TEMPORAL DYNAMICS OF EASTERN OYSTER LARVAL ABUNDANCE IN GREAT BAY ESTUARY, NEW HAMPSHIRE

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ABSTRACT Eastern oyster *Crassostrea virginica* populations have been declining steadily over the past several decades across the North American East coast. The Great Bay Estuary (GBE), located in New Hampshire, is experiencing this loss and restoration efforts have been put into effect. This paper characterizes larval abundances of settled spat and two early stages of *C. virginica*, D-hinge and veliger, in GBE from 2018 to 2020. Abundances are compared based on date of sampling, year, collection site, and the physicochemical data recorded on each sampling date. It was found that overall, D-hinge larval abundances have declined significantly from 2018 to 2020, whereas veliger abundances have remained steady or increased. Although the physicochemical factors are known to play a role in larval abundance, very little significance was found, suggesting future study may need to be modified to include a broader range of factors (e.g., more temporal sampling). This study indicates that both D-Hinge, veliger, and spat settlement occur in GBE before when sampling traditionally has started (June), suggesting an earlier than previously thought first spawn of *C. virginica* in GBE. This finding can be used to enhance restoration efforts as it suggests that spat brought in to augment current sites of active restoration should be released earlier in the season and that recruitment devices should be deployed before the previously thought first spawn of each season.

KEY WORDS: *Crassostrea virginica*, Great Bay Estuary, eastern oyster

INTRODUCTION

Drastic declines in eastern oyster *Crassostrea virginica* abundance (Kirby 2004, Beck et al. 2011, Rick et al. 2016) have led to a loss of ecosystem services provided throughout its North American range. As "ecosystem engineers," services provided by *C. virginica* have been estimated to be worth \$5,500–\$99,000 ha^{-1} y^{-1} (Grabowski et al. 2012). Specifically, healthy oyster reefs aid in mediating nutrient cycles through nitrogen removal (Higgins et al. 2011, 2013), provide complex habitats for an array of organisms (Beck et al. 2011), augment fishery resources (Grabowski & Peterson 2007, Scyphers et al. 2011), and help to regulate ecosystem processes through top-down control of phytoplankton (Coen et al. 2007). Additionally, healthy *C. virginica* populations influence ecological processes across gradients, driving food-web dynamics through direct and indirect effects on species interactions and changes to biomass across trophic levels (Grabowski et al. 2020). Consequently, the continued decline of oyster populations is a cause for concern and could prove to be catastrophic for some estuarine ecosystems (Coen et al. 2007, zu Ermgassen et al. 2013, Kaplan et al. 2016), particularly estuaries in northern New England. For example, the Gulf of Maine is currently one of the fastest warming regions on Earth, where sea surface temperatures increased at a rate of 0.26°C y^{-1} between 2004 and 2012 (Mills et al. 2013). Such rapid change can facilitate both biotic and abiotic changes that may be contributing to the decline in oyster abundance in the Great Bay Estuary (GBE) of New Hampshire.

In the face of the declining New England and specifically in New Hampshire (NH) oyster populations, efforts toward oyster reef restoration have intensified (Grizzle & Ward 2016). Oyster reefs previously existed throughout much of New Hampshire's estuaries, which include GBE, but current oyster populations in New Hampshire are less than 10% of their numbers in the 1980s (Grizzle & Ward 2016). These declines have been attributed to disease, anthropogenic impacts, lack of natural shell for larval setting, and sedimentation leading to the burial of shell (Grizzle & Ward 2016). Current shellfish restoration efforts in GBE have demonstrated some successes. Spat on shell have been reared at the University of New Hampshire and deployed onto GBE restoration sites as part of The Nature Conservancy oyster restoration efforts after which it was observed that approximately 5.8×10^4 oyster spat recruited to a 1-ha reef constructed in the mouth of the Lamprey River (Konisky et al. 2011). Another restored site in GBE near Lamprey River has shown indications of natural recruitment (Grizzle & Ward 2016). A recent study has demonstrated the potential for larval recruitment of oysters in the GBE, suggesting that recruitment is more favorable at sites that are in proximity to existing natural reefs with established adult oysters (Atwood & Grizzle 2020).

