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Memorandum

To	George Meservey, Director of Planning & Community Development
CC	Betsy Shreve, AICP, AECOM Project Director Eric Karplus, Science Wares, Inc. Jennifer Doyle-Breen, AECOM
Subject	**Town of Orleans, MA** **Water Quality and Wastewater Planning** **Task 12.1.B.1 - Lonnie's (Kescayo-Gansett) Pond Oyster Aquaculture Demonstration Project Year 3 Report - Final**
Project Number	60476644
From	Thomas Parece, P.E., AECOM Project Manager
Date	July 2, 2019

Executive Summary

The 2018 Demonstration Year at Lonnie's Pond occupied approximately 0.37 acres and provided a third consecutive year of documentation for nitrogen removal through uptake by growth, removing an estimated 60 kg of nitrogen using floating gear deployed in approximately 16,200 square feet. About 61 percent of the growing area was dedicated to second year grow-out of oysters that had been raised during the 2017 demonstration year starting as 2 to 3 mm seed purchased in early May 2017. The remaining 39 percent of the growing area was used to grow out a new order of 2 to 3 mm seed purchased in early May 2018. The Y1 oysters were transferred from floating spat bags to 6 mm diamond mesh floating bags at a point when a fast-growing subpopulation representing approximately 37 percent of the initial seed purchase was expected to be retained in the 6mm bags.

A significantly higher efficiency of nitrogen uptake through growth was observed in a new growing area established in the northeastern part of the pond. There was also evidence suggesting that nitrogen removal rates are higher over deeper water areas in the southern region of the pond than over shallow water areas in the southern region of the pond.

Nitrogen content for Y1 oysters of approximately 0.31 percent ± 0.046 percent of harvest weight was observed for 2018, consistent with previous end-of-season values of 0.30 percent ± 0.031 percent in 2017 and 0.32 percent ± 0.069 percent in 2016. Higher nitrogen content for Y2 oysters of approximately 0.40 percent ± 0.054 percent of harvest weight was observed in 2018, compared with 0.32 percent ± 0.026 percent in 2017 and 0.30 percent ± 0.042 percent in 2016. Significant mortality of Y2 oysters accounting for approximately 28 percent of the population was observed during 2018, and may have been partly due to the large number of runt animals (approximately 58 percent of the population but only 36 percent of the biomass at the start of the 2018 season) carried over from the 2 to 3 mm seed grown out as Y1 oysters during the 2017 demonstration year. During 2018, the highest nitrogen removal rate of 234 kg of nitrogen per acre of deployed gear was achieved by Y1 oysters.

It is anticipated that targeting a stocking density of approximately1,000 Y1 oysters per bag in all 2,040 bags distributed among two fields in the northeastern section of the pond and two fields in the southwestern section of the pond will make it possible to achieve the nitrogen removal target of 75 kg, which is equivalent to 200 kg of nitrogen removed per acre of deployed floating gear. If 2 to 3 mm seed is purchased in early May and grown out in floating spat bags, it is expected that 30 to 35 percent of the seed purchase will have desirable characteristics for achieving efficient nitrogen removal in a high density floating gear configuration in Lonnie's Pond.

The UMass Dartmouth School for Marine Science and Technology (SMAST) continued their denitrification studies in 2018 and these studies were supported by growing out the Y1 oysters from 2017 as Y2 oysters in 2018.

1. Technical Memorandum Organization and Background

This Technical Memorandum for the Kescayo-Gansett (Lonnie's) Pond Oyster Aquaculture Demonstration (Tech Memo) documents the installation details and data collected during the 2018 Demonstration. This Tech Memo also provides an analysis of the data and a summary of key findings from all three years of the Demonstration Project.

A. Memorandum Organization

Section contents are as follows:

- Section 1 describes the Technical Memorandum Organization and summarizes the specific purposes of the Lonnie's Pond Oyster Aquaculture Demonstration Project (Lonnie's Pond Demonstration);
- Section 2 presents the system and techniques that were used for installing the 2018 demonstration project in the field, and describes how this installation was operated and maintained throughout the 2018 growing season; overwintering data from the winter of 2017-18 are also presented;
- Section 3 presents the 2018 data collection methodology and results obtained for growth, mortality and nitrogen-content of the oysters that were grown;
- Section 4 describes what was done with the oysters the end of the 2018 growing season;
- Section 5 presents the conclusions and recommendations from this demonstration project; and
- Section 6 summarizes the references utilized as part of the demonstration project.

Two appendices are included at the end of the document:

- Appendix A shows the field layouts at various stages in the project; and
- Appendix B presents additional oyster growth data.

B. Purpose of the Lonnie's Pond Demonstration

The three-year Lonnie's Pond Demonstration was designed to provide the data needed to support the use of shellfish within the Town's Amended Comprehensive Wastewater Management Plan (ACWMP). Information on growth rates at various stocking densities and age cohorts, nitrogen uptake, denitrification, field logistics and stakeholder perspectives was needed to guide appropriate design and implementation plans. In addition, the Massachusetts Department of Environmental Protection (MassDEP) must approve shellfish cultivation as part of the Town's ACWMP and has stated that three years of data are needed to fully quantify the extent to which oysters can make a measurable difference in water quality parameters for regulatory purposes.

This three-year demonstration was designed to confirm the suitability of Lonnie's Pond for growing oysters and provide reliable documentation of the amount of nitrogen that can be removed by uptake as a result of oyster growth. A strategy has been developed that supports calculation of the nitrogen removed by oyster growth using direct measurements as shown in Equation (1):

$$kg\ Nitrogen\ Removed = (\ Outgoing\ Weight - Incoming\ Weight\ ) * (\ N\ \%\ of\ Weight\ ) \qquad (1)$$

All the quantities in Equation (1) can be directly measured each year, providing reliable documentation of actual nitrogen removal from an estuary by oyster growth. The *N% of Weight* for equation (1) must calculated in a way that is relevant to the incoming and outgoing weights, which means that the harvest weights of live animals should be obtained before they are sent to the lab for analysis, after the same amount of cleaning, draining, and drying that is applied when the *Outgoing Weight* and *Incoming Weight* for equation (1) is obtained.

The area occupied by gear used to grow the oysters can also be directly measured, making it possible to calculate the amount of nitrogen removed per unit area as shown in Equation (2)

$$kg\ Nitrogen\ removed\ per\ Acre = (\ kg\ Nitrogen\ removed\ ) \,/\, (\ Acres\ Occupied\ by\ Gear\ ) \qquad (2)$$

The nitrogen removed per acre calculated as shown in Equation (2) is a useful planning number for other sites, especially if it has been measured from a large deployment area. Although nitrogen removal rates via direct uptake from oyster cultivation may be site-specific, the results from Lonnie's Pond are expected to be useful for planning related to other impaired sections of Pleasant Bay because the conditions found in Lonnie's Pond are similar to other areas such as Meetinghouse Pond, The River, Arey's Pond, Namequoit River and Paw Wah Pond.

The UMass Dartmouth School for Marine Science and Technology (SMAST) is also measuring the extent to which oysters cause enhanced denitrification in Lonnie's Pond. Findings on denitrification enhancement from the 2016 and 2017 growing seasons are included in separate reports by SMAST (Howes et. al. 2017, Howes et. al. 2018), and results from the 2018 growing season are to be presented in an SMAST report scheduled to be finalized in June 2019.

A key focus of this last year of the three-year demonstration project was to assess whether nitrogen removal rates per unit area of deployed gear were consistent as the deployment area was scaled up to occupy twice the area that had been used in 2017. A significant portion of the project area (approximately 61 percent) was devoted to growing out second year oysters that had been raised as first year oysters during 2017. This second year oyster population included both oysters that had been fast-growing as first year oysters in 2017 (approximately 37 percent of population and 64 percent of biomass) as well as oysters that had been slow-growing as first year oysters in 2017.

2. Field Details for the 2018 Demonstration

A. 2017-2018 Overwintering

Approximately 399,000 oysters were raised as Y1 oysters during the 2017 growing season, starting as 2 to 3 mm seed purchased in early May 2017. These oysters were kept in 6 mm bags and suspended on bottom racks in Lonnie's Pond over the winter of 2017-2018. The oysters were submerged on December 19, 2017 thru December 21, 2017 and resurfaced on April 20, 2018 thru April 22, 2018. After resurfacing, each bag was emptied onto a table, and dead shells were removed and counted. A total of 7,046 dead shells were collected, corresponding to a mortality rate of 1.77 percent. This is similar to what was experienced for the 2016-2017 overwintering period. Table 1 below summarizes the overwintering mortalities experienced during the two overwintering seasons of the demonstration project. Figure 1 gives a graphical presentation of the results for first year oysters and may suggest that larger biomass per overwintered bag appears to be associated with increased mortality. Even so, the worst-case overwintering mortality rates observed were quite low.

Table 1. Summary of Overwintering Mortality Rates

Overwintering Season	Description	Total Bags Processed	Estimated Live Population	Average Mortality
2016-2017	Market ready oysters entering their third year of growth in 2017, relayed back to Town of Falmouth	525	113,000	10.9 percent ±4.85 percent
2016-2017	Oysters originally from Town of the Falmouth entering their second year of growth in 2017	30	11,200	4.56 percent ±2.33 percent
2016-2017	Oysters originally from Cape Cod Oyster entering their second year of growth in 2017	225	57,800	1.31 percent ±0.690 percent
2017-2018	Oysters grown in Lonnie's Pond from 2-3mm seed, Runts at approximately 600/bag	67	40,000	1.25 percent ±1.25 percent
2017-2018	Oysters grown in Lonnie's Pond from 2-3mm seed, Runts at approximately1,000/bag	190	190,000	1.70 percent ±1.59 percent
2017-2018	Oysters grown in Lonnie's Pond from 2-3mm seed, Studs at approximately 600/bag	172	103,000	1.30 percent ±1.08 percent
2017-2018	Oysters grown in Lonnie's Pond from 2-3mm seed, Studs at approximately 1,000/bag	66	66,000	2.97 percent ±2.75 percent

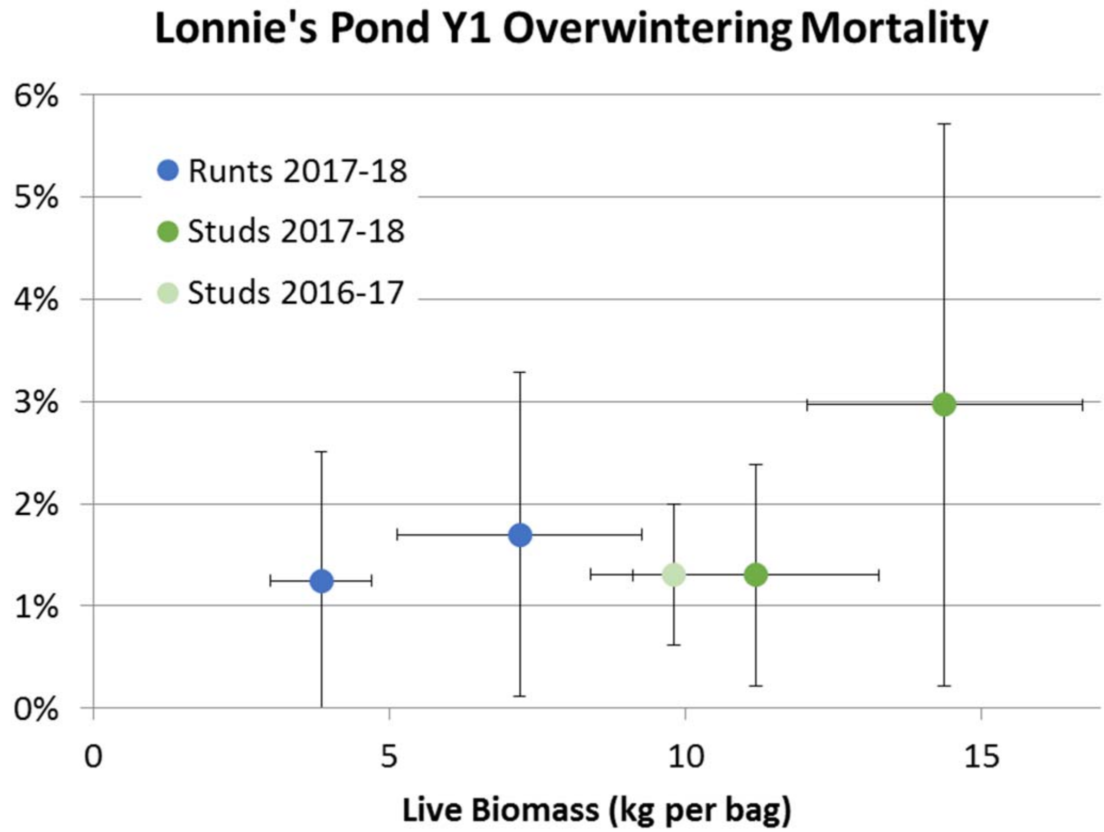


Figure 1. First Year Oyster Overwintering Mortality as a Function of Live Biomass per Bag for 2016-2017 and 2017-2018 Overwintering Seasons

B. Overall Configuration

The 2018 demonstration included the following two groups of oysters:

- 2,185,000 new first year oysters ordered as 2-3mm seed in early May 2018; and
- 392,000 overwintered oysters that were Y1 oysters in 2017 and deployed as Y2 oysters entering their second year of growth in 2018.

The above groups of oysters were deployed in four growing areas, referred to as fields, described in Table 2. The layout of the fields in Lonnie's Pond is shown in Figure 2, with additional details provided in Appendix A. Each bag in each field was assigned a specific ID comprised of two letters identifying the field (SW, NW, NE, SE) as well as a row and column identifier. Each field had 17 columns spaced on 4 foot centers, with the westernmost column designated as A and the easternmost column designated as Q, and 30 rows spaced on 2 foot centers, with the southernmost row designated as 1 and the northernmost row designated as 30.

In previous years of the demonstration it was observed that growth in bags at the corners and along outside edges of a high density field had the fastest growth rates. It was also observed that there is still significant growth after the end of the critical period (starting September 1). In an effort to even out the biomass increase per bag, during the measurements made at the end of the critical period, bags in the outermost positions were moved to the innermost positions, and bags in the innermost positions were moved to the outermost positions. Additional information about this process is provided in Appendix A.

