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Short communication

Remote set of oyster (*Crassostrea virginica*) in various aquaculture gear

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ABSTRACT

Remote-setting of oyster, *Crassostrea virginica*, larvae from the disease-resistant Haskin NEH line is performed every other year in Delaware to supply small-scale oyster enhancement efforts, citizen oyster gardening program. Over the past four remote sets (2009, 2011, 2013, 2015), three types of shell containment gear were utilized to monitor and compare the setting efficiency rate and post-set survival. Shell containment gears included diamond, plastic mesh bags (2009, 2011), wire baskets (2011, 2015), and plastic aquaculture trays (2013, 2015). Average setting efficiency was estimated at 28%, 23%, 17%, and 29% in the respective 2009, 2011, 2013, and 2015 remote sets while gear specific settling efficiency (%) include 28% for mesh bags in 2009, 22% for mesh bags and 33% for wire baskets in 2011, 17% for aquaculture trays in 2013, and 30% for aquaculture trays and 22% for wire baskets in 2015. Settling efficiency was lowest in 2013 using aquaculture trays compared to other years while aquaculture trays in 2015 and wire baskets in 2011 resulted equally higher settling efficiency. For small-scale growers, the stacked aquaculture trays are advantageous for several reasons: reducing handling time, uniform shell distribution within tanks, environmentally friendly alternative, and ease of cleaning detritus between shell layers.

Introduction

The eastern oyster, *Crassostrea virginica*, is a keystone species with the well-documented ability to provide ecological services, environmental enrichment, and commercial value (Coen et al., 2007). Oyster reefs provide valuable habitat for many ecologically and economically important species and in benthic and intertidal habitats (Harding and Mann, 2001). Oysters drive benthic-pelagic coupling by filtering suspended particles from the water, and their deposits enrich benthic communities and increase carbon sequestration (Newell, 2004). Their bioactivity and structure lead to a greater abundance and diversity of other aquatic species (Harding and Mann, 1999).

The Delaware Inland Bays consist of three interconnected bodies of water in southeastern Sussex County. The bays drain a watershed of about 829 km^2 that is rapidly undergoing development. They are very shallow (average depth 1.5 m), poorly flushed by tidal movement, and are especially sensitive to decreased water quality conditions. For >30 years, the bays have had an extremely low abundance of naturally recruiting the Eastern oyster, *C. virginica* (Erbland and Ozbay, 2008; Kendall et al., 2007). This decline can be attributed to overharvesting, habitat degradation, poor water quality, and disease (Wilberg et al., 2011; Ozbay et al., 2009; Ewart and Ford, 1993; Newell, 1988). Oyster populations in the Delaware Inland Bays have decreased by over 95% of their population by the 1950s (Ewart, 2013). By 1978, there was no more available seed oyster supply or production. Between overfishing and disease outbreaks, oysters are virtually nonexistent to control eutrophication and nutrients. While nitrogen is an essential nutrient in aquatic ecosystems, excess nitrogen could lead to problems such as eutrophication and deterioration of water quality in the Delaware Inland Bays (Chaillou et al., 1996). According to research by Rose et al. (2015), nitrogen removal by shellfish farms was a more favorable solution per acre than Best Management Practices (BMPs) for agricultural and storm water runoff. Therefore, oyster aquaculture should be considered as a possible solution to be implemented in order to reduce nutrient loading and prevent eutrophication in the Delaware Inland Bays.

In response to the drastic decline in oyster populations, conservation organizations in the coastal eastern states have developed community shellfish culturing commonly referred to as “oyster gardening”, to help mitigate the loss of oyster populations. Oyster gardening instills a strong sense of environmental stewardship in

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the community while locally providing the ecological services of the oyster stocks (Rossi-Snook et al., 2010). As enhancement and restoration of the eastern oyster move forward, it is important to understand the overall contributions and impacts that oysters may impart in the surrounding environment.

Since 2003, the Delaware Inland Bays have been home to a small community-based oyster restoration program to help rebuild the locally decimated oyster stock (Marenghi et al., 2009, 2010). Oyster gardening in Delaware has become a popular shellfish aquaculture practice for homeowners with waterfront property, and it has developed into an integral part of the ecological restoration efforts for the bays. This program requires a new cohort of oyster spat on shell biennially to distribute to the oyster gardeners (Rossi-Snook et al., 2010).

To provide spat on shell to volunteer growers in this program, a remote set is performed every other year to allow oyster larvae to settle on cultch in a closed tank system (Ozbay et al., 2013, 2009). Remote setting, a technique used to produce oyster seed, was developed in the U.S. Pacific Northwest in the late 1970′s and early 1980′s (Webster and Merritt, 2013). It consists of two phases: (1) setting, where larvae attach to a setting material (cultch) such as oyster shell; and (2) a nursery period where newly set oysters (spat) are grown in protected areas until they are ready for planting (Bohn et al., 1995).

Determining the successfulness of the remote set process is often neglected in small-scale programs, yet gathering this critical information can inform managers of the approximate number of spat distributed in this program or likewise in a commercial business. The aim of this study was to estimate the number of settled spat on shell in our program, the spat setting efficiency, and the spat survival rate during the nursery period for the different gear types. We hypothesized 1) no significant difference would be in average larval setting efficiency between the years and 2) no significant difference would be in average larval setting efficiency among the gears employed in each year.