As a result of increasing carbon dioxide into the atmosphere, which the ocean absorbs, pH is declining and causing acidic water conditions (Cai et al. 2011). This process, known as ocean acidification, negatively affects early developmental stages and metamorphosis in shellfish (Miller et al. 2009, 2020, Mabardy et al. 2015). Larval recruitment to wild populations is critical to sustainable restoration of eastern oysters, and this recruitment ultimately depends on production of larvae that survive to metamorphosis and settle on reef surfaces. But currently, spatial and temporal preferences of larval dispersal in GBE remain largely unknown and understanding larval dispersal will help streamline possible strategies to successfully restore healthy

oyster populations in GBE (Brumbaugh et al. 2006, Rodriguez-Perez et al. 2020). There are a variety of threats that could affect the successes of oyster larval reproduction and settlement (NOAA 2007) including suitable settling habitat, temperature, acidification, disease, predation, overharvesting, and extreme fluctuations in physicochemical variables (pH, temperature, salinity, *etc.*). As a result, it is imperative to discuss and view oyster restoration efforts as hierarchies depending on the quantities of larvae and the source(s) of stress. This present study aims to (1) reveal the dynamics of abundance of *Crassostrea virginica* larvae in GBE by examining annual changes in larval abundance and (2) ascertain whether there are differences in settlement across representative sites where oysters previously were abundant. These data will provide a baseline understanding of the reproductive output of eastern oysters in the GBE, help ascertain possible differences in oyster larval abundance among sites, and provide insight that will aid oyster restoration efforts in the GBE by optimizing the larval season, deployment of culch at the best sites for recruitment.

MATERIALS AND METHODS

Great Bay Estuary

The GBE system, located along the New Hampshire–Maine border within the Gulf of Maine region, has unique characters that make it difficult to simply extend knowledge from other North American Midatlantic estuaries. The tidal range is 2–4 m, tidal currents are greater than 2 m s^{-1} in the channels at maximum ebb and flood, and at low tide, as much as 50% of GBE is exposed as low-lying mudflats. The surface area of GBE is approximately 55 km^2 (Trowbridge 2007), the volume is 156×106 m^3 and 235×106 m^3 for low and high tides, respectively, and the tidal prism is 79×106 m^3 (Trowbridge 2007, Swift & Brown 1983). The Bay is fed small volumes of fresh water by seven rivers where fluxes are determined by precipitation and runoff, and except during high flow events (storms and ice off), freshwater input only contributes 2% of the tidal prism (Short 1992). The generally small freshwater fluxes and strong tidal mixing results in weak or negligible stratification (except very close to the river mouths) and during periods of little rainfall the salinities at the center of GBE are nearly equal to the Gulf of Maine proper, contributing to the unique ecological dynamics of GBE (Mills et al. 2009).

Study Area

In 2018 to 2019, four sites were sampled throughout the study: Woodman's Point (WP), Nannie Island (NI), the Lamprey River (LR), and Squamscott River (SR). In 2020, the study design was modified to examine the relationship of oyster larval abundance and settlement at naturally occurring reefs and an oyster farm. Consequently, the 2020 sites included two of the 2018 to 2019 (WP and NI) and two new sites, Adams Point (AP) and an oyster farm (OF), which encompass a broad area of the GBE (13 km^2, Fig. 1), reflecting conditions throughout the entirety of the estuary during the sampling period.

Experimental Design

In 2018 and 2019, horizontal surface larval tows using a 64-µm mesh net were conducted at four sites within GBE approximately once per week with three larval tows, each lasting approximately 5 min at 0.5–1.0 m sec^{-1}. In both years, sampling began the last week in June and lasted through the end of August. In 2020, replicate tows were conducted ~0.3 m below the surface for approximately 2 min at 0.5–1.0 m sec^{-1}, resulting in an average of 38 m^3 of water sampled per tow. Samples collected from larval tows were preserved with formalin sucrose (Haney & Hall 1973).

