Striped Blenny	Feather Blenny	Naked Goby	Green Goby	Mummichog	Striped Killi	Eel	Grass shrimp	Amphipods	Worms	Mud Crab	Blue Crab	Anenome	Mussel
1	**Messitte**	51	12							10	347	7	3	1		
		44	5							151	387	4	5			
		35	5							114	228	12	3	1		1
	Sum	**130**	**22**							**275**	**962**	**23**	**11**	**2**		**1**
2	**Nutter**	75	3							18	560	1	5	1		2
		32	5							68	450	27	5	1		4
		48	3		1					32	514	27	2	1		2
	Sum	**155**	**11**		**1**					**118**	**1524**	**55**	**12**	**3**		**8**
3	**Crenshaw**	48	1							78	792	4			2	
		8								13	778	15	1	1		
		40								23	660	18	3	1		
	Sum	**96**	**1**							**114**	**2230**	**37**	**4**	**2**	**2**	
4	**Gardiner**	14	27			1	2	1		7	492	3	2			2
		64	2				2			132	368		3			
		46	1					1		28	93		5			2
	Sum	**124**	**30**			**1**	**4**	**2**		**167**	**953**	**3**	**10**			**4**
5	**Williams**	46	1					1	1	24						4
		64	1							90	33	7				
		Missing														
	Sum	**110**	**2**					**1**	**1**	**114**	**33**	**7**				**4**
6	**Cook**	15					3			81	1250		2			
		40		1						87	236	3	1			
		31					1	2	2	142	289	3	4			
	Sum	**86**		**1**			**4**	**2**	**2**	**310**	**1775**	**6**	**7**			
7	**Harmon**	29	2							35	634	12	1	1		
		62	1							77	160	1	1			
		29								54		1				
	Sum	**120**	**3**							**166**	**794**	**14**	**2**	**1**		
9	**Maddox**	62								2	780	6				
		57								4	1476			1		
		66								20	123	6	2			
	Sum	**185**								**26**	**2379**	**12**	**2**	**1**		
10	**Brown**	37	4							6	650	18	11			1
		26	1		1					5	686	51	7			
		46	4							2	936	4	2			
	Sum	**109**	**9**		**1**					**13**	**2272**	**73**	**20**			**1**
TOTALS		**1115**	**78**	**1**	**2**	**1**	**8**	**5**	**3**	**1303**	**12922**	**230**	**68**	**9**	**2**	**18**
MEAN		**42.9**	**3.0**	**0.0**	**0.1**	**0.0**	**0.3**	**0.2**	**0.1**	**50.1**	**497.0**	**8.8**	**2.6**	**0.3**	**0.1**	**0.7**

Table 3. All organisms found in all float bags with non-living oyster shell at all home owner sites during the study.

		Skillet fish	Striped Blenny	Feather Blenny	Naked Goby	Green Goby	Mummichog	Striped Killi	Eel	Grass shrimp	Amphipods	Worms	Mud Crab	Blue Crab	Anonome	Mussel
1	**Messitte**	53	4							112	198	5	1			5
		42	4		2					181	256	4	2			2
		48	2				3			107	232	1	1			3
	Sum	**143**	**10**		**2**		**3**			**400**	**686**	**10**	**4**			**10**
2	**Nutter**	23			1					47	845	4	7	1		4
		59	2				2			114	560	6	4			12
		21	1							146	635	5	7	1		4
	Sum	**103**	**3**		**1**		**2**			**307**	**2040**	**15**	**18**	**2**		**20**
3	**Crenshaw**	51								33	328	12	1	1		
		45								119	324	11	5			3
		19	1							75	278	15	3	1		
	Sum	**115**	**1**							**227**	**930**	**38**	**9**	**2**		**3**
4	**Gardiner**	32	4				7			21	2592	24	6			
		39								26	712	6	9			
		39								50	1620	6	5			1
	Sum	**110**	**4**				**7**			**97**	**4924**	**36**	**20**			**1**
5	**Williams**	34								8	84	26	1	3		
		21	4							22	103	22	2	0	1	1
		12							1	1	138	16	1	1		
	Sum	**67**	**4**						**1**	**31**	**325**	**64**	**4**	**4**	**1**	**1**
6	**Cook**	37	1				1			67	512	2	10			3
		Lost- vandalized														
		Lost- vandalized														
	Sum	**37**	**1**				**1**			**67**	**512**	**2**	**10**			**3**
7	**Harmon**	19			1					78	580	8				
		39	1		1				1	172	224	2	1			
		39	1							98	212	2	3			
	Sum	**97**	**2**		**2**				**1**	**348**	**1016**	**12**	**4**			
9	**Maddox**	34								2	690	33	1			
		44								-	888	17	2			
		27								1	550	14				
	Sum	**105**								**3**	**2128**	**64**	**3**			
10	**Brown**	23	2							8	459	8	4			
		45	4							26	107	5	8			
		19	1		2					38	422	2	1			
	Sum	**87**	**7**		**2**					**72**	**988**	**15**	**13**			
TOTALS		**864**	**32**	**0**	**7**	**0**	**13**	**0**	**2**	**1552**	**13549**	**256**	**85**	**8**	**1**	**38**
MEAN		**35**	**1.3**	**0**	**0.3**	**0**	**0.5**	**0**	**0.1**	**62.1**	**542**	**10**	**3.4**	**0.3**	**0**	**1.5**

