Eastern Oysters *Crassostrea virginica* settle near inlets in a lagoonal estuary: Spatial and temporal distribution of recruitment in mid-Atlantic Coastal Bays (Maryland, USA) (#71727)

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Eastern Oysters *Crassostrea virginica* settle near inlets in a lagoonal estuary: Spatial and temporal distribution of recruitment in mid-Atlantic Coastal Bays (Maryland, USA)

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Background. Declines of the Eastern oyster *Crassostrea virginica*, and its numerous ecological benefits have spurred oyster restoration initiatives. Successful restoration of a self-sustaining oyster population requires evaluating the temporal and spatial patterns of recruitment (settlement and survival) of oyster larvae in the target waterbody. Restoration of the eastern oyster population in the Maryland Coastal Bays (MCBs), U.S.A., a shallow lagoonal estuary, is of interest to federal and state agencies, but the location and timing of natural recruitment is not known.

Methods. We assessed the spatial and temporal variation in oyster larval recruitment throughout the MCBs using horizontal ceramic tiles and PVC plates. Newly settled oyster larvae (recruits) were monitored biweekly from June to September 2019 and 2020 at 12 sites in the MCBs and a comparison site in Wachapreague, Virginia. Water quality parameters (temperature, salinity, dissolved oxygen, turbidity, and pH) were also measured. The objectives of this study were to determine 1) the most effective substrate design for monitoring oyster recruitment, 2) the spatial and temporal distribution of oyster larval recruitment in the MCBs, and 3) patterns in oyster larval recruitment that would be applicable to other lagoonal estuaries.

Results. 1) Ceramic tiles were more effective than PVC plates for recruiting oyster larvae; 2) Peak settlement began during the period from late June through July, and oyster recruitment was greatest at sites closest to the Ocean City and Chincoteague inlets; 3) Areas near broodstock that have long flushing rates to retain larvae, may provide the best environments for recruitment of oysters to lagoonal estuaries.

Discussion. As the first study on oyster larval recruitment in the MCBs, our results provide insight into their spatial and temporal distribution, methods that can serve as a foundation for future recruitment studies in other lagoonal estuaries, and baseline data that can be used to inform stakeholders and evaluate the success of oyster restoration projects in MCBs.

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Abstract

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Subjects: Ecology, Natural Resource Management, Conservation Biology

Keywords: Oyster, *Crassostrea virginica*, Larval recruitment, Settlement, Restoration, Ecosystem services, Lagoonal estuary, Maryland, Mid-Atlantic.

Introduction

Coastal lagoonal estuaries account for 13% of the coastline worldwide (McAvoy & Clancy, 1994). As an inhabitant of coastal lagoonal estuaries along the Atlantic shoreline of the United States, the Eastern oyster *Crassostrea virginica* (Gmelin 1791), serves as a keystone species that provides invaluable ecosystem services (Sanjeeva Raj, 2008) such as filtering water (Wilber & Clarke, 2010), cycling nitrogen (Jiang et al., 2020), conducting benthic-pelagic coupling (Maryland Department of Natural Resources, 2004), providing habitat (Harding & Mann, 2001), sequestering carbon and nitrogen (Smyth, Geraldi & Piehler, 2013; Fodrie et al., 2017), and protecting shorelines (Piazza, Banks & La Peyre, 2005). Despite its ecological importance, however, anthropogenic stresses (e.g., increased shoreline development, habitat destruction, pollution, water quality degradation) (Lotze et al., 2006; Worm et al., 2006), overharvesting (Kirby, 2004), and disease (Harvell et al., 1999; Beck et al., 2011) have contributed to its decline.

Remnant populations of wild eastern oysters exist in intertidal areas of the Maryland Coastal Bays (MCBs), a two-inlet lagoonal estuarine system along the east coast of the United States in the Mid-Atlantic. Current populations have declined dramatically from historic levels due to overharvesting and lasting effects from the creation of the Ocean City Inlet during a hurricane in 1933. This inlet introduced changes in salinity and hydrodynamics within the MCBs as well as new diseases, predators, and competitors (Maryland Department of Natural Resources, 2004; Jesien et al., 2009; Kang et al., 2017). Shellfish surveys conducted by the Maryland Department of Natural Resources in the MCBs since 1993 have never found natural oysters on the former oyster bars of the MCBs. Instead, oyster shells are deteriorating, becoming fouled, and buried in sediment (Maryland Department of Natural Resources, 2004). Though no viable

natural oysters exist in subtidal areas of the MCBs, small populations of oysters have settled on anthropogenic structures in intertidal areas (Maryland Department of Natural Resources, 2004; Jesien et al., 2009) especially in the general vicinity of the two inlets.

Population declines and habitat losses for *C. virginica* have inspired federal, state, and non-governmental agencies to pursue restoration efforts to reestablish the species in native waters (Chesapeake Bay Program, 2000). Successful oyster restoration projects have been conducted in lagoonal estuaries along the east coast of the United States at various scales, among other locations. Restoration efforts in the Delaware Inland Bays, which encompass Rehoboth Bay, Indian River Bay, and Little Assawoman Bay, include smaller restoration projects. Volunteers in the Delaware Oyster Gardening Program grow oysters for two years on private docks after which they are utilized for research or restoration purposes (Reckenbeil & Ozbay, 2014). In addition, federal and state agencies are restoring native eastern oyster populations and habitats in 10 tributaries throughout Maryland and Virginia (by 2025) as part of the 2014 Chesapeake Bay Watershed Agreement. Of the five tributaries targeted in Maryland, 788 acres of oyster reefs have been restored since 2014, with a goal of 1,439 acres by 2025.

An oyster restoration project in the MCBs would help improve its poor water quality (Jesien et al., 2009), create hard substrate habitat, and provide additional ecosystem services (Maryland Department of Natural Resources, 2004; Jesien et al., 2009). Restoration of Eastern Oyster populations in the MCBs had been discussed among federal, state, NGO, and academic partners prior to 2013 (B. Stevens, 2018, pers. comm.)

Natural recruitment of wild oyster larvae aid in restoration success by supplementing restoration efforts (Schulte & Burke, 2014). However, the spatial and temporal distribution of wild oyster larvae was not known. Prior to restoration initiatives, it is crucial to evaluate the

spatial and temporal recruitment patterns of wild oyster larvae, in addition to their growth and survival over multiple years in order to determine the feasibility, scale, and location of a restoration effort (Kennedy et al., 2011; Soniat et al., 2012; Casas, La Peyre & La Peyre, 2015). Additionally, an assessment of the best methods for measuring recruitment is needed to guide future studies in other waterbodies.

The Maryland Department of Natural Resources (MD DNR) has conducted an annual survey of shellfish populations in the MCBs since 1993 through their Shellfish Monitoring and Assessment Program. The purpose of their survey is to collect data on shellfish populations in the MCBs, to guide management and policy decisions related to conservation. Shellfish populations assessed include Eastern Oysters, Hard Clams *Mercenaria merceneria*, Softshell Clams *Mya arenaria*, Stout Razor Clams *Tagelus plebeius*, and Bay Scallops *Argopecten irradians* (Maryland Department of Natural Resources). Since 1993, natural oysters in subtidal areas of the MCBs have been dead and no recruitment observed. Instead, oyster shells from remnant oyster bars such as Yates Oyster Bars, are deteriorating, becoming fouled, and buried in sediment (Maryland Department of Natural Resources, 2004). Though no viable natural oysters exist in subtidal areas of the MCBs, small populations of oysters have settled on anthropogenic structures in intertidal areas, especially around the southern (Chincoteague) inlet (Maryland Department of Natural Resources, 2004; Jesien et al., 2009).

This study was conducted throughout the MCBs, located along the Mid-Atlantic coast of the United States between the Delmarva Peninsula (spanning the states of Delaware, Maryland, and Virginia) and the Atlantic Ocean (Fig. 1) (Dennison et al., 2016). The MCBs system is a shallow lagoonal estuary that encompasses a 453 km^2 watershed and comprises six bays (ranging from north to south): Assawoman Bay, Saint Martin River, Isle of Wight Bay, Sinepuxent Bay,

Newport Bay, and Chincoteague Bay (Maryland Department of Natural Resources, 2004; Krantz et al., 2009). MCBs is a two-inlet system with Ocean City Inlet in the north and Chincoteague Inlet in the south. It has an average depth of 1.5 m, but approximately 3 m at Ocean City Inlet and 4 m at Chincoteague Inlet (Dennison et al., 2016; Kang et al., 2017; Oseji, Fan & Chigbu, 2019). As a shallow estuary, it is well-mixed and highly productive with little to no salinity or thermal gradients (Bricker et al., 2009; Oseji, Fan & Chigbu, 2019).