Table 2. Summary of Oysters Deployed for 2018 Season

Field	Description	Estimated Initial Population	Growout Area (sq.ft.)	Initial Density (kg/sq.ft.)	Initial Deployment Dates
SW	Y2 oysters started as 2-3mm seed in May 2017 in Lonnie's Pond	Runt: 102,000 Stud: 68,000	4,080	0.44	4/20/18 – 4/23/18
NW	Y2 oysters started as 2-3mm seed in May 2017 in Lonnie's Pond	Runt: 0 Stud: 77,000	2,840	0.47	4/20/18 – 4/23/18
	Y1 Oysters as 2-3mm seed	389,000	1,120	0.0033	5/11/18
NE	Y2 oysters started as 2-3mm seed in May 2017 in Lonnie's Pond	Runt: 125,000 Stud: 20,000	2,992	0.45	4/20/18 – 4/23/18
	Y1 Oysters as 2-3mm seed	378,000	1,088	0.0036	5/18/18
SE	Y1 Oysters as 2-3mm seed	1,418,000	4,080	0.0033	5/11/18

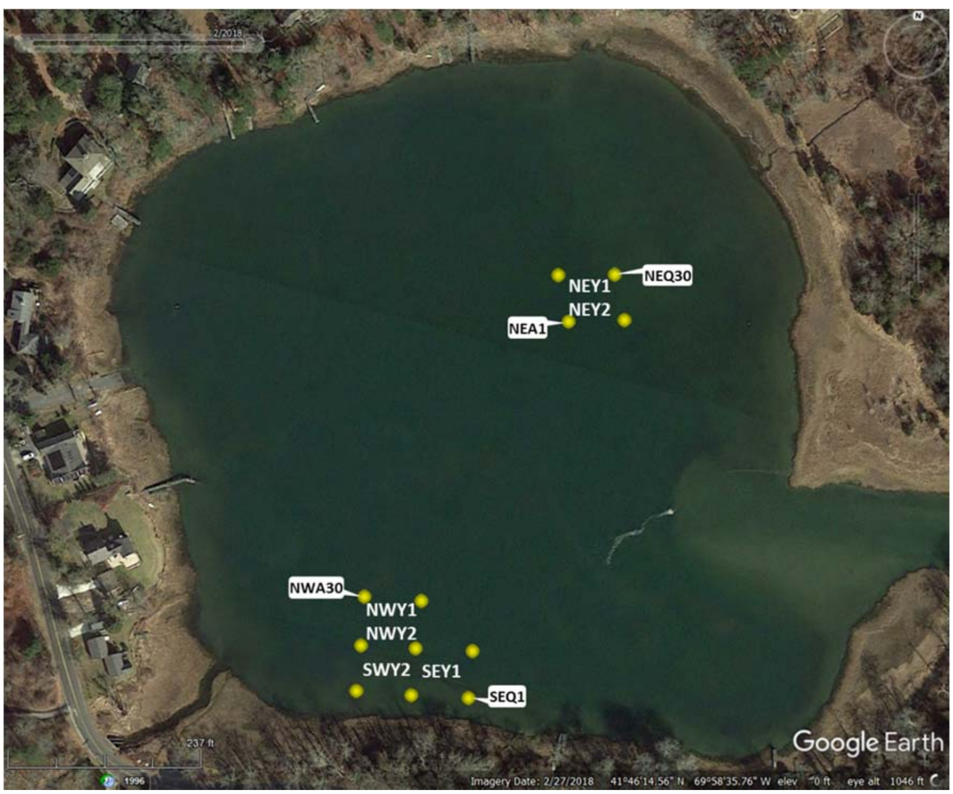


Figure 2. Layout of Growing Areas in Lonnie's Pond for the 2018 Growing Season Along With Some Representative Bag IDs

C. Y1 Oyster Deployment

From results obtained during the 2017 demonstration shown in Figure 3 below, it was observed that the highest nitrogen removal efficiency per unit area was achieved by stocking first year oysters that exhibited rapid growth during the first 6 to 8 weeks of the growing season at density targeting 1,000 oysters per bag at the end of the growing season. As shown in Figure 4 below, it was also observed that this population accounted for approximately 37 percent of the initial seed purchase. Consequently the intent for the 2018 demonstration was to place a large enough seed order to target 1,000 fast-growing oysters per bag, and select out the fast-growing subpopulation within the first 8 weeks of the growing season by transferring the animals from fine-mesh spat bags to 6 mm diamond mesh bags at a time when the slower-growing animals would pass through the bag mesh and drop to the bottom, while the faster-growing animals would be retained inside the diamond mesh bags.

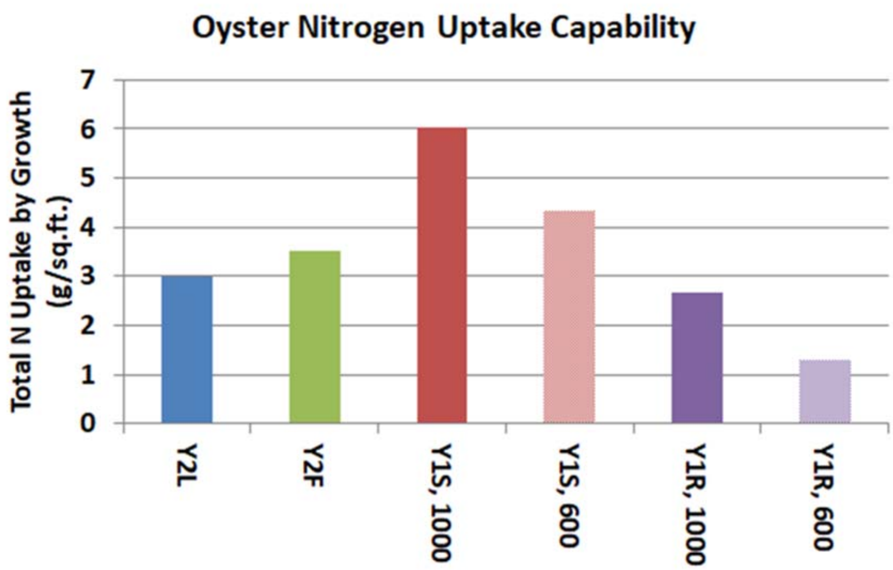


Figure 3. Comparison of the Nitrogen Uptake Capability of the Configurations of Oysters Monitored During the 2017 Demonstration Season. Reproduced from 2017 Report Figure 12.

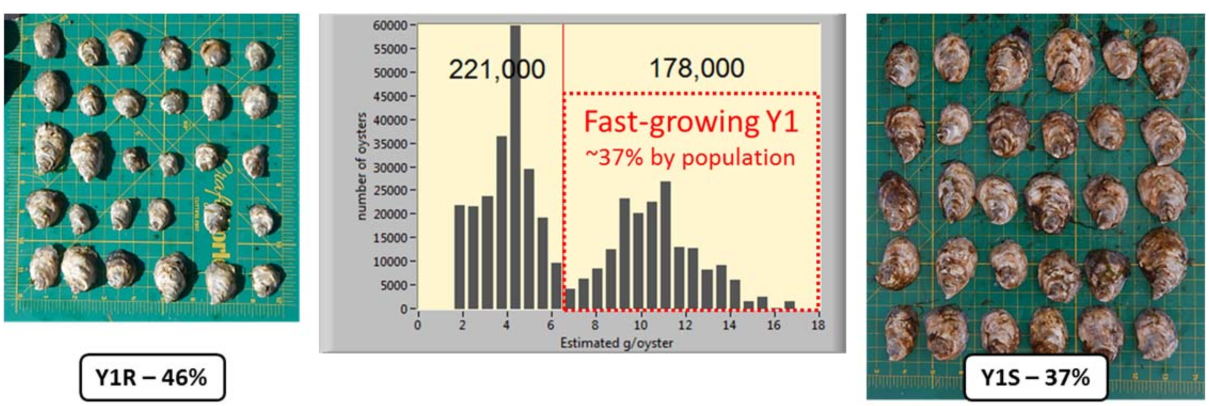


Figure 4. Histogram of Average Individual Weights in Y1 Population in Mid-September 2017. Reproduced from 2017 Report Figure 10 with Red Annotation Added. Estimated g/oyster is Projected for the End of the Growing Season.

Because oysters are ordered from the hatchery by population and not biomass, the number of oysters that must be ordered to end up with a population of 1,000 fast-growing oysters per bag is 1,000 / 0.37 = 2,700 oysters per bag. Based on the results presented in Appendix C of the 2017 report, it was also considered worthwhile to explore whether a higher stocking density of 1,200 fast-growing oysters would provide a higher level of nitrogen removal per unit area while still resulting in a viable intermediate seed size animal at the end of one growing season. The calculation for the 2 to 3 mm oyster seed order is shown in Table 3. This seed was received in two shipments a week apart, shown in Figure 5.

Table 3. Y1 seed order calculations for 2018 season

Target Fast-Growing Population per 6mm Bag	Required 2-3mm Seed Order per 6mm Bag	Number of 6mm Bags	Total 2-3mm Seed Order
1,000	2,700	646	1,744,000
1,200	3,240	136	441,000
		Total:	2,185,000

5/11/18: 10.95kg @ 160/g ≈ 1,750,000 5/18/18: 2.58kg @ 169/g ≈ 435,000

Figure 5. Y1 2 to 3 mm Seed for the 2018 Growing Season, as Received from Mook Seafarms

Because there were no upweller resources available for this project, the strategy of using floating spat bags was further refined for the early stage grow-out of the 2 to 3 mm seed. A new bag design was developed in 2018 that has a rectangular internal floating frame constructed of ¾-inch pipe size SDR21 pvc tubing cemented together with 3-way corners as shown in Figure 6. The thin-wall high pressure PVC tubing was filled with ¾-inch nominal diameter closed-cell foam cord to ensure that water infiltration which might occur if the assembly is damaged or the integrity of the seals degrades over time would not result in a negatively buoyant assembly. The tubing frames were designed so that 10 frames could be conveniently nested and held together with six zip ties in an efficient shape for transport. The net buoyancy of the floating spat bag assembly, including the spat bag, PVC sliders, connecting lines and clips is approximately 1.1 kg, which should be able to float oysters with a harvest weight of approximately 2.7 kg.

The 2018 floating spat bag design was easier to assemble, fill, empty, maintain, transport, and store than the design used for the 2017 season. It is also expected to have significantly greater longevity as there is no exposed foam. Assuming a 10 year lifetime, replacing the outside bag every 3 years, and a deployment stocking density of approximately 8,200 oysters, the cost of materials and labor for fabrication is under $0.50 per 1,000 oysters deployed, which is believed to be substantially less than the cost of an upweller. An added benefit is that the floating spat bags can be deployed in the same format as other floating bags, and there is no need for a power source. Cleaning takes about a minute per bag using a scrub brush and optional spray down pump, and typically needs to be done 10 to 15 times before the oysters can be transferred into 6mm bags. This represents a cost of $0.60-$0.90 per 1,000 oysters deployed if labor costs are $30/hr.

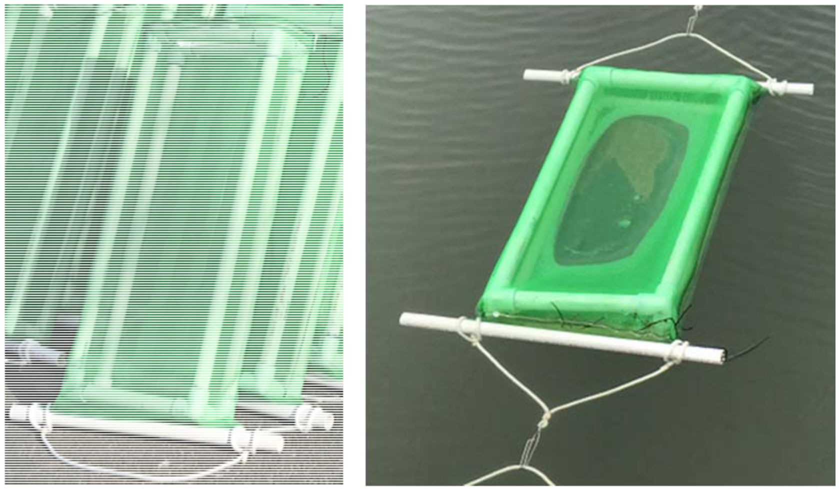


Figure 6. Floating Spat Bag Assembly Developed for the 2018 Deployment Ready for Filling (left) and Deployed in the Water with Initial Stocking of Approximately 8,100 2 to 3 mm Seed (right)

When the water temperature and food resource is adequate, Y1 seed oysters can be deployed at high population stocking densities. The strategy used for the 2018 growing season was to deploy enough 2 to 3 mm seed in one floating spat bag to target filling of three 6mm diamond mesh bags, and to place this in such a way that each floating spat bag initially has access to approximately 15 square feet of water. The stocking densities were matched to the incoming seed orders and the plan to deploy at stocking densities targeting 1,000 and 1,200 fast-growing oysters per bag. The initial deployment densities are shown in Table 4, and Figure 7 shows the layout of the preliminary deployment into the SE field as of May 23, 2018. On May 29, 2018 the floating spat bags were spread out to also occupy the Y1 areas in the NW and NE fields, and from this time until the Y1 oysters were transferred to 6 mm diamond mesh bags on the dates shown in Table 5, each floating spat bag had access to approximately 24 square feet of water.

Table 4. Floating Spat Bag Stocking Densities for 2018 Season

Target Fast-Growing Population per 6mm Bag	Required 2-3mm Seed for Three 6mm Bags	Number of Spat Bags	Total 2-3mm Seed
1,000	approximately 8,110	216	1,750,000
1,200	approximately 9,460	46	435,000
		Total:	2,185,000

Figure 7. Initial Deployment of 263 Floating Spat Bags Shown on May 23, 2018 Containing Approximately 2,185,000 Y1 Oysters in SE field, Adjacent to Y2 Oysters in the SW and NW Fields

The floating spat bags were cleaned as needed, working from a kayak as shown at the top of Figure 8. First the bag was flipped, then it was scrubbed with a bristle brush, then it was manually submerged to rinse it off, and sometimes sprayed with a battery-operated wash down pump. Fouling shown on the bag in foreground on the bottom left of Figure 8 accumulated over 5 days in early July, a few days before being split into 6 mm diamond mesh bags. The bag appears as shown in the foreground at the bottom right of Figure 8 after approximately one minute of cleaning. Bags in the background of the bottom row pictures of Figure 8 have not been cleaned since the last maintenance cycle, showing how exposure to sun and air helps clean the fouling in between cycles.

Figure 8. Floating Spat Bags Being Cleaned From a Kayak

When the Y1 oysters had grown enough in the spat bags so that the number of fast-growing oysters that would be retained in a 6mm bag would achieve the target stocking density, the floating spat bags were processed using the system shown in Figure 9.

