Galveston Bay Foundation Oyster Shell Recycling Program Phase 4: Sun Curing Research & Collaboration

GLO Contract No. 22-045-005-D102

FINAL REPORT
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I. Project Summary

To reestablish hard substrate in Galveston Bay, the Galveston Bay Foundation (GBF) partners with restaurants to collect shucked oyster shells. The shells are transported in recycling receptacles by GBF staff to upland storage sites where they are stockpiled and sun-cured for a minimum of six months. The recycled shells are then returned to the bay through shoreline protection projects, reef creation projects and reef enhancement initiatives such as volunteer oyster gardening.

During CMP Grant Cycle 26, 160 tons of oyster shells were recycled through GBF's Oyster Shell Recycling Program (OSRP). The collection of these shells was conducted in Galveston from January 2022 through August 2022 and in the Houston and Clear Lake regions from February 2022 through August 2022. During this time, all recycled oyster shell was stockpiled at one of two curing sites: Red Bluff or Moody Gardens. The shells will continue to be stored at the respective curing sites where they will be turned intermittently to allow for proper sun curing before being returned to the Bay. The shells will be utilized in GBF's Volunteer Oyster Gardening efforts or returned to Galveston Bay through (separately funded) oyster reef restoration projects.

CMP Grant Cycle 26 funding allowed GBF to continue the expansion of the shell recycling operations to the inner loop of Houston as well as on Galveston Island. Due to the expansion effort that began in 2021, GBF has continued to increase shell recycling capacity which resulted in another record-breaking year. A total of 242 tons of oyster shell was recycled in 2022, which is the most oyster shell recycled in a single year since the OSRP began in 2011!

The CMP Grant Cycle 26 also funded the 2022 oyster gardening season during which 126 volunteers, managing a single garden or multiple gardens, monitored and cared for their gardens throughout the summer and early fall to promote successful oyster recruitment and growth on the recycled shell. As a result, approximately 7,555 oysters were recruited in the volunteers' 410 gardens. These new oysters were introduced onto restoration reefs in October and November 2022 under separate grant funding.

During Cycle 26, GBF partnered with the Environmental Institute of Houston at the University of Houston-Clear Lake (UHCL) and the Honors College at the University of Houston (UH) to determine the sun curing time needed to reduce the risk of infecting native oysters with the parasite *Perkinsus marinus*, otherwise known as "Dermo" which is a spore forming protozoan that impacts the fitness and longevity of Eastern Oysters, while not delaying the use of the material in reef restoration projects. Oysters were placed in either the interior or on top of sun-curing piles and co-located with temperature and relative humidity sensors. Half of the oysters deployed in sun-curing piles were sampled for Dermo via the standard fluid thioglycollate method. The other half were monitored for tissue decomposition. All oysters were surveyed for eight months, and Dermo intensity decreased by more than 80% following the first week of deployment at the curing site, however, presence of Dermo persisted the entire study period.

II. Background Information

Oyster reefs are a vital component of a healthy estuary. Oysters filter contaminants from the water, protect shorelines, stabilize sediment, and provide habitat and food sources for other aquatic species. Unfortunately, oyster reefs are the most threatened marine habitat worldwide. Studies show that over 85% of oyster habitat has been lost on a global scale (Beck et al, 2011). In Galveston Bay, over 60% of the oyster reefs have been damaged, primarily due to decades of heavy exploitation combined with multiple storm events, particularly Hurricanes Ike and Harvey (Hons and Robinson, 2010). Prior to 2008, Galveston Bay yielded 90% of the oyster harvest in Texas (Haby et al, 2009). However, the severe sediment deposition resulting from Hurricane Ike smothered oyster reefs across the bay system and eliminated a large portion of the hard substrate required for oyster development.

To help replenish hard substrate in the bay and support oyster reef restoration efforts, GBF partnered with local restaurant owner Tom Tollett of Tommy's Restaurant and Oyster Bar in 2011 and began recycling oyster shells. Before GBF's Oyster Shell Recycling Program began, oyster shells were discarded along with other restaurant waste and sent to a landfill. To avoid the disposal of this vital resource, GBF has established partnerships with local restaurants and research partners to collect their shucked oyster shell. The reclaimed shell is returned to Galveston Bay to serve as new oyster habitat, thus enhancing the local oyster populations.

With the assistance of CMP funding, GBF has expanded the OSRP from the pilot stage (Phase 1) with only one shell recycling partner through an initial expansion phase (Phase 2). During the evaluation phase (Phase 3), a Strategic Development Plan (SDP) was created with the goal of assessing alternative recycling methods to achieve a more sustainable program. The SDP led GBF to expand shell recycling operations to the inner loop of Houston to increase the volume of shell recycled. The second expansion phase (Phase 4) was initiated with the purchase of new recycling equipment (the dump truck) in the spring of 2021 followed by the first shell collection in the inner loop of Houston in May 2021. Phase 4 continued into Cycle 26 with an additional seven recycling partners added to the OSRP. As of August 2022, GBF has secured a total of 29 active shell recycling partners.

With the expansion of GBF's shell recycling operations, the amount of shell collected and stockpiled is rapidly increasing. Therefore, GBF initiated a research partnership with (UHCL) and (UH) to ensure proper sun curing methods are in place before returning recycled shell back into Galveston Bay. There are currently no standardized curing procedures for restoration efforts to follow, however many recycling groups sun-cure oyster shells for up to six months. During Cycle 26, UHCL and UH conducted a study to help determine the sun curing time needed to reduce the risk of infecting native oysters with a parasite known as Dermo, while not delaying the use of the material in reef restoration projects.

III. Project Implementation

A) Task 1: Oyster Shell Collection and Curing Site Management

A.1 Oyster Shell Recycling Metrics

A total of 160 tons of recycled oyster shells were collected with Cycle 26 funds. This shell was collected in Galveston from January 2022 through August 2022 and in the Houston and Clear Lake regions from February 2022 through August 2022. During this time, GBF and Moody Gardens staff collected oyster shell from a total of 29 shell recycling partners on a weekly basis to relieve them of their shell waste. GBF staff collected shell from recycling partners in the Clear Lake and Houston region while Moody Gardens staff collected shell from recycling partners in the Galveston region.

Throughout the week, restaurant/lab staff deposited shucked oyster shells in recycling receptacles. GBF and Moody Gardens staff transported the containers of shell to one of two curing sites where all shell was stockpiled for future use in reef restoration efforts. GBF and Moody Gardens staff followed the Sun Curing Protocol established in CMP Cycle 24 to ensure all recycled shell will be fully sun-cured prior to being returned to Galveston Bay.

The expansion of the OSRP began in 2021 (under Cycle 24) with the purchase of a heavy-duty truck equipped with a dump bed and bin lift (the dump truck). The dump truck facilitated the expansion of GBF's shell recycling operations to the inner loop of Houston and allowed for the addition of more restaurant partners, growing the OSRP from 10 to 21 recycling partners in one year.

This report captures all shell recycling under Cycle 26 which lasted from January 2022 to August 2022. During Cycle 26, four Houston restaurants and three Galveston restaurants became shell recycling partners. As of August 31, 2022, GBF was actively recycling shell with 29 partners (Table 1). The expansion is ongoing under Cycle 27, with an additional restaurant partner added in Galveston, two restaurants added in Clear Lake, and six restaurants added in Houston as of August 2023.

In 2022, under separate funding, a new trailer was purchased for Moody Gardens staff to utilize to assist with the OSRP operations in Galveston. A second trailer was purchased in 2022, under separate funding, to replace the trailer used by GBF staff for the OSRP operations in Clear Lake.

Please refer to Table 2, Chart 1, and Chart 2 below for the shell collection numbers and associated graphs, as well as Appendix A for a map of active shell recycling partners during Cycle 26. Please note, oyster shell tonnage is based on an average weight of 182 pounds of shell per 32-gallon bin and 30 pounds of shell per five-gallon bucket and is subject to a variance of approximately five percent.

Table 1: Active Oyster Shell Recycling Partners

Shell Recycling Start Date	Shell Recycling Partner	Region
March 2011	Tommy's Restaurant & Oyster Bar	Clear Lake
August 2013	The Aquarium (Kemah)	Clear Lake
November 2013	Crazy Alan's Swamp Shack (Kemah)	Clear Lake
October 2015	Capt. Benny's Seafood (Gulf Freeway)	Houston
June 2016	Tookie's Seafood	Clear Lake
January 2018	BLVD Seafood	Galveston
June 2019	Crazy Alan's Swamp Shack (Baybrook)	Clear Lake
March 2020	Sam's Boat (Seabrook)	Clear Lake
October 2020	Barge 295	Clear Lake
November 2020	Fisherman's Wharf	Galveston
February 2021	Seafood Safety Lab at TAMUG	Galveston
March 2021	Kritikos Grill	Galveston
April 2021	BB's Tex-Orleans (Webster)	Clear Lake
April 2021	Loch Bar	Houston
May 2021	Bludorn	Houston
June 2021	Eunice	Houston
June 2021	La Lucha	Houston
July 2021	BB's Tex-Orleans (Heights)	Houston
July 2021	BB's Tex-Orleans (Upper Kirby)	Houston
July 2021	State of Grace	Houston
November 2021	Fish Tales	Galveston
December 2021	Goode Company Seafood (Westpark)	Houston
January 2022	Gaido's Seafood Restaurant	Galveston
January 2022	Shuck's Tavern & Oyster Bar	Galveston
January 2022	Cajun Greek	Galveston
March 2022	Acme Oyster House	Houston
March 2022	BB's Tex-Orleans (Oak Forest)	Houston
June 2022	Flying Fish	Houston
July 2022	Mambo Seafood (Edgebrook)	Houston

Table 2: Tonnage of Oyster Shells Recycled under Cycle 26

MONTH/YEAR	OYSTER SHELL RECYCLED (TONS)		
	Galveston Region	Clear Lake Region	Houston Region
January 2022	2.74	N/A*	N/A*
February 2022	3.12	9.81	5.92
March 2022	5.02	11.08	8.8
April 2022	5.82	9.92	10.83
May 2022	3.62	8.62	12.1
June 2022	3.78	7.01	10.35
July 2022	3.56	6.96	11.47
August 2022	1.26	6.60	11.38
Total	**28.92**	**60.00**	**70.85**
Grand Total	***159.77 tons of shell recycled from Jan. 2022 - Aug. 2022***		

**Shell recycling for the Clear Lake and Houston Region was conducted under Cycle 25 through January 2022.*

Chart 1: Tonnage of Oyster Shells Recycled under Cycle 26

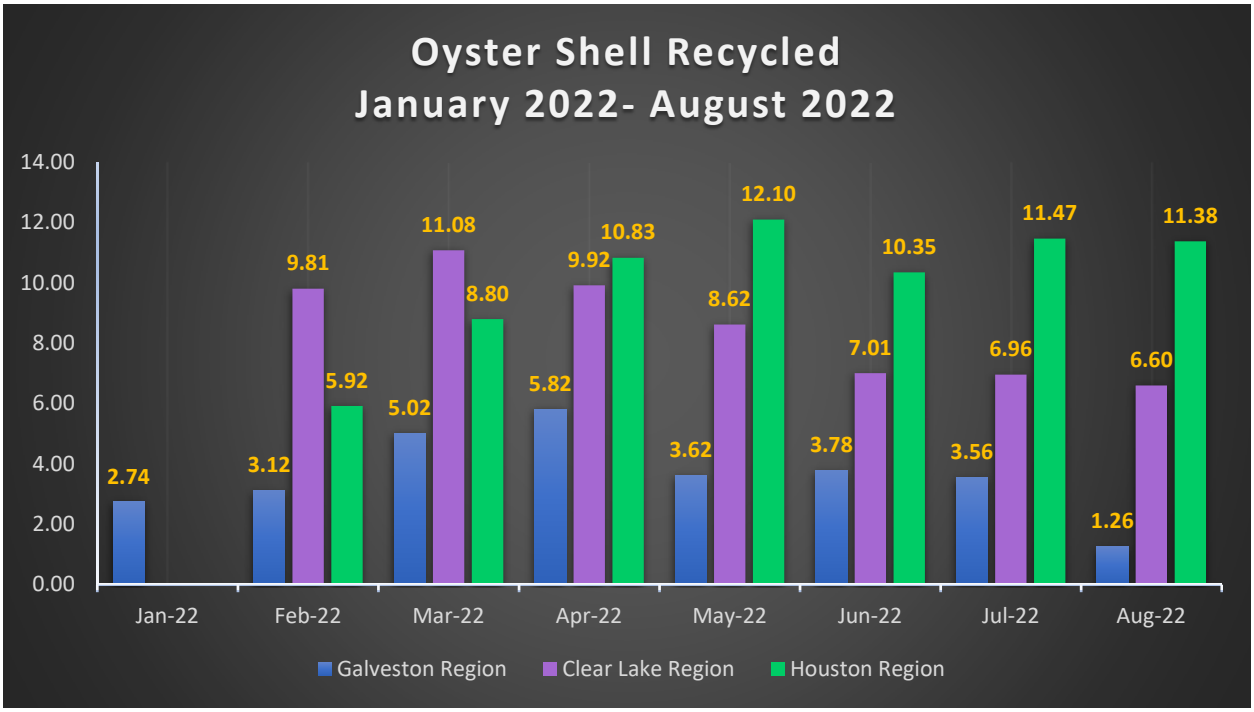
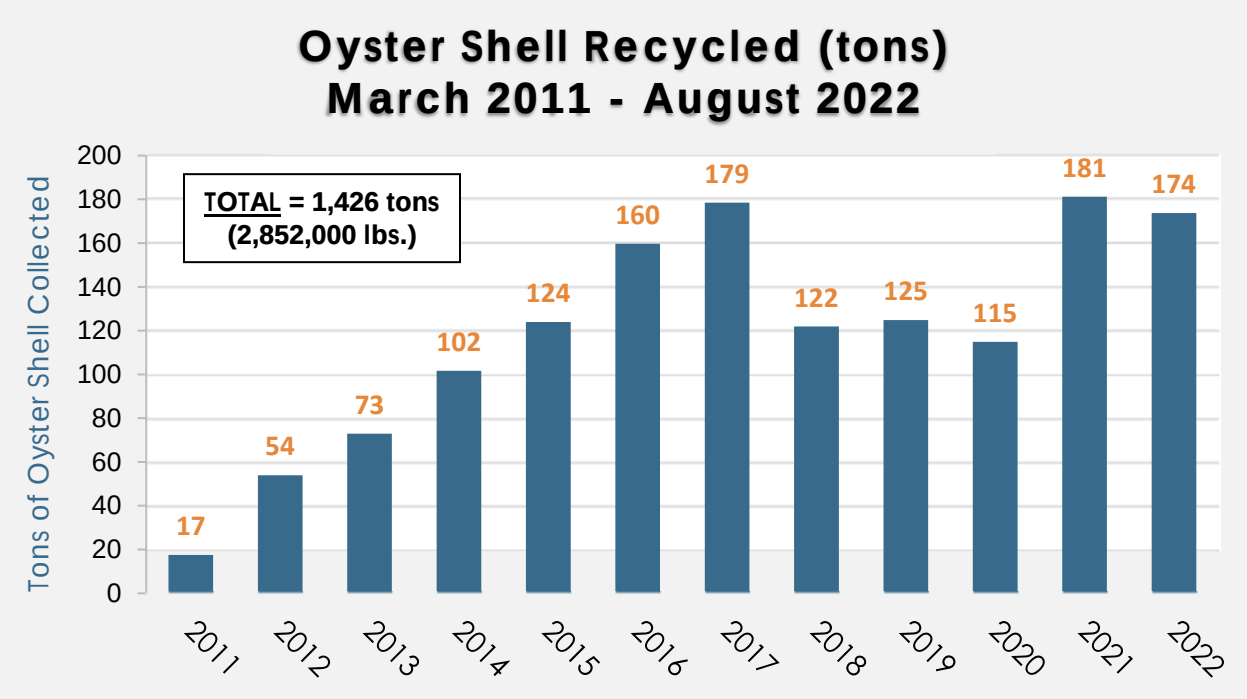


Chart 2: Tonnage of Oyster Shells Recycled to Date



**In 2022, 14 tons were recycled during Cycle 25 and 160 tons were recycled during Cycle 26.*

A.2 Curing site data management, maintenance updates, and photos

To track the amount of oyster shell recycled and where it is stockpiled, GBF and Moody Gardens staff maintain Microsoft Excel spreadsheets in which the date of collection, source of shell (e.g., recycling partner name or special event), amount of shell, curing site name, pile location, and pile rotation is recorded. GBF and Moody Gardens staff also document the date a shell pile is turned during the sun curing process and when cured shell is transported off the curing site property for restoration projects. This allows GBF to maintain an estimate of total shell available for use in reef restoration projects.

During the grant cycle, a total of 160 tons of recycled oyster shell was delivered to two curing sites: Red Bluff and Moody Gardens (Table 3). Due to the close proximity with the Clear Lake and Houston partners, the majority of the shell was deposited at Red Bluff. Red Bluff is also the largest property and can therefore accommodate the largest volume of shell. All shell collected from recycling partners on Galveston Island was delivered to the curing site located on Moody Gardens' property. The Texas City curing site was not utilized during this grant cycle.

Table 3: Tonnage of Shells Delivered to Curing Sites during Cycle 26

Curing Site	Oyster Shell Onsite (Tons)
Red Bluff	130.85
Moody Gardens	28.92
TOTAL	**159.77**

GBF staff developed a Sun Curing Protocol in 2020 to standardize and improve the sun curing process. Shell at different stages of sun curing (Phase 1 – Active Collection; Phase 2 – Curing; Phase 3 – Cured) is kept in individual piles separated by a 10-foot buffer. This allows GBF to track which shell is available for use in restoration projects. To better accomplish this, staff have divided sections of Red Bluff, Texas City, and Moody Gardens to monitor each phase of the curing process more precisely.

During Cycle 26, GBF staff managed and maintained two curing sites for shell storage: Red Bluff and Moody Gardens. No maintenance was required for the Texas City curing site in 2022. Red Bluff is leased from the Port of Houston Authority. Per the lease terms, GBF is responsible for all maintenance and management. As a 1.5-acre property, more time and effort is required to ensure the site meets standards for proper and efficient shell curing. GBF staff performed regular mowing and vegetation management for access, as well as shell turning, moving, and piling to comply with GBF's Sun Curing Protocol. In years past, this site was taken out of circulation during the rainy season. However, continuous improvements now allow for year-round use of Red Bluff. The Moody Gardens curing site requires minimal maintenance due to concrete road access. Moody Gardens staff maintains the shell piles according to GBF's Sun Curing Protocol. GBF staff assists Moody Gardens with maintenance of the shell piles when needed, for example when fully cured shell was relocated to a reef restoration site.

Please refer to Table 4 for the curing site maintenance log and Appendix A for photos of the curing sites.

Table 4: Log of Curing Site Maintenance during Cycle 26

Date	Curing Site	Maintenance Conducted
1/8/2022	Moody Gardens	Cured shell moved from Pile D1 to Pile A2
1/13/2022	Red Bluff	Smoothed out the road base with the tractor along main road of property
4/7/2022	Red Bluff	Cured shell moved from Pile G1 and J1 to Pile E1; Partially cured shell at Pile L1 was rotated
4/8/2022	Moody Gardens	Cured shell moved from Pile B2 to Pile A2
6/22/2022	Red Bluff	Mowed
7/6/2022	Red Bluff	Re-staked and roped off Piles C, E, and F because the t-posts were removed when fully cured shell was hauled off to restoration site
7/16/2022	Moody Gardens	Cured shell moved from Pile C2 to Pile A2
9/8/2022	Moody Gardens	Fully cured shell from Pile A2 was moved to Sweetwater
9/14/2022	Red Bluff	Mowed
9/26/2022	Red Bluff	Sprayed herbicide on property with ATV
10/27/2022	Moody Gardens	Cured shell moved from Pile D2 to Pile A3

B) Task 2: Partnerships and Community Outreach

B.1 Restaurant Database

In 2014, GBF staff created a Restaurant Database to identify all seafood restaurants serving oysters in the Houston, Clear Lake, and Galveston regions. Each year, GBF staff review and update the Restaurant Database to analyze new, potential shell recycling partners. The updated 2022 Restaurant Database is included in Appendix B.

Through analysis of restaurant location and menu items, GBF staff identified six restaurants in the Clear Lake region and five restaurants in the Galveston region as priority future partners to pursue. Based on the Houston list, GBF staff identified six restaurants that are in or near the inner loop. Of these 17 restaurants, two to three have high potential to join the OSRP during Cycle 27. GBF staff initiated communications with Famous Crab, Field & Tides, Jackie's Brickhouse, Opus Bistro & Steakhouse, The Rouxpour, and Valdo's Seafood House but have not been successful with receiving a response. GBF staff will continue to reach out to potential partners in hopes of adding them to the OSRP in the near future. Sammy G's District 70 BBQ & Grill located in Clear Lake is planning on opening in September/October of 2023 and are interested in joining the OSRP once they open.

B.2 Notes from Galveston Bay Oyster Workgroup Meetings and Participant List

GBF staff attended the Galveston Bay Oyster Workgroup meeting which was led by the Texas Parks and Wildlife Department (TPWD) in November 2022. This meeting was assembled to bring together state agencies, universities, engineers, and conservation non-profits to discuss guidance for reef restoration in Galveston Bay, facilitate research, provide updates on current projects, and identify funding and partnership opportunities. Support from these parties benefited not only the OSRP's efforts, but also TPWD's oyster fishery management goals. Such collaboration will result in a more harmonious approach to sustaining the Galveston Bay oyster population both as a fishery and as a vital ecological component of the estuary. The Galveston Bay Oyster Workgroup will meet annually going forward.

Please refer to Appendix B for the notes from the Galveston Bay Oyster Workgroup Meeting. Please note a participant list was not available because the meeting was conducted through Zoom, however a total of 25 attendees were documented.

B.3 Photos of the Houston Oyster Festival

GBF originally planned to launch the expansion of the OSRP in April 2020 with the Inaugural Houston Oyster and Seafest. Due to the COVID-19 pandemic, the festival was postponed in 2020 and again in April 2021. The festival was then rescheduled for June 2022 and took place during Cycle 26. The Houston Oyster and Seafest allowed GBF to reach new and larger audiences in the greater Houston area. A total of 442 people attended the event. All proceeds from the festival benefitted the OSRP and all discarded shells generated at the event were recycled by GBF. It is proposed that this annual festival can provide at least the baseline funding required to sustain minimum shell recycling operations. The second annual Houston Oyster and Seafest occurred in April 2023 during Cycle 27 and the third festival is scheduled for April 2024.

Please refer to Appendix B for photos of the Inaugural Houston Oyster and Seafest.

B.4 List of Presentations, Exhibits/Events, Conferences, etc.

Throughout 2022, GBF conducted nearly all outreach events in person including presentations and boothing activities, one-time shell recycling events, and volunteer oyster garden creation and collection events. Table 5 includes a list of all outreach activities that occurred in 2022.

In addition to these outreach activities, the OSRP received a variety of media exposure throughout 2022. In April, GBF staff and the owner of Tommy's Restaurant and Oyster Bar were interviewed live by a Houston FOX 26 reporter for Earth Day. The link to the report can be found here: https://www.fox26houston.com/news/oyster-shell-recycling-program-created-in-galveston. Also in April, Houston's KPRC 2 aired the OSRP's segment that was filmed in January 2022. The link to the segment can be found here: https://www.click2houston.com/news/local/2022/04/26/galveston-bay-foundation-using-oysters-to-rebuild-reef-in-galveston/.

In July, GBF staff collected footage from two volunteer oyster gardeners' residents to feature their efforts with the Oyster Gardening Program for a social media post (Appendix B, Figures 10-11). The Oyster Gardening Program was also featured on ABC13 in July and can be viewed at: https://abc13.com/oyster-gardening-galveston-bay-foundation-reefs-shell-recycling/12074587/. In August, the story and video collected by GBF staff featuring the two volunteer oyster gardeners was posted. The link to the feature can be found here: https://galvbay.org/tiki-island-oyster-gardening/.

Please refer to Appendix B for photos of outreach efforts.

Table 5: 2022 Outreach Activities

No. of Events	Date	Activity	Description	Type of Outreach	Participants
1	2/26/22	Austin Oyster Festival	One-time shell recycling event and oyster program booth	Booth/Recycling	900-950
2	4/22/22	Space Center Houston Planet Earth Celebration	Oyster program booth set up for Space Center Houston's Earth Day celebration	Boothing	3,593
3	4/22/22	Live interview with Houston's FOX 26 news	Live interview regrading oyster program for Earth Day	Interview	N/A
4	4/23/22	Oyster Olympics for BB's Tex-Orleans	One-time shell recycling event for BB's oyster shucking contest	Recycling	~150
5	4/26/22	Report with Houston's KPRC 2 news	Interview regarding the oyster program; filmed in Jan. 2022	Interview	N/A
6	5/7/22	Moody Gardens Oyster Gardening Creation Event	Volunteers constructed their gardens and took them home to hang on their piers	Community Outreach	9
7	5/14/22	Bay Day Festival	Oyster program booth set up for GBF's Bay Day event at the Kemah Boardwalk	Boothing	~500-1,000
8	5/17/22	Dickinson Oyster Gardening Creation Event	Volunteers constructed their gardens and took them home to hang on their piers	Community Outreach	10
9	5/17/22	GBF's Annual Meeting	Oyster program booth set up for GBF's Annual Meeting	Boothing	60-70
10	5/21/22	Tiki Island Oyster Gardening Creation Event	Volunteers constructed their gardens and took them home to hang on their piers	Community Outreach	31
11	5/28/22	San Leon Oyster Gardening Creation Event	Volunteers constructed their gardens and took them home to hang on their piers	Community Outreach	12
12	6/4/22	Houston Oyster and Seafest	Shell recycling fundraising event for GBF's Oyster Shell Recycling Program	Booth/Recycling	442
13	6/9/22	Beach City Oyster Gardening Creation Event	Volunteers constructed their gardens and took them home to hang on their piers	Community Outreach	8
14	6/11/22	Galveston Oyster Gardening Creation Event	Volunteers constructed their gardens and took them home to hang on their piers	Community Outreach	9
15	6/14/22	Bayou Vista Saltwater Garden Club	Oyster Gardening Program presentation at the Saltwater Garden Club meeting in Bayou Vista	Presentation	~25
16	6/21/22	Bayou Vista Oyster Gardening Creation Event	Volunteers constructed their gardens and took them home to hang on their piers	Community Outreach	37
17	8/13/22	Friends of Galveston Island State Park	Oyster Shell Recycling Program presentation	Presentation	30
18	8/26/22	Galveston Island Park Board	Oyster Shell Recycling Program presentation	Presentation	20
19	9/13/22	Friends of Moody Gardens	Oyster Shell Recycling Program presentation	Presentation	15
20	10/15/22	Clear Lake Shores, Dickinson, League City, San Leon, Seabrook Oyster Garden Collection Event	Gardening collection, spat counts, spat transplants via volunteer assistance	Volunteer Event	17
21	10/29/22	Bayou Vista, Hitchcock, Omega Bay Oyster Garden Collection Event		Volunteer Event	42
22	11/5/22	Galveston, Jamaica Beach, Pirates Cove, Tiki Island Oyster Garden Collection Event		Volunteer Event	33
23	11/15/22	Galveston Bay Oyster Workgroup Meeting	Virtual meeting with restoration partners to discuss ongoing/future shell recycling and reef restoration activities	Regional Workgroup Meeting	25
24	11/15/22	Baytown, Beach City Oyster Garden Collection Event	Gardening collection, spat counts, spat transplants via volunteer assistance	Volunteer Event	11
25	12/5/22	Poster Session at Restore America's Estuaries Summit	Poster presenting the expansion of the Oyster Shell Recycling Program (virtual and in-person)	Boothing	~300-500
26	12/7/22	Presentation at Restore America's Estuaries Summit	Oyster Shell Recycling Program presentation: Expansion of Shell Recycling Operations During the COVID-19 Pandemic (virtual and in-person)	Presentation	~30

B.5 Outreach Materials

To inform and educate restaurant patrons about the Oyster Shell Recycling Program, approximately 30 rack cards were distributed to all new restaurant partners added during Cycle 26. A total of seven window clings were distributed to new restaurant partners as well. The window clings were displayed on entry doors to identify each restaurant as a participant of the OSRP. To further advertise active shell recycling partners, GBF updated the trailer sign (located on the back gate of the oyster shell recycling trailer) with recycling partner logos each time a new restaurant joined the OSRP. New signs depicting the OSRP process were also installed onto the sidewalls of the new trailer purchased under separate funding in 2022. The truck wraps and trailer signage on GBF's recycling equipment also provide continuous advertisement as these vehicles are driven throughout the community three times a week during shell collections.

