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RESTOCKING NATURAL BEDS WITH REMOTE-SET DISEASE-RESISTANT OYSTERS IN CONNECTICUT: A FIELD TRIAL

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ABSTRACT Oyster production in Connecticut historically has had large fluctuations due to two major factors: disease epizootics caused by a protozoan parasite multinucleate sphere X (*Haplosporidium nelsoni*), and long periods without successful natural sets. A field trial was performed to investigate whether the remote set of oysters can be used to restock natural beds during mortalities caused by disease epizootics and lack of recruitment due to periods without sets. Disease-resistant oysters were remote set in a hatchery and placed either in or without biodegradable nets on a natural bed. Aged, clean cultch was used as the control, and the growth, mortality, predation, fouling, and prevalence of disease were monitored for two growing seasons. The oysters grew to market size (3 inches, 76.2 mm) in 15 mo. Predation, due mainly to Atlantic oyster drills *Urosalpinx cinerea*, and overgrowth of oysters caused a mortality of 72% in the oyster seed during the first growing season. More abundant wild oysters set but fewer fouling organisms landed on the remote-set shells than on the clean cultch. To evaluate the effect of siltation on mortality, the oysters were left on the natural bed instead of being transplanted to growing areas, as is standardly done in commercial shellfishing operations. During the second growing period, siltation caused additional mortality until only 1.5% of the oysters deployed were alive. The method of restocking natural beds in Connecticut with remote-set oyster seed for use by the oyster industry looks promising due to the fast growth of the oyster seed. This experiment also demonstrates the challenges presented by nonharvest oyster restoration activities due to significant siltation-associated mortality.

KEY WORDS: oysters, *Crassostrea virginica*, restocking, remote-set, restoration

INTRODUCTION

The oyster industry has significant historic and economic value to Connecticut (MacKenzie 1996). The State currently has approximately 16,000 acres of natural beds and 70,000 acres of privately leased, franchised or town-owned growing areas. Historically, oyster *Crassostrea virginica* (Gmelin, 1791) production in Connecticut has had large fluctuations due to two major biological factors: disease epizootics caused by a protozoan parasite MSX, *Haplosporidium nelsoni* (Sunila et al. 1999), and long periods without successful natural oyster sets for the use of the shellfishing industry (Loosanoff 1966).

Oyster bottom aquaculture in Connecticut is based on leased grounds to which seed oysters from natural beds are transplanted. These natural beds are designated for the sole purpose of aquaculture. To catch oyster set, cultch (old oyster and clamshell) is broadcast on natural beds at the beginning of each summer. The cultch is turned over to remove the silt and to enhance recruitment, and new dock-dried shell is added yearly. Using small hand dredges from boats, seed oystermen harvest the small oysters from the natural beds, and the product is then transplanted to individually leased growing areas (Getchis et al. 2006). On average the oyster set peak occurs around July 20 each year, albeit another, rarer, peak may occur later in fall (Loosanoff 1966). To meet public health criteria set by the U.S. Food and Drug Administration/Interstate Shellfish Sanitation Conference, National Shellfish Sanitation Program, growing areas and market oysters must be tested and must be below a certain fecal coliform level. Consequently, oysters are transplanted from natural beds, often located in prohibited or restricted relay areas, to approved growing areas for a prescribed period prior to harvest (FDA 2011).

Oyster sets in Long Island Sound are irregular (Loosanoff 1966), but when a successful natural set lands on seed beds in Connecticut, it sustains the oyster industry for several years. Unlike more southern estuaries such as Chesapeake Bay, where oyster seed sets yearly (Tarnowski 2007), several years may pass without sets in Long Island Sound. A prolonged period of no sets and low standing stocks lasting for two decades from 1947 to 1967 resulted in oyster industry of Connecticut being qualified for resource disaster funds in 1967 (USFWS 1969).

A major outbreak of *Haplosporidium nelsoni*, or multinucleate sphere X (MSX), occurred in Connecticut in 1997 to 1998 causing high mortalities of oysters, a 76% decrease in market harvest, and significant economic losses for the shellfishing industry (Sunila et al. 1999a). A previous major MSX-associated mortality event occurred in the mid-1980s (Sunila & Visel 2015). Epizootic prevalence of MSX in oysters in Connecticut were reported for the first time in 1960 (Haskin & Andrews 1988), and cyclic high MSX prevalence and related mortalities have occurred ever since.

Attempts to demonstrate direct transmission of MSX have been unsuccessful; consequently it is speculated that another host may exist, acting either as a reservoir for infective stages or as an intermediate host for transmission (Burreson & Ford 2004). The uncertainty in the transmission method, the fast progression of the disease, and high mortalities of oysters make the disease difficult to effectively manage. Although the natural-set-based oyster industry is relatively defenseless against periodic mortalities caused by MSX epizootics and the lack of seed during periods of poor sets, hatchery-raised MSX-resistant

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oyster seed can provide an annual supply of seed and ensure better survival of oysters during disease outbreaks.

Hatchery-raised oysters also suffered significant mortalities during the 1997 to 1998 epizootics in Connecticut. High prevalences of *Haplosporidium nelsoni* and mortalities of hatchery-raised oysters in bottom cultures in New Haven Harbor (Sunila et al. 1999) as well as in an upweller system in Clinton (Sunila et al. 2000) were reported. At the time, disease-resistant oysters were not commonly in use by aquaculturists in Connecticut. Consequently, after the 1997 outbreak, Bureau of Aquaculture, Department of Agriculture of the State of Connecticut started a selective breeding program in collaboration with oyster industry partners to create an MSX-resistant, fast-growing oyster strain (Sunila et al. 1999b). In Connecticut, *H. nelsoni* occurs as a coinfection with another haplosporidian parasite, *Haplosporidium costale* or seaside organism (Sunila et al. 2002), which, together with environmental conditions, creates a unique selection pressure for Long Island Sound oysters. The oyster strain, "Clinton," was tested in common-garden experiments at several sites in southern New England and performed as well as, or better than, NEH, a patented strain considered an industry standard for disease resistance (Rawson et al. 2010).

Massive efforts to restore historical oyster reefs during the past two decades were attempted in Maryland and Virginia in the Chesapeake Bay (Kennedy et al. 2011) to maintain the fishery and/or to provide ecosystem services. Many of these ventures were based on the assumption that restored oyster reefs become self-sustaining, spontaneously growing units, a goal which basic patterns in oyster recruitment and mortality render unrealistic (Mann & Powell 2007). Other published reports concerning eastern oyster restoration include evaluation of oyster reef restoration efforts and success in Texas, Louisiana, Mississippi, Alabama, and Florida in the northern Gulf of Mexico (La Peyre et al. 2014) and in Florida in the southern Gulf of Mexico (Volety et al. 2014). Natural sets in these more southern estuaries area yearly event are more frequent and abundant than in Long Island Sound, where oyster sets are irregular. Consequently, the use of hatchery-raised seed provides an alternative for restocking seed beds in Connecticut.