In addition to the revision of sites, the field season protocol was modified to include a longer period of seasonal sampling (June 2020 to November 2020) and deployment of recruitment devices. A total of 12 recruitment devices were created by placing $n = 3$ ceramic tiles (each $55 \times 3 \times 0.6$ cm) enclosed within a $75 \times 50 \times 55$ cm coated metal wire cage, with $n = 3$ devices per site situated approximately 15 m apart. Recruitment devices deployed in May were monitored at weekly intervals, field and weather condition dependent. One tile was removed from one of the replicate recruitment cages at each of the four sites weekly and was replaced with a new seasoned ceramic tile. The third cage remained undisturbed until the study concluded to ascertain the total number of spat settled cm^2 over the season. At the end of the 2020 sampling period, all three spat collectors were retrieved at sites NI and WP, but as a result of storms that occurred throughout the season, only one recruitment device was recovered from sites AP and OF.

Physicochemical Data

To assess the potential relationships among abiotic factors and oyster larval abundance, physicochemical data (pH, salinity, and temperature) were retrieved for each sampling date from the National Oceanic and Atmospheric Administration (NOAA) National Estuarine Research Reserve System (NERRS) data buoy, which monitors water quality, nutrient/pigment, and meteorological data in real-time using a System-Wide Monitoring Program. The real-time data were accessed using the data graphing and export system for the Great Bay station from their Centralized Data Management Office website (Fig. 2). Because of the aforementioned unique circulation of GBE, data provided by the NERRS buoy have been shown to reflect environmental conditions of GBE as a whole (Pennock 2007).

Enumeration of Larvae

Enumeration for each of the 3 y followed the same basic microscopy protocol. Formalin-preserved plankton samples were filtered through a set of brass sieves retaining 45–106 µm, then diluted with 200 mL of filtered seawater before enumeration via light microscopy. Three well-mixed 1-mL replicate subsamples were applied to Sedgewick Rafter Counting Chambers and then enumerated under ×4 magnification with an Olympus CX31 microscope. Oyster larvae were identified based upon shell shape, umbo character, and velum. For only the 2020 samples, all size fractions were returned to the original container and reconstituted in formalin-seawater after which samples were filtered through a 177-µm sieve to isolate oyster larvae from other larger biological material then enumerated using an automated FlowCam (Yokogawa Fluid Imaging Technologies). A 600× flow cell was used to process six 5-mL replicates per sample. A capture filter set from 10 to 300 µm (Baldwin &
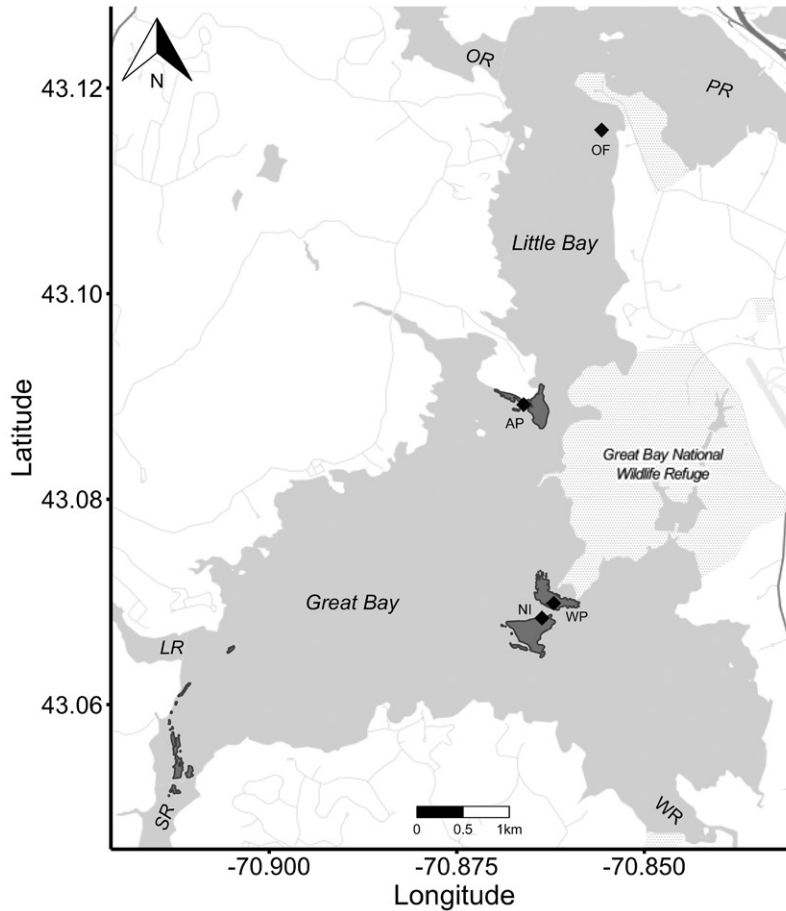
Figure 1. Map of Great Bay Estuary showing four sites (black diamonds) where larval tows were conducted and spat collectors were deployed. Natural reefs are shown as dark gray areas. Sites Nannie Island (NI), Woodmans Point (WP), Squamscott River (SR), and Lamprey River (LR) were sampled in 2018 and 2019. Those sampled in 2020 were NI, WP, and Adams Point (AP), located at natural oyster reefs, and OF located at an oyster farm. SR: Squamscott River, LR: Lamprey River mouth, OR: Oyster River, PR: Piscataqua River, WR: Winnicut River are shown on the map for reference. Not shown are Bellamy River, and Salmon Falls and Cocheco Rivers that feed into the Piscataqua River.
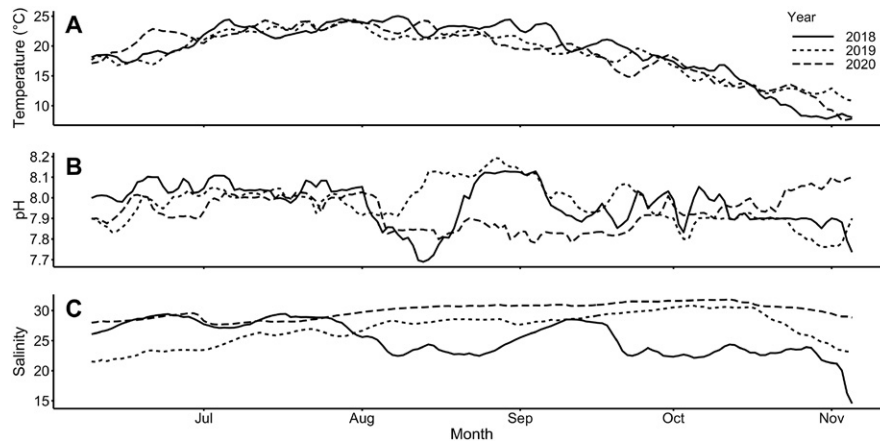