and the number of individuals of each species. We analyzed the results of this experiment by separating fish from colonizing invertebrates. Eight different species of fish were found in both living and non-living bags. In both treatments (Live and dead shell), skilletfish (*Gobiesox strumosus*) were by far the most numerous fish and distantly followed by striped blenny (*Chasmodes bosquianus*). The living oyster bags had significantly ($P<0.01$) more skilletfish and striped blennies (mean = 42.9/bag and mean =3.0/bag, respectively) compared to bags with non-living shell (mean = 35.0 skilletfish/bag and mean = 1.3 striped blennies/bag). Other fish that are common, oyster reef-dwelling or shallow water–dwelling were represented in small numbers.

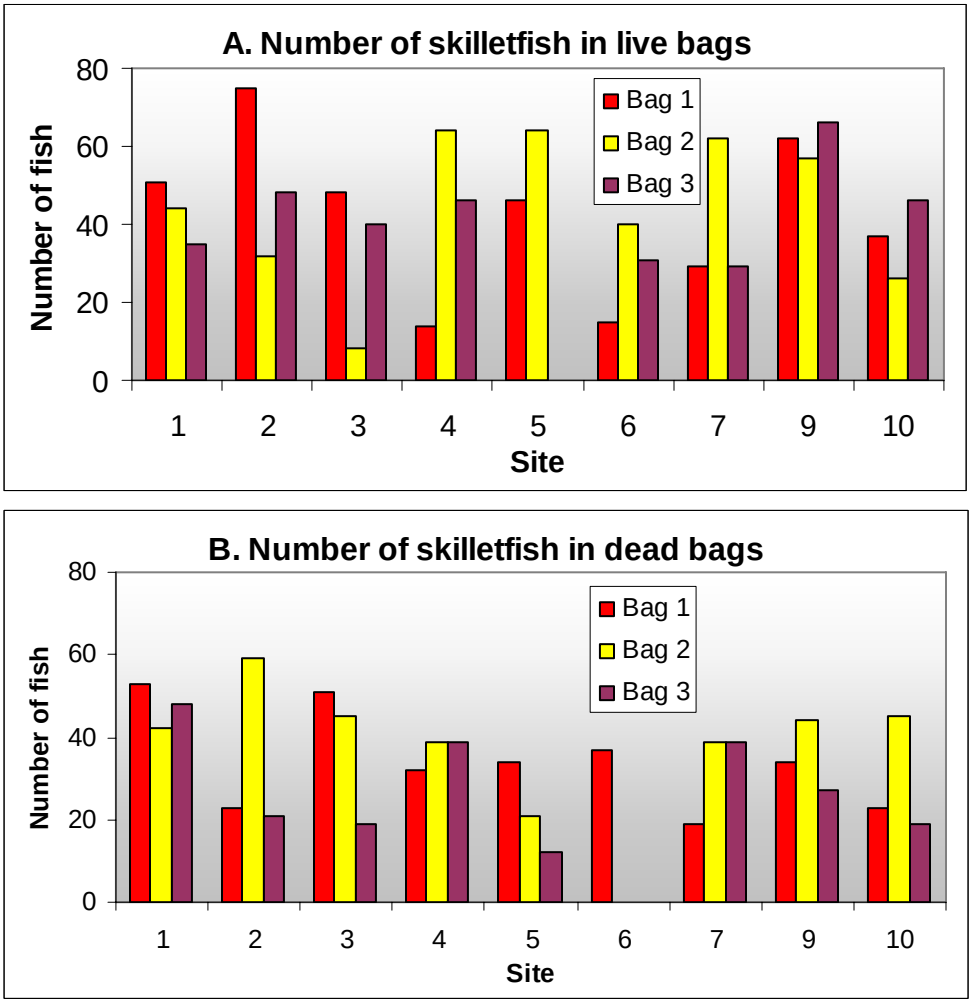


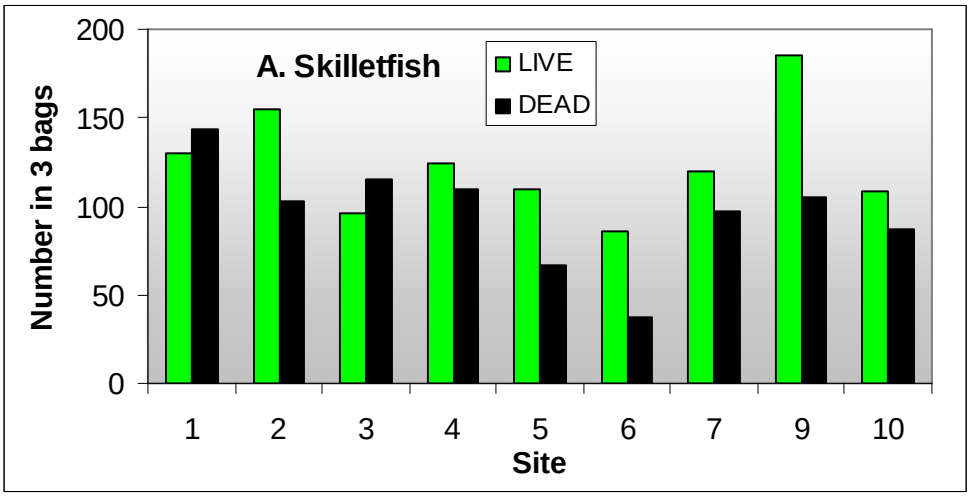
Figure 5. Number of skilletfish (*Gobiesox strumosus*) found in bags containing A. living oyster shell and bags containing B. dead oyster shell and sorted by site.

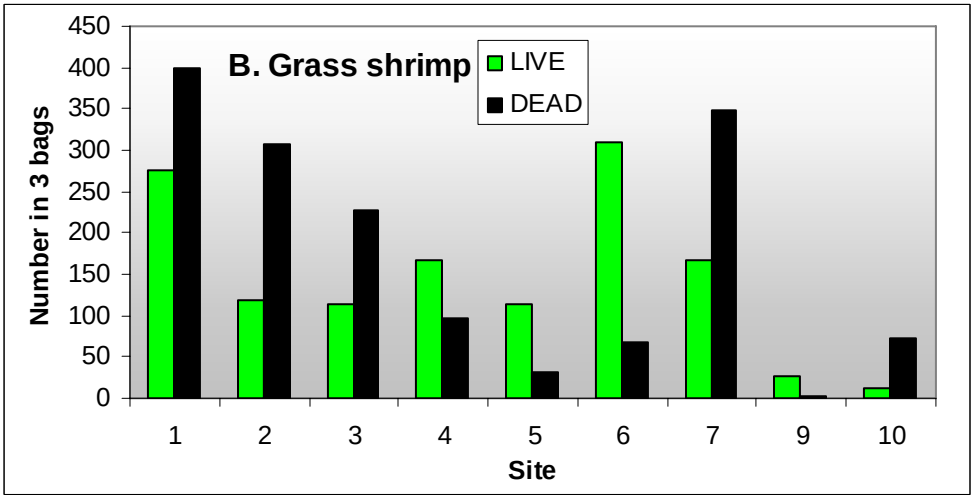
To determine if there was a significant difference in the number of skillet fish found in the different bags and at the different sites, we conducted a two-way ANOVA, that indicated that there was a significant ($P<0.01$) difference between the number of skillet fish in living bags compared to dead bags, but there was no significant difference between sites (Figure 5). Indeed, Figure 5 shows the remarkable similarity of all bags at all sites according to treatment (A. living shell and B. non-living shell). This indicates that bags with living shell were relative consistent with higher numbers of skilletfish at all sites compared to the consistently lower number of skilletfish in dead bags.

Therefore, conditions from down-river (Figure 1), Maddox (site 9) and Brown (site 10), were consistent with and not statistically different from the sites furthest up-river Harmon (site 7). We, therefore, concluded that there was no significant difference due to salinity or other environmental factors between the sites. Likewise, there were no significant differences due to the date of sampling because bags sampled early (6/27/2007) at Williams-site 5 –were not significantly different from those sampled last (9/21/2007) at Messitte-site 1, Gardiner-site 4, and Cook-site 6).