Materials and methods

Total experimental shell volume

The remote sets were performed following Delaware Oyster Gardening protocol in the summers of 2009, 2011, 2013, and 2015 (July-August in years 2009, 2011, and 2013; and August-September in 2015). "Haskins NEH" disease-resistant strains oyster larvae from The Rutgers University Haskins Shellfish Research Laboratory, Port Norris, NJ were used for the experiment. This later remote set in 2015 was due to the unavailability of larvae prior to August. One million oyster larvae were shipped from Haskin Shellfish Research Laboratory to the University of Delaware Hatchery Outdoor Flow-through Facility in Lewes, DE in a chilled Styrofoam container with ice packs to maintain the temperature of 5–10 °C. Oyster larvae were initially filtered on screens and placed in a nylon mesh to form a bundle to keep larvae damp at the shellfish lab for shipping according to FAO Protocol (2020). Those larvae were added to the tanks after they were acclimated to the water quality conditions in the flow-through system. Sun-bleached shell was power washed and then placed into containers. In 2009, shell was contained in 225 vinyl diamond braided-mesh bags (oyster netting). In 2011, 180 vinyl mesh bags and 19 vinyl-coated wire baskets (14-gauge 25 mm square wire) were utilized to hold shell. In 2013, cultch was placed on 54 stacked, plastic aquaculture trays (Dark Sea Stackable Trays, 68.5 cm * 68.5 cm * 8.9 cm). In 2015, shell was placed into 42 aquaculture trays with 6.35 cm PVC spacers placed between the trays, and in nine baskets (35.6 cm * 35.6 cm * 15.2 cm). In order to maximize the amount of cultch available, the baskets were stacked three high and were placed in areas of open water in the tank between the side of the tank and the stacks of trays. The amount of shell contained in each gear type was calculated based on the amount of shell that displaced a known amount of water according to John Ewart (Delaware Sea Grant, Fisheries Expert, personal communication).

$$Amount\ of\ shell\ contained = X\ mesh\ bags\ x\ 2L\ displaced\ water/mesh\ bag \tag{1}$$

$$\begin{aligned} &Amount\ of\ shell\ contained = X\ wire\ baskets\ x\ 17.62L \\ &\quad or\ 5L\ displaced\ water/wire\ basket \end{aligned} \tag{2}$$

$$\begin{aligned} &Amountof\ shell\ contained = X\ aquaculture\ trays\ x \\ &\quad 5L\ displaced\ water/aquaculture\ tray \end{aligned} \tag{3}$$

For example, each mesh bag contained enough shell to displace 2L of water. Therefore, the total amount of shell was determined according to Equation (1). The displacement of shell in wire baskets was 17.62L in 2013 and 5L in 2015, respectively (Equation (2)). The volume of water displaced by shell in aquaculture trays was 5L (Equation (3)). For years where more than one gear type was used, the amount of shell per gear type was summed to determine how much shell by volume was used in each experiment. The total amounts are listed in Table 1. Our experimental strategy was asymmetrical and included mesh bags only in 2009, mesh bags and wire baskets in 2011, aquaculture trays only in 2013, and wire baskets and aquaculture trays in 2015 due to non-availability of all three gears for each experimental year.

Remote set designs

In 2009, shell in bags were all placed on the bottom of the tank (Fig. 1a). Baskets were utilized in 2011 to elevate some shell off the bottom of the tank (Fig. 1b). In 2013, aquaculture trays containing shell were stacked on a center pole. The 2015 design was established based on the success of the 2013 aquaculture tray set. Instead of stacking trays on top of each other, spacers were engineered by cutting 4 PVC tubes to 6.35 cm and placing them between trays at each of the four corners of each tray (Fig. 1c). Stacked baskets were placed in open spaces between the aquaculture trays to help maximize the amount of cultch available for setting.

Water was pumped directly from the Broadkill River into the 5,678 L tank at an approximate rate of 11,356 L/h (i.e. 2 full exchanges per hour). Additional aeration was supplied to the tank via a piston-based aerator to facilitate the circulation of water throughout the system. Several days of water circulation allowed a biofilm to develop on cultch prior to larvae being added to the tank. "Haskins NEH" disease-resistant strains oyster larvae from The Rutgers University Haskins Shellfish Research Laboratory, Port Norris, NJ were added to the tank on *7–2-2009, 6–29-2011, 7–9-2013,* and *8–12-2015*, and a dark tarp was placed over the tank to reduce sunlight. Unicellular marine algae with various species naturally found including Chaetoceros, Skeletonema, Isochrysis, Chlorella, Tetraselmis species ranging in terms of total Chlorophyll-*a* concentration from 5.7 to 12.8 μg/L contained in inflowing water exclusively served as a food source for the larvae/spat. Larvae usually attach to the cultch and metamorphose into spat within 24 h of being added to the tank (Helm et al., 2004). After a settlement period of 48–72 h, water circulation to the tank was resumed. In 2009, 1.056 M larvae were either diploid (824 K) or triploid (232 K), and there was no way to tell which variety settled onto shell. The 2011, 2013, and 2015 cohorts contained 1 M diploid larvae. After a nursery period of three weeks in the

Table 1
Total shell used by volume for each gear type, average survival (%), setting efficiency (%) and number of spat settled for each gear type and in each experimental year. Initial larval introduced was 1 million larvae. S denotes significant different ($P \le 0.05$) between the gear type in the year and year that is different than other year based on the average setting efficiency.