Figure 2. Physicochemical data from 2018, 2019, and 2020. Data include (A) temperature, (B) salinity, and (C) pH.

Newell 1995) allowed capture of images of organisms in the expected size range of oyster larvae. To further optimize the detection of oyster larvae, images of putative oyster larvae were filtered using a library created from known oyster larvae. Results of the two enumeration methodologies in 2020 were compared and contrasted, allowing for quality assurance. Both Sedgewick Rafter and FlowCam enumeration for all samples were performed by several individual workers and FlowCam counts were validated by having three readers assess saved images of oyster larvae. At least two readers needed to come to
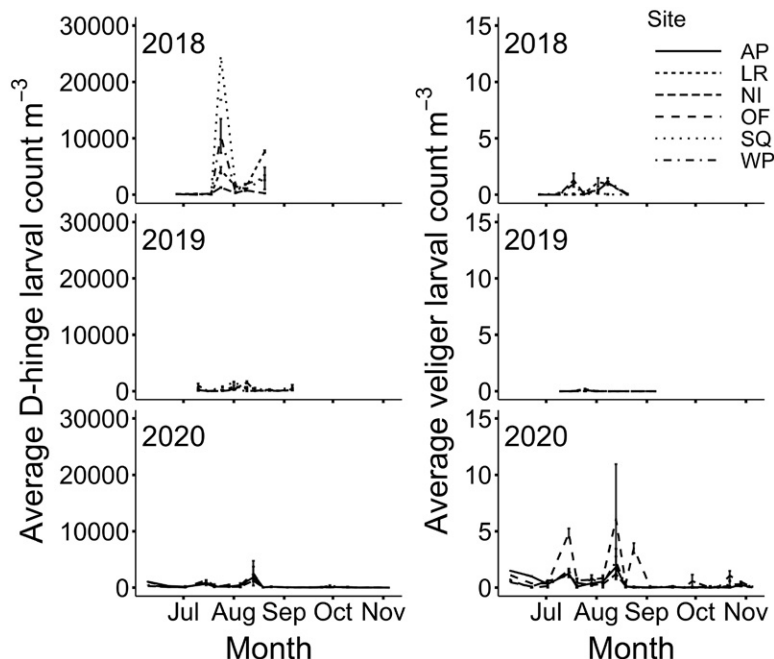
Figure 3. *Crassostrea virgincia* **D-hinge and veliger larval abundances in (A and B) 2018, (C and D) 2019, and (E and F) 2020. Standard error bars are shown with each data point.**