Please refer to Appendix B for depictions of outreach materials. Please note the updated signs for the new trailer used for the OSRP operations were purchased in 2022 under separate funding.

C) Task 3: Volunteer Oyster Gardening

C.1 Photos of Oyster Gardening Events

Cycle 26 funded the 2022 oyster gardening season which began in the spring of 2022. In the summer of 2022, GBF hosted seven Oyster Garden Creation Events. The events were held in Moody Gardens, Dickinson, Tiki Island, San Leon, Beach City, and Bayou Vista. Volunteers and GBF staff worked together to build over 400 oyster gardens. All volunteers were given the option of three garden types: bags, stringers, or cages. A total of 449 oyster gardens were suspended from piers, docks, and bulkheads into Galveston Bay by August 2022 (Table 6). Refer to the Annual Gardening Report in Appendix C for photos of the oyster gardening events.

C.2 Texas Parks and Wildlife Department Introduction Permit

Please refer to Appendix C which contains the approved Texas Parks and Wildlife Department Introduction Permit.

C.3 Oyster Gardening Metrics

A total of 449 oyster gardens were suspended off piers, docks, and bulkheads at 126 bayfront homes located within 15 bayfront communities in 2022 (Table 6). Volunteers contributed through spring garden creation efforts, ongoing oyster garden monitoring during the summer, and fall garden collection efforts. Volunteers were instructed to rinse their gardens weekly to help reduce biofouling and predation. Weekly maintenance also allowed volunteers to inspect their gardens for new oyster growth.

In October and November of 2022, GBF staff coordinated the collection of the oyster gardens through five community events in Bayou Vista, Beach City, Galveston, San Leon, and Tiki Island. Volunteers delivered their gardens to these locations where GBF staff received the gardens, documented new oyster growth, and prepared the gardens for transport. Volunteers unable to attend a community event were encouraged to arrange with a neighbor attending the collection event to deliver their gardens. If the

volunteer could not coordinate delivery with a neighbor, GBF staff collected the volunteer's gardens in each community the day of the collection event. The number of gardens deployed at the beginning of the season (449) was greater than the number of gardens collected at the end of the season (410). The decrease in the number of gardens collected was due to the loss of gardens from storms, potential theft, or not being able to get in contact with the volunteer at the time of the Garden Collection Events in the fall.

Table 6: Oyster Garden Creation and Deployment

Community	Volunteer Homes	Bags Deployed	Cages Deployed	Stringers Deployed	TOTAL Gardens Deployed
Bayou Vista	32	67	17	24	108
Baytown	6	16	0	0	16
Beach City	9	19	0	9	28
Clear Lake Shores	1	1	1	1	3
Dickinson	2	0	3	3	6
Galveston	14	30	14	7	51
Hitchcock	8	15	4	21	40
Jamaica Beach	3	1	3	5	9
League City	1	1	1	1	3
Omega Bay	7	11	5	4	20
Pirates Cove	4	3	7	3	13
San Leon	8	25	3	6	34
Sea Isle	4	9	2	1	12
Seabrook	1	1	1	1	3
Tiki Island	26	25	33	45	103
TOTALS:	**126**	**224**	**94**	**131**	**449**

Table 7: Oyster Garden Collection and Oyster Recruitment

Community	Gardens Collected	Total Oysters Recruited	Avg. Oysters per Garden
Bayou Vista	85	98	1
Baytown	12	3	0
Beach City	28	53	2
Clear Lake Shores	3	16	5
Dickinson	6	0	0
Galveston	46	405	9
Hitchcock	36	262	7
Jamaica Beach	7	4	1
League City	3	0	0
Omega Bay	18	15	1
Pirates Cove	13	206	16
San Leon	31	599	19
Sea Isle	12	304	25
Seabrook	3	1	0
Tiki Island	107	5,589	52
TOTALS:	**410**	**7,555**	**18**

Thanks to the 126 dedicated oyster gardening households, approximatley 7,555 oysters were recuirted in the oyster gardens (Table 7). These oysters were transplanted onto restoration reefs in October and November 2022 under separate grant funding to help improve the local oyster population. For additional information about the 2022 oyster gardening season, please refer to Appendix C which contains the complete Annual Gardening Report.

D) Task 4: Sun Curing Research

During Cycle 26, GBF partnered with UHCL and UH, particularly Dr. Jenny Oakley and her lab at UHCL, to research the sun curing time needed to reduce the risk of infecting native oysters with Dermo, a parasite that negatively affects oysters, while not delaying the use of the material in reef restoration projects.

The parasite *Perkinsus marinus*, otherwise known as "Dermo" is a spore forming protozoan that impacts the fitness and longevity of Eastern Oysters. Dermo can be transmitted from one infected oyster to another, so sun-curing is used to minimize the prevalence of Dermo before reclaimed shells are returned to an estuary. There are currently no standardized curing procedures for restoration efforts to follow, however many groups sun-cure oyster shells for up to six months.

During the study conducted by Oakley et al. (2023), whole oysters were placed in either the interior or on top of sun-curing piles and co-located with temperature and relative humidity sensors. Half of the oysters deployed in sun-curing piles were sampled for Dermo via the standard fluid thioglycollate method. The other half were monitored for tissue decomposition. All oysters were surveyed for eight months. Dermo intensity decreased by more than 80% following the first week of deployment at the curing site, however, presence of Dermo persisted the entire study period (eight months). The temperature was more consistent and generally cooler, while humidity was also more consistent but generally higher in the interior of the piles. Dermo was not detected in the interior of the piles after the sixth week post-deployment. Tissue decomposition was more rapid in the interior of the piles, with more than half of the shells having no tissue present after the fifth week post-deployment.

The impact of wild animals on the sun-curing process was tested in this study using fenced and unfenced piles. Initial analysis of game camera photos conducted by Smith et al. (2023) suggested several types of wildlife interact with the oyster piles including feral hogs, vultures, opossums, deer, coyote, and songbirds. The number of interactions at unfenced piles was much higher than at fenced piles and the highest number of interactions for feral hogs occurred in the first week post-deployment, while the highest number of interactions by vultures occurred in the third week post-deployment (Smith et al. 2023). Within the first week of deployment oysters deployed at the top of the unfenced piles were depredated by feral hogs which resulted in the removal of all oyster tissue for those affected oysters and therefore the assumed removal of Dermo.

These preliminary results can be used to inform future studies and improve restoration practices by updating sun-curing best practices to minimize the introduction of Dermo into local estuaries from reef restoration projects. The fact that there are feral hogs that are habituated to shell dumping and are utilizing the tissue as a food source at the Red Bluff Curing Site provides a benefit by removing decaying tissue, effectively removing the Dermo infection. However, it is unknown if Dermo can survive the digestive tract of a feral hog, and if so, if it can remain viable in the hog feces. Because the oyster tissue from the tops of the piles was effectively gone due to depredation by feral hogs within two weeks of deployment, the researchers assumed that oysters at the tops of the piles were free of Dermo after two weeks. The oysters in the interior of the piles had no Dermo infection detected after the sixth week of deployment. Depending on the demand for oyster shells, the results of this study suggest that resource managers and practitioners that have active depredation of oyster tissue at the top of their piles, to the extent that tissue is quickly removed, cure their recycled oyster shell material for a minimum of three months if the shell is deployed during "warm-weather" months (April – September). For oysters deployed during "cold-weather" months, the results suggest continuing the current practice of six months deployment with a mechanical rotation at three months be continued until additional studies can be

completed to better understand the seasonal component and determine how temperature may impact the decomposition of the oyster tissue and subsequent Dermo infection prevalence. Should the feral hog population cease to exist on the Red Bluff Curing Site property, the tissue decomposition and Dermo infection of oysters on the top of the piles is expected to increase, and the researchers recommend returning to the cold-weather curing protocol.

D.1 Executed Agreement Between GBF and UH

Upon execution of the agreement between GBF and UH in January 2022, UH began to conduct a study to help determine the sun curing time needed to reduce the risk of infecting native oysters with a parasite known as Dermo, while not delaying the use of the material in reef restoration projects.

D.2 Executed Agreement Between GBF and UHCL

Upon execution of the agreement between GBF and UHCL in April 2022, UHCL began to conduct a study to help determine the sun curing time needed to reduce the risk of infecting native oysters with a parasite known as Dermo, while not delaying the use of the material in reef restoration projects.

D.3 Photos of Assembled Shell Piles in Preparation for Experiment

Please refer to Appendix D for the photos of the assembled shell piles constructed for the sun curing experiment.

D.4 Collected Data

Collected data from the sun curing experiment is available upon request. Please see the complete report in Appendix D for the synthesis of the data collected.

D.5 Report Analyzing Experiment Results

Please refer to Appendix D for the complete report analyzing the sun curing experiment results.

IV. Results

GBF utilized Cycle 26 funds to continue the OSRP's expansion to the inner loop of Houston. During Cycle 26, 160 tons of oyster shell were collected, and seven new shell recycling partnerships were secured. All oyster shells collected during Cycle 26 are currently undergoing the sun curing process. Upon completion of the sun curing process, these shells will be utilized in GBF's Volunteer Oyster Gardening Program and oyster reef restoration efforts.

Due to the Houston expansion and new partnerships in Galveston, GBF recycled a record-breaking tonnage of shell in 2022, a total of 242 tons. Based on this new record in 2022, GBF is now collecting an average of 20 tons of oyster shell per month. As of August 2023, GBF has recycled a total of 1,667 tons of oyster shells since the inception of the OSRP.

Cycle 26 also funded the 2022 oyster gardening season. One hundred and twenty-six volunteers participated in oyster gardening during 2022 and helped grow 7,555 oysters which were transplanted onto restoration reefs under separate grant funding.

During Cycle 26, GBF partnered with UHCL and UH to determine the sun curing time needed to reduce the risk of infecting native oysters with Dermo, while not delaying the use of the material in reef restoration projects. Dermo intensity decreased by more than 80% following the first week of deployment at the curing site, however, presence of Dermo persisted the entire eight-month long study period. The temperature was more consistent and generally cooler, while humidity was also more consistent but generally higher in the interior of the piles. Dermo was not detected in the interior of the piles after the sixth week post-deployment. Tissue decomposition was more rapid in the interior of the piles, with more than half of the shells having no tissue present after the fifth week post-deployment.

V. Lessons Learned

Shell Recycling Operations

GBF plans to continue recruiting additional shell recycling partners as capacity allows. The threshold for expansion will be dictated by the shell-hauling capacity of the recycling equipment (dump truck, landscape trailer, Moody Gardens' equipment), storage capacity at the curing sites, and/or funding availability. To date, neither the shell-hauling equipment nor the curing sites have exceeded capacity. The largest expenses documented thus far are associated with travel, vehicle/equipment maintenance, and staff time (salary and fringe).

The dump truck maintenance requires a significant amount of funds in the event the equipment becomes damaged or needs to be replaced due to normal wear and tear. Fortunately, thanks to federal and state grants and private donations through individuals or corporations, GBF has been able to fund the maintenance costs associated with the dump truck thus far. To save on cost GBF staff perform the necessary maintenance whenever possible. GBF staff also established a plan on how to conduct the Houston region shell collections when the dump truck is out of commission to keep the normal schedule in place as much as possible.

Thanks to Moody Gardens, three additional partners were secured on the Island under Cycle 26. To continue the addition of new recycling partners, GBF purchased a small utility trailer to be used by Moody Gardens staff to haul a larger quantity of oyster shell so multiple trips are no longer needed. Unfortunately, during the tourist season Moody Gardens staff find it difficult to maneuver the trailer in some areas along the route. If the Galveston shell recycling operations continue to expand, additional staff and alternative equipment will be required in the future.

Four more Houston restaurant partners were added to the OSRP during Cycle 26 and as of August 2023 the OSRP has a total of 19 Houston region restaurant partners. To not exceed hauling capacity for the dump truck GBF staff focused less on securing new restaurant partnerships for the Houston region. New Houston restaurant partners are added to the OSRP if the restaurant contacted GBF and adding them to the OSRP is feasible or if the restaurant is located directly on the current established route. The Houston shell collections are conducted on Mondays, Wednesdays, and Fridays. Due to the weekend Mondays have the heaviest loads, and at times the dump truck almost reaches max capacity. Therefore, when a new restaurant partner is interested in joining the OSRP it is recommended they receive shell collections on Wednesdays or Fridays to lessen the load on Mondays.

During Cycle 27, GBF staff will update the OSRP's Strategic Development Plan to evaluate the shell recycling operations expansion to determine the future of the OSRP. GBF staff plan to estimate when the OSRP will reach max capacity for shell recycling operations to be conducted solely by GBF staff and how to move forward when max capacity has been achieved. GBF staff will reference other oyster shell recycling programs that recycle large quantities of shell to help determine how the OSRP will plan for the future.

Volunteer Oyster Gardening

To streamline the Garden Creation Events and reduce expenses next season, GBF staff plan to decrease the number of events held at the beginning of the season when volunteers meet to learn about the

Volunteer Oyster Gardening Program and build their gardens to take home. For 2022 seven Oyster Garden Creation Events were held which required a significant amount of time, labor, and cost for GBF staff. Instead of hosting events for each individual bayfront community, the proposed plan for next season will be to host events at Moody Gardens in Galveston as well as GBF's headquarters in Kemah. Hosting the Garden Creation Events at Moody Gardens will help to reduce the number of events while still offering a central location to meet for volunteers located in the lower Galveston Bay region. Likewise, hosting the Garden Creation Events at the GBF headquarters will also help to reduce the number of events while still offering a central location to meet for the mid Galveston Bay region volunteers. A Garden Creation Event will still be held at GBF's Trinity Bay Discovery Center in Beach City for volunteers located in the upper Galveston Bay region. All the Garden Creation Events will be offered to any volunteer no matter what bayfront community they reside in. For volunteers that cannot attend any of the events, it will be recommended they arrange to have a neighbor pick up gardens for them at one of the events or they can schedule a pickup time to receive their gardens at GBF's headquarters. The GBF headquarters is central to all participating communities and an in-person, scheduled pick-up will provide an opportunity for the volunteers to meet with GBF staff and receive proper instructions.

To reduce the manual labor and time requirements at the Dickinson Bay Reef introduction site, GBF staff plan to change the transportation method next season. After all the spat from the gardens of the volunteers located in Clear Lake Shores, Dickinson, League City, San Leon, and Seabrook had been counted and recorded, the spat and shells were transferred to 5-gallon buckets. From the volunteer's residence that hosted the Garden Collection Event, the buckets were then transported by kayak to the Dickinson Bay Reef where the spat and shells were placed onto the reef. While the water conditions were favorable and the kayaking trip was manageable, it was agreed by both the volunteers and GBF staff that having a boat available to assist with the transplanting would be a better option for next season to help reduce the manual labor of loading and unloading the kayaks to/from the water. Next season, GBF staff plan to coordinate the event with either a volunteer boat owner or utilize GBF's boat if the staff that operate the boat are available.

Sun Curing Research

The sun curing research study showed depredation by feral hogs and vultures impacts oysters at the tops of sun-curing piles. Oysters were deployed in the same plastic mesh that's used for GBF's oyster gardening bags. The bags were attached to wire cable in an attempt to avoid losing the study oysters, but the feral hogs were able to rip through the bags and remove the bailing wire to access the oyster tissue. This was important to the study design as one of the goals was to determine the impact that wildlife has on the sun-curing process. Future studies should consider using a sturdier container that will allow the oysters to be exposed to the ambient environment at the top of the piles but protect them from depredation as not all sun-curing sites have feral hogs, or the same wildlife present.

Additionally, the development of the tissue condition categories was a work-in-progress as the tissues throughout the initial weeks of deployment were observed. There were not previously defined condition categories beyond the initial "plump" and "shrunken" as defined by Ray (1966). As a result, the field team had to spend significant time in the field together standardizing the evaluation of these categories, and re-evaluation using photos in the initial weeks was required after the categories were finalized. Future studies may use these categories to standardize the process of documenting tissue degradation in oysters deployed in sun-curing piles.

VI. References

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VII. Appendix

APPENDIX A: Task 1

- Task 1 Photographs
- Map of Shell Recycling Locations

APPENDIX B: Task 2

- Task 2 Photographs
- Restaurant Database
- Galveston Bay Oyster Workgroup Meeting Notes
- Oyster Shell Recycling Program Outreach Materials

APPENDIX C: Task 3

- Texas Parks and Wildlife Department Introduction Permit
- Annual Gardening Report

APPENDIX D: Task 4

- Task 4 Photographs
- Report Analyzing Experiment Results

APPENDIX A
Task 1 Deliverables

OYSTER SHELL COLLECTION AND CURING SITE MAINTENANCE

Task 1
Photographs

Figure 1. Fully cured shell in the background and partially cured shell that was just rotated in the foreground at Red Bluff curing site

Figure 2. Re-staked and roped off shell piles at Red Bluff curing site

Figure 3. GBF staff relocating fully cured shell from Moody Gardens curing site to GBF's Sweetwater Preserve

Task 1
Map of Shell Recycling Locations

Oyster Shell Recycling Locations

Project Name: Oyster Shell Recycling Program (OSRP)	
Project Location: Harris & Galveston County, TX	
Image Source: ESRI World Street Map	
Projection: NAD 1983, UTM Zone 15N	
Date Drawn: 9/13/2023	Drawn by: H.Leija

GALVESTON BAY FOUNDATION

1725 Highway 146, Kemah, TX; (281) 332-3381

APPENDIX B
Task 2 Deliverables

PARTNERSHIPS AND COMMUNITY OUTREACH

Task 2
Photographs

Figure 1. Booth at Austin Oyster Festival

Figure 2. Booth at Space Center Houston Planet Earth Celebration

Figure 3. Booth at Bay Day

Figure 4. Inaugural Houston Oyster and Seafest

Figure 5. Inaugural Houston Oyster and Seafest

Figure 6. Inaugural Houston Oyster and Seafest

Figure 7. Inaugural Houston Oyster and Seafest

Figure 8. Inaugural Houston Oyster and Seafest

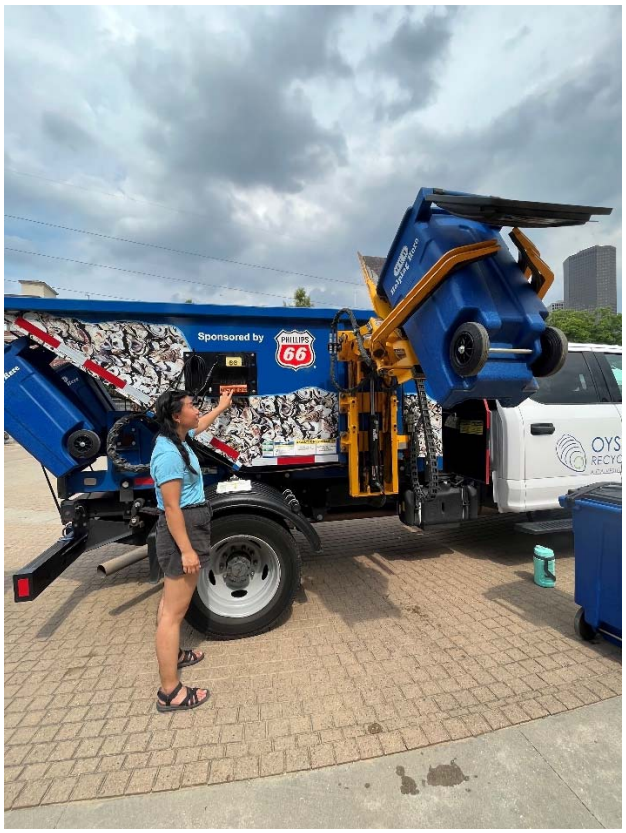


Figure 9. Inaugural Houston Oyster and Seafest


Figure 10. GBF staff filming volunteer oyster gardeners for social media feature

Figure 11. GBF staff filming volunteer oyster gardeners for social media feature

OYSTER SHELL RECYCLING PROGRAM
A GALVESTON BAY FOUNDATION PROGRAM

Expansion of the Galveston Bay Foundation's Oyster Shell Recycling Program

Shannon Batte, Galveston Bay Foundation, Kemah, TX

Oyster Shell Recycling Process

- The Galveston Bay Foundation's (GBF) Oyster Shell Recycling Program partners with local restaurants to collect shucked oyster shells.

- The shells are transported weekly by staff to curing sites where the shells are properly quarantined on land for a minimum of six months in preparation for reuse in local oyster reef restoration projects.

- The fully cured recycled oyster shells are returned to the bay through intertidal and subtidal reef restoration efforts, shoreline protection projects, and GBF's Volunteer Oyster Gardening Program.

Program Expansion

With the assistance of Coastal Management Program funds and private donations, GBF was able to purchase a one of a kind heavy duty truck equipped with a dump bed and bin lift to facilitate the expansion of shell recycling efforts to the inner loop of Houston in May 2021. As of October 2022, GBF has secured 14 restaurant partners in Houston, resulting in 123 tons of oyster shells recycled from the Houston region.

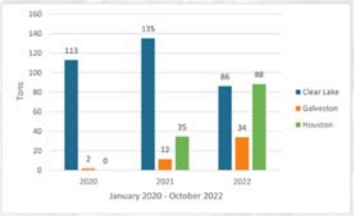
2022 Recycling Locations

The program has expanded to 31 recycling partners and three curing sites, steadily increasing the tonnage of shells recycled on an annual basis. GBF has managed this program since 2011, collecting over 1,400 tons of oyster shells as of October 2022. Of this amount, approximately 840 tons have been incorporated in restoration efforts in Galveston Bay.

Moody Gardens Partnership

Thanks to a new partnership with Moody Gardens, secured during the pandemic in October 2020, the Oyster Shell Recycling Program also expanded on Galveston Island. Moody Gardens staff collect oyster shells from eight recycling partners in the Galveston region. Currently GBF does not have the funding, staff, or equipment to facilitate weekly shell collections on Galveston Island, therefore the collaboration with Moody Gardens made oyster shell recycling possible for Galveston restaurants. Moody Gardens also provides space on their property to store the recycled shell throughout the curing process, thus further reducing costs for GBF.

Oyster Shell Recycled Per Region



As a result of the Houston expansion and the Moody Gardens partnership, the amount of oyster shells recycled increased significantly in 2021 and 2022, exceeding all previous years of oyster shell recycled.

Scan me to learn more
or visit galvbay.org/oysters

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Figure 12. Poster for Restore America's Estuaries Summit

Task 2
Restaurant Database

RESTAURANT DATABASE
Clear Lake Region

No. of Restaurants	Restaurant Name	Location	Oyster Items on Menu		Oysters Purchased (Sacks per week?)	Contacted?	Interested?	Restaurant Phone #
			Raw (Y/N)	Cooked				
1	Barge 295	Seabrook	Y	5	10-12 sacks/wk	Yes	Current Partner	(281) 549-7603
2	BB's Tex-Orleans	Webster	Y	0	5 sacks/wk	Yes	Current Partner	(281) 767-9644
3	Bou-Shay's	Bacliff	Y	2		No		(832) 864-2862
4	Captain Benny's Seafood	Deer Park	Y	4		No		(281) 476-1513
5	Crafty Crab	League City	Y	1		No		(281) 849-9000
6	Crazy Alans Swamp Shack	Kemah	Y	4	~3 sacks/wk	Yes	Current Partner	(281) 334-5000
7	Crazy Alans Swamp Shack	Friendswood	Y	4	~3 sacks/wk	Yes	Current Partner	(832) 284-4895
8	East Star Chinese Buffet	Webster	Y	0		No		(281) 280-8822
9	Floyd's Cajun Seafood and Steakhouse	Webster	Y	6	~35 sacks/wk	Yes	Current Partner	(281) 332-7474
10	Flying Dutchman	Kemah	Y	4		Yes	*Past partner*	(281) 334-7575
11	Gilhooley's Restaurant	San Leon	Y	4		Yes	No	(281) 339-3813
12	Jackie's Brickhouse	Kemah	Y	1		Yes	No response yet	(832) 864-2459
13	La Costa Seafood Grill	Alvin	Y	3		No		(281) 824-4384
14	LA Crawfish	Webster	Y	3		No		(832) 905-5154
15	LA Crawfish	Baytown	Y	3		No		(832) 479-8081
16	LA Crawfish	Pasadena	Y	3		No		(832) 288-4494
17	Landry's Seafood House	Kemah	Y	1		Yes	*Past partner*	(281) 334-2513
18	Little Daddy's Gumbo Bar	League City	Y	2		No		(281) 524-8626
19	Main St Bistro	League City	Y	0		No		(281) 332-8800
20	Mambo Seafood	Baytown	Y	0		No		(832) 926-7551
21	Marais	Dickinson	Y	4		No		(281) 534-1986
22	Monument Inn	La Porte	Y	0		No		(281) 479-1521
23	Noah's Ark Bar & Grill	Bacliff	Y	4		No		(281) 339-2895
24	Opus Bistro & Steakhouse	League City	Y	4		Yes	No response yet	(281) 334-0006
25	Pappas Seafood House	Webster	Y	1		Yes	Need to talk to corporate	(281) 332-7546
26	Perry's Steakhouse & Grille	Friendswood	Y	0		Yes	Maybe	(281) 286-8800
27	Pier 6 Seafood & Oyster House	San Leon	Y	4		Yes	No	(281) 339-1515
28	Sammy G's District 70 BBQ & Grill	El Lago	Y	Unsure		Yes	Yes, once restaurant opens in 9/2023	

RESTAURANT DATABASE
Clear Lake Region

No. of Restaurants	Restaurant Name	Location	Oyster Items on Menu		Oysters Purchased (Sacks per week?)	Contacted?	Interested?	Restaurant Phone #
			Raw (Y/N)	Cooked				
29	Sam's Boat	Seabrook	Y	0		Yes	Current Partner	(281) 326-7267
30	Schafer's Coastal Bar & Grille	Clear Lake Shores	Y	3		Yes	Yes, but don't serve oysters on regular basis	(281) 532-6860
31	The Aquarium Restaurant	Kemah	Y	0		Yes	Current Partner	(281) 334-2521 (Bio and Edu Dept.) (281) 334-9010 (Restaurant)
32	The Reef Seafood House	Texas City	Y	0		No		(409) 945-6151
33	The Rouxpour	Friendswood	Y	4		Yes	Yes, but no response yet	(281) 480-4052
34	TJ Reed's Flippers	Dickinson	Y	2		No		(832) 340-7340
35	Tommy's Restaurant & Oyster Bar	Houston	Y	5		Yes	Current Partner	(281) 480-2221
36	Tookie's Seafood	Seabrook	Y	6	40-60 sacks/wk	Yes	Current Partner	(281) 942-9445
37	Topwater Grill	San Leon	Y	5		Yes	*Past partner*	(281) 339-1232
38	Valdo's Seafood House	Seabrook	Y	4		Yes	No response yet	(281) 326-3866