Year	Total Number of each Gear Type	Shell Displacement (L)	Total Shell by Volume (L)	Spat Survival (%)	Setting Efficiency (%)	Estimated Number of Spat Settled (*1000)
2009	225 mesh bags	2	450	85	28	297
2011	180 mesh bags19 wire baskets*199*	2	360	90	22	200
	total	18	335	95	33^S	32
			695	*93*	*23*	*232*
2013	54 aquaculture trays	5	270	95	17^S	170
2015	42 aquaculture trays	5	210	82	30^S	247
	9 wire baskets	5	45	97	22	38
	51 total		*255*	*88*	*29*	*285*

Fig. 1. Pictures of aquaculture **a.** bags, **b.** baskets, and **c.** trays used during the remote set practices. For each type of gear, clean oysters shells as seen in the pictures were put for larvae to settle in the tank. *Photo credit to Brian Reckenbeil (1a) and Laurieann Phalen (1b,c).*

remote set tank, the spat were large enough to count. Samples of shell were collected from random locations within the tank to ensure cultch would not come from one clustered location. Shells were inspected for the presence of live and dead spat (oysters < 25 mm). Spat were counted as dead if 1) the right valve was loose to the touch, 2) both valves were still articulated but the oyster was empty, and 3) the oyster body was attached, but the right valve was missing or crushed. In 2009, 2011, 2013, and 2015, spat scars were counted but not classified as dead because of the uncertainty of whether or not the scar may have been an artifact on the cultch from a prior set. Percentage settling efficiency was calculated by total estimated spats divided by total larvae settle per gear, multiple by 100 as provided in Equation (4).

$$\%Setting\ Efficiency = (total\ estimated\ spats/\ total\ larvae\ settle\ per\ gear)x100 \tag{4}$$

Meritt and Webster (2012) used the number of shells instead of total volume those shell can occupy (in this study) to calculate remote set spat per shell and later for the entire tank. However, our settling efficiency calculation was similar to what they provided in their extension publication where spat produced divided by larvae in the tank.

Samples consisted of shell from randomly selected vinyl mesh bags (n = 9) in 2009, mesh bags (n = 6) and wire baskets (n = 6) in 2011, plastic trays (n = 9) in 2013, and both plastic trays (n = 9) and wire baskets (n = 9) in 2015. In 2009 and 2011, each sample consisted of cultch that filled an 8L container by dry weight, which amounted to 2L of displaced water to provide a normalized shell amount by volume. This volume of shell approximately equated to the amount contained in each mesh bag, which were later distributed to oyster gardeners. In 2013, each sample was comprised of enough shell to displace 2L of water, and the number of shells was counted to determine the number of spat per shell. All 2013 samples were nearly at the 8L volume of dry weight as filled in the previous two years (within +/−5 shells). In 2015, samples that displaced 2L of water were examined.

Water quality

Water quality data (temperature, dissolved oxygen, salinity, and pH)) were collected and averaged on a monthly basis using YSI 556 Multiprobe (Xylem Inc., Yellow Springs OH), Chlorophyll-a using AquaFlash Handheld Active Fluorometer (Turner Designs, San Jose, CA) and total suspended solids analyzed according to Boyd and Tucker (1992). Parameters like dissolved oxygen concentration was confirmed with the University of Delaware Citizen Monitoring Program data (2019) collected at the PEL Dock station, ~80 m away.

Data analyses

Three outcomes were desired for this project: mean spat settled per gear type, survival of settled larvae, and setting efficiency onto shell in each gear type. In order to determine mean spat per gear type, spat in each sample were counted and then multiplied by the number of bags, baskets, or trays used. Survival was calculated simply as the number of live spat-on-shell counted divided by total spat counted (alive and dead) multiplied by 100. The setting efficiency was calculated as the percent of spat-on-shell counted as per the total larval input at the beginning of the experiments. When two gear types were used, the larval availability per gear type was identified as a percentage. That is, if 180 bags and 19 baskets were used, 90.4% of the larvae was assumed to be available for settlement on bags (180 bags/199 different gear *100), while 9.6% of larvae was allotted to settlement in baskets. The percent data was transformed to normalize the data for the number of spat settled before analyses, and mean values were calculated and compared according to normalized data using Microsoft Excel software. Two-way ANOVA was used to determine significance between the remote set methods within the same year and between the years and interaction effects for spat set efficiency (IBM SPSS Statistics Inc.). We also compared the setting efficiency of oyster larvae in water column in 2015 to find if there were any differences in setting efficiency. Each of the measured water quality parameters (temperature, dissolved oxygen, salinity, pH, total suspended solids, and Chlorophyll-*a*) were analyzed using one-way ANOVA across setting events to evaluate the variation in ambient environmental conditions during these experiments (IBM SPSS Statistics Inc.). During our analysis, we found 2009 water quality parameters were different than other remote set years. In all statistical comparisons, responses were considered significant at $P \leq 0.05$.

Results

Estimated number of spat settled

The mean number of spat in each gear type varied between years and gear types. In 2009, estimated total spat counts equaled 297,113 in mesh bags. The two gear types in 2011 yielded an estimated total of 231,581 spats for both bags and baskets, specifically 200,250 spat setting into bags while 31,331 spats settled into the baskets. An estimated 170,073 spat settled into aquaculture trays in 2013. In 2015, estimated total spat counts equaled to 284,781 spat. Out of this total, 246,635 were spats in the trays while 38,146.5 spat settled into baskets (Table 1). The comparison of the settlement of settled spat regarding placement in the water column is provided for year 2015 (Fig. 2) and the highest settlement is recorded for the bottom column.

