Oyster Reef Restoration and Monitoring, Wellfleet, MA

Draft Final Report

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Submitted by:

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I. Introduction

The wild oyster reefs of Wellfleet were once so extensive that they presented navigation hazards, prompting Samuel de Champlain to name Wellfleet Harbor "Port aux Huitres" (Oyster Harbor) when he explored the area in 1606. As recently as the 1970s, state fisheries biologists counted 1000 bushels of oysters on the flats around Lieutenant Island (Curley et al 1972). Overharvest and disease took their toll on the natural reefs (Beck et al 2011), and the famous Wellfleet oyster is now mostly a farm product, grown in plastic mesh bags from purchased hatchery seed. With the disappearance of the wild reefs, many of the services they provide have also gone, including benthic-pelagic coupling (Kellogg et al 2011, Willcox 2009), water filtration (Grizzle et al 2008), shoreline stabilization (Piazza et al 2005), and providing habitat for a wide array of finfish and invertebrates, (Coen et al 2007, Peterson et al 2003, Harding and Mann 2001). While states to our south have long recognized the importance of wild reefs and have been working to restore natural oyster populations for decades, Massachusetts has not developed any significant restoration programs, with state regulators instead focusing on management of aquaculture shellfish. This project fills a longstanding need for shellfish restoration research in Massachusetts.

The **goal** of this project was to restore an oyster reef on tidal flats owned by Mass Audubon off Lieutenant Island. Our **objectives** were to catch a natural set of oysters, monitor the growth and survival of the oysters to determine which of three treatments (shell culch, Reef Balls, or Oyster Castles) worked best for catching and growing wild oysters, survey other organisms present, monitor the oysters for disease, and involve the community at all steps of the process.

Our measures of success include:

1) High density and survival of oysters growing on the reef. A successful reef will have multiple generations of oysters growing together, ideally covering all surfaces of the reef materials.
2) Increased diversity and abundance of invertebrates, fish, and birds at the restoration site relative to two control sites.
3) A clear permitting track for future shellfish restoration projects in MA.

Because this restoration is the first of its kind in New England, the lessons learned from our research will inform both the expansion of this project as well as any future oyster restorations in terms of:

- The best materials to use for attracting a set of oysters
  - Which materials survive winter conditions and sedimentation better
  - Which type of culch grows oysters better
- The best way to arrange those materials to ensure long-term survival of the reef
- How biodiversity changes with reef development compared to reference sites
- Levels of two common oyster diseases on the wild reef
- And, in 2012 and beyond, whether harvest is compatible with maintaining a self-sustaining reef.

II. Methods

Restoration involved deploying three common oyster growth substrates (culch, Reef Balls, Oyster Castles) in a nine-block experimental design with treatments randomly assigned within each block. The blocks are 60 feet long with 20 foot sub-blocks containing each treatment. A map of the layout of the blocks at the site is included with supporting materials below.

Both Reef Balls and Oyster castles are made from special concrete mixes pH balanced to mimic natural reef materials. Reef Balls were purchased from Reef Innovations, Inc of St. Cloud, Florida, through the Reef Ball Foundation (reefball.org). Oyster Castles were provided at no cost by Allied Concrete of Charlottesville, Virginia. Shell culch was a mix of purchased surf clam shells and a small amount of oyster shell provided by Shellfish Promotion and Tasting (SPAT), organizers of Wellfleet OysterFest.

Monitoring Activities

i. Oyster abundance, survival, and demography: Oyster abundance, survival, and demography are being measured annually in the spring to assess winter survival and again in the fall after recruitment. The number of individual oysters is counted in a 0.25 m^2 quadrat placed on the culch, and total oysters are counted on a subset of the Reef Balls and Oyster Castles in each replicate block. The size distribution of oysters is estimated by measuring the length of 25 individual oysters within the 0.25 m^2 quadrat or on an individual reef ball or castle to the

nearest mm. To facilitate comparisons between treatments with very different shapes, arrangements, and surface areas, we converted the average counts of oysters on the balls and castles into the theoretical density of oysters possible based on how many Reef Balls and Oyster Castles could fit into a square meter.

ii. Biodiversity:

a. Organisms on the surface of the reef and the sediment. The biological community that develops in the restored oyster reef is being compared to a nearby bare tidal flat of similar sediment and elevation ("sandy reference area") and to an existing natural oyster bed ("rocky reference area"). The method uses 20 randomly placed 1 m^2 quadrats within which a smaller 0.25m^2 quadrat is nested. The percent cover of sessile organisms on the surface of the sediment or reef (e.g., barnacles, oysters, and macroalgae) are recorded within each 1 m^2 quadrat using the Braun-Blanquet cover classes. The number of individuals of mobile fauna (e.g., crabs, snails) and sessile organisms (not counting encrusting macroalgae) within nested 0.25 m^2 quadrat is also recorded. The sampling is carried out mid-summer, when most organisms are active.

b. Infauna. The abundance of infauna in the reef and reference areas is surveyed using 20 randomly placed cores (10 cm diameter, depth of 20 cm) in the restoration area and the two reference areas. Sampling is done concurrently with the surface quadrats described above. These cores were used to characterize the sediment characteristics of the sites in year one. To assess micro scale variation in invertebrate abundance related to the presence of the restoration substrates, in 2010 cores were also taken at three locations at each treatment: within the treatment, the outside edge, and three feet outside the treatment. The cores are brought back to the lab and sieved through a 0.5 mm screen. The organisms are then picked out and preserved in alcohol for later identification by a taxonomic expert.

c. Nekton. Various methods were tested for quantifying fish use of the restoration area, including throw traps, minnow traps, snorkeling and beach seines. Lift-netting was the most promising method, and a list of fish species on the reef and adjacent unrestored area was generated with this method in 2011.

d. Birds. During April and May and again from July through October, an observer with a spotting scope counted birds in the restoration area and the nearby sandy reference area for ten minutes at low tide on each sample day. During the time that diving ducks are present (November through May), counts were made at high tide. The observer stays far enough away from each area so as not to disturb the birds, but close enough to allow identification.

iii. Sedimentation: We anticipated that the restored reef would act as a sediment trap. We will therefore examine the effect of the restored reef on sedimentation and the types of sediments. The particle size distribution was determined in year one by collecting sediment cores (see 4b above), separating the sediments through a series of sieves into different particle size groups, and then drying and weighing each group. The sediments will be characterized again after three years of oyster growth to determine the effect of the restoration on the sediments. A series of rebar sections were installed in 2009 as references to monitor any accumulation or loss of sediments over time.

iv. Oyster Diseases: We examine the oysters in our reef for the presence of Dermo and MSX annually. This aspect of the restoration is under the supervision of Dr. Roxanna Smolowitz of Roger Williams University. A minimum of 75 randomly selected individual oysters were collected once a year and examined for the diseases. Dermo testing has been carried out by staff and volunteers in the Wellfleet Bay Wildlife Sanctuary lab. For MSX, samples are sent out for histopathology testing by Dr. Smolowitz.