The objective of this experiment was to test whether natural oyster beds in Connecticut could be restocked using disease-resistant remote-set oyster seed, thereby supplying oyster industry of Connecticut with seed during a potential future MSX-epizootic and/or absence of natural sets. The oyster seed was monitored for two growing seasons for survival, growth, predation, fouling, and disease.

MATERIALS AND METHODS

Experimental Site

A private town shellfish lease in Westport, CT, near the mouth of the Saugatuck River was used as the experimental natural bed (Lease 366, 41′06.147° N, 73′22.210° W). Water depth at the site was about 2 m mean low water. The area is classified as conditionally restricted relay seasonally closed due to a mooring field in the vicinity. Prior to deployment of the remote-set oysters and cultch, the selected area on the sea bottom was cleared of all biota using a commercial oyster dredge (1 m wide, 4 cm mesh, 4 cm teeth). Organisms removed by the dredge were brought onto the deck and identified to provide a baseline assessment of benthic epifauna inhabiting the area. Fouling organisms and oyster predators were listed, and all dredged organisms were relocated to a nearby private shellfish lease. The dredged site was then divided into three 9-m long rows with approximately 3 m between rows. The plot and the rows were marked with cinderblocks, bamboo poles, and a flagged buoy indicating the project code.

Hatchery Phase

Oysters from broodstock repository in Cedar Island Marina in Clinton, CT, were brought to the Noank Aquaculture Cooperative hatchery in Noank where they were conditioned and spawned. The oyster strain used in this study was "Clinton" (F5). Oysters were brushed clean and put in the broodstock tank in ambient temperature seawater, and conditioned for 6 wk, and spawned.

While the conditioning and larvae culture were proceeding, aged shell (82% oyster shell, 18% hard clam shell) of various sizes was prepared for cultch. The shell was placed in tote boxes and washed with fresh water, then set on a large tarp to dry in the sun for 2 days. Shell was then funneled into plastic mesh bags (44 bags total, 1.3 cm mesh, 25 cm diameter, cut to 1-m tubes from the roll and ends tied). The bagged shell was placed overnight in a 3.7×1.2-m tank filled with aerated, ambient temperature, filtered seawater in the hatchery and 4 million D-stage larvae were added over a period of 6 days to set.

After 4 wk in the hatchery, the bagged set-on-shell (SOS) was deployed into four coated wire mesh cages ($1.4 \times 0.9 \times 1.2$ m, 25.4 mm mesh size) in bay water. The cages were designed to keep the oysters elevated 15 cm off the bottom and to limit predation. The cages were lifted two to three times a week, and the bags were rinsed with seawater to wash away sediment buildup and predators. The bags were also flipped to assure even flow to all sides of the cultch. After about 6 wk in this setup, the numbers of oysters in the bags were estimated by counting the number of shells per bag from three bags (300, 298, and 307) and the number of set was then counted from 30 shells (10 shells from each bag). Then the bags were transported to the field site for deployment.

Deployment

The experiment comprised three groups: (1) SOS without nets, (2) a control of clean cultch, and (3) set-on-shell in biodegradable nets (SSBN) (Fig. 1A, B). Oysters were deployed on the experimental site for three consecutive days during low tide. The first day (June 12, 2012), SOS from 21 hatchery bags was deployed off the side of a small seed boat. The SOS was released from the plastic mesh bags (Fig. 1A) and spread along the designated row between bamboo stakes. The next day, an equal volume of clean cultch consisting of aged oyster shell originating from the Bureau of Aquaculture shell pile in Milford, CT, was scattered in the middle row. The third group, 23 bags of SOS, was repacked into biodegradable netting and was then lowered onto the last row of the plot (Fig. 1B).

Two types of mussel seeding socking nets (Mussock International Ltd., Christchurch, New Zealand) were chosen for the experiment: a strong cotton net (54 meshes around the circumference; Fig. 1B), and a 60/40 polyester-cotton blend. The nets were knotted on both ends to form 60-cm-long tubes.

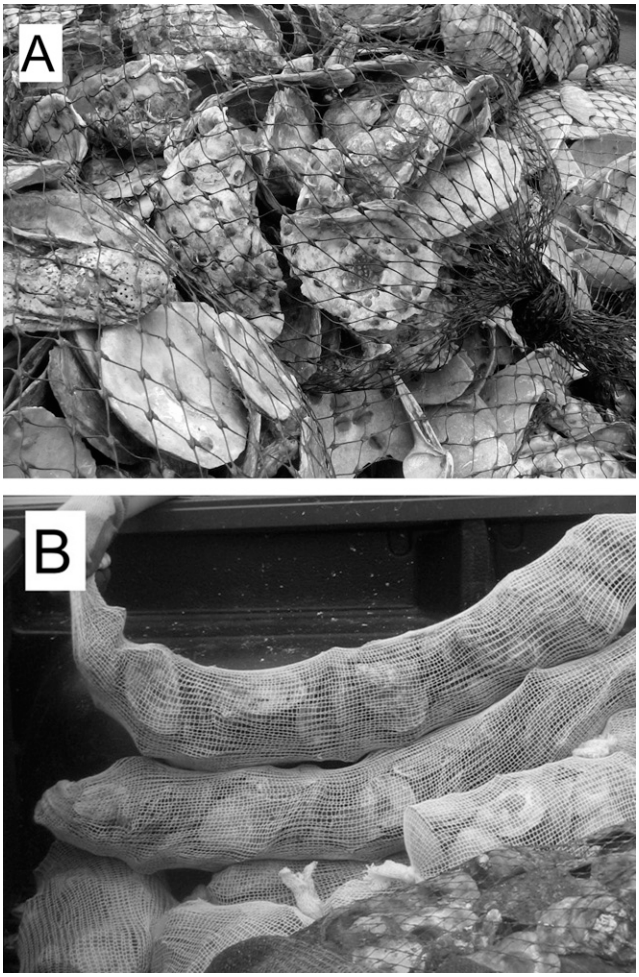

Figure 1. SOS. (A) A 4-mm oyster set (on the average 27 oysters/shell) ready for deployment. (B) SOS packed in mussel seeding socking.

Testing of Nets

For the experiment, samples of biodegradable nets from different companies were considered and samples were requested. Materials included jute (Outsidepride, Independence, OR), BioGrid, OxyGrid polypropylene (Conwed, Minneapolis, MD), coconut, straw, straw-coconut mix (East Coast Erosion Blankets, Bernville, PA), coir, coconut fiber (Eco Fabriks, LLC., Gaithersburg, MD), resin netting (Dzolv Products Ltd., O'Connor, Western Australia), and mussel socking. The mussel socking was selected for this experiment because it was the only material available strong enough for packing the oyster shell in and resistant to tearing by the sharp shell edges. Additionally, it was available in tube form and already used in aquaculture applications. Technical data about degradation times of the products in sea water were not available. Samples of the mussel socks were filled with oyster shell, enclosed in oyster seed bags within wire cages, and submerged to the bottom in Milford Harbor. The nets were checked weekly from June 20, 2012, until September 6, 2012, for degradation. Temperature and salinity at this site were recorded.