Despite these characteristics, however, the MCBs have long flushing rates, e.g., the amount of time it takes for water to be replaced by water exchange through the inlets and freshwater inputs (nine days in Isle of Wight Bay to 63 days in Chincoteague Bay) (Pritchard, 1960; Thomas et al., 2009). The MCBs also have uneven circulation with well-flushed areas and high current velocities near the inlets that decrease with distance from the inlets (Krantz et al., 2009); the only sources of "new" water (inlets and freshwater input) account for approximately 7.5% of the volume in the MCBs daily (Pritchard, 1960). Well-flushed areas also have better water quality than areas in or close to tributaries. The uneven distribution of well-flushed areas in combination with input from non-point sources can cause nutrient enrichment that leads to poor water quality (Bricker et al., 2009; Dennison et al., 2016; Oseji, Fan & Chigbu, 2019).

We conducted the first study of recruitment of oyster larvae in the MCBs. From June to September in 2019 and 2020, we assessed the spatial and temporal distribution of recruitment using three sampler types (ceramic arrays, PVC arrays, and PVC collectors) that utilized PVC plates or ceramic tiles. Specific objectives were to 1) determine which sampler type was most effective for recruitment, 2) determine the spatial and temporal distribution of oyster larval recruitment in the MCBs, and 3) identify patterns in oyster larval settlement that would be applicable to other lagoonal estuaries.

Materials & Methods

Site Selection

Twelve sites in the MCBs (Fig. 1, Table 1) (10 sites in Maryland and two in Virginia) were selected based on several factors including geographic location, proximity to inlets, salinity (S= 18 – 39), bottom type, depth, historical water quality data, and expert recommendations. Historical water quality data were provided by the National Park Service (NPS) (2016 – 2018), Maryland Department of Natural Resources (MDNR) (1999 – 2019), and Maryland Coastal Bays Program (MCBP) (2013 – 2015). Final selected sites (n = 12) included three currently monitored for water quality by local agencies: DNR XDN4312 (site St. Martin River), DNR TUV0021 (site Turville Creek), and NPS ASSA 2 (site Verrazano Bridge). Sites had a range of bottom sediments from coarse sand to silt (Mid-Atlantic Ocean Data Portal, 2021) and were defined as either Pier or Bay Sites depending on whether sampling equipment was attached to a shore-based pier or placed in open water.

An additional study site for sampling gear comparison (substrate material and design) was established in Wachapreague, Virginia, at the Virginia Institute of Marine Science (VIMS) Eastern Shore Laboratory (ESL), where VIMS conducts a recruitment study on oyster larvae.

Sampler types and study site locations

"Settlement" in this study is defined as an oyster larvae cementing itself to a substrate, thereby becoming sessile (Connell, 1985), while "recruitment" refers to settlement in addition to survival for a time frame defined by the investigator (Bushek, 1988). In this study, we define "recruitment" as recently settled oyster larvae or recruits that survived on settlement substrate for up to two weeks (Rimler, 2014).

Recruitment of oyster larvae was monitored using two different types of sampler designs (collectors or arrays) containing either PVC plates or ceramic tiles (10.16 cm x 10.16 cm). PVC collectors consisted of a cage made of plastic-coated wire (dimensions: 22.86 cm x 22.86 cm x 53.34 cm) with 1.5 in^2 apertures containing PVC plates. PVC collectors were built to our specifications by Ketcham Traps (New Bedford, MA). Each collector contained three PVC plates suspended horizontally using bungee cords at 35.6 cm, 40.6 cm, and 45.7 cm above the substrate and was weighted with two bricks placed in the bottom (Fig. 2A). PVC plates were custom cut to be 12.70 cm x 13.97 cm. Plates were drilled in four corners and sanded on both sides with 100 grit sandpaper in a cross-hatched pattern to simulate the rugosity of the outside of an oyster shell to enhance settlement (Beiras & Widdows, 1995). An outer border of 6.35 mm on two sides and 12.7 mm on two sides was scored to define a counting area of exactly 10.16 cm x 12.7 cm (129 cm^2).

Arrays consisted of a 30.5 cm nylon threaded rod (0.95 cm diameter) on which three center-drilled PVC plates or ceramic tiles (10.2 cm x 10.2 cm x 0.7 cm, or 103 cm^2) were positioned and separated by 5 cm sections of 1.25 cm PVC pipe (Fig. 2B; Fig. 2C). Ceramic tiles were arranged with the unglazed side facing downwards. This design was similar to arrays used by VIMS (Ross & Synder, 2020) though our design included weights (two bricks) below, and a small float (buoy) above, to keep the plates suspended in the water column at a fixed height off the bottom. PVC collectors were deployed in 2019 and 2020 and arrays (both PVC and ceramic) were deployed only in 2020. Because chemical cues have been suggested to induce settlement (Pawlik, 1986), approximately 90% of plates and tiles were conditioned in seawater for 8–24 hours prior to deployment; the remaining were not due to time constraints.

In 2019, we compared recruitment rates between our PVC collector design and the VIMS ceramic arrays at the VIMS ESL pier. Three PVC collectors were suspended next to VIMS arrays made of ceramic tiles. This comparison was made to determine 1) if PVC plates were as effective as ceramic tiles and 2) if a potential lack of recruitment on the PVC plates in the MCBs was due to the presence of fewer oyster, or collector design, or PVC plates. Results from 2019 suggested that ceramic tiles were more suitable for monitoring oyster larval settlement. Therefore, in 2020, ceramic tile and PVC arrays employing the VIMS design were added to sites in the MCBs where oyster larval recruitment was observed during the previous year.

Field Sampling

PVC collectors and arrays were either attached to lines suspended from a shore-based pier (Pier site) or attached to a surface line suspended by buoys between each collector (Bay site) (Fig. 3A; Fig. 3B). Between June and September 2019 and 2020, PVC plates and ceramic tiles were replaced biweekly at the sites for a total of five times per site, or five "swaps". In 2019 only PVC collectors (n = 531 plates) were deployed, whereas in 2020, PVC collectors (n = 333 plates), PVC arrays (n = 225 plates), and ceramic tiles (n = 225 ceramic tiles) were deployed. In 2020, samplers could not be deployed at the planned five swap dates or at all 13 sites because of novel COVID-19 restrictions and transportation issues.

In 2019, three PVC collectors (three replicates), each containing three PVC plates (n = 9) were deployed at all 13 sites. In 2020, PVC collectors were deployed at 11 of the 13 sites, while three PVC arrays and three ceramic arrays were added to six of those sites (designated as "primary sites"). These six sites included five sites where oyster larvae settled in 2019, in addition to site Mills Island that was recommended by watermen. At all 13 sites in 2019, PVC plates (total n = 117) were collected and replaced biweekly. In 2020, at six primary sites (DNR

Pier, Island Mark 12, Mills Island, Guys Point, Queen Sound, and Wachapreague), PVC plates in PVC collectors (n = 54), PVC plates in PVC arrays (n = 54), and ceramic tiles in ceramic arrays (n = 54) were collected and replaced biweekly. At the remaining sites including Greys Creek, Verrazano Bridge, South Point, Public Landing, and Taylor Landing, only PVC collectors (n = 54) with PVC plates were used. Note that the sampling units for this study were the individual PVC plates or ceramic tiles, so n values refer to the number of plates or tiles in all cases that were deployed biweekly. Not all plates and ceramic tiles could be retrieved, however, due to being lost in the field, removed etc.

Environmental data were also measured using a Xylem ProDSS Multiparameter Water Quality Meter (Xylem, Yellow Springs, OH) that was positioned above the sediment. Environmental parameters measured included temperature (°C), salinity, dissolved oxygen (mg/l), pH, and depth (m). Turbidity was measured as secchi disk depth (m). Field experiments were approved by the Maryland Department of Natural Resources under Scientific Collection Permit numbers SCP201964 and SCP202091.