Percent survival

Survival is the ratio of counted live spat to total spat counted. The survival percentage was never below 82% for any experimental treatment (Table 1). The greatest survival of 97% was recorded in 2015 in wire baskets. A value of 82% was recorded in 2015 with the aquaculture trays at the end of the experimental season while similar trays yield higher survival of 95% in 2013. Survival rate for spats are recorded much higher than it has been found in the past.

Total setting efficiency

Setting efficiency is the ratio of all counted spat (both dead and alive) to total number of larvae added into the tank. Average setting efficiency was estimated as 28%, 23%, 17%, and 29% in the respective 2009, 2011, 2013, and 2015 remote sets. Settling efficiency (%) for various gear types include 28% for mesh bags in 2009, 22% for mesh bags and 33% for wire baskets in 2011, 17% for aquaculture trays in 2013, and 30% for aquaculture trays and 22% for wire baskets in 2015.

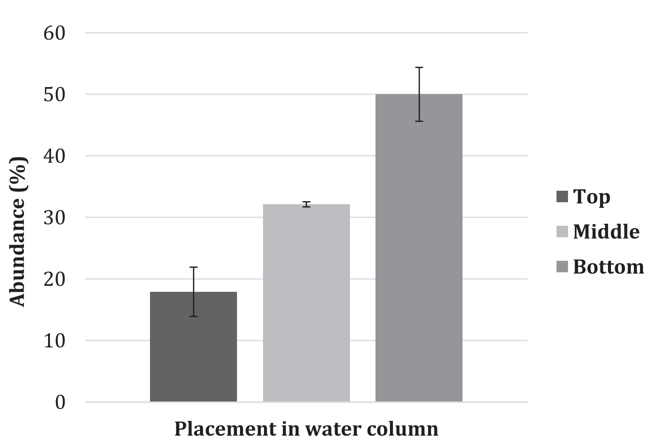

Fig. 2. This graph shows % spat settlement ± SD based on basket placement in the water column during 2015 experiment. Darkest bars represent baskets near the top of the water column, while lightest bars are at the bottom. The letters a, b, and c denote significant differences ($P \leq 0.05$) in % spat settlement between the water columns.

Total setting efficiency of larvae was greatest in the wire baskets in 2011, with an efficiency of nearly 33% (Table 1). The lowest setting efficiency of 17% was recorded in aquaculture trays in 2013. However, aquaculture trays had greater setting efficiency than the wire baskets in 2015 (30% vs. 22%). We found significant difference in average spat set efficiency between 2013 other years (2009, 2011 and 2015, $P \leq 0.05$). There were differences in spat setting efficiency between the wire baskets and trays ($P \leq 0.05$) used in 2015. There were also significant differences in spat set efficiency between mesh bags and wire baskets in 2011. While we recorded differences between gear types, some of the gear types were more abundant for our use from one year to another made our analysis and data interpretation difficult. Based on the existing in data on the larval setting efficiency in Maryland, overall, only all gear types yield acceptable spat setting efficiency while wire baskets and trays seem to yield higher spat set efficiency in this study. Spat settled on gear and tank walls were not enumerated. Based on our results, we rejected our null hypotheses that we found 2013 setting efficiency being lower than average set efficiency of other three years and difference was recorded between the gear types used in 2011 and 2015. In 2015, greatest percentage of spat set were found in baskets at the bottom of the tank compared to the baskets locate in the middle and surface water columns (Fig. 2).

Water quality

A One-way ANOVA (Table 2) showed a significant difference in temperature (°C), salinity and dissolved oxygen values between remote set years. Major differences in water quality parameters were recorded between 2009 and other years. Difference in water quality parameters between 2011, 2013, and 2015 were not significant ($P > 0.05$). With exception of year 2009, average dissolved oxygen was above 5 mg/L recommended lowest limit (Boyd and Tucker 1992). No significant difference was found in pH, total suspended solids or Chlorophyll-*a* values. Total suspended solids were well below the maximum recommended in the water column for aquaculture (<80 mg/L) according to Swann (1999). pH was monitored at the adjacent water source for remote set tanks ranged between 7.2 and 7.8 and remained constant, ~7.6–7.7 during the warmer months of this study (late May, June, July, August, September, and early October). According to Kennedy et al. (1996), the optimal pH range for *C. virginica* is 6.75 to 8.75 with overall pH range is 6.0 to 9.0 oysters are found. Average Chlorophyll-*a* values were at moderate algae level for 2009 (between 2.6 and 7.2 μg/L) and at elevated algae levels for other years (between 7.3 and 35 μg/L) according to Wood-Pawcatuck Watershed Association (2016).

Discussion

Remote set design

Containment of shell is a necessity in the remote set process for two reasons: 1) ease of transport of shell into and out of the tank, and 2) to allow for sufficient water circulation in the tank, which should allow larvae to penetrate into all shell crevices (Bohn et al., 1995, Congrove et al., 2009). Otherwise, shell would be dumped in the tank, and larvae would only penetrate the top 7.5 cm–15 cm of shell (Congrove et al., 2009). Therefore, a tank

Table 2
One-way ANOVA of water quality parameters. S denotes significant differences ($P \leq 0.05$) for water quality parameters among the experimental years. Major differences in water quality parameters were recorded between 2009 and other years. Difference in water quality parameters between 2011, 2013, and 2015 were not significant ($P > 0.05$).