Two types of netting were deployed. The first type was 100% cotton net (the number of meshes around the circumference 54: the English cotton count of the yarn 2/20: lay flat, or tightness of the diameter of the socking in mm 330), and the other type was 60:40 cotton:polyester with the same circumference and mesh size as mentioned earlier.

Monitoring

Oysters were monitored monthly from June to November for two growing seasons for growth, mortality, and the presence of fouling organisms and predation. Temperature and salinity at the site bottom were recorded during each sampling event. At the end of the second growing season, samples were taken for pathology. Oysters were collected by snorkeling at low tide from July through November 2012 and June to November 2013. Approximately 30 shells were collected per sampling date from each row. For the two experimental groups, the 30 shells collected refer to the cultch on which the hatchery oysters set. The number of live oysters per shell was counted to document mortality (initial set was 27 oysters/shell). It was specifically noted if mortality was caused by predation (i.e., drill holes apparent) or siltation (dark, mucky accumulation on shells), when empty shells were available for observation. From each sample, 100 live oysters were then measured for maximum length to the nearest millimeters using calipers. This measure gives the distance between the umbo and posterior growing margin and is also referred to as shell height (Carriker 1996). The thirty cultch shells from the control group were examined for potential natural set, and the number and length of natural-set oysters per sample was recorded separately. Fouling organisms and predators found on the samples were also identified and recorded. After measurement, the oysters were replaced onto the site.

Hurricane Sandy hit the coast of Connecticut on October 29, 2012, and during the sampling on November 21, only 10 shells per sample were collected by oyster tongs. During the last sampling in November 2013, an oyster seed dredge was used to collect samples and examined as above. To compare parasite prevalence, thirty mature oysters from the oyster group with nets and another sample from the group with no nets were taken for pathology. A control sample of market size oysters (year class 2010) representing natural-set oysters was collected from a nearby shellfish lease (Westport 358), because the scarcity of natural set on the experimental cultch row did not allow for an adequate sample size (30) for pathology. The samples for pathology were fixed in Davidson's fixative, and a sample from the rectal–anal area was placed in Ray's fluid thioglycollate medium for detection of *Perkinsus marinus*. Samples were processed using standard histological methods by embedding tissues in paraffin, and 5-µm sections were stained with hematoxylin–eosin (Howard et al. 2004). Results were presented as prevalences (%) and the intensity of *P. marinus* as weighted prevalence (intensity of infection rated from 0 to 5 according to the number of enlarged hypnospores on the slide, and the sum divided by the number of animals in the sample, 30).

Using StatGraphics Plus software results were compared with analysis of variance for sizes in pathology samples and Mann–Whitney (Wilcoxon) test (W) for growth and mortality measurements.

RESULTS

Predators and Fouling

Species dredged from the bottom of the lease prior to deployment and species migrating to the lease during the

experiment are listed in Table 1. The lease had not been actively used, and consequently, the species prior to deployment represented natural recruitment to the site.

Predators were detected already during the first sampling, after the deployment of the seed, when *Panopeus herbstii* and *Urosalpinx cinerea* were observed in association with the oyster seed both with and without bags. Oyster drill egg cases were also noted on the oyster shell with seed. During the second sampling, in August 2012, the first oyster drill holes were observed in many of the seed oysters in biodegradable netting. Egg cases of *Eupleura caudata* were also observed but not as frequently as those of *U. cinerea*. During the next samplings and until the end of the first growing period, *U. cinerea* was observed predating on the oyster seed. New egg cases on the shells and new drill holes in the seeds were observed.

During the second growing season, June and July, samplings, oyster drills were constantly seen in association with the seed oysters and egg cases and drill holes were apparent. In August of the second growing season, several specimens of *Hemigrapsus sanguineus* were observed for the first time during this experiment. At the same time, large specimens of *Urosalpinx cinerea* were still trying to prey on the oysters, which at this time were already 72.69 and 76.46 mm, without nets and with nets, respectively. During the end of the second growing period, *Ilyanassa obsoleta* were seen in the samples with oysters. Green crabs *Carcinus maenas* were only observed during the last sampling in November 2013.

The most prevalent predator throughout the experiment was *Urosalpinx cinerea*. There were no markings on the shells consistent with crab predation at any time; however, drill holes were present in increasing numbers, and in September of the second growing period most of the oysters showed drill holes consistent with *U. cinerea*.

Figure 2 presents succession of biofouling organisms on cultch. Biofouling communities consisted of *Crepidula fornicata*, *Crepidula plana*, *Anomia simplex*, barnacles (which were not identified to species during samplings), *Schizoporella unicornis*, *Hydroides dianthus* (Serpulidae), *Anadara ovalis*, and *Microciona prolifera*.

The first species to set was *Crepidula fornicata*, which were present on cultch at high numbers during the first sampling. Some *Crepidula plana* and a light barnacle set were also detected. During August sampling, *C. plana* dominated, and *Anomia simplex* were also present. In September, *Schizoporella unicornis* were observed, and in October, *Hydroides dianthus* were also observed.

During the second growing season, *Anadara ovalis* and *Microciona prolifera* joined the species listed above colonizing on cultch. The control cultch was almost completely covered by fouling organisms by September of the first season.

Shell with oyster set on, regardless of whether it had been in biodegradable netting or without netting, received many fewer fouling organisms than the cultch. All the species listed above eventually set on shell with oyster seed, but later than on the

TABLE 1.

List of species dredged from the experimental site prior to deployment of remote-set oysters and species observed during the experiment.