Laboratory processing

In the laboratory, any sediment on the plates was gently rinsed and brushed off, then organisms including oyster larvae, barnacles, serpulid worms, and bryozoans were counted under a dissecting microscope. Data were collected differently depending on the substrates or the types of animals being counted. All animals within the PVC border were quantified, while only oyster larvae on the underside of the ceramic tile were quantified. Oyster larvae identification was conducted after confirmation by P. Ross at VIMS. Oyster larvae were counted on the upper side (A side) and underside (B side) of the PVC plates in PVC collectors in 2019 and 2020 for consistency between years and to identify differences in preferential side settlement. Conversely,

oyster larvae were only counted on the B side of the PVC plates and ceramic tiles in the array samplers in 2020 to replicate the VIMS methods (Ross & Synder, 2020) and because the textures of the tiles were different on either side (i.e. glazed A side, unglazed B side). Lastly, fouling organisms were enumerated in both 2019 and 2020 to determine their distribution (data not presented in this manuscript).

Statistical Analysis

We tested for differences in oyster recruitment among 1) six primary sites and 2) three collector types. To maintain consistency between PVC and ceramic arrays, recruitment data collected only from the B side of PVC collectors was included in the analyses, unless stated otherwise. Because of differences in the surface areas of the PVC plates and ceramic tiles, raw oyster counts (c) were adjusted to a standardized area of 100 cm^2 (referred to hereafter as *StndCounts*) using the formula:

$$StndCount = 100 \cdot c/A$$

where A = area of PVC plate or ceramic tile. Normality of *StndCounts* was tested quantitatively using Shapiro-Wilk's normality test and visually with Quantile-Quantile plots and histograms. The distribution of *StndCounts* was deemed non-normal and transformed to help meet the assumptions of parametric statistical tests (see below). Due to a combination of many small and fewer large values, *StndCounts* were log-transformed after adding 1 to accommodate 0 values: $\log_{10}(StndCounts + 1)$. Although this did not completely normalize the *StndCounts*, the $\log_{10}(StndCounts + 1)$ transformation did improve their distribution.

Data were analyzed using RStudio version 3.6.3, with the "MASS" and "mgcv" packages (R Core Team, 2020). Two separate generalized linear models (GLMs) were used to test for differences in oyster larval recruitment by 1) spatial distribution and 2) sampler type among the

six primary sites. Because raw counts were corrected to a standardized area (O'Hara & Kotze, 2010), $\log_{10}(StndCounts + 1)$ was chosen as the response variable and GLMs were fitted using the Gaussian distribution with an Identity link function. Post Hoc Tukey's honestly significant difference (HSD) tests were performed to identify which sites or sampler types differed statistically from one another. A Mann-Whitney U test was also used to examine for a significant difference between recruitment at sites DNR Pier and Wachapreague in 2020 while nonparametric Wilcoxon signed-rank tests were performed to identify any significant differences between recruitment on PVC collectors at sites (i.e., sites DNR Pier and Wachapreague) that experienced high recruitment in both years.

Results

Sampler types

Ceramic arrays were the most effective collector type for assessing oyster larval recruitment at all sites (Fig. 4). The number of oyster larvae recruits was significantly greater, by two orders of magnitude, on Ceramic arrays than on either PVC arrays (Tukey's HSD test, $P<0.001$) or PVC collectors (Tukey's HSD test, $P<0.001$) (Fig. 5).

Within the PVC collectors deployed in 2019, 37% of oyster larvae settled on the A side and 63% on the B side, whereas in 2020, 29% settled on the A side and 71% on the B side. Considering both years, 32% of oyster larvae settled on the A side and 68% on the B side of PVC collectors.

Spatial distribution

There were consistent spatial patterns in recruitment for PVC collectors (bottom, B side only) deployed in 2019 and 2020 (Fig. 6A, Fig. 6B). In 2019 and 2020, sites DNR Pier and Wachapreague exhibited the greatest recruitment in comparison to the remaining sites. No

279 significant difference in recruitment occurred between sites DNR Pier and Wachapreague in
280 2019 (Mann-Whitney U test, U = 958.00, $P = 0.60$), though in 2020 the difference was
281 marginally non-significant (Mann-Whitney U test, U = 861.00, $P = 0.06$).

282 Settlement on PVC collectors was five times greater in 2020 than in 2019 at both sites
283 DNR Pier and Wachapreague, although inter-annual differences were significant only for site
284 Wachapreague (Wilcoxon signed-rank test, Z = 2.60, $P< 0.01$) but not for site DNR Pier
285 (Wilcoxon signed-rank test, Z = 1.09, $P = 0.28$). No recruitment occurred on the underside or B
286 side at site Island Mark 12 and Mills Island for PVC collectors in both years. However, a single
287 oyster larvae settled on the upper (A) side at site Island Mark 12 in southern Sinepuxent Bay in
288 2019. Little recruitment occurred on PVC collectors at sites Guys Point and Queen Sound in both
289 years and recruitment was slightly less in 2020 than 2019. When considering both the A and B
290 side of PVC plates, oyster larvae were found in 2020 at all sites where they occurred in 2019, in
291 addition to site Verrazano Bridge in 2020. Oyster larvae were not identified at this site in 2019,
292 so only three PVC collectors were deployed in 2020, and three oyster larvae were observed on
293 the B side of PVC plates collected on 12 August 2020.

294 PVC and ceramic arrays deployed solely in 2020 exhibited certain spatial patterns that
295 occurred on the PVC collectors as well (Fig. 6A, Fig. 6B). Within the MCBs, the greatest
296 recruitment on PVC arrays over the entire field season occurred at site DNR Pier (66 total oyster
297 larvae in 2020, mean 1.57 ± 4.30 s.d.), while the greatest recruitment on ceramic arrays in the
298 MCBs occurred at site Queen Sound (6682 total oyster larvae in 2020, mean 278.40 ± 282.11).
299 For ceramic arrays, site Queen Sound received six times the total number of oyster larvae than
300 site DNR Pier, which had the second highest recruitment (1173 total oyster larvae in 2020, mean
301 29.33 ± 49.78). Sites Island Mark 12 and Mills Island received little to no recruitment on both

array types. PVC arrays at site Guys Point received more recruitment than PVC arrays at sites Mills Island and Queen Sound, whereas ceramic arrays at site Guys Point received less recruitment than ceramic arrays at site Queen Sound. Recruitment on ceramic arrays at site Wachapreague was less than half that at site Queen Sound, but approximately three times that of site DNR Pier.

For ceramic arrays in the MCBs during 2020, the sites closest to the inlets (sites DNR Pier and Queen Sound) had the greatest recruitment of all sites. Settlement (i.e. mean standardized count per plate/tile) was 2.62 orders of magnitude greater on Ceramic arrays than on PVC collectors for DNR Pier, and 4.14 orders greater at Queen Sound, respectively (Fig. 6B). A Mann-Whitney U test was also used to examine for a significant difference between recruitment at sites DNR Pier and Queen Sound between Ceramic arrays and PVC arrays in 2020. Significant difference in recruitment occurred between Ceramic and PVC arrays at site DNR Pier (Mann-Whitney U test, $U = 1081.5$, $P = 0.008$, effect size (r) = 0.29), and Queen Sound (Mann-Whitney U test, $U = 412$, $P < 0.001$, effect size (r) = 0.61. In considering both years and all sampler types, our results within the MCBs suggest that oyster larvae are more likely to be found at sites near inlets, rather than further away from them. Tukey HSD tests comparing recruitment at the six primary sites considering all collector types combined revealed that there were significant differences between multiple pairs of sites (Fig. 7; Table 2). Moreover, the greatest recruitment occurred at sites DNR Pier (near Ocean City Inlet), Queen Sound (near Chincoteague Inlet), and Wachapreague.

Temporal distribution

PVC collectors were deployed in both 2019 and 2020 to compare temporal distribution patterns in recruitment between years (Fig. 8). Because only PVC plates were used in both 2019

and 2020, we compared raw counts of oyster larvae per PVC plate, rather than *Stndcounts.* No settlement occurred at sites other than the six primary sites. Of those sites, settlement on the B side occurred in both years at sites DNR Pier, Guys Point, Queen Sound, and Wachapreague, while sites Island Mark 12 and Mills Island received little to no recruitment. One recruit settled on the A side of a plate at site Island Mark 12 on 25 July 2019, while no recruits were observed at site Mills Island in either 2019 or 2020. In the MCBs, settlement occurred from 9 July to 9 August 2019 and 2 July to 11 August 2020. In 2019, settlement on PVC collectors began in the same week at site Wachapreague and the MCBs sites except Island Mark 12. In 2020, settlement at sites Wachapreague and Queen Sound occurred earlier than the remaining primary sites (3 July 2020).