Parameters	2009 Mean	2011 Mean	2013 Mean	2015 Mean	F	df	P-value
Temperature (°C)	26.10	24.90	22.40	22.70	10.20	14	0.00164^{S}
Salinity (g/L)	23.80	30.30	29.70	29.60	56.90	14	0.000000549^{S}
Dissolved oxygen (mg/L)	3.80	6.40	5.90	6.20	26.10	14	0.0000264^{S}
Total suspended solids (mg/L)	76.4	76.80	55.90	51.20	1.11	14	0.386865
pH	7.50	7.65	7.60	7. 70	1.31	14	0.089416
Chlorophyll-*a* (μg/L)	5.70	10.10	12.80	12.20	4.14	14	0.034398^{S}

with uniformly distributed shell should yield in greater numbers of spat settlement.

Estimated number of spat settled

When comparing all gear types across all years, the largest total number of spat settled in bags are in 2009. This was the only gear type available for spat settlement in 2009. In 2011, cultch in mesh bags contained on average more spat than in the wire baskets. There was a decrease in the estimated number of spat settled in bags from 2009 to 2011. This may be a result of cultch container placement. While cultch bags lined the bottom of the tank in both years, baskets of shell were placed on top of the bags. Larvae may not have been able to readily access to the bags as they did in 2009. When comparing the bags to the wire baskets in 2011, the settlement of the larvae was most probably affected by setting away from the light near the surface (where the baskets were located) and toward the darker areas of the tank (where the bags were located). Our remote set experiment was primarily conducted during the months of June and July with the longer daylight averaging from 13.5 to 15.5 h however; larval tanks were covered to block the sunlight into the tanks. Shaw et al. (1970a), Shaw et al. (1970b) found that, "the setting of mature *C. virginica* larvae is encouraged by darkness and partially inhibited by light" due to negative phototropism. Similar to Shaw et al. (1970a), Shaw et al. (1970b), dark or low light conditions have been found yielding higher settlements rates for *C. virginica* in the studies by Lillis et al. (2013) and Ritchie and Menzel (1969).

The increase in number of spat settled in baskets from 2011 to 2015 could be attributed to the baskets' placement throughout the entire water column, rather than near the surface. Comparing the settlement of spat settled (living plus scars) based on basket placement in the water column (top, middle, bottom) in 2015, greatest percentage were found in baskets at the bottom of the tank (Fig. 2).

In 2013, trays were stacked on top of one another. In between each tray of cultch was an empty tray. This alternating design may have limited the access that larvae had to the cultch in the filled trays. In 2015, the use of stacked trays with PVC spacers allowed ample water flow between all shells and allowed larvae access to any shell in the trays. These trays had almost 18% more spat settlement than baskets also used in 2015. The surface area for each tray was 3 times greater than that of each basket, which may result in larvae finding cultch with greater ease to settle upon.

Percent survival

Bag in 2009 and aquaculture trays in 2015, had the lowest percent of survival. This may be attributable to either the cultch becoming compacted inside the bags allowing insufficient water flow to travel through to the spat or difference in larval quality and health located in the trays. However, aquaculture trays in 2013 and wire baskets in 2011 and 2015 had very high survival, (95%, 95% and 97%, respectively) (Fig. 1a,b,c).

Along with the previous years, in 2015, a secondary natural set that occurred in the Broadkill River was obvious, and thus increased the abundance of spat on shell within the tank. No effort was made to differentiate cohorts, as any spat, which were alive or dead, was counted, as they are subsequently utilized in the oyster gardening program. According to NJ DEP (2020), oyster spawning begins when the water temperature reaches 77°F and continue throughout the summer. The first spawning typically occurs in when water temperature reaches 77°F (25 °C). Subsequent spawns commonly occur throughout the summer until early-September. We observed and more likely monitored second sets spats on the clean shells in Delaware. Throughout the study years, we saw a second set of smaller spat on the cultch or on the tank walls and gears but we only recorded the sets (more likely including second sets) on the oyster shells in Delaware. This more likely lead to an overestimating in our setting efficiency, survival, and overall total spats throughout the study years.

Occasionally, individual shells from the various gear types contained over 30 spat. Wallace et al. (2008) stated that when using whole oyster shells as cultch, the initial "set" should have 10 to 30 early spat per shell. Successful remote sets yield 20–30 spat per shell and good environmental conditions should allow three to five oysters per shell to reach market size. Shells containing five to ten spat are ideal (Bohn et al., 1995), as too many oysters would cause a competition for space and result in the death of more oysters over time. Additionally, in 2015 there was an average of 7 spat per shell from the entire cultch samples used in the study, making it ideal for oyster growth.

Setting efficiency

The setting efficiency for this study ranged on annual average from 17% to 29%, which is consistent with other remote set efficiency studies performed at Virginia Institute of Marine Science (VIMS), which ranged from 0% to 24% (Congrove, 2009) and 5% to 30% in Maryland (Parker et al., 2011). It was also over the 5% minimum amount that Congrove (2009) determined would provide economical remote set production of spat-on-shell.