	Prior to deployment	During the experiment
Phylum	**Species**	**Species**
Mollusca	Eastern oyster, *Crassostrea virginica*	Eastern oyster, *Crassostrea virginica*
	Northern quahog, *Mercenaria mercenaria*	–
	Blue mussel, *Mytilus edulis*	–
	Ribbed mussel, *Geukensia demissa*	–
	Atlantic jackknife clam, *Ensis directus*	–
	Blood ark, *Anadara ovalis*	Blood ark, *Anadara ovalis*
	Common jingle, *Anomia simplex*	Common jingle, *Anomia simplex*
	Common Atlantic slippersnail, *Crepidula fornicata*	Common Atlantic slippersnail, *Crepidula fornicata*
	Eastern white slippersnail, *Crepidula plana*	Eastern white slippersnail, *Crepidula plana*
	Northern moon snail, *Euspira heros*	–
	Atlantic oyster drill, *Urosalpinx cinerea*	Atlantic oyster drill, *Urosalpinx cinerea*
	–	Thick-lip drills, *Eupleura caudata*
	–	Mud dog whelk, *Ilyanassa obsoleta*
Arthropoda	Northern rock barnacle, *Semibalanus balanoides*	Barnacles
	Ivory barnacles, *Balanus eburneus*	Barnacles
	Atlantic mud crab, *Panopeus herbstii*	Atlantic mud crab, *Panopeus herbstii*
	Asian shore crab, *Hemigrapsus sanguineus*	Asian shore crab, *Hemigrapsus sanguineus*
	Common spider crab, *Libinia emarginata*	–
	Blue crab, *Callinectes sapidus*	–
	Horseshoe crab, *Limulus polyphemus*	–
	–	Green crab, *Carcinus maenas*
Bryozoa	–	Single horn bryozoan, *Schizoporella unicornis*
Echinodermata	Forbes sea star, *Asterias forbesi*	–
Annelida	Clam worm, *Alitta virens*	–
	–	Limy tube worms, *Hydroides dianthus*
Porifera	–	Red beard sponge, *Microciona prolifera*
Chordata	Lined seahorse, *Hippocampus erectus*	–
	Striped searobin, *Prionotus evolans*	–

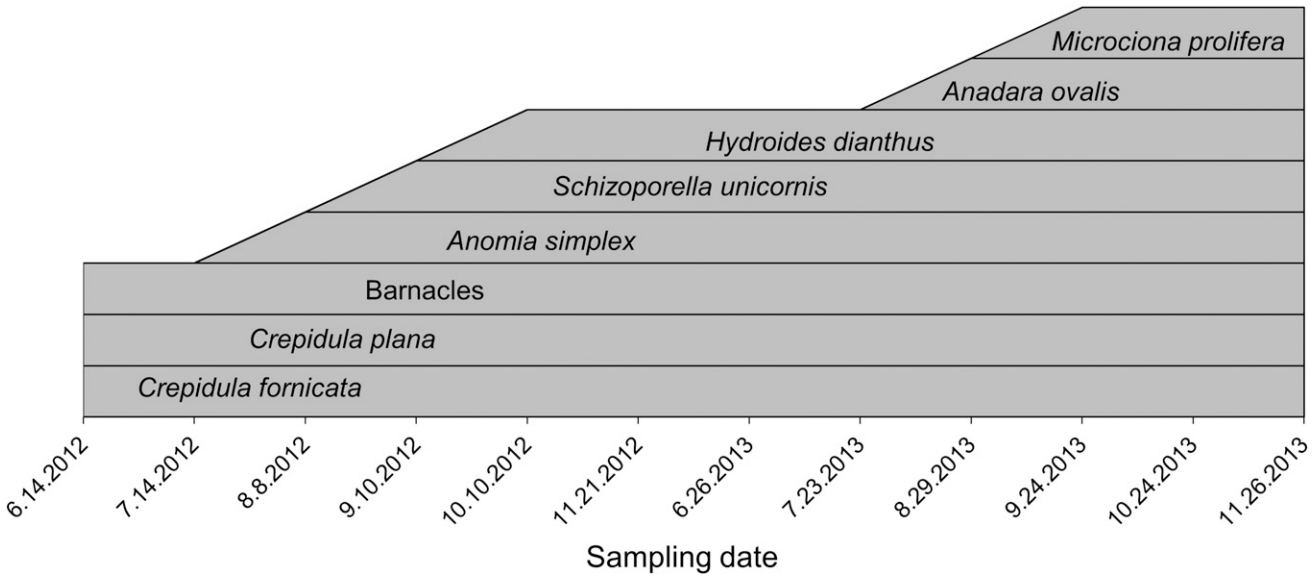

Figure 2. Succession of fouling organisms on cultch deployed to experimental natural bed and observed for two growing seasons.

cultch. During the first sampling, very light *Crepidula fornicata* set was detected, but *Crepidula plana* and barnacles were not present until during August sampling. Limy tube worms *Hydroides dianthus* were not present until in November of the second growing season, a year later than on the cultch, and *Anadara ovalis* were detected a month later on shells with oysters than on the cultch. Shells occupied with the oyster seed remained almost free of fouling organisms, and only limited fouling was detected between the seed oysters.

Growth

The growth of the oysters during the experiment is presented in Figure 3. The temperature and salinity data is presented in Table 2.

The oyster seed was 3.77 mm (SD = 1.84, range = 1–8 mm) at the time of the deployment, and at the end of the experiment, the length of the oysters without bags was 90.46 mm (SD = 13.12, range = 69–142 mm) and with biodegradable netting was 90.27 mm (SD = 12.27, range = 65–127 mm) with no statistical difference between the groups (W = 3,576.5, P = 0.877, NS).

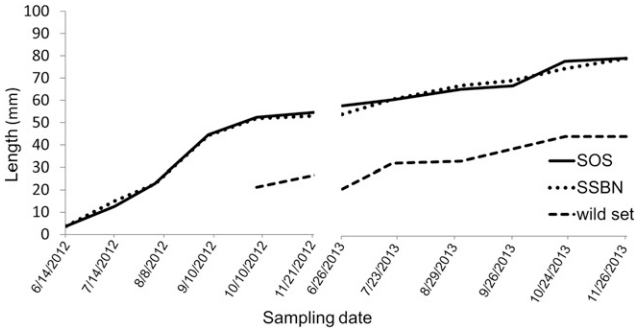

Figure 3. Growth of remote-set oyster seed *Crassostrea virginica* on an experimental natural bed during two growing seasons. SOS = set-on-shell, SSBN = set-on-shell in biodegradable netting, wild set refers to set on control cultch.

The sizes during individual sampling times were not normally distributed with skewness and kurtosis because of smaller-size specimens dominating with a few very large individuals. The average monthly growth rate was 5.09 mm.

During the first season, the growth rate was very fast until the middle of October, after which it slowed down. There was not much growth during winter and spring months between November and June samplings. The majority of the oysters (67%) reached the 3-inch market size (76.2 mm) in September of their second growing season, 15 mo after the deployment.

The first time new natural set was observed on the cultch was in September of the first growing season (0.03/shell), and more set was observed during the October (0.17/shell) and November (0.2/shell) samplings. Natural set also landed on the oyster shells with hatchery-raised seed during the experiment, which was reflected as a decrease in growth and an increase in survival in graphical data. From October onward, the sizes of natural set on cultch were measured, and the same size classes from the

TABLE 2.

Temperatures and salinities at the bottom of the experimental natural oyster bed on shoreline of Connecticut during the 2-y experiment.