At all sites in the MCBs, settlement in 2019 and 2020 began in early to mid-July, excluding site Island Mark 12 at which settlement occurred only in late-July of 2019. Settlement continued until late July and early August during both years. Earliest settlement within the MCBs occurred at site Queen Sound, but sampling equipment at that site disappeared after 30 July 2020 due to a storm, which prevented further data collection. Settlement began slightly earlier at site Wachapreague than in the MCBs, in late June (2019) and early July (2020), and extended longer, until late August in 2020. At site DNR Pier, near Ocean City Inlet, two settlement peaks were observed in both 2019 and 2020 and occurred within approximately the same week of each year.

Discussion

This is the first recruitment study for oyster larvae in the MCBs, and the resulting spatial and temporal distribution patterns can provide insight into evaluating restoration initiatives and serve as a foundation for future recruitment studies in other lagoonal estuaries. We assessed the

recruitment distribution of oyster larvae at 12 sites within the MCBs and a site for sampling gear comparison, using PVC plates and ceramic tiles that were monitored biweekly in summer 2019 and 2020. This study resulted in four significant findings: 1) ceramic tiles received significantly greater recruitment than PVC plates, 2) new recruits settled in the greatest numbers at sites that were closest to Ocean City and Chincoteague inlets, as opposed to sites further within the bays, 3) settlement occurred between late June and early July into mid-August, which was consistent with previous studies at similar latitudes (Shaw, 1967; Kennedy, 1980), and 4) the spatial and temporal patterns of settlement were essentially identical in both 2019 and 2020, although recruitment was four to five times greater in 2020. This study can supplement ongoing data collection (e.g., surveys of fish, shellfish and submerged aquatic vegetation, water quality, and current drift monitoring) to gain a broader understanding of the MCBs and provide baseline data upon which to build. Notably, it may guide stakeholders in evaluating the decision to potentially pursue an oyster restoration project within the MCBs and similar lagoonal estuaries.

Sampler types and plates

Oyster larvae exhibit preferential settlement (Keough & Downes, 1982), which was apparent in this study, because they settled significantly more on ceramic arrays in 2020 than any other collector type. Despite our PVC plates being sanded with 100 grit sandpaper, the ceramic tiles did have greater rugosity, making it easier for oyster larvae to attach (Marques-Silva et al., 2006). In addition, ceramic tiles are alkaline (Reig et al., 2013) and oyster larvae are more likely to settle when exposed to ammonia, which is alkaline (Coon, Fitt & Bonar, 1990). Preferential settlement on ceramic tiles rather than PVC was also evident in the study by Chuku et al. (2020), who compared monthly recruitment of the West African mangrove oyster *Crassostrea tulipa* among five substrates (coconut shell, oyster shell, nylon mesh, PVC slats, and ceramic tile) in

four lagoonal estuaries in Ghana. Ceramic tiles had the greatest monthly settlement in three of the four estuaries, and PVC slats had the greatest in the fourth; recruitment on ceramic tiles was not significantly different than on PVC slats (Chuku et al., 2020).

Consistently for both years and overall, more oyster larvae settled on the B side (bottom, ~70% of the time) than on the A side (top) of the plates in the PVC collectors. This is the opposite of what was observed in a study by Kennedy (1980) in which oyster larvae most commonly settled on the upper side of plates (78% - 99%). However, more investigators have found that larvae preferentially settle on the underside of substrates, which is what we expected would happen in the present study (Chuku et al., 2020; Crisp, 1967; Grossman, Grossman, Barber, Gamblewood, & Crosby, 2020; Kenny et al. 1990; Marques-Silva et al., 2006; Pit & Southgate, 2003; Poirier et al., 2019). Michener and Kenny (1991) found that oyster recruitment was five times greater on the underside of plates than on the upper side. Greater settlement on the underside of plates is attributed to oyster larvae being negatively phototropic thus avoiding direct sunlight and seeking shaded areas (Michener & Kenny, 1991; Baker, 1997) and reduced sedimentation (Bahr & Lanier, 1981). Sedimentation can bury new recruits and prevent recruitment altogether with only a few millimeters of sediment accumulation (Thayer et al., 2005; Wilber & Clarke, 2010). Not only does sedimentation inhibit recruitment (Michener & Kenny, 1991; Ortega & Sutherland, 1992; Jordan-Cooley et al., 2011; Quan et al., 2017), but it increases mortality (Wilber & Clarke, 2010), limits growth, (Housego & Rosman, 2016), and prevents metamorphosis (Tamburri et al., 2008). Biofilms are effective at inducing oyster settlement (Tamburri, Zimmer-Faust & Tamplin, 1992; Zhao, Zhang & Qian, 2003; Su et al., 2007; Campbell et al., 2011), but the effect varies with length of conditioning and rugosity of the substrate (Taylor, Southgate & Rose, 1998; Devakie & Ali, 2002; Zhao, Zhang & Qian, 2003; Su

et al., 2007; Tamburri et al., 2008; Bellou et al., 2020). Therefore, our use of sanded PVC plates may have counteracted the need for conditioning.

Spatial distribution

Although settlement and recruitment behaviors can be difficult to measure *in-situ*, our results show a spatial distribution trend of greater recruitment at sites near Ocean City (DNR Pier) and Chincoteague Inlet (Queen Sound), suggesting those are more attractive locations for oyster settlement than sites further away from inlets. Additionally, broodstock live close to those inlets. Both Sydney rock oyster *Saccostrea glomerata* and invasive Pacific oysters *Crassostrea gigas* in Port Jackson Estuary, Australia, had greater settlement at sites nearer the Pacific Ocean than in the upper channel (Scanes et al., 2016). The Port Jackson Estuary exhibited similar spatial distribution patterns to that of the MCBs, with more oyster larvae being observed closer to the interface between the estuary and ocean.

The James River is well-flushed with high freshwater discharge, which reduces larval residency time prior to settling (two to three weeks). Andrews (1983) noted it was not surprising that the greatest settlement occurred in late summer, when there was lower freshwater discharge. The Delaware Bay is a large estuary that has lower freshwater discharge and shallow flats, which contribute towards greater settlement (Andrews, 1983). The MCBs also have lower freshwater discharge and high flushing close to Ocean City and Chincoteague inlets. Flushing rates in the individual sub-bays vary greatly, e.g. from 9 days in Isle of Wight Bay to 63 days in Chincoteague Bay (Pritchard, 1960). This implies that oyster larvae are retained longer, thus have a longer period of time to settle in Chincoteague Bay than in Isle of Wight Bay. The longer retainment period in Chincoteague Bay supports the greater settlement observed near

Chincoteague Inlet (Maryland Department of Natural Resources, 2004). Retention within a system correlates to recruitment success (Norcross & Shaw, 1984).

Circulation in the MCBs is primarily driven by winds and tides (Kraines et al., 1999; Herrling & Winter, 2015). A hydrodynamic model by Kang et al. (2017) demonstrated that the northward flow of water through the MCBs is primarily wind-driven, except when wind speeds are weak (e.g., 3 m/s), at which times it becomes tidally driven. Tidal cycles however, drive circulation patterns near Ocean City and Chincoteague inlets (Wells, Hennessee & Hill, 2002). The MCBs have a distinct seasonal wind pattern, with prevailing winds blowing from the southwest in the summer from the Bermuda High pressure system and winds blowing from the northeast in the winter. Circulation patterns in the MCBs may also be influenced by the shape of the estuary's basin and bathymetry, or depth (Lee & Valle-Levinson, 2012) since the shallow basin and winds in the MCBs can alter wave dynamics (Mao & Xia, 2018). Strong turbulence from waves can cue oyster larvae to sink, increasing their proximity to suitable substrate in which to attach (Fuchs et al., 2013). Wheeler (2015) found a positive relationship between high average flow acceleration and diving of oyster larvae. Eastern oyster larvae primarily swim vertically in a loose helix (Hidu & Haskin, 1978) and infrequently swim horizontally (Kennedy et al., 1996, chap. 10). Therefore, currents are the primary mode of transport to habitat (Brown, Jackson & Brooks, 2000). Small, tidal currents are strongest at the inlets, causing turbulence and cuing larvae to sink closer to habitat. The turbulence from wind and tidal circulation could be a reason for the observed spatial distribution near the inlets.