III. Results

Summary of Key Results

A population of between 60,000 and 250,000 oysters has been restored to the project area since the materials were placed in 2009. A clear winner emerged from the three restoration substrates tested - the Oyster Castles were the only substrate to maintain their structural integrity and to show a net increase in their oyster population each year. Invertebrate abundance and diversity has measurably increased on the project site relative to control sites and shorebird

use of the reef area has also increased. American Oystercatchers, a species of management concern in Massachusetts, now regularly feed on blue mussels that are growing at the site as a result of this project. Preliminary data indicate fish may be preferentially using the reef relative to adjacent unrestored areas, including tautog, cunner, killifish, and even squid. While we expect benefits in terms of improved water quality and increased spat recruitment on nearby commercially harvested flats, it is not possible to measure the impact of our project on these factors due to the overwhelming influence of tidal flushing in the area relative to the scale of the project.

Results to date have been communicated to various audiences, including the town of Wellfleet Shellfish Committee, the Cape Cod Natural History Conference, the New England Estuarine Research Society, the International Shellfish Restoration Conference in South Carolina, and the Northeast Aquaculture Conference and Exposition. Our advisory meetings included many who would be the players in any future restoration efforts in Wellfleet and around the Cape. Restoration biologists from multiple states have contacted us about our results, and they are particularly interested in the performance of the Reef Balls and Oyster Castles. Among the most important results of this project is clarification of what had been a thoroughly confusing permitting process for this unprecedented project for the state. There is currently no regulatory allowance for shellfish spawning sanctuaries in Massachusetts – all projects must be opened to harvest after three years under current law, despite strong evidence in the literature that harvest is not compatible with sustainable reefs (e.g. Powers 2009, Schulte et al 2009)). Since the restored reef cannot be completely protected from harvest, we have negotiated an experimental harvest plan to study the effect of different harvest levels (0%, 50%, and 100% of legal oysters) on the reef. Harvest is scheduled for summer of 2012.

A. Oyster Abundance and Survival

The materials were deployed in June of 2009, and by late summer it was clear the reef had caught a heavy set of spat. Fall oyster density surveys showed that roughly 250,000 young oysters were growing on the reef, with the culch having the highest density and abundance of the three treatments heading into the reef's first winter. Spring surveys in 2010 showed high winter mortality, particularly on the culch, reducing the overall number of oysters to around 60,000.

Surprisingly, at least 52% of the Reef Balls had broken apart over the winter, likely due to improper formulation of the concrete for surviving the freezing and thawing typical of a winter on intertidal flats in Massachusetts. None of the Oyster Castles showed any signs of breaking down. Much of the culch was dispersed by the tides or covered in sand over a two year period, burying the growing oysters and negatively affecting survival. The culch always suffered the highest winter mortality, ranging from 66 – 89% each year.

Treatment	2011 Winter Mortality (%)		
	2010	2009	Average
Culch	90	89	89.5
Reef Balls	53	46	49.5
Castles	63	57	60.0

Table 1. Winter mortality of one year-old (2010 class) and two year-old (2009 class) oysters in spring of 2011. High culch mortality is likely due to sand burying the oysters.

Good summer recruitment brought the total number of oysters on the reef close to 200,000 by fall of 2010. As of 2011 the total number of oysters on the reef is around 130,000, with the Oyster Castles having emerged as the clear winner among the three treatments in every respect: total oysters, oyster density, and average size (Table 2). In addition, the castles are the only of the three treatments to show a net increase in oysters each year (Figure 1). A separate experiment begun in 2010 sought to determine whether oyster shell or surf clam shell made the best culch for attracting and maintaining a population of oysters (Table 4). The results showed that surf clam shell caught a higher density of spat in the first year, and maintained its structural integrity better than oyster shell when exposed to tides and storms over several months. By the second year one of the oyster treatments had completely washed away. Densities were similar on the two culch types in 2011, but the surf clam shell held twice as many total oysters.

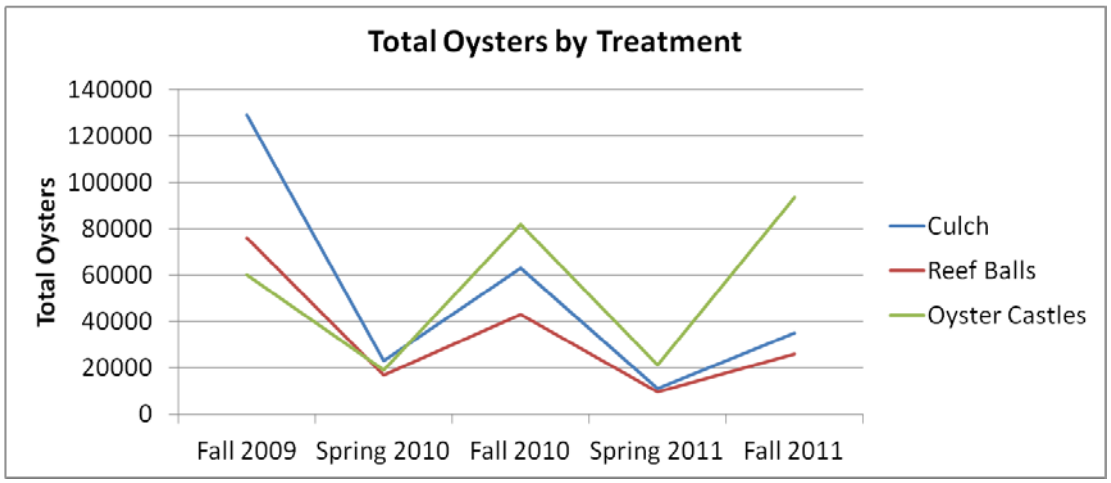


Figure 1. Estimated total oysters on the substrates through time. Winter mortality was heavy in both 2009 and 2010. The oyster castles continue to support the most oysters of the three treatments. Only the castles have shown an increase in total oysters each year.

