

Direct Setting of Eyed Larvae: *In situ* spat-on-shell

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## Larvae Overboard on Shell

Experiments were conducted in hopes of developing techniques whereby an individual, with a minimum of equipment and labor, can utilize eyed larvae to profitably induce strike on appropriate oyster leases. The best-case scenario would be to utilize shell already present on the lease. Adding clean shell produced better results.

### Dudley Biddlecomb's experiments using larvae from Mike Congrove

Four experiments were conducted. On 08/15/13 four PVC poles were installed in a square with 25 foot sides in about 4 feet of water (37 48.73N, 76 19.00W) near where wire cylinders were subsequently installed (lease #9773). Shell was dredged from the surrounding area of the lease and dumped between the poles.

Three wire cylinders 4 ½ feet in diameter and 4 feet tall were constructed using 1" x 1" vinyl-coated, galvanized, welded wire mesh. The bottom three feet were covered with filter cloth (Dura-Shield from Walmart). On 08/21/13 two cylinders were staked side-by-side in three feet of water in Cranes Creek (Photo #1) and one cylinder staked near the head of a small arm of Reason Creek (37 50.32N, 79 17.78W – lease #15032).

Ten bushels of shell from Purcell's Seafood were dumped in one of the cylinders at Cranes Creek and in the cylinder at Reason Creek. The other cylinder at Cranes Creek received 18 bags, each containing about ½ bushel of shell.

The next day, on 08/22/13 at high slack tide under calm conditions, 9 million triploid larvae provided by Mike Congrove were added to all four experiments. These were the same larvae used in Cockrell's spat-on-shell tank. 6 million were dispersed on the mound of dredged shell and one million were dispersed in each of the wire cylinders. Larvae suspended in water were slowly poured into a funnel at one end of a PVC pipe with a 1 meter-wide "T" at the other end into which holes had been drilled. The "T" was moved over and in contact with the shell or bags. It was intended that the "T" deliver larvae directly to the bottom and disperse them over a wider area than if only the pipe was used.

On 08/27/13 the filter cloth was removed and only two oysters had attached to the cloth.

On 10/22/13 the experiments were terminated and the shell from the wire cylinders tonged out and dispersed on the leases.

**1)** The Cranes Creek wire cylinder with 10 bushels of loose shells had very few shells with no strike. There were no differences between shells on top and those just above the mud, or about 9 inches below the top of the shell. A sample from the top was photographed (Photo #2) and counted:

Number of strike	0	1	2	3	4	5	6	7	8	9	10
Number of shells	6	18	3	3	9	12	8	6	4	2	1

Shells with more than 10 strike: 11, 11, 11, 12, 12, 13, 13, 13, 13, 16, 16

Summary: 420 strike on 86 shells. 78 (19%) of the strike were on the outside of the shell. The ploidy of five shells from the bottom of the pile just above the mud was determined and all were triploid.

**2)** The Cranes Creek wire cylinder with 9 bushels of bagged shells had relatively uniform strike within all the bags no matter their location and there was little difference between the center of the bags and the outside. A typical bag had 1.7 strike per shell on 56% of the shells.

**3)** Three samples from the surface of the shell between the poles were tonged and counted:

Number of strike	0	1	2	3	4	5	7	# strike	#shells
Number of shells	84	13	10	8			1	64	116
	154	23	10	3	1	1		61	192
	110	50	20	8	2	2		132	192

Summary: 257 strike on 500 shells. Samples from within the area of the poles as well as samples obtained by tonging between 25 and 40 feet outside the poles (Shown in Photo #3) were all diploid. Many of the shells had strike ranging in size from about one centimeter to several centimeters, suggesting the natural strike took place over the summer. The shell was obviously sufficiently clean for strike to occur. It is puzzling why no triploids were found and insufficient sampling is a possibility.