The MCBs are characterized as being “microtidal” since tidal exchange is limited to Ocean City Inlet and Chincoteague Inlet. Although the tidal excursion of the MCBs is unknown, similar lagoonal estuary systems have tidal excursions of 2.7 km in Haulover Canal connecting

Mosquito Lagoon and Indian River lagoon (Smith, 1993), 2 km both for Little Egg Harbor and Barnegat Bays (Chant, 2001), and 1.02 km to 8.25 km depending on the site proximity to Fort Pierce Inlet within the Indian River lagoon (Smith, 1983). Tidal excursion refers to the distance between low water and high water, in which a particle travels. It is a measurement to describe the movement of particles such as larvae and pollutants, within a tidal cycle (Ji, 2008). A coupled biological-physical transport model by Kim et al. (2013) simulated that larger tidal excursions during a tropic tide caused greater larval dispersion. We hypothesize that the spawning adult oysters are within a 1 km – 8 km range of settlement sites in the MCBs. Perhaps the coupling of long flushing rates (especially in Chincoteague Bay) and tidal circulation through the inlets, contributed to greater settlement.

The differences in spatial distribution of oyster larvae could be influenced by the amount of space occupied by fouling organisms and their physical size on the PVC plates and ceramic tiles (Sebens, 1982). For example, a relationship between physical space and survival between two species was illustrated by Sebens (1982) who found that colonies of octocoral *Alcyonium siderium* (Verrill, 1922) within quadrats with sea squirt *Aplidium* for 17 months survived if the average diameter of the octocoral was 11 mm (±5.3 mm, n = 53) but disappeared or was overgrown by sea squirts if the diameter of octocoral was 5.3 mm (±4.6 mm, n = 8) and 4.5 mm (±3.6 mm, n = 37) respectively. The results suggested that when animals take up greater space, they have a greater chance of survival.

This may translate to small oyster larvae that can be overgrown by animals such as barnacles or worm tubes based on their small diameter. From observations in this study, the PVC plates at specific sites that were dominated by barnacles and worm tubes did not have oyster larvae (unpublished data). Conversely, branching bryozoans cover little space and were prevalent

at site Wachapreague with heavy oyster recruitment and little to no barnacle settlement. Therefore, the presence of barnacles or worm tubes on PVC plates or ceramic tiles grown over two weeks, could reduce the amount of space available for settlement. If settlement occurs, current fouling organisms may overgrow the larvae and reduce their survival. Thus, oyster larvae may have been deterred from settling in sites with high barnacle and worm tube counts or where other conditions were unsuitable for settlement.

Temporal distribution

The eastern oysters in this study region typically spawn from June through October (Haven & Fritz, 1985). Samplers showed little variation in peak timing between 2019 and 2020 (< 10 days apart) and all settlement peaks began in July in both years. Our results showed that oyster larvae settled between late-June and mid-August, which was expected based on settlement timing reported by previous studies at similar latitudes in the Mid-Atlantic (Shaw, 1967; Kennedy, 1980; Haven & Fritz, 1985; Capelle et al., 2020; Ross & Synder, 2020). Although monitoring in our study did not continue into late September, it has been documented by other studies that peaks do occur during that time (Haven & Fritz, 1985).

Conclusions

The aims of this project were to 1) determine which collector type was most effective for recruitment 2) determine the spatial and temporal distribution of oyster larvae in the MCBs and 3) identify patterns in oyster larvae settlement that would be applicable to restoration efforts in the MCBs and other lagoonal estuaries. Our results revealed that ceramic tiles received significantly greater recruitment than PVC plates, suggesting ceramic tiles are more suitable for recruitment and should be used in future recruitment studies. Oyster recruitment was greatest at

sites in well-flushed areas near Ocean City Inlet and Chincoteague Inlet, closest to the ocean. Finally, similar spatial and temporal patterns of recruitment were observed in both 2019 and 2020, and these were similar to those described by previous studies at similar latitudes in the Mid-Atlantic. As the first oyster larval recruitment study in the MCBs, this study filled important knowledge gaps and served as a baseline for future recruitment studies within the MCBs and other lagoonal estuaries. This study provided information that will be useful to state and federal agencies in making informed decisions about restoration initiatives.

Recommendations

Prior to pursuing oyster restoration in the MCBs and other lagoonal estuaries we recommend: 1) selecting a restoration site that is in close proximity to broodstock, has a long flushing rate, and circulation patterns that retain larvae, 2) utilizing oyster shells as substrate for preliminary recruitment studies and/or restoration projects (if oyster shells are not accessible, ceramic tiles can be used as an alternative), 3) establishing a restoration site prior to or in early June (in the Mid-Atlantic) to ensure wild oyster larvae settle during peak time, 4) conduct additional research on the current state of parasites and diseases to ensure survival and growth of oysters, and 5) establish a monitoring program to assess progress and address environmental changes (see Kennedy et al., 2011 for a more thorough review). Additional studies would provide insight into the potential success of an oyster restoration project. Future work is needed to assess the growth and survival of newly recruited oyster larvae, examine the effects of overwintering on juvenile and adult oysters, and identifying the presence and impact of diseases and predators. Partnering with local agencies as well as oyster farmers and watermen would aid in the collection of necessary data.

For any oyster restoration project, monitoring is recommended prior, during, and after restoration to assess the reef habitat, the organisms living on the reef, and interactions among organisms (Thayer et al., 2005). This is important so adjustments can be made if needed and the progress of the restoration can be observed over time. Kennedy et al. (2011) developed a set of recommendations for future restoration initiatives after studying restoration and monitoring projects of 12 agencies. They suggested that monitoring a restored oyster reef should include observations of oyster abundance, mean size, recruitment, disease, and mortality. In addition, new recruits can be monitored to understand recruitment surrounding the reef as well by deploying ceramic tile collectors (Thayer et al., 2005). Additional factors such as water quality and disease presence should also be evaluated (Thayer et al., 2005).

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Figure 1

Study area map.

Map of study area depicting the locations of 12 coastal bay sites in Maryland and one in Virginia where three sampler types were deployed from June to September 2019 and 2020 to assess the distribution of oyster larvae. Circles indicate sites in which three collector types were suspended from a pier while squares indicate sites where three collector types were set on a floating buoy line. Inset shows the location of the study area within the Delmarva Peninsula (USA).