	Oysters/unit (Actual)		Oysters/Square Meter (Theoretical)	Total Current Oysters	Avg. Size (mm)
	2011 Class	***Older***			
Culch	74/m2	7/m2	81	12000	31
Reef Balls	85/ball	22/ball	428	26000	40
Castles	137/castle	39/castle	1584	93000	45
			TOTAL:	**131000**	

Table 2. Density and abundance of oysters by treatment, fall 2011. Theoretical densities were calculated to allow comparisons among the treatments, and reflect the density of oysters likely if the maximum number of reef balls and oyster castles were placed per square meter. Oysters over 55mm were classified as "older" and represent the 2010 and 2009 year classes combined.

	Oysters/unit			
Treatment	**2010**	**2009**	**TOTAL**	**Total Current**
Culch	12.5	4.5	17/ m^2	11,016
Reef Balls	21	18	39/ball	9,477
Castles	26	14	40/castle	21,240
				TOTAL: 41733

Table 3. Density and abundance of one year-old (2010 class) and two year-old (2009 class) oysters in spring of 2011, illustrating the greatly reduced population typical of the spring counts. Units are oysters per square meter, ball, or castle.

	% Dead	Density Live Oysters	Total Live Oysters
Oyster Shell	0.69	440	3212
Surf Clam Shell	0.66	448	6541

Table 4. Density and abundance of oysters as a function of culch type. Pure treatments of oyster shell and surf clam shell culch were placed in the restoration area in 2010 to determine which culch type catches a better set of young oysters. After one year, densities of live oysters growing on the shells were similar, though the total number of oysters remaining was much higher on the surf clam piles, likely because the heavier shells of the surf clams better resisted being washed away by the tidal currents.

B. Biodiversity

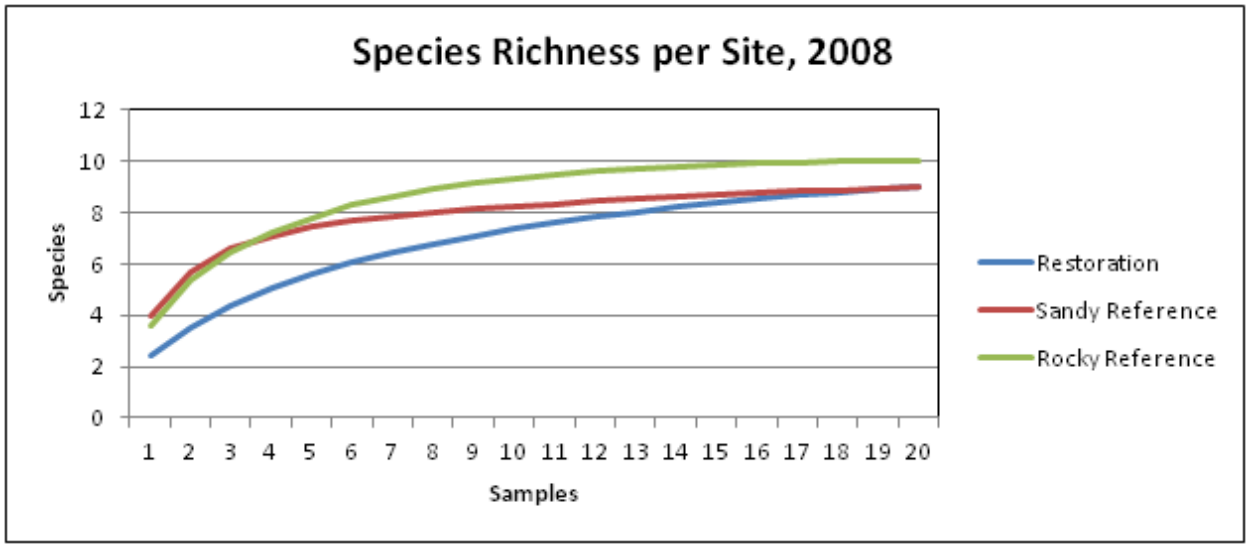
Surface Fauna

The number of organisms counted in the surface quadrats increased in the restoration area relative to the two reference areas between 2008 and 2011. Species richness was examined using resample-based species accumulation curves showing the number of species as a function of the number of samples in each area (Figure 2). In 2008, the existing oyster flat ("rocky reference area") had the highest species richness of the three areas, but by 2011 the restoration area had the highest richness. Much of the surface fauna data are still being analyzed.

Infauna

Many samples have yet to be identified to species, but the number of animals per core was quantified for the restoration area and the two reference areas. Between 2008 and 2009, the number of invertebrates per core increased in the restoration area relative to the reference areas (Figure 3). The rocky reference area had the highest number of organisms per core in 2008, but the number of organisms counted in the restoration area increased dramatically in the year following the start of the project. Cores were also taken in three locations relative to each treatment: within the treatment, at the outer edge, and 3 feet outside the treatment. The goal was to look for small scale patterns in benthic invertebrate abundance that reflected the influence of the reef materials. We had noticed that polychaetes were often easy to find both at the edges of treatments and among the culch, and this sampling scheme sought to quantify these patterns. More species per core were noted in the edge samples and in the culch samples. Average species per core was higher inside the treatments than in a bare sandy area three feet outside the treatment.

a.



