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

Madeline A Farmer [Corresp., 1] , **Sabrina A Klick** [2] , **Daniel W Cullen** [1] , **Bradley G Stevens** [1]

[1] Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, Maryland, United States

[2] Southeast Watershed Research Laboratory, USDA-ARS, Tifton, Georgia, United States of America

Corresponding Author: Madeline A Farmer
Email address: madelinea.farmer6@gmail.com

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 measurements collected included temperature, salinity, dissolved oxygen, pH, and turbidity. 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 slow 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.

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

6 Madeline A Farmer[1], Sabrina A Klick[2], Daniel W Cullen[1], Bradley G Stevens[1]

8 [1] Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, MD,
9 21853, USA
10 [2] USDA-ARS, Southeast Watershed Research Laboratory, Tifton, GA, 31793, USA

12
13 Corresponding Author:
14 Madeline Farmer
15 3133 Cobb Hill Lane, Oakton, Virginia, 22124, USA
16 Email address: madelinea.farmer6@gmail.com

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

51   Coastal lagoonal estuaries account for 13% of the coastline worldwide (McAvoy &

52 Clancy, 1994). The Eastern oyster, *Crassostrea virginica* (Gmelin 1791), is an inhabitant and

53 keystone species of coastal lagoonal estuaries along the Atlantic shoreline of the United States.

54 Important ecosystem services (Sanjeeva Raj, 2008) provided by the Eastern oyster are water

55 filtration (Wilber & Clarke, 2010), nitrogen cycling (Jiang et al., 2020), benthic-pelagic coupling

56 (Maryland Department of Natural Resources, 2004), habitat formation (Harding & Mann, 2001),

57 carbon and nitrogen sequestration (Smyth, Geraldi & Piehler, 2013; Fodrie et al., 2017), and

58 shoreline protection (Piazza, Banks & La Peyre, 2005). Despite the Eastern oyster's ecological

59 importance, anthropogenic stresses such as increased shoreline development, habitat destruction,

60 pollution, water quality degradation (Lotze et al., 2006; Worm et al., 2006), overharvesting

61 (Kirby, 2004), and disease (Harvell et al., 1999; Beck et al., 2011) have contributed to its

62 decline.

63   Population declines and habitat losses of the Eastern oyster have inspired federal, state,

64 and non-governmental agencies to pursue restoration efforts to reestablish the species in native

65 waters (Chesapeake Bay Program, 2000). Successful oyster restoration projects have been

66 conducted in lagoonal estuaries along the east coast of the United States at various scales and

67 locations. For example, the restoration efforts in the Delaware Inland Bays, which encompass

68 Rehoboth Bay, Indian River Bay, and Little Assawoman Bay, include smaller restoration

69 projects. Volunteers in the Delaware Oyster Gardening Program grow oysters for two years on

70 private docks to be utilized for research or restoration purposes (Reckenbeil & Ozbay, 2014). In

71 Maryland and Virginia, federal and state agencies are restoring native Eastern oyster populations

72 and habitats in 10 tributaries (by 2025) as part of the 2014 Chesapeake Bay Watershed

73   Agreement. Of the five tributaries targeted in Maryland, 788 acres of oyster reefs have been

74   restored since 2014, with a goal of 1,439 acres by 2025. Restoration of Eastern oyster

75   populations in the Maryland Coastal Bays (MCBs) have also been discussed among federal,

76   state, NGO, and academic partners prior to 2013 (B. Stevens, 2018, pers. comm.), However, the

77   spatial and temporal distribution of wild oyster larvae in the MCBs was not known.

78   Surveys on historical oyster bars and adult Eastern oyster populations in the MCBs have

79   been conducted as part of the annual shellfish population surveys by the Maryland Department of

80   Natural Resources (MD DNR) through their Shellfish Monitoring and Assessment Program.

81   Remnant populations of wild Eastern oysters exist in intertidal areas of the MCBs, a two-inlet

82   lagoonal estuarine system along the east coast of the United States in the Mid-Atlantic. The

83   Eastern oyster populations have declined dramatically from historic levels due to overharvesting

84   and lasting effects from the creation of the Ocean City Inlet during a hurricane in 1933. This inlet

85   introduced changes in salinity and hydrodynamics within the MCBs as well as new diseases,

86   predators, and competitors (Maryland Department of Natural Resources, 2004; Jesien et al.,

87   2009; Kang et al., 2017). Shellfish surveys have never found natural oysters on the former oyster

88   bars of the MCBs since 1993. Instead, oyster shells are deteriorating, becoming fouled, and

89   buried in sediment (Maryland Department of Natural Resources, 2004). Although no viable

90   natural oysters exist in subtidal areas of the MCBs, small populations have settled on

91   anthropogenic structures in intertidal areas near Ocean City Inlet and southern Chincoteague Bay

92   where subtidal oyster farms also exist (Maryland Department of Natural Resources, 2004; Jesien

93   et al., 2009). Since the annual shellfish surveys only provide data on adult oyster populations, no

94   information on the locations of settled oyster larvae exists, so the spatial and temporal

95   distribution of wild oyster larvae still remains unknown in the MCBs.

96   Prior to restoration initiatives, it is crucial to evaluate the spatial and temporal recruitment

97   patterns of wild oyster larvae and their growth and survival over multiple years to determine the

98   feasibility, scale, and location of a restoration effort (Kennedy et al., 2011; Soniat et al., 2012;

99   Casas, La Peyre & La Peyre, 2015). Additionally, mapping the locations of wild oyster larvae is

100   important because natural recruitment supplements restoration efforts and aids in the success of

101   restoration projects (Schulte & Burke, 2014). Therefore, we conducted the first study of

102   recruitment of oyster larvae in the MCBs and assessment of the best methods for measuring

103   recruitment to guide future studies in other waterbodies. From June to September in 2019 and

104   2020, we assessed the spatial and temporal distribution of recruitment using three sampler types

105   (ceramic arrays, PVC arrays, and PVC collectors) that utilized PVC plates or ceramic tiles.

106   Specific objectives were to 1) determine which sampler type was most effective for recruitment,

107   2) determine the spatial and temporal distribution of oyster larval recruitment in the MCBs, and

108   3) identify patterns in oyster larval settlement that would be applicable to other lagoonal

109   estuaries. Identifying natural recruitment of wild oyster larvae can aid in restoration success by

110   supplementing restoration efforts (Schulte & Burke, 2014). An oyster restoration project in the

111   MCBs would help improve its poor water quality (Jesien et al., 2009), create hard substrate

112   habitat, and provide additional ecosystem services (Maryland Department of Natural Resources,

113   2004; Jesien et al., 2009).

114

Materials & Methods

116   **Study Area**

117   This study was conducted throughout the MCBs, located along the Mid-Atlantic coast of

118   the United States between the Delmarva Peninsula (spanning the states of Delaware, Maryland,

119   and Virginia) and the Atlantic Ocean (Dennison et al., 2016). The MCBs system is a shallow

120   lagoonal estuary that encompasses a 453 km² watershed comprised of six bays ranging from

121   north to south: Assawoman Bay, Saint Martin River, Isle of Wight Bay, Sinepuxent Bay,

122   Newport Bay, and Chincoteague Bay (Maryland Department of Natural Resources, 2004; Krantz

123   et al., 2009). The MCBs is a two-inlet system with Ocean City Inlet in the north and

124   Chincoteague Inlet in the south. It has an average depth of 1.5 m, but approximately 3 m at

125   Ocean City Inlet and 4 m at Chincoteague Inlet (Dennison et al., 2016; Kang et al., 2017; Oseji,

126   Fan & Chigbu, 2019). As a shallow estuary, it is well-mixed and highly productive with little to

127   no salinity or thermal gradients (Bricker et al., 2009; Oseji, Fan & Chigbu, 2019).

128   The MCBs have varied flushing rates, the amount of time it takes for water to be replaced

129   by water exchange through the inlets and freshwater inputs, which range from 9 days in Isle of

130   Wight Bay to 63 days in Chincoteague Bay (Pritchard, 1969; Thomas et al., 2009). Another

131   characteristic of the MCBs is uneven circulation with high current velocities near the inlets that

132   decrease with distance from the inlets (Krantz et al., 2009). The only sources of "new" water

133   (inlets and freshwater input) account for approximately 7.5% of the volume in the MCBs daily

134   (Pritchard, 1960). Well-circulated areas have better water quality than areas in or close to

135   tributaries. The uneven distribution of well-circulated areas in combination with input from non-

136   point sources can cause nutrient enrichment that leads to poor water quality (Bricker et al., 2009;

137   Dennison et al., 2016; Oseji, Fan & Chigbu, 2019).

138   **Site Selection**

139   Historical water quality data from the National Park Service (NPS; 2016 – 2018),

140   Maryland Department of Natural Resources (MDNR; 1999 – 2019), and Maryland Coastal Bays

141   Program (MCBP; 2013 – 2015) were used to guide the selection of study sites. From the

142   historical water quality data, twelve sites in the MCBs (10 sites in Maryland and two in Virginia)

143   were selected based on several factors including geographic location, proximity to inlets, salinity

144   (18 – 39 ppt), bottom type, depth, historical water quality data, and expert recommendations

145   (Fig. 1, Table S1). The study sites (n = 12) included three currently monitored for water quality

146   by local agencies: DNR XDN4312 (site: St. Martin River), DNR TUV0021 (site: Turville

147   Creek), and NPS ASSA 2 (site: Verrazano Bridge). Sites had a range of bottom sediments from

148   coarse sand to silt (Mid-Atlantic Ocean Data Portal, 2021) and were defined as either Pier or Bay

149   Sites depending on whether sampling equipment was attached to a shore-based pier or placed in

150   open water. An additional study site for sampling gear comparison (substrate material and

151   design) was established in Wachapreague, Virginia, at the Virginia Institute of Marine Science

152   (VIMS) Eastern Shore Laboratory (ESL), where VIMS conducts a recruitment study on oyster

153   larvae.