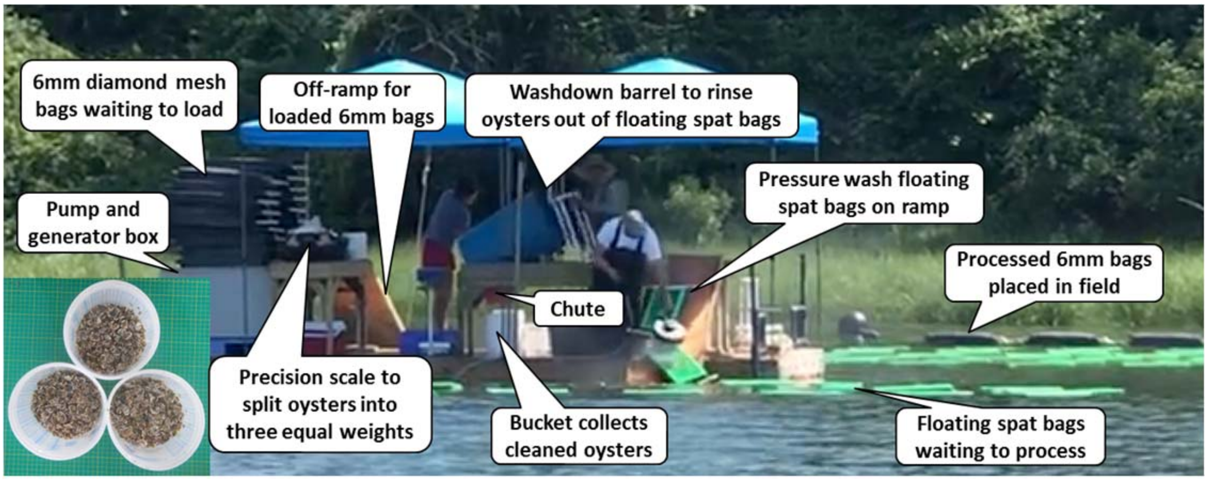


Figure 9. Floating Work Platform Configured for Splitting Y1 Oysters from Floating Spat Bags into 6 mm Diamond Mesh Bags

Each floating spat bag was cleaned using an electric pressure washer, then opened and emptied into a wash-down barrel where the oysters were rinsed out of the bag and off the PVC frame. The cleaned spat bags were rolled and stored in a tote, and the frames were stacked for subsequent removal from the work area. The oysters were dumped out of the wash-down barrel, through a chute, and into a bucket in which they were further rinsed to remove algae. The cleaned oysters were then placed into three buckets with drain holes and allowed to drain for at least two minutes. After draining, the contents of the three buckets were adjusted using a precision scale to equally divide the oysters. The split weight was recorded, and the oysters were then put into 6mm diamond mesh bags with ID tags, and were placed in the field in a specific order. The 6 mm bags were flipped as needed to control biofouling, which could be as often as every 6 to 7 days during July and August.

It was expected that the best time to perform the above operation would be when the weight placed in each 6mm diamond mesh bag was around 0.36 kg when targeting 1,000 oysters per 6mm bag, and around 0.43 kg when targeting 1,200 oysters per 6 mm bag. This is based on the assumption that oysters will be retained in a 6mm diamond mesh bag when they weigh approximately 0.25 g each, and that approximately 37 percent of the initial stocking population will account for approximately 69 percent of the total weight. These percentages are taken from the histogram data shown in Figure 4. It was anticipated that this stage of growth was going to be reached around the first week of July in 2018, when measurements of all bag weights at the start of the critical period were to be made. Since extremely rapid growth was observed in the NE field, and there were some close followers in the NW and SE fields, a decision was made to split about half the field early and the other half late, as shown in Table 5. Population estimates made at the end of critical period suggested that approximately 38 percent of the initial stocking population had been retained. Subsequent population estimates made at the end of the growing season suggested that 29 percent of the initial stocking population had been retained.

Table 5. Dates Y1 Oysters were Transferred from Floating Spat Bags to 6 mm Diamond Mesh Bags

Dates	Area	Number of 6mm Bags	Average Weight per 6mm bag (kg)
6/19/18	NEY1	136	0.22
6/20/18	NWY1	136	0.15
6/21/18	SEearly	136	0.20
7/14-15/18	SElate	412*	0.55

* Four bags were placed in western end of row NW22

D. Y2 Oyster Deployment

The oysters that were grown from 2 to 3 mm seed in 2017 were overwintered in bags suspended from racks on the bottom of Lonnie's Pond during the winter of 2017-2018. As shown in Figure 4, these oysters had been characterized as faster-growing 'Studs' and slower growing 'Runts' based on their growth rate in during the 2017 growing season. After overwintering, there were approximately 392,000 of these oysters with characteristics shown in Table 6.

Table 6. Summary of Y2 oysters resurfaced for 2018 season

Category	Estimated Population	Fraction of total population	Fraction of total biomass	Average Individual (g/oyster)
Runt	227,000	58%	36%	7.22
Stud	165,000	42%	64%	17.4

The populations described in Table 6 were distributed according to weight into each bag in the Y2 growing areas as designated in Table 7. Because the runt oysters were significantly lighter per individual, their initial stocking density per bag was much higher. There was an intentional effort to keep the deployed biomass per unit area consistent. The NW population was stocked at a slightly higher initial density as it was the last field populated, and a conservative estimate of the required stocking density was made to ensure that there would not be a shortage of bags. A consequence was that the NW22 row ended up not being populated with any Y2 oysters, and the NW21 row had two empty bags at positions A and B. This accounts for the slightly smaller Y2 growout area in the NW field than in the NE field.

Table 7. Summary of Y2 oysters deployed for 2018 season

Field	Growout Area (sq.ft.)	Initial Density (kg/sq.ft.)	Estimated Initial Runt Population	Estimated Initial Stud Population	Estimated Runts per Bag	Estimated Studs per Bag
SW	4,080	0.44	102,000	68,000	545	211
NW	2,840	0.47	-	77,000	-	217
NE	2,992	0.45	125,000	20,000	465	190
Total	9,912		227,000	165,000		

The method for loading the 2018 bags was to empty out an overwintered bag from the 2017 season, pull out the dead oysters, weigh the live oysters, then weigh out the desired mass of live oysters and place it in a 2018 bag, which was tagged and placed into the field in a specific order. For example for the SW field, 3.51 kg of oysters were placed in each bag, which occupied an 8 square foot area, resulting in an initial density of 0.44 kg/sq.ft. In some cases there were enough oysters in an overwintered bag to fill multiple 2018 bags, and in other cases there were not enough oysters in an overwintered bag to fill a single 2018 bag. The ID of the overwintered bag that was the primary source of oysters for each 2018 bag was noted to provide information for tracking whether the bag had runts or studs in it. In many cases, a 2018 bag included oysters from more than one overwintered bag.

3. Measuring Oyster Growth

A. Data Collection Methodologies

Oyster Weights

A key goal of the 2018 demonstration was to evaluate how scaling up the deployment area affected growth rates. For this reason, each deployed bag was weighed at the following points:

- Initial deployment;
- Start of Critical Period (July 1);
- End of Critical Period (August 31); and
- End of season.

Due to the number of bags in the field, time required for processing, and available staff and equipment resources, each measurement required several days. To the extent possible, measurements at the start and end of the Critical Period were performed so that the range of measurement dates was centered on the target date. For report calculations, linear interpolation was used to estimate weights on the exact critical period dates of July 1 and September 1.

During initial deployment of Y1 oysters into floating spat bags, a suitable mass of oysters (typically approximately 50 g per floating spat bag) was weighed out for each bag using a portable battery powered scale with 100 g capacity and 0.01 g readability.

When Y1 oysters were split out from one floating spat bag into three 6mm diamond mesh bags, a portable NTEP Legal-For-Trade IP67 rated battery powered scale with 3 kg capacity and 1 g readability was used.

Weights for oysters in 6mm diamond mesh bags were obtained on a rechargeable-battery powered IP69 rated platform scale with 60 kg capacity and 0.01 kg readability.

When estimates of average individual weights of oysters were being obtained, a batch of 50 Y1 oysters or two batches of 25 Y2 oysters were counted out and weighed on a portable NTEP Legal-For-Trade IP67 rated battery powered scale with 3kg capacity and 1g readability.

Weights were recorded along with the associated bag ID by hand on a water resistant notepad attached to a foam float for safety in case wind carried the pad onto the water. Photos of the oysters were taken with a waterproof digital camera mounted at a fixed height above the processing table.

Nitrogen Content and Mortality

Tracking nitrogen content and mortality with high time resolution was not considered important for two reasons:

- the nitrogen removal calculation in equation (1) only requires a percent nitrogen measurement at the time of removal; and
- the anticipated project format for commercial operation in 2019 and beyond involves leaving first year oysters in for the full growing season, so the final population is all that matters from a commercial perspective.

For the reasons above and because of the low mortality rates experienced in previous years, the original intent was to only assess mortality at the end of the growing season. However, a noticeable amount of mortality was observed in the Y2 population in July and August. As a result, intermediate assessments were performed as time permitted. For the Y2 bags, a full mortality assessment was performed at the end of the critical period (around August 31). When mortality was assessed, the dead oysters were removed and weighed, and the remaining live oysters were also weighed. After obtaining field weights of the dead oyster shells, the shells were air dried for a minimum of 4 weeks and then their dry weight was obtained. The ratio of the total field weight of the dead shells to the total dry weight of the shells was used as described below to calculate the nitrogen content of the dead oyster shells.

The calculation for nitrogen content of the shells of dead oysters can be made using Equation (3):

$$kg\ Nitrogen\ in\ Shells = (\ Field\ Weight\ of\ Shells\ ) * (\ N\ \%\ of\ Shell\ Field\ Weight\ ) \quad (3)$$

As with Equation (1), all the quantities in Equation (3) can be directly measured each year, providing reliable documentation of actual nitrogen removal from an estuary by oyster growth. The *N% of Shell Weight* for Equation (3) must be calculated in a way that is relevant to the *Field Weight of Shells*. This was done by measuring the ratio in Equation 4 of the field weight of the dead shells to the dry weight of the dead shells after they were allowed to air dry on land for a minimum of one month:

$$Dry\ to\ Field\ Shell\ Ratio = (\ Dry\ Weight\ of\ Shells\ ) / (Field\ Weight\ of\ Shells\ ) \quad (4)$$

The N % of Shell Field Weight can then be calculated as shown in Equation (5)

$$N\%\ of\ Shell\ Field\ Weight\ = (\ Dry\ to\ Field\ Shell\ Ratio\ ) * (\ N\ \%\ of\ Dry\ Shells\ ) \quad (5)$$

The quantity *N % of Dry Shells* is part of the normal lab analysis performed on live oysters, so no additional lab analysis is required if the *Dry to Field Shell Ratio* is measured. This measurement may not be necessary if mortality is low, because the nitrogen content of small quantities of shell may not be significant enough to merit calculation.

At the end of the growing season, a sample of 25 oysters from each field and population group described in Table 2 was processed according to the method described in Appendix D of the 2017 report.

B. Presentation of Results

Figure 9 shows the nitrogen removed by growth of oysters during the 2018 growing season in Lonnie's Pond, normalized per acre of deployed floating gear and averaged by field (NW, NE, SE, SW) and class (Y1, Y2). The error bars are calculated using only the fractional error of the nitrogen content measurement. The total nitrogen removal data is also presented in Table 8.

Table 8. Total kg N Removed per Acre by Field and Age Class for 2018 Growing Season

Field	kg N removed per acre
NWY1	174 ± 22.9
NWY2	139 ± 13.7
NEY1	234 ± 36.7
NEY2	232 ± 29.8
SEY1	130 ± 18.1
SWY2	135 ± 19.7

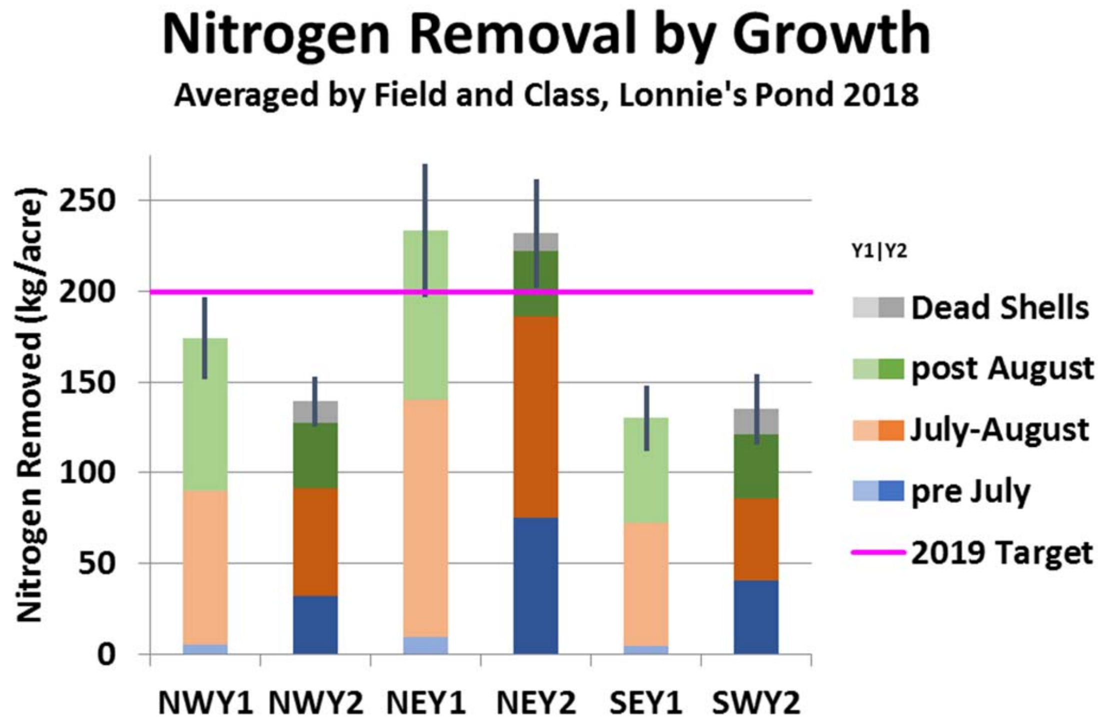


Figure 9. Nitrogen Removal per Acre of Floating Gear for 2018, Averaged by Field and Class

The following key findings about the 2018 growing season can be observed in Figure 9:

- the NE field was significantly more efficient than the other three growing fields;
- the fields overlapping or abutting the areas used in both previous years of the demonstration were least efficient at removing nitrogen through growth;
- Most of the nitrogen removal from growth of Y1 oysters occurs after July 1;
- Most of the nitrogen removal from growth of Y2 oysters occurs before August 31; and
- There was a non-trivial contribution from the nitrogen content in the shells of Y2 animals that died due to higher Y2 mortality during the 2018 growing season than was experience during the 2016 and 2017 growing seasons.

It has been observed in other projects (Howes, 2019) that oysters deployed in an impaired estuary tend to feed more aggressively on the outgoing ebb tide. This may be because phytoplankton populations have had an opportunity to build up while the estuary fills, so food concentrations are highest while the estuary drains. It may be that the tide cycle in Lonnie's Pond delivers phytoplankton more efficiently to the NE field than to the other fields.