LEGEND
Current Partner
Priority for Shell Recycling
Contact for Houston Oyster Festival
Low Priority

RESTAURANT DATABASE

Galveston Region

No. of Restaurants	Restaurant Name	Location	Oyster Items on Menu		Oysters Purchased (Sacks per week?)	Contacted?	Interested?	Restaurant Phone #
			Raw (Y/N)	Cooked				
1	Black Pearl Oyster Bar	Galveston	Y	4		No		(409) 762-7299
2	BLVD Seafood	Galveston	Y	3		Yes	Current Partner	(409) 762-2583
3	Cajun Greek	Galveston	Y	0	10 sacks/wk	Yes	Current Partner	(409) 744-7041
4	Fish Tales	Galveston	Y	0		Yes	Current Partner	(409) 762-8545
5	Fisherman's Wharf	Galveston	Y	0	14-20 sacks/wk	Yes	Current Partner	(409) 765-5708
6	Gaido's/Nick's Kitchen & Beach Bar	Galveston	Y	8	20-60 sacks/wk	Yes	Current Partner	(409) 761-5500
7	Grand Galvez Bar & Grill	Galveston	Y	Unsure	5-10 sacks/wk 3 or 4 big weekends around holidays	Yes	Current Partner	(409) 765-7721
8	Katie's Seafood House	Galveston	Y	2		Yes	Yes	(409) 765-5688
9	Kritikos Grill	Galveston	Y	0	2 sacks/wk	Yes	Current Partner	(409) 539-5915
10	Landry's Seafood House	Galveston	Y	1		No	Maybe	(409) 744-1010
11	Little Daddy's Gumbo Bar	Galveston	Y	2		No		(281) 524-8626
12	Number 13	Galveston	Y	0		Yes	*Past partner*	(409) 572-2650
13	Saltwater Grill	Galveston	Y	3		No		(409) 762-3474
14	Shuck's Tavern & Oyster Bar	Galveston	Y	3	5 sacks/wk from Prestige 8-10 sacks/wk from east coast	Yes	Current Partner	(409) 444-1700
15	Willie G's Seafood & Steaks	Galveston	Y	1		No		(409) 762-3030

LEGEND
Current Partner
Priority for Shell Recycling
Contact for Houston Oyster Festival
Low Priority

RESTAURANT DATABASE
Houston Region

No. of Restaurants	Restaurant Name	Location	Oyster Items on Menu		Oysters Purchased (Sacks per week?)	Contacted?	Interested?	Restaurant Phone #
			Raw (Y/N)	Cooked				
1	1751 Sea and Bar	Houston	Y	2		No		(832) 831-9820
2	A'Bouzy	Houston	Y	1		No		(713) 722-6899
3	Acadian Coast	Houston	Y	3		No		(713) 432-9651
4	Acme Oyster House	Houston	Y	2	200-300 sacks/wk	Yes	Current Partner	(346) 571-2071
5	B&B Butchers & Restaurant	Houston	Y	1		No		(713) 862-1814
6	B.B. Lemon	Houston	Y	0		No		(713) 554-1809
7	BB's Tex-Orleans	Houston- Briargrove	Y	0		No	No	(713) 339-2566
8	BB's Tex-Orleans	Houston- Heights	Y	0	10-15 sacks/wk	Yes	Current Partner	(713) 868-8000
9	BB's Tex-Orleans	Houston- Montrose	Y	0		Yes	Current Partner	(713) 524-4499
10	BB's Tex-Orleans	Houston- Upper Kirby	Y	0	8 sacks/wk	Yes	Current Partner	(713) 807-1300
11	BB's Tex-Orleans	Houston- Pearland	Y	0		No	Yes	(832) 856-3200
12	BB's Tex-Orleans	Houston- Oak Forest	Y	0	6 sacks/wk	Yes	Current Partner	(832) 318-6533
13	Bludorn	Houston	Y	2	28 sacks/wk	Yes	Current Partner	(713) 999-0146
14	Brasserie 19	Houston	Y	1		No		(713) 524-1919
15	Brennan's of Houston	Houston	Y	2		No		(713) 522-9711
16	Cajun Kitchen	Houston	Y	4		No		(281) 495-8881
17	Captain Benny's Seafood	Houston	Y	4	20 sacks/wk	Yes	Current Partner	(713) 643-0589
18	Captain Benny's Seafood	Houston	Y	4		No		(713) 666-5469
19	Captain Benny's Seafood	Stafford	Y	4		No		(281) 498-3909
20	Captain Benny's Seafood	Houston	Y	4		No		(713) 680-1828
21	Captain Tom's Seafood & Oyster	Houston	Y	0		No		(713) 451-3700
22	Caracol	Houston	Y	1		No		(713) 622-9996
23	Chilos Seafood & Oyster Bar	Houston	Y	No menu online		No		(713) 947-8700
24	Christie's Seafood & Steaks	Houston	Y	2		No		(713) 978-6563
25	Crafty Crab	Pearland	Y	1		No		(832) 856-1111
26	Crafty Crab	Houston (FM 1960 Rd)	Y	1		No		(832) 680-1111
27	Crafty Crab	Houston (Fondren Rd)	Y	1		No		(713) 820-6888
28	Crafty Crab	Houston (Jersey Village)	Y	1		No		(832) 856-5656
29	Crafty Crab	Houston (Westheimer Rd)	Y	1		No		(832) 810-3333
30	Drunken Oyster	Spring	Y	0		No		(832) 843-6196
31	Eddie V's Prime Seafood	Houston- West Ave	Y	4		No		(713) 874-1800
32	Eddie V's Prime Seafood	Houston- City Centre	Y	4		No		(832) 200-2380

RESTAURANT DATABASE
Houston Region

No. of Restaurants	Restaurant Name	Location	Oyster Items on Menu		Oysters Purchased (Sacks per week?)	Contacted?	Interested?	Restaurant Phone #
			Raw (Y/N)	Cooked				
33	Eugene's Gulf Coast Cuisine	Houston	Y	5		No		(713) 807-8889
34	Eunice	Houston	Y	2	40 sacks/wk	Yes	Current Partner	(832) 491-1717
35	Famous Crab	Houston	Y	3		Yes	Yes, but no response	(281) 484-2722
36	Field & Tides	Houston	Y	1		No	No response	(713) 861-6143
37	Fish City Grill	Pearland	Y	1		No		(713) 340-1493
38	Fish City Grill	Sugarland	Y	1		No		(281) 494-3474
39	Flora	Houston	Y	0		No		(713) 360-6477
40	Floyd's Cajun Seafood and Steakhouse	Sugar Land	Y	6		No		(281) 240-3474
41	Floyd's Cajun Seafood and Steakhouse	Pearland	Y	6		No		(281) 993-8385
42	Flying Fish	Houston	Y	4	9 sacks/wk	Yes	Current Partner	(713) 377-9919
43	Frank's Americana Revival	Houston	Y	Unsure		Yes		(713) 572-8600
44	Georgia James	Houston	Y	1		No		(832) 241-5088
45	Good Vibes Coastal Kitchen	Pearland	Y	1		No		(832) 569-4141
46	Goode Company- Seafood	Houston- Westpark	Y	4	45 sacks/wk	Yes	Current Partner	(713) 523-7154
47	Harold's	Houston- Heights	Y	1		Yes	Maybe	(713) 360-6204
48	Hometown Seafood Company	Pearland	Y	4		No		(281) 416-5419
49	Hugos	Houston				Yes	Yes	(713) 524-7744
50	Julep	Houston	Y	Unsure		No		(832) 371-7715
51	Kata Robata	Houston	Y	0		No		(713) 526-8858
52	LA Crawfish	Houston- Greenway	Y	3		No		(832) 767-1533
53	LA Crawfish	Houston- Memorial	Y	3		No		(713) 461-8808
54	LA Crawfish	Houston- Langwood	Y	3		No		(832) 491-1121
55	LA Crawfish	Houston- Wallisville Rd & Beltway 8	Y	3		No		(281) 416-5352
56	LA Crawfish	Katy	Y	3		No		(346) 251-5902
57	LA Crawfish	Pearland	Y	3		No		(832) 781-4946
58	LA Crawfish	Houston- Gulfgate	Y	3		No		(832) 804-6901
59	LA Crawfish	Missouri City	Y	3		No		(281) 208-7759
60	La Lucha	Houston	Y	3	100 sacks/wk	Yes	Current Partner	(713) 955-4765
61	Liberty Kitchen & Oysterette	Houston- River Oaks	Y	2		No		(713) 622-1010
62	Liberty Kitchen & Oysterette	Houston- Memorial	Y	2		No		(713) 468-3745
63	Little's Oyster Bar	Houston	Y	Unsure		No		(713) 522-4595
64	Loch Bar	Houston- River Oaks District	Y	5	30 sacks/wk	Yes	Current Partner	(832) 430-6601

RESTAURANT DATABASE
Houston Region

No. of Restaurants	Restaurant Name	Location	Oyster Items on Menu		Oysters Purchased (Sacks per week?)	Contacted?	Interested?	Restaurant Phone #
			Raw (Y/N)	Cooked				
65	Low Tide Kitchen & Bar	Houston- Spring Branch	Y	2		Yes	Current Partner	(713) 360-6304
66	Mambo Seafood	Houston- 45S & Edgebrook	Y	0	20-50 sacks/wk	Yes	Current Partner	(713) 946-0000
67	Mambo Seafood	Houston- 290 & Tidwell	Y	0		No		(713) 462-0777
68	Mambo Seafood	Houston- 45N & West Rd	Y	0		No		(281) 820-3300
69	Mambo Seafood	Houston- Airline & Tidwell	Y	0		No		(713) 691-9700
70	Mambo Seafood	Houston- Gessner & Long Point	Y	0		No		(713) 465-5009
71	Mambo Seafood	Houston- Hillcroft & Bellaire	Y	0		No		(713) 541-3666
72	Mambo Seafood	Houston- I-10 & Federal	Y	0		No		(713) 637-0553
73	Mambo Seafood	Katy	Y	0		No		(832) 391-6644
74	Mannie's Seafood	Houston	Y	2		No		(713) 641-5003
75	Marcos Seafood & Oyster Bar	Houston	Y	0		No		(713) 946-1168
76	Mastro's Steakhouse	Houston	Y	1		No		(713) 993-2500
77	McCormick & Schmick's Seafood & Steaks	Houston- Town & Country Village	Y	4		Yes		(713) 465-3685
78	McCormick & Schmick's Seafood & Steaks	Houston- Uptown Park, Galleria	Y	4		Yes		(713) 840-7900
79	McCormick & Schmick's Seafood & Steaks	Houston- Downtown	Y	4		Yes		(713) 658-8100
80	Musaafer	Houston	Y	1		No		(713) 242-8087
81	Nancy's Hustle	Houston	Y	Unsure (New to menu)	4-5 sacks/wk	Yes	Current Partner	(346) 571–7931
82	Navy Blue	Houston	Y	1	20-30 sacks/wk	Yes	Current Partner	(713) 347-7727
83	Orleans Seafood Kitchen	Katy	Y	1		No		(281) 646-0700
84	Ostioneria Michoacan Seafood and Oyster Bar	Houston- #11	Y	1		No		(713) 921-1800
85	Ostioneria Michoacan Seafood and Oyster Bar	Houston- #1	Y	1		No		(281) 999-3995
86	Ostioneria Michoacan Seafood and Oyster Bar	Houston- #3	Y	1		No		(713) 330-4419
87	Ostioneria Michoacan Seafood and Oyster Bar	Houston- #4	Y	1		No		(281) 447-5061
88	Ostioneria Michoacan Seafood and Oyster Bar	Houston- #5	Y	1		No		(713) 974-6828
89	Ostioneria Michoacan Seafood and Oyster Bar	Woodlands- #6	Y	1		No		(281) 292-6811
90	Ostioneria Michoacan Seafood and Oyster Bar	Houston- #7	Y	1		No		(713) 463-5410
91	Ostioneria Michoacan Seafood and Oyster Bar	Houston- #8	Y	1		No		(281) 877-8855
92	Ostioneria Michoacan Seafood and Oyster Bar	Houston- #15	Y	1		No		(281) 477-7697
93	Ostioneria Michoacan Seafood and Oyster Bar	Houston- #16	Y	1		No		(832) 672-4139
94	Pappadeaux Seafood Kitchen	Houston- Hobby Airport	Y	1		No		(713) 847-7622
95	Pappadeaux Seafood Kitchen	Houston- Galleria	Y	1		No		(713) 782-6310
96	Pappas Bros. Steakhouse	Houston- Galleria	Y	0		No		(713) 780-7352

RESTAURANT DATABASE
Houston Region

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			Raw (Y/N)	Cooked				
97	Pappas Seafood House	Houston- Aldine Bender	Y	1		No		(281) 999-9928
98	Perry's Steakhouse & Grille	Houston- Champions	Y	0		No		(281) 970-5999
99	Perry's Steakhouse & Grille	Katy	Y	0		No		(281) 347-3600
100	Perry's Steakhouse & Grille	Houston- Memorial City	Y	0		No		(832) 358-9000
101	Perry's Steakhouse & Grille	Houston- River Oaks	Y	0		No		(346) 293-8400
102	Perry's Steakhouse & Grille	Sugar Land	Y	0		No		(281) 565-2727
103	Perry's Steakhouse & Grille	Woodlands	Y	0		No		(281) 362-0569
104	Ragin' Cajun	Houston- The Original	Y	1		No		(713) 623-6321
105	Relish Restaurant & Bar	Houston	Y	1		No		(713) 599-1960
106	Riel	Houston	Y	1		No		(832) 831-9109
107	Sam's Boat	Pearland	Y	0		No		(713) 436-0201
108	Sam's Boat	Houston	Y	0		No		(713) 781-2628
109	State of Grace	Houston	Y	1	~80 sacks/wk	Yes	Current Partner	(832) 942-5080
110	Steak 48	Houston	Y	0		No		(713) 322-7448
111	The Annie Café & Bar	Houston	Y	0		No		(713) 804-1800
112	The Chalet at Rosie Cannonball	Houston	Y	0		No		(832) 380-2471
113	The Crawfish Pot & Oyster Bar	Houston	Y	2		No		(713) 360-6547
114	The Oceanaire	Houston	Y	1		Yes		(832) 487-8862
115	The Original Ninfa's	Houston- Navigation	Y	1		No		(713) 228-1175
116	The Original Ninfa's	Houston- Uptown	Y	1		No		(346) 335-2404
117	The Pearl Restaurant & Bar at The Sam Houston Hotel	Houston	Y	3		No		(832) 200-8817
118	The Rouxpour	Sugarland	Y	4		No		(281) 240-7689
119	The Rouxpour	Katy	Y	4		No		(281) 394-5013
120	The Rustic	Houston	Y	2		No		(832) 321-7775
121	Tiny Champions	Houston	Y	0	2-4 sacks/wk	Yes	Current Partner	(713) 485-5329
122	Tobiuo Sushi & Bar	Katy	Y	1		No		(281) 394-7156
123	Toulouse	Houston	Y	1		No		(713) 871-0768
124	Traveler's Table	Houston	Y	3		Yes	Yes, but no response	(832) 409-5785
125	Truluck's Seafood Steak & Crab House	Houston	Y	1		No		(713) 783-7270
126	Truluck's Seafood Steak & Crab House	Woodlands	Y	1		No		(281) 465--7000
127	Turner's	Houston	Y	1		No		(713) 804-1212
128	Weights + Measures	Houston	Y	1		No		(713) 654-1970

RESTAURANT DATABASE
Houston Region

No. of Restaurants	Restaurant Name	Location	Oyster Items on Menu		Oysters Purchased (Sacks per week?)	Contacted?	Interested?	Restaurant Phone #
			Raw (Y/N)	Cooked				
129	Willie G's	Houston	Y	8		No		(713) 840-7190
130	Winnie's	Houston	Y	1	~10 sacks/wk	Yes	Current Partner	(713) 520-0660
131	Xochi	Houston	Y	1		No		(713) 400-3330

LEGEND
Current Partner
Priority for Shell Recycling
Contact for Houston Oyster Festival
Low Priority

Task 2
Galveston Bay Oyster Workgroup Meeting Notes

GALVESTON BAY OYSTER WORKGROUP
MEETING NOTES
Tuesday, November 15, 2022
10:00am – 12:00pm
Via Zoom
25 Attendees

A) Introductions & Housekeeping

Affiliation: subgroup of the GBEP-NRU Subcommittee led by Lindsey Lippert

Co-Chairs: Bill Rodney & Haille Leija

Workgroup Goals

a. Provide technical guidance for reef restoration in Galveston Bay
    - Site selection, design, materials, monitoring, BMPS
b. Facilitate research
    - Define gaps in research related to Galveston Bay oysters
    - Establish partnerships to fill those gaps
c. Identify funding & partnership opportunities

B) Galveston Bay Project Updates

a. TNC – Trinity Bay Reef (Kathy Sweezey)
    - Plan to finish construction sanctuary reef in summer 2023 (at least 2 ac?)
    - New funding source – Center Point Energy and Cummings
    - Alternative materials may be used (recycled, 3d printed, etc.)
    - Testing diff. reef heights
    - Need partner to monitor
b. TPWD – Trinity Bay Reef (Bill Rodney)
    - La Nina has likely had a influence on spat recruitment
    - 3-4 cohorts
    - Density = 500-600 oysters/m^2; 700 spat/m^2
    - Additional year of closure for the harvestable reefs
c. TPWD – East Bay project – NEW (Bill Rodney)
    - NRDA Trustees – GLO is the leads; Freese & Nichols did the design
    - Construction to start in 2023
    - Subtidal reef (similar to Trinity Bay Sanctuary Reef) ~ 6 ac
    - Unfishable materials
    - Intertidal reef ~ 19 ac
d. TPWD – general updates
    - Good spat set and recruitment within the last year (Chris Steffen)
    - Habitat Assessment Model – slowly rotating through the bays to complete mapping; each project takes 2-3 years; aim to update each bay system every 10 years
    - Funds received for new sanctuary reefs in Copano Bay

e. GLO (Diana Ramirez)
    - GLO is lead on NRDA project in East Bay
    - CEPRA has a few other oyster projects in the works
f. DSHS (Kirk Wiles)
    - Not much rain so very little bacteria except for TX 6 due to the rainfall on last night
g. NRCS (Russell Castro)
    - Pursuing cost share with producers beginning oyster aquaculture operations in Copano Bay
    - Want to address living shorelines as well – maybe cost share for coastal boundary surveys?
    - Want to participate in reef restoration
    - CCA & GBF interested in partnering on these efforts
h. CCA (Shane Bonnot)
    - Reach out to CCA in 2023 for funding support
i. NRDA (Kim Biba)
    - Working on East Bay project with GLO
j. USFWS – no representatives present
k. TAMUG – Dollar Bay Reef Restoration Monitoring (Laura Jurgens)
    - TAMUCC & TAMUG
    - 5 years of monitoring
    - Constructed with different reef mound densities (narrow vs. broad)/mound spacing (20’ vs. 40’) to determine efficacy of mounds
l. TAMUG – Dermo Surveys (Laura Jurgens)
    - Restarting dermo surveys in Galveston Bay
    - Currently a student project
    - Initial data indicates there is a significant amount of dermo in Galv. Bay
    - Working on acquiring data on dermo prevalence in different parts of the Bay by mid-2023
    - No relationship with Oyster Sentinel/Webpage – nothing has been done with this data since 2015; old oyster shell budget model/tool
b. Freese & Nichols (Dave Buzan)
    - Monitoring oyster growth on Matagorda Bay Living Shoreline
    - USFWS project – shoreline protection via DU (Freese is assisting with permitting); miles of rock substrate along GIWW
    - Need documentation & BMPs for monitoring oyster growth on breakwaters – reference GLO’s Coastal Study
    - Group discussion – need to ID basic methodologies to know if breakwaters truly provide oyster substrate (quantitative vs. qualitative data); Laura J. – shouldn’t be difficult to get a good quantitative design on rip-rip, just need calm water and visibility to use quadrat sampling

C) Hurricane Harvey Oyster Reef Restoration Project

Evan Pettis, TX Parks & Wildlife Department

- TPWD's restoration with Hurricane Harvey funds:
  - 1104 ac – bagless dredging
  - 50 ac – dealer led cultch placement
  - 606 ac – contracted cultch placement
- Dollar Bay Reef
  - Heavily degraded but a lot of shell/hash to support restoration
  - Restored Oct. 2021 (closed until Nov. 2023)
  - $215/CY^3 limestone
  - Footprint = 13.5 ac (restored mosaic = 35 ac)
  - TAMUG/TAMUCC – research/monitoring partners
  - Cultch Mounds
    - Diameter = 10 ft
    - Height = 2ft
    - Spacing = 20' and 40' (center to center)
  - Monitoring = acoustic mapping & patent tongs; will continue sampling after reef opens to harvest
  - Low salinity = low recruitment immediately after construction
  - Evidence of barge-traffic & shrimper impacts
  - Now excellent recruitment & salinity level
- Other reef designs being studied:
  - Grass Island – mounds vs flats
  - Keller Reef – 3" vs. 6" layer of cultch (flats)
  - Resignation Reef – alternative cultch materials – shell on rock & vice versa
  - Josephine's Reef – 1' vs. 2' mound heights (have not exceed 3' b/c of water depths but possible in the future
- All monitoring is conducted biannually
- Typical material used = limestone or river rock <4"

D) Use of Recycled Oyster Shell in Reef Restoration

Haille Leija, Galveston Bay Foundation

*see attached slides

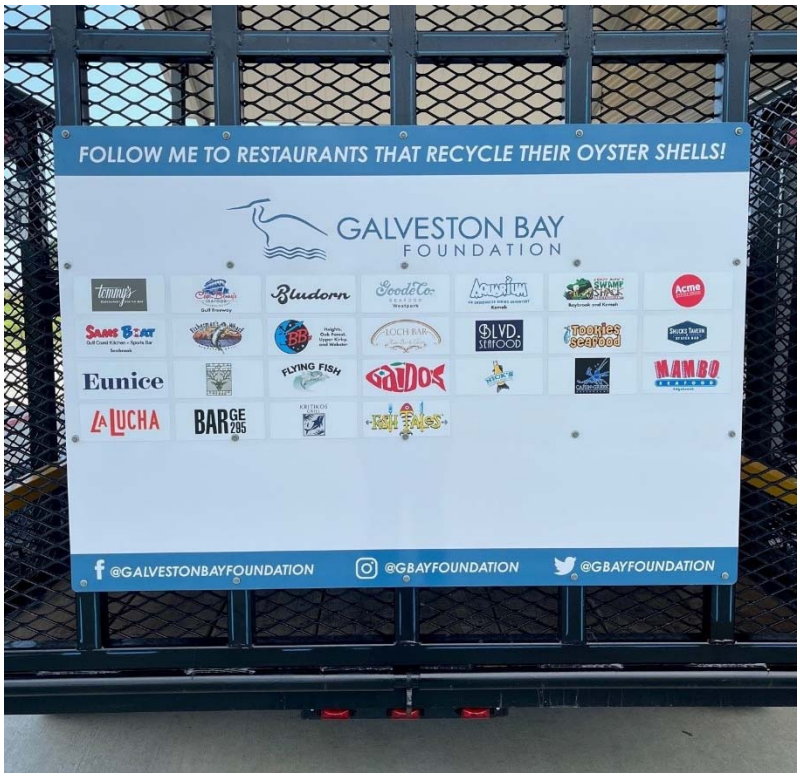
E) Discussion/Planning

a. Contact Carl Sepulveda (carl.sepulveda@freese.com) with Freese & Nichols – use of oyster shell for capping sediment
b. Future Presentation topics:
   i. Breakwater oyster growth
   ii. Monitoring of large cultch (ex: Trinity Bay Sanctuary Reef – measuring vol)
   iii. Diver quadrats
   iv. Monitoring BMPs in general
c. Annual meetings moving forward – November, before the holiday

Task 2
Oyster Shell Recycling Program Outreach Materials

Rack Cards

Window Clings



Back Gate Trailer Sign

Sidewall Trailer Sign

APPENDIX C
Task 3 Deliverables

VOLUNTEER OYSTER GARDENING

Task 3
Texas Parks and Wildlife Department Introduction Permit

TEXAS PARKS & WILDLIFE

Life's better outside.®

November 4, 2022 IP_UC_I1042022a

Haille Leija
1725 Highway 146
Kemah, TX 77565

Dear Ms. Leija,

TPWD regulates the introduction and stocking of fish, shellfish, and aquatic plants into the public water of the state (Texas Parks and Wildlife Code §12.015, 12.019, 66.015, and Texas Administrative Code §57.251-259). Specifically, TAC Title 31 Part 2 Subchapter C Rule §57.252 requires a permit for the introduction of fish, shellfish, and aquatic plants into public waters.

Your application and required documentation have been received and reviewed in accordance with TPWD regulations. Your request to relocate and introduce native Eastern oysters (*Crassostrea virginica* spat up to ~2") sourced from the Galveston Bay Foundation Oyster Gardening Program oyster garden shell bags in the West Galveston Bay system has been approved. Please review the attached special permit conditions for this project.

This letter constitutes an approval to relocate and introduce Eastern oyster, *Crassostrea virginica*, into Galveston Bay at the locations listed on the attached table and map. Oysters must be from the approved source (garden bags at the locations in Exhibit A) and can only be placed at the locations shown in Exhibit B. Oysters grown in the West Galveston Bay communities (see Exhibit A) must only be placed at West Bay restoration sites (Jones Bay, Sweetwater West Bay, Sweetwater Lake) and cannot be placed onto any of the reefs in Galveston Bay north of Texas City Dike (Exhibit B). This permit is valid for one year beginning on November 4, 2022. You must contact this Department (Claire Iseton, Claire.Iseton@tpwd.texas.gov) at least 7 days prior to stocking and include TPWD Oyster Transport Chain of Custody (TPWD form PWD1439F) for each stocking event. A copy of this letter and the Oyster Transport Authorization must be in your possession at the time of the stocking.

I appreciate your efforts to cooperate and fulfill the requirements of this law.