154   **Sampler types**

155   "Settlement" in this study is defined as an oyster larvae cementing itself to a substrate,

156   thereby becoming sessile (Connell, 1985). "Recruitment" refers to settlement in addition to

157   survival for a time frame defined by the investigator (Bushek, 1988; Roegner & Mann, 1995).

158   We defined "recruitment" as recently settled oyster larvae or recruits that survived on settlement

159   substrate for up to two weeks as described in Rimler (2014). Recruitment of oyster larvae was

160   monitored using two different types of sampler designs (collectors or arrays) containing either

161   PVC plates (12.70 cm x 13.97 cm) or ceramic tiles (10.16 cm x 10.16 cm).

162   PVC collectors consisted of a cage made of plastic-coated wire (22.86 cm x 22.86 cm x

163   53.34 cm) with 1.5 in^2 apertures containing PVC plates and built to our specifications by

164   Ketcham Traps (New Bedford, MA). Each collector contained three PVC plates suspended

165 horizontally using bungee cords at 35.6 cm, 40.6 cm, and 45.7 cm above the substrate and was

166 weighted with two bricks placed in the bottom (Fig. 2A). PVC plates were custom cut to be

167 12.70 cm x 13.97 cm but had an outer border that confined a counting area to 10.16 cm x 12.7

168 cm. Plates were drilled in four corners and sanded on both sides with 100 grit sandpaper in a

169 cross-hatched pattern to simulate the rugosity of the outside of an oyster shell to enhance

170 settlement (Beiras & Widdows, 1995). An outer border of 6.35 mm on two sides and 12.70 mm

171 on two sides was scored to define a counting area of exactly 10.16 cm x 12.7 cm (129 cm^2). The

172 border was defined to ease plate removal and reduce the risk of dislodgement of organisms

173 because the plates could not be picked up comfortably using one hand width.

174   Arrays consisted of a 30.5 cm nylon threaded rod (0.95 cm diameter) on which three

175 center-drilled PVC plates or ceramic tiles (10.2 cm x 10.2 cm x 0.7 cm, or 103 cm^2) were

176 positioned and separated by 5 cm sections of 1.25 cm PVC pipe (Fig. 2B; Fig. 2C). Arrays did

177 not have a surrounding wire mesh cage. Ceramic tiles were arranged with the unglazed side

178 facing downwards. This design was similar to arrays used by VIMS (Ross & Synder, 2020) but

179 modified with weights (two bricks) below, and a small float (buoy) above, to keep the plates

180 suspended in the water column at a fixed height off the bottom. Ceramic tiles did not have a

181 border, like the PVC plates, because they were smaller and could easily be picked up on the sides

182 using one hand, without risk of dislodging organisms. PVC collectors were deployed in 2019 and

183 2020 and arrays (both ceramic and PVC) were deployed only in 2020. Because chemical cues

184 have been suggested to induce settlement (Pawlik, 1986), approximately 90% of plates and tiles

185 were conditioned in seawater for 8–24 hours prior to deployment; the remaining were not due to

186 time constraints.

187   In 2019, we also carried out an observational study at site Wachapreague at the Virginia

188   Institute of Marine Science (VIMS) Eastern Shore Laboratory to compare VIMS ceramic arrays

189   and our PVC collector design. Three PVC collectors were suspended next to VIMS arrays made

190   of ceramic tiles. This comparison was made to determine 1) if PVC plates were as effective as

191   ceramic tiles and 2) if a potential lack of recruitment on the PVC plates was due to the presence

192   of fewer oyster, collector design, or plate substrate. Due to the results of this study and low

193   counts in 2019 on PVC plates vs. ceramic tiles we added ceramic array designs to six sites in

194   2020 as described below. Results from 2019 suggested that ceramic tiles were more effective for

195   monitoring oyster larval settlement. Therefore, ceramic and PVC arrays employing the VIMS

196   design were added in 2020 to the sites where oyster larval recruitment was observed during

197   2019. Although PVC collectors were less suitable for recruitment than ceramic tiles, they were

198   deployed again in 2020 to compare spatial and temporal distribution between years and evaluate

199   potential patterns.

200   **Field Sampling**

201   PVC collectors and arrays were either attached to lines suspended from a shore-based

202   pier (Pier site) or attached to a surface line suspended by buoys between each collector (Bay site)

203   (Fig. 3A; Fig. 3B). The PVC plates and ceramic tiles were replaced biweekly at the sites for a

204   total of five times per site, or five "swaps" from June – September of 2019 and 2020. In 2019,

205   three PVC collectors (three replicates), each containing three PVC plates ($n = 9$) were deployed

206   at all 13 sites. At all 13 sites in 2019, PVC plates (total $n = 117$) were collected and replaced

207   biweekly ($n = 531$ plates total). During 2020, PVC collectors ($n = 333$ plates), PVC arrays ($n =$

208   225 plates), and ceramic tiles ($n = 225$ ceramic tiles) could not be deployed at the same five swap

209 dates nor at all 13 sites from 2019 due to COVID-19 restrictions and transportation issues.

210 Therefore, the "swap dates" typically occurred later and fewer sites were sampled in 2020 than

211 in 2019.

212   In 2020, PVC collectors were deployed at 11 of the 13 sites, while three PVC arrays and

213 three ceramic arrays were added to six of those sites (designated as "primary sites"). These six

214 sites included four sites where oyster larvae settled in 2019 (Fig. S1), in addition to site Mills

215 Island and Island Mark 12 that were recommended by watermen. Among the six primary sites

216 (DNR Pier, Island Mark 12, Mills Island, Guys Point, Queen Sound, and Wachapreague), PVC

217 plates ($n = 54$) in PVC collectors, PVC plates ($n = 54$) in PVC arrays, and ceramic tiles ($n = 54$)

218 in ceramic arrays were collected and replaced biweekly. Only PVC collectors with PVC plates

219 were used at the remaining sites including Greys Creek, Verrazano Bridge, South Point, Public

220 Landing, and Taylor Landing. Not all plates and ceramic tiles could be retrieved, however, due

221 to being lost in the field, removed etc.

222   Environmental data were also measured using a Xylem ProDSS Multiparameter Water

223 Quality Meter (Xylem, Yellow Springs, OH) that was positioned above the sediment.

224 Environmental parameters measured included temperature (°C), salinity, dissolved oxygen

225 (mg/l), pH, and depth (m). Turbidity was measured as secchi disk depth (m). Field experiments

226 were approved by the Maryland Department of Natural Resources under Scientific Collection

227 Permit numbers SCP201964 and SCP202091.

228 **Laboratory processing**

229   PVC plates and ceramic tiles collected in the field were transported to the laboratory at

230 the University of Maryland Eastern Shore in CD containers to prevent abrasion among plates.

231 Sediment on the plates was gently rinsed and brushed off and a dissecting microscope was used

232   to count oyster larvae. PVC material rugosity was consistent on both sides, while ceramic tiles

233   had a smooth glazed top and a rough unglazed bottom. Oyster larvae were counted on the

234   underside of the PVC plates and ceramic tiles to replicate the methods used by the Virginia

235   Institute of Marine Science (VIMS; Gloucester, VA, USA; (Ross & Synder, 2020) and because

236   of the texture differences. Oyster larvae identification was conducted after confirmation by P.

237   Ross at VIMS.

238   **Statistical Analysis**

239   All statistical analyses were performed in R version 4.2.2 (R Core Team, 2022) and the

240   graphics were generated using the "ggplot2" R package (Wickham, 2016). Histogram plots and

241   the Shapiro-Wilk test for normality showed a non-normal distribution for the oyster larval count

242   data. Kruskal-Wallis rank sum tests (R Core Team, 2022) followed by Dunn's post hoc multiple

243   comparison tests (Ogle et al., 2022) were used to determine differences ($\alpha = 0.05$) among sites

244   and sampler types. Due to the high percentage of zeros in the larval counts during 2019 (95%)

245   and 2020 (42%), the data was subset to counts ≥ 1 to determine statistical differences among

246   sites and sampler types.

247   Densities of the larval counts (larval count per cm^2 = larval counts from each PVC plate

248   or ceramic tile/area of PVC plate or ceramic tile) were calculated to generate density maps for

249   spatial data visualization. Densities adjusted for plate size differences between ceramic (10.16

250   cm x 10.16 cm) and PVC plates (10.16 cm x 12.7 cm).

251   Larval counts and water quality measurements from all sites 2019 ($n = 496$) and 2020 (n

252   = 830) were combined into one dataset to examine potential relationships. The larval counts from

253   plates and sampler types were summed to obtain independent observations of larval counts and

254   water quality measurements from sampling time points within each site ($n = 13$). The

255 correlations between the larval counts and water quality measurements in the combined ($n = 58$)

256 and 2020 ($n = 47$) dataset were evaluated using principal component analysis (PCA). The

257 significance of these correlations was tested using the Kendall Tau-b (τ_B) rank correlation

258 method ($\alpha = 0.05$; McLeod, 2011). This correlation method is appropriate when data contains

259 non-normal distributions, tied ranks, and outliers (Croux & Dehon, 2010; Alfons, Croux, &

260 Filzmoser, 2017; Akoglu, 2018). The PCA plot was generated using the "prcomp" function in

261 the FactoMineR package (Kassambara and Mundt, 2020), which uses singular value

262 decomposition to examine covariances and correlations between the observations. The factoextra

263 package (Lê et al., 2008) was used to evaluate the eigenvalues to determine the highest

264 percentages of variance retained by each principal component.