agreement to validate the total count of oyster larvae present in a given sample.

Analyses

Oyster larval data for 2018 and 2019, pediveliger and D-hinge oyster larvae m^{-3}, were determined by microscopy ($n = 12$) and averaged for the year. Counts of both D-hinge and veliger oyster larvae m^{-3} determined by microscopy ($n = 3$) and the FlowCam ($n = 5$) were averaged per site, and GBE as a whole, corresponding with their respective sampling dates. Analyses of variance were conducted in R (v 3.6.3, R Core Team 2020) to assess the suitability of combining data for each year. Although veligers can be positively identified as oyster larvae, it is not possible to distinguish oysters from other bivalves at the D-stage, leading to a probable inflation of oyster counts at the D-stage because those counts also can include mussels and clams. To account for zero-inflation and peak spawning periods resulting in peak larval abundances, the median abundance from each year was compared among years. Average counts were log ($x + 0.05$) transformed to meet normality and homogeneity assumptions. Shapiro–Wilks test for normality and Bartlett's test were used to examine normality and homogeneity, respectively. Multivariate analyses of variance tests were performed to test for differences in D-hinge and veliger counts among years. A Tukey's honestly significant difference (HSD) test was performed *post-hoc* among sampling years for both D-hinge and veliger. A Kruskal–Wallis test was performed to test for differences in the number of spat cm^{-2} settled among dates. Model selection of explanatory variables was performed using the "dredge" function in the "MuMIn" package (Barton 2015). Regression models were performed using temperature, salinity,

and pH as the independent variables if they were found to be significant during the model selection process, and D-hinge and veliger counts as the dependent variables. All statistical analyses were done in R (v 3.6.3, R Core Team 2020).