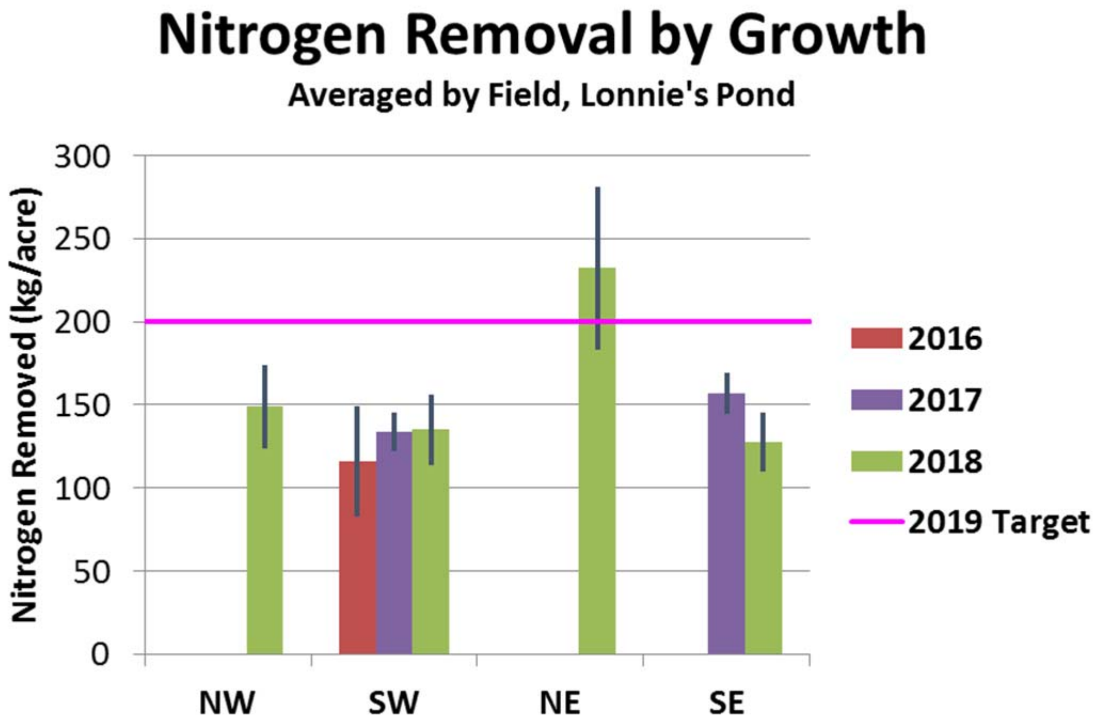
It appears that growing conditions in Lonnie's Pond are more favorable in the northern and eastern areas, in particular the NE field but also the northern portion of the NW field and northern and eastern portions of the SE field. Maps of growth per unit area and a comparison of growing in innermost bags relative to outermost bags are presented in Appendix B.

Figure 10 shows a comparison of nitrogen removal by growth of oysters for all three demonstration years, grouped by field and by age class. This data is also presented in Table 9. A composite diagram showing the relative placement of the fields is shown in Figure A-6. The 2019 target was exceeded in the NE field but not reached in any of the other fields. Part of the reason for the underperformance of the other fields was the high level of mortality in the Y2 population, which has been partly attributed to the large number of small runt animals in the deployed population. Another reason was that the split operation transferring Y1 oysters from floating spat bags into 6mm diamond mesh bags resulted in stocking densities that were lower than what was intended. It is believed that waiting an additional 1 to 2 weeks, accompanied by more frequent cleaning of the floating spat bags during this time, would have resulted in final population densities closer to the targets of 1,000 and 1,200 oysters per bag at the end of the growing season. The achievability of the 2019 target is discussed in the Conclusions and Recommendations section.

Table 9. Total kg N removed per acre by field (top) and age class (bottom) for 2016, 2017, and 2018 growing seasons.

	kg N removed per acre		
Field	**2016**	**2017**	**2018**
NW			149 ± 25.1
SW	116 ± 33.1	134 ± 11.7	135 ± 21.2
NE			232 ± 49.0
SE		157 ± 12.4	128 ± 17.7

	kg N removed per acre		
Age Class	**2016**	**2017**	**2018**
Y1	90.2 ± 18.6	157 ± 15.2	154 ± 23.4
Y2	128 ± 16.9	134 ± 11.7	154 ± 20.4



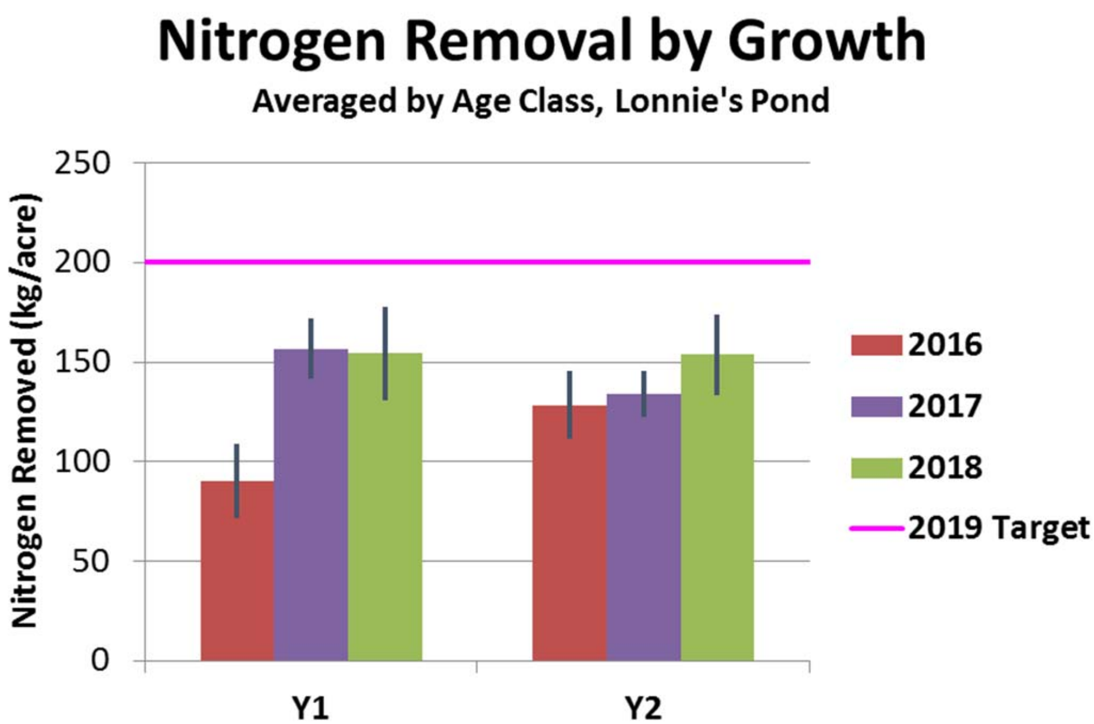


Figure 10. Nitrogen Removal per Acre of Floating Gear for all Three Demonstration Years, Averaged by Field (top) and Age Class (bottom)

The nitrogen content of samples of oysters from each field and age class at the end of the growing season is shown in Figure 11. This data is also presented in Table 10. The error bars represent the combined variation in the sum of the shell and tissue contributions to the overall nitrogen content. Notable observations are as follows:

- The Y2 oysters appear to have a significantly higher concentration of nitrogen in both the shell and tissue when compared to the Y1 oysters. This difference was observed only in 2018, and not in prior years; and
- The faster growing NE oysters appeared to have slightly lower nitrogen concentrations than their slower growing counterparts. For example, NEY2 has lower average nitrogen concentrations compared to either NWY2 or SWY2. Similarly, SEY1, which began in the SE field but finished at a faster rate of growth along the southern edge of the NE field, and NEY1 have a lower average nitrogen concentration compared to SEY1 and NWY1, respectively. The lower overall nitrogen concentration is more than overcome by the faster growth rate, such that faster growth rates result in more efficient nitrogen removal even if the nitrogen concentration is a smaller fraction of the harvest weight. There was nothing observed that suggested the lower nitrogen concentration might be associated with a weaker shell.

Table 10. Nitrogen Content of Oysters as a Percent of Harvest Weight from Each Field and Age Class

Field	N % of Harvest Weight
NWY2	0.41 ± 0.043
NEY2	0.39 ± 0.052
SWY2	0.42 ± 0.066
NWY1	0.32 ± 0.042
NEY1	0.30 ± 0.049
SEY1	0.31 ± 0.043
SEY1ne	0.29 ± 0.051

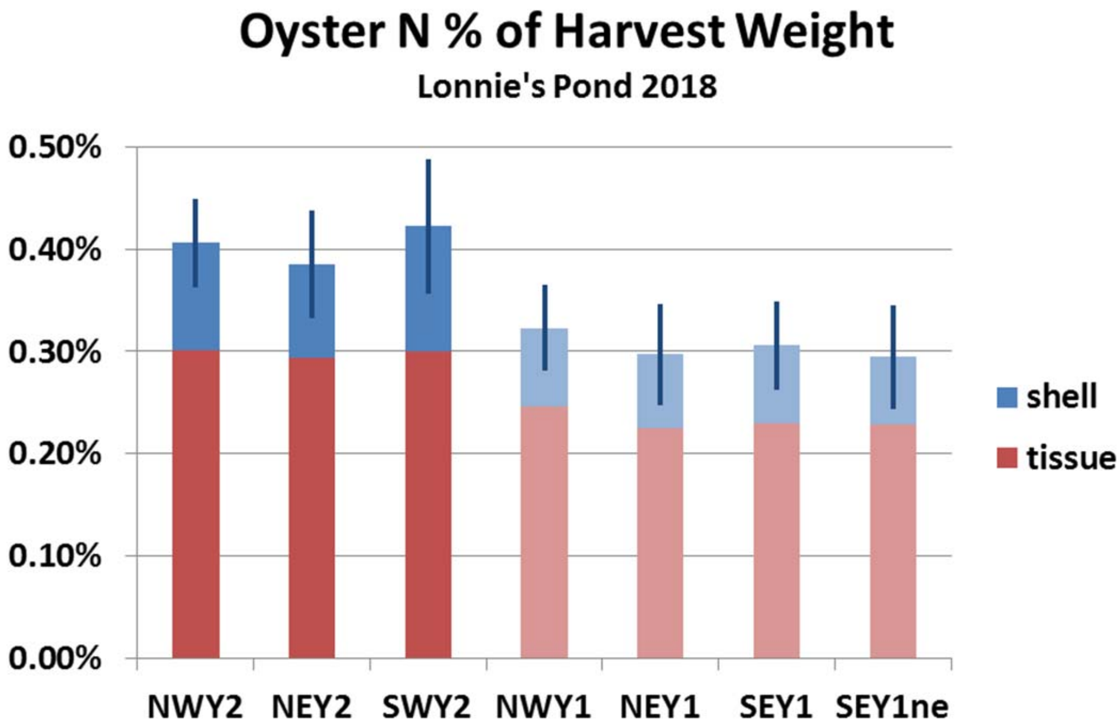


Figure 11. Nitrogen Content of Oysters as a Percent of Harvest Weight from Each Field and Age Class

The nitrogen content for each age class of oysters during each project year is shown in Figure 12. The Y1 nitrogen content appears to be consistent across all project years. The Y2 nitrogen content in 2018 was significantly higher than in previous years. As shown in Figures 8 and 9 on p.11 of the 2017 demonstration year report, the nitrogen content was monitored as a function of time over the course of the growing season. The level of variation observed for Y2 oysters during 2017 does not argue for expecting the increased concentration observed in Y2 oysters in 2018. However, the 2017 Y2 oysters were initially raised in an upweller by Cape Cod Oyster in May and June of 2016 and were selected out of the upweller for fast growth before being moved to Lonnie's Pond to grow out as Y1 oysters in 2016. The 2018 Y2 oysters had been raised as Y1 oysters in 2017 in floating spat bags in Lonnie's Pond, and during that season they did exhibit a change in nitrogen content from approximately 0.41 percent to 0.33 percent of harvest weight over the post-August part of the growing season, though this occurred over the course of approximately 90 days compared with 30 days that were available for such a drop in 2018.

It is possible that variation in nitrogen content is a feature of the oysters obtained from Mook Seafarms, but there is limited evidence to support this conclusion because the time dependence of the nitrogen content was only monitored over the course of the 2017 season. All the oysters used in 2016, 2017, and 2018 were diploids. Even if there was evidence to suggest a higher nitrogen content earlier in the season, removing Y1 oysters before they have finished growing, especially when a significant portion of their growth occurs at the end of the growing season, would likely cancel out the benefit of the higher nitrogen concentration. Furthermore, the survivability of the animals is expected to be lower if they are moved to a land-based overwintering facility when the water temperatures are warmer.

Table 11. Averaged Nitrogen Content as a Percent of Harvest Weight of Oysters for Each Age Class for 2016, 2017 and 2018 Growing Season

	N percent of Harvest Weight		
Age Class	**2016**	**2017**	**2018**
Y1	0.32 ± 0.066	0.32 ± 0.031	0.31 ± 0.046
Y2	0.30 ± 0.040	0.30 ± 0.026	0.40 ± 0.054

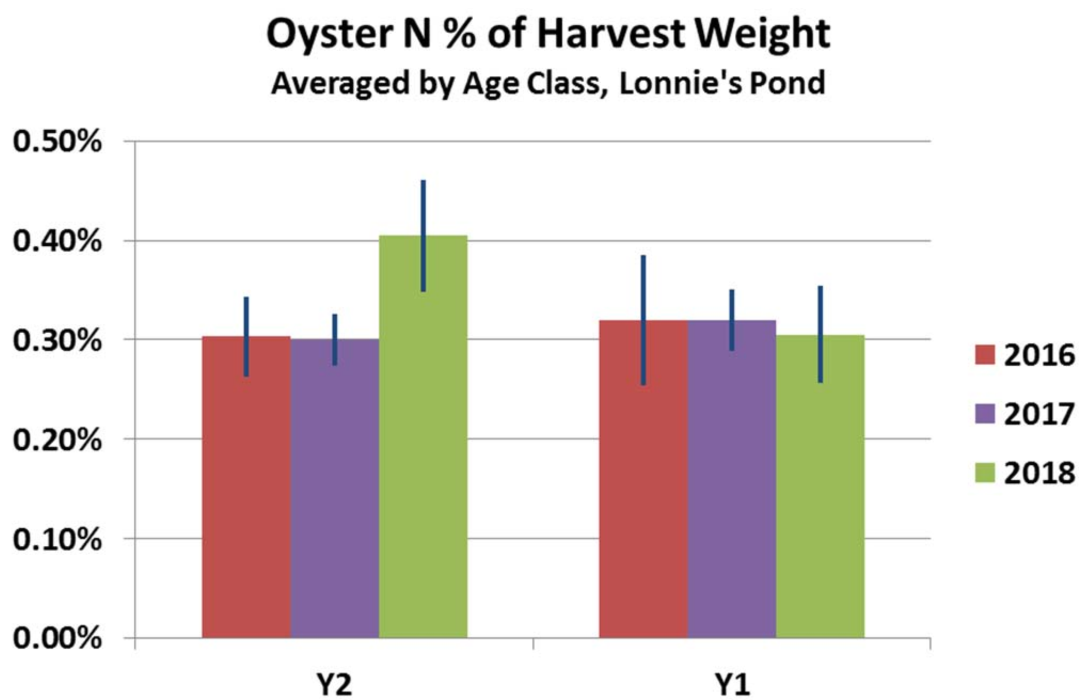


Figure 12. Averaged Nitrogen Content as a Percent of Harvest Weight of Oysters for Each Age Class for 2016, 2017 and 2018 Growing Seasons