265 To determine the influence of sampler type, site location, and sample timing on larval

266 recruitment, the oyster larval counts from 2019 and 2020 were used to generate a generalized

267 linear mixed model (GLMM) with the "glmmTMB" R package (Brooks et al., 2017). Models

268 were run with a zero-inflated Poisson (ZIP) regression due to the high percentage of zeros

269 (84.5%) in the dataset. The Akaike information criterion (AIC) values from each model (mi)

270 were used to calculate a second-order bias correction estimator (AIC$_C$). A model was chosen

271 based on the AIC$_C$ values and quality checks provided by the "DHARMa" R package (Fig. S2;

272 Hartig, 2022).

273

274 **Results**

275 **Sampler types**

276 The presence of oyster larvae varied by sampler type and site during 2020 with many

277 zero counts (absence) within sites (Fig. 4A). Ceramic arrays were the most effective sampler

278   type for assessing oyster larval recruitment at the six primary sites in the MCBs. Oyster larvae

279   settled on 2% - 33% of the ceramic tiles and on 1% - 14% of the PVC plates (Fig. 4A).

280   Additionally, the settlement of oyster larvae was observed more frequently on the ceramic arrays

281   (116 times) than on the PVC arrays (65 times) among the sites (Fig. 4B; H (2) = 7.054, p =

282   0.029). The median larval counts were also significantly higher on ceramic arrays (13.5 ± 18.53

283   [MAD]) compared to PVC collectors (1 ± 0 [MAD]) and PVC arrays (3 ± 2.97 [MAD]; Fig. 5A;

284   H (2) = 34.393, p < 0.0001). The ceramic arrays had larval counts up to 930 compared to the

285   PVC collectors and arrays with counts up to 32 and 93, respectively (Fig. 5B). The oyster larvae

286   on the ceramic arrays were observed most frequently at DNR Pier (18), Guys Point (24), Queen

287   Sound (16), and Wachapreague (45; Fig. 4B). The median larval count (447 ± 189.78 [MAD])

288   was significantly higher at Queen Sound compared to the other five sites, but maximum larval

289   counts >100 were observed at Queen Sound, DNR Pier, and Wachapreague (Fig. 5C, 5D; H (5)

290   = 68.855, p < 0.05).

291   **Spatial distribution**

292   PVC collectors were deployed during 2019 and 2020 to compare distribution patterns in

293   oyster recruitment between years. No oyster settlement occurred at sites other than the six

294   primary sites during 2019 and 2020, except one occurrence at North Verrazano Bridge during

295   August 2020 (Table 1). Of the six primary sites, oyster settlement occurred in both years at DNR

296   Pier, Guys Point, Queen Sound, and Wachapreague. Little to no recruitment occurred at Island

297   Mark 12 and Mills Island (Table 1). Spatial patterns of recruitment on PVC collectors were

298   consistent between 2019 and 2020 because all sites with settlement in 2019 also received

299   settlement in 2020 (Fig. 6A). Total settlement density from all sites on the PVC collectors was

300   greater during 2020 (3.07 larvae per cm^2) than 2019 (0.37 larvae per cm^2). At DNR Pier,

301 settlement density was five times greater on the PVC collectors during 2020 (0.50 larvae per

302 cm^2) than 2019 (0.11 larvae per cm^2; Fig. 6A). Additionally, total settlement density at

303 Wachapreague (2.62 larvae per cm^2) from 2019 and 2020 was almost 18 times greater than

304 settlement at DNR Pier (0.61 larvae per cm^2). Inter-annual differences were only significant at

305 Wachapreague ($H(1) = 4.95$, $p = 0.0204$) but not at DNR Pier or the primary sites. No

306 recruitment occurred at Island Mark 12 and Mills Island for PVC collectors during both years.

307 Recruitment on the PVC collectors was low at Queen Sound in both years and recruitment was

308 slightly less during 2020 (0.03 larvae per cm^2) than 2019 (0.07 larvae per cm^2; Fig. 6A).

309 Recruitment at Guys Point (0.05 larvae per cm^2) remained the same for both years.

310   The spatial distribution of settlement densities from the ceramic arrays were compared to

311 the settlement densities from the PVC arrays, which were only deployed during 2020 at the six

312 primary sites (Fig. 6B). Overall, total settlement density was greater on ceramic arrays (109

313 larvae per cm^2) than PVC arrays (0.92 larvae per cm^2; $H(1) = 96.291$, $p < 0.0001$). Within the

314 MCBs, the greatest larval density on ceramic arrays occurred at Queen Sound (66.81 larvae per

315 cm^2), Wachapreague (29.31 larvae per cm^2), and DNR Pier (11.73 larvae per cm^2) in 2020 over

316 the entire field season (Fig. 6B). Among the six primary sites, settlement density on the ceramic

317 arrays was greatest at the sites closest to the inlets (DNR Pier and Queen Sound). Lastly, Island

318 Mark 12 (0.01 larvae per cm^2) and Mills Island (0.21 larvae per cm^2) received little recruitment

319 on both array types (Fig. 6B).

320 **Temporal distribution**

321   In 2019, settlement on PVC collectors began in early to mid-July at site Wachapreague

322 and the MCBs sites, except Island Mark 12 (Fig. 7). In 2020, settlement at sites Wachapreague

323 and Queen Sound occurred earlier than the remaining primary sites (3 July 2020; Fig. 7D, 7E).

324 At all sites in the MCBs, settlement in 2019 and 2020 generally began in early to mid-July, but

325 settlement only occurred in late-July of 2019 at Island Mark 12 (Fig. 7). Settlement continued

326 until late July and early August during both years. Earliest settlement within the MCBs occurred

327 at site Queen Sound, but sampling equipment at that site disappeared after 30 July 2020 due to a

328 storm, which prevented further data collection (Fig. 7D). Settlement began slightly earlier at site

329 Wachapreague than in the MCBs, in late June (2019) and early July (2020), and extended longer,

330 until late August in 2020 (Fig. 7E). At site DNR Pier, near Ocean City Inlet, two settlement

331 peaks were observed in both 2019 and 2020 and occurred within approximately the same week

332 of each year (Fig. 7A).

333 **GLMM model for larval recruitment prediction**

334   Several ZIP GLMMs were tested and compared against a null model to determine the

335 best fit model for predicting larval counts based on sampler type (St), site location (Si), and

336 sample timing represented as swap number (Sw; Table 2). The m_4 model contained sampler type,

337 site location, and sample timing (swap number) as fixed effects but was eliminated due to a

338 probability (w_i) <0.05, higher AIC_C value, and failing quality checks (outlier test and

339 Kolmogorov-Smirnov test for uniformity of the residuals). Models m_6 and m_7 results had the

340 lowest AIC_C values and uniquely contained the plate levels within the samplers and line number,

341 the location of the sampler on the pier or buoy line, as a nested random effect. Model m_7 was

342 chosen because it contained both site location and sample time as a fixed effects and resulted in

343 the lowest AIC_C value, a probability >0.05, and passed model quality checks (Table 2; Fig. S2).

344 The chosen model predicted the highest larval counts to occur at DNR pier, Queen Sound, and

345 Wachapreague sites and during swaps 2 and 3 which represented the time period between late

346 June and mid-August (Fig. S3).

347 **Environmental effects**

348   The Principal Component Analysis (PCA) plot showed potential relationships between

349 oyster larval counts and water quality measurements of temperature, salinity, dissolved oxygen

350 (DO), pH, and turbidity. The principal components (PC-1 and PC-2) calculated from the water

351 quality variables and larval counts explained 59% of the variance observed in the dataset (Fig.

352 8). Results of the Kendall Tau-b (τ_B) rank correlation tests showed that larval counts had a

353 significant positive correlation with salinity ($\tau_B = 0.49$, z = 6.545, $p < 0.0001$) and negative

354 correlations with DO ($\tau_B = -0.17$, z = -2.237, $p < 0.05$) and pH ($\tau_B = -0.19$, z = -2.453, $p < 0.05$).

355 Average salinity ranged from 24 - 32 ppt among sites and was highest (>30 ppt) at DNR pier,

356 Mills Island, Guys Point, Queen Sound, and Wachapreague (Table 1). At these 5 sites, the

357 average pH and DO were 7.6 - 8.1 and 4.6 - 6.6 mg O L^{-1}, respectively (Table 1). No correlations

358 were found between the larval counts and temperature or turbidity in the PCA analysis. The

359 average temperature and turbidity among these five sites ranged 22 - 30°C and 0.3 - 1 m,

360 respectively (Table 1).

361 # Discussion

362 **Sampler types**

363   Oyster larvae exhibit preferential settlement (Keough & Downes, 1982), indicated by

364 significantly more counts on the ceramic arrays in 2020 than any other sampler type. Despite our

365 PVC plates being sanded with 100 grit sandpaper, the ceramic tiles had greater rugosity, making

366 it easier for oyster larvae to attach (Marques-Silva et al., 2006). In addition, ceramic tiles are

367 alkaline (Reig et al., 2013) and oyster larvae are more likely to settle when exposed to ammonia,

368 which is alkaline (Coon, Fitt & Bonar, 1990). Preferential settlement on ceramic tiles rather than

369 PVC was also evident in the study by Chuku et al. (2020), who compared monthly recruitment of

370   the West African mangrove oyster *Crassostrea tulipa* among five substrates (coconut shell,

371   oyster shell, nylon mesh, PVC slats, and ceramic tile) in four lagoonal estuaries in Ghana.

372   Ceramic tiles had the greatest monthly settlement in three of the four estuaries, and PVC slats

373   had the greatest in the fourth; recruitment on ceramic tiles was not significantly different than on

374   PVC slats (Chuku et al., 2020). In addition, biofilms are effective at inducing oyster settlement

375   (Tamburri, Zimmer-Faust & Tamplin, 1992; Zhao, Zhang & Qian, 2003; Su et al., 2007;

376   Campbell et al., 2011), but the effect varies with length of conditioning and rugosity of the

377   substrate (Taylor, Southgate & Rose, 1998; Devakie & Ali, 2002; Zhao, Zhang & Qian, 2003; Su

378   et al., 2007; Tamburri et al., 2008; Bellou et al., 2020).