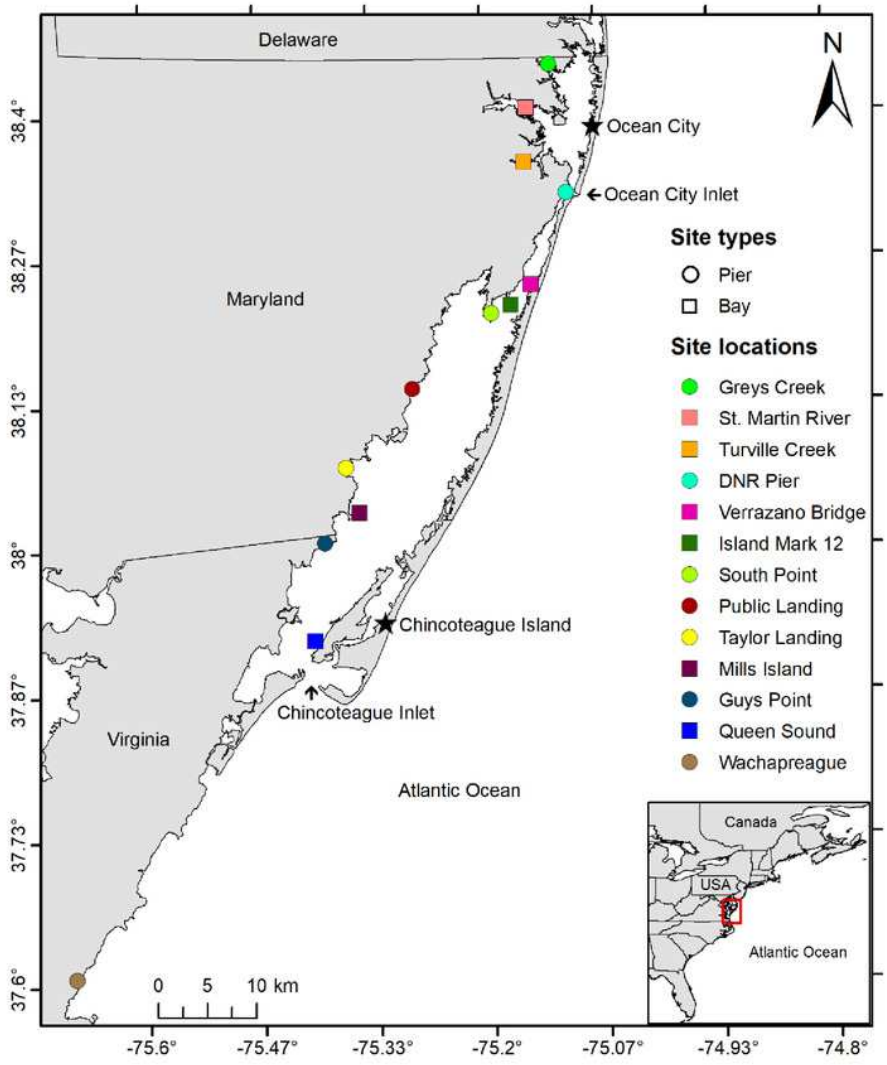

Delaware
Maryland
Virginia
Atlantic Ocean
Ocean City
Ocean City Inlet
Chincoteague Island
Chincoteague Inlet
N
Site types
Pier
Bay
Site locations
Greys Creek
St. Martin River
Turville Creek
DNR Pier
Verrazano Bridge
Island Mark 12
South Point
Public Landing
Taylor Landing
Mills Island
Guys Point
Queen Sound
Wachapreague
Canada
USA
Atlantic Ocean
0 5 10 km
38.4°
38.27°
38.13°
38°
37.87°
37.73°
37.6°
-75.6°
-75.47°
-75.33°
-75.2°
-75.07°
-74.93°
-74.8°

Table 1(on next page)

Characteristics of 13 coastal bay sites.

Sites chosen to monitor the distribution of oyster larvae from June to September 2019 and 2020. Data about bottom type were obtained from the Mid-Atlantic Ocean Data Portal. Mean salinity and depth (m) were calculated from data collected in 2019 and 2020.

Table 1 **Characteristics of 13 coastal bay sites.** Sites chosen to monitor the distribution of oyster larvae from June to September 2019 and 2020. Data about bottom type were obtained from the Mid-Atlantic Ocean Data Portal. Mean salinity and depth (m) were calculated from data collected in 2019 and 2020

						Temp (°C)		Salinity		Depth (m)	
ID	Site name	Site type	Site location	Lat	Long	Mean ± sd	n	Mean ± sd	n	Mean ± sd	n
GC	Greys Creek	Pier	Tributary of Assawoman Bay	38.45	-75.12	28.80 ± 1.22	10	24.86 ± 2.09	10	0.61 ± 0.09	7
SM	St. Martin River	Bay	St. Martin River	38.41	-75.15	27.79 ± 2.18	4	26.58 ± 0.91	4	0.95 ± 0.13	3
TC	Turville Creek	Bay	Turville Creek	38.36	-75.15	28.40 ± 1.83	4	26.62 ± 1.14	4	0.69 ± 0.25	4
DP	DNR Pier	Pier	Sinepuxent Bay	38.33	-75.10	22.54 ± 2.04	10	30.40 ± 1.29	10	2.81 ± 0.60	7
VB	Verrazano Bridge	Bay	Sinepuxent Bay	38.24	-75.14	27.16 ± 1.04	7	29.07 ± 1.36	7	1.09 ± 0.29	7
IM	Island Mark 12	Bay	Sinepuxent Bay	38.22	-75.17	26.84 ± 1.70	7	28.69 ± 1.82	7	0.87 ± 0.14	7
SP	South Point	Pier	Sinepuxent Bay	38.22	-75.19	28.43 ± 1.11	10	28.97 ± 2.00	10	0.91 ± 0.12	8
PL	Public Landing	Pier	Chincoteague Bay	38.15	-75.29	28.72 ± 0.98	10	28.20 ± 2.08	10	0.76 ± 0.12	8
TL	Taylor Landing	Pier	Chincoteague Bay	38.08	-75.36	28.81 ± 1.89	10	29.35 ± 2.21	10	0.78 ± 0.16	7
MI	Mills Island	Bay	Chincoteague Bay	38.03	-75.35	26.82 ± 1.49	8	30.88 ± 1.65	8	0.88 ± 0.15	6
GP	Guys Point	Pier	Chincoteague Bay	38.01	-75.39	29.61 ± 1.01	8	32.35 ± 4.65	8	1.20 ± 0.25	10
QS	Queen Sound	Bay	Chincoteague Bay	37.92	-75.40	26.61 ± 1.92	8	30.96 ± 1.25	8	1.25 ± 0.14	6
W	Wachapreague	Bay	Burtons and Bradford Bay	37.61	-75.69	27.76 ± 1.54	10	31.69 ± 2.07	10	1.44 ± 0.59	9

Figure 2

Sampler types used to monitor recruitment of oyster larvae at 13 sites to assess their distribution:

(A) PVC collector, B) Ceramic array, and (C) PVC array.

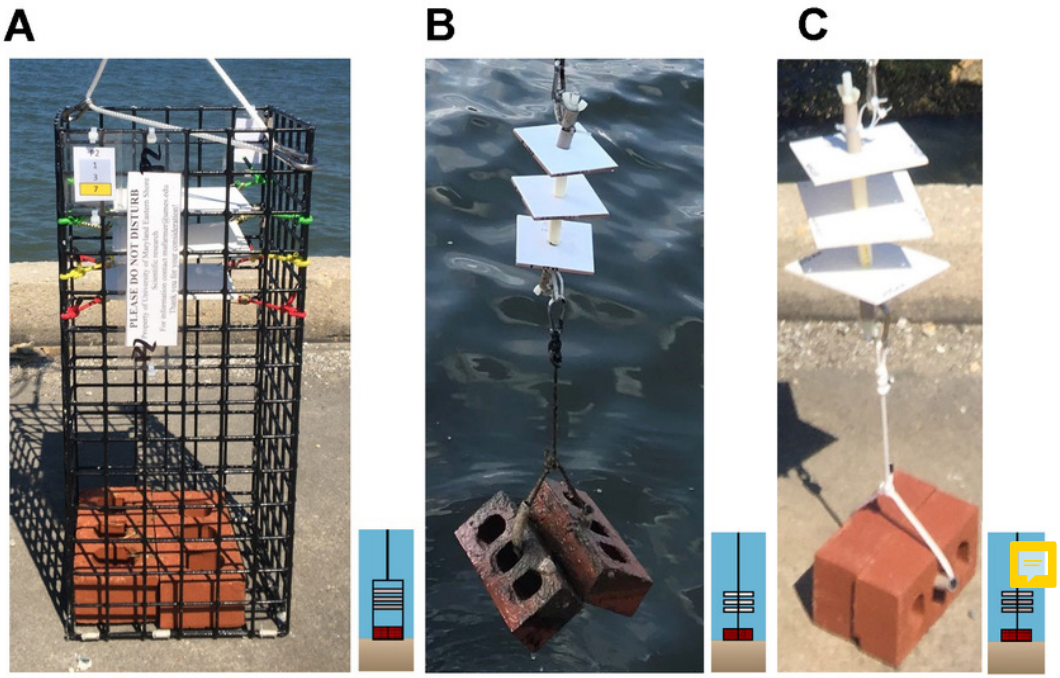

A
B
C
PLEASE DO NOT DISTURB

Figure 3

Diagrams of samplers deployed at coastal bay site locations:

(A) Pier site in which ceramic arrays (white), PVC arrays (gray), and PVC collectors (gray, in rectangles) were suspended from a shore-based pier and (B) Bay site in which samplers were suspended from a floating buoy line. At both site locations, each sampler type had three replicates that were positioned in random order. In 2019, all 13 sites included three PVC collectors. In 2020, six primary (sites DNR Pier, Island Mark 12, Mills Island, Guys Point, Queen Sound, and Wachapreague) included all three sampler types (ceramic arrays, PVC arrays, and PVC collectors), while the remaining sites included three PVC collectors.