Date	Temperature (°C)	Salinity
06.12.2012	20.3	25.5
07.14.2012	24.4	26.7
08.08.2012	24.4	25.8
09.10.2012	24.1	26.4
10.10.2012	18.0	27.2
11.21.2012	9.5	27.0
06.26.2013	19.2	25.9
07.23.2013	24.0	25.5
08.29.2013	24.0	26.5
09.26.2013	19.4	27.5
10.25.2013	14.0	27.4
11.28.2013	5.5	28.3

shell with seed were omitted from the growth and mortality data. Histograms demonstrate the presence of a bimodal distribution of the growth data where the wild set comprised a second minor peak (Fig. 4).

Mortality

The average number of oyster set/shell prior to deployment was 27 (SD = 14.34, minimum = 10, maximum = 71). As the total number of bags in the experiment was 44 and the average number of shells/bag was 301.67, the total number of shells in the experiment was 13,273.48. The total number of oyster seed in this experiment was 358,384 and the setting efficiency was 8.96% (358,383 oysters set from 4 million eyed larvae), Figure 1A.

Mortality of the oysters is depicted in Figure 5. The beginning value of the seed on each oyster shell (27.17) is expressed as 100% and the monthly decrease thereafter is expressed as declining percentage. New set was omitted from the graph by counting the number of new set from the growth data and deducting their number from the total counts.

Because of predation by *Urosalpinx Cinerea*, there was high mortality during the first 2 mo. The biodegradable netting protected the seed-on-shell during the first month, and survival in July was significantly higher ($W = 714.5$, $P < 0.0001$) in oysters in the netting (24.37, SD = 12.83) in comparison with oysters without nets (13.50, SD = 10.08). This benefit was lost when the bags started to disintegrate and oyster drill predation decreased the number of surviving seed oysters to the same level as in oysters without netting (with netting 9.16, SD = 3.51, without netting 11.27, SD = 6.35; $W = 222.5$; $P = 0.365$, NS). Overgrowth caused by the high number of oyster seed on each shell also contributed to the mortality.

After the August sampling of the first growing season, mortality suddenly decreased, and the number of surviving oysters remained relatively stable until winter. The size of the oysters was about 23 mm at the cessation of the oyster drill predation (SOS mean 22.83, SD = 6.53, SSBN 22.65, SD = 6.76).

On October 29, 2012, hurricane Sandy hit the coastline of Connecticut. During the next sampling, more sedimentation was observed at the sampling site; however, this was not reflected in increased mortality during November sampling.

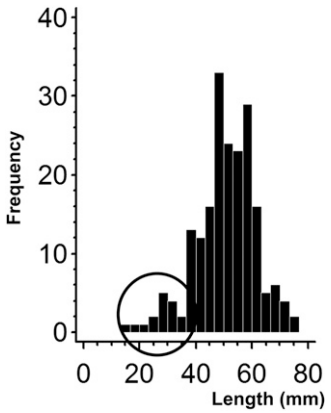

Figure 4. Histogram of the size distribution of oysters in the end of the first growing season (October 2012). Wild set comprise an additional peak circled on the left.

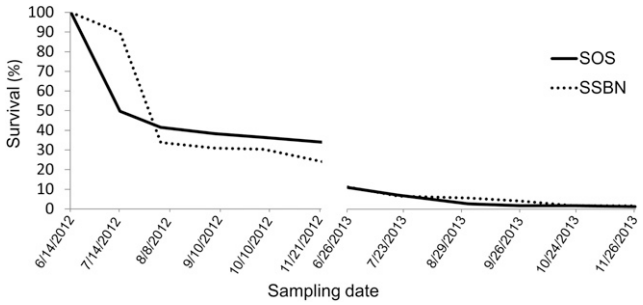

Figure 5. Mortality of remote-set oyster seed *Crassostrea virginica* on an experimental natural bed during two growing seasons. SOS = set-on-shell, SSBN = set-on-shell in biodegradable netting.

During the second growing season, mortality steadily continued due to siltation at the lease, characterized by dead oysters covered by black mucky accumulation. At the end of the experiment, only 7,168 individuals, less than 2%, of the oysters planted on the lease were alive with no significant difference between oysters grown in biodegradable netting and oysters without nets ($W = 127.5$; $P = 0.74$, NS).

The set on cultch was scarce and only a few individuals were observed among the heavy fouling, but more wild oysters set on shells with the remote-set seed. The average number of wild set landing on the shells ($n = 30$ shells) during the experiment was 5.58 (SD = 7.09) for oyster shell in biodegradable netting, 4.36 (SD = 6.58) for shell without nets, and 2.0 (SD = 1.66) for cultch (Spearman's rank correlation $P = 0.006$).

Testing of Nets

Degradation of the nets was tested in the beginning of the first growing season by filling the netting with shell and placing these units into seed bags. The seed bags were put in metal cages (80 × 55 × 8 cm), suspended to the bottom and monitored frequently. After only 12 days, the cotton netting was showing signs of degradation. After 4 wk, the cotton netting was quite degraded and holes were present. The 60:40 netting appeared much thinner. The cotton netting was completely degraded after 6 wk in seawater; simultaneously, the 60:40 net appeared very thin, but maintained its integrity. After 2 mo, the 60:40 netting showed large holes and was approximately 50% degraded. Observations of the netting degradation are summarized in Table 3.

Pathology

In the final sampling, size of the oysters in the group with nets was 95.47 mm (SD = 13.55), without nets 96.53 mm (SD = 11.49) and in the sample from the control lease was 88.77 mm (SD = 9.32); the sample from the control lot being significantly smaller than the two other groups ($F = 3.96$, $P = 0.02$). The majority of the shucked oyster meats in each group were fat with from one to three watery specimens in each group (Fig. 6). There was *Polydora websteri* infestation inside the shells in each group: 77% prevalence in the oysters with nets, 93% with no nets, and 27% in oysters from the control lot. There was heavy biofouling with *Crepidula fornicata* and *Crepidula plana* in each group: prevalence was 70% in the group with nets, 90% with no nets, and 50% in the oysters from the control lot. Of the oysters

TABLE 3.

Degradation of mussel socking in seawater.