379   **Spatial distribution**

380   Although settlement and recruitment behaviors can be difficult to measure *in-situ*, our

381   results show a spatial distribution trend of greater recruitment at sites near Ocean City (DNR

382   Pier) and Chincoteague Inlet (Queen Sound), suggesting those are more attractive locations for

383   oyster settlement than sites further away from inlets. Additionally, broodstock live close to those

384   inlets. Settlement of both Sydney rock oyster *Saccostrea glomerata* and invasive Pacific oysters

385   *Crassostrea gigas* in Port Jackson Estuary, Australia was greater at sites nearer the Pacific Ocean

386   than in the upper channel (Scanes et al., 2016). Spatial distribution patterns in the Port Jackson

387   Estuary were similar to those of the MCBs with more oyster larvae being observed closer to the

388   interface between the estuary and ocean.

389   The MCBs have lower freshwater discharge, varied flushing rates, and high velocity

390   water near Ocean City and Chincoteague inlets. Flushing rates in the individual sub-bays vary

391   greatly, e.g. from 9 days in Isle of Wight Bay to 63 days in Chincoteague Bay (Pritchard, 1969;

392   Lung, 1994). This implies that oyster larvae are retained longer, thus have a longer period of

393   time to settle in Chincoteague Bay than in Isle of Wight Bay. The longer retainment period in

394   Chincoteague Bay supports the greater settlement observed near Chincoteague Inlet (Maryland

395   Department of Natural Resources, 2004). Retention within a system correlates to recruitment

396   success (Norcross & Shaw, 1984).

397   A hydrodynamic model by Kang et al. (2017) demonstrated that the northward flow of

398   water through the MCBs is primarily wind-driven, except when wind speeds are weak (e.g., 3

399   m/s), at which times it becomes tidally driven. However, near Ocean City and Chincoteague

400   inlets, tidal cycles drive circulation patterns (Wells, Hennessee & Hill, 2002). The MCBs have a

401   distinct seasonal wind pattern, with prevailing winds blowing from the southwest in the summer

402   due to the Bermuda High pressure system and winds blowing from the northeast in the winter.

403   Circulation patterns in the MCBs may also be influenced by the shape of the estuary's basin and

404   bathymetry, or depth (Lee & Valle-Levinson, 2012) since the shallow basin and winds in the

405   MCBs can alter wave dynamics (Mao & Xia, 2018). Strong turbulence from waves can cue

406   oyster larvae to sink, increasing their proximity to suitable substrate in which to attach (Fuchs et

407   al., 2013). The strong turbulence at the inlets could be a reason for the observed spatial

408   distribution near the inlets.

409   The MCBs are characterized as being "microtidal" since tidal exchange is limited to

410   Ocean City Inlet and Chincoteague Inlet. Although the tidal excursion of the MCBs is unknown,

411   similar lagoonal estuary systems have tidal excursions of 2.7 km in Haulover Canal connecting

412   Mosquito Lagoon and Indian River lagoon (Smith, 1993), 2 km both for Little Egg Harbor and

413   Barnegat Bays (Chant, 2001), and 1.02 km to 8.25 km depending on the site proximity to Fort

414   Pierce Inlet within the Indian River lagoon (Smith, 1983). Tidal excursion refers to the distance

415   between low water and high water in which a particle travels. It is a measurement to describe the

416   movement of particles such as larvae and pollutants within a tidal cycle (Ji, 2008). A coupled

417   biological-physical transport model by Kim et al. (2013) simulated that larger tidal excursions

418   during a tropic tide caused greater larval dispersion. We hypothesize that the spawning adult

419   oysters are within a 1 km – 8 km range of settlement sites in the MCBs. Perhaps the coupling of

420   flushing rates (slowest in Chincoteague Bay), proximity and tidal circulation through the inlets

421   may have contributed to greater settlement.

422   **Temporal distribution**

423   The Eastern oysters in this study region typically spawn from June through October

424   (Haven & Fritz, 1985). Samplers showed little variation in peak timing between 2019 and 2020

425   (< 10 days apart) and all settlement peaks began in July in both years. Our results showed that

426   oyster larvae settled between late-June and mid-August, which was expected based on settlement

427   timing reported by previous studies at similar latitudes in the Mid-Atlantic (Shaw, 1967;

428   Kennedy, 1980; Haven & Fritz, 1985; Capelle et al., 2020; Ross & Synder, 2020). Although

429   monitoring in our study did not continue into late September, it has been documented by other

430   studies that peaks do occur during that time (Haven & Fritz, 1985).

431   **Environmental effects**

432   Temperature and salinity are known to have an influence on oysters throughout their life

433   cycle (Hori, 1933; La Peyre et al., 2013). Although there was no correlation found between

434   larval counts and temperatures, settlement was greatest between 22 and 26 °C at DNR Pier, Guys

435   Point, Queen Sound, and Wachapreague (data not shown). These temperatures were within the

436   optimal range (20 and 32.5 °C) for oyster larval growth (Calabrese and Davis 1970) and the

437   ambient water temperatures (20 and 30 °C) that induce adult oysters to spawn (Horn Point Oyster

438   Hatchery, 2021). Previous studies observed increased settling with thermal shock (Lutz, Hidu &

439   Drobeck, 1970; Hidu & Haskin, 1971). Perhaps the mixing and change in temperature between

440   the warmer bay water and cooler ocean water from the inlets may have contributed to settlement.

441   The optimal salinity ranges of 12.00 – 28.00 (Dame, 1996) and 15.00 – 20.00 (Barnes et

442   al., 2007) have been reported for oyster growth. Our results are consistent with Nelson (1923),

443   who observed the greatest abundance of straight-hinge larvae at stations in the most saline and

444   lower portion of Barnegat Bay, New Jersey. This is contrary to laboratory experiments by Hidu

445   and Haskin (1971), in which oyster larvae were not stimulated to settle with an increase in

446   salinity. Highest larval settlement occurred at sites with average salinities >30 ppt over the 2019-

447   2020 period, which is just above the optimal range for oyster growth. Salinity had the strongest

448   and most significant correlation with larval counts. Although these sites were also located closest

449   to the inlets, where salinity is naturally higher, there are other factors such as current flow and/or

450   tidal excursion that can influence settlement.

451   The negative correlations between larval counts, pH, and DO were significant, but the

452   correlation coefficient was weak (<0.20). For oysters, the ideal range in pH and DO for growth is

453   6.75 to 8.75 (Calabrese & Davis, 1966; Clark & Gobler, 2016) and 7 mg O L^{-1}, respectively

454   (Clark & Gobler, 2016). The average pH at sites DNR pier, Mills Island, Guys Point, Queen

455   Sound, and Wachapreague fell within the desired range for oyster larvae, but not for DO.

456   Interestingly, the site with the lowest average DO was at site Wachapreague (4.6 mg O L^{-1}),

457   which had the second greatest larval counts. This further corroborates the negative correlation

458   between larval counts.

459

460   **Conclusions**

461   This is the first recruitment study for oyster larvae in the MCBs, and the resulting spatial

462   and temporal distribution patterns can provide insight into evaluating restoration initiatives and

463   serve as a foundation for future recruitment studies in other lagoonal estuaries. We assessed the

464   recruitment distribution of oyster larvae at 12 sites within the MCBs and a site for sampling gear

465   comparison, using PVC plates and ceramic tiles that were monitored biweekly in summer 2019

466   and 2020. This study resulted in four significant findings: 1) ceramic tiles received significantly

467   greater recruitment than PVC plates; 2) new recruits settled in the greatest numbers at sites that

468   were closest to Ocean City and Chincoteague inlets, as opposed to sites further within the bays;

469   3) settlement occurred between late June and early July into mid-August, which was consistent

470   with previous studies at similar latitudes (Shaw, 1967; Kennedy, 1980); and 4) the spatial and

471   temporal patterns of settlement were essentially identical in both 2019 and 2020, although

472   recruitment was four to five times greater in 2020. This study can supplement ongoing data

473   collection (e.g., surveys of fish, shellfish, submerged aquatic vegetation, water quality, and

474   current drift monitoring) to gain a broader understanding of the MCBs and provide baseline data

475   upon which to build. Notably, it may guide stakeholders in evaluating the decision to potentially

476   pursue an oyster restoration project within the MCBs and similar lagoonal estuaries.

477   **Recommendations**

478   For any oyster restoration project, monitoring is recommended prior, during, and after

479   restoration to assess the reef habitat, the organisms living on the reef, and interactions among

480   organisms (Thayer et al., 2005). This is important so adjustments can be made if needed and the

481   progress of the restoration can be observed over time. Examples of restoration techniques used in

482   the Mid-Atlantic and other areas include creating 3-dimensional structures with vertical relief

483   that emulate natural oyster reefs. These consist of clutch, which is a material (e.g. shells, shell

484   fragments, limestone, concrete, etc.) in which oyster larvae settle (Kurz, 2012). Clutch is

485   deposited on the sediment or a foundation then are piled to make the vertical structure

486   (Luckenbach, Mann & Wesson, 1999; O'Beirn et al., 2000; Brumbaugh & Coen, 2009). Other

487   methods include pre-cast limestone or concrete structures (e.g. oyster castles or oyster balls) and

488   oyster shells in bags (Olander et al., 2020; Virginia Institute of Marine Science, 2023).

489   Partnering with local agencies as well as oyster farmers and watermen would aid in the

490   collection of necessary data.