We separated all invertebrates found into 7 categories. Of all the invertebrates, amphipods were by far the most numerous in both types of bags with a mean of 497 individuals found in each living shell bag and a mean of 542 individuals found in each dead shell bag. Time restrictions did not allow us to identify and analyze amphipods by species. The second most numerous invertebrate was grass shrimp (*Palaemonetes pugio*) with a mean of 50.1 and 62.1 individuals in each living and non-living bag, respectively. Interestingly, the numbers of invertebrates in each bag with non-living shell was higher than the number in those bags with living shell. It is likely that the non-living shell created more refuges from invertebrate predators because of the openings between the two shell halves, and invertebrates may have selectively chosen dead shell over live shell for hiding. However, we can not state this conclusively since the results were not statistically different. It also could be true that the larger number of fish in the living shell bags reduced the number of invertebrates in living shell bags, but again we did not conduct experiments to test this hypothesis. Yet, it is important to note that the community of both fish and invertebrates that developed over time in bags was similar to that found on the bottom of the St. Mary's River, and that there were similar numbers in bags regardless of the treatment (living or dead).

We also examined the biodiversity data by comparing the total number of A. skilletfish, B. grass shrimp, and C. amphipods in all living bags at each site and in all dead bags at each site (Figure 6). Because we lost all six bags at site 8, one living bag at site 5, and two dead bags at site 6) (Figure 5), we were not able to conduct statistical analyses for the data given in Figure 6. However, the graphic representation of the results in Figure 6 clearly shows, again, that skilletfish in live bags out numbered skilletfish in dead bags at almost every site, and that the reverse trend was true for grass shrimp at most sites. The pattern for amphipods was less clear, and the results were probably heavily influenced by experimental error as amphipods are quite small and could have been easily lost from bags during sampling.





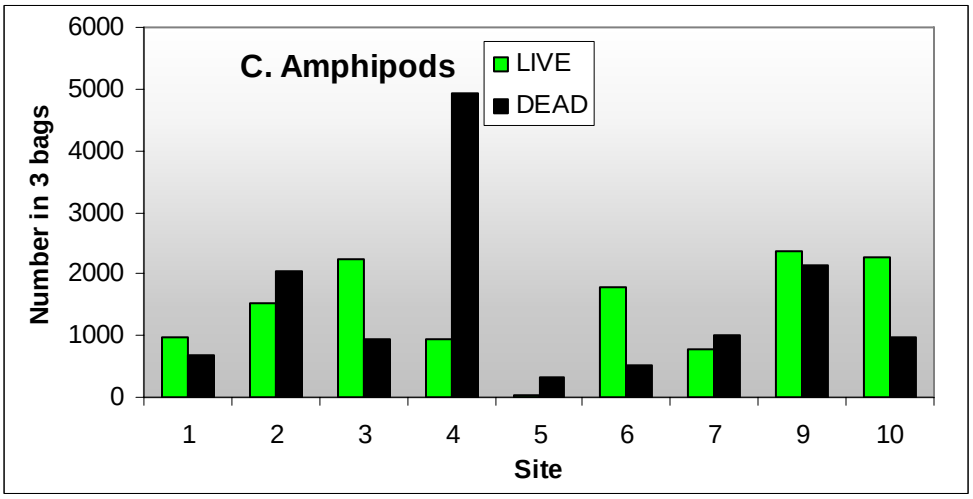


Figure 6. Total number of A. skilletfish, B. grass shrimp, and C. amphipods in all living bags and all dead bags at each site. Note that Site 5 had only 2 live bags at the end of the study and that Site 6 had only 1 dead bag remaining at the end of the study, so the total number of organisms at both these sites is substantially reduced.