Date	Temperature (°C)	Salinity	Condition of nets	
			Cotton	60:40
06.20.2012	21.3	26.0	Good	Good
07.12.2012	27.0	25.0	Degrading, holes	No holes
07.19.2012	23.4	23.4	Very degraded, holes	Thinning, no holes
07.26.2012	23.0	26.6	Almost completely degraded	Thinning, no holes
08.01.2012	24.5	26.0	Completely degraded	Very thin, no holes
08.09.2012	26.6	26.6	Completely degraded	Very thin, large holes
08.16.2012	24.4	24.5	Completely degraded	50% degraded, large holes
09.06.2012	24.3	27.6	Completely degraded	75% degraded, large holes

Oyster shells were enclosed in two types of socking, placed in seed bags, and descended in metal cages to the bottom and visually evaluated once a week.

from the control lot, 20% had biofouling by *Anomia simplex*, 3% on the oysters from the group with no nets and none in the group with nets. Further, 7% of the oysters with nets or with no nets had red-beard sponge, *Microciona prolifera*, on the shells but none on the oysters from the control lot. There were pea crabs (*Pinnotheres ostreum*) in 10% of the oysters with nets, in 20% in oysters with no nets, and in 3% in oysters from the control lot. Prevalences of fouling organisms did not significantly vary between the groups (SSBN versus SOS: $W = 16$, $P = 0.53$; SSBN versus control: $W = 9.5$, $P = 0.06$, NS; and SOS versus control: $W = 9.5$, $P = 0.34$, NS).

All three oyster samples had a high-prevalence, low-intensity infection with *Perkinsus marinus*. Prevalence was 77% and weighted prevalence 0.9 in the oyster sample with biodegradable net, 87% and 0.8 in the sample without nets, and 73% and 0.8 in the oyster sample from the control lot with no significant differences between the groups ($H = 0.88$; $P > 0.05$, NS, respectively). In 10% of the oysters with nets and in 3% of the oysters with no nets and oysters of the control lot, *Haplosporidium nelsoni* infection was present.

There were prokaryotic inclusions (*Rickettsia*-like organisms) in 7% of the oysters with nets, 3% with no nets, and none in the oysters from the control lot. Turbellaria were detected in the intestines of 3% of the oysters with nets, in 7% of oysters with no nets, and in none in oysters from the control lot. Ciliates (Ancistrocomidae) were found on the gills, stomachs, and intestines in all groups at low prevalences. All oysters processed for histology presented resting, indeterminate gonads.

DISCUSSION

This study described the deployment of remote-set oyster seed on a natural bed in Connecticut with 2-y monitoring for survival, growth, fouling, predation, and disease. The experiment was performed at only one site, whereas the 100-mile shoreline of Connecticut offers several different ecosystems with variable sediment types, depths, and plant and animal communities. Major oyster industry in Connecticut is located in the western end, where hard bottom provides natural oyster habitat, and the location of the experimental lease can be considered as ''typical'' for the industry as it is traditionally practiced. Also, estuarine animal communities vary between years, and different results may be achieved during different years of study. The events and progression of the variables in the study offer a ''snap shot'' of what was happening on this particular site during this particular period.

Many projects use triploids as remote-set eastern oyster seed (Congrove et al. 2009, Murray & Hudson 2011) for better growth and survival. The rationale for using a diploid, local, disease-resistant oyster strain in the present project was based on the fast growth of the strain and its ability to spawn and proliferate in this region. Diploid oysters, unlike triploids, are fertile. Most of the commercial oyster aquaculture in Connecticut is based on wild set. Private beds (leases and grants) produce 60% of the seed and public, natural beds between 30% and 40% of the available seed. Only 10% of the seed originates from hatcheries (Getchis et al. 2006). Spawning time in oysters has a strong genetic component. In general, the further south the oysters are collected from the earlier the gametes will mature (Thompson et al. 1996). Barber et al. (1991) compared native and inbred oysters from Delaware Bay and Long Island Sound oyster stocks in Delaware Bay to study whether differences in the timing of reproduction in different oyster stocks are genetic or adaptive. The Long Island Sound inbred strain maintained the difference in the timing of gonadal development even after six generations (23 y) in Delaware Bay. Consequently, the use of a local, disease-resistant, diploid strain produces seed not only for the potential end user of the remote set, but also serves the wild-set-based oyster industry by releasing oyster larvae as potential spat fall on natural seed beds.

The oysters grew fast during the experiment reaching an average of 90.4 mm by the end of the experiment and market size of 3 inches (76.2 mm) only 15 mo after the deployment of 4-mm seed (Fig. 3). For commercial operations using wild set, it takes 3–4 y for oysters in Connecticut to reach market size. The Clinton strain was selected for fast growth and, according to this experiment, the production cycle was reduced 2- to 3-fold by using a fast-growing hatchery strain in comparison with using wild set. Dégremont et al. (2012) compared the growth of triploid and diploid eastern oyster strains at different sites in the Virginia part of the Chesapeake Bay for two growing seasons. On average, the triploid strains grew 66.5 mm from the initial shell height of 27.5 mm during the 23-mo experiment, and the

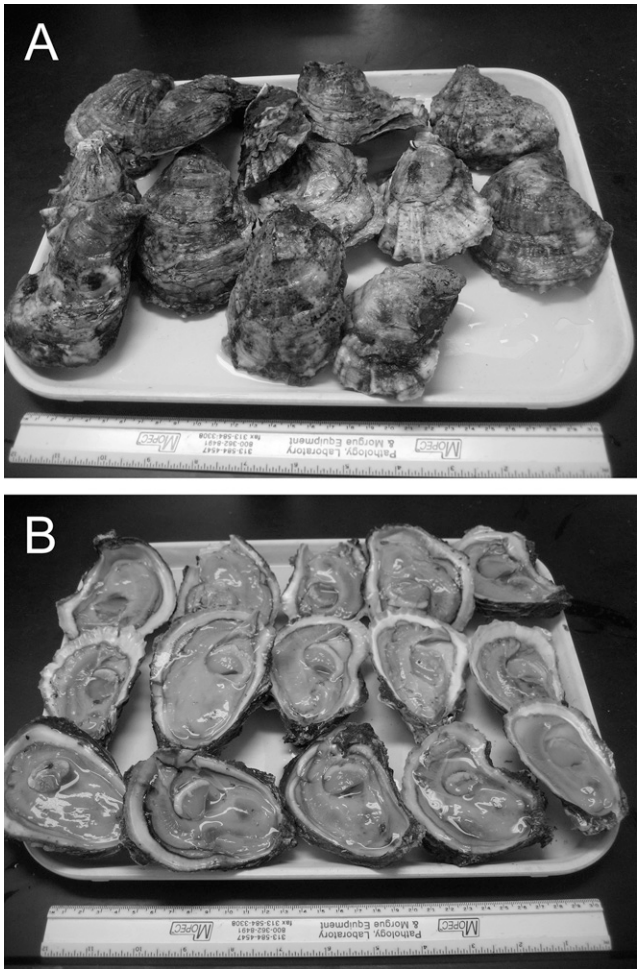

Figure 6. Remote-set oysters *Crassostrea virginica* after two growing seasons on a natural bed. (A) Whole oysters with left valve up. (B) Shucked oysters with right valve removed.

diploid strains grew 53.5 mm from the initial shell length of 21.5 mm. Interestingly, the oysters in the present study grew 86.6 mm from the initial length of 4 mm during the 17-mo experiment, thereby outgrowing the triploid oysters in the Chesapeake Bay study by 23.2% and the diploid strains by 38.2% regardless of the shorter time in the field. In another study, Harding (2007) compared the growth of a disease-resistant diploid strain DEBY and triploid oysters at a York River site in the Virginia area of the Chesapeake Bay. Total growth of the triploids during the 16.4-mo experiment was 32.6 mm and of the diploid DEBY oysters was 36.9 mm. The period of the grow-out in the field was comparable to the present study, and the Clinton oysters outgrew the triploids by 62.4% and the DEBYs by 63.1%.