491   For setting up a larval recruitment and settlement monitoring study, ceramic arrays would

492   be the best sampler to use in the short term (a spawning season), prior to restoration -- to

493   evaluate the location and time to establish a restoration project. The ceramic arrays should be

494   utilized during at least two spawning seasons to determine if there are spatial and temporal

495   patterns in settlement. However, oyster shells should be prioritized for use in restoration efforts

496   over ceramic substrate, because of the protein periostracum that is present on oyster shells

497   (Crisp, 1967), chemical cues released from conspecifics (Tamburri, Zimmer-Faust & Tamplin,

498   1992), and the contoured surface (Taylor, Southgate & Rose, 1998) but ceramic tile is an

499   alternative if oyster shells are not readily accessible. Natural recruitment of wild oyster larvae aid

500   in restoration success by supplementing restoration efforts (Schulte & Burke, 2014). Burke and

501   Schultz (2014), concluded that restored reefs with planted spat and adults, recruited greater

502   populations and in greater densities than unenhanced reefs because wild settlement rose two to

503   three orders of magnitude. The additional larvae and adults provide additional substrate for wild

504   oysters in which to settle (Southworth & Harding, 2014).

505   Based on the results of our study, we recommend the following for site selection prior to

506   pursuing oyster restoration in the MCBs and other lagoonal estuaries: 1) selecting a restoration

507   site that is in close proximity to broodstock, has a slow flushing rate, and circulation patterns that

508   retain larvae; 2) utilizing oyster shells as substrate for preliminary recruitment studies and/or

509   restoration projects (if oyster shells are not accessible, a ceramic array design as seen in this

510   study, can be used as an alternative); 3) establishing a restoration site prior to or in early June (in

511   the Mid-Atlantic) to ensure wild oyster larvae settle during peak time; 4) conduct additional

512   research on the current state of parasites, overwintering, and diseases to ensure survival and

513   growth of oysters; and 5) establish a monitoring program to assess progress and address

514   environmental changes (Recommendations further described in Kennedy et al., 2011).

515

516   **Acknowledgements**

517   This paper is in honor of Daniel Cullen, who was an incredible friend, mentor, and

518   colleague. He is missed greatly.

519   We would also like to thank our boat Captains for their assistance in transportation to our

520   study sites as well as the National Park Service, Maryland Department of Natural Resources, and

521   Maryland Coastal Bays Program in guiding the location of study sites. We are grateful to

522   Elizabeth North, Mitchell Tarnowski, and the anonymous reviewers for providing comments to

523   improve this manuscript. Special thanks to Steve Doctor and Paige Ross for sharing their

524   expertise and University of Maryland Eastern Shore students for assisting in the field and

525   laboratory. Lastly, we thank Cara C Schweitzer at the NOAA NMFA-Office of Habitat

526   Conservation for her feedback on the statistic models.

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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). Sites include Greys Creek (GC), St. Martin River (SM), Turville Creek (TC), DNR Pier (DP), Verrazano Bridge (VB), Island Mark 12 (IM), South Point (SP), Public Landing (PL), Taylor Landing (TL), Mills Island (MI), Guys Point (GP), Queen Sound (QS), and Wachapreague (W).

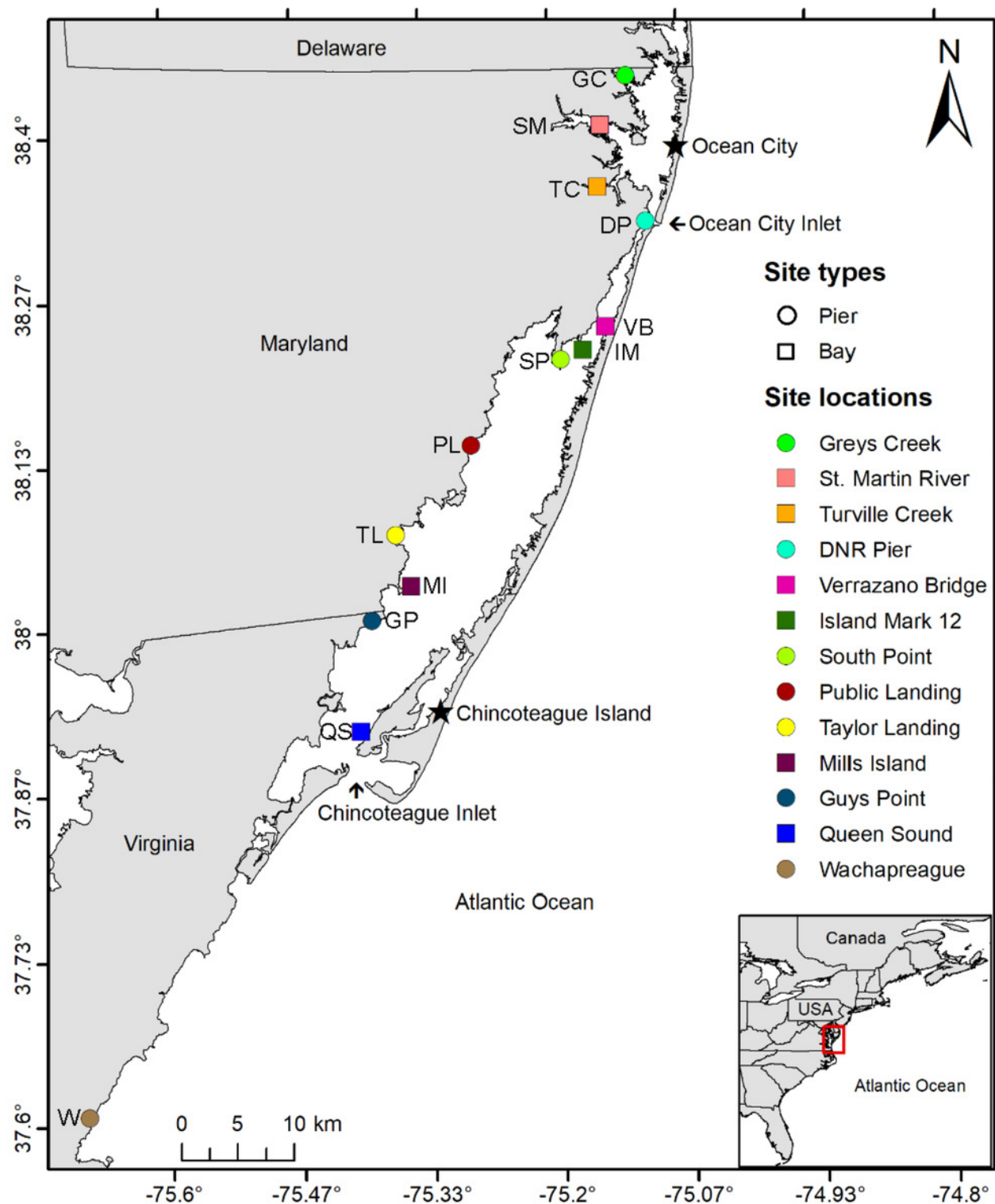



Figure 2

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

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

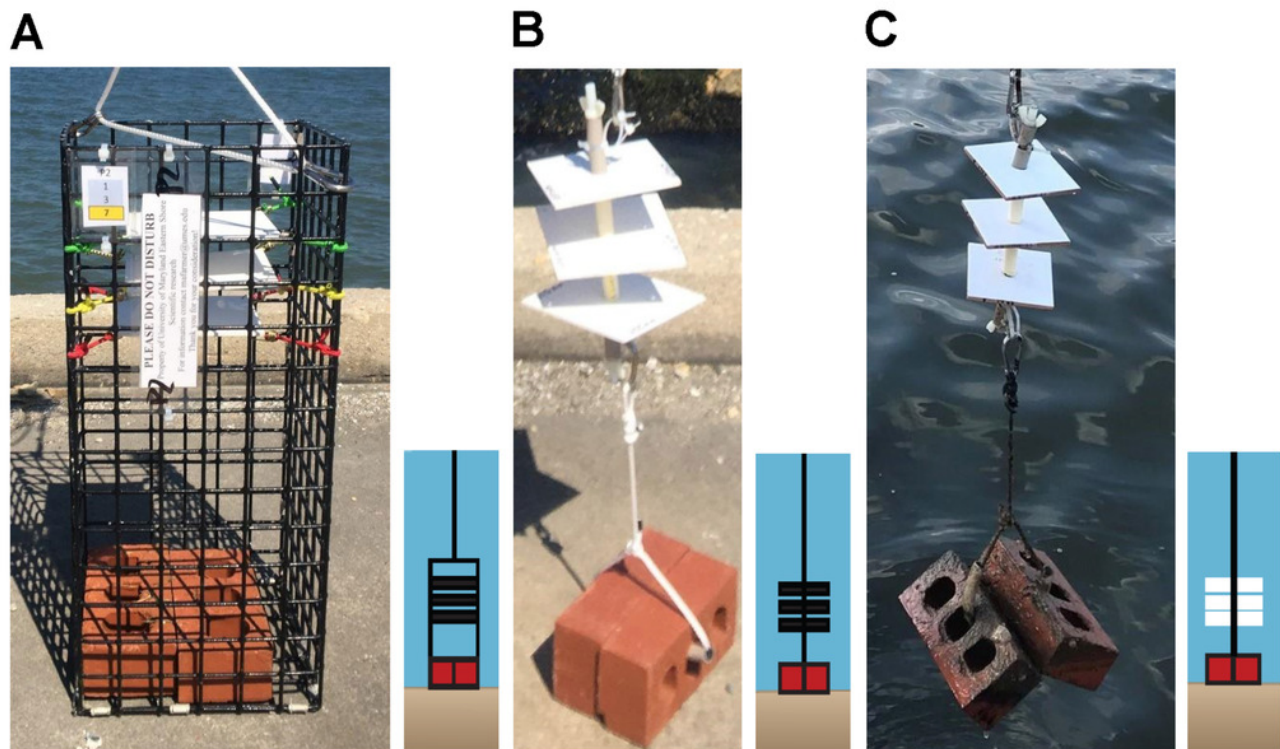



Figure 3

Diagrams of samplers deployed at coastal bay site locations.

(A) Pier site in which ceramic arrays (white), PVC arrays (black), and PVC collectors (black, in rectangles) were suspended from a shore-based pier. (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.