4. 2018 End-of-Demonstration Oyster Relay

On October 25 thru 28, 2018 and November 8 thru 10, 2018, the Y2 oysters were relayed to a recreational harvest area in Pleasant Bay near the Orleans-Chatham border on Route 28. Each bag was processed individually to separate out and weigh the dead oysters and weigh the living oysters before they were transferred into totes. During the November 8-10 period, two batches of 25 live oysters were randomly selected from each bag and the total weight of these counted oysters was recorded. The weight of the live oysters in each bag was divided by this average individual weight to calculate an estimated stocking density. This information was used to calculate the histogram of average individual weight for the population relayed in November 2018 shown in Figure 13. A line described by Equation (6) was fitted to the Oyster Live Weight vs Shell Length data presented in Figure 8 of the 2016 report and was used to estimate oyster size from the average individual weight shown in Figure 14. The presence of both runts and studs can be seen clearly in the histogram, with the runts accounting for approximately 42 percent of the population instead of 58 percent as they had at the beginning of the season. Based on the histogram shown in Figure 14, it was estimated that approximately 75 percent of the relayed population were legal for harvest, which would be 213,000 of the total estimated relay of approximately 282,000.

$$Oyster\ Size\ (mm) = 22.2 * (\ Oyster\ Harvest\ Weight\ in\ g\ )^{0.3532} \quad (6)$$

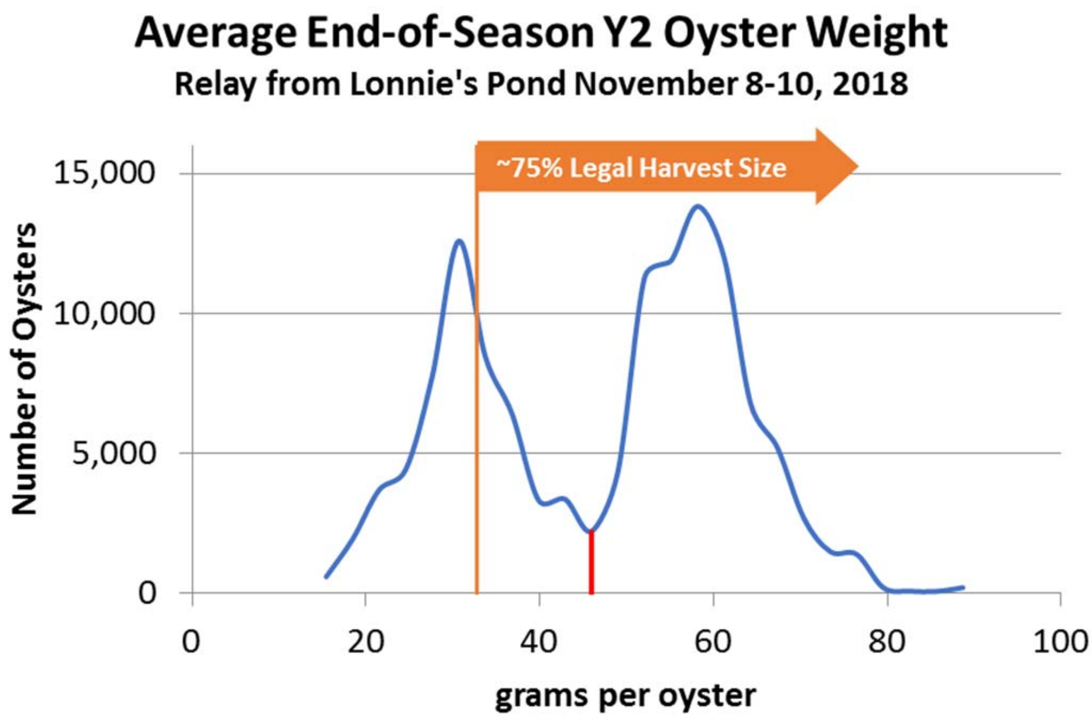


Figure 13. Histogram of Average Individual Weight for Y2 Population Relayed in November 2018

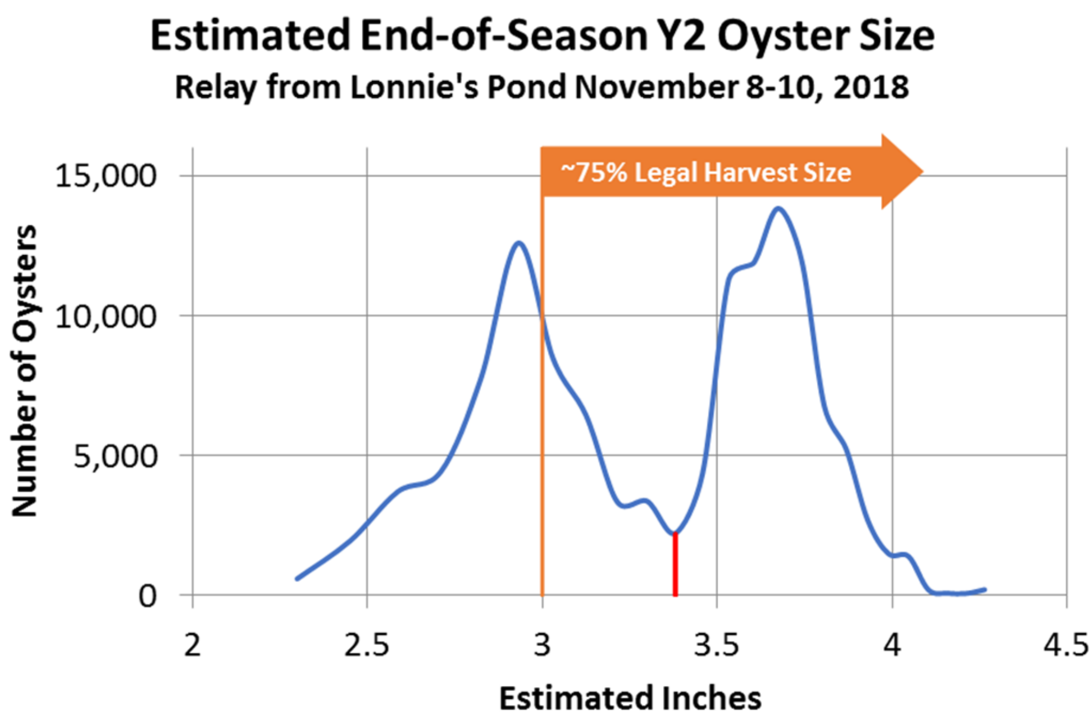


Figure 14. Histogram of Estimated Oyster Size for the Y2 Population Relayed in November 2018

Table 12 presents a summary of the disposition of Y2 oysters for the 2016, 2017 and 2018 growing seasons. The increased mortality in 2018 compared with 2016 and 2017 may be due to carrying over a significant fraction of runts from the 2017 Y1 population that was grown out in spat bags from 2 to3 mm seed. At deployment, these runts accounted for approximately 58 percent of the population but only 36 percent of the biomass, and their average individual weight was approximately 40 percent of the average individual weight of the studs. By the end of the growing season, these runts accounted for approximately 42 percent of the total population, and it was estimated that 65 percent of them were below the legal harvest size of 3-inch.

Table 12. Disposition of Y2 Oysters Grown Out in 2016, 2017 and 2018, by Population

	Percent of Initial Deployment Population		
Disposition	**2016**	**2017**	**2018**
Survived Overwintering	95	--	--
Relayed at End	--	95	72
Harvest Size (3”) at End	88	88	54

On December 14 thru 17,2018 the Y1 oysters were relayed to a land-based overwintering facility operated by the Town of Brewster. Each bag was processed individually to weigh the living oysters before they were transferred into totes. A nominal two red totes of dead oyster shells with a dry weight totaling less than 20 kg were collected from the entire Y1 deployment. The nitrogen content of these shells is less than 4g and accounts for less than 0.02 percent of the total nitrogen removed by the Y1 oysters.

A batch of 50 live oysters were randomly selected from each bag and the total weight of these counted oysters was recorded. The weight of the live oysters in each bag was divided by this average individual weight to calculate an estimated stocking density. This information was used to calculate the histogram of average individual weight for the population relayed in November 2018 shown in Figure 15.

The population estimates for each bag were added together to calculate that approximately 639,000 Y1 oysters were relayed, representing approximately 29 percent of the initial seed purchase. Equation (6) was used to estimate oyster size from the average individual weight and the results are shown in Figure 16. It is believed that if overwintered properly, approximately 98 percent of this population would be an appropriate size for sale as intermediate seed at the start of the growing season, if grown under a non-municipal propagation scenario. In this scenario, the largest 10 percent of the animals are likely to reach market size before July of the next growing season, and thus could have significant value per piece. It may be risky to sort them just before storage because of the risk of damage. Survival after sorting is likely to be much higher if done at the start of the next growing season.

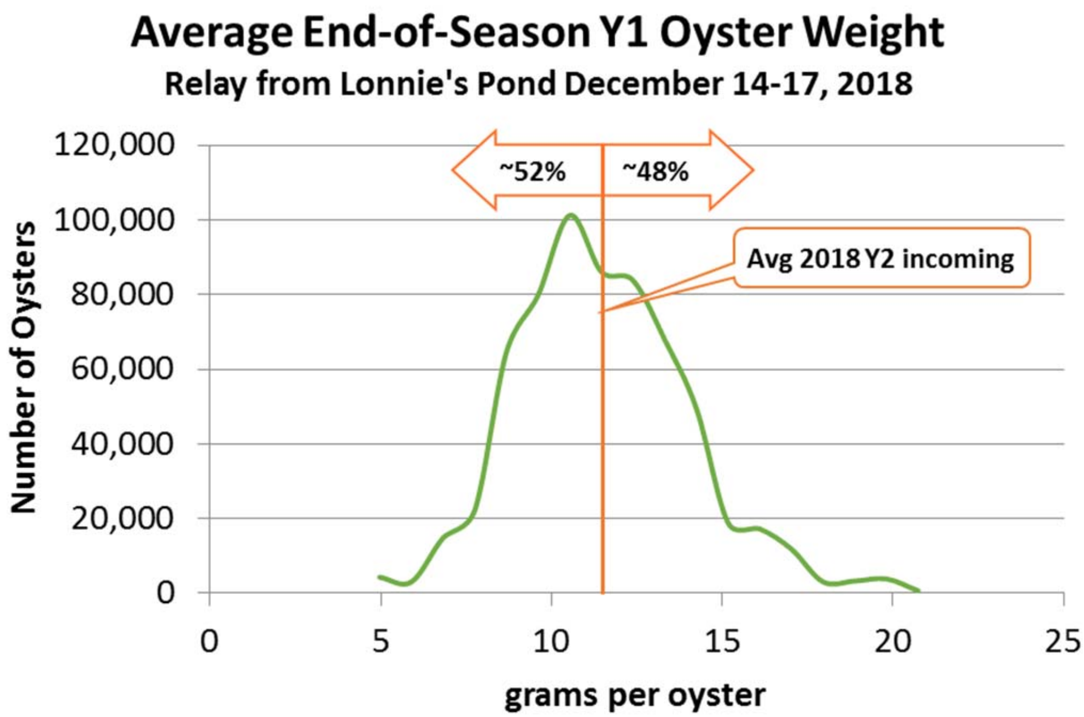


Figure 15. Histogram of Average Individual Weight for the Y1 Population Relayed in December 2018

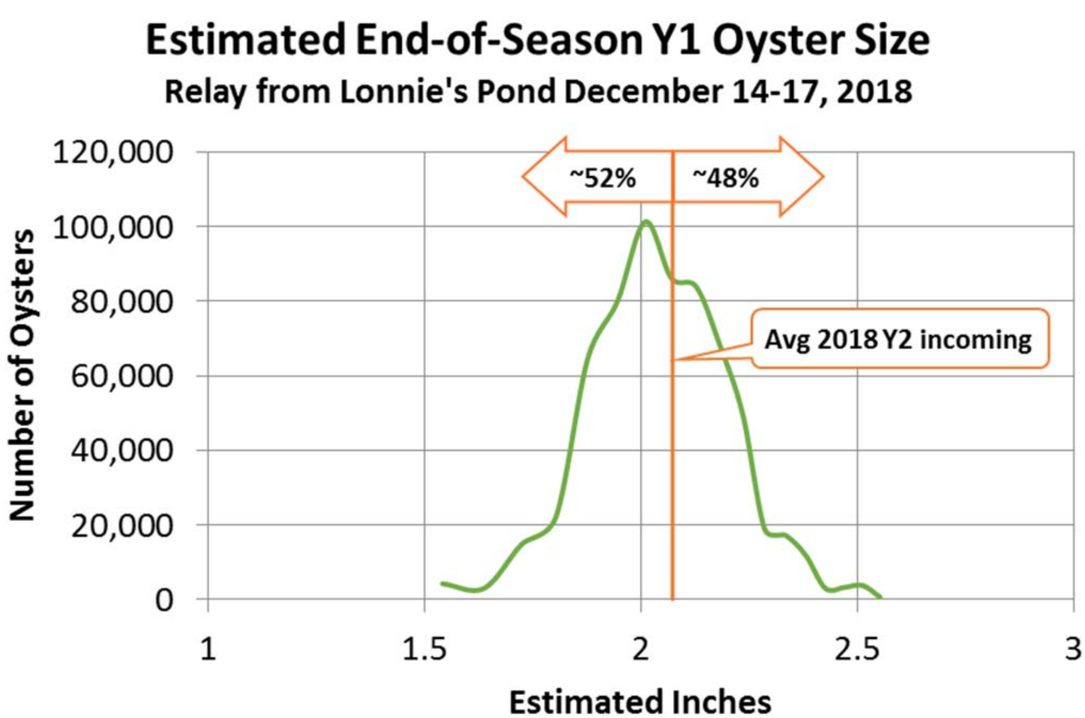


Figure 16. Histogram of Estimated Oyster Size for the Y1 Population Relayed in December 2018

Table 11 presents a summary of the disposition of the Y1 oysters grown from 2 to 3 mm seed in 2017 and 2018. There were no oysters corresponding to this grow-out strategy during the 2016 season as the Y1 oysters for that season had been preselected as studs coming out of a commercially operated upweller at a different site. The results from 2017 and 2018 suggest that 30 to 35 percent of the oysters that come in a 2 to 3 mm seed purchase in early May will have desirable characteristics for achieving efficient nitrogen removal in a high density floating gear configuration.