Sincerely,

Claire Iseton

Claire Iseton
Ecosystem Leader
Ecosystem Resource Assessment Team
1502 FM 517 East
Dickinson, TX 77539

CI:cicc: Jackie Robinson, Emma Clarkson, Lindsay Campbell, IFpermits

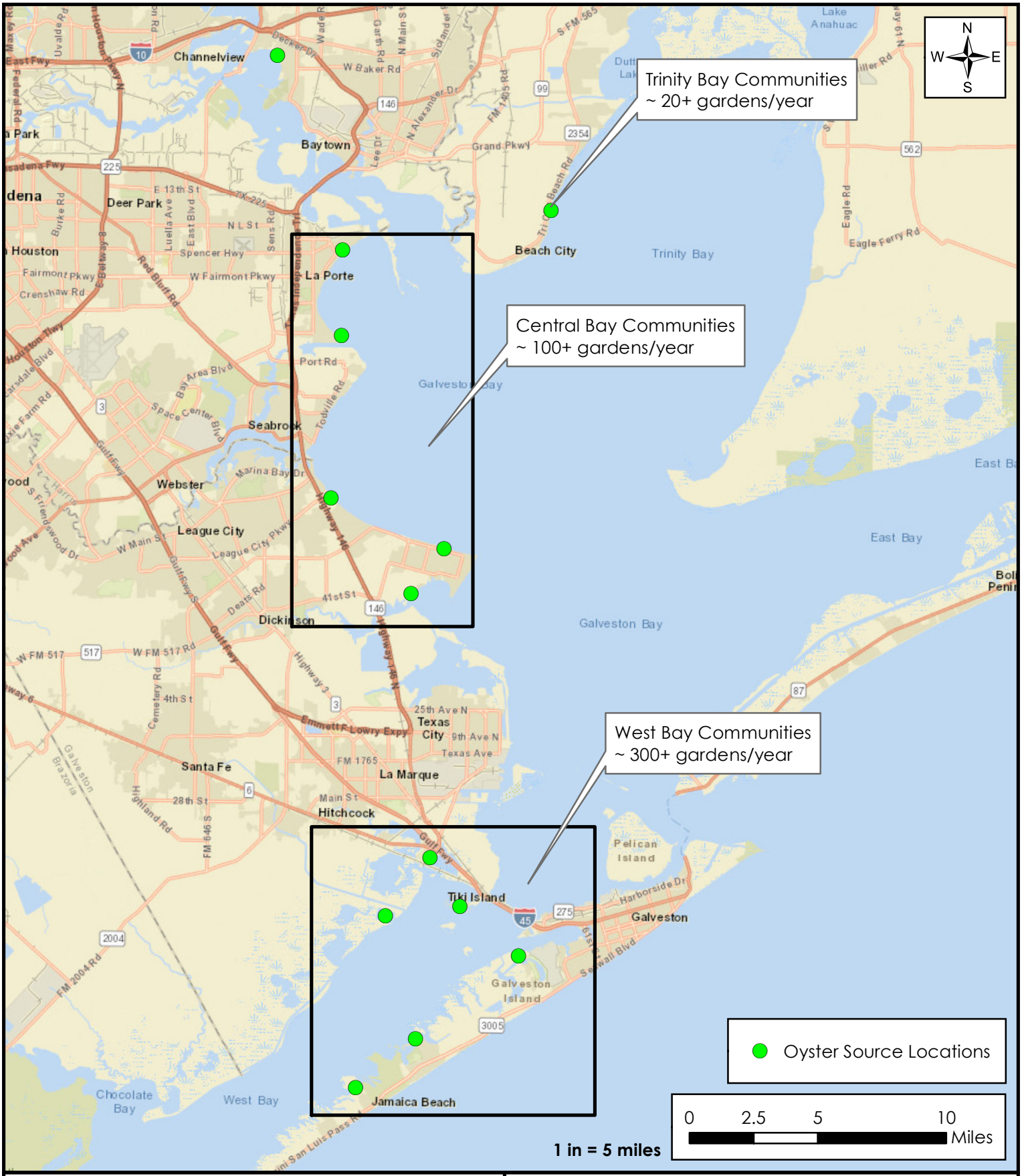
4200 SMITH SCHOOL ROAD
AUSTIN, TEXAS 78744-3291
512.389.4800
www.tpwd.texas.gov

TEXAS PARKS AND WILDLIFE DEPARTMENT PERMIT TO INTRODUCE FISH, SHELLFISH, OR AQUATIC PLANTS INTO PUBLIC WATERS SPECIAL PERMIT CONDITIONS

The following conditions are precedent to the issuance of this permit (IP_UC_11042022a)

1. This permit is valid until November 4, 2023.
2. The applicant should adhere to TPWD guidelines for introducing the application species.
3. The introduced specimen will be Eastern Oyster (*Crassostrea virginica*) seed <2" in size grown in oyster garden bags in the Galveston Bay system. Oysters grown in West Galveston Bay (see "West Bay Communities" on Exhibit A) MUST ONLY be placed on reefs in West Galveston Bay (including Jones Bay reefs, Sweetwater West Bay reefs, and Sweetwater Lake reefs) and cannot be stocked at the Galveston Bay reefs (north of the Texas City Dike).
4. The applicant is responsible for procuring all necessary permits (Scientific Collector's permit), CWA Section 404 permit (U.S. Army Corps of Engineers Water Quality (Texas Commission of Environmental Quality) and leases (Texas General Land Office Surface Lease) necessary to conduct the proposed research.
5. The permittee shall provide written notice to the appropriate TPWD field contact of the plans for each introduction activity at least one week prior to the scheduled introduction. Notice should include the Oyster Transport Chain of Custody Document (TPWD Form PWD 1439A). Permittee must receive written approval from TPWD seven (7) days prior to any transfer of oysters or seed between sites or imported from an out-of-state hatchery. Upon completion of the research, all experimental materials (Cultch, oysters, silt curtains, anchors, etc.) will be removed from the research site. The permittee shall provide the appropriate TPWD field contact with a notice of final research completion.
6. All oyster transports must conform to the TPWD Oyster Biosecurity Protocols
7. Upon completion of each introduction effort, the permittee shall provide the appropriate TPWD field contact with a notice of completion that includes the date of completion, number of oysters introduced and a map illustrating the introduction site of the oysters.
8. The permittee shall provide TPWD with research or monitoring reports, and any publications produced from this research.

EXHIBIT A - OYSTER SOURCE LOCATIONS



Proposed Source Locations (2021-2022)	
Project Name: Oyster Introductions	
Project Location: Galveston Bay & adjacent Sub-bay Systems	
Image Source: ESRI World Street Map	
Projection: NAD 1983, UTM Zone 15N	
Date Drawn: 11/4/2021	Drawn by: H.Leija

GALVESTON BAY FOUNDATION

1725 Highway 146, Kemah, TX; (281) 332-3381

EXHIBIT B - OYSTER INTRODUCTION LOCATIONS

Proposed Introduction Sites (2021-2022)	
Project Name: Oyster Introductions	
Project Location: Galveston Bay & adjacent Sub-bay Systems	
Image Source: ESRI World Street Map	
Projection: NAD 1983, UTM Zone 15N	
Date Drawn: 11/4/2021	Drawn by: H.Leija

GALVESTON BAY FOUNDATION

1725 Highway 146, Kemah, TX; (281) 332-3381

Proposed Oyster Introduction Sites
2022-2023

Site Name		Project Title	Waterbody	Latitude	Longitude
1	Baytown Nature Center - Burnet Bay	BNC Oyster Shell Breakwater	Burnet Bay	29.757529	-95.049517
2	Baytown Nature Center - Crystal Bay	BNC Reef Restoration & Shoreline Protection	Crystal Bay	29.745466	-95.05293
3	Dickinson Bay Reef	Dickinson Bay Reef Restoration	Dickinson Bay	29.469668	-94.948274
4	Jones Bay Reefs	Oystercatcher Habitat Restoration	Jones Bay	29.297379	-94.939588
5	Kemah Headquarters Reef	Kemah Living Shoreline	Galveston Bay	29.532518	-95.008263
6	Kemah Boardwalk Reef	TPWD Kemah Reef	Galveston Bay	29.547365	-95.016610
7	Morgan's Point Reef	J.Lodge Reef	Galveston Bay	29.666415	-94.995543
8	San Leon Reefs	TPWD San Leon Reef	Galveston Bay	29.499133	-94.922980
9	Sweetwater Lake	Sweetwater Lake Oyster Shell Breakwater	Sweetwater Lake	29.254973	-94.880110
10	Sweetwater West Bay	Sweetwater W. Bay Oyster Shell Breakwater	West Galveston Bay	29.269409	-94.891607
11	Trinity Bay Discovery Center	TBDC Living Shoreline	Trinity Bay	29.714685	-94.85427
12	Trinity Bay Reef	Galveston Bay Sustainable Oyster Reef Restoration	Trinity Bay	29.638823	-94.864224

Crassostrea virginica

TEXAS PARKS & WILDLIFE

Life's better outside.®

Commissioners

Arch "Beaver" Aplin, III
Chairman
Lake Jackson

Dick Scott
Vice-Chairman
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James E. Abell
Kilgore

Oliver J. Bell
Cleveland

Paul L. Foster
El Paso

Anna B. Galo
Laredo

Jeffery D. Hildebrand
Houston

Robert L. "Bobby" Patton, Jr.
Fort Worth

Travis B. "Blake" Rowling
Dallas

Lee M. Bass
Chairman-Emeritus
Fort Worth

T. Dan Friedkin
Chairman-Emeritus
Houston

Carter P. Smith
Executive Director

November 4, 2022

IP_UC_11042022b

Haille Leija
1725 Highway 146
Kemah, TX 77565

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Sincerely,

Claire Iseton

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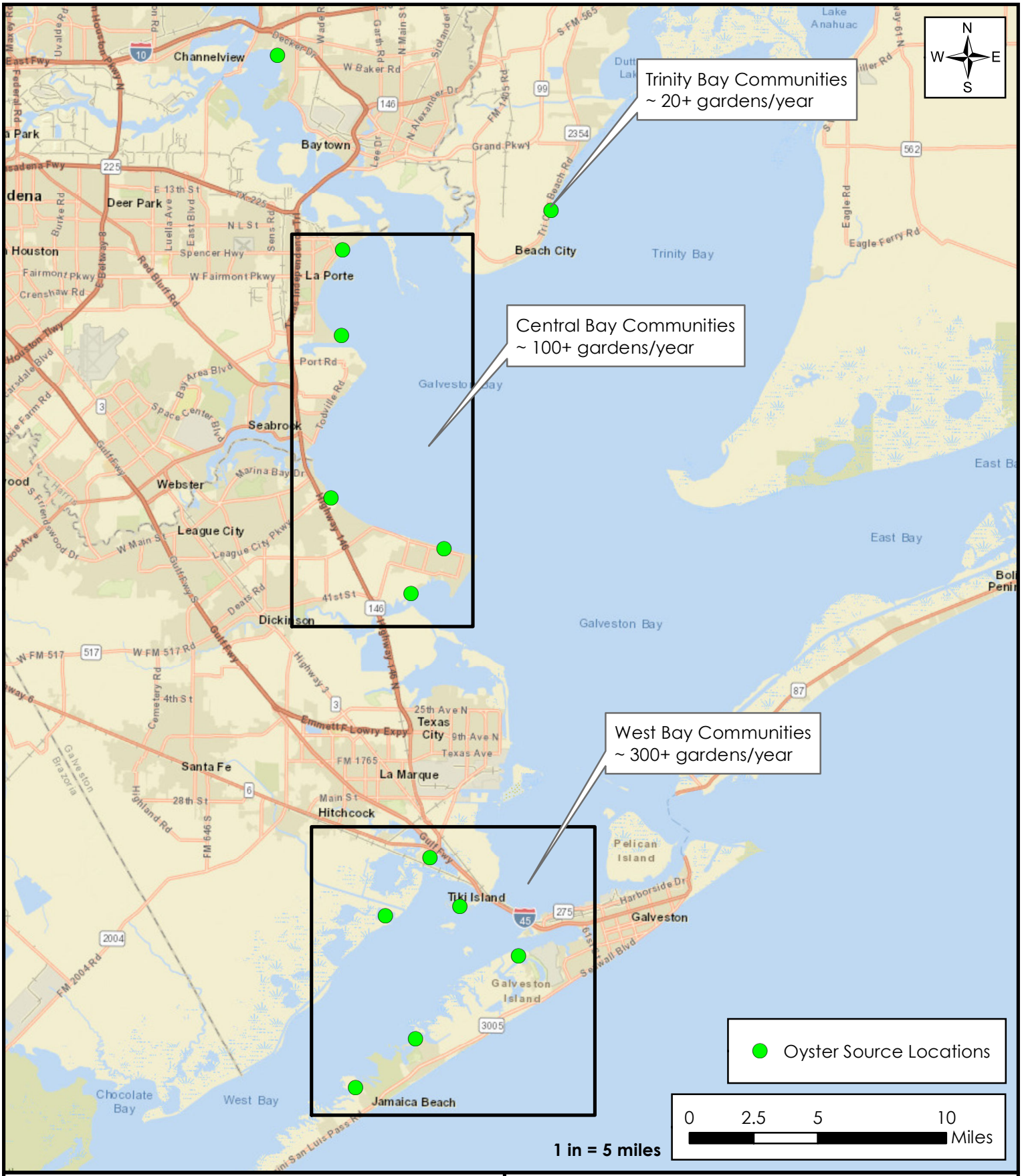
AUSTIN, TEXAS 78744-3291
512.389.4800
www.tpwd.texas.gov

TEXAS PARKS AND WILDLIFE DEPARTMENT PERMIT TO INTRODUCE FISH, SHELLFISH, OR AQUATIC PLANTS INTO PUBLIC WATERS SPECIAL PERMIT CONDITIONS

The following conditions are precedent to the issuance of this permit (IP_UC_11042022b)

1. This permit is valid until November 4, 2023.
2. The applicant should adhere to TPWD guidelines for introducing the application species.
3. The introduced specimen will be Eastern Oyster (*Crassostrea virginica*) seed <2" in size grown in oyster garden bags in the Galveston Bay system. Oysters grown in Central and Upper Galveston Bay (see "Central Bay Communities" and "Trinity Bay Communities" on Exhibit A) MUST ONLY be placed on reefs in Central and Upper Galveston Bay (see Exhibit B) and cannot be stocked at the West Galveston Bay reefs (south of the Texas City Dike).
4. The applicant is responsible for procuring all necessary permits (Scientific Collector's permit), CWA Section 404 permit (U.S. Army Corps of Engineers Water Quality (Texas Commission of Environmental Quality) and leases (Texas General Land Office Surface Lease) necessary to conduct the proposed research.
5. The permittee shall provide written notice to the appropriate TPWD field contact of the plans for each introduction activity at least one week prior to the scheduled introduction. Notice should include the Oyster Transport Chain of Custody Document (TPWD Form PWD 1439A). Permittee must receive written approval from TPWD seven (7) days prior to any transfer of oysters or seed between sites or imported from an out-of-state hatchery. Upon completion of the research, all experimental materials (Cultch, oysters, silt curtains, anchors, etc.) will be removed from the research site. The permittee shall provide the appropriate TPWD field contact with a notice of final research completion.
6. All oyster transports must conform to the TPWD Oyster Biosecurity Protocols
7. Upon completion of each introduction effort, the permittee shall provide the appropriate TPWD field contact with a notice of completion that includes the date of completion, number of oysters introduced and a map illustrating the introduction site of the oysters.
8. The permittee shall provide TPWD with research or monitoring reports, and any publications produced from this research.

EXHIBIT A - OYSTER SOURCE LOCATIONS



Proposed Source Locations (2021-2022)	
Project Name: Oyster Introductions	
Project Location: Galveston Bay & adjacent Sub-bay Systems	
Image Source: ESRI World Street Map	
Projection: NAD 1983, UTM Zone 15N	
Date Drawn: 11/4/2021	Drawn by: H.Leija

GALVESTON BAY FOUNDATION

1725 Highway 146, Kemah, TX; (281) 332-3381

EXHIBIT B - OYSTER INTRODUCTION LOCATIONS

Proposed Introduction Sites (2021-2022)	
Project Name: Oyster Introductions	
Project Location: Galveston Bay & adjacent Sub-bay Systems	
Image Source: ESRI World Street Map	
Projection: NAD 1983, UTM Zone 15N	
Date Drawn: 11/4/2021	Drawn by: H.Leija

GALVESTON BAY FOUNDATION

1725 Highway 146, Kemah, TX; (281) 332-3381

Proposed Oyster Introduction Sites
2022-2023

Site Name		Project Title	Waterbody	Latitude	Longitude
1	Baytown Nature Center - Burnet Bay	BNC Oyster Shell Breakwater	Burnet Bay	29.757529	-95.049517
2	Baytown Nature Center - Crystal Bay	BNC Reef Restoration & Shoreline Protection	Crystal Bay	29.745466	-95.05293
3	Dickinson Bay Reef	Dickinson Bay Reef Restoration	Dickinson Bay	29.469668	-94.948274
4	Jones Bay Reefs	Oystercatcher Habitat Restoration	Jones Bay	29.297379	-94.939588
5	Kemah Headquarters Reef	Kemah Living Shoreline	Galveston Bay	29.532518	-95.008263
6	Kemah Boardwalk Reef	TPWD Kemah Reef	Galveston Bay	29.547365	-95.016610
7	Morgan's Point Reef	J.Lodge Reef	Galveston Bay	29.666415	-94.995543
8	San Leon Reefs	TPWD San Leon Reef	Galveston Bay	29.499133	-94.922980
9	Sweetwater Lake	Sweetwater Lake Oyster Shell Breakwater	Sweetwater Lake	29.254973	-94.880110
10	Sweetwater West Bay	Sweetwater W. Bay Oyster Shell Breakwater	West Galveston Bay	29.269409	-94.891607
11	Trinity Bay Discovery Center	TBDC Living Shoreline	Trinity Bay	29.714685	-94.85427
12	Trinity Bay Reef	Galveston Bay Sustainable Oyster Reef Restoration	Trinity Bay	29.638823	-94.864224

Crassostrea virginica

Task 3
Annual Gardening Report

ANNUAL OYSTER GARDENING REPORT

TASK 3 DELIVERABLE

Project Name: Galveston Bay Foundation Oyster Shell Recycling Program Phase 4: Sun Curing Research & Collaboration

GLO Contract No: 22-045-005-D102

Deliverable: Task 3 – Volunteer Oyster Gardening

Due Date: 09/30/2023

I. PROJECT DESCRIPTION

Since 2012, the Galveston Bay Foundation (GBF) has fostered relationships in bayfront communities to "garden" oysters. Waterfront homeowners in these communities volunteer as oyster gardeners and suspend mesh bags, lines (stringers), or cages containing recycled oyster shells ("oyster gardens") from their piers, docks, or bulkheads to recruit oyster larvae. The oyster gardens are submerged in the bay during the spawning season, approximately May through November. Volunteers monitor and care for the oyster gardens throughout the summer and early fall to promote successful growth of baby oysters (spat) recruited on the recycled shell. In the fall, GBF staff collect the oyster gardens and spread the shells and new oysters on nearby restoration reefs to enhance the local oyster population. The volunteers not only learn about the lifecycle of the Eastern oyster and the importance of oyster reefs in the Galveston Bay ecosystem, but they are also exposed to a variety of marine life that find shelter in the oyster gardens. Furthermore, oyster gardening volunteers have the opportunity to participate in citizen science through GBF's oyster recruitment studies.

II. SUMMARY OF 2022 VOLUNTEER OYSTER GARDENING

a) Oyster Garden Creation Events

To reduce the annual supply needs and allow the focus of the Garden Creation Events to be education, GBF suggested volunteers limit the number of oyster gardens managed by each household to a maximum of three. As the number of participating volunteers has increased, the time commitment for staff has risen dramatically, particularly in the fall when gardens are collected. Documentation of oyster growth in each individual garden is a time-consuming process. Therefore, reducing the number of gardens per volunteer will reduce expenses associated with staff time while continuing to facilitate the collection of valuable data. Overall, the volunteers were content with managing up to three gardens. A couple dedicated volunteers have their

grandchildren participate in the gardening process, therefore they requested to build enough gardens for each of their grandchildren, which was accepted. A few volunteers were persistent with building more than three gardens, so while it was suggested to limit the number of gardens to three it was not strictly enforced.

To accommodate additional volunteers in more bayfront communities, GBF staff hosted seven Oyster Garden Creation Events in the summer of 2022. The events were held in Moody Gardens, Dickinson, Tiki Island, San Leon, Beach City, and Bayou Vista (Table 1). Originally the event on June 11th was scheduled to be held at a volunteer's bayfront home in Galveston, however due to the host's health concerns the event was relocated to Moody Gardens. Volunteers and GBF staff worked together to build over 400 oyster gardens (Table 2 and Figures 1-2). At these events, volunteers were also educated on the oyster gardening process and oyster reef ecology. All volunteers were given the option of three garden types: bags, cages, or stringers. A total of 449 oyster gardens were suspended off piers, docks, and bulkheads at 126 bayfront homes in 2022 (Table 2).

Table 1: Garden Creation Events

Garden Creation Event Location	Event Date	Volunteer Attendees
Moody Gardens	5/7/22	9
Dickinson	5/17/22	10
Tiki Island	5/21/22	31
San Leon	5/28/22	12
Beach City	6/9/22	8
Moody Gardens	6/11/22	9
Bayou Vista	6/21/22	37
Total		**116**

Table 2: Oyster Garden Creation

Community	Volunteer Homes	Bags Deployed	Cages Deployed	Stringers Deployed	TOTAL Gardens Deployed
Bayou Vista	32	67	17	24	108
Baytown	6	16	0	0	16
Beach City	9	19	0	9	28
Clear Lake Shores	1	1	1	1	3
Dickinson	2	0	3	3	6
Galveston	14	30	14	7	51
Hitchcock	8	15	4	21	40
Jamaica Beach	3	1	3	5	9
League City	1	1	1	1	3
Omega Bay	7	11	5	4	20
Pirates Cove	4	3	7	3	13
San Leon	8	25	3	6	34
Sea Isle	4	9	2	1	12
Seabrook	1	1	1	1	3
Tiki Island	26	25	33	45	103
TOTALS:	**126**	**224**	**94**	**131**	**449**

b) Oyster Garden Monitoring

Throughout the remainder of 2022, volunteers monitored their gardens for oyster recruitment. Volunteers were instructed to rinse their gardens weekly to help reduce biofouling and predation. Weekly maintenance also allowed volunteers to inspect their gardens for new oyster growth. GBF staff sent out maintenance and monitoring reminders via email and Facebook to help support the volunteers throughout the gardening season. Facebook posts and regular emails also provided an opportunity for questions and answers, further supporting volunteers in their gardening efforts.

To capture the volunteers' time committed to monitoring and maintaining their oyster gardens, GBF staff created an online form (https://www.emailmeform.com/builder/form/18cW804zms50) to allow volunteers to log their hours monthly. GBF staff sent out monthly reminders via email containing a link to the form. While this method helps improve documentation of volunteer hours, only a small portion of the volunteers utilized the online form.

In 2022, volunteers were encouraged to document their oyster recruitment throughout the season, rather than solely in the fall at the collection events. A link to an online data form (https://www.emailmeform.com/builder/form/Lv550hdRQj4k6r) was provided to all volunteers to submit their oyster recruitment data as often as they would like. Submitting the data was not

a requirement, but available for those who wished to participate. Approximately ten volunteers submitted their oyster recruitment data throughout the season.

c) Oyster Garden Collection

In the fall of 2022, GBF staff coordinated the collection of the oyster gardens through five community events. The first event was held at a volunteer's home in San Leon on October 15, 2022. The second event was held at Bayou Vista's City Pavilion on October 29, 2022. The third and fourth events were both held on November 5, 2022, one was at a volunteers' home in Tiki Island and the other was at Moody Gardens in Galveston. The fifth event was held at GBF's Trinity Bay Discovery Center in Beach City on November 15, 2022. Volunteers delivered their gardens to these locations where GBF staff received the gardens, documented new oyster growth, and prepped the gardens for transport (Figures 3-6).

Volunteers unable to attend a community event were encouraged to arrange with a neighbor attending the collection event to deliver their gardens. If the volunteer could not coordinate delivery with a neighbor, GBF staff collected the volunteer's gardens in each community the day of the collection event.

Thanks to the dedicated volunteers across 126 bayfront homes who participated in oyster gardening in 2022, approximately 7,555 oysters were recruited in the oyster gardens (Figures 7-11). Please note, the total number of oysters documented in each garden includes both live and recently dead oysters to provide an estimate of overall recruitment (Table 3). These oysters were introduced onto restoration reefs in October and November 2022 under separate grant funding (Figures 12-13). Table 4 shows the total number of oysters and the total cubic yards of oyster shells transplanted at each restoration site. Please note, GBF holds permits via the Texas Parks and Wildlife Department, Texas General Land Office, and U.S. Army Corps of Engineers to introduce oysters and shell into Galveston Bay and the respective sub-bay systems. These permits are available upon request.

The number of gardens deployed at the beginning of the season (449) was greater than the number of gardens collected at the end of the season (410). The decrease in the number of gardens collected was due to the loss of gardens from storms, potential theft, or not being able to get in contact with the volunteer at the time of the Garden Collection Events in the fall.

Table 3: Oyster Garden Collection and Oyster Recruitment

Community	Gardens Collected	Total Oysters Recruited	Avg. Oysters per Garden
Bayou Vista	85	98	1
Baytown	12	3	0
Beach City	28	53	2
Clear Lake Shores	3	16	5
Dickinson	6	0	0
Galveston	46	405	9
Hitchcock	36	262	7
Jamaica Beach	7	4	1
League City	3	0	0
Omega Bay	18	15	1
Pirates Cove	13	206	16
San Leon	31	599	19
Sea Isle	12	304	25
Seabrook	3	1	0
Tiki Island	107	5,589	52
TOTALS:	**410**	**7,555**	**18**

Table 4: Oyster Introductions

Date of Introduction	CY of Shell Transplanted	Total Oysters Introduced	Source Location	Introduction Location	
				Bay/Sub-bay	***Project Site***
10/15/22	0.17	517	Clear Lake Shores Dickinson League City San Leon Seabrook	Dickinson Bay	Dickinson Bay Oyster Reef
10/29/22	0.36	669	Bayou Vista Hitchcock Omega Bay	West Galveston Bay	Sweetwater Lake Oyster Shell Breakwater (Sec. B)
11/05/22	0.23	6,084	Galveston Jamaica Beach Pirates Cove Sea Isle Tiki Island	West Galveston Bay	Sweetwater Lake Oyster Shell Breakwater (Sec. D)
11/15/22	0.17	56	Baytown Beach City	Trinity Bay	TBDC Living Shoreline
11/15/22	0.01	130	Sea Isle	West Galveston Bay	N/A
11/30/22	0.02	99	San Leon	Central Galveston Bay	TPWD San Leon Reefs
	0.97	**7,555**	**Please note, all oyster introductions were conducted under separate funding.*		

III. FINDINGS & LESSONS LEARNED

a) Community Assessment

In 2022, the communities of Tiki Island, Sea Isle, San Leon, and Pirates Cove documented the highest amount of oyster growth in their gardens while the other communities observed lower levels of oyster recruitment. Tiki Island led the way with an average of 52 oysters per garden and the Sea Isle oyster gardens contained an average of 25 oysters per garden. San Leon had an average of 19 oysters per garden and Pirates Cove had an average of 16 oysters per garden. Galveston and Hitchcock had slightly lower recruitment with an average of nine and seven oysters per garden, respectively. Clear Lake Shores, Beach City, Bayou Vista, Omega Bay, and Jamaica Beach experienced the lowest recruitment with an average of five or less oysters per garden (Chart 1). For all 15 communities, an overall average of 18 oysters per garden was recorded.

Compared to 2021, the San Leon oyster gardens had higher recruitment levels, specifically for the waterfront homes along E Bayshore Dr., which have piers located directly in Galveston Bay. In 2022, GBF staff documented 599 oysters in the San Leon gardens, which resulted in an average of 19 oysters per garden.