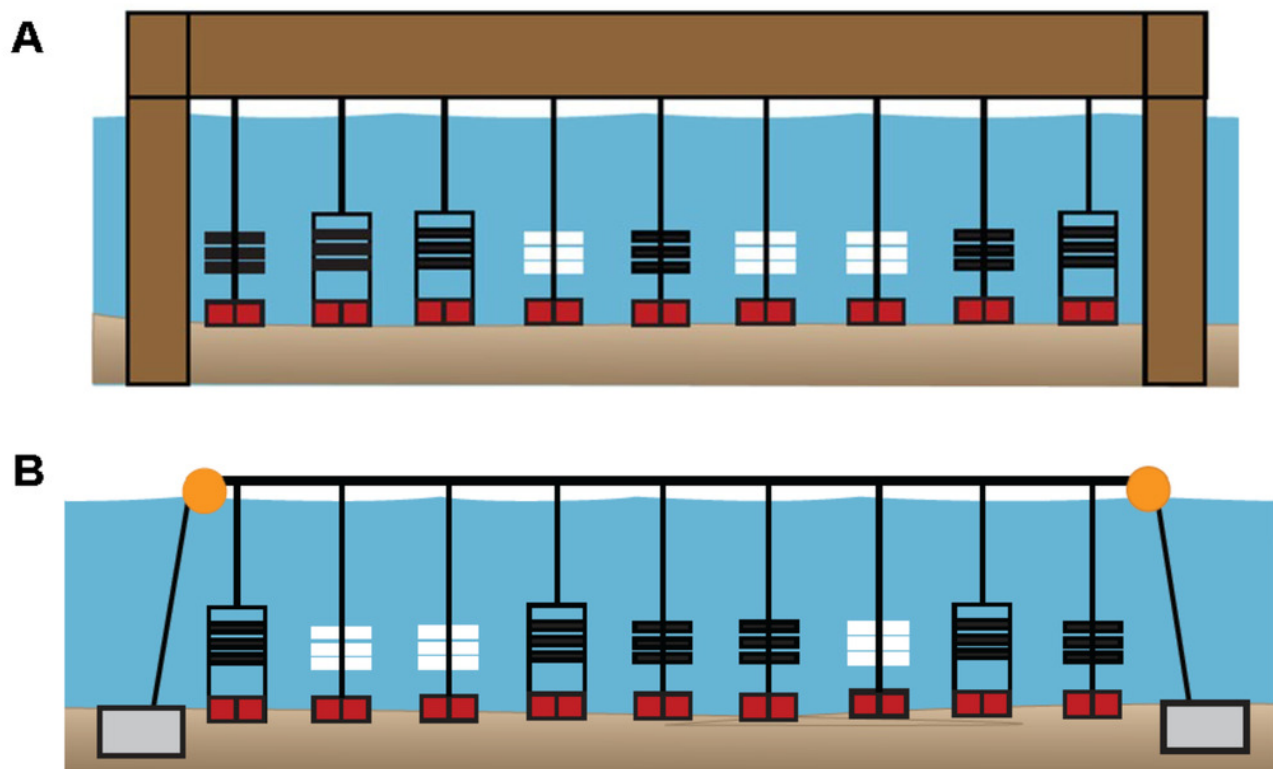



Figure 4

The proportion of the absence and presence of oyster larvae and the number of times oyster larvae was present on the collection devices at each site during 2020.

The larval count data was used to calculate the presence and absence of oyster larvae on collection devices from each site. (A) The proportion of oyster larvae that was absent (gray) or present on ceramic arrays (CA; green), PVC arrays (PA; orange), and PVC collectors (PC; purple). Sites and total number of observations were DNR Pier (n = 127), Island Mark 12 (IM; n = 45), Mills Island (MI; n = 113), Guys Point (GP; n = 132), Queen Sound (QS; n = 72), and Wachapreague (W; n = 137). (B) The number of times (frequency) that oyster larvae was observed on the types of collection devices.

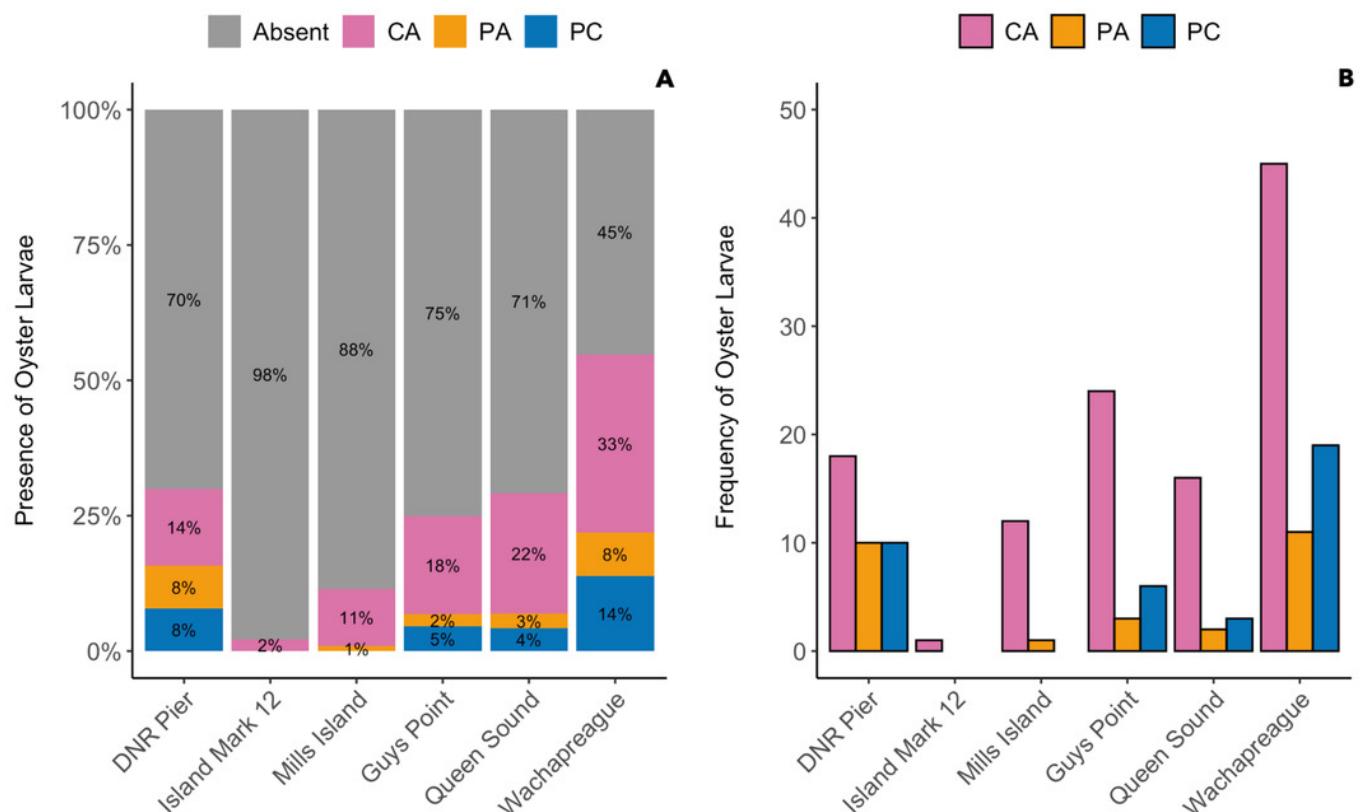



Figure 5

Comparison of median and maximum larval counts to sampler types and sites during 2020.

Data was pooled by either sites or sampler types to compare the median and maximum larval counts (≥ 1). (A-B) Counts from collection devices of ceramic arrays (CA; n = 116), PVC arrays (PA; n = 27), and PVC collectors (PC; n = 38). (C-D) Counts from sites of DNR Pier (DP; n = 18), Island Mark 12 (IM; n = 1), Mills Island (MI; n = 12), Guys Point (GP; n = 24), Queen Sound (QS; n = 16), and Wachapreague (W; n = 45). Bars above median counts represent standard error (SE). The letters above the SE bars denote significant differences (p < 0.05) between median larval counts.