Table 11. Disposition of Y1 Oysters Grown from 2 to 3 mm Seed in 2017 and 2018, by Population

	Percent of Initial Seed Purchase	
Disposition	**2017**	**2018**
Runt after August	46	--
Stud after August	37	38
Stud at End	34	29
Not retained	17	71

Figure 17. Views of Y1 Oysters Relayed Out of Lonnie's Pond on December 14-17. 2018, Including a Look Inside the Overwintering Storage Facility at the Relay Destination

5. Conclusions and Recommendations

Significant observations from the 2018 growing season are as follows:

- The NE field , which had not been used in previous seasons, supported substantially higher growth rates for both Y1 and Y2 oysters;
- The average nitrogen content for Y1 oysters across the 2016, 2017 and 2018 growing seasons has been 0.32 percent ± 0.008 percent of harvest weight;
- Deploying four fields each containing 510 bags in an approximately 60-foot by 68-foot area is compatible with other uses of the pond;
- Growth rates in the innermost bags of the above fields can be 20 to 26 percent lower than growth rates in the outermost bags. Moving innermost bags to the outermost positions around the middle of the growing season in late August can help keep growth more even across the full population, as can swapping oysters from lower productivity fields into higher productivity fields;
- Y1 stocking densities of 1,000 oysters per bag are considered optimal in terms of nitrogen removal and commercial value of final product; and
- If 2 to 3 mm seed is purchased in early May, grown out using floating spat bags, and transferred around early July to 6mm diamond mesh bags, about 30 percent of the population will be retained and have desirable characteristics for achieving efficient nitrogen removal in a high density floating gear configuration. If overwintered properly, about 98 percent of this population could be sold as intermediate seed at the start of the following growing season if propagation did not occur under a municipal propagation permit.

Recognizing that the intent is to have a commercial contractor deploy Y1 oysters for the 2019 growing season, the following recommendations are made:

- Deploy Y1 oysters in four fields, each containing 510 bags in an approximately 60-foot by 68-foot area with stocking densities targeting 1,000 oyster per bag at the end of the growing season;
- Place the above fields in the areas shown in Figure18. This is to take advantage of the higher productivity conditions identified during the 2018 season in the northern region of the pond and deeper sections of the southern region of the pond;
- If oysters are ordered as 2 to 3 mm seed and raised in floating spat bags
    a. the initial order should be approximately 5,510,000 and they can be deployed into 680 floating spat bags in early May
    b. the floating spat bags should be flipped and cleaned every 4 days or less, especially at the end of June and in early July
    c. during early July, the seed in each floating spat bag should be divided into three equal weight portions, each of which can be placed in a 6mm diamond mesh bag
- If oysters are brought in from an upweller, the initial deployment should occur as soon as the target population is big enough to be retained in a 6mm diamond mesh bag, which is typically at a weight of 0.25 kg for 1,000 oysters;
- The 6mm bags should be maintained by flipping regularly, typically once a week during July and August; and
- The oysters should remain in the pond until the growing season is substantially complete, typically around the middle of December.

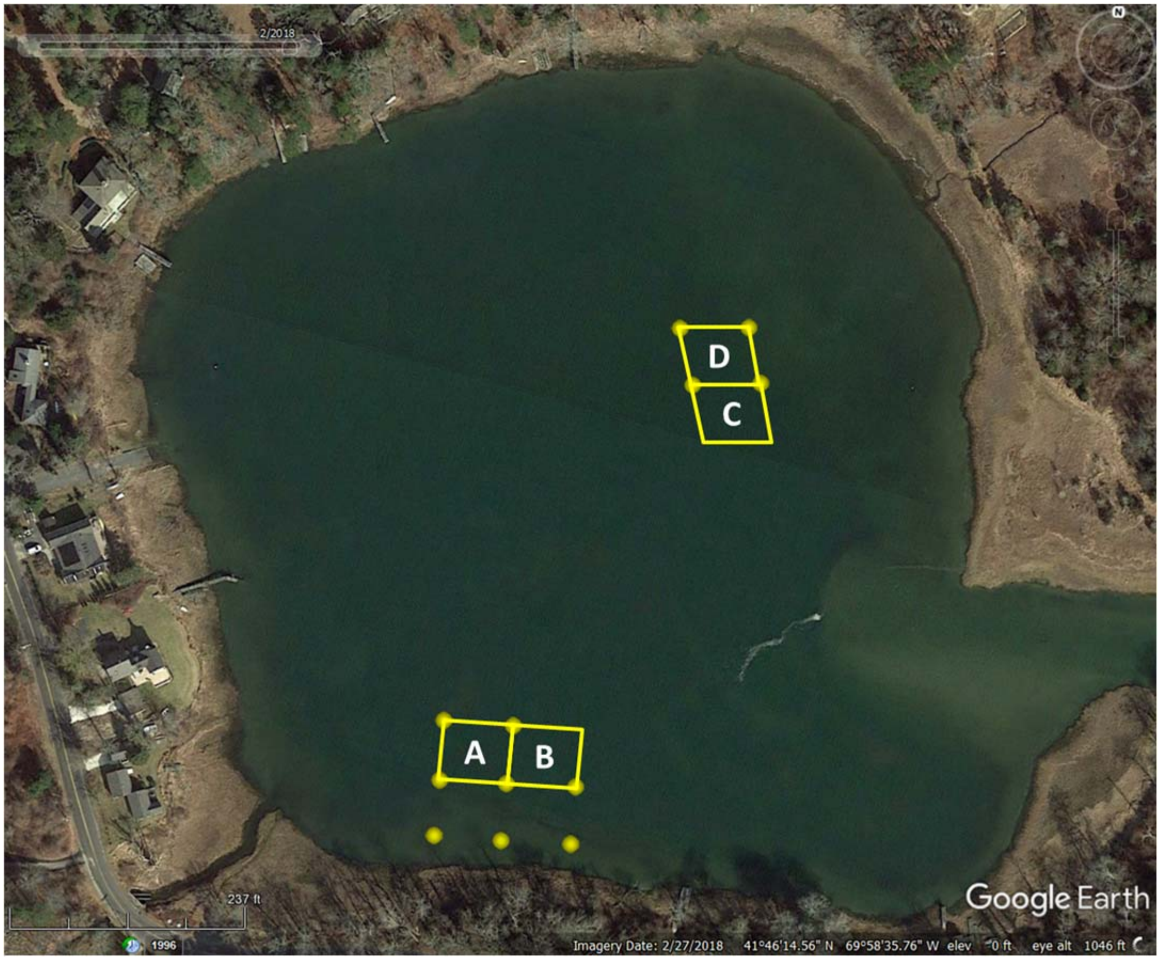


Figure 18. Recommended Feld Locations for 2019 Growing Season

The target nitrogen removal for 2019 is 75 kg across a deployment of 2,040 bags stocked at an average of 1,000 oysters at the end of the growing season for a total of 2.04 million oysters. Given that each bag occupies 8 square feet in the proposed layout, the target nitrogen removal of 75 kg corresponds to an annual removal rate of 200 kg of nitrogen per acre. Assuming a nitrogen content of 0.32 percent, the required biomass increase over the course of the season to remove 75 kg of nitrogen in 2.04 million oysters is 11.5 g per oyster. Starting with 2 to 3 mm seed deployed in early May, the average weight increase per relayed Y1 oyster at the end of 2018 season was 11.5 g. If the incoming weight of each oyster is 0.25g coming from an upweller, then the average oyster weight will need to reach 11.75 g by the end of the growing season. At 1,000 oysters per bag, this is a biomass increase of 11.5 kg per bag.

For Y1 oysters deployed in the areas recommended in Figure 18, the biomass increase averaged 10.9 kg per bag with an average population of 938 oysters per bag. If the population is increased to 1,000 oysters per bag the biomass increase is expected to be 11.6 kg, which exceeds the target of 11.5 kg increase per bag.

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Appendix A

Field layouts

Lonnie's Pond 2018 Demonstration Year

The figures below show the dates the areas were used during the 2018 demonstration year. Figure A-2 shows the dominant configuration during the Critical Period including July and August. Figure A-3 shows the dominant configuration for post-Critical Period growth.

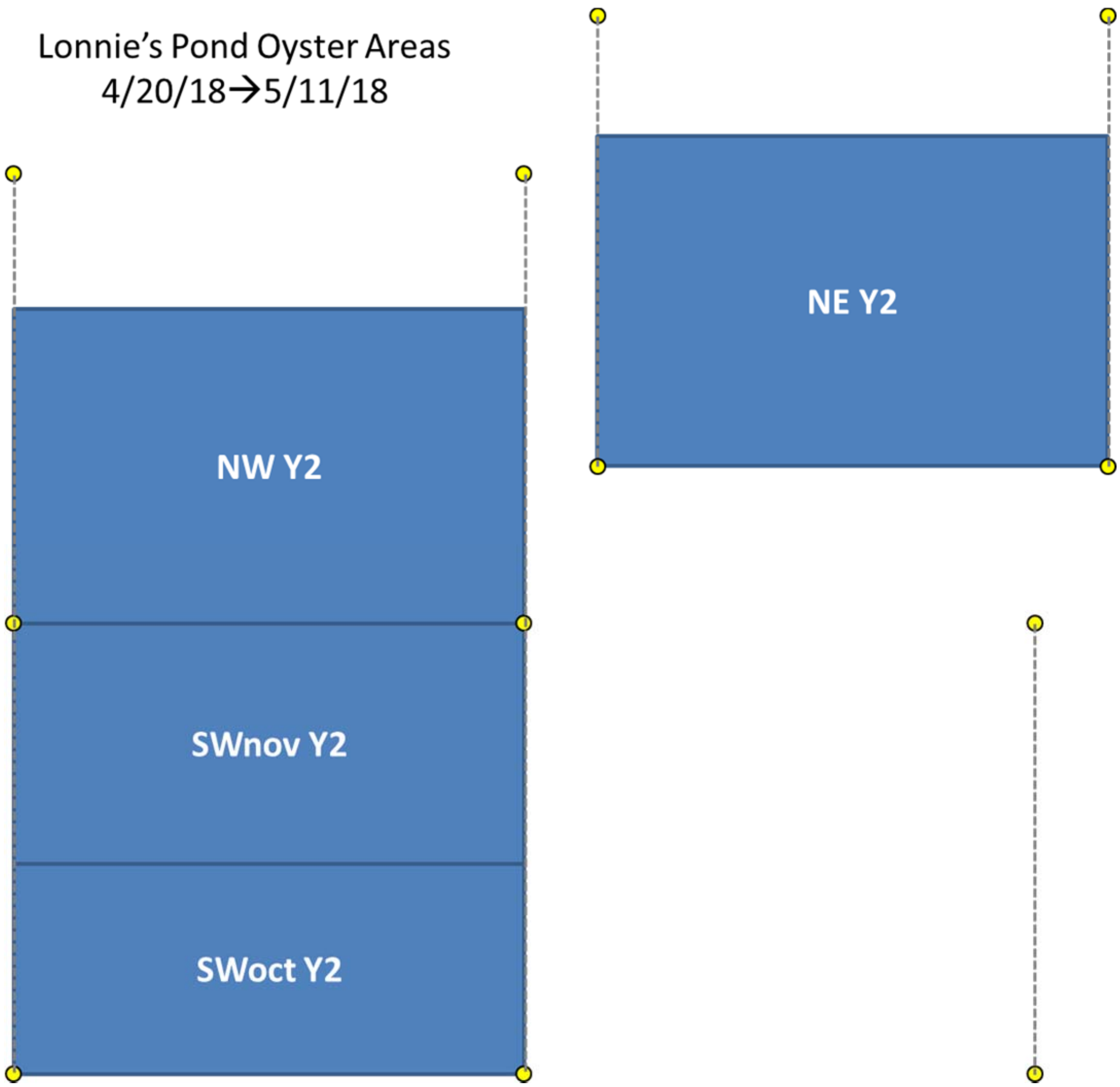

Figure A-1. Field layout for 4/20/18-5/11/18 showing initial deployment of Y2 oysters.

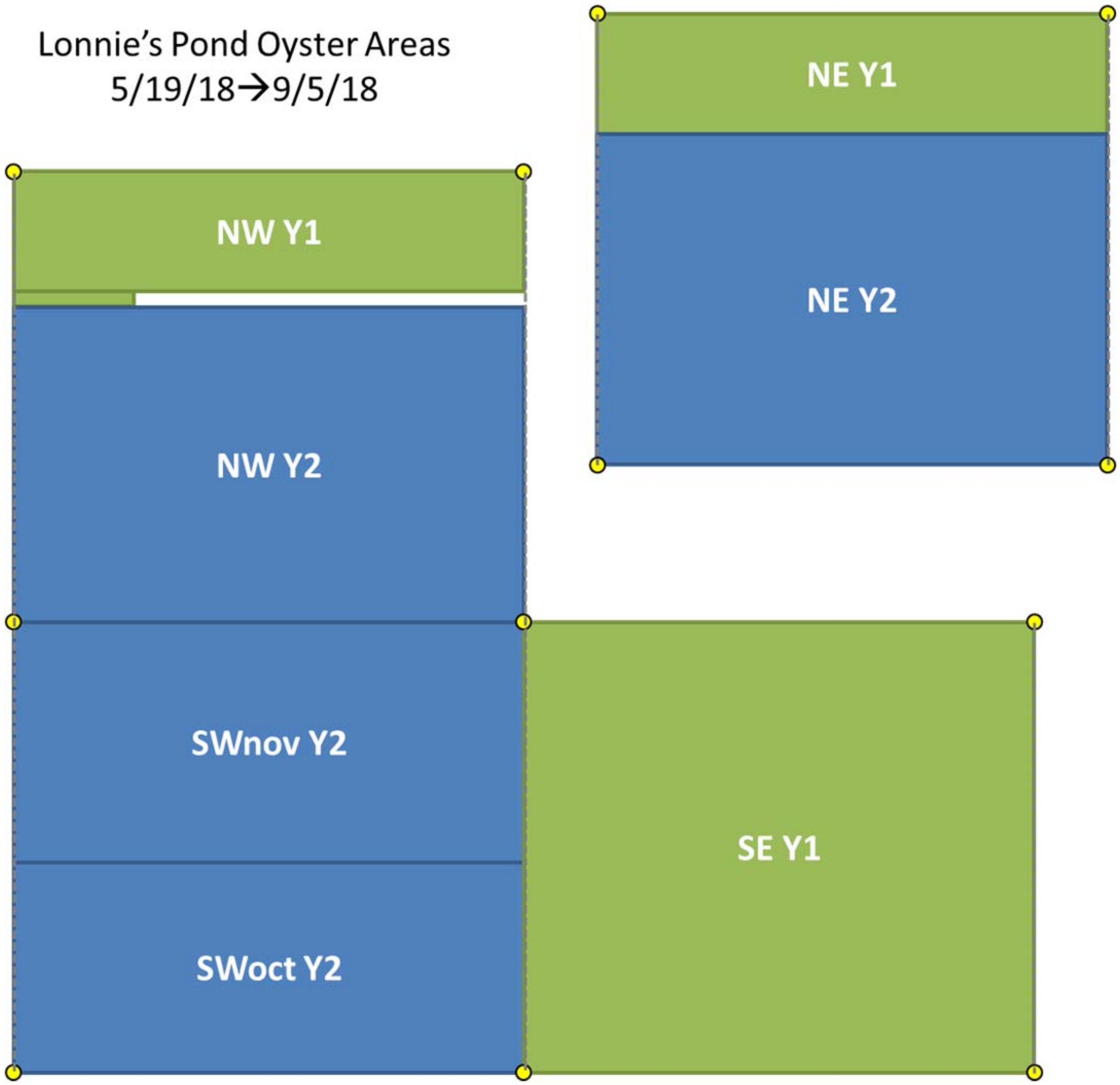

Figure A-2. Field layout for 5/19/18-9/5/18 showing areas with Y1 and Y2 oysters deployed.