Results from the surface analysis of oysters retrieved from home owner's docks

The five oysters taken randomly from each of the five remaining bags on the June 27th (Williams dock) and from all six bags on the August 1st (Maddox dock) sampling dates were examined and assessed for surface colonization. Although we made a total of 75 and 90 stereoscope views of 25 and 30 oysters, respectively, at both the Williams and Maddox sites (15 oysters from live bags and 15 oysters from dead bags), the results were inconclusive. We found that bryozoans (*Membranipora tenuis*) were, by far, the dominant surface colonizer of the living and non-living shells. Frequently, 70-100% of the viewing field would be covered by an individual bryozoan colony. In addition, oyster surfaces and our three timed views of each shell frequently contained barnacles (*Chthamalus fragilis, Balanus eburneus,* and *Balanus improvisus*), and/or mud whip worms (*Polydora ligni*). We counted bristle worms (polycheates) when we encountered them in our fields of view, but we did not identify them as common glass worm (*Nereis succinea*), red-gilled mud worm (*Scolecolepides viridis*) or glassy tube worm (*Spiochaetopterus oculatus*), although all were present. Because this analysis was tedious and took a long time to complete, we abandoned the surface analyses for the sites sampled after August 1st.

The results of statistical tests (t-tests) that sought to find significant differences between living and dead shells were inconclusive. Even though the statistical tests did not show significant differences between the two treatments, we did find that the surface colonization results were similar to the fish and invertebrate biodiversity results. It is clear that oyster shell that is contained in bags and fastened to floats develops a complex community of surface-dwelling organisms that is similar to a community that would be associated with naturally occurring oyster bars.

5) Discussion and Adaptive Management

In summary, our experiment confirms that:

- In controlled experiments at CBFL, farm-raised Circle C oysters in bags out performed bags that contained dead shell in filtering and removing material from the water. This may seem like a nonsensical result, but dead oyster shells are colonized by organisms that filter water just as oysters do. If this filtration is not assessed, then the results of other experiments can be misintepreted.

- In controlled experiments individual farm-raised Circle C oysters removed significantly greater amounts of sediments and algae from St. Mary's River water than did individual oysters from native oyster beds in the St. Mary's River.

- Bags of oysters and bags of equivalent amounts of dead shells in bags were suspended on different floats located at home owners docks. We expected that the bags with living oyster shell would attract a different community of organisms compared to the dead bags. However, this was not the case; there was not

difference between living and dead bags. Therefore, the presence of living oysters does not make a significant difference in attracting organisms.

- However, the community of organisms found in both living oyster bags and dead shell bags was quite similar to the community of organisms that would be associated with natural oyster bottom. Therefore, floating aquaculture bags provides a good habitat and refuge for other organisms in the estuary (mostly oyster bed-dwelling fish and invertebrates).

Some of the experiments conducted in this project were highly successful and conclusive while other experiments were less definitive. The most important scientific finding of this study was that suspended bags of selectively-bred, Circle C oysters in the St. Mary's River behave very much like native oysters do in the bottom oyster beds. They attract the same surface-dwelling organisms, as well as motile invertebrates, and fish that one would find in bottom oyster communities. Whether the biological diversity of float and natural bottom communities are the same or different was not within the scope of this project and was not assessed here. It seems that the structure of oyster shells with in bags provides a good and complex habitat for colonization, regardless of whether the shell is alive or not. Therefore, floats promote and sustain oyster reef biological diversity.

We also found, to our considerable surprise, that non-living shell (and its associated surface colonizing community) filters significant amounts of St. Mary's River water to remove TSS. Living oysters, however, compared to their non-living counterparts, have a significantly great capacity to remove TSS from the water column. While we expect that the water quality of the St. Mary's River could be drastically improved by using oyster aquaculture techniques and floats, it is unclear how many floats would be necessary to purge the river of unwanted algae and sediments. Inorganic sediments washing into the river as a consequence of erosion and storm events need to be controlled in other ways.

It is also clear that selectively bred, Circle C oysters are not the panacea for all the ills that befall the native *Crassostrea virginica* oyster because winter mortality was significant in this project. This mortality also thwarted our attempts to study Circle C oyster growth in the St. Mary's River. While the reasons for the death of our oysters on floats remains obscure, we do know that using oysters on aquaculture floats has its own set of idiosyncratic problems. For example, native oysters are largely immune to the problem of long exposure to freezing air temperatures, but oysters stranded on floats during extremely low tides will likely be killed.

As regards public outreach, we can claim that we converted ten dockowners on the river into private oyster growers. Commercial potential is limited because of heavy regulatory pressures that limit profitability, and the possibility of negative reactions if too many floats are deployed, but some increase may be possible. We demonstrated our form of oyster aquaculture at the association's annual River Fest, publicized the experiment on

our website and printed newsletter, and will widely distribute our comprehensive final report to NFWF plus a brief video.

Literature Cited

SADAR, M. 2002. Turbidity Instrumentation - An overview of today's available technology, Turbidity and other sediment surrogates workshop, Reno, NV.