RESULTS

Baselines for oyster larval abundance were observed across all 3 y. In 2018, there was a mean occurrence of 2,293 D-hinge m^{-3} (SE 548, range 75.8–10,165) with a median of 985 D-hinge m^{-3}, and 0.29 veliger larvae m^{-3} (SE 0.08, range 0.001–0.8) with a median of 0.056 veliger larvae m^{-3} per sampling date. In 2019, a mean occurrence of 325 D-hinge m^{-3} (SE 74.8, range 18.5–764), median of 265 D-hinge m^{-3}, 0.01 veliger larvae m^{-3}(SE 0.0024, range 0–0.07), and median of less than 0.001 veliger larvae m^{-3} was observed per sampling date. In 2020, there was a mean occurrence of 273 D-hinge m^{-3} (SE 85.9, range 1.18–2,009), median of 83.7 D-hinge m^{-3}, 0.532 veliger larvae m^{-3} (SE 0.198, range 0.003–2.86, Fig. 3), and median of 0.301 veliger larvae m^{-3} per sampling date. Analysis of variance demonstrated no significant difference among sites for each corresponding date. Multivariate analysis of variance showed significant differences in the number of larvae observed across sampling years for D-hinge and veliger larvae (F = 14.501, $P < 0.001$, Fig. 4). Model selection indicated that salinity and pH were not significant; temperature was the only significant variable associated (positively) with D-hinge oyster larval abundance ($P < 0.001$, adj. $R^2 = 0.5086$, Fig. 5). Furthermore, none of the variables examined were associated with veliger abundance. The quantity of spat settled [Chi-square = 66.329, degrees of freedom (*df*) =3, $P < 0.001$, Fig. 6] differed significantly by date.
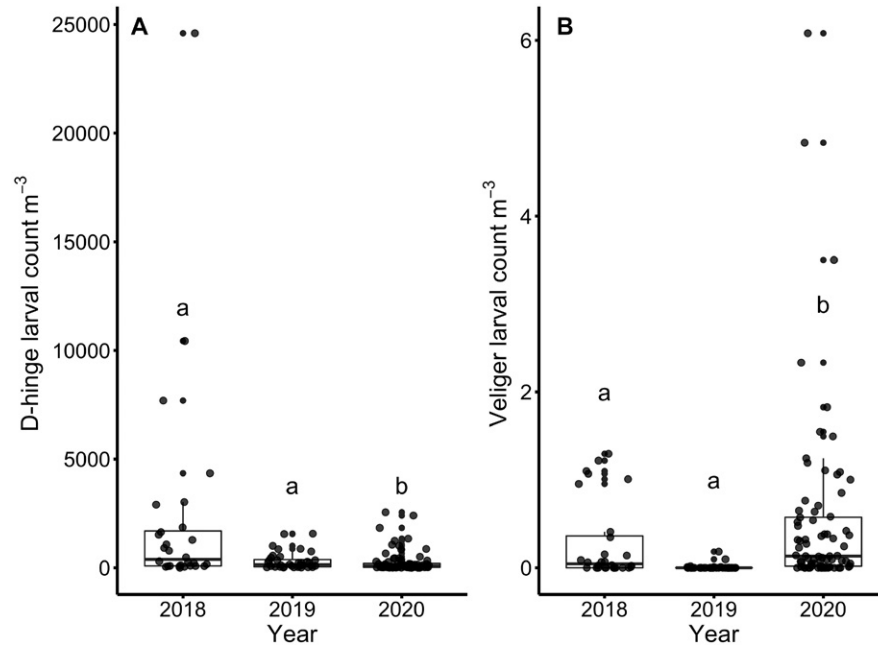
Figure 4. *Crassostrea virginica* **larval counts (A) D-hinge and (B) veliger collected from Great Bay Estuary, NH in 2018, 2019, and 2020. Black circles denote observations of larval counts during a given sampling period. Midlines within each boxplot represent median values, and the hinges indicate the first and third quartile ranges (25th and 75th percentiles).**
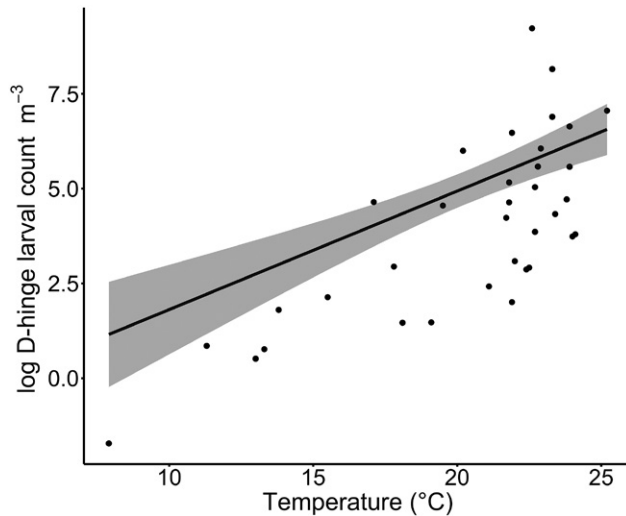

Figure 5. Regression model of D-hinge oyster larvae and temperature (°C) with corresponding trendline and 95% confidence intervals shown in gray.