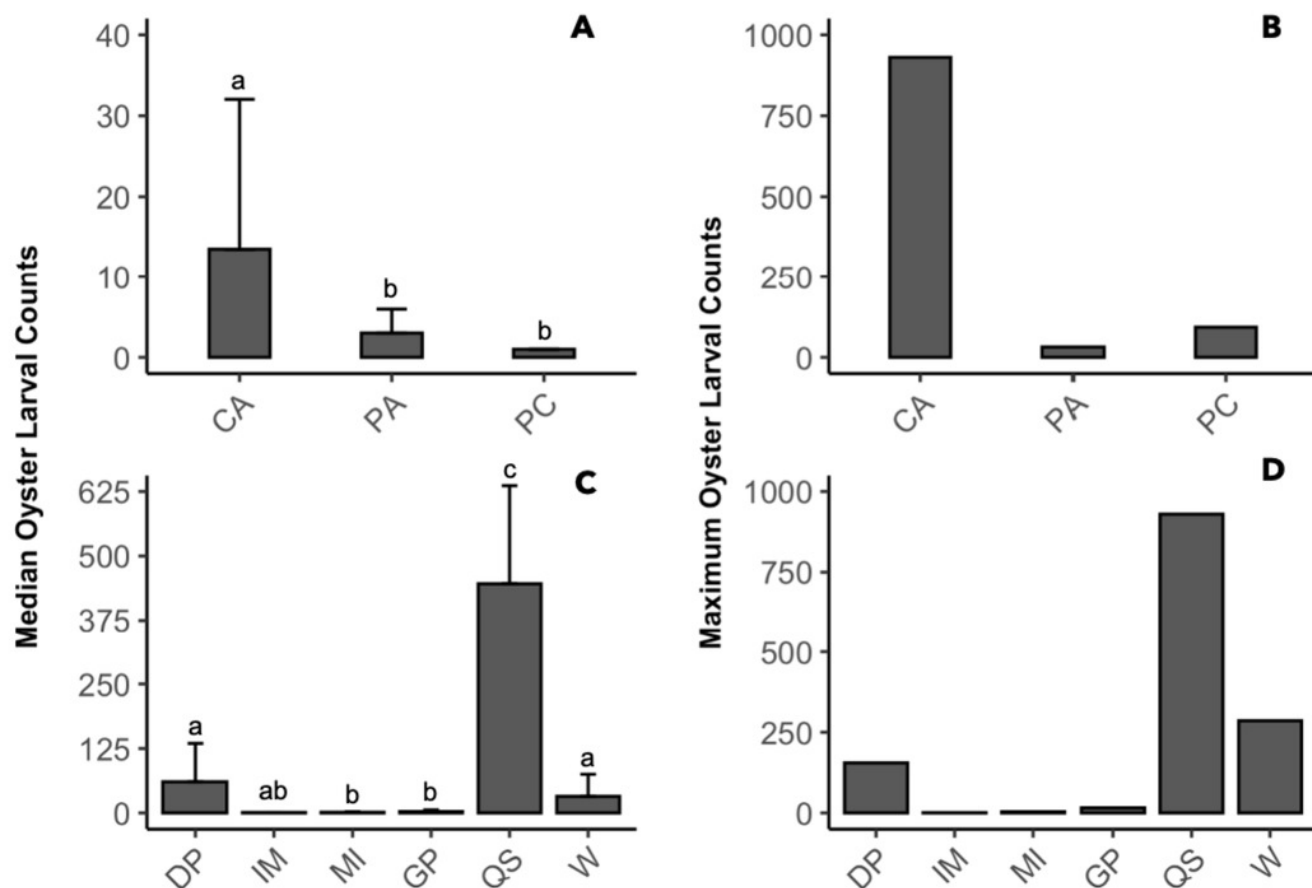



Figure 6

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

(A) Density of oyster larvae per plate over entire field season on PVC collectors in 2019 and 2020. (B) Density oyster larvae per plate over entire field season on PVC arrays and ceramic arrays in 2020. Values refer to density of median oyster larvae counts from the underside of a ceramic tiles or PVC plates. Six primary sites included DNR Pier (DP), Island Mark 12 (IM), Mills Island (MI), Guys Point (GP), Queen Sound (QS), and Wachapreague (W).

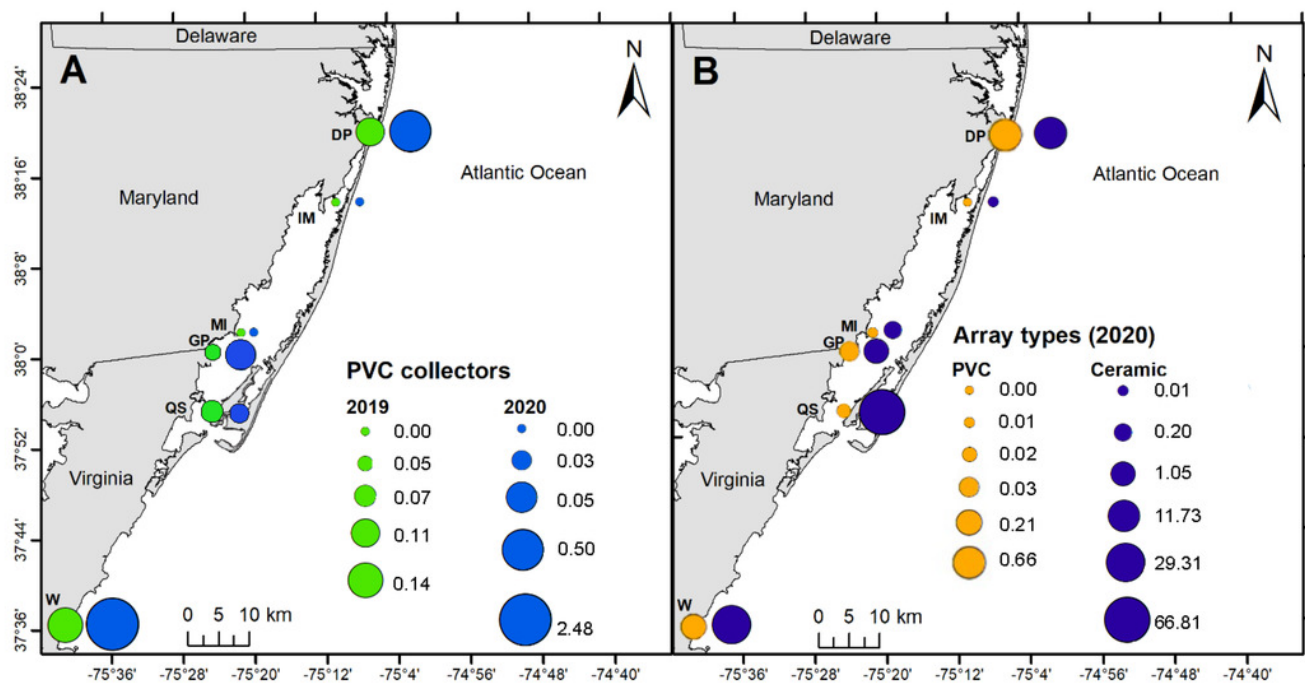



Figure 7

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. Sites (A) DNR Pier. (B) Island Mark 12. (C) Guys Point. (D) Queen Sound. (E) Wachapreague. Note scales of y-axes differ.