When looking at gear type, setting efficiency was the highest for wire baskets in 2011 (33%), followed by aquaculture trays in 2015 (30%), and bags (28%) in 2009. Total setting efficiency (regardless of gear type) was the highest of any year in 2015 (29%). It is possible that other unforeseen factors may have aided in a more successful total set in 2015 or may have just been ideal environmental condition and food source. When comparing setting efficiency for gear type in 2015, two-way ANOVA resulted significant difference between wire baskets and aquaculture trays. Setting efficiency in aquaculture trays in 2013 was lower than in 2015. This increase may be attributed to the differences in how the trays were deployed. In 2013, there were no spacers between the trays, while in 2015 spacers were used. The spacers may allow more surface area of cultch for the spat to access.

Remote set efficiency was relatively higher for the Delaware remote set studies compared to other remote set studies in the Mid-Atlantic region (Congrove et al., 2009; Webster and Merritt 2011). This is mainly due to weekly flushing and washing of the tank and remote set gear. Due to weekly tank flushing, oyster spat were housed longer in the tank before deployment. By flushing and cleaning the gear, we removed any sediment in the tank and allowed continuous water flow through the gears and food supplies for the spat (John Ewart – personal communication).

Water quality

Differences in temperature, salinity, and dissolved oxygen values are expected between remote set years due to differences in weather. Dissolved oxygen values of the Broadkill River were not a major concern as additional aeration was provided to the system to maintain adequate circulation in the tank. Although receiving water dissolved oxygen level was below the recommended minimum of 5 mg/L in 2009, with additional aeration in the remote set tank, this level was kept well above 5 mg/L (Boyd 1998). Lund (1972) found that 22–34 ppt was the optimum salinity range for remote set. Mean salinities for the remote set years fall within this optimum range for oyster larvae. Henderson (1983) demonstrated that the success of setting increased as the salinity increased from 15 to 30 ppt. Webster and Meritt (2011) reported that while oyster larvae can survive somewhat wide temperature ranges, they prefer temperatures between 25 and 30 °C. Bohn et al. (1995) state that the most successful oyster larvae set occurs between 26.7 and 32.2 °C. We did not find a clear relationship between mean temperature and survival, spat settlement, or setting efficiency. Most incoming water quality parameters from Broadkill River were ideal for oyster larvae and spat growth while additional aeration and water exchange helped maintain the system at optimum capacity.

Feasibility of gear type

It cannot be unequivocally determined if the different gear utilized in this remote set study affected the estimated number of spat settled or setting efficiency. Because of this uncertainty, the practicality of each gear type was examined. The criteria for this focused on labor intensiveness of the gear used (the input) in relation to the number of spat settled and the setting efficiency (the output). Bohn et al. (1995) found that limiting the labor and effort put into the handling of cultch during a remote set is all-important to not negatively affect profitability for the oyster grower.

Bags were determined to be very labor intensive to fill and handle. Because of the shape and diameter, they had a reduced surface area for larvae settlement. They were hard to keep clean (from mud deposition), and the shell became packed tight within the bags from stacking, which reduced the flow of water into them. It was not practical to remove them from the tank for cleaning.

Wire baskets were used in 2011 to elevate shell off the bottom and were found to be easier to clean than the nylon bags. Wire baskets were used in 2015 to fill in "empty spaces" between the aquaculture trays. Rinsing the cultch in the baskets was labor intensive, as the baskets needed to be removed from the tank with a boat hook, rinsed, and replaced.

In 2013, aquaculture trays were purchased, and their ease of use in the remote set process was evaluated. The trays were stacked one on top of another on a center pole. Seven poles were used, and each held up to 14 trays. In between each cultch-filled tray was an empty tray that was used as a spacer. Trays were able to be "fit" more easily into the tank than baskets and were easier to handle. When PVC spacers were used between the trays in 2015, more trays of shell were used. This design also better facilitated washing the shell to prevent the settlement of mud on the shell.

It was determined that stacked trays with PVC spacers worked best with this small-scale set-up and will be used for future remote sets because they: 1) require less handling time than mesh bags and baskets, 2) distribute shell more uniformly within the tank, 3) are more environmentally friendly (used multiple sets/years), 4) made washing sediments off of the shells easier, and 5) yielded a high setting efficiency (second highest with 30% settling efficiency to the wire baskets in 2011 with 33% settling efficiency) and average of spat set when compared to other gear types.

Aquaculture of eastern oysters was approved in 2013 for the Inland Bays for the first time since the early 1970s, and Delaware was the last state on the eastern shore to do so. However, details about policy are still being worked out, and potential oyster farmers have been enduring frequent resistance from the public. Wild oysters in this area are poorly studied due to extremely low numbers, but restoration efforts have been occurring for the past few decades.

It is hoped that these results in concert with other findings obtained over the years in the oyster gardening program will provide data to help potential commercial oyster growers successfully raise oysters and establish within the Delaware Inland Bays. Future research should be directed towards the improvement of larval settlement rates achieved in this study. Several chemical cues may induce oyster settlement (Anderson, 1996; Grant, 2009), so exploring the addition of chemicals into the tank to facilitate this process may be helpful. In addition, possibly adding a layer of living oysters may release the natural chemicals in which dead, sun-bleached shells do not release (Wallace et al. (2008)). In a similar study conducted by Aideed et al. (2014) on pearl oyster *Pinctada margaritifera* growth and reproduction, authors stated the importance of detailed investigations for pearl oyster reproduction ecology to improve overall understanding of reproductive behavior in the wild and their implications on natural populations and applications in expected cultivation of *P. margaritifera*, with similarly limited information we have experienced in our research effort. It is equally important to understand the oyster population genetics and genetic diversity to advance aquaculture practices in the Delaware Inland Bays.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical Statement

This project is conducted according to the policy set by Delaware State University for the laboratory research. As a mollusk, only oysters were used in this study and no permit required. No other animals under the American Association for Laboratory Animal Science, Institutional Animal Care and Use Committee (IACUC) was used in this study.