Figure 6. *Crassostrea virginica* **spat settlement m^{-2} and SE observed in 2020 in Great Bay Estuary (GBE).**

DISCUSSION

Temporospatial Patterns of Oyster Spawning and Recruitment Activity

The lack of spatial variation in larvae or recruitment across the different sites suggests that spatial effects experienced across different sites in the Bay are likely uniform. Prior studies of eastern oyster recruitment in GBE and in other northern estuaries indicate that recruitment activities peak between late July and mid-August (Ayer et al.1970, Kennedy et al. 1996), and that spawning occurs from June to October (Cox & Mann 1992, Haven & Fritz 1985, Mann et al. 2014). This study revealed that oyster l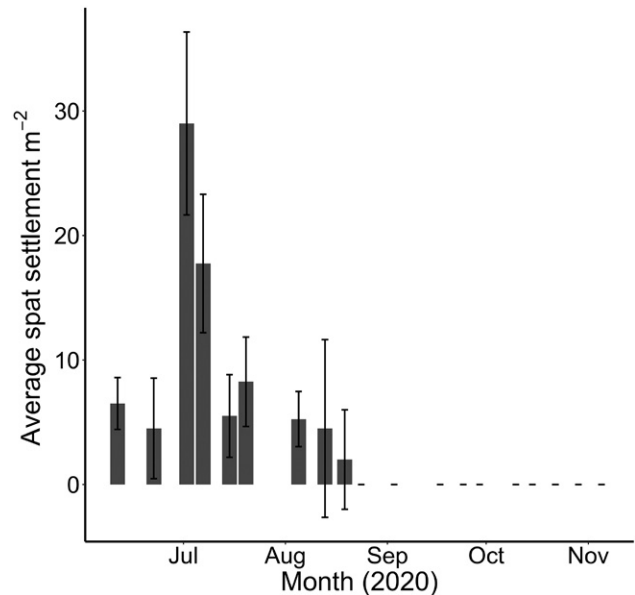arvae likely were present in waters before sampling began in June as oyster larvae already were abundant in early June, which is indicative of prior spawning activity. Furthermore, larvae continued to be observed in November. Unlike reports from other estuaries (Carriker 1951, Dekshenieks et al. 1996), salinity did not appear to have an impact on larval abundance in this study. Oyster settlement was observed in mid-June, peaked in early July, and ceased around mid-August. Spat settlement in mid-June suggests that eastern oysters in GBE likely spawn by late May, assuming prior data

are correct for approximately 32 days to settlement (Carriker 1951, Dekshenieks et al. 1993). This informs us that restoration efforts should occur earlier in the season than they currently are conducted. Depositing oysters earlier in the season will allow for a longer growing season and potential for larger oysters. Despite the continued presence of larvae in the water column, spat were not observed after August on the experimental collection tiles. The lack of set is notable because while oyster larvae follow a generally uniform distribution in the water column, more mature larvae are known to inhabit the lower levels of the water column (Carriker 1951) and should therefore be available to set. As a result, future studies would benefit from incorporation of hydrodynamic modeling to ascertain how oyster larvae move and settle within GBE, especially with regard to determining optimal sites for reef restoration.

Data from 2018 to 2020 show a significant decrease in oyster D-hinge larval abundances in GBE (Fig. 4) and veliger larval counts that are exceedingly low, 10–100 times lower than oyster larval abundances in other estuaries (Carriker 1951, Andrews 1982, Wahyudin & Yamamoto 2020, Christo et al. 2021). To obtain a measurement of local recruitment, The Nature Conservancy has placed recruitment devices at or adjacent to five native oyster reefs and three restored reefs since 2018. Although spat did recruit to the devices, results were variable, consistently low at all sites within Great Bay proper, and there was relatively more recruitment at the Lamprey and Squamscott sites (Laferriere & Group 2021). The GBE 2018 to 2020 spat set (generally <10 spat m^{-2}) is half the magnitude observed at these GBE sites in 2013 to 2015 by Eckert (2016) and is two to three orders of magnitude lower than observations for *Crassostrea* settlement in other estuaries along the North American Atlantic coast (VIMS 1986, Powell et al. 2009, Narvaìez et al. 2012, Peters et al. 2017, Southworth & Mann 2020). Thus, the dearth of veligers is the most likely cause of spatfall failure in GBE.