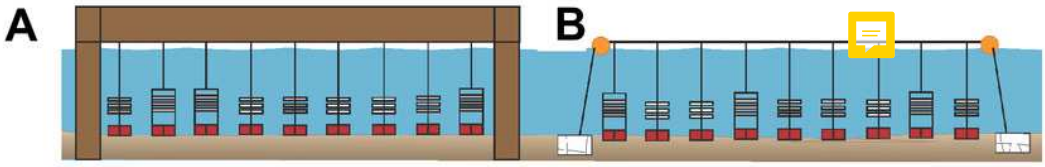

A
B

Figure 4

Comparison of Log_{10}(*StndCounts* +1) for three sampler types at six primary coastal bay sites.

The collector types include (A) Ceramic arrays, (B) PVC arrays, and (C) PVC collectors. All three collector types were deployed from June to September 2020 to monitor the distribution of oyster larvae at the six primary coastal bay sites. PVC collectors were deployed at the remaining sites. *StndCount* = Oyster larvae counts (c) multiplied by 100/A, where A = area of the PVC plate or ceramic tile. The bolded horizontal bars represent the median.

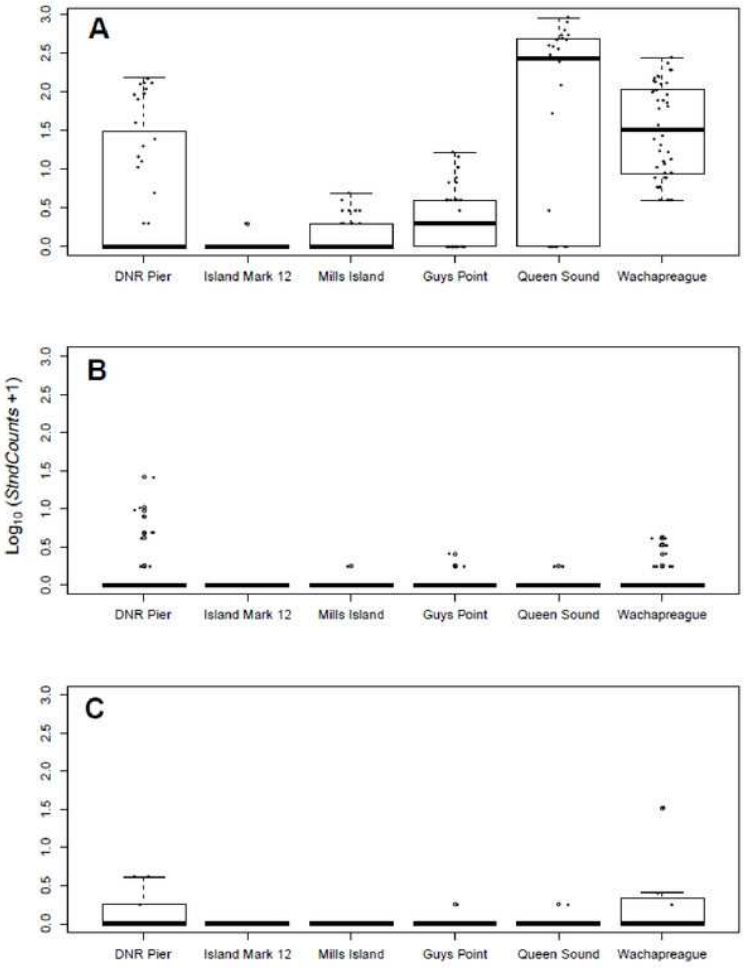

A
B
C
Log10 (StndCounts +1)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
DNR Pier
Island Mark 12
Mills Island
Guys Point
Queen Sound
Wachapreague

Figure 5

Comparison of cumulative Log_{10}(*StndCounts* +1) totaled from June to September 2020 to compare three sampler types at six primary coastal bay sites.

Three collector types were used to determine which was the most successful in recruiting oyster larvae. Letters indicate similar groups as determined by Tukey's honestly significant difference (HSD) test. Open circles indicate the vertical position of outliers; filled circles are observed data (jittered to prevent overlap). Ceramic arrays (n= 202), PVC arrays (n = 197), PVC collectors (n = 236). The bolded horizontal bars represent the median.

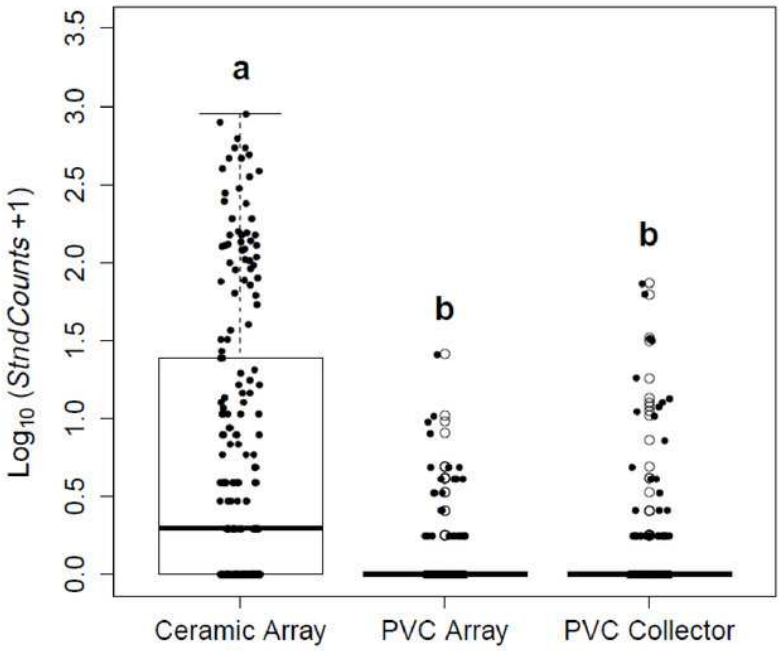

a
b
b
Log10 (StndCounts +1)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Ceramic Array
PVC Array
PVC Collector

Figure 6

Map of spatial distribution of oyster larvae that settled on three sampler types at six primary coastal bay sites:

(A) Mean (with standard deviation) oyster larvae per plate over entire field season on PVC collectors in 2019 and 2020; (B) Mean (with standard deviation) oyster larvae per plate over entire field season on ceramic and PVC arrays in 2020. Values refer to oyster larvae counts from the underside of a plate or tile standardized to an area of 100 cm^2: Counts (n) x 100/A, where A = plate or tile area. Six primary sites included site DNR Pier (DP), Island Mark 12 (IM), Mills Island (MI), Guys Point (GP), Queen Sound (QS), and Wachapreague (W).