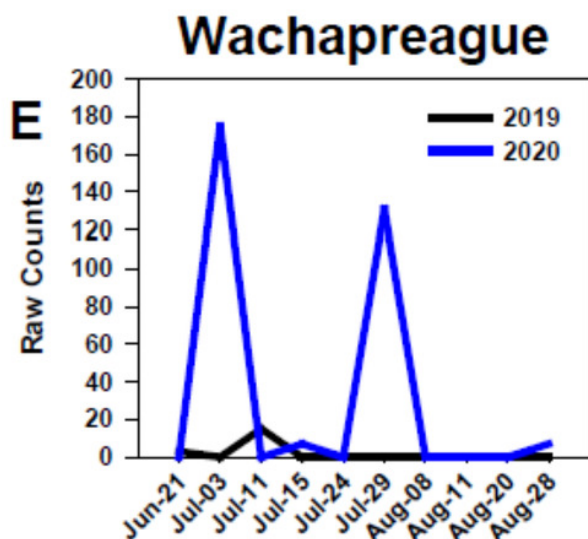
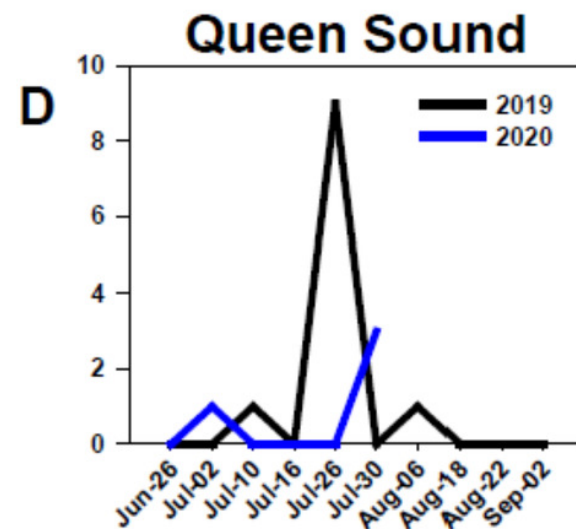
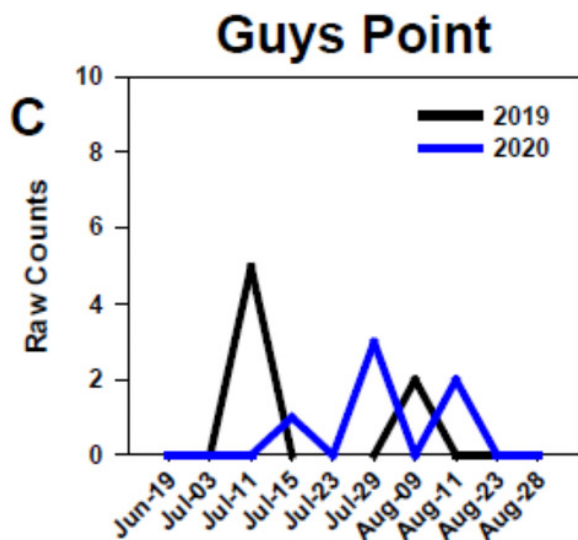
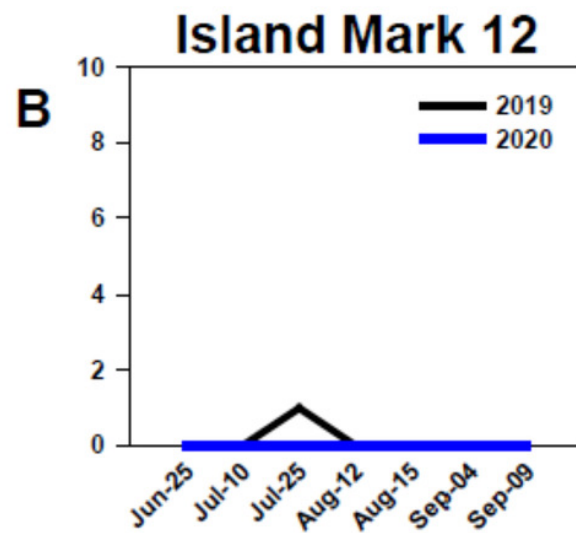
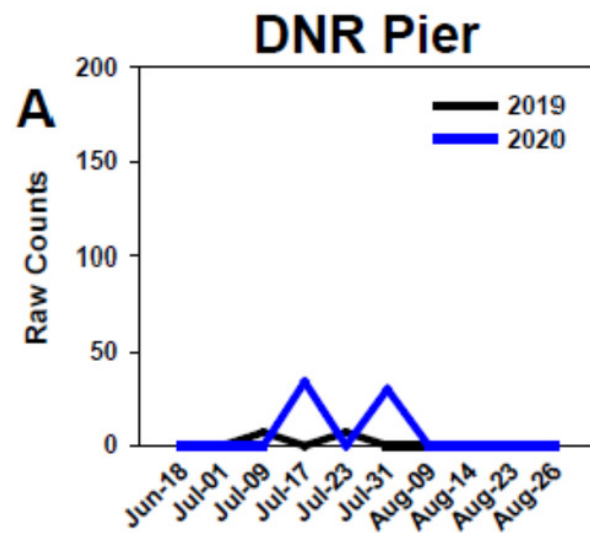
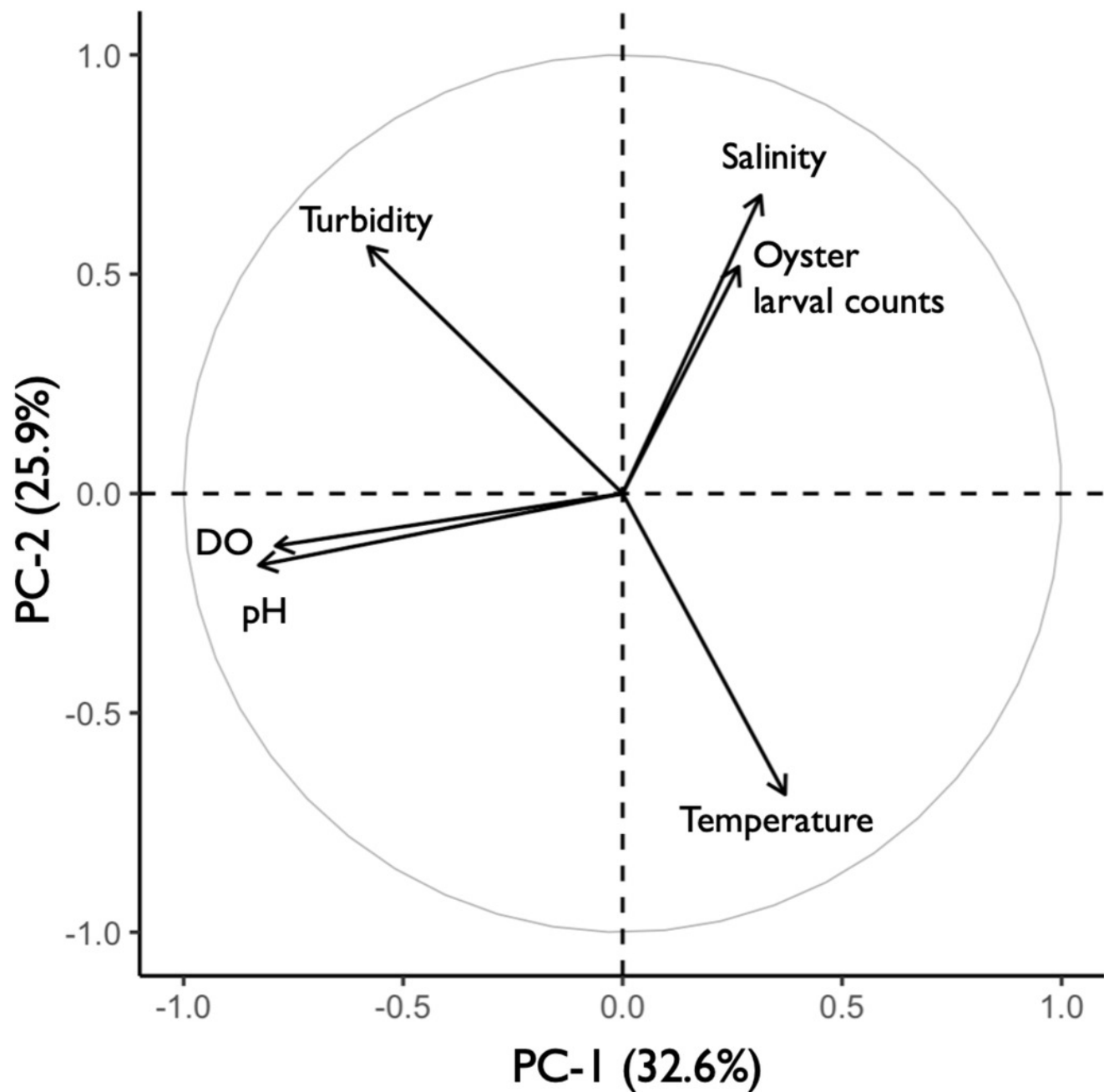



Figure 8

Principal component analysis (PCA) of oyster larval counts and water quality parameters.

PCA plot showing the potential correlations between oyster larval counts and water quality measurements of salinity, turbidity, dissolved oxygen (DO), pH, and temperature (°C). The correlations between larval counts and water quality measurements were obtained by combining the 2019 ($n = 58$) and 2020 ($n = 47$) datasets with all 13 sites.

Total oyster larval counts and water quality measurements of sites during 2019 and 2020.

Larval counts were summed to calculate totals and water quality measurements of depth, temperature, salinity, pH, dissolved oxygen, and turbidity. Values were averaged (mean ± standard deviation) by site across years and time points.

Site	Site type	n	Total Larval Counts	Depth (m)	Temperature (°C)	Salinity (ppt)	pH	Dissolved oxygen (mg O L^{-1})	Turbidity (m)
Greys Creek	Pier	10	0	0.61 ± 0.09	28.80 ± 1.22	24.86 ± 2.09	7.65 ± 0.41	4.19 ± 2.80	0.44 ± 0.12
St. Martin River	Bay	10	0	0.95 ± 0.13	27.79 ± 2.18	26.58 ± 0.91	7.85 ± 0.33	6.54 ± 0.81	0.45 ± 0.05
Turville Creek	Bay	8	0	0.69 ± 0.25	28.40 ± 1.83	26.62 ± 1.14	7.77 ± 0.43	6.96 ± 1.01	0.45 ± 0.13
DNR Pier	Pier	7	1374	2.81 ± 0.60	22.54 ± 2.04	30.40 ± 1.29	8.10 ± 0.38	6.64 ± 0.76	0.95 ± 0.28
Verrazano Bridge	Bay	4	3	1.09 ± 0.29	27.16 ± 1.04	29.07 ± 1.36	8.18 ± 0.47	7.57 ± 1.28	0.61 ± 0.12
Island Mark 12	Bay	8	1	0.87 ± 0.14	26.84 ± 1.70	28.69 ± 1.82	8.14 ± 0.31	7.02 ± 0.81	0.49 ± 0.11
South Point	Pier	7	0	0.91 ± 0.12	28.43 ± 1.11	28.97 ± 2.00	8.08 ± 0.33	6.42 ± 1.12	0.43 ± 0.20
Public Landing	Pier	10	0	0.76 ± 0.12	28.72 ± 0.98	28.20 ± 2.08	7.99 ± 0.31	6.54 ± 0.94	0.49 ± 0.15
Taylor Landing	Pier	8	0	0.78 ± 0.16	28.81 ± 1.89	29.35 ± 2.21	7.94 ± 0.34	5.75 ± 1.42	0.37 ± 0.11
Mills Island	Bay	10	22	0.88 ± 0.15	26.82 ± 1.49	30.88 ± 1.65	7.96 ± 0.36	5.93 ± 0.94	0.42 ± 0.12
Guys Point	Pier	10	125	1.20 ± 0.25	29.61 ± 1.01	30.93 ± 1.48	7.87 ± 0.26	6.12 ± 1.99	0.59 ± 0.27
Queen Sound	Bay	4	6912	1.25 ± 0.14	26.61 ± 1.92	30.96 ± 1.25	8.01 ± 0.39	6.41 ± 0.79	0.54 ± 0.09
Wachapreague	Bay	9	3391	1.44 ± 0.59	28.26 ± 1.22	32.22 ± 1.29	7.52 ± 0.12	4.00 ± 0.63	0.38 ± 0.11

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Comparison of ZIP generalized linear mixed models (m_0–m_7) corresponding to the different variables tested for oyster larval settlement prediction.

Model	Variables	df	logLik	AIC_C	Δ_i	w_i
m_0	L ~ 1	2	-18161.9	36327.9	+30624.8	0
m_1	L ~ Sw + (1|ST) + (1|Si) + (1|N)	9	-3217.5	6453.1	+750.0	<0.001
m_2	L ~ Si + (1|ST) + (1 | Sn) + (1|N)	17	-3205.7	6445.9	+742.8	<0.001
m_3	L ~ Si + Sw + (1|ST) + (1|N)	20	-3189.8	6420.2	+717.1	<0.001
m_4	L ~ Si + Sw + ST + (1|N)	21	-3181.5	6405.6	+702.5	<0.001
m_5	L ~ Si + Sw + (1|ST) + (1|N) + (1|L)	21	-3014.8	6072.3	+369.2	<0.001
m_6	L ~ Si + (1|Sw) + (1|ST) + (L|N)	22	-2842.0	5728.8	+25.7	<0.001
m_7	**L ~ Si + Sw + (1|ST) + (L|N)**	**25**	**-2826.1**	**5703.1**	**-**	**0.99**

1 df = degrees of freedom; Loglik = log-likelihood; AIC_C = corrected AIC value; Δ_i = difference between each
2 model and the best selected model; and wi = probability that a given model provided is the best fit for the data.
3 Variables: L = oyster larvae counts; Si = site; Sw = swap number represented sampling time; ST = sampler
4 type; L= plate level on the sampler; N= line number represented the position of the sampler on the pier or buoy
5 line. Model m_7 was selected to be the best fit model.
6