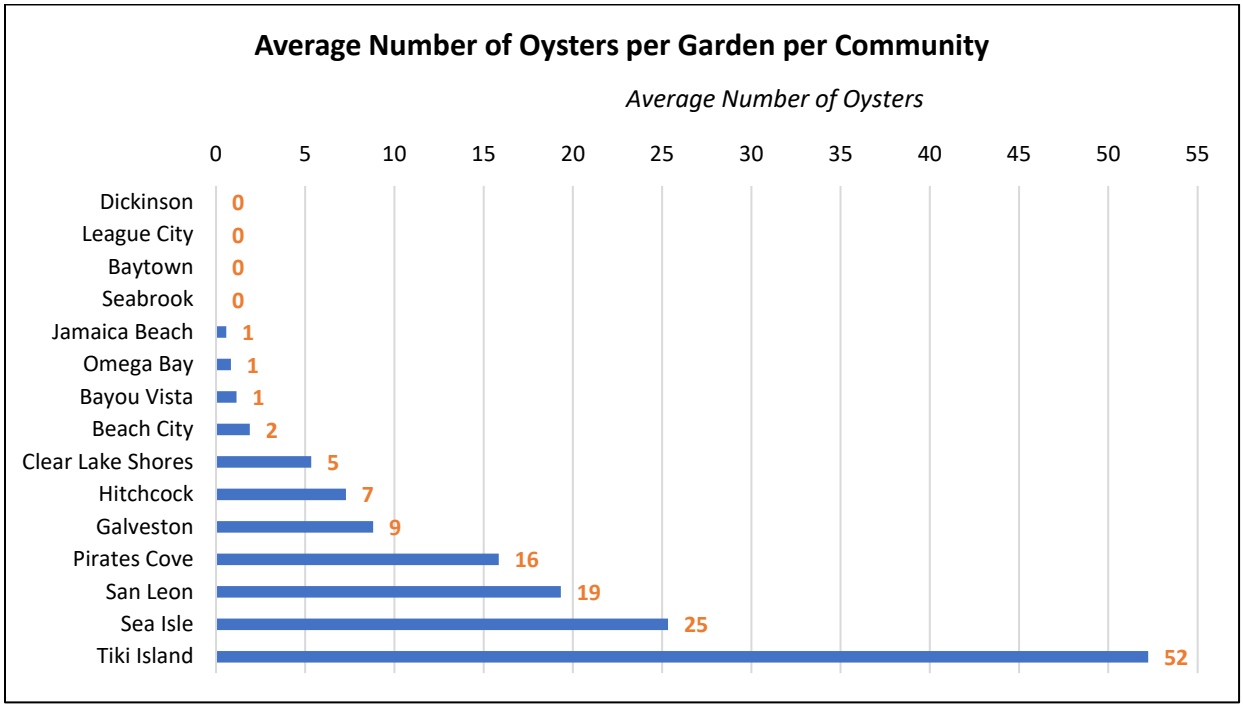
In Bayou Vista, most of the oyster growth was observed along the marsh edge, off Blue Heron Street and along Highland Bayou, whereas the gardens within the canals were overgrown with

dark false mussels (Figure 10). Within Bayou Vista, the highest amount of oyster recruitment was observed in the oyster gardens located at the end of Houston Dr.

The communities in central and northern Galveston Bay received relatively lower oyster recruitment. Two new waterfront communities in this region attempted oyster gardening for the first time in 2022, specifically in Baytown and Clear Lake Shores. For Baytown, only three oysters were recruited out of the 12 gardens collected. However, 16 oysters were documented within the Clear Lake Shores gardens, which resulted in an average of five oysters per garden.

Using web-based ArcGIS, GBF staff created an online map displaying every household that has participated in oyster gardening from 2020 through 2022 along with their spat counts and spat averages for each year. The link to the online map (https://arcg.is/08azKm) was shared with the volunteers via email. Positive feedback was received from the volunteers, and they enjoyed viewing the data depicted on a map. The link to the map was also shared with GBF's contact at Texas Parks and Wildlife Department.

Chart 1: Oyster Recruitment per Community



b) Garden Type Assessment

Since 2018, GBF has utilized three different garden types, bags, stringers, and cages (Figures 14-16), and has continued to assess the pros and cons of each. For 2022, GBF staff modified the construction of the stringer gardens slightly by utilizing weed eater cord instead of metal wire. At the oyster garden collection events in the fall when the oyster shells are removed from the stringer, at times, the shells were very difficult to remove from the metal wire. In 2021, a volunteer used weed eater cord to assemble stringer gardens and at the collection event GBF

staff observed the oyster shells slid off the weed eater cord effortlessly compared to the metal wire. A spool of weed eater cord is also less expensive than metal wire.

GBF staff also modified the construction of the cage gardens somewhat by utilizing mesh material with one-inch squares rather than the previous mesh material that had one and a half inch squares. Some oyster shells would fall through the one and a half inch squares, therefore the one-inch squares are a more suitable size for containing the oyster shells inside the cage during the gardening season.

Oyster growth documentation in 2022 indicates cages had the highest levels of oyster recruitment and oyster retention with an average of 48 oysters per cage. The bags and stringers had similar levels of oyster recruitment and retention with an average of 11 and 8 oysters per garden respectively (Chart 2). These results are consistent with observations made in 2021 (Chart 3), indicating the cages may be more effective in oyster recruitment and retention.

As suggested in the 2021 Annual Oyster Gardening Report, it is proposed that the larger openings in the cages provide more water flow than the bags, thus allowing oyster larvae to easily enter the cages to come in contact with the recycled shells. It appears the stringers have limited room for oyster larvae to attach to the recycled shells because of the way the shells are stacked on top of each other on the weed eater cord. The bags are difficult to rinse and often capture heavier loads of sediment, thus covering viable shell and potentially preventing larvae attachment. An additional benefit of the cages is their ability to be reused for at least one to two years whereas bags and stringers are single use only.

While these findings point to cages as the most effective garden type, additional data is needed to confirm this conclusion. GBF plans to continue to offer all three garden types to volunteers as long as funding allows.

Chart 2: Oyster Recruitment in Different Garden Types in 2022

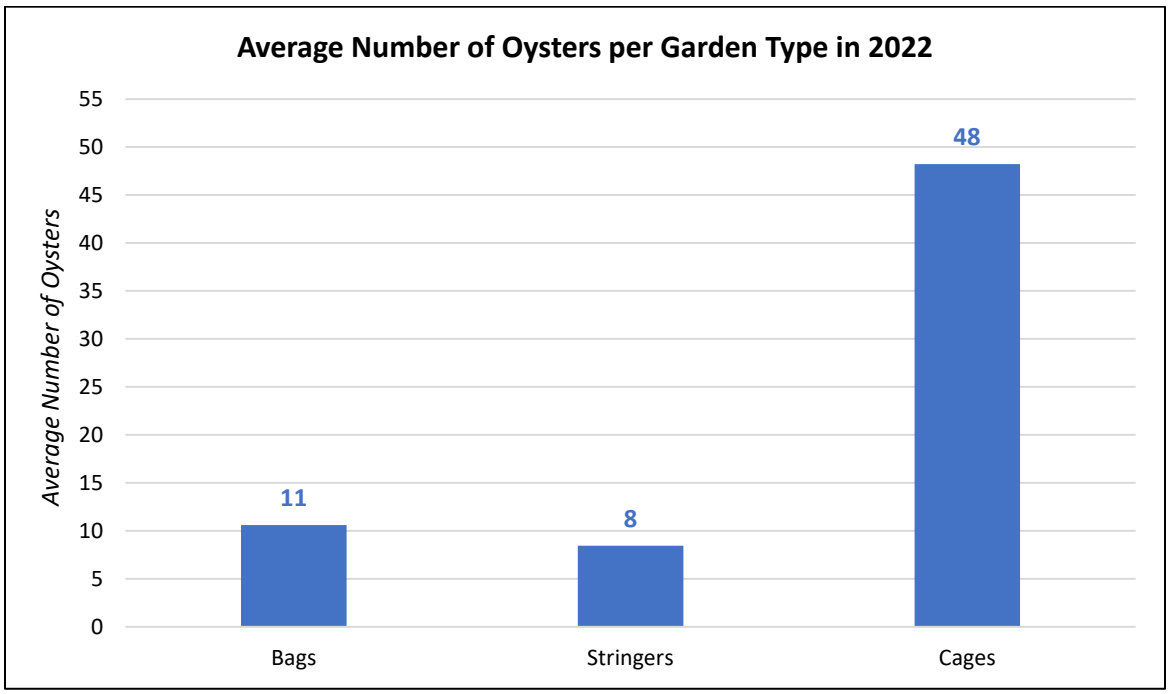
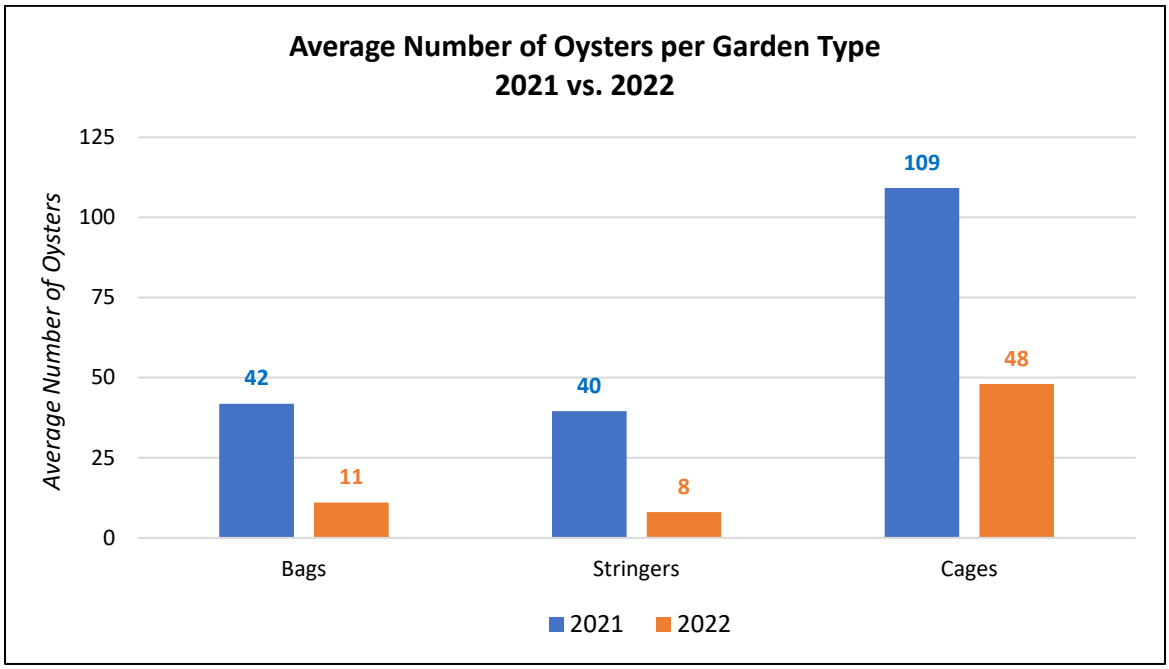


Chart 3: Oyster Recruitment in Different Garden Types 2021 vs. 2022

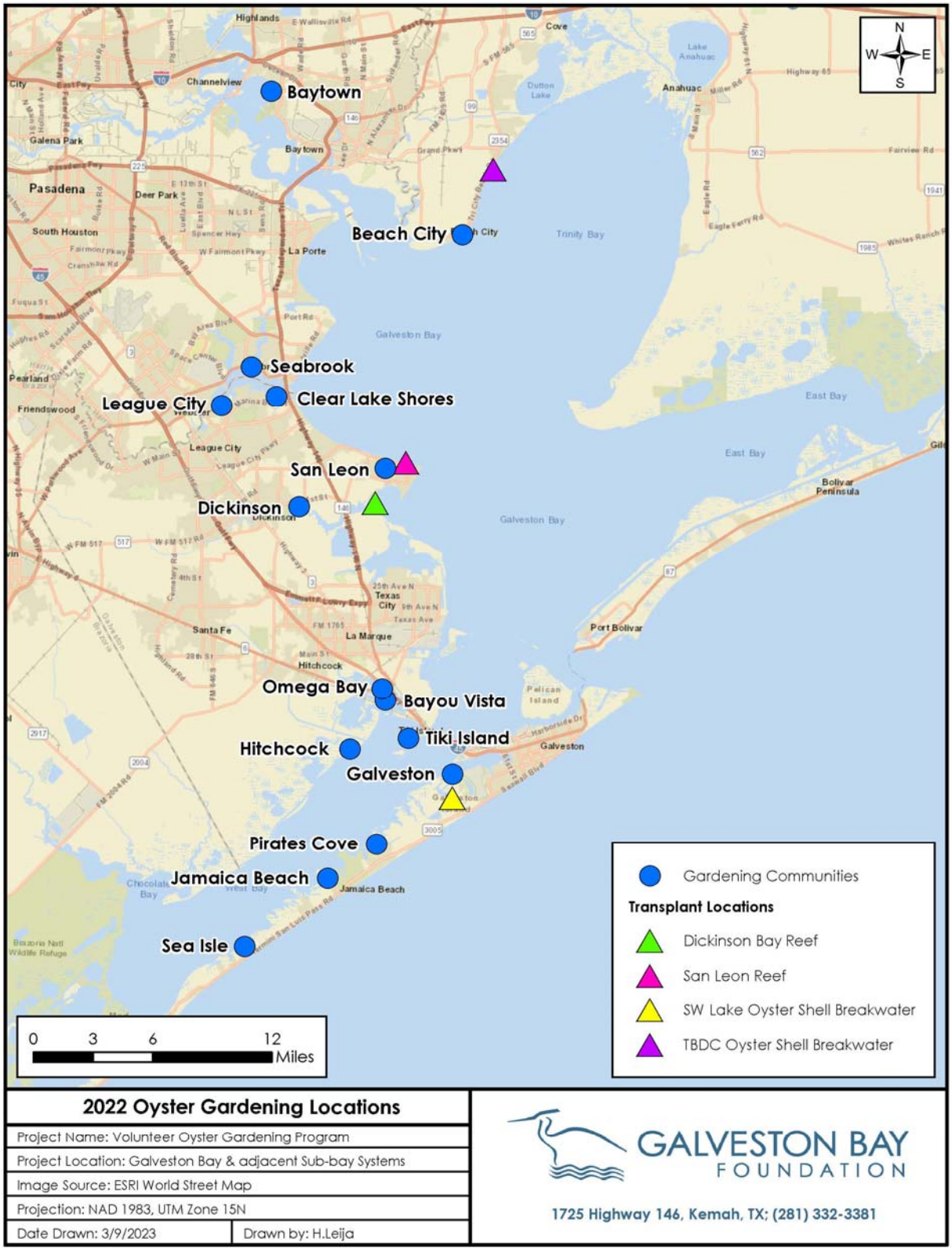


c) Considerations for Future Oyster Gardening

To streamline the Garden Creation Events and reduce expenses next season, GBF staff plan to decrease the number of events held at the beginning of the season when volunteers meet to learn about the Volunteer Oyster Gardening Program and build their gardens to take home. For 2022 seven Oyster Garden Creation Events were held which required a significant amount of time, labor, and cost for GBF staff. Instead of hosting events for each individual bayfront community, the proposed plan for next season will be to host events at Moody Gardens in Galveston as well as GBF's headquarters in Kemah. Hosting the Garden Creation Events at Moody Gardens will help to reduce the number of events while still offering a central location to meet for volunteers located in the lower Galveston Bay region. Likewise, hosting the Garden Creation Events at the GBF headquarters will also help to reduce the number of events while still offering a central location to meet for the mid Galveston Bay region volunteers. A Garden Creation Event will still be held at GBF's Trinity Bay Discovery Center in Beach City for volunteers located in the upper Galveston Bay region. All the Garden Creation Events will be offered to any volunteer no matter what bayfront community they reside in. For volunteers that cannot attend any of the events, it will be recommended they arrange to have a neighbor pick up gardens for them at one of the events or they can schedule a pickup time to receive their gardens at GBF's headquarters. The GBF headquarters is central to all participating communities and an in-person, scheduled pick-up will provide an opportunity for the volunteers to meet with GBF staff and receive proper instructions.

To reduce the manual labor and time requirements at the Dickinson Bay Reef introduction site, GBF staff plan to change the transportation method next season. After all the spat from the gardens of the volunteers located in Clear Lake Shores, Dickinson, League City, San Leon, and Seabrook had been counted and recorded, the spat and shells were transferred to 5-gallon buckets. From the volunteer's residence that hosted the Garden Collection Event, the buckets were then transported by kayak to the Dickinson Bay Reef where the spat and shells were placed onto the reef (Figure 12). While the water conditions were favorable and the kayaking trip was manageable, it was agreed by both the volunteers and GBF staff that having a boat available to assist with the transplanting would be a better option for next season to help reduce the manual labor of loading and unloading the kayaks to/from the water. Next season, GBF staff plan to coordinate the event with either a volunteer boat owner or utilize GBF's boat if the staff that operate the boat are available.

IV. PROJECT LOCATION MAP



V. PROJECT PHOTOGRAPHS

Figure 1. Volunteers building cage oyster gardens at Garden Creation Event (Spring 2022)

Figure. 2. Volunteers building stringer and bag oyster gardens at Garden Creation Event (Spring 2022)

Figure 3. Volunteers documenting oyster growth at Garden Collection Event (Fall 2022)

Figure 4. Volunteers and GBF staff documenting oyster growth at Garden Collection Event (Fall 2022)

Figure 5. Galveston volunteer with spat on shell from her oyster garden (Fall 2022)

Figure 6. Volunteers and GBF staff documenting oyster growth at Garden Collection Event (Fall 2022)

Figure 7. Oyster growth on a single recycled shell from a Tiki Island oyster garden (Fall 2022)

Figure 8. Oyster growth on a single recycled shell from a San Leon oyster garden (Fall 2022)

Figure 9. Spat on recycled oyster shell from a Beach City oyster garden (Fall 2022)

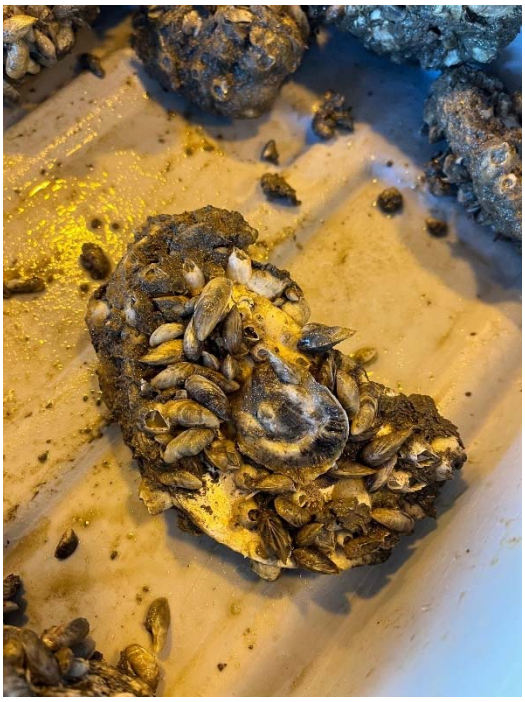


Figure 10. Oyster shell from a Bayou Vista garden encrusted with barnacles, mussels, and one oyster (Fall 2022)

Figure 11. Oyster growth on recycled shell from a Galveston oyster garden (Fall 2022)

Figure 12. A volunteer placing oysters and recycled shell onto Dickinson Bay Reef Restoration Project site (Fall 2022)

Figure 13. Volunteers placing oysters and recycled shell onto Sweetwater Lake Oyster Shell Breakwater (Fall 2022)

Figure 14. Bag oyster garden

Figure 15. Stringer oyster garden

Figure 16. Cage oyster garden

APPENDIX D
Task 4 Deliverables

SUN CURING RESEARCH

Task 4
Photographs

Figure 1. Assembled fenced shell pile (without chicken wire) in preparation for sun curing experiment

Figure 2. Assembled unfenced shell pile in preparation for sun curing experiment

Figure 3. Assembled fenced shell pile (with chicken wire) in preparation for sun curing experiment

Figure 4. Aerial view of all four shell piles for sun curing experiment

Task 4
Report Analyzing Experiment Results

Dermo infection (*Perkinsus marinus*) in Sun-Cured Oyster Shells; Informing Oyster Restoration in Texas

Final Report

EIH Final Report #EIH23-012
Date: September 15, 2023

Prepared by the Environmental Institute of Houston at the University of Houston – Clear Lake and the University of Houston, Honors College, in cooperation with the Galveston Bay Foundation.

University of Houston Clear Lake

Environmental Institute of Houston

TEXAS COASTAL MANAGEMENT PROGRAM

NOAA NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION U.S. DEPARTMENT OF COMMERCE

GALVESTON BAY FOUNDATION

HONORS

Environmental Institute of Houston

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Prepared in cooperation with and for the Galveston Bay Foundation

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Pass-through Entity Federal Award Identification # (FAIN): 22-045-005-D102

Subaward #: GBF-UHCL-2021-001

Recommended citation: Oakley, J.W., G. Guillen, M. Hanke, S. Batte, and H Leija. 2023. Dermo infection (*Perkinsus marinus*) in Sun-Cured Oyster Shells; Informing Oyster Restoration in Texas, Final Report. (Report No. EIH23-012). Prepared for the Galveston Bay Foundation (FAIN: 22-045-005-D102, Subaward #: GBF-UHCL-2021-1). 38 pages.

Contents

List of Figures

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List of Abbreviations

EIH	Environmental Institute of Houston
FAIN	Federal Award Identification Number
GBF	Galveston Bay Foundation
GLM	Generalized Linear Model
GLO	Texas General Land Office
NOAA	National Oceanic and Atmospheric Administration
OSP	Office of Sponsored Programs
OSRP	Oyster Shell Recycling Program
RFTM	Ray's Fluid Thioglycollate Method
TPWD	Texas Parks and Wildlife Department
UH	University of Houston
UHCL	University of Houston-Clear Lake

EXECUTIVE SUMMARY

Oyster reefs provide critical functions for a healthy coastal ecosystem in Galveston Bay, Texas. Oyster reefs have declined to a fraction of their historical coverage resulting in reef restoration becoming a focus for resource managers, commercial oyster industry, researchers, and NGOs. Oyster reef restoration is accomplished by introducing hard substrate, typically in the form of reclaimed shucked shells (or cultch), back into the local environment to be colonized by native spat. The Galveston Bay Foundation's Oyster Shell Recycling Program began in 2011 and they currently collect discarded oyster shells from 35 recycling partners. Recycled oyster shells should be sun-cured (or quarantined) prior to use in restoration projects because they can harbor invasive species and disease. Dermo infection, caused by the spore-forming protozoan parasite *Perkinsus marinus* is a density-dependent limiting factor to oyster population growth. Currently sun-curing recommendations are based on a single study conducted in South Carolina in 2002. With the increasing interest and number of oyster restoration projects this project was developed to investigate current best practices to assess the risk of infecting native oyster reefs with Dermo infection through restoration projects.

This study was purposefully designed to test a "worst-case scenario" for Dermo infection in sun-cured oysters in Texas. Oysters with elevated Dermo infection were obtained and deployed whole, either in the interior or top of four experimental shell piles, to demonstrate the sun-curing process of un-shucked oysters that may enter the recycling pathway. Two of the piles were fenced to limit access by wildlife and two were left unfenced. Oysters were individually numbered and tracked throughout the study. Half of the oysters were tracked for Dermo infection using the Ray's Fluid Thioglycollate Method, while the other half were evaluated for tissue decomposition using percent coverage of tissue and tissue condition categories. Oysters were deployed a total of 35 weeks from October 2022 to June 2023. Temperature and relative humidity monitors were co-located with deployed oysters. The piles were also monitored using game cameras for the first 6 weeks of deployment to assess potential disturbance due to foraging wildlife.

Oysters deployed on the tops of the unfenced piles were depredated by feral hogs within the first 31 hours of deployment. There was a significant difference in the tissue condition and decomposition between the oysters deployed on the tops of the fenced and unfenced piles. Temperatures were higher and more variable on top of the piles, while relative humidity was generally higher in the interior of the piles. Oysters in the interior of the piles were slower to desiccate compared to those at the top of the piles, but once desiccated the oysters in the interior of the piles continued to degrade. More decomposing insects, such as maggots, were observed in association with interior oysters, and they had a lower percent cover of tissue compared to those at the top of the piles. There was a significant decrease in the Dermo infection intensity after the first week of deployment and throughout the study. Oysters in the interior of the piles had significantly less Dermo infection intensity than the top of the piles. No dermo infection was detected in the interior of the piles after the 6th week of deployment, while it was detected on the top of the piles until the 31st week of deployment.

This study used oysters with a historically high initial level of Dermo infection collected from Confederate Reef, which is currently closed to harvest. It is likely that commercially sourced oysters that typically end up in the recycling pathway would have lower background Dermo infection levels, but this hypothesis should be tested further. It is unknown how frequently un-

shucked oysters are found in the recycled shell materials, but oyster recycling staff have observed them regularly while collecting shells. Future audits of oysters entering the recycling pathway (from both commercial and retail sources) should be conducted to quantify the amount of tissue entering the curing piles. For the curing site used in this study, the presence of a robust feral hog population seems to help to remove oyster tissue resulting in expedited curing treatment, but not all sun-curing locations have feral hog populations. Most of the tissue of oysters deployed in the interior of the piles was gone by the 16th week, which corroborates results from the previous study on which current recommendations are based. Alternatively, most oysters deployed at the top of the fenced piles had tissue remaining through the 35th week of our study. The previous study did not evaluate oysters on top of the piles. It was thought that UV light and lower relative humidity levels helped to speed up tissue decomposition and *P. marinus* mortality rates, but our results bring this into question. It is understood that the decomposition rate is positively correlated with higher temperatures. Similar to the 2002 study, we found that the interior temperature was generally lower than the external temperature of the piles. However, we found the oysters deployed in the interior of the piles decomposed more quickly. Therefore, perhaps other factors may have a higher influence on decomposition such as humidity and insect interaction than temperature.

While Dermo infection intensity ratings were typically low after the first week of deployment, *P. marinus* is known to be able to infect an oyster with as few as ten cells. *P. marinus* is and has historically been found in all bays and estuaries in the northern Gulf of Mexico, so there is no concern for introducing *P. marinus* through restoration efforts into an area in Texas where it does not already exist. Background Dermo infection levels in Texas are high relative to much of the northern Gulf of Mexico and Dermo infection reduces growth and reproduction of infected oysters. Oyster spawning season extends from late Spring through early Fall and the success of an oyster restoration project is typically measured by the recruitment of spat, and the growth of the reef post-restoration. Therefore, to aid in the success of a restoration project the reef substrate material should not contribute to local sources for *P. marinus* exposure to newly recruited oysters. To this end, timing the deployment of the recycled shell to the beginning of the non-spawning season could ensure that should residual tissue remain, there is ample time for it to break down, and any released *P. marinus* dies before new spat settles at the restoration site. The viability of the *P. marinus* spores observed throughout this study is unknown. Future laboratory-based studies to expose uninfected oysters to the desiccated but infected tissues from oysters gathered at the sun-curing site are needed to determine the viability and risk level of *P. marinus* associated with the recycled shell material.

Perkinsus marinus is not the only risk associated with the use of recycled oyster shells for restoration projects, however it was the only risk evaluated in this study. While there are a variety of treatments that can be used to sterilize the recycled shells such as heat treatment, and freshwater, bleach, or acid soaks these are not logistically reasonable for large-scale shell recycling programs. Dermo infection is monitored across the northern Gulf of Mexico by a variety of organizations, but consistent monitoring in Galveston Bay has not occurred since 2010. The results of this study suggest that resource managers and practitioners that have active depredation of oyster tissue at the top of their piles, as seen in this study, may consider curing their shell material for a minimum of 3 months provided that is deployed for curing during "warm-weather" months (April – September). Shell deployed for curing during "cold-weather" months, should continue to follow existing recommendations of curing for 6 months due to the reduced rate of tissue degradation during cold-weather months.