The Y1 oysters in the southern 8 rows of the SE field as well as the areas of the NW and NE fields were moved from floating spat bags to 6mm diamond mesh bags on 6/19/18-6/21/18. The rest of the Y1 oysters in the SE field were moved from floating spat bags to 6mm diamond mesh bags on 7/15/18-7/17/18.

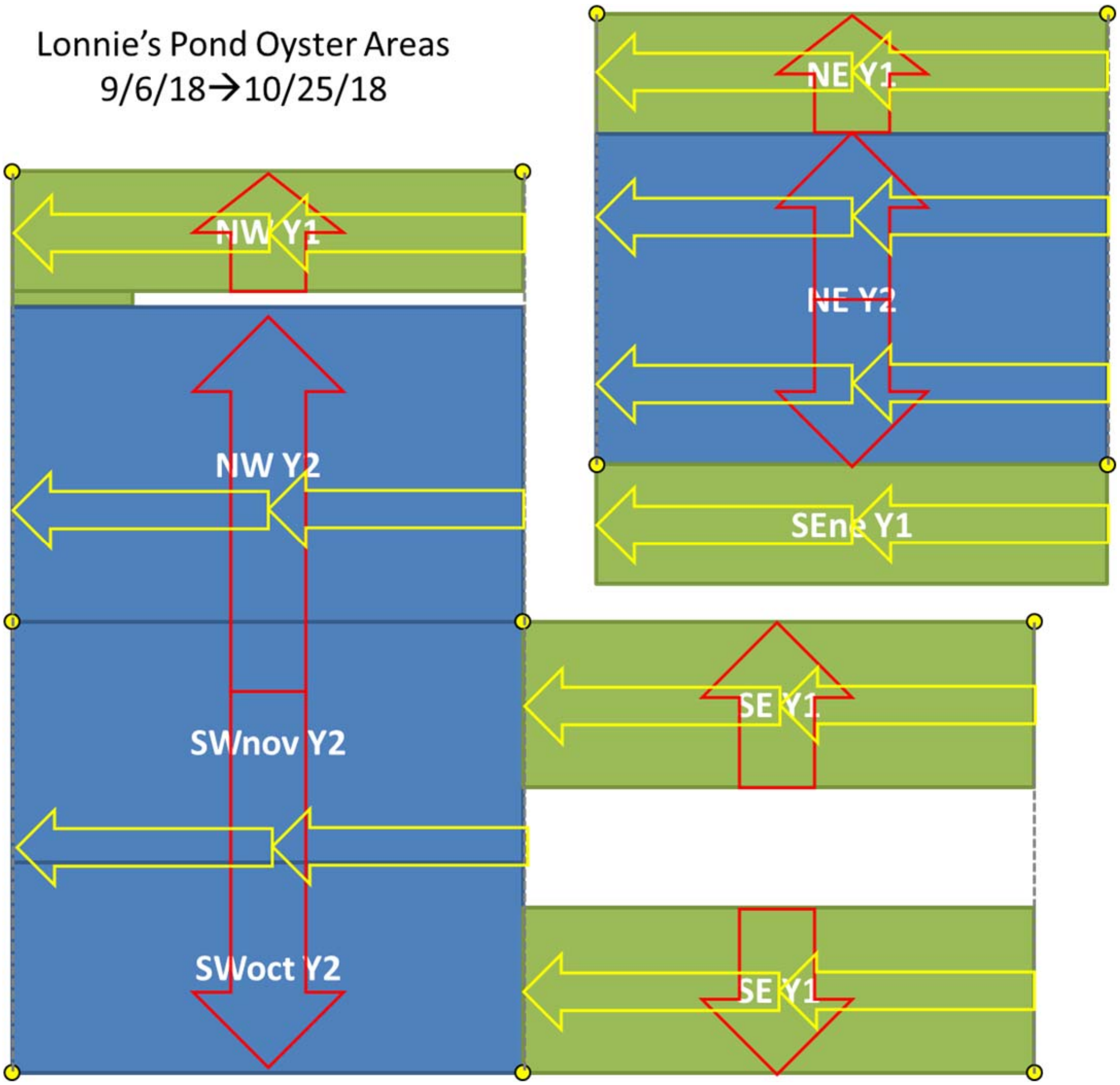

Figure A-3. Field layout for 9/6/18-10/25/18

Four of the centermost rows (13-16) and four of the outermost rows (1,2,29,30) of the SE field were transferred to the NE field to assess whether the slower growth rate in the SE field was a function of differences in growing conditions or characteristics of oysters. In the transition between the layout of Figure A-2 and Figure A-3 the position of the bags in each row was shifted so the bags in the outermost positions were moved to the innermost positions, and the bags in the innermost positions were moved to the outermost positions. This was accomplished by

- Moving the central rows to the outside edges as shown by the red arrows, so NE11 becomes NE1, NE20 becomes NE2, NE9 becomes NE3, etc.
- Shifting columns I through Q westward to occupy columns A through I, and columns A through H eastward to occupy columns J through Q as shown by the yellow arrows.

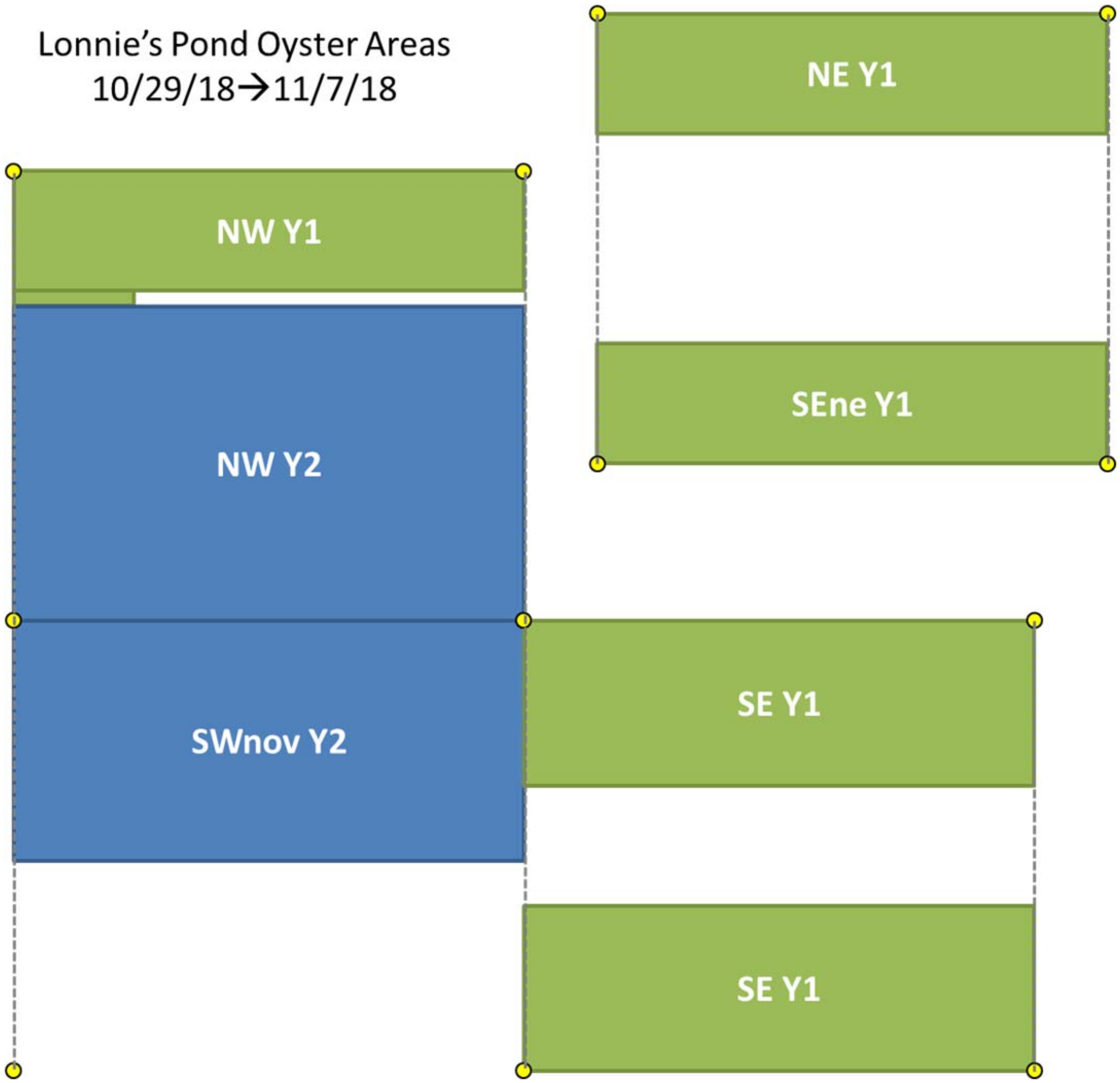

Figure A-4. Field layout for 10/29/18-11/7/18 showing configuration after the first group of Y2 oysters was relayed to a recreational harvest site.

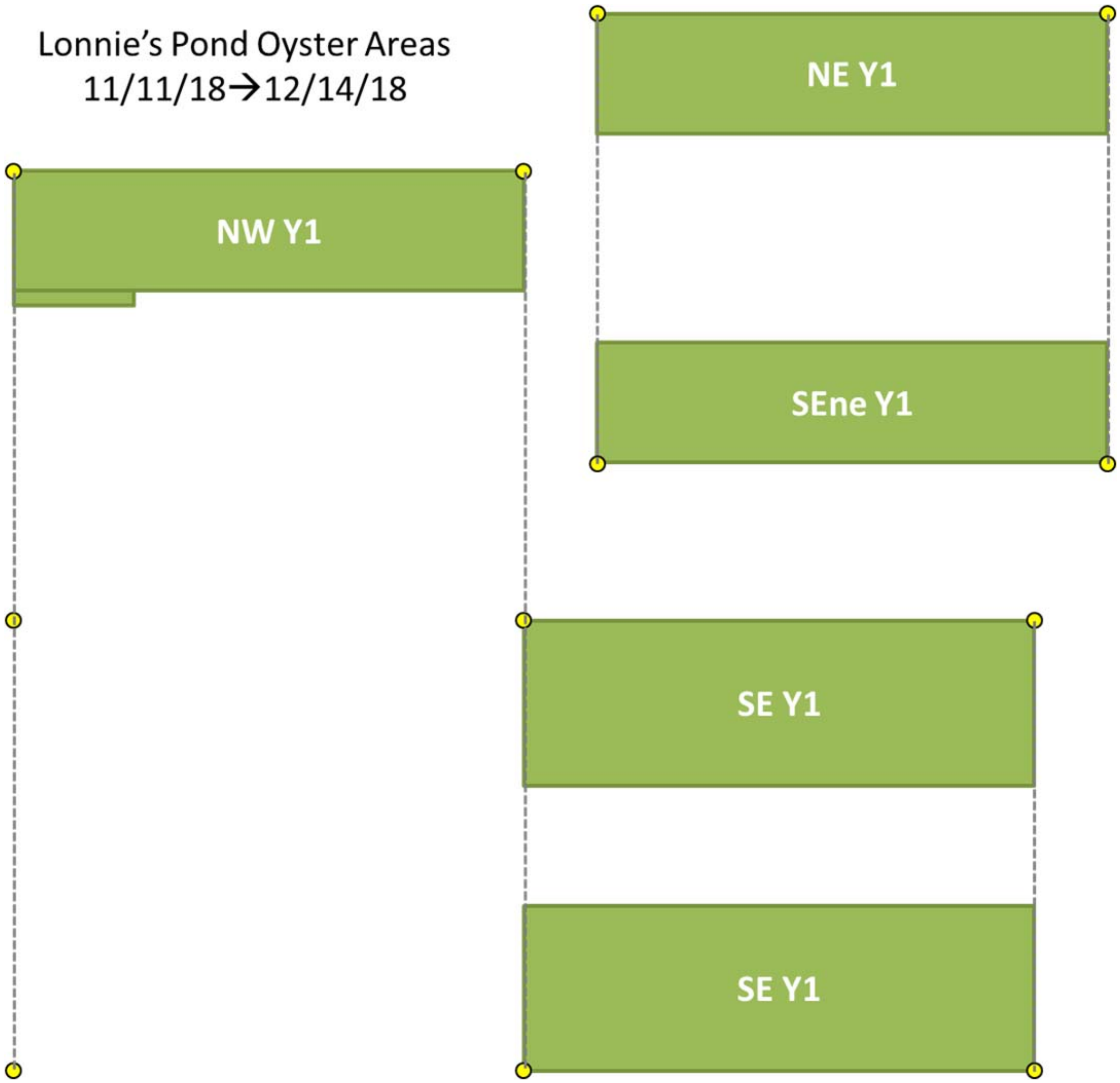

Figure A-5. Field layout for 11/11/18-12/14/18 showing configuration after the second group of Y2 oysters was relayed to a recreational harvest site.

By 12/17/18, all floating gear had been removed from the project area.

A composite diagram of the areas used in all three demonstration years is shown in Figure A-6.