Oyster growth depends on the culture gear; the growth on bottom being the slowest and rope cultures being the fastest (Mallet et al. 2013, Walton et al. 2013). In the present experiment, oysters were deployed directly on the bottom to simulate the standard culture practice in Connecticut. The different growth rates in different gears must, however, be kept in mind when comparing published growth rates of oysters. It should also be noted that the growth was not linear (Fig. 3), but was very fast in the late summer, early fall of the first growing period and then slowed down. Kraeuter et al. (2007) reviewed growth rates of eastern oysters from different locations. The growth rates were also expressed as mm/mo, which in the present experiment was 5.09 mm/mo. Interestingly, when comparing the present results with published results based on bottom culture starting from seed and two growing seasons from data by Kraeuter et al. (2007), similar fast growth rates were noted in southern locations such as Louisiana (4.94 mm/mo), Galveston Bay, TX (4.83 mm/mo), Pensacola, FL (3.96 mm/mo), and Charleston Area, SC (3.96 mm/mo). Publications reporting growth of oysters in Long Island Sound are rare. Matthiessen and Davis (1992) compared the growth rates of chemically induced (cytochalasin B) triploid oysters with diploids from the same parent stock for two growing seasons deployed in pearl nets hanging from a long line in Ocean Pond, Fishers Island, and Fishers Island Sound in Long Island Sound. The triploid oyster grew better in both locations; the final shell height of the oysters being 65.8 mm (3.86 mm/mo) for the triploids and 57.4 mm (3.34 mm/mo) for the diploids in Ocean Pond, and 95.4 mm (5.71 mm/mo) for the triploids and 72.0 mm (4.25 mm/mo) for the diploids in Fishers Island Sound.

The following factors contributed to the mortality of the oysters (Fig. 5): predation by Atlantic oyster drills, overgrowth by oysters, and siltation. Oyster drills caused an initial decrease of about 60%–70% in the oyster seed that is discussed in more detail in the paragraphs below. The setting of an average of 27 oysters/shell was too high and caused overgrowth and mortality, the extent of which could not be measured. The number of set/shell can be regulated by alternating the number of oyster larvae deployed in relation to the amount of cultch used. Details about optimizing the proportions of larvae and cultch are discussed in the manuals by Bohn et al. (1995), who recommend final setting between 5 and 25 eyed larvae/shell and Congrove et al. (2009), who advise adding 100 eyed larvae to the tanks per each shell. Setting success rates vary greatly, and the excess setting density in the present paper was based on the attempt to produce enough oyster set for the measurement requirements of this experiment rather than trying to optimize the proportions for commercial use.

Siltation caused significant mortality of the oysters during the second growing season (Fig. 5). During standard culture practices in Connecticut, oysters are transplanted from natural beds to growing areas until market size. The experimental lease was classified as conditionally restricted relay, and during a commercial operation the oysters would be transplanted to growing areas between November 1 and November 15. Natural beds are closer to the mouths of the rivers and the accompanying siltation. Growing areas are on harder bottom and closer to the open waters of the Sound. A decision was made to leave the oysters on the natural bed for the period of two growing seasons to determine the effects of siltation on mortality.

Many oyster restoration projects and proposals are based on nonharvest and consequently nontransplant oyster beds, which leaves the oysters exposed to siltation for an extended period. There are limited data on the effects of siltation on oysters, and opinions range from the claim that oysters feed only in clear waters to that of oysters being unaffected by highly turbid waters. Increased concentrations of suspended materials can induce a reduction in pumping rate, a clogging

of the gill apparatus, a subsequent reduction in growth rate, and death. Some of the data are based on experiments measuring filtration rates, shell movements and the formation of pseudofeces, or measuring different physiological parameters in oysters deployed in cages next to dredging sites (Shumway 1996). There are no mortality data prior to the present work about the destiny of oysters placed in a natural seed bed exposed to continuous siltation from a river, a common characteristic of all natural beds in Connecticut. The practice of transplantation has been refined by decades of experience by the oyster industry to produce high yield and low mortality. Survival of the oysters on the date of potential transplantation to the growing areas in November, after the oyster drill and overgrowth caused mortalities, was 33.6% and 22.96% in oysters without bags and in oysters in biodegradable netting, respectively, and in the end of the second growing season, the survival was 1.3% and 1.74% in oysters without bags and in oysters in biodegradable netting, respectively. The significant siltation-associated mortality makes a strong argument against nonharvest oyster restoration activities in Connecticut.

The organisms that harm the oysters can be divided into three classes: (1) predators, which feed on the oyster; (2) fouling organisms or competitors, which occupy the same ecological niche as the oyster and can cause harm indirectly by competing for the available food and living space; and (3) parasites, which live within the tissues (Arakawa 1990).

Several species of known oyster predators (Table 1) such as *Urosalpinx cinerea*, *Panopeus herbstii*, *Euspira heros*, *Callinectes sapidus*, *Hemigrapsus sanguineus*, and *Asterias forbesi* were present at the experimental site prior to the experiment starting and were removed by dredging before the oyster seed was deployed. Aforetime the first sampling, Atlantic oyster drills and Atlantic mud crabs had already found their way to the oyster seed. During the later samplings, *Eupleura caudata* (only egg cases observed), Asian shore crabs, *Ilyanassa obsoleta*, and *Carcinus maenas* were observed. Conversely, the moon snails, blue crabs, or sea stars never returned to the site. In the case of the sea stars, this was not unexpected, because starfish populations fluctuate with years of great abundance usually followed by relative scarcity (Galtsoff 1964).

The most important predator in this experiment, based on the observations of adult specimens, egg cases, and characteristic drill holes in dead oyster seed, was the Atlantic oyster drill. Oyster drill predation caused a 60%–70% mortality of the oyster seed during the first growing season (Fig. 5), which suddenly ended in August when the oysters reached a size of 23 mm (Fig. 3). This is in accordance with Galtsoff (1964) who reported that there are many locations in Long Island Sound, and in other regions, where drills commonly kill 60%–70% of the seed oysters. According to MacKenzie (1981), oyster drills (*Urosalpinx cinerea* and *Eupleura caudata*) cause an estimated 33% loss of seed oysters during their first summer in Long Island Sound.