INTRODUCTION AND BACKGROUND

The Eastern Oyster (*Crassostrea virginica*) is a species of oyster native to Texas. Oyster reefs are in decline with an estimated 85% loss world-wide (Beck et al. 2011) and 60-80% loss locally, in Galveston Bay (GBF 2023). Healthy oyster reefs are an important component of Texas Bays providing numerous ecosystem services such as shoreline stabilization, water filtration, habitat creation, and it is one of Texas' most economically important fisheries (Beck et al. 2011, Bidegain et al. 2017, Coen et al. 2007, DePiper et al. 2017, Grabowski et al. 2012) (Figure 1). However, reefs face a myriad of natural and anthropogenic stressors. Natural pressures on oyster populations include predation (Grabowski et al. 2012, Hill and Weissburg 2013, Hanke et al. 2017), sedimentation (Du et al. 2019, Hanke et al. 2021, Saoud and Rouse 2000) extreme weather events (Du and Park 2019, Hanke et al. 2022), and disease (Craig et al. 1989). Whereas anthropogenic stressors on oyster populations are mainly derived from overfishing, habitat loss, and pollution (Beck et al. 2011, Jackson et al. 2001, Worm et al. 2006). Resource managers, academics, and non-governmental organizations work together to address these threats through regulation and restoration.

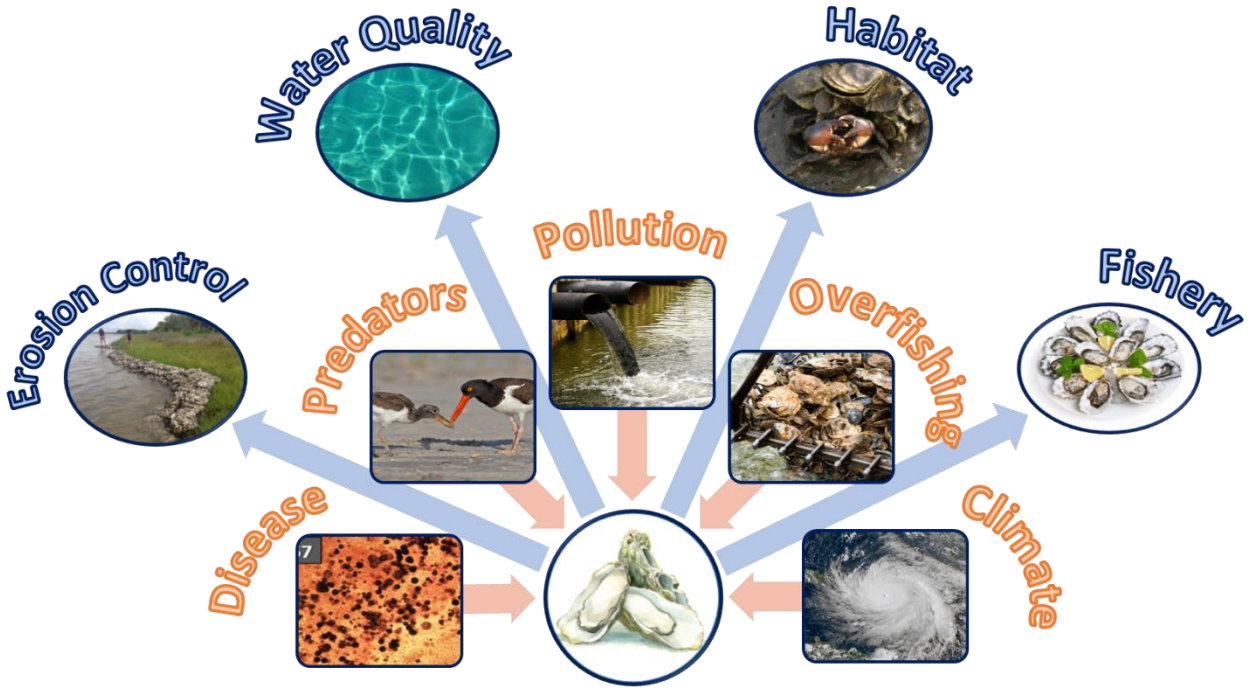

Figure 1. Eastern Oysters (*Crassostrea virginica*) provide many ecosystem services (blue arrows), but they also face threats (orange arrows).

Oyster reef restoration can be accomplished by introducing hard substrate, typically in the form of reclaimed oyster shells (or cultch), back into the local environment to be colonized by native spat (Coen and Luckenbach 2000). The Galveston Bay Foundation (GBF) gathers shells from local seafood restaurants through the Oyster Shell Recycling Program (OSRP) for reuse in reef restoration in Galveston Bay, Texas (GBF 2022) (Figure 2). The Galveston Bay Foundation piloted the OSRP in 2011 with a single restaurant. Over the last decade, GBF has expanded its operations and now collects an average of 150 tons (300,000 pounds) of shells per year from over 36 restaurants ranging from the Inner Loop of Houston to Galveston Island. To date, GBF

has collected over 1,650 tons (3,300,000 pounds) of oyster shell and returned approximately 840 tons of these recycled shells to Galveston Bay to help replenish hard substrate and sustain the local oyster population. The Galveston Bay Foundation's shell-based reef restoration and shoreline protection efforts have resulted in 0.80 acres of oyster habitat creation (Laroche et al. 2022) and 2,600 linear feet of shoreline protection (Hanke et al 2022). With the goal of acquiring larger volumes of shell to support larger reef restoration efforts, it is imperative to test and validate these sun-curing procedures. The information derived from this study will help ensure that clean and safe shell is returned to Galveston Bay and other state waters. With any conservation effort, it is important to make sure practitioners are not inadvertently introducing or increasing disease in native reefs.

Figure 2. Schematic illustrating the oyster shell recycling pathway through the Galveston Bay Foundation's Oyster Shell Recycling Program (OSRP) and some example images of each step. a. photo of oyster recycling bins from participating restaurants that are picked up by the OSRP. b. photo of the recycling bins being emptied at the sun-curing site c. photo of a dump truck load of recycled shell being emptied at the sun-curing site. d. photo of a large-scale oyster restoration using sun-cured oyster shells, and e. photo of a volunteer oyster restoration event where bags of the sun-cured oysters are placed back into the bay.

Dermo disease is caused by the spore-forming protozoan parasite *Perkinsus marinus*. Oysters can become infected when they ingest any life stage of *P. marinus* (Volety and Chu 1994) (Figure 3). Once ingested, *P. marinus* proliferates within the tissues of the oyster host. It can be transmitted from an infected oyster to surrounding oysters either through excretion or when decomposing tissue from dead oysters release spores into the water column (Bidegain et al. 2017). Dermo infection rates are highest when the water is warm and salinity is high, so late summer tends to be the peak of *P. marinus* loading in Texas bays (Calvo et al. 2003, Craig et al. 1989, Silvy et al. 2020). Dermo infection does not harm people that ingest the oysters, but the infection can impair oyster growth and reproduction, eventually causing mortality. Because Dermo infection can be transferred from decomposing oyster tissue, many restoration programs

are mandated to quarantine or sun-cure before re-introducing recycled oyster shell back into the bay.

Recycled oyster shells may harbor invasive species and disease-causing organisms (including *P. marinus*), therefore the OSRP currently follows best practices recommended by the Texas Parks and Wildlife Department (TPWD) which includes a minimum of six months of land-based sun-curing. The current best practices are based off of a study conducted by Bushek et al (2004) in South Carolina, which used oysters from a reef in Galveston Bay (Confederate Reef) with historically high levels of Dermo infection. This study demonstrated Dermo infection prevalence declined significantly after one month and was virtually eliminated after three months (Bushek et al. 2004). To expand on the limited previous work evaluating Dermo infection persistence in sun-cured oysters, this project was developed to track Dermo infection presence, prevalence, and intensity in sun-cured oysters with considerations for location within the pile and the influence of foraging wildlife.

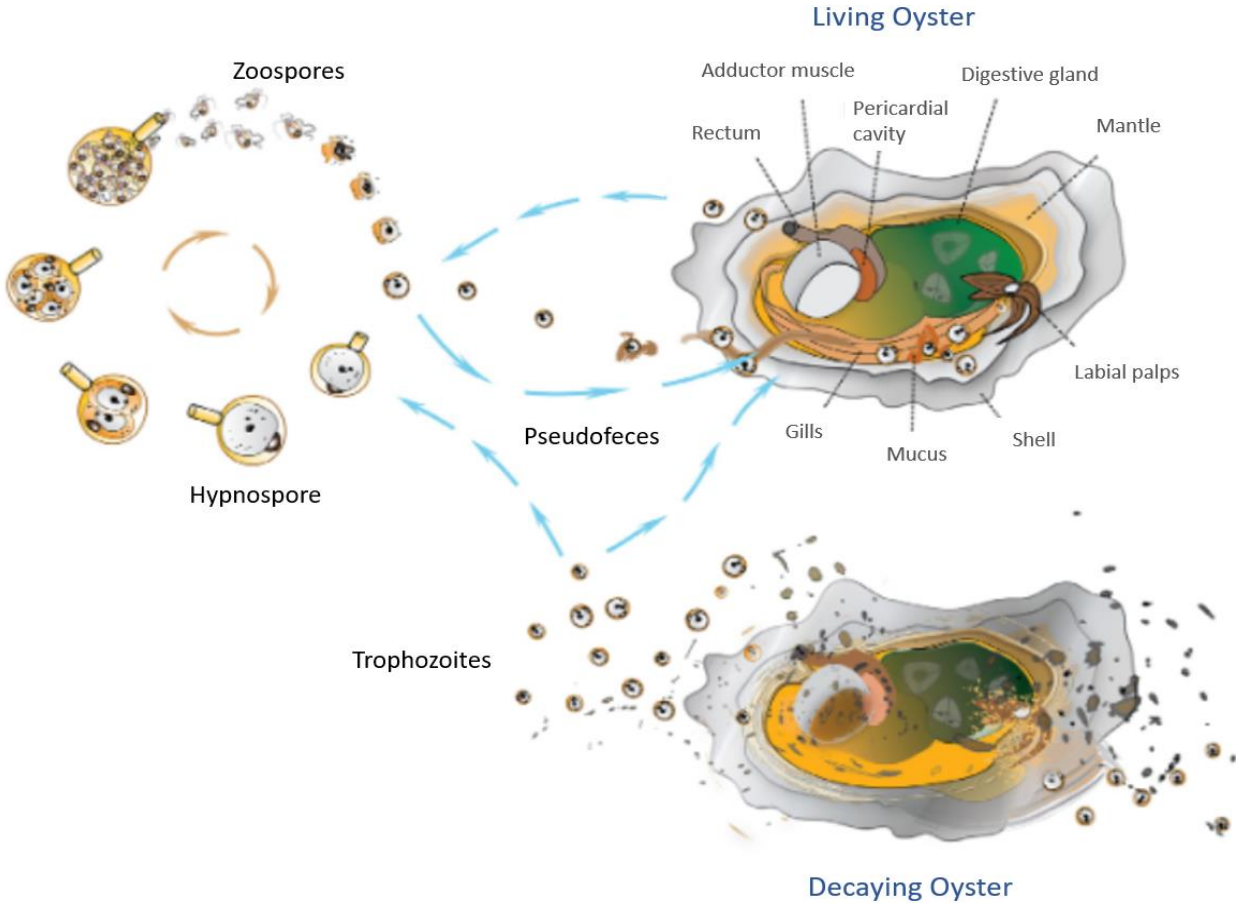

Figure 3. Lifecycle and infection mechanisms of Dermo infection (*Perkinsus marinus)* in Eastern Oyster (*Crassostrea virginica*) adapted from: Fernández et al. 2018

Objectives

The objectives of the study were to:
1) track the prevalence and severity of Dermo infection in sun-cured oysters,

2) evaluate the influence of location of oysters within curing piles on Dermo infection prevalence and severity, and
3) evaluate the impact from wildlife foraging during the sun-curing process.

METHODS

This study was purposefully designed to test a "worst-case scenario" for Dermo infection in sun-cured oysters in Texas. Oysters with elevated Dermo infection were used and deployed whole to demonstrate the sun-curing process of un-shucked oysters that may enter the recycling pathway.

Study Site

Oysters were collected from Confederate Reef in Galveston Bay on October 6, 2022. This reef has historically high Dermo infection rates (Silvy et al. 2020) and was sampled at the end of the summer. Oysters were processed the same day as collection. Once processed (see Field Methods section below for detailed processing steps), oysters were kept on ice overnight and deployed at the GBF's Red Bluff Curing Site (Figure 4) on the following day, October 7, 2022. The GBF created four replicate piles approximately 6 feet wide by 3 feet tall of recycled oyster shells collected through their OSRP. Two of the piles were fenced (piles A & C in Figure 4) and two were left unfenced (piles B & D in Figure 4) to evaluate potential influence by wildlife access. Fenced piles were surrounded by four-foot high, 4-gauge wire fence panels with four-inch square mesh and then reinforced by a layer of chicken wire to exclude smaller animals.

Field Methods

Initial processing consisted of cleaning the exterior of the oysters using a stiff hand-held brush and fresh water and knocking off other shell fragments or spat. When clean, oysters were measured (length and width). Then the oysters were shucked (i.e., the abductor muscle was detached from the lid only) and the oyster was tilted to allow water to drain from the open shell. The shucked and drained oysters were weighed, and initial tissue condition was recorded for each oyster. Initial tissue condition was categorized as either shrunken (e.g., small, dehydrated appearance) or plump (e.g., round, lush, creamy color) based on Ray (1966). Additional tissue condition categories were added after the first week of deployment and included "liquified", "desiccated", and "no tissue" (Figure 5). A 5-mm biopsy punch was used to take a sample of the mantle tissue which was placed in a prepared vial with 10mL of NaCl Thioglycollate medium inoculated with Chloromycetin/Nystatin solution and incubated in the dark at room temperature for 7 days (per Ray 1966).

Oysters were individually numbered, and the shells were closed around the tissue with bailing wire and deployed in either the interior or top of one of four replicate piles of recycled oyster shell at the GBF Red Bluff Curing Site. This was done to mimic a situation where a whole un-shucked oyster was included in the shell recycling material. After initial deployment, half of the oysters (n = 40) from each deployment location were sampled for Dermo infection ("Dermo" oysters) and the other half were sampled for tissue condition and decomposition ("Tissue" oysters) weekly for the first six weeks, then every other week for six months, and once a month for two more months (covering a total of 8 months deployment). To monitor pile status, game cameras (HyperFire 2, Reconyx, Holmen, Wisconsin, USA) were set to take three photos, one

second apart when motion was detected. Game cameras were deployed for the first 6-weeks of the study and downloaded during each weekly check (Figure 4).

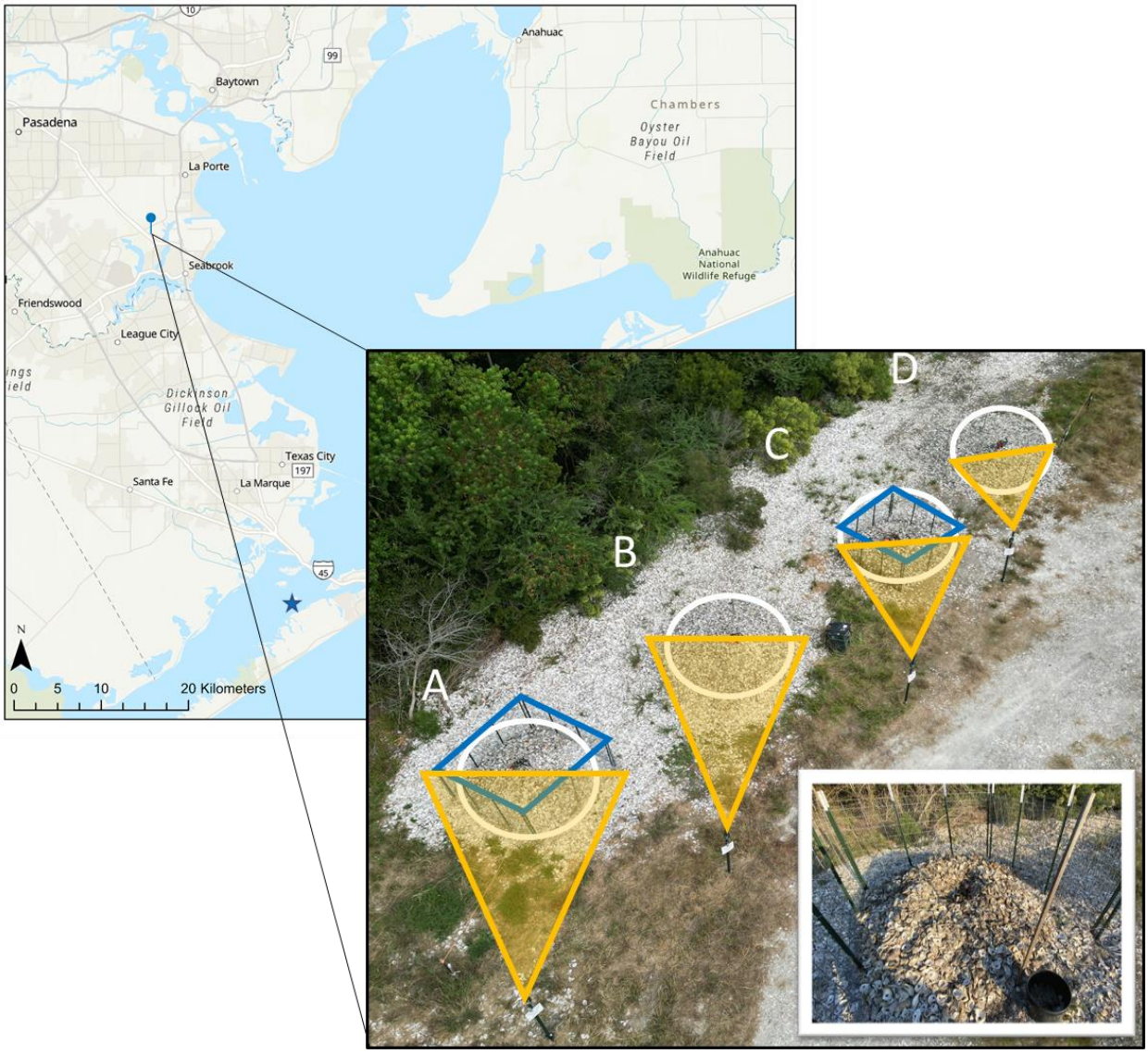

Figure 4. Map of Red Bluff curing site utilized by the Galveston Bay Foundation, and site of the sun-curing experimental piles. Aerial image showing the four experimental piles, A and C were fenced (blue squares), and B and D were unfenced. Game cameras were deployed for the first 6-weeks of the study to document wildlife interactions (yellow triangles = approximate field of view of game cameras). Inlayed photo of a fenced pile with the center dug out to deploy the interior oyster treatment. Blue star on map indicates location of Confederate Reef where oysters were procured for the study.

At each check, all "Dermo" oysters were evaluated for tissue condition (Figure 5). If tissue was present, a 5-mm biopsy punch was used to take a sample for Dermo infection analysis. Additionally, at each check all "tissue" oysters were weighed and percent cover of tissue on the shell and tissue condition was recorded. Temperature and relative humidity sensors (U23-001 HOBO Pro v2, Onset, Bourne, Massachusetts, USA) were co-located with each group of oysters in the interior of the piles and deployed on the top of pile C to capture the ambient conditions.

Figure 5. Examples of the five tissue condition categories used to describe decaying oyster tissue deployed at GBF's sun-curing site from the on-going Texas General Land Office study by GBF and UHCL.

Laboratory Methods

Oyster tissue samples were evaluated following Ray's Fluid Thioglycollate Method (RFTM) after being incubated for 7 days (Ray 1966). The tissue was removed from the incubation vial, macerated on a glass slide, then stained with Lugol's solution and covered with a cover slip. Samples were viewed under a dissecting microscope (5x power), *P. marinus* spores were counted, and a Dermo infection intensity rating was assigned using the Mackin (1961) scale, as modified by Craig et al. (1989) which ranges from 0 (e.g., no *P. marinus* spores detected) to 5 (e.g., nearly 100% of the tissue is comprised of hypnospores) (Figure 6).

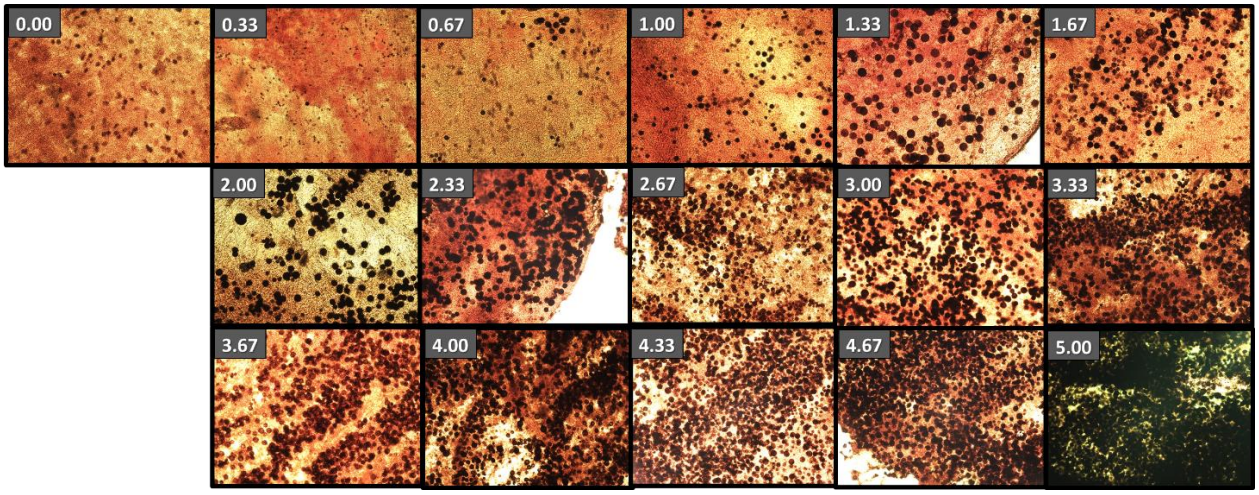

Figure 6. Examples of tissue pathology slides showing the range of Dermo intensity rating using the Ray's Fluid Thioglycollate Method (RFTM).

Data Analyses

All data were tested for normality prior to statistical analysis (Shapiro and Wilk 1965). If data were determined to be non-normal, nonparametric statistical methods described below were used. Statistical analyses were conducted using R Studio (2022.07.2 build 576). The relationship between the presence or absence and intensity of Dermo infection and categorical variables were evaluated using either the Kruskal-Wallis Rank Sum Test (Myles and Wolfe 1973) with subsequent post-hoc Pairwise Wilcoxon Rank Sum Test (when applicable) or a binomial Generalized Linear Model (GLM) for detection prediction analysis (R package *pscl*). For all statistical tests, we used $\alpha = 0.05$ to determine statistical significance. All means are reported ±1 SE, unless otherwise noted. We used a Friedman Rank Sum Test (Myles and Wolfe 1973) to evaluate repeated measures of Dermo infection intensity by study week.

RESULTS

A total of 96 oysters were collected from Confederate Reef in West Bay, Galveston Bay (29.26349˚ N, -94.91654˚ W - WGS84) and processed on October 6, 2022. The water temperature at the time of collection (9:35 am) was 25.4 deg C and salinity was 26.37 psu. A sub-set of 80 of the collected oysters were utilized in the sun-curing study. Average length was 93.7 mm ± 1.26 and the average total (shell and tissue) weight after being shucked and drained was 161.5 ± 4.88 g (Figure 7).

Deployed oysters used were live at the time of shucking and initial tissue condition was recorded with 51% ($n = 41$) as plump, and 49% ($n = 39$) as shrunken with a minimum of 50% coverage by the tissue. Additionally, 35% ($n = 28$) were observed to be “milky” in color indicating development for spawning, while 65% ($n = 52$) were “watery”. Forty of the oysters were used to track the prevalence and intensity of Dermo infection, two of which were below legally harvestable size (76.03 and 75.98 mm). We included these smaller oysters in the study because of their elevated Dermo infection intensity rating of 1.0. The average initial Dermo infection intensity was 0.9665 ± 0.08 (Figure 8). There was no correlation between length of oyster and Dermo infection intensity ($F = 1.193$, $p = 0.2775$, one-way ANOVA).

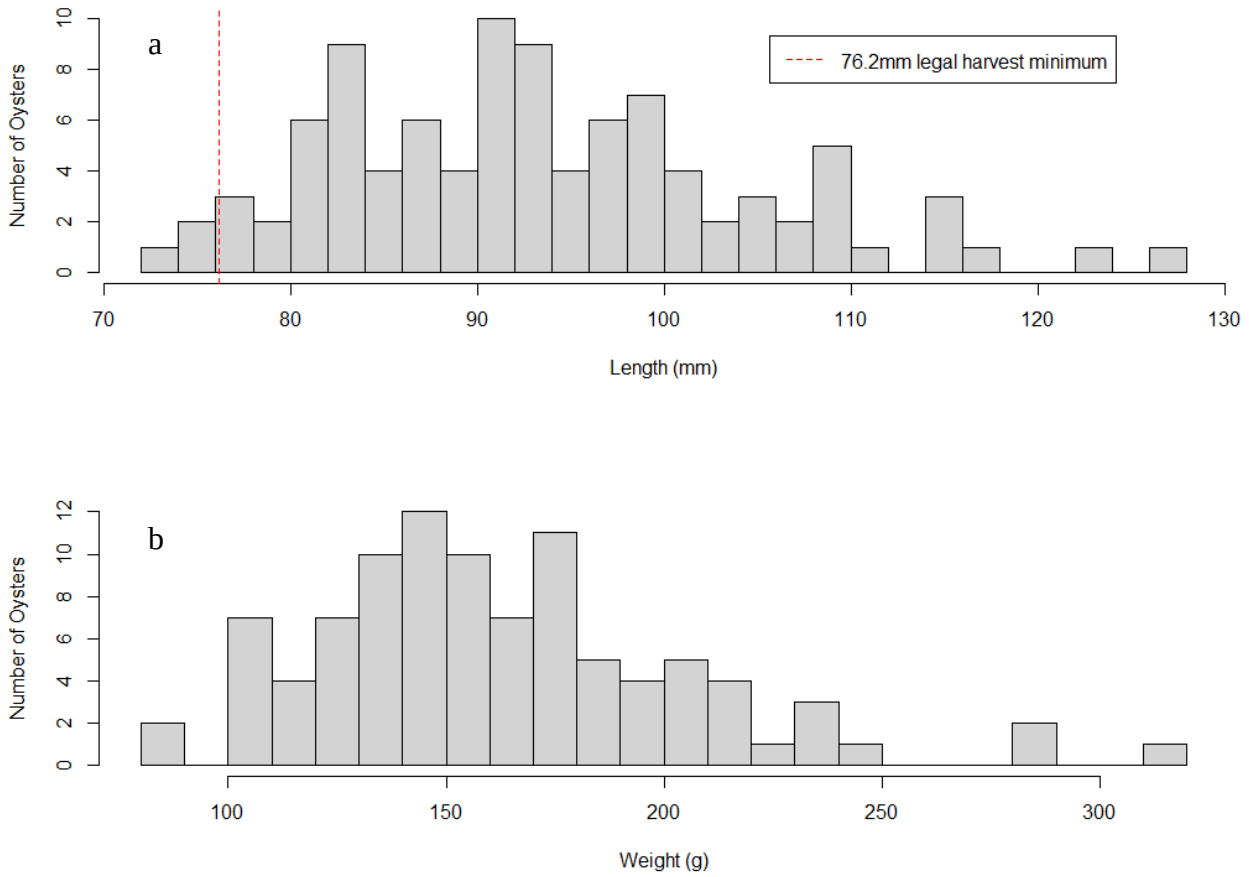

Figure 7. Histograms illustrating the frequency of oysters by a) length (mm) and b) post-shuck weight of the shell and tissue (g) for the 80 oysters collected from Confederate Reef in West Bay, Galveston Bay and used in the Sun-Curing project.