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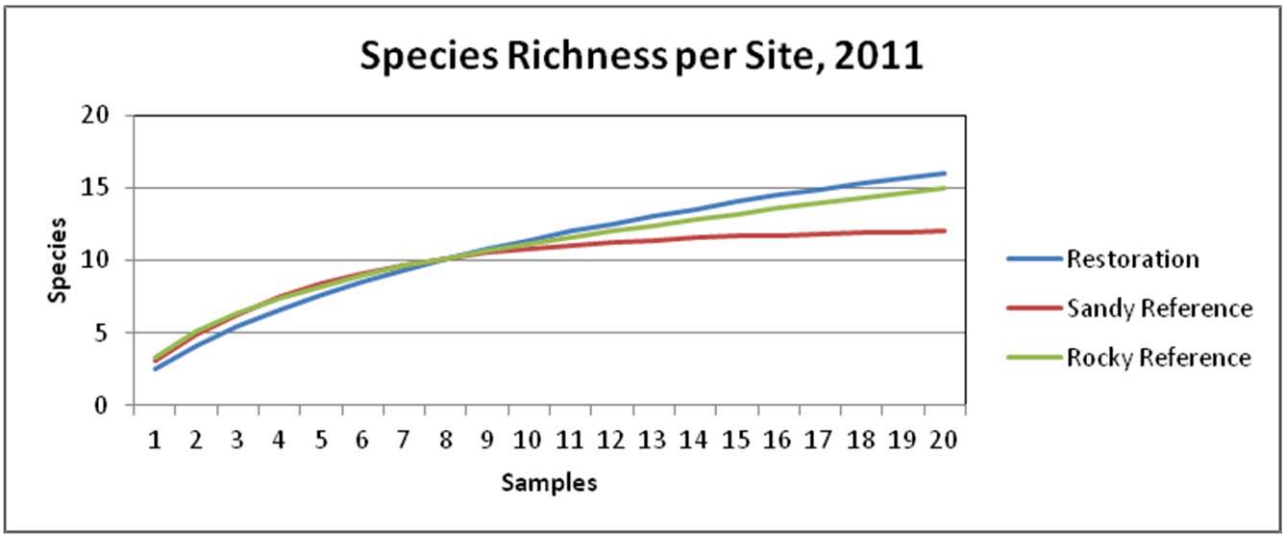
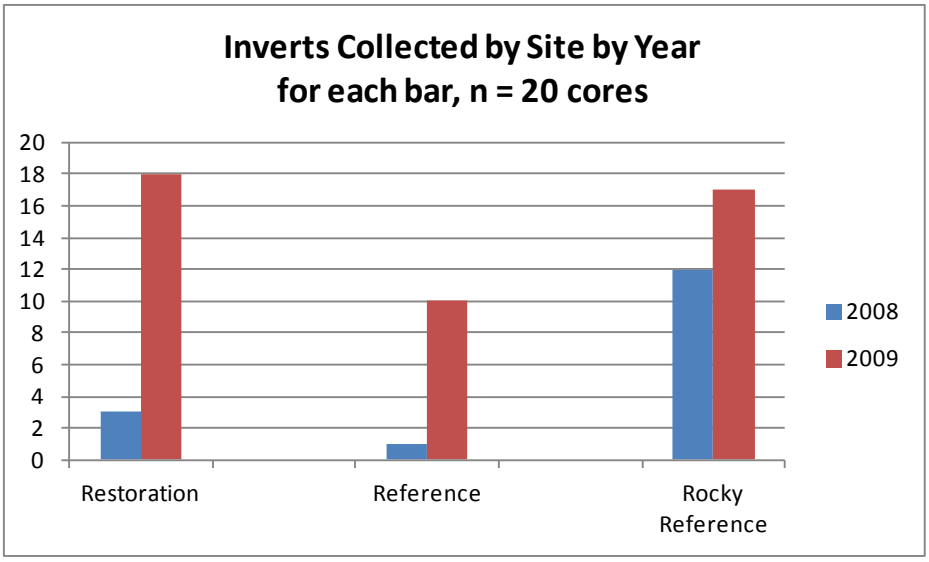


Figure 2. Biodiversity of surface fauna. Species richness presented in the form of accumulation curves (number of species of surface organisms as a function of the number of quadrats sampled) for the three survey areas in a) 2008 and b) 2011.

a.



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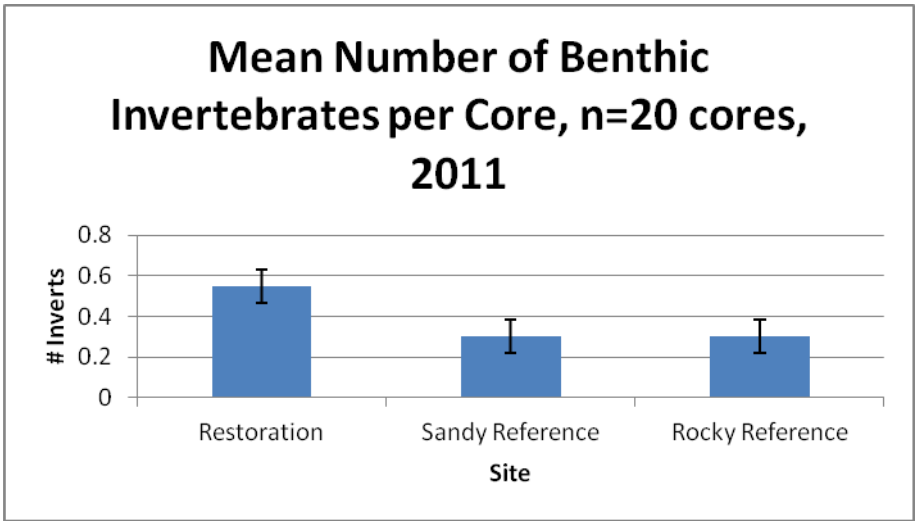

Figure 3. Mean (+1 SD) individuals of benthic invertebrates per sediment core increased in the restoration area relative to control sites between 2008 and 2009 (a), and were higher than in both reference areas in 2011 (b).

	Sample Location			
Treatment	Within	Edge	Outside	Average
Balls	1.67	1.33	0.89	1.30
Castles	1.22	1.22	1.33	1.26
Culch	2.33	3.00	1.56	2.30
Average	1.74	1.85	1.26	

Table 5. Mean invertebrate species per sample for cores taken at three locations at each treatment: within the treatment, the outside edge of the treatment, and three feet outside the treatment. The culch averaged more species per core than the other treatments, and more species per sample were found in the edge samples compared with the other locations.