References

Aideed, M.S., Ahmed, B.A., Mukhaysin, A.A., 2014. Existence, growth and reproduction of pearl oyster *Pinctada margaritifera* in Hadhramout coast/Gulf of Aden. Egyptian Journal of Aquacultic Research 40 (4), 473–481. https://ac.els-cdn.com/S1687428514001058/1-s2.0-S1687428514001058-main.pdf?_tid=f87466e2-04c9-4ab5-82b8-3c35c33dec30&acdnat=1543806463_b9b186e9931eccc515d7666ac5afca60.

Anderson, M.J., 1996. A chemical cue induced settlement of Sydney rock oysters, *Saccostrea commercialis*, in the laboratory and in the field. Ecology and Evolution 190, 350–358.

Bohn, R.E., Webster, D.W. and Meritt, D.W., 1995. Producing oyster seed by remote setting. Northeastern Regional Aquaculture Center, University of Massachusetts, Dartmouth, MA. NRAC Bulletin No: 220. pp.11.

Boyd, C.E., 1998. Water quality for pond aquaculture. international center for aquaculture and aquatic environments – Fish-culture. In: Alabama Agricultural Experiment Station. Auburn University, Auburn AL 36849 USA, p. 37.

Boyd, C.E., Tucker, C.S., 1992. Water quality and pond soil analyses for aquaculture. Auburn agricultural experimental station. Auburn University, Auburn, AL 36849 USA, p. 183.

Chaillou, J.C., Weisberg, S.B., Kutz, F.W., DeMoss, T.E., Mangiaracina, L., Magnien, R.…Summers, J.K., 1996. Assessment of the ecological condition of the Delaware and Maryland coastal bays. US Environmental Protection Agency, Office of Research and Development, Washington, DC, p. 118.

Coen, L.D., Brumbaugh, R.D., Bushek, D., Grizzle, R., Luckenbach, M.W., Posey, M. H.…Tolley, S.G., 2007. Ecosystem services related to oyster restoration. Marine Ecology Progress Series 341 (2007), 303–307.

Congrove, M.S., 2009. A bio-economic feasibility model for remote setting: potential for oyster aquaculture in Virginia Master of Science Thesis. Virginia Institute of Marine Science, VA, p. 117.

Congrove, M.S., Wasson, J.A. and Allen, S.K., 2009. A Practical Manual for Remote Setting in Virginia. VIMS Marine Resource Report No. 2009-1. 25 pp.

Erbland, P.J., Ozbay, G., 2008. A Comparison of the macrofaunal communities inhabiting a *Crassostrea virginica* oyster reef and oyster aquaculture gear in the Indian River Bay, Delaware. Journal of Shellfish Research 27 (4), 757–768.

Ewart, J. W. and Ford. S.E., 1993. History and Impact of MSX and Dermo Diseases on Oyster Stocks in the Northeast Region. Northeastern Regional Aquaculture Center Fact Sheet No. 200. University of Massachusetts Dartmouth, North Dartmouth, MA. 8pp.

Ewart J.W., 2013. Shellfish Aquaculture in Delaware's Inland Bays: Status, Opportunities and Constraints. Inland Bays Shellfish Aquaculture White Paper. Delaware Sea Grant Program, College of Earth, Ocean and Environment (CEOE), University of Delaware. Lewes, DE. pp. 43.

FAO, 2020. Part 6 Hatchery operation: culture of spat in remote setting site, in the hatchery and in nurseries. http://www.fao.org/3/y5720e/y5720e0b.htm

Grant, M., 2009. Chemical induction of settlement in larvae of the eastern oyster *Crassostrea virginica* (Gmelin) Master of Science Thesis. University of Maryland Center for Environmental Science, College Park, Maryland, p. 60.

Harding, J.M., Mann, R., 1999. Fish species richness in relation to restored oyster reefs, Piankatank River, Virginia. Bulletin of Marine Science 65 (1), 289–300.

Harding, J.M., Mann, R., 2001. Oyster reefs as fish habitat: Opportunistic use of restored reefs by transient fishes. Journal of Shellfish Research 20 (3), 951–959.

Helm, M.M., Bourne, N. and Lovatelli, A., 2004. (comp./ed.) Hatchery culture of bivalves. A practical manual. FAO Fisheries Technical Paper. No. 471. Rome, FAO. 177pp.

Kennedy, V.S., Newell, R.I.E., Able, A.F., 1996. The eastern oyster. University of Maryland, Sea Grant Publications, College Park, MD. xvi +734.

Kendall, L.R., Ewart, J.W., Ulrich, P.N. and Marsh, A.G. 2007. Low incidence and limited effect of the oyster pathogen dermo (*Perkinsus marinus*) on an artificial reef in Delaware's Inland Bays. Estuaries and Coasts: Journal of Estuarine Research Federation 30:154–162. https://doi.org/10.1007/BF02782975.

Lillis, A., Eggleston, D.B., Bohnenstiehl, D.R., 2013. Oyster larvae settle in response to habitat-associated underwater sounds. PLoS ONE 8, (10). https://doi.org/10.1371/journal.pone.0079337 e79337.