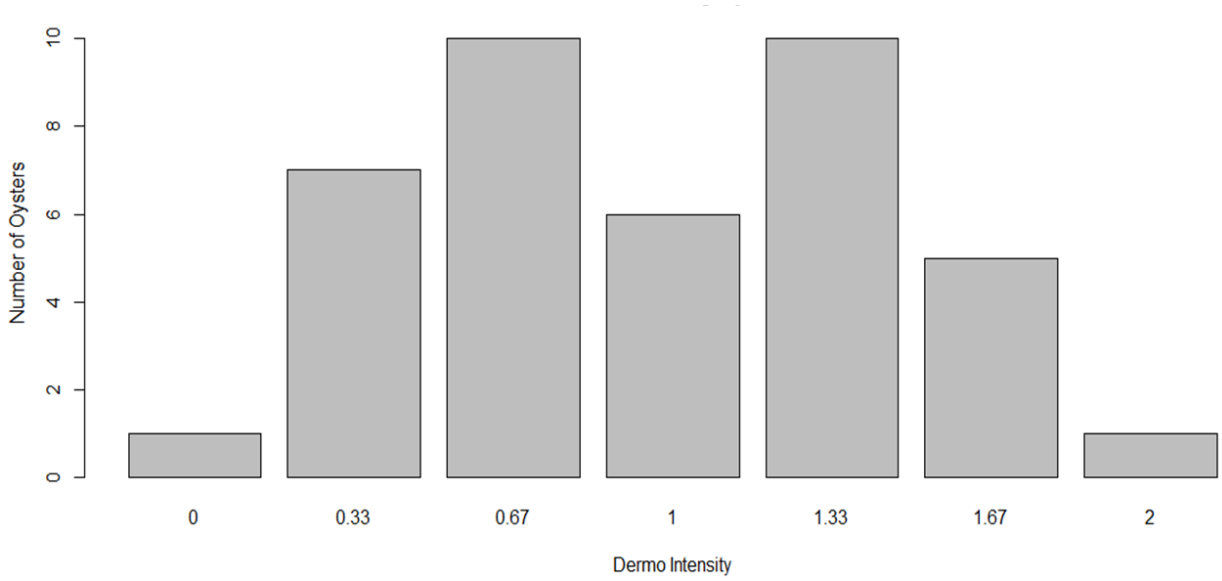

Figure 8. Distribution of initial dermo infection intensity scores for the 40 oysters used to track dermo infection prevalence and intensity.

Oysters were deployed in the experimental curing piles on October 7, 2022, and the experiment ran for 35 weeks, through June 8, 2023. Some of the oysters deployed at the top of the piles were removed from the experiment due to depredation by feral hogs. These oysters are denoted as "N/A" tissue condition in Figure 10. As a result, there were only four oysters sampled for tissue in the tops of the unfenced piles after the first week of deployment and only one after the second week. Further analysis of tissue condition only included oysters deployed in fenced piles as a result. Camera traps deployed at each pile captured initial interaction between the deployed oysters and feral hogs which occurred just 3 hours after deployment. Depredation of study oyster tissue by feral hogs, occurred just 31 hours after initial deployment (Figure 9). During some oyster sampling visits the research team could hear the feral hogs in the nearby tree line and they would occasionally appear to seemingly check to see if the coast was clear for them to scavenge any new oyster shell.

While there were wildlife interactions observed for fenced piles (primarily from vultures) deployed oysters were not compromised/depredated. Through camera trap footage review it was clear that the impact from wildlife to the unfenced piles only affected the top of the pile and there was no physical disturbance to the oysters deployed in the interior of the piles. Oysters that were depredated and the shells were not recovered were assumed to have zero percent tissue cover. While some "Dermo" oysters were depredated we failed to detect any statistically significant difference in Dermo infection intensity between oysters deployed at the top of fenced piles versus not fenced piles (chi-squared = 0.1878, p = 0.6647, Kruskal-Wallis rank sum test), therefore all piles were pooled for further Dermo infection analyses.

The temperature (˚F) and relative humidity (%) recorded throughout the deployment time varied on a diurnal cycle as well as a seasonal cycle, as the experiment ran from early Fall through early Summer (Figure 11 & Figure 12). Temperatures were higher and more variable on top of the piles, while relative humidity was generally higher in the interior of the piles. In fact, following rain events it was not uncommon for the relative humidity in the interior of the piles to stay at or near 100% for days or even weeks. The 19 sampling events spanned a wide range of temperature and relative humidity conditions with the highest recorded being 49.2 ˚C (120.6 ˚F) and 100% and the lowest recorded being -8.8 ˚C (16.2 ˚F) and 14.9% respectively.

Figure 9. Unfenced piles B and D when wildlife interaction compromised deployed study oysters 31 hours post-deployment**.**

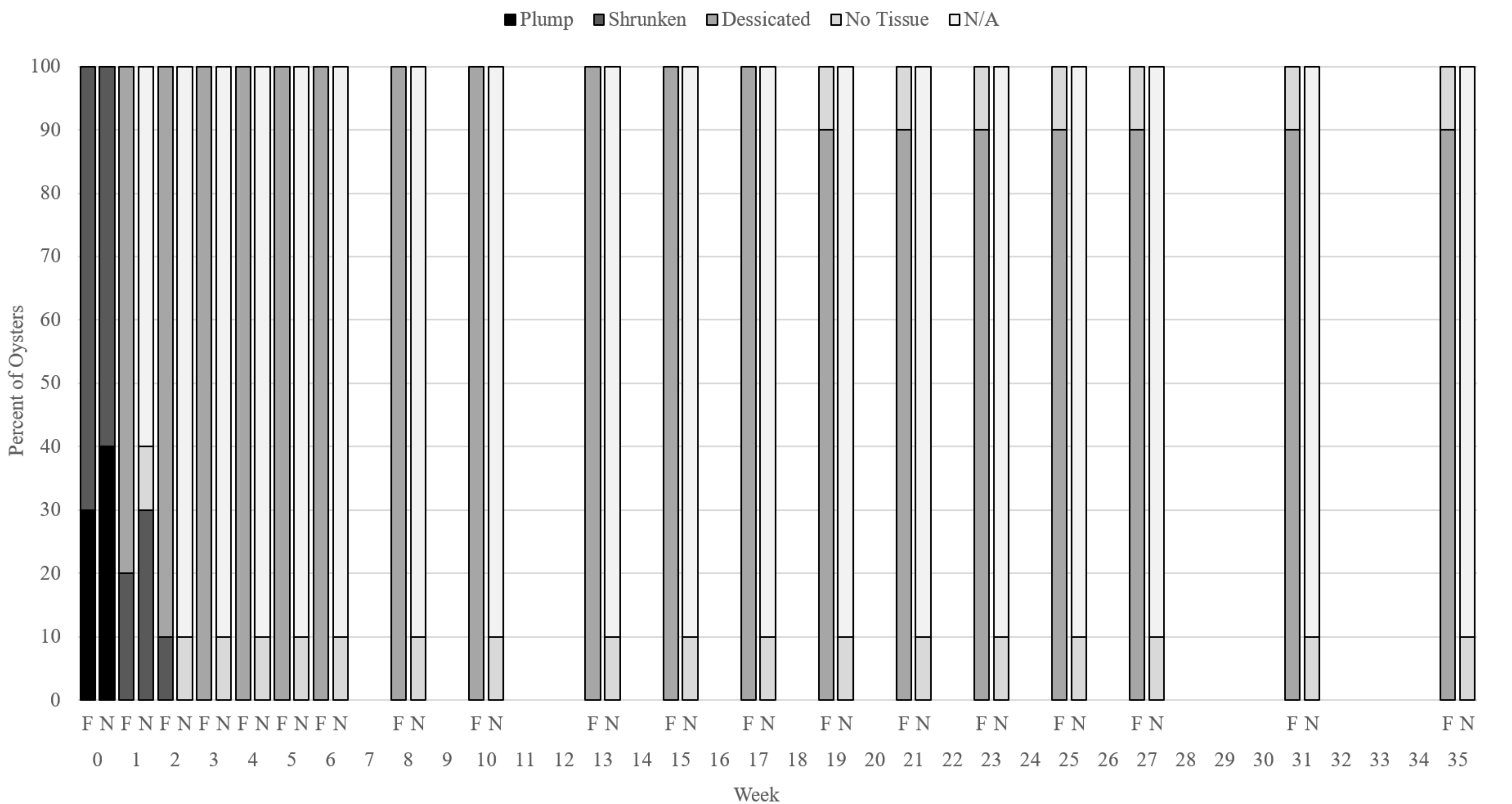

Figure 10. The percentage of "tissue" oysters deployed on the tops of the piles by tissue condition category by sampling week and whether the pile was fenced = "F", or not = "N". N/A represents oysters that were depredated by feral hogs and therefore no longer trackable for tissue condition.

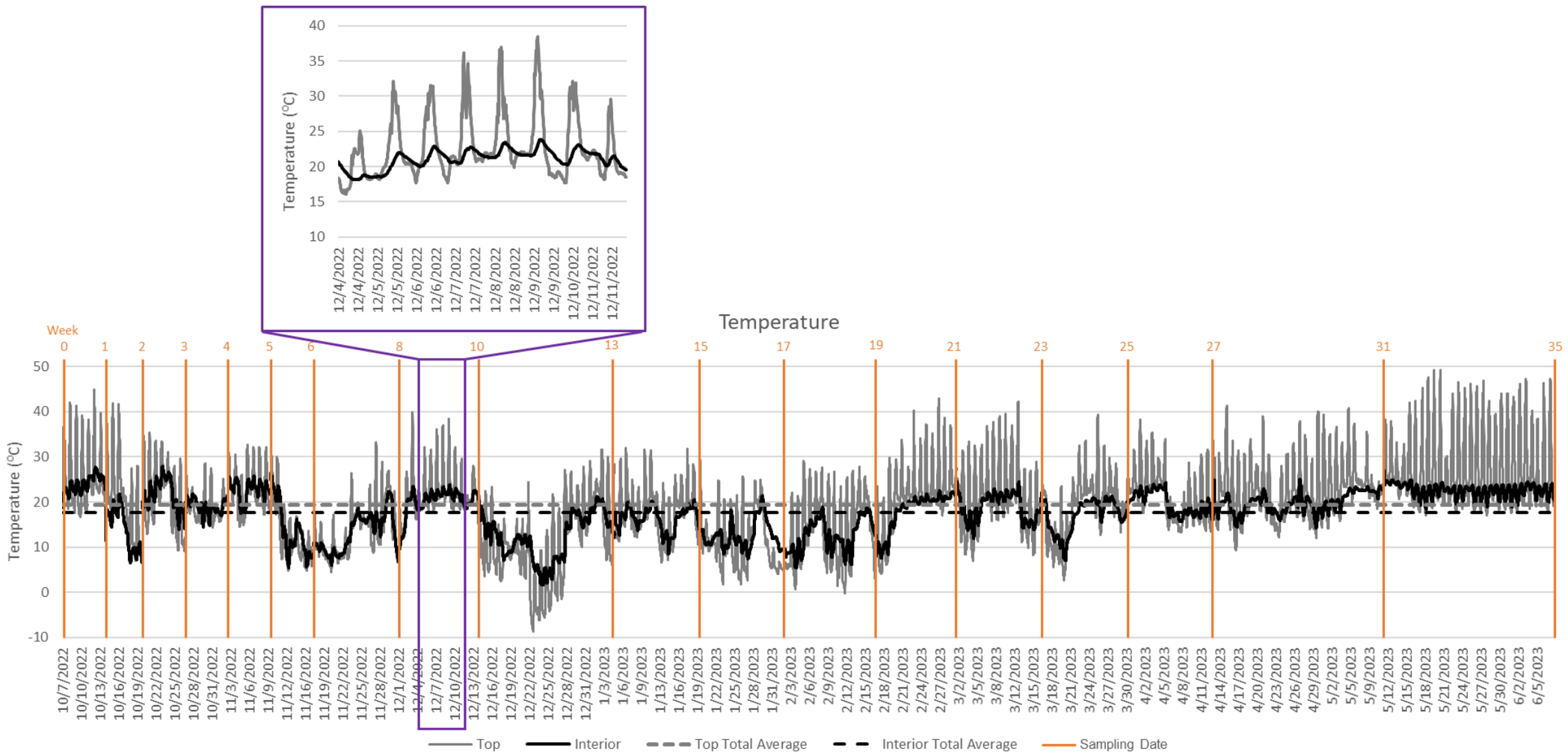

Figure 11. Time series of temperature data as the average of the sensors co-located in the interior of each of the four experimental piles (black line) with the sensor located at the top of pile C (grey line - ambient conditions). Dashed black line is the total overall mean temperature inside of the piles and the dashed grey line is the total overall average temperature on top of the piles for the duration of the study. Orange bars indicate sampling dates. Purple border inlayed graph shows an expanded view of temperature data to demonstrate the difference in diurnal variability inside versus on top of the piles.

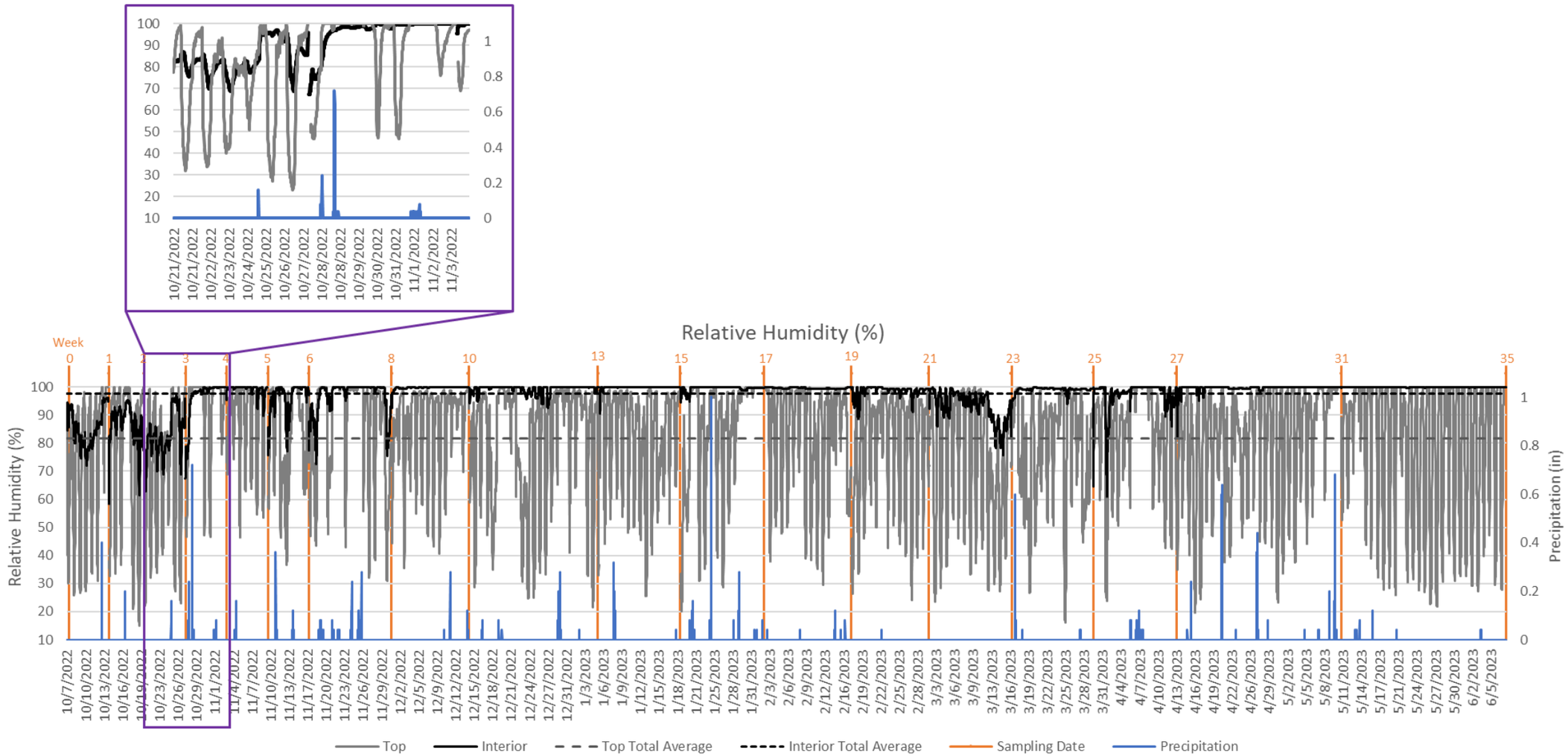

Figure 12. Time series of relative humidity data as the average of the sensors co-located in the interior of each of the four experimental piles (black line) with the sensor located at the top of pile C (grey line - ambient conditions). Dashed black line is the total overall mean relative humidity inside of the piles and the dashed grey line is the total overall average relative humidity on top of the piles during the duration of the study. Blue bars illustrate precipitation in inches. Orange bars indicate sampling dates. Purple-border inlayed graph shows an expanded view of relative humidity data to demonstrate the difference in diurnal variability inside versus on top of the piles.

Oysters in the interior of the piles were slower to desiccate compared to those at the top of the piles, but once desiccated the oysters in the interior of the piles continued to degrade and had a lower percent cover of tissue throughout the study compared to those at the top of the piles (Figure 13). A sub-set of the oysters in the interior of the piles became liquified (e.g. Figure 5) during weeks 1 through week 3. This liquified tissue condition state of decomposition was not observed in the oysters deployed at the top of the piles; those oysters transitioned directly to a desiccated state. At week 4, all remining tissue was in the desiccated condition and at week 6 the percent of oysters with no tissue as well as the percent cover of tissue for the oysters that had desiccated tissue present stabilized until week 19 (Figure 13). At week 19 we see an increase in oysters with no tissue (decrease in oysters with tissue condition desiccated) regardless of location, and this sampling week corresponds with the first period with consistent warming (Figure 11). The next observed reduction in percent of oysters with no tissue for the interior oysters occurred at week 31 which corresponded with elevated relative humidity levels (Figure 12).

Anecdotally, the research team noted the presence of maggots (as either pupae or larvae) as well as other insects, all of which were more prevalent in oysters deployed in the interior of the piles. There were 97 occurrences of oysters with maggots observed in the interior of the piles, while the top of the piles only had 47 occurrences of oysters with maggots observed. Maggots were most prevalent in the first week of deployment and their presence dropped off through week 13 of deployment (Figure 14). There also appeared to be a relationship with the presence of maggots and the Dermo infection intensity. Dermo infection intensity was significantly lower for oysters that had no maggots observed (chi-squared = 43.082, $p < 0.0001$, Kruskal-Wallis rank sum test) (Figure 15) with the probability of maggots present being highest 68% with a Dermo infection intensity of 1.0, and lowest (10%) with a Dermo infection intensity of 0 ($z = 5.12$, $p < 0.0001$, Generalized linear model).The mechanism(s) driving this correlation is(are) unknown.

Dermo infection intensity significantly decreased after the first week of deployment regardless of location (top versus interior) within the pile (x^2 (18) = 301.68, $p < 0.0001$, Friedman test with pairwise Wilcoxon signed-rank test; p-adjusted using the Bonferroni multiple testing correction method). Throughout the study (week 1 – week 35) there was a significant decrease in Dermo infection intensity measured for oysters deployed in the interior of the piles compared to the top of the piles (chi-squared = 10.086, $p = 0.0015$, Kruskal-Wallis test) with no Dermo infection detected in the interior of the piles after week 6 (Figure 16). Dermo infection continued to be detected in tissue sampled from the oysters deployed at the top of the piles through the 31st week of deployment. Individual tissue condition and Dermo infection intensity for each oyster by week are summarized in Appendix A.

All oysters, including the "tissue" oysters were sampled for Dermo infection at the initial (week 0) and at the end (week 35) and the only oyster that was positive for Dermo infection (lowest intensity rating = 0.33) at week 35 was a "tissue" oyster deployed in the top of pile C. Interestingly, during the initial Dermo test, no Dermo infection was detected for this oyster.

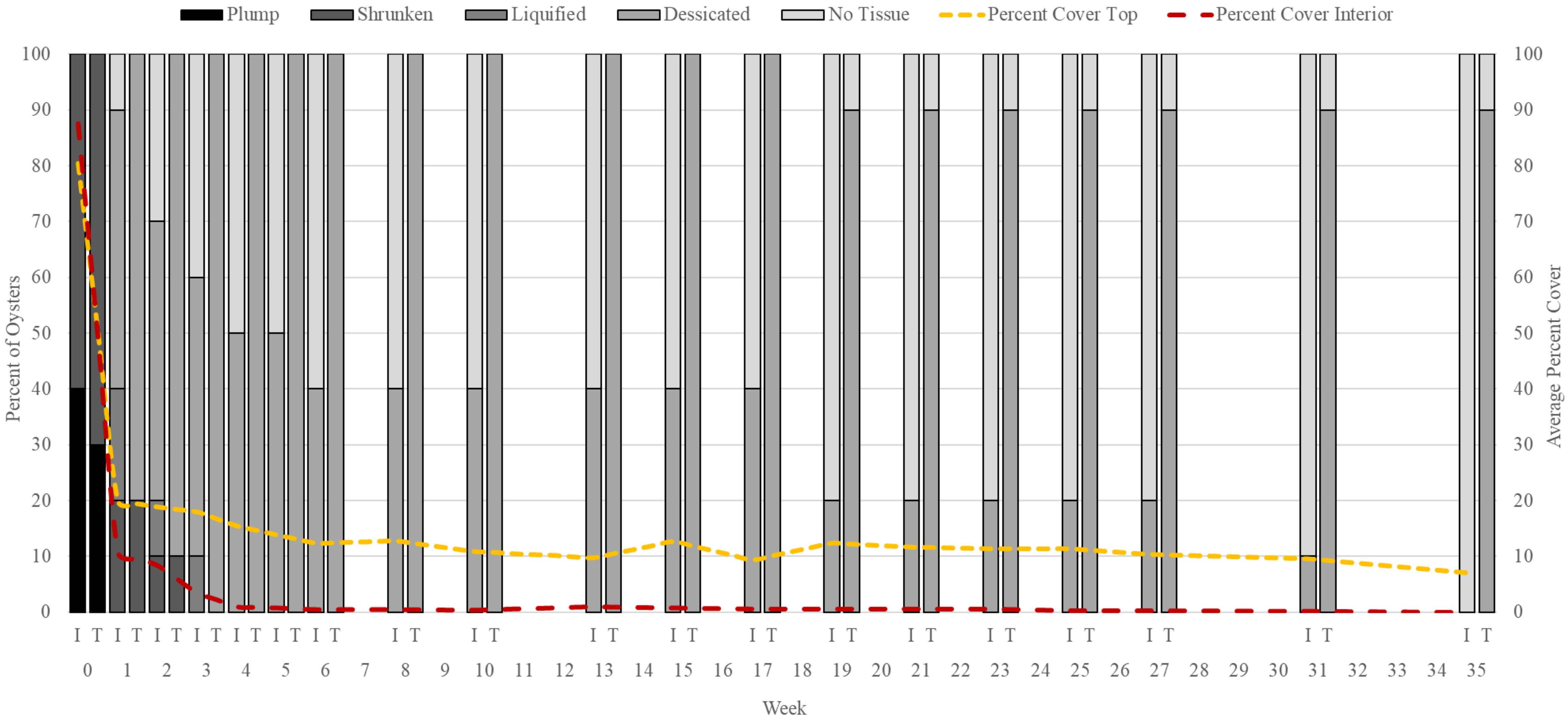

Figure 13. Percent of oysters deployed in fenced piles by tissue condition category of oysters monitored for tissue condition by sampling week and oyster deployment location. The average percent of the oyster shell that was covered by tissue by week and location are plotted (yellow short-dash line = oyster deployed at the top of the fenced piles, and red long-dash line = oysters deployed at the interior of the fenced piles).

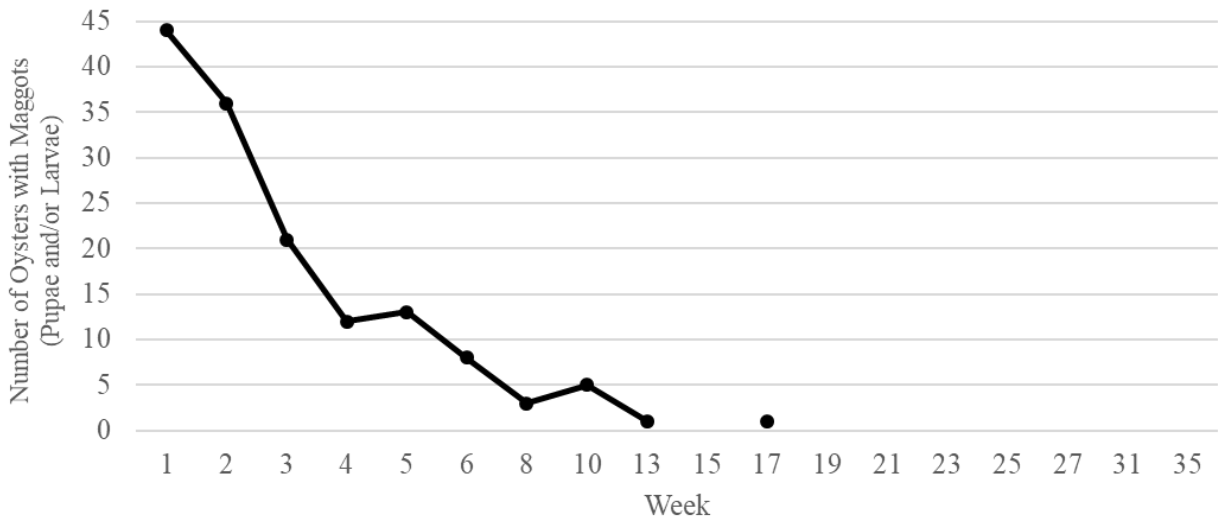

Figure 14. Number of oysters with maggots (pupae and or larvae) observed by week in all oyster piles combined.

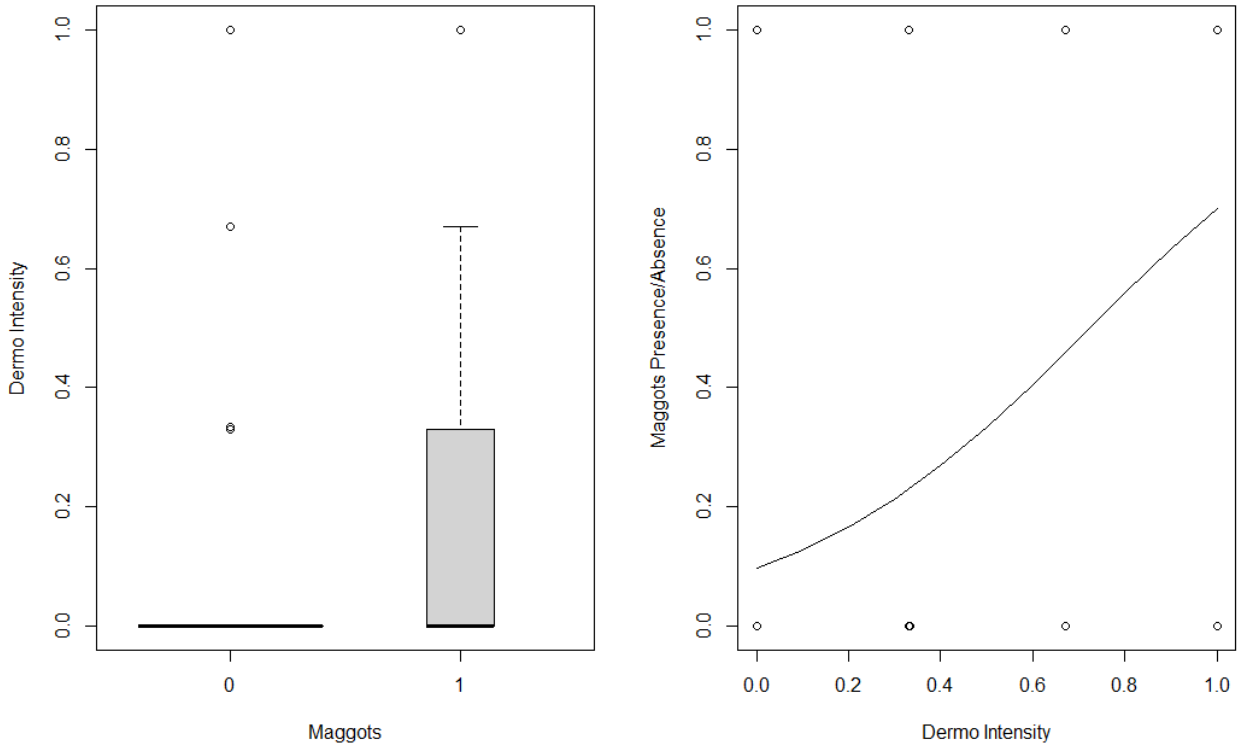

Figure 15. a) Boxplot of Dermo infection intensity for oysters with maggots observed (1) versus not observed (0). b) Fitted binomial Generalized Linear Model (GLM) applied to the probability of maggots being present by the Dermo infection intensity with detection probability curve.