Birds

Shorebird use of the restoration area increased noticeably over the course of the project. Ruddy turnstones, a species favoring hard substrates and loose shells, are often seen feeding directly on the reef. Willets and gulls feed routinely in the small pools that developed on the north side of the treatment blocks. At least two pairs of American oystercatchers, which nest nearby, feed daily during the summer on the dense concentrations of blue mussels that are growing on the site as a result of the project. Common eiders, which feed on mussels and crabs, and common loons, which feed on small fish, showed a small increase over the course of the project during the high tide surveys, though sample sizes were small.

	Mean Birds/Visit	
	Common Eider	Common Loon
2008-2009	0.2	0.2
2009-2010	0.4	0.6
2010-2011	0.7	0.7

Table 6. Abundance of two common diving waterfowl likely to benefit from increased crab, fish, and shellfish abundance on the restored reef increased slightly over the course of the project, though sample sizes were always low.

a. Mean Birds per visit	
Reference	Restoration
19.8	42.4

b. Mean Species per visit	
Reference	Restoration
3.2	4.4

Table 7. Mean number of birds (a) and species (b) per visit for shorebird surveys, data combined across years.

Nekton

Lift nets (Rozas 1992, Wenner 1996) were deployed over all three treatment types and the adjacent sandy reference area. Results from the three treatments were combined since the primary goal was comparing the restored reef with an unrestored reference area. Nets consisted of a 68′ by 7′ beach seine stretched around a frame of 10′ pvc pipes. Photos can be seen in the following online gallery: Lift net fish sampling, Summer 2011. There were few tides during the summer allowing sufficient time to use this method, so sampling opportunities were limited. Multiple attempts failed when strong tidal currents dislodged the gear. The results are approximate due to the resulting low and uneven sample sizes (more samples were collected over the reef), but they suggest that more fish, in terms of both species and individuals, may use the reef compared with an unrestored sand flat nearby.

	Species/Sample	Individuals/Sample
Restoration (n=5)	3.8	821
Reference (n=2)	2.5	621

Table 8. Number of species and individual nekton per sample collected using lift-nets over the restored reef and an adjacent unrestored sand flat.

C. Sedimentation

Nine sediment monitoring stations (locations can be seen in Figure 5) were checked periodically to measure the sand level relative to when the stations were installed in 2009. PVC monitoring stations had been installed prior to 2009 but did not survive the winter, so we replaced them with rebar sections marked with a length of rope and a float. Of these, two disappeared by 2010. On average the seven remaining stations showed erosion, with a cumulative loss of 8 cm of sand when data were summed across all stations. However, stratification was strong, with high levels of *accretion* in the more seaward and middle stations adjacent to the restoration area, and strong *erosion* in the

more landward three stations. The accretion in the lower stations is consistent with our observations of accumulating sand on the experimental treatments.

	Restoration	Sandy
Alewife	96	
Atlantic	3827	1223
Striped Killifish	102	1
Winter	1	
Cunner (juv)	6	
Butterfish	39	
Shore Shrimp	++	++
Sand Shrimp	++	++
Squid	4	
Sea Robin (juv)	1	
Northern	5	4
Tautog (juv)	1	
Bay Anchovy	1	

Table 9. List of nekton species trapped during lift net sampling in 2011. Shrimp were abundant in all samples but were not counted. Uneven sample sizes prevent statistical comparison between treatment and control, but the list of species found on the restored reef and reference area is presented for general interest.

STATION	**Change**
Deep3	28
Deep2	4
Deep1	20
Mid3	25
Mid2	5
Mid1	lost
Shallow3	-80
Shallow2	-10
Shallow1	lost

Table 10. Sediment monitoring stations and the change in sand level since fall 2009. Deep stations are closer to the restoration area, while shallow stations are closer to land (see Figure 6).

D. Disease

Dermo

A sample of roughly 76 oysters from the restored reef was tested in each year using Thioglycollate methods (Ray 1952). Oyster tissue was sampled, incubated in a sterilized growth medium for a week, then examined under a microscope for presence of the disease organism. Upper and lower refer to location in the restoration area, with upper referring to the three most landward treatments and lower referring to the three most seaward treatments. Results were similar in 2009 and 2010 – less than half of the animals showed infection, and infection intensities were always low as indicated by few disease spores per slide.

	Upper	Lower
Intensity	0.00	0.00
Prevalence	0.42	0.39
# infected	16	15

Table 11. Results from Dermo testing of oysters from the upper and lower reef in 2011. Results indicated about 40% of the animals showed infection (prevalence), and the infection intensities were very low as indicated by few spores noted per sample (intensity). Infections this light are believed to be well below the mortality threshold for this disease. Results in other years were similar.

MSX

No MSX was detected by Dr. Roxanna Smolowitz in 164 animals collected from the restoration site in 2010 and 2011. See Appendix A. Methods are described in Kim et al (2006).

IV. Summary of Community Involvement and Outreach Activities

- We convened Project Advisory Committee 3-4 times during project development in 2008-2009 to discuss Experimental Design prior to submittal to Division of Marine Fisheries. These meetings included regular attendance by 7-8 individuals of the Wellfleet shellfishing community, who are members of the Committee and helped to develop the consensus-based Experimental Design, which was approved by the Division of Marine Fisheries. Discussions included pros and cons of various experimental substrates.
- Each October we had a table at the Wellfleet OysterFest, with over 20 - 30,000 people estimated to attend over these two day annual events celebrating the

Wellfleet Oyster and the community of fishermen who harvest them. We also presented lectures about the project at two of the last three OysterFests.

- Presentation by Mark Faherty at annual New England Estuarine Research Society meeting in Provincetown, MA in November 2010.
- Mark presented a poster at the International Shellfish Restoration Conference in Charleston, South Carolina in November 2010.
- Mark presented at the Northeast Aquaculture Conference and Expo in Plymouth, MA
- Mark Faherty and Boze Hancock of TNC presented at a panel discussion at the Mass Association of Conservation Commissions meeting at The College of the Holy Cross in Worcester on March 5.
- Mark presented a talk on the project at the South Shore Natural Science Center in Norwell, MA. The audience included many interested in restoring oysters in the North River estuary and Duxbury Bay.
- Boze Hancock gave a public lecture on oyster restoration at Wellfleet Bay Wildlife Sanctuary in August 2008 discussing the historical extent of oyster reefs, the reasons for their disappearance, quantitative data on the ecological and economic services the reefs provide, and examples of successful restorations in other states, ultimately linking all of this to the Wellfleet project.
- Our education staff teaches a lesson on oyster reef habitat and oyster reef restoration in Eastham and Wellfleet schools.
- A guided tour of the reef was offered as an auction item during our "Wild Wild Wellfleet" Fundraiser in 2010, and the winning bidders included the publishers of Edible Cape Cod magazine and other local food experts.
- We offered a guided tour for the Lieutenant's Island Homeowners Association. The restoration site is on the west side tidal flats adjacent to some of the homes on the island.
- We continue to offer many public walks to the restoration site in addition to the various speaking engagements at conferences and festivals covered elsewhere in the document.