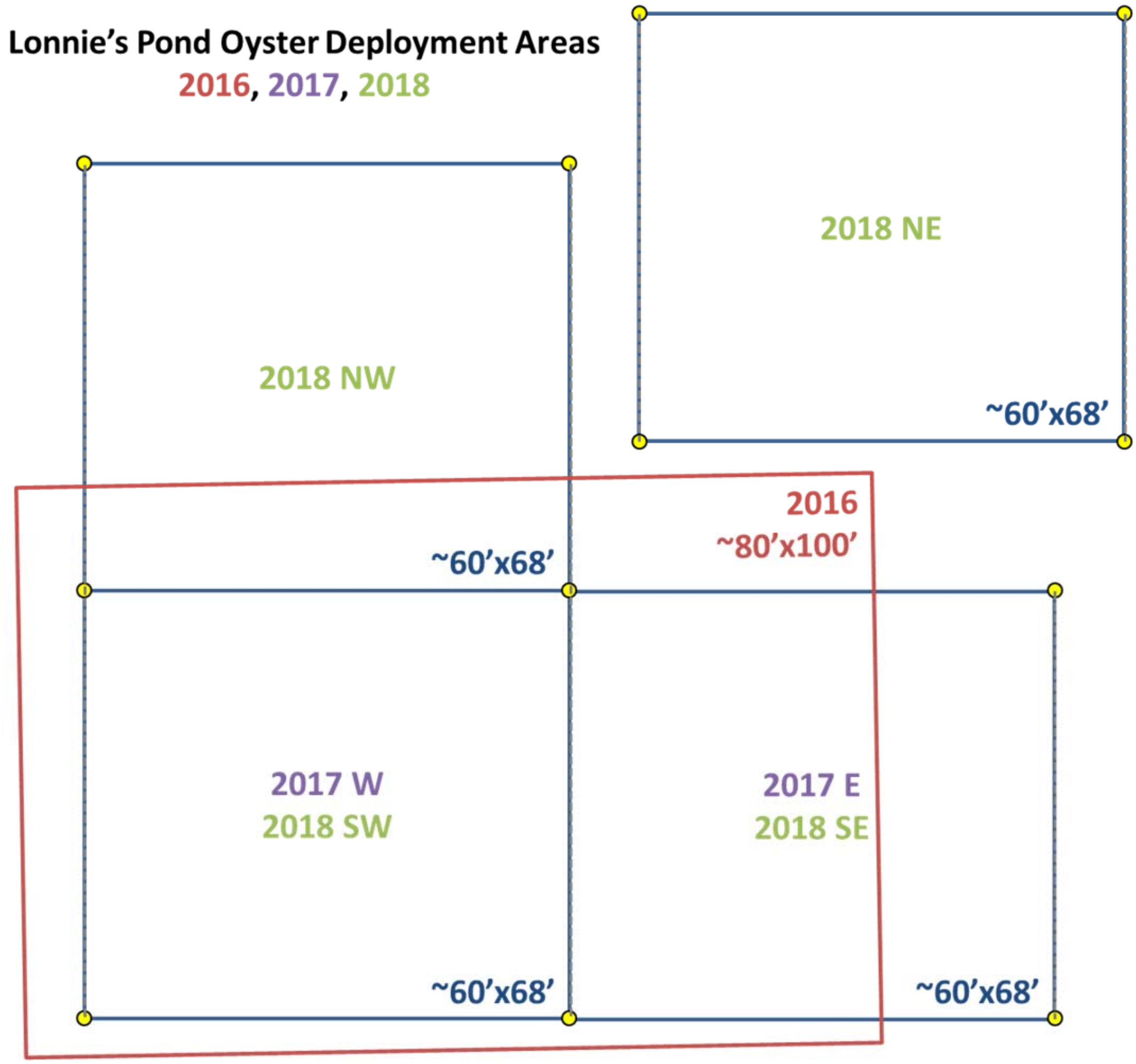

Figure A-6. Composite diagram of deployment areas used in all three years of demonstration.

Appendix B

Oyster Growth Details

Lonnie's Pond 2018 Demonstration Year

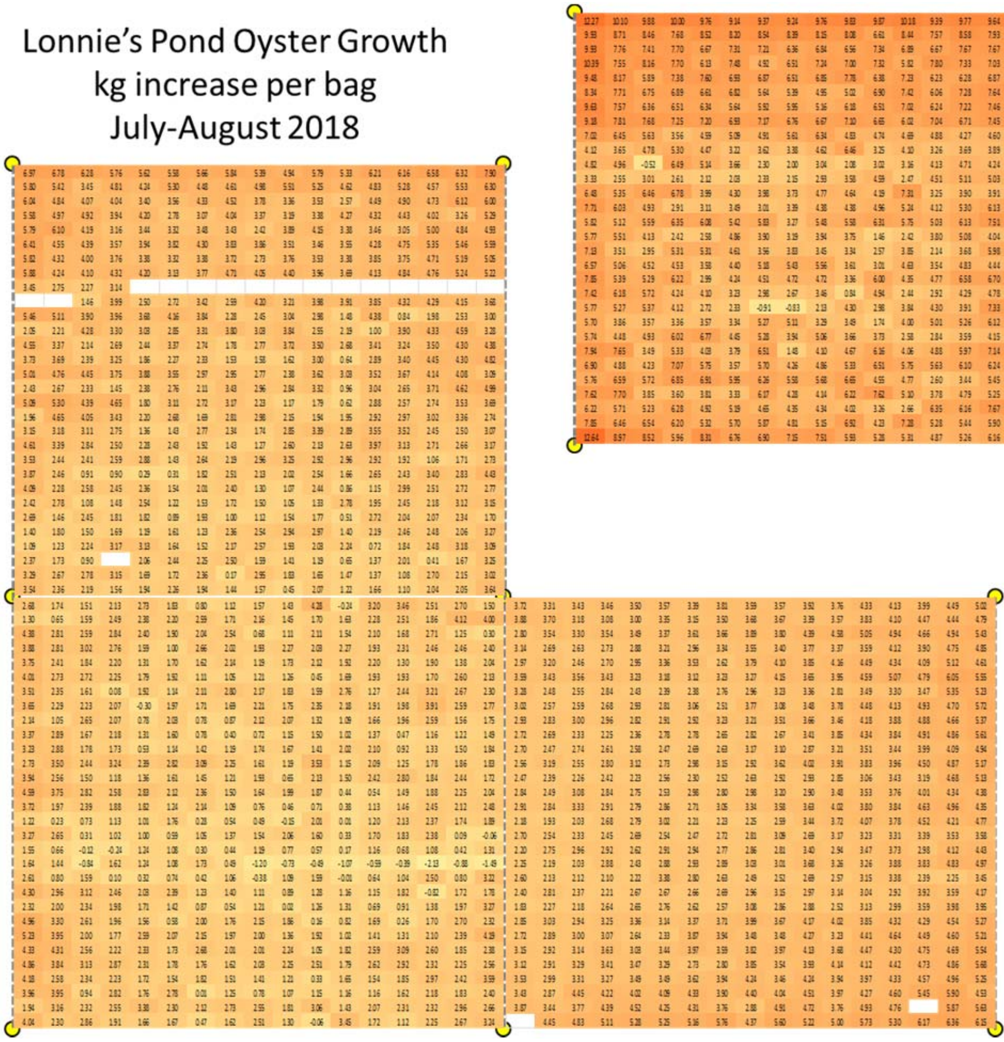

Figure B-1. Average biomass increase in kg per bag during July and August 2018

Table B-1. Ratio of Edge bag to Middle bag average biomass increase during July and August 2018.

Field	Y1 Middle:Edge	Y2 Middle:Edge
NW		0.67
SW	0.78	0.77
NE	0.74	
SE	0.80	0.72

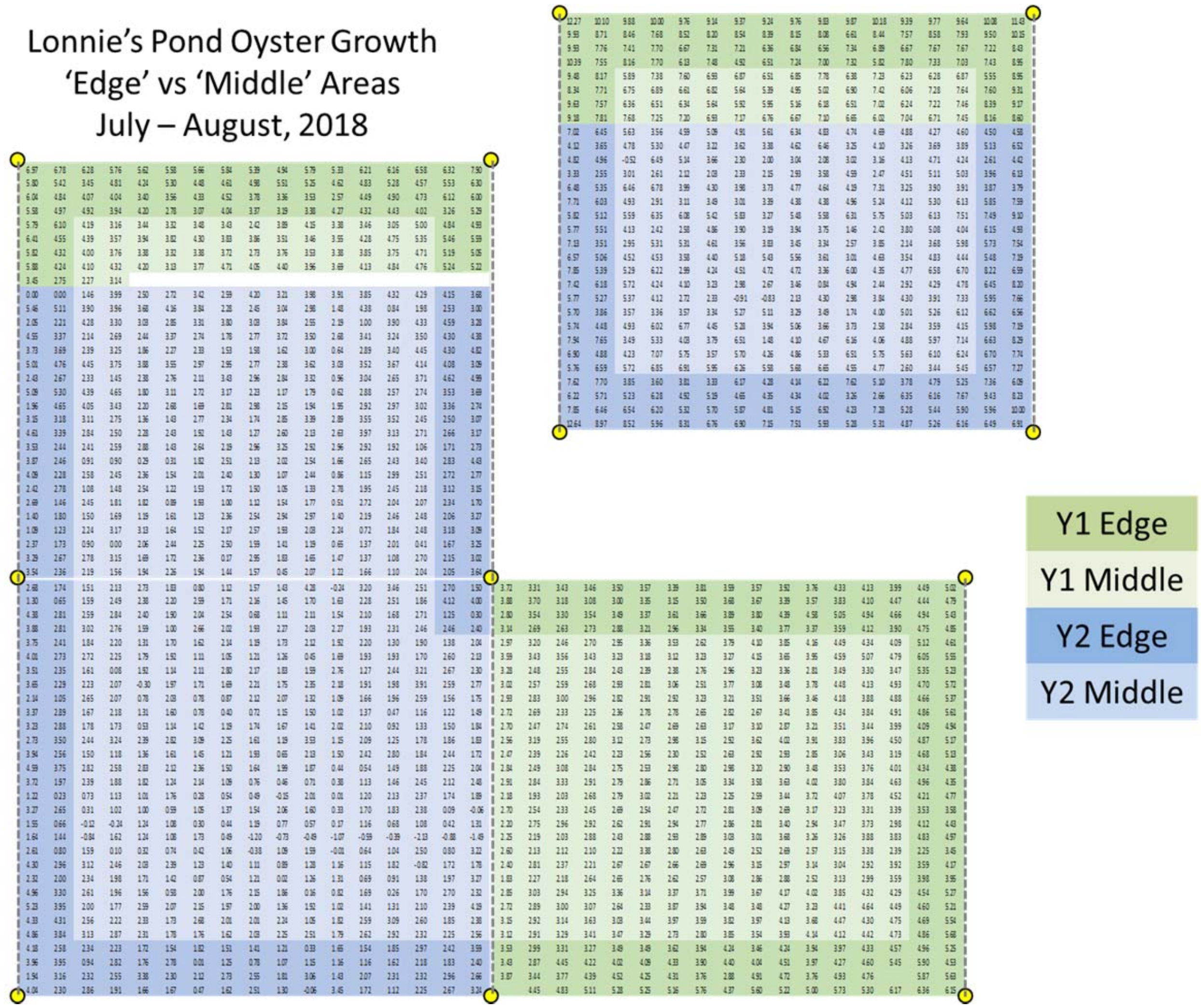

Figure B-2. Designation of Edge and Middle bags for ratios presented in Table B-1.

The progress of average oyster biomass density for various groups of oysters is shown in Figure B-3. The following important features of this data can be observed:

- the NE field supported the highest average biomass density of approximately1.8 kg/sq.ft. which corresponds to over approximately14 kg of live oysters per bag.
- The southern portion of the SW field (SWY2oct) and the southern portion of the SEY1 field, which are closest to shore and in the shallowest water, supported the lowest average biomass density of approximately0.97 kg/sq.ft. which corresponds to approximately7.8 kg of live oysters per bag.
- The northern part of the SW field (SWY2nov), and the NWY2 field which are over deeper water supported an intermediate biomass density of approximately1.2 kg/sq.ft. which corresponds to approximately9.6 kg of live oysters per bag. The growth rate for these two fields decreased noticeably in the middle of the critical period and never recovered.

- The SEY1ne population, which was moved out of the SE field to the southern edge of the NE field 5 days after the end of the Critical Period. From the time of this move to the end of the growing season, the SEY1ne oysters that were moved grew approximately40% faster, at an average rate of approximately5.6 g/sq.ft./day, than the SEY1 oysters that remained in the SE field, which grew at an average rate of approximately4.0 g/sq.ft./day. This suggests that the growing conditions associated with the field location were the dominant factor controlling the rate of growth of the oysters.

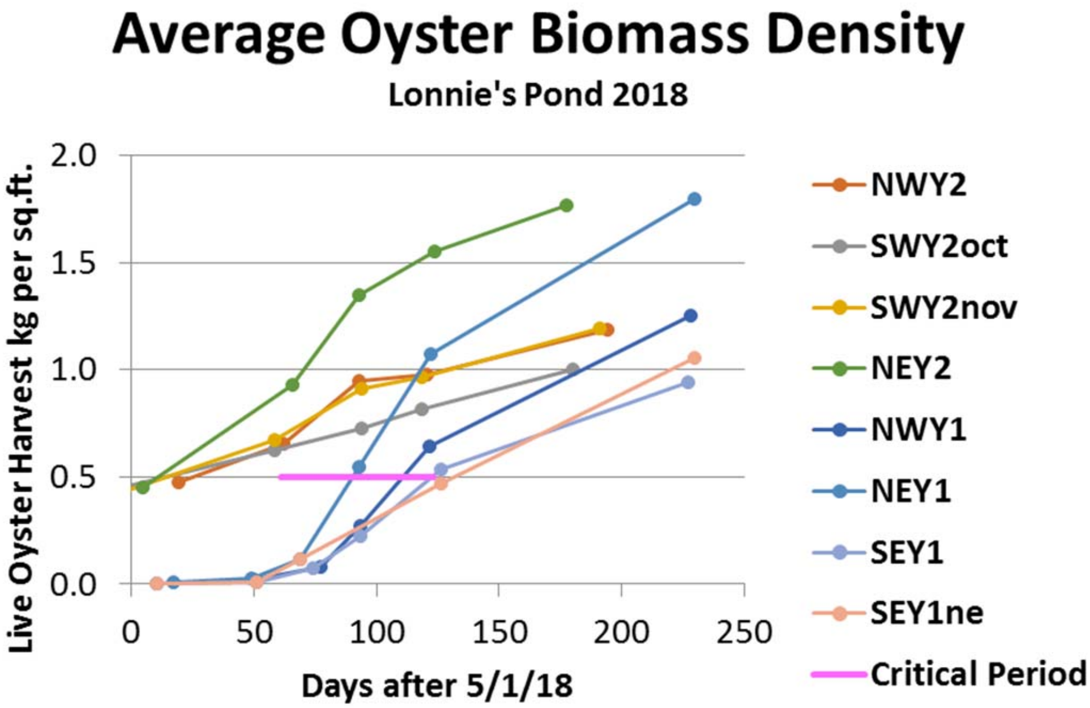

Figure B-3. Progress of average oyster biomass density for various groups of oysters.

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