**4)** The Reason Creek wire cylinder with 10 bushels of loose shell also had relatively uniform strike top to bottom. The strike was smaller and less abundant than at Cranes Creek, probably because of less circulation near the head of the small arm of Reason Creek. The count from a sample from the middle of the cylinder was:

Number of strike	0	1	2	3	4	5	6	7	8	9	10
Number of shells	98	45	25	42	1	6	4	1	3	2	3

Shells with more than 10 strike: 11, 13

Summary: 382 strike on 232 shells.

The best results were obtained on the loose shell in the wire cylinder at Cranes Creek, an area with good water circulation. This experiment should be repeated with variable amounts of shell and larvae. The larval density, 1 million larvae added to 10 bushels of shell, was three times larger than in the spat-on-shell tank, 7 million larvae added to 200 bushels of shell, and both may be excessive. Experiments should also be done comparing recently shucked shell with shell “seasoned” for longer periods of time, or even over winter. The effect of fouling by biofilms on shell in the water for different lengths of time should also be investigated. The wire cylinders can probably be removed after a few days rather than leaving them in place for several months. Larvae only remain in a downweller or under static circulation in a spat-on-shell tank for three or four days. Rather than removing all the shell after several months as was done with these experiments, some of the shell piles that remain after removing the wire cylinder should be sampled and then left undisturbed, possibly even until the next growing season.

This technique, the “poor man’s portable spat-on-shell” should be repeated multiple times using a hard, preferably shelled, bottom as a “nursery.” The cylinder, or cylinders arranged around a numbered stake, should be deployed, filled with shell and then larvae added. After about four days the cylinders should be moved and the process repeated. The experiments should be designed to determine how to maximize strike while minimizing the labor and the number of larvae. The oysters can either be dispersed on the lease or moved out of the “nursery.”

The bags at Cranes Creek were similar to the loose shell at Reason Ck. 1% strike from 1 million larvae yields 10,000 oysters. If half of the oysters are recovered and marketed, about 16 bushels would be worth more than the cost of larvae plus labor, fuel, etc. More strike at Cranes Creek than at Reason Creek in cylinders with loose shell suggests water quality/circulation must be considered as a variable.

Unfortunately, the mounded shell produced the worst results, for reasons that are not clear since natural strike of a variety of sizes was present. This experiment should be repeated with shell cleaned as much as possible by either dunking the dredge several times and/or washing the shell with a stream of water in the dredge or on the culling board. Experiments should also be conducted using containment cylinders on top of the mounded shell, and by releasing the larvae the day after the shell is dredged.

### **Experiment at Cockrells railway with larvae from grant to Myles Cockrell**

In early-mid July 1,200 bushels of shell from Purcells Seafood were spread on about ¾ acre of Cockrell's lease adjacent to the railway (37 53.93N, 76 18.04W, lease #18627) and east of the spat-on-shell tank. 6M triploid larvae were put overboard the afternoon of 07/25/13 under calm conditions, just after larvae from the same batch were added to the spat-on-shell tank. The larvae, suspended in water, were dribbled into a funnel attached to a weighted hose as the skiff was poled around the area. We had hoped to add the larvae within a few days of the shell being spread, but larvae were not available due to hatchery problems and deployment was delayed about ten days. On 08/05 after the spat-on-shell tank was terminated, over 100 shells were tonged and examined, and one shell with multiple strike was found. We tonged again on 08/15 and found no strike even when we tried to get down into the pile.

On 08/22 we dribbled another 6M triploid larvae in the same area under calm conditions, again using larvae from the same batch added to the spat-on-shell tank. This time we used "T"-shaped manifolds to spread the larvae out. One PVC manifold was attached to a hose whereas the other was all PVC pipe, which worked better in the shallow water because it could be used to pole the skiff around. Some of the shell still had clean surfaces.

Shells were retained from each of the 6 spat-on-shell tank cycles in 2013 and kept in running water in a trough at the railway. Photo #4 was taken on 10/28/13 and shows strike on shells from the spat-on-shell tank started on the same date larvae were put overboard on Cockrell's lease. Some of the strike on larvae deployed 08/22 are smaller than those deployed 07/25, but there is overlap in size.

On 10/26/13 about a dozen samples were taken using tongs. All samples contained at least one shell with strike.