V. Supporting Materials

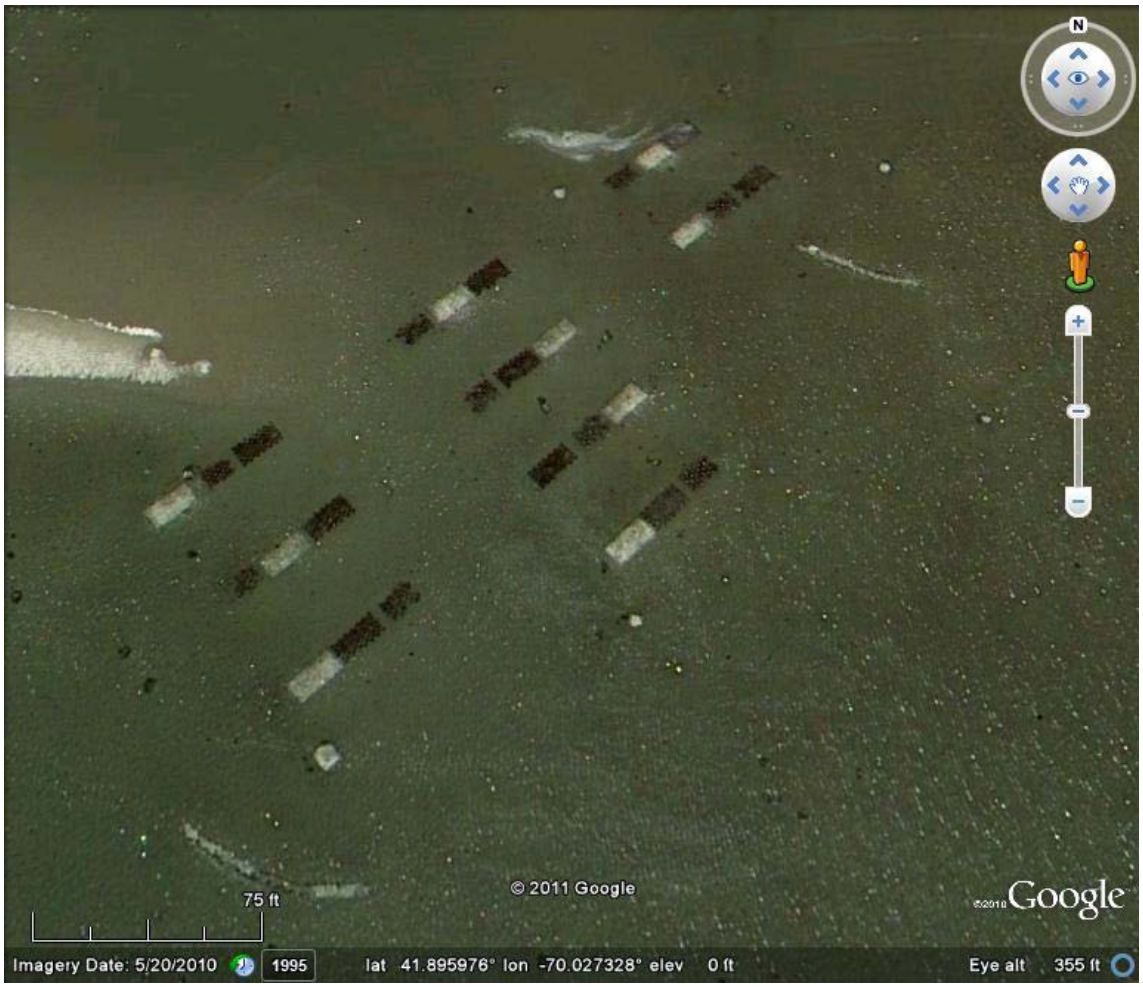

Figure 4. Google Earth image of the restoration area showing the 9 experimental blocks. Culch (white areas), Reef Balls (smaller sub-rectangles, 27 per block), and Oyster Castles can be distinguished in the photo.