At all sites, the cages containing oyster settling tiles were noted to have oyster drills, *Urosalpinx*, and green crabs, *Carcinus maenas*. Although there are studies showing predation of oyster drills and green crabs on eastern oysters (Poirier et al. 2017), they are not likely predators of oyster spat/larvae. Lack of appropriate settling area is an additional likely option to explain the low numbers of spat. At OF (an oyster farm site), it was noted that from mid-August to the start of November, large quantities of tunicates, *Botryllus schlosseri* (established, Yund et al. 2015) and *Botrylloides violaceus* (invasive), colonized the recruitment devices. Previous studies have shown that colonial ascidians inhibit settlement by other species near or on their tunics (e.g., Dijkstra et al. 2008). Furthermore, colonies can fuse to create large colonies that occupy a greater amount of contiguous space, further limiting the amount of bare space for oyster settlement (Westerman et al. 2009). Ascidians have limited capacity to regulate salt concentrations, which limits their distribution to areas with higher (>20) salinity (Dijkstra et al. 2008, Lambert et al. 2018) and may mitigate their potential effects on settlement at river mouths where salinity is slightly lower. Finally, nutrition has long been acknowledged as a factor in larval period and settlement success (Stanley & Sellers 1986) and it is known that eastern oysters consume phytoplankton selectively based on size and the chemical quality of the food particles (Baldwin

1995, Weissberger & Gilbert 2021). Future studies focused on the composition and size ranges of phytoplankton communities may shed further light on the lack of spat.

Effect of Physicochemical Parameters on Larval Abundance

Of the physicochemical changes that were recorded, only temperature appeared to drive the phenology and densities of D-hinge larvae in GBE. Increases in salinity were slightly associated with a decline in veligers but were not otherwise informative. These results concur with other studies that found equivocal correlations among spawning activity, settlement, water temperature, and salinity (Carriker 1951, Cox & Mann 1992, Kim et al. 2010, Narvaìez et al. 2012). This study reveals a lengthening of the reproductive phenology of the eastern oyster in GBE that quite possibly is related to increased temperature as other studies have shown that elevated temperatures have resulted in shorter planktonic development, longer growth, and altered reproduction of ectothermic species (e.g., Kimmel & Newell 2007, Dijkstra et al. 2017). Consequently, it is important to begin future sampling by April, possibly earlier. This will provide a more complete understanding of the relationships between physicochemical factors and oyster larval abundance in GBE.

Importance of a Baseline

Oyster restoration in estuarine ecosystems is necessary for living resources management and is one of many approaches focused on reduction in the negative effects of eutrophication by top-down filter feeding excess phytoplankton (Fulford et al. 2007) but cannot alone alleviate the effects of algal blooms (Pomeroy et al. 2006, 2007). The larval abundance data presented here can help focus oyster restoration efforts where they are most likely to lead to the resurgence of the GBE oyster population. This baseline knowledge of the occurrence and distribution of oyster larvae within GBE can be used in concordance with NH Fish and Game Review of Oyster Data published online each year, which tracks adult oyster numbers in GBE. In other estuaries (Chesapeake Bay, VA, Cedar Point, AL, and Hiroshima Bay, Japan), despite a focus on restoration efforts, similar dramatic declines in successful oyster restoration efforts have been observed (Rothschild et al. 1994, Kim et al. 2013, Wahyudin & Yamamoto 2020). It has been shown that optimal spat settlement correlates with a 1- to 2-wk period after the peak larval abundance for the season (van den Brink et al. 2020), demonstrating the necessity to be able to determine the peak larval abundance and ensure appropriate setting substrate. Studies have shown that climate change has the potential to cause mismatches between phytoplankton blooms and spawning phenology in certain fishes (Asch et al. 2019, McQueen & Marshall 2017), and it is likely that these impacts do not apply exclusively to fish taxa. Thus, given the low possibility that physicochemical characteristics are the driver of reduced larval abundance, restoration efforts in GBE should consider factors other than simple physicochemical variation (e.g., toxic phytoplankton blooms, nutritious phytoplankton, quantities of larvae relative to the proximity of adult oyster reefs) to better comprehend how eastern oysters are responding to these interacting factors in New England.

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