<u>number of strike</u>	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>strike/shell</u>
multiple samples	200	18	4	2	0.14
worst single sample	30	2	1		0.09
best single sample	12	3	5	2	0.86

Most of the strike was slightly larger than was true of the shells saved in the trough (Photo #4). This is not unexpected as growth in the "wild" was likely better than

growth in the trough. It is not possible to determine which larval release was responsible for the strike. The results from the 07/25/13 run in the spat-on-shell tank were worse than any other of the six cycles in 2013. All five samples analyzed for ploidy, shown in Photo #3, were triploid.

Assuming 720,000 shells were deployed (1,200 bushels \* 600 shells/bushel), that there are 0.1 strike/shell and that half the strike can be recovered and marketed, then 120 bushels of oysters are potentially harvestable, worth more than the cost of the larvae. Dredging this area in the future will determine the number of marketable oysters. It is possible that larvae flushed out of the spat-on-shell tank when raw water was added could have contributed to the observed strike.

### Summary

Here is a comparison between the five experiments and the spat-on-shell tank:

	% no strike	strike/shell	% strike*
wire cylinder, loose shell, Cranes Ck	2	5.4	3
wire cylinder, bags, Cranes Ck	44	1.7	1
mounded shell, Cranes Ck – natural strike	69	0.5	
wire cylinder, loose shell, Reason Ck	42	1.7	1
Cockrell shelled bottom	80	0.3	0.1
Spat-on-shell tank 07/25/13	48	1.4	3
Spat-on-shell tank 08/22/13	35	4.0	9

\* % strike is number of shells (600/bushel) \* strike per shell / number of larvae

The results from the wire cylinder at Cranes Creek are as good as results obtained in the spat-on-shell tank, albeit with a higher larval density. Even under the best of conditions, in a downweller striking on microcultch, the percent strike is rarely better than about 20%. Taking into account the capital investment and labor, the results are very encouraging. The direct application of larvae in wire cylinders containing clean shell, the “poor man’s portable spat-on-shell,” should be pursued as should direct application of larvae to mounds of clean shell. Increasing the number of eyed larvae released into the wild under circumstances that favor their attachment constitutes a strategy for increasing Bay oyster populations that is much less expensive than the construction of “sanctuary” reefs or widespread spat-on-shell deployment. Release of hatchery-raised eyed larvae on appropriate bottom is preferable to relying on unreliable and sporadic natural set. The cost of larvae can be reduced in the future, unlike the cost of shell, fuel or labor. More importantly, this strategy can potentially affect the economics of many people who hold appropriate leases. The potential for much more widespread distribution of (preferably fertile) oysters is obviously advantageous.

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## Photographs



Photo #1: Wire cylinders being installed in Cranes Creek on 08/21/13. Note the filter cloth, just visible above the water. Photo courtesy of Karen Hudson.



Photo #2: A sample tonged from the top of the Cranes Creek wire cage containing loose shell on 10/22/13. The shells have been turned concave up. Note the strike knocked off the shells by rough handling during tonging. Five samples from shells about nine inches below the top of the shell pile, just above the mud, were all triploid.



Photo #3: Samples submitted for ploidy 11/7/13. "MC" is from Cockrells' shelled ground. The strike is larger than the strike on shells from the spat-on-shell tank held in the trough at the railway, shown in Photo #4. All five samples were triploid. "CAGE" is from the bottom of the shell in the wire cylinder from Cranes Creek. All five samples were triploid. "DB" is from outside the 25 ft. x 25 ft. area where the shell was concentrated by dredging at Cranes Creek. All five samples were diploid. Strike is larger on surface samples (MC and DB) than from below the surface (Cage).



Photo #4: Shell saved from the spat-on-shell tank when it was emptied, held in a trough of running water at Cockrell's railway. Larvae were added to both the tank and to the shell on Cockrell's lease on the dates noted and the tank was emptied about two weeks later. Photo taken 10/28/13. Compare to sample "MC" in Photo #3, taken 10/26/13.