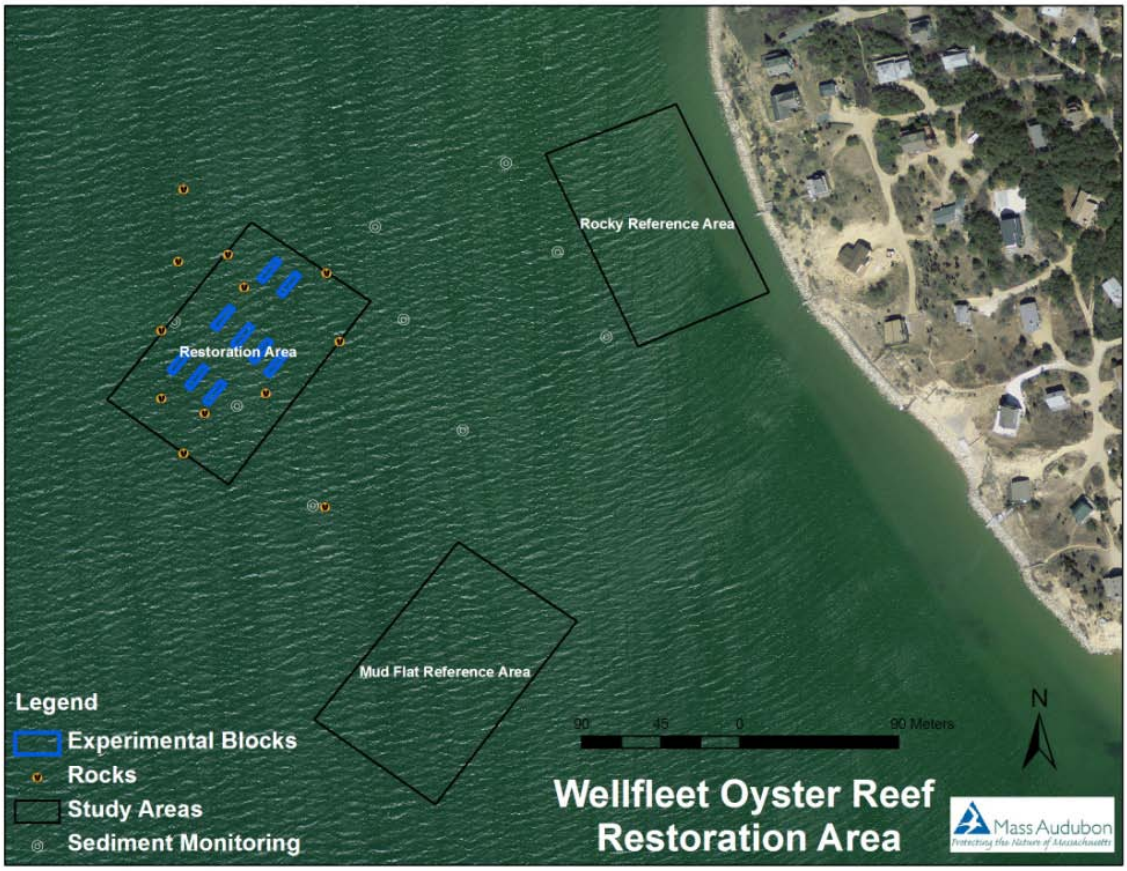

Figure 5. Map of the larger study area showing the restoration area and two reference areas. Biodiversity data was collected in the two reference areas in addition to the restoration area.

Figure 6. Top photo shows a block with the three randomly assigned treatments just after set-up. The bottom photo shows the extensive, oysterless sand flats that dominated the site before restoration.

Figure 7. Top and Bottom: Oyster Castles, the most successful of the three treatments, covered in oysters of multiple ages. October 2011.

Figure 8. Top and Bottom: While most of the culch was dispersed or buried, patches remain that support adult oysters. October 2011.

Figure 9. Top: A Reef Ball covered in oysters. Bottom: Some of the roughly 75% of Reef Balls showing partial to complete disintegration. October 2011.

Earned Media Coverage:

National coverage on NPR's "Living on Earth Series"

10/17/09 Cape Cod Times: Article about Wellfleet Oyster Fest which also mentions the oyster restoration.

11/24/09 Provincetown Banner: Story on the State of Wellfleet Harbor Conference, including our oyster reef talk

Mish Michaels of WBZ-TV Boston visited the project site and the story aired on July 1 during the evening news.

Photo Galleries:

The Reef in October 2011

Lift net fish sampling, Summer 2011

The Reef in October 2010

Post-winter Reef, March 2010

American Oystercatchers on the Reef, 2010

Oysters Growing on Experimental Materials, September 2009

Setting up the Reef, June 2009

Culch Pilot Study Set Up, 2008

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Appendix A. MSX Disease Testing Results, 2011

Submitted by Dr. Roxanna Smolowitz, Roger Williams University Aquatic Diagnostic Laboratory

Oysters were divided into four groups: upper one-year olds, lower one-year olds, upper two-year olds, lower two-year olds. No evidence of MSX was found, but full official results are included for completeness. Dr. Smolowitz also noted histological evidence of Dermo infections consistent with our lab results presented above.

SHELLFISH PATHOLOGY REPORT
RWU Case No. 3717
Date of Report: Dec. 22, 2011
Source of Animals: M. Faherty, Audubon Society
Species: Crassostrea virginica
Location of Collection: Wellfleet Bay Wildlife Sanctuary/upper 1 yr olds
Collection Date: 10/12/2011
Collected By: M. Faherty
Date Processed at RWU: 10/12/2011
No. of animals examined grossly: 25
No. of animals examined microscopically: 25
No. of animals examined using Thioglycollate culture techniques: 0

Gross description of animals:

a. Weight: averaged 35.8 g

b. Shell height: averaged 73 mm

c. Gross appearance: recent set spat attached to shell surface (affects weight measurement)

1. Animals processed histologically were:

a. randomly selected from the upper 1 year olds

2. Histological Findings:

a. Parasites:

Group	Parasite Species	Percent infected
Bacteria	Chlamydia/Rickettsia	0
Fungi		0
Protozoa	QPX	0
	Dermo (*Perkinsus* sp.)	24%
	MSX	0
	SSO	0
Metazoa		0
Helminths		0
Arthropoda		0

b. Neoplasia

	Hematopoietic Leukemia	0
	Gonadal Tumor	0
	Other tumor:	0

c. Other histological findings:

6/25 animals showed rare Dermo organisms in tissues of the gill and digestive gland. Associated inflammation is mild. One female shows a probable microsporidial infection (similar findings to those found in *Steinhausia* sp. infections) in the retained eggs in the inflamed gonadal tubules.
Gross and histological Summary:

Dermo identification using histology is less sensitive than methods of detection using Thioglycollate culture techniques. Microsporidial infections of oyster eggs is considered an incidental finding.

Comments:

Only mild Dermo infections were noted histologically. No MSX infections were identified.

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SHELLFISH PATHOLOGY REPORT
RWU Case No. 3718
Date of Report: Dec. 22, 2011
Source of Animals: M. Faherty, Audubon Society
Species: Crassostrea virginica
Location of Collection: Wellfleet Bay Wildlife Sanctuary, lower 1 year olds
Collection Date: 10/12/2011
Collected By: M. Faherty
Date Processed at RWU: 10/12/2011
No. of animals examined grossly: 23
No. of animals examined microscopically: 23
No. of animals examined using Thioglycollate culture techniques: 0

Gross description of animals:

a. Weight: averaged 38.2 g

b. Shell height: averaged 65.5mm

c. Gross appearance: Many show new spat cemented to the surface of the animals examined

Histological Evaluation:

1. Animals processed histologically were:

a. randomly selected from the lower 1 year olds

2. Histological Findings:

a. Parasites:

Group	Parasite Species	Percent infected
Bacteria	Chlamydia/Rickettsia	0
Fungi		0
Protozoa	QPX	0
	Dermo (*Perkinsus* sp.)	9%
	MSX	0
	SSO	0
Metazoan		0
Helminths (polycheate)		4%
Arthropoda		0

b. Neoplasia

	Hematopoietic Leukemia	0

Gonadal Tumor	0
Other tumor:	0

c. Other histological findings:

2/23 animal show very low Dermo infections with very mild associated inflammation. One animal shows a cross section of a turbellarian-like organism in the stomach lumen. One animal shows a focal pocket of inflammation associated with a possible section of a polycheate in the area between the shell and mantle.