Although mortality due to oyster drill predation decreased in August of the first growing season, drills were present throughout the experiment. The drills were attached to the shells and even attempting to bore the shells of adult oysters that reached the average size of 86 mm in the end of the second growing season, however without much success as evidenced by the dynamics of the mortality graph in Figure 5. Interestingly, Lord and Whitlatch (2012) demonstrated that inducible defenses of the eastern oyster allows them to produce thicker shells in response to the threat of predation by *Urosalpinx cinerea*. The cue involved is likely chemical, not tactile, because the oyster drills and oysters were kept spatially segregated within the containers during the 2-mo experiment.

Although predatory crabs were frequently observed in the present study in association with the oyster seed, no characteristic predation marks of crabs (chipped or crushed shells) were observed at any sampling. Asian shore crab predation on oysters depends on the size of the seed; seed under 10 mm becoming more likely prey (Brousseau et al. 2001). Also, other decapod crabs prefer smaller size bivalve seed regardless of the higher dietary value in larger prey (Juanes 1992). This is due to the mechanical cost of predation; decapod crustacean predators are constrained from maximizing net energy intake rates when feeding on hard-shelled bivalve prey because of the probability of incurring damage as prey strength (and size) increases. Seed-on-shell is harder for crabs to manipulate than cultchless seed, and may provide further disadvantage in energy budget as described by Juanes (1992). Crabs (*Callinectes sapidus* and *Panopeus herbstii*) chemically induce changes in the shells of eastern oysters, which make the shells more difficult to crush. Oysters reared in the presence of blue and mud crabs were less susceptible to predation than those maintained in no-predator controls in feeding assays (Robinson et al. 2014). Either, or both, mechanisms described above may have helped the oyster seed escape significant crab predation during this experiment.

The netting provided a significant benefit for survival ($W = 714.5$, $P < 0.0001$) during the first sampling (Fig. 5), but the benefit was lost along with the degradation of the nets. The biodegradable mussel socking is available in different proportions of cotton and polyester; however, degradation rates of the materials in seawater were not available prior to deployment in this experiment. The cotton net was preferred because of its well-established biodegradability; however, the cotton–polyester netting also completely degraded. Stronger cotton–polyester nettings were not used for fear of leaving potentially nonbiodegradable material on the experimental site to tangle oyster dredges during commercial harvests. Most of the information about biodegradation of fabrics originates from land-based experiments and concern degradation rates in anaerobic landfills (Li et al. 2010). Cotton, as well as polyester, is degraded with microbial action to CO_2 and H_2O; the efficiency and rate depending on pH, temperature, moisture, and oxygen content of the environment (Tokiwa et al. 2009). Dionne et al. (2006) tested a cotton:polyester netting (50:50) for another aquaculture application to deter diving ducks from mussel lines. Their experiment did not last long enough to produce a definite degradation time for the netting and they concluded that a new blend of cotton and polyester also needs to be tested to ensure that the protective layer biodegrades on an appropriate time scale, a conclusion that can also be drawn based on the present experiment.

Fouling organisms and the succession with which they settled on cultch is presented in Figure 2. Species originating from five different Phyla consisted of slipper shells, blood arks, jingle shells, red beard sponge, barnacles, bryozoan, and tube-building worms. The fouling community was constantly changing as new species settled and older ones were preyed upon. According to Galtsoff (1964), oyster drills show preference to

barnacles and "usually stop drilling oysters if a rock covered by barnacles is placed by." Fouling was effective, and all available surface area on control cultch was occupied by the fouling community before September of the first growing season. In the remote-set shell, oysters outcompeted other fouling organisms in a first-come-first-serve order.

Published information concerning fouling organisms on oyster shell is scarce. Beaven (1947) described fouling organisms on cultch in the Chesapeake Bay based on observations of shell on natural beds and experimental clean shell in wire cages. The fouling assemblages consisted principally of Bryozoa, barnacles, mussels, *Molgula*, sponges, tube-building worms, folliculinids, *Crepidula*, hydroids and algae. In a number of instances, one or more of these organisms completely covered all exposed surfaces of planted shells in a comparatively short period leaving no suitable areas for spat attachment. It was concluded that such fouling may, at times, be a major factor in determining the success of spat fall. The effectiveness of fresh verses previous year's cultch was compared, and new cultch caught 4.46 times more oyster set than cultch from the prior year.

Fresh cultch is usually deployed annually on seed beds in Connecticut by the shellfishing industry during the first week of July. The period for deploying cultch is based on information about the timing of oyster sets (Loosanoff 1966), which is on average on July 20, and the timing of sets of fouling organisms, such as barnacles, which set early spring. As fouling organisms compete with eastern oysters for the space on cultch, and if the space that would have otherwise been available to oyster larvae is already occupied by other organisms, the spat will fail. Consequently, it is important to optimize the time of deploying the cultch for optimum catching of oyster set. Although set was scarce during this experiment, the shell with remote-set oyster seed received more oyster set than cultch. This difference may be an important method for propagating more set and offsetting the initial cost of remote setting.

There was a low-prevalence (3% and 10%) infection of *Haplosporidium nelsoni* (MSX) in the oyster samples, and their prevalence cycles peaks every 5–7 y (Ford & Tripp 1996). The present experiment was not performed during a significant epizootic of MSX (previous peak was in 2009; Sunila & Visel 2015) and consequently did not provide the challenge to demonstrate a potential difference between the disease-resistant Clinton strain and the native oysters. The major emphasis in disease-resistant oyster project in Connecticut has been in developing resistance to MSX and seaside organism. All the samples also had a high-prevalence, low-intensity infection by *Perkinsus marinus* (dermo) with no statistically significant differences. This is characteristic of oyster stocks of Connecticut, where *P. marinus* intensities remain so low until market size that no significant dermo-associated mortality occurs before the oysters are harvested (Sunila 1997).

CONCLUSIONS AND RECOMMENDATIONS

The growth of the oysters was fast, and market size was reached in 15 mo. Survival after initial oyster drill predation and overgrowth was on the average 28%. Mortality could potentially be lowered by avoiding overgrowth by adding 1 million eyed larvae into the setting tank instead of the 4 million used in this experiment for the same amount of cultch (13,273 shells). A stronger cotton–polyester predator netting can be used; however, the degradation time should be tested. Remote set promoted natural set and deterred fouling. Additional siltation-associated mortality could have been avoided by transplanting the oyster to deeper-water growing areas after the first growing season. The method of using remote-set oysters to restock natural beds in Connecticut for the use of the oyster industry during prolonged periods of no sets seems feasible. The method of using remote-set oysters could potentially turn unproductive natural beds into seed beds and thus boost oyster production of Connecticut. The significant siltation-associated mortality and the irregularity of oyster sets demonstrate that the self-perpetuating nonharvest oyster restoration activities in natural beds of Connecticut may not be feasible.

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