Lund, D.S., 1972. Laboratory studies on setting of the Pacific oyster (*Crassostrea gigas*) Master of Science Thesis. Oregon State University, Eugene, OR, p. 85.

Meritt, D and D. Webster. 2013. Classroom in the Hatchery. In Advances in Aquaculture Hatchery Technology 1st Edition (G. Allan and Gavin Burnell, editors). 30 Chapters. Woodhead Publishing Ltd., Cambridge UK. pp. 596-625, total book 680p. eBook ISBN: 9780857097460.

Marenghi, F., Ozbay, G., Erbland, P., Rossi-Snook, K., 2010. Comparison of species composition, diversity and abundance in sub-tidal and floating oyster (*Crassostrea virginica*) gear habitat from Delaware's Inland Bays. Aquaculture International 2010, 1–11. https://doi.org/10.1007/s10499-009-9273-3.

Marenghi, F., Ozbay, G., Rossi-Snook, K., Chalabala, E.J., 2009. Restoration program in inland bays improves ecosystems as oyste populations recover. Global Aquaculture Advocate, 16–17.

Meritt, D., Webster, D., 2012. Remote Setting Systems. University of Maryland. Maryland Cooperative Extension Publication, p. 16. https://extension.umd.edu/sites/extension.umd.edu/files/_docs/programs/aquaculture/Rem%20Set%20text%20Apr%2012.pdf.

Newell, R.I.E., 1988. Ecological changes in Chesapeake Bay: are they the result of overharvesting the American oyster, *Crassostrea virginica?*. In: Lynch, M.P., Krome, E.C. (Eds.), Understanding the Estuary: advances in Chesapeake Bay research. Chesapeake Research Consortium, Publication 129 CBP/TRS 24/88, Gloucester Point, VA, pp. 536–546.

NJ DEP, 2020. Shellfish Mitigation Account Shellfish Enhancement Program. Five Year Conceptual Plan. New Jersey Department of Environmental Protection Division of Fish and Wildlife, Marine Fisheries Administration Bureau of Shellfisheries Delaware Bay Region Office/Atlantic Coast Region Office, pp. 26. https://www.state.nj.us/dep/opi/assets/final-for-web-shellfish-mitigtation-account-mou-five-year-plan.pdf.

Ozbay, G., Marenghi, F., Rossi-Snook, K., 2009. delaware oyster gardening and restoration – A cooperative effort. 2009 YSI Inc. YSI Application Note, YSI Aquaculture 0409 A571, p. 2.

Ozbay, G., Reckenbeil, B., Marenghi, F., Erbland, P., 2013. Eastern Oyster (*Crassostrea virginica*) aquaculture and diversity of associated species. In: Turner, J.P. (Ed.), Oysters: biology, consumption, and ecological importance. NOVA Science Publishers, Hauppauge, NY, p. 128. ISBN: 978-1-62948-808-0.

Parker, M., Dill, S., Webster, D., Meritt, D., 2011. Contained culture cost analysis "Economy, Employment, Environment". Oyster aquaculture technology series AGNR-EM-14-07. University of Maryland Extension, Clinton, MD, p. 13.

Ritchie, T.P., Menzel, R.W., 1969. Influence of light on larval settlement of American oysters. Proceedings of National Shellfisheries Association 59, 116–120.

Rose, J.M., Bricker, S.B., Ferreira, J.G., 2015. Comparative analysis of modeled nitrogen removal by shellfish farms. Marine Pollution Bulletin 91 (1), 185–190.

Rossi-Snook, K., Marenghi, F., Ozbay, G., 2010. Oyster (*Crassostrea virginica*) gardening for restoration in Delaware Inland Bays. Aquaculture International 2010, 1–6. https://doi.org/10.1007/s10499-009-9271-5.

Shaw, R., Arnold, D.C., Stallworthy, W.B., 1970a. Effects of light on spat settlement of the American Oyster (*Crassostrea virginica*). Journal of the Fisheries Research Board of Canada 27, 743–748.

Shaw, R., Arnold, D.C., Stallworthy, W.B., 1970b. Effects of light on spat settlement of the American Oyster (*Crassostrea virginica*). Journal of the Fisheries Research Board of Canada 27 (1970), 743–748. https://doi.org/10.1139/f70-07.8.

Swann, L., 1999. A fish farmer's guide to understanding water quality. Department of Animal Sciences, Illinois-Indiana Sea Grant Program, Purdue University, West Lafayette, IN, p. 11. https://www.extension.purdue.edu/extmedia/as/as-503.html. [Accessed by November 10, 2018].

Wallace, R.K., Waters, P., Rikard, F.S., 2008. Oyster hatchery techniques. southern regional aquaculture center. SRAC Publication 4302, 6 pp.

Webster, D., Meritt, D., 2011. Remote setting systems. Oyster aquaculture technology series. Oyster aquaculture technology series AGNR-AO-11-05. University of Maryland Extension, Queenstown, MD, p. 18.

Wilberg, M.J., Livings, M.E., Barkman, J.S., Morris, B.T., Robinson, J.J., 2011. Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. Marine Ecology Progress Series 436 (2011), 131–144.

Wood-Pawcatuck Watershed Association, 2016. Chlorophyll Data. Wincheck Pond, Hope Valley, RI. 2 pp. http://www.wpwa.org/waterdata/Chlorophyll/Chl_4040.PDF. [Accessed by November 10, 2018].