Gross and histological Summary:

Dermo identification using histology is less sensitive than methods of detection using Thioglycollate culture techniques. Turbellarian infestation in the lumen of the stomach are incidental finding when only 1 or 2 are identified in histology sections. The occurrence of a pocket of inflammation between the mantle and shell associated with a polycheate worm indicates the probable polycheate borrowed through the shell into the underlying tissue. This is rare, but does occasionally happen. Usually the oyster "walls off" the intrusion into its soft tissues and in most cases is not severely affected. Polycheate (mud worm) infestations of oyster shells are common.

Comments:

Only mild Dermo infections were noted histologically. No MSX infections were identified.

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SHELLFISH PATHOLOGY REPORT
RWU Case No. 3719
Date of Report: Dec. 22, 2011
Source of Animals: M. Faherty, Audubon Society
Species: Crassostrea virginica
Location of Collection: Wellfleet Bay Wildlife Sanctuary, upper 2 year olds
Collection Date: 10/12/2011
Collected By: M. Faherty
Date Processed at RWU: 10/12/2011
No. of animals examined grossly: 19
No. of animals examined microscopically: 19
No. of animals examined using Thioglycollate culture techniques: 0

Gross description of animals:

a. Weight: averaged 62.1 g

b. Shell height: averaged 94.3 mm

c. Gross appearance: Many show new spat cemented to the surface of the animals examined

Histological Evaluation:

1. Animals processed histologically were:

a. randomly selected from the upper 2 year old population

2. Histological Findings:

a. Parasites:

Group	Parasite Species	Percent infected
Bacteria	Chlamydia/Rickettsia	0
Fungi		0

Protozoa	QPX	0
	Dermo (*Perkinsus* sp.)	10.5%
	MSX	0
	SSO	5%
Metazoa		0
Helminths (polycheate)		
Arthropoda		0

b. Neoplasia

Hematopoietic Leukemia	0
Gonadal Tumor	0
Other tumor:	0

c. Other histological findings:

2/19 animals were noted to contain low levels of dermo in gill and the body. 1/19 contained one SSO-like organisms in the gill connective tissues
1/19 animals showed multifocal infestation with Sphenophyra-like protozoans causing hyperplasia and mild necrosis of associated gill water tubular epithelium
1/19 animals showed a turbellarian flatworm cross-section in the gastric lumen
2/19 showed multifocal inflammation of digestive gland ducts but no cause was noted.

Gross and histological Summary:

Dermo identification using histology is less sensitive than methods of detection using Thioglycollate culture techniques. It is likely that the 2 animals that showed inflammation of the digestive gland were also infected by Dermo organisms that were

not seen histologically. Turbellarian infestation in the lumen of the stomach are incidental finding when only 1 or 2 are identified in histology sections. Splenophra-like (ciliate) infestation of the gills is not uncommon, but hyperplasia to the extent seen in this animal in association with that infestation is uncommon. This finding suggests that more organisms were present in life and that they were washed out of the tubules during processing (they are not firmly attached to the epithelium). Because only one animal appeared affected by the Splenophra ciliates and the areas of infestation were not numerous, this is considered an incidental finding. One SSO-like organism was identified in one animal. This is an extremely low level infection. SSO is not directly infective and so this infection may not become a problem in following years. But the population should be closely watched for any mortality in the coming May/June time period. The organisms are only abundant and cause mortality if there are many spread to the oysters from the other host (no one knows what that other host may be at this time).

Comments:

Only mild Dermo infections were noted histologically. No MSX infections were identified. One SSO infected animal was identified.

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SHELLFISH PATHOLOGY REPORT
RWU Case No. 3720
Date of Report: Dec. 22, 2011
Source of Animals: M. Faherty, Audubon Society
Species: Crassostrea virginica
Location of Collection: Wellfleet Bay Wildlife Sanctuary, lower 2 year olds
Collection Date: 10/12/2011
Collected By: M. Faherty
Date Processed at RWU: 10/12/2011
No. of animals examined grossly: 25
No. of animals examined microscopically: 25

No. of animals examined using Thioglycollate culture techniques: 0

Gross description of animals:

a. Weight: averaged 52.3 g

b. Shell height: averaged 79.8 mm

c. Gross appearance: Many show new spat cemented to the surface of the animals examined

Histological Evaluation:

1. Animals processed histologically were:

a. randomly selected from the lower 2 year olds

2. Histological Findings:

a. Parasites:

Group	Parasite Species	Percent infected
Bacteria	Chlamydia/Rickettsia	0
Fungi		0
Protozoa	QPX	0
	Dermo (*Perkinsus* sp.)	20%
	MSX	0
	SSO	0
Metazoan		0

Helminths (polycheate)

Arthropoda	0

b. Neoplasia

Hematopoietic Leukemia	0
Gonadal Tumor	0
Other tumor:	0

c. Other histological findings:

5/25 animals show Dermo infections histologically. An additional 4/25 animals show inflammation that is suggestive of Dermo infections, but no organisms were identified in these animals histologically. 2/25 Show mild inflammation in the gonadal tubules with low numbers of degenerative eggs (no *Steinhausia* sp. organisms were noted in these animals).

Gross and histological Summary:
Dermo identification using histology is less sensitive than methods of detection using Thioglycollate culture techniques. Mild degeneration of retained eggs were noted in the gonadal tubules. Early forms of *Steinhausia* sp. are often not identified, but degeneration of eggs in the gonadal tubule lumens post-spawning can be unrelated to *Steinhausia* sp. infection.

Comments:
Only mild Dermo infections were noted histologically. No MSX or SSO infections were identified.

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