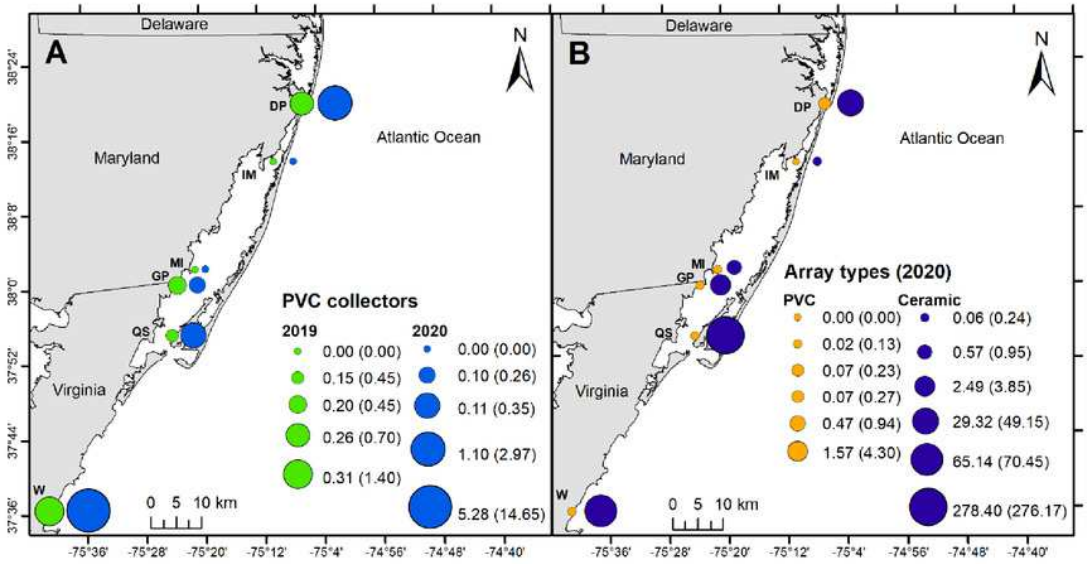

A
Delaware
Maryland
Virginia
Atlantic Ocean
DP
IM
MI
GP
QS
W
N
0 5 10 km
PVC collectors
2019
0.00 (0.00)
0.15 (0.45)
0.20 (0.45)
0.26 (0.70)
0.31 (1.40)
2020
0.00 (0.00)
0.10 (0.26)
0.11 (0.35)
1.10 (2.97)
5.28 (14.65)
B
Array types (2020)
PVC
0.00 (0.00)
0.02 (0.13)
0.07 (0.23)
0.07 (0.27)
0.47 (0.94)
1.57 (4.30)
Ceramic
0.06 (0.24)
0.57 (0.95)
2.49 (3.85)
29.32 (49.15)
65.14 (70.45)
278.40 (276.17)
38°24'
38°16'
38°8'
38°0'
37°52'
37°44'
37°36'
-75°36'
-75°28'
-75°20'
-75°12'
-75°4'
-74°56'
-74°48'
-74°40'

Figure 7

Comparison of cumulative $Log_{10}(StndCounts + 1)$ from all sampler types combined among six primary coastal bay sites.

The three sampler types included ceramic arrays, PVC arrays, and PVC collectors. All three sampler types were deployed sites from June to September 2020 to study the distribution of oyster larvae at six primary coastal bay sites. *StndCount* **=** Oyster larvae counts (n) multiplied by 100/A, where A = PVC plate or ceramic tile area. Open circles indicate the vertical position of outliers; filled circles are observed data (jittered to prevent overlap). The bolded horizontal bars represent the median.

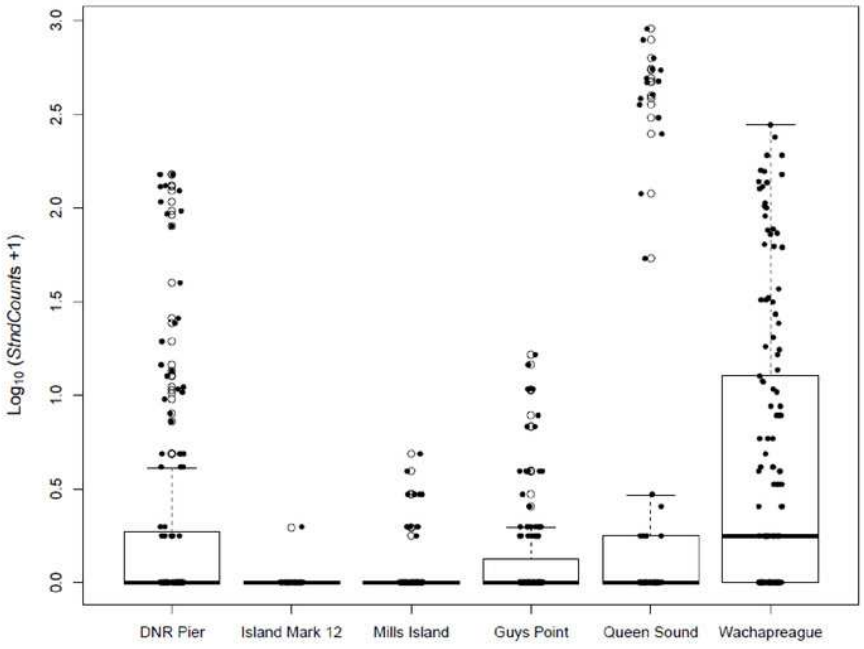

Log10 (StndCounts +1)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
DNR Pier
Island Mark 12
Mills Island
Guys Point
Queen Sound
Wachapreague

Table 2 (on next page)

Results of Tukey's honestly significant difference (HSD) test.

Tukey's HSD test results for significant differences in $\log_{10}(StndCounts + 1)$ among the six primary coastal bay sites considering all collector types. Six primary sites include site DNR Pier (DP), Island Mark 12 (IM), Mills Island (MI), Guys Point (GP), Queen Sound (QS), and Wachapreague (W). P-values indicated as * < 0.05, ** < 0.01, *** <0.001.

1

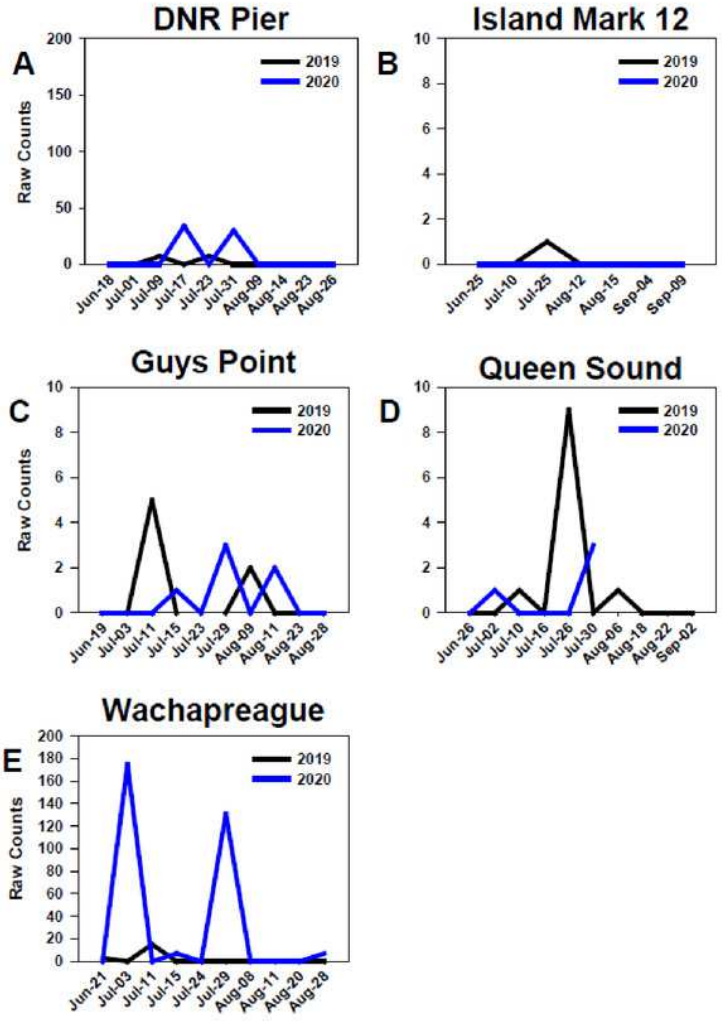
2 Table 2 **Results of Tukey's honestly significant difference (HSD) test.** Tukey's HSD test results for significant differences in $\log_{10}(StndCounts + 1)$ among the six primary coastal bay sites considering all collector types. Six primary sites include site DNR Pier (DP), Island Mark 12 (IM), Mills Island (MI), Guys Point (GP), Queen Sound (QS), and Wachapreague (W). P-values indicated as * < 0.05, ** < 0.01, *** <0.001.

	DNR Pier	Island Mark 12	Mills Island	Guys Point	Queen Sound	Wachapreague
DNR Pier						
Island Mark 12	0.0236 *					
Mills Island	0.0036 **	0.999				
Guys Point	0.0839	0.8331	0.878			
Queen Sound	0.0716	<0.001 ***	<0.001 ***	<0.001 ***		
Wachapreague	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	0.9713	

Figure 8

Temporal distribution of oyster larvae at coastal bay sites.

Comparison of recruitment on PVC collectors at five sites from June to September 2019 and 2020. Figures display raw counts of oyster larvae counted on the underside of PVC plates within PVC collectors. Site Mills Island was excluded because settlement occurred only on the A side of a single PVC plate. Sites (A) DNR Pier, (B) Island Mark 12 , (C) Guys Point , (D) Queen Sound , (E) Wachapreague . Note scales of y-axes differ.


DNR Pier
A
Raw Counts
2019
2020
Island Mark 12
B
Guys Point
C
Queen Sound
D
Wachapreague
E