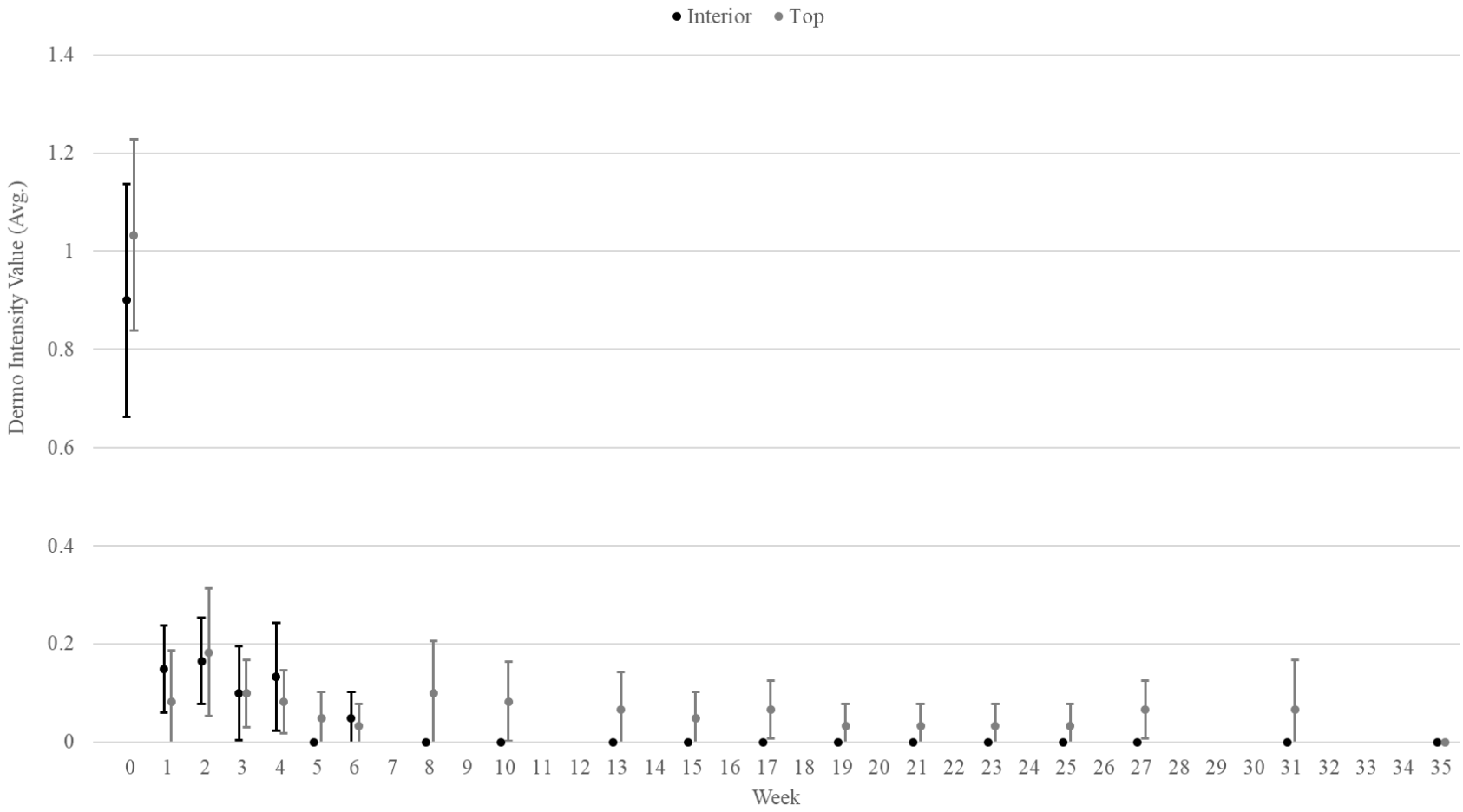

Figure 16. Average dermo infection intensity values by week and location for all piles. Error bars represent 95% confidence interval.

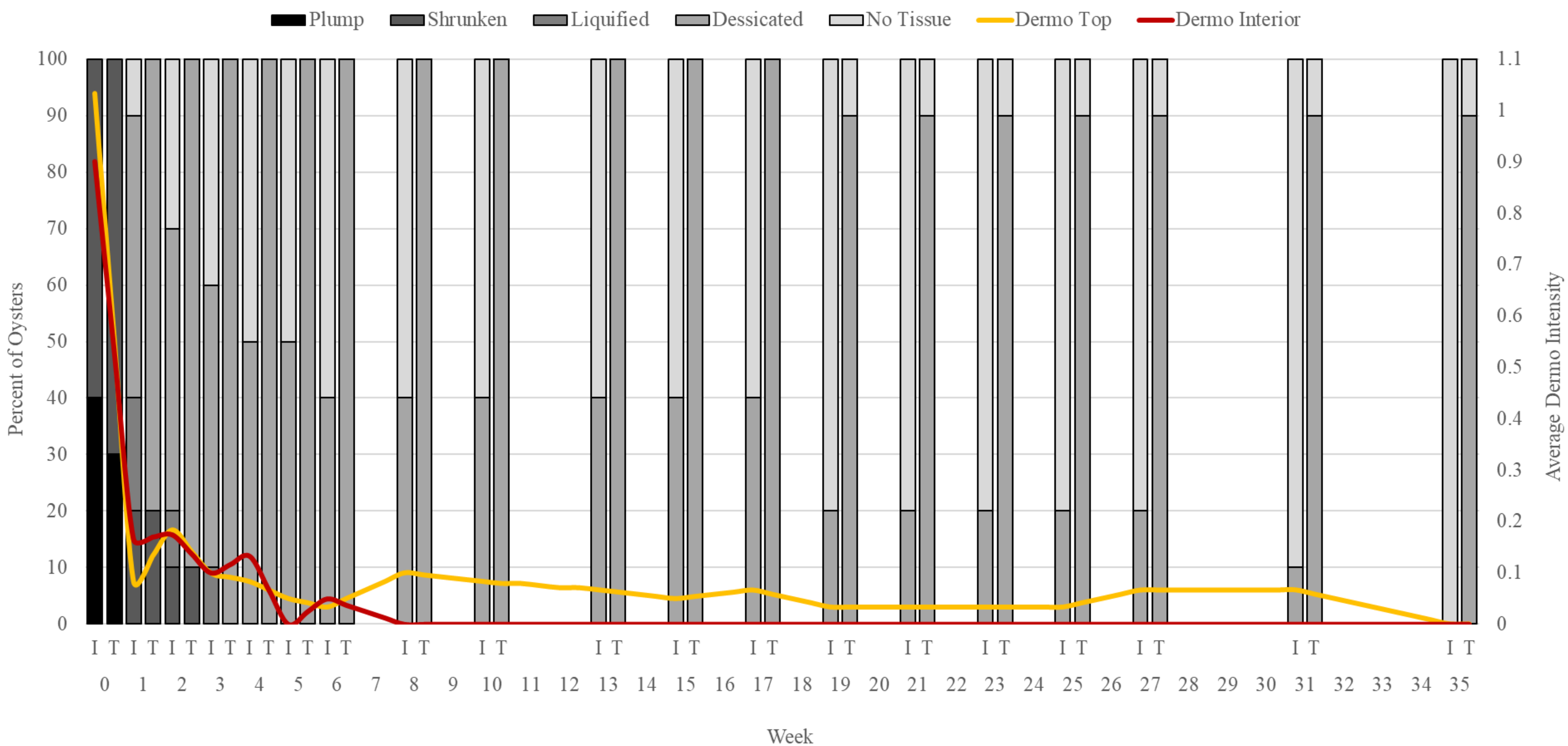

Figure 17. Percent of oysters monitored for tissue condition in fenced piles by tissue condition category by sampling week and oyster deployment location. The average Dermo infection intensity for oysters from all piles by week and location are plotted (yellow line = oyster deployed at the top, and red line = oysters deployed at the interior).

DISCUSSION

This study was designed to test the worst-case-scenario by including oysters with a high initial level of Dermo infection, and deploying them wired-closed, simulating whole un-shucked oysters deployed in the sun-curing piles. Dermo infection in commercially harvested oysters that could end up in the oyster recycling pathway are not regularly monitored because Dermo infection poses no threat for human consumption. A preliminary study by Schubert and Hanke (2023) that evaluated Dermo infection in commercially sourced oysters found that 21-22% of oysters tested were positive for Dermo infection, while the oysters used to monitor Dermo infection in the study had an initial positivity rate of 97.5%. Furthermore, the oysters deployed in this study were meant to mimic whole un-shucked oysters that were included in the recycling process. It is unknown how frequently un-shucked oysters are found in the recycled shell materials, but it is reasonable to assume that it can happen, particularly if a restaurant is unable to sell oysters before their holding time. There are few references to un-shucked oysters that have been observed in shell piles in the literature (Bushek 1997, Bushek 1998, Bushek et al. 2004), and GBF OSRP staff have made note of un-shucked oysters on many occasions while collecting shell (personal communication: S. Batte, GBF).

The impact of wild animals on the sun-curing process was tested in this study using fenced and unfenced piles. Initial analysis of game camera photos conducted by Smith et al. (2023) suggests that a number of types of wildlife interact with the oyster piles including feral hogs, vultures, opossums, deer, coyotes, and songbirds. The number of interactions at unfenced piles was much higher than at fenced piles and the highest number of interactions for feral hogs occurred in the first week post-deployment, while the highest number of interactions by vultures occurred in the third week post-deployment (Smith et al. 2023). Within the first week of deployment oysters deployed at the top of the unfenced piles were depredated by feral hogs which resulted in the removal of all oyster tissue for those affected oysters and therefore the assumed removal of *P. marinus*. Feral hogs have quickly spread globally and have well documented negative impacts on the environment including but not limited to, competition with and predation of native species, habitat damage, disease transmission, and fecal bacteria in local waterways (Massei et al. 2011). We may have found the one positive impact that feral hogs can have on the environment, consumption of decaying oyster tissue at sun-curing sites; however, we recognize that this is not a sufficiently redeeming quality to allow their continued habitation. For the Red Bluff curing site, the presence of a robust feral hog population helps to remove oyster tissue and subsequent *P. marinus* resulting in potentially expedited treatment, but not all sun-curing locations have feral hog populations, and their detrimental impacts to the surrounding environment probably outweigh their help consuming rotting oyster tissue. There were also instances of feeding observed by vultures and opossums (although it is possible the opossums were feeding on the associated insects). Their impact was restricted to just the oysters deployed on the top of the piles; game camera footage suggests that all these wild animals only access the top few inches of the oyster piles.

The temperature and relative humidity sensors that were co-located with the deployed oysters helped to characterize the differences in the ambient conditions that the oysters were exposed to in the interior of the piles compared to the top of the piles. These data may help explain the differences in tissue decomposition and Dermo infection presence and prevalence between these

two deployment locations. Historically sun-curing recommendations for quarantining recycled oysters to be used in restoration projects were aimed at making sure the shell was exposed to the sun for UV irradiation to damage/degrade *P marinus*, as has been shown in laboratory-based studies (Ford et al. 2001, Buschek and Howell 2000). Our results indicate that the interior of the piles was more humid and supported a more consistent (yet lower) temperature environment compared to the top of the piles which mirror results presented in Buschek et al. (2004).

Tissue decomposition was more rapid for oysters deployed in the interior of the piles. Also, some oysters deployed in the interior of the piles experienced a liquification decomposition stage, which seemed to correspond with high maggot presence and resulted in loss of tissue when the liquified material leaked out of the oyster shells or were consumed by insects making it unsampleable at the next check. Anecdotally the research team noted the presence of maggots as well as other insects; all of which were more prevalent in oysters deployed in the interior of the piles. These insects may play an important role in the degradation of oyster tissue in curing piles. The potential relationship between the Dermo infection and the presence of maggots may be a result of timing, as both the maggot presence and the Dermo infection intensity was higher in the earlier weeks of the study and then declined.

There appeared to be continuous tissue decomposition through the first three weeks of deployment, then there was little to no change from week 4 through week 17 which corresponds to November through February when temperatures were the lowest during the deployment. The rate of decomposition may have been higher if the oysters were deployed in warmer months. Because most recycled oyster shell for the GBF OSRP comes from restaurants, the volume of recycled shell is variable throughout the year, but there tends to be a peak in March each year with elevated levels through August (personal communication: S. Batte, GBF). Similar to Bushek's 2004 study, most (greater than 50%) of the tissue in the deployed oysters in the interior of the piles was gone by the 16th week, which was the end of their study, but alternatively the majority of oysters deployed at the top of the piles had tissue remaining throughout the 35th week of our study (top deployment location was not evaluated in Bushek et al. 2004).

Much of the oyster shell collected through the GBF's OSRP comes from participating restaurants, and it is not uncommon for un-shucked, uneaten oysters, and/or shell with varying amount of tissue remaining to be included in with the recycled shells (personal communication: S. Batte, GBF). Other studies have found that desiccated oyster tissue found in shell piles exhibited Dermo infection (Bushek et al. 1994, Bushek et al. 2004). Additional studies to better evaluate the background levels of Dermo infection present in oysters that enter recycling pathways are needed. Understanding the presence and intensity of Dermo infection in oysters that can be recycled will help to evaluate the underlying source level of Dermo infection in sun-cured oysters. An audit of the amount of tissue present in a typical load of recycled shell, with quantification of the number of un-shucked oysters would be helpful in extrapolating the amount of tissue and therefore Dermo infection present in recycled shell piles.

We observed a significant decrease in the presence and prevalence of Dermo infection after the first week of deployment. While the intensity ratings for these tissues were typically low (average Dermo infection intensity of 0.33), studies have shown that infection can be initiated with as few as ten cells (Valiulis 1973, Bushek et al. 2004) and an overall infective dose

estimated at 50 cells (Bidegain et al. 2016). *Perkinsus marinus* can survive for 3 to 14 days in seawater (Chu et al. 2002, Chu and Lund 2006). Transmission of *P. marinus* has been shown to be highest from dead/decaying oysters during periods of high temperatures when oyster die-offs are occurring from Dermo infection (Calvo et al. 2003). As temperatures rise and extend for longer periods throughout the year due to global warming, Dermo infection and transmission rates are expected to increase (Craig et al. 1989).

There was a significant difference in the Dermo infection presence and prevalence depending on where they were deployed with the oysters in the interior of the piles having no Dermo infection detected after week six. While we refer to some analyses in terms of the presence and absence of Dermo infection, we cannot confirm absence, rather in these cases, we did not detect Dermo infection in the tissue sample that was used during that sampling event. The GBF OSRP currently sun-cures their recycled oyster shells for 6 months and mechanically mixes the piles after 3 months of curing. The experimental piles included in this study were not mechanically turned. Additionally, the experimental piles used in this study were relatively small (~6 ft wide by ~3 ft tall) and previous work suggests that the size and shape of the shell pile during sun-curing may alter the decomposition of tissue and subsequent Dermo infection (Bushek et al. 2004). Typical sun-curing piles at the GBF OSRP Red Bluff site are spread out flat, up to two feet tall to increase the proportion of oysters exposed to the top/sun. It was thought that UV light and lower relative humidity levels helped to speed up the decomposition and *P. marinus* mortality rates (Bushek et al. 2004, Diggles 2020, Diggles et al. 2021) but our results do not support this hypothesis. Bushek et al. 2004 suggested that tissue decomposition rates are likely to decrease with an increase in shell pile size, but they did not test oysters at the top of the piles. It is understood that the decomposition rate is positively correlated with higher temperatures and similar to our study they found that the interior temperature was generally lower than the external temperature of the piles. However, we found that the oysters deployed in the interior of the piles actually decomposed more quickly than those deployed on the tops of the piles; therefore, perhaps other factors have a higher influence on decomposition such as humidity and insect interaction than temperature. Anecdotally researchers noticed that once the tissue became desiccated the insect interactions decreased and decomposition slowed. It would be interesting to involve an entomologist in future studies to investigate the interactions between the insects and decomposition of the oyster tissue at the sun-curing site.

Perkinsus. marinus is and has historically been found in all bays and estuaries in the northern Gulf of Mexico (Craig et al. 1989). Consequently, there is no concern for introducing *P. marinus* through restoration efforts into an area in Texas where it does not already exist. Background Dermo infection levels in Texas are high relative to much of the northern Gulf of Mexico (Craig et al. 1989). Dermo infection reduces growth and reproduction of oysters (Dittman et al. 2001). Oyster spawning season extends from late Spring through early Fall when water temperatures are elevated. The success of an oyster restoration project is typically measured in the recruitment of spat, and the growth/size of the reef/oysters post-restoration. Therefore, to aid in the success of a restoration project, the reef substrate material should not contribute to the local source for *P. marinus* exposure to newly recruited oysters. To reduce this risk, timing the deployment of the recycled shell to the beginning of the non-spawning season (cooler temperatures) should ensure that if any residual desiccated tissue remains, there is ample time for it to break down and any released *P. marinus* die before new spat settles at the restoration site.

The viability of the spores observed throughout this study is unknown. Bushek et al. (2004) attempted to monitor the viability of the *P. marinus* in oysters deployed in sun-curing piles and they suggest that the parasites likely did not enlarge during the RFTM incubation period, bringing their viability into question. Future laboratory-based studies to expose uninfected oysters to the desiccated but infected tissues from oysters gathered at the sun-curing site is needed to determine the viability and risk level of the recycled shell material.

Typically, the RFTM requires that a tissue sample is collected from the mantle of the oyster for analysis, however depending on the decomposition pathway and rate, identifying or discerning the tissue types or even if something is in fact oyster tissue became difficult. Therefore, samples were taken from any available tissue using best professional judgment. It is unknown how the types of tissue sampled may have impacted our ability to observe the Dermo infection present in the remaining tissue as a whole.

Perkinsus marinus is not the only risk of using recycled oyster shells for restoration projects. With the global seafood market and the popularity of boutique oyster bars and restaurants, oyster shells that enter the recycling pathway can come from nearly anywhere in the world. There can be non-native polychaetes, algae, sponges, tunicates, gastropods, viruses, bacteria, and protozoans associated with raw and discarded oyster shells (Diggles 2021). While there are a variety of treatments that can be used to sterilize the recycled shells such as heat treatment, and freshwater, bleach, or acid soaks these are not logistically reasonable for large-scale shell recycling programs (Diggles 2021, Bushek 2000). Sun-curing or desiccation for 4 to 6 months remains the preferred method to treat large volumes of recycled shell. Our results support previous studies recommendations that the prevalence of Dermo infection is correlated to the decomposition rates of tissue (Bushek et al. 2004). Therefore, we expect that deployment of recycled oyster shell in hotter and wetter months will help decomposition happen more quickly after initial deployment, and result in more rapid declines in the potential for additional Dermo infection in wild oysters.

Recommendations

Dermo infection is monitored across the northern Gulf of Mexico by a variety of organizations and reported to the Oyster Sentinel database (https://data.oystersentinel.cs.uno.edu/). There has not been any Dermo infection monitoring in Galveston Bay since 2015, and no consistent monitoring since 2010. There is a need for year-round monitoring of Dermo infection in oysters of Galveston Bay as seasonal cycles of infection and associated environmental variables can aid in existing oyster reef management, and restoration strategies as well as help researchers and managers understand the potential impacts of declining freshwater inflow and increasing salinity and water temperatures on Dermo infections.

The fact that there are feral hogs that are habituated to shell dumping and are utilizing the tissue as a food source at the Red Bluff Curing Site provides a benefit by removing decaying tissue, effectively removing the Dermo infection. However, it is unknown if *P. marinus* can survive the digestive tract of a feral hog, and if so, if it can remain viable in the hog feces. Because the

oyster tissue from the tops of the piles was effectively gone due to depredation by feral hogs within 2 weeks of deployment, we assumed that oysters at the tops of the piles were free of *P. marinus* after 2 weeks. The oysters in the interior of the piles had no Dermo infection detected after the 6th week of deployment. Depending on the demand for oyster shells, the results of this study suggest that resource managers and practitioners that have active depredation of oyster tissue at the top of their piles, to the extent that tissue is quickly removed, cure their recycled oyster shell material for a minimum of 3 months as long as the shell is deployed during "warm-weather" months (April – September). For oysters deployed during "cold-weather" months, the results suggest continuing the current practice of 6 months deployment with a mechanical rotation at 3 months be continued until additional studies can be completed to better understand the seasonal component and determine how temperature may impact the decomposition of the oyster tissue and subsequent Dermo infection prevalence. Should the feral hog population cease to exist on the Red Bluff Curing Site property, the tissue decomposition and Dermo infection of oysters on the top of the piles is expected to increase, and we recommend returning to the cold-weather curing protocol.

Lessons Learned

Our study showed depredation by feral hogs and vultures impacts oysters at the tops of sun-curing piles. We deployed oysters in the same plastic mesh bags that GBF uses for its oyster gardening (GBF 2023) and attached the bags to wire cable in an attempt to avoid losing the study oysters, but the feral hogs were able to rip through the bags and remove the bailing wire to access the oyster tissue. This was important to our study design as one of our goals was to determine the impact that wildlife has on the sun-curing process. Future studies should consider using a sturdier container that will allow the oysters to be exposed to the ambient environment at the top of the piles but protect them from depredation as not all sun-curing sites have feral hogs, or the same wildlife present.

Additionally, the development of the tissue condition categories was a "work-in-progress" as we observed the tissues throughout the initial weeks of deployment. We did not have previously defined condition categories beyond the initial "plump" and "shrunken" as defined by Ray (1966). As a result, the field team had to spend significant time in the field together standardizing the evaluation of these categories, and re-evaluation using photos in the initial weeks was required after the categories were finalized. Future studies may consider using these categories to standardize the process of documenting tissue degradation in oysters deployed in sun-curing piles.

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Appendix A – Data Summary Table

Summary table of Dermo intensity values and tissue condition category by oyster by week of the study. Data are organized by the wildlife access type (fenced or not fenced), the pile (A, B, C, or D), location of deployment within the pile (interior or top), the data type being monitored (Dermo or Tissue), and the oyster number. The top row of data for each oyster number displays the tissue condition category by week (P = plump, S = shrunken, L = liquified, D = desiccated, NT = no tissue, and N/A = not sampled because it was removed from the study due to depredation). The second row of data for each oyster number is the Dermo intensity by week, note: Dermo intensity was measured for all oysters at their initial deployment (week 0), and for all oysters with tissue remaining at week 35, otherwise only "Dermo" oysters were monitored for Dermo intensity each week of the study.

Wildlife Access	Pile	Location	Oyster Type	Oyster No	Week 0	1	2	3	4	5	6	8	10	13	15	17	19	21	23	25	27	31	35
Fenced	A	Interior	Dermo	2365	P	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0.33	0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2373	P	L	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3953	P	S	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					1.67	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4032	P	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0.33	0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4046	S	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.33	0.67	0.33	0.67	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2326	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2358	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2370	P	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3955	S	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3971	S	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Top	Dermo	2359	P	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					1.33	0.33	0	0.33	0	0.33	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0
				2360	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					1	0	0.67	0	0.33	0	0.33	1	0.67	0.67	0.33	0.33	0	0	0	0	0.33	1	0
				3952	P	L	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					1	0	0.33	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0
				3954	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT
					1.33	0	0	0	0	0	0	0	0	0	0.33	0	0	0	0.33	0	0	0	0
				4041	S	L	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT
					1.67	1	1	0.33	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2343	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				2357	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3742	S	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3750	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3960	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0

Appendix A Cont.

Wildlife Access	Pile	Location	Oyster Type	Oyster No	Week																	
					0	1	2	3	4	5	6	8	10	13	15	17	19	21	23	25	27	31	35
Not Fenced	B	Interior	Dermo	2335	P	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2342	P	L	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3745	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4036	P	S	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4050	P	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2368	P	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3962	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3966	P	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3972	P	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4048	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Top	Dermo	2331	S	L	S	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT
					1	0	0	0	0	0	0	0	0	0.33	0	0.33	0	0.33	0	0	0	0	0
				2345	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					1.33	0.33	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0
				2361	S	L	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					0.33	0	0	0.33	0	0	0.33	0	0	0.33	0	0.33	0	0.33	0	0	0.33	0	0
				3958	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4035	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	0	0	0.33	0	0	0	0.33	0.33	0	0.33	0	0	0	0.33	0.33	0.33	0	0
			Tissue	2347	P	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2348	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2363	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4040	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4042	P	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix A Cont.

Wildlife Access	Pile	Location	Oyster Type	Oyster No	Week																		
					0	1	2	3	4	5	6	8	10	13	15	17	19	21	23	25	27	31	35
Fenced	C	Interior	Dermo	2329	P	L	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT
					0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2374	S	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4027	P	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1	0	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4028	P	S	D	D	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4038	S	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.33	0.33	0.33	0.67	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2333	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2340	P	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3957	S	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3975	P	S	S	L	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4033	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Top	Dermo	2341	P	L	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT
					0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2346	P	S	S	S	S	S	S	L	L	D	D	D	D	D	D	D	D	NT	NT
					0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3961	S	L	L	L	L	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3970	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0
				3973	P	L	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT
					0.67	0	0	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2334	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					N/A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.33
				2354	S	S	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3951	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3959	S	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				4039	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0

Appendix A Cont.

Wildlife Access	Pile	Location	Oyster Type	Oyster No	Week																	
					0	1	2	3	4	5	6	8	10	13	15	17	19	21	23	25	27	31	35
Not Fenced	D	Interior	Dermo	2351	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					2	0.33	0.67	0	0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0
				2371	S	S	L	L	L	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				2372	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					0.67	0	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				3965	P	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					1.33	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0
				4049	S	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					1.67	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0
			Tissue	2337	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				2339	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				3728	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
				4034	P	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4037	S	S	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Top	Dermo	2330	P	S	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					1.33	0	0.33	0	0	0	0	0	0	0	0	0.33	0.33	0	0	0	0	0	0
				2338	P	D	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					1.67	0	0.67	0.33	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0
				2375	P	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT
					0.67	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0.33	0	0
				3968	S	S	D	L	L	D	D	D	D	D	D	D	NT	NT	NT	NT	NT	NT	NT
					1	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				4029	S	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	NT	NT	NT
					1.67	0	0	0	0	0	0	0.33	0	0	0	0	0.33	0	0	0.33	0	0	0
			Tissue	2349	S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2350	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					1.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				2353	P	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				3967	S	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				4047	P	S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
					0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-