Development of an Improved Oyster for Massachusetts Shellfish Farmers: Field Test of Seedless Oysters

Final Report

(*Submitted by William Walton & Diane Murphy – April 25, 2011*)

Triploidy has been widely implemented at a commercial-scale by producers of the Pacific oyster, *Crassostrea gigas,* in a number of regions around the world, but adoption of this method has been slower by commercial producers of the eastern oyster, *C. virginica*. In response to increasing interest in triploid oysters by Massachusetts shellfish farmers, we conducted a three year study, funded by the Massachusetts Department of Agricultural Resources. The third year was provided (at no-cost) to provide an additional year of data.

To evaluate the potential performance of naturally-induced triploid *C. virginica* (which are approved for human consumption whereas chemically-induced triploids are not) in the southern New England region, we began a comparative field test of triploid and diploid oysters at twelve shellfish farms along the Massachusetts coast in 2008. These twelve shellfish farms represented growing areas in Cuttyhunk, Ipswich, Provincetown, Orleans, Dennis, Eastham, Wareham, Barnstable, Onset, Wellfleet Harbor, South Wellfleet, and Chatham (Fig.1). All study sites were maintained from 2008 through 2010, except for Ipswich which dropped out in 2009 due to difficulties within the town and in 2010 Orleans was lost due to unanticipated winter mortality.


Fig.1. Massachusetts study sites

In both 2008 and 2009, participating shellfish farmers took summer delivery of paired diploid and triploid seed, spawned and reared at ARC in Dennis, MA. Seed were raised in standard commercial oyster plastic mesh bags (stocked in the first year at 500/bag and 150/bag in the second year), positioned in vinyl-coated wire racks (Fig.2) placed on-bottom. Seed and gear were maintained by the participating shellfish farmers throughout the study. .

Triploids and diploids survived, on average, equally well. For all growth and condition results, there was a very strong effect of site; the effect of ploidy depended heavily on where the oysters were being raised. In the first year of grow-out, there was not a clear difference between the triploids and diploids, though at a small subset of sites triploid seed were larger than the paired diploid seed. In the second year of grow-out of the 2008 crop, at a subset of sites the average daily change in the various shell dimensions, dry shell weight, and tissue dry weight were significantly larger for triploids compared to the paired diploids. Analyses indicated that triploids had heavier shells and more meat (per millimeter of shell height) than paired diploids, again at a subset of sites. When we examined condition index over the growing season, we observed a trend for triploids to equal or exceed the condition of paired diploids. The only measure where diploids appeared to outperform paired triploids was in terms of the 'fan' shape of the oyster (shell length/shell height, where a higher number indicates a broader oyster). At a subset of sites, diploids had significantly higher ratios compared to paired diploids (though even the lowest ratios observed appeared to be acceptable to the market).

Final results from the field study, which concluded after the 2010 growing season, indicate that triploid oysters have the *potential* for large advantages in terms of growth and condition. Due to the large site-to-site variability, though, it is recommended that a side-by-side trial be run before large-scale purchases.

Methods

In 2008 we initiated a test of native triploid oyster (*Crassostrea virginica*) growth and survival compared to diploid oysters at twelve sites (Research Farms) throughout the Cape Cod region – representing a wide variety of water bodies and growing conditions. This field experiment component concluded fall 2010.

In addition, temperature loggers were placed at each site coincident with the deployment of the triploid oysters. Temperature loggers were periodically removed to download stored data before redeployment. These data were compiled and made available as Excel spreadsheets to the Research Farm site operator(s).

We asked Research Farm participants to treat all oyster bags within an experiment the same way. For example, when moving any of the triploid oysters up to a larger mesh bag, all the diploid oysters in that experiment also had to be moved up to that larger mesh. Similarly, overwintering could be done by any method they chose but we asked that 1) all the bags be treated the same way and 2) they be able to know which bags went where (e.g., knowing that a given bag was a top rack raised triploid treatment). Color-coded plastic cable ties were used to identify bags and cage slots to ensure all bags remained in their appropriate locations throughout the study.


Fig.2. Oysters were raised in on-bottom wire racks.

For the oyster ploidy experiments, we wanted to ensure that all the bags were starting the second growing season at identical densities. Therefore, we asked each participant to take time in the spring of 2009 and 2010 to hand count 150 live oysters in each of the ploidy bags. This was done one bag at a time so that there was no mixing among bags and oysters were counted without selecting for size or appearance. If there were less than 150 in a bag, that bag was marked and the starting number recorded. Any remaining oysters were theirs to do with as they please.

1. **1.** Paired triploid and diploid spawn produced at ARC (Dennis, MA)
   - Triploids spawned with eggs from MA broodstock and tetraploid sperm provided by 4C's Breeding Technologies
     - 2008 – VA males
     - 2009 – NJ resistant stock males
     - 2008 – spawn within 2 days
     - 2009 – simultaneous spawn, half-siblings

2. Seed provided to growers as 'retained on a 6 mm sieve' in mid-July
   - 2008 – 3,000 diploids + 3,000 triploids (also ~5,000 'extra' triploids)
   - 2009 – 3,000 diploids + 3,000 triploids (also ~5,000 'extra' triploids)

3. Size of seed at deployment:
   - 2008, triploid seed were significantly larger than the diploid seed (t-test, <0.01)
     - 11.0 + 2.8 vs. 7.7 + 2.4 mm shell length
   - 2009, no significant difference
     - 10.37 + 0.2 mm shell length

4. All seed deployed in 6 mm mesh bags, arranged in a Latin square design in wire racks at two levels (top, bottom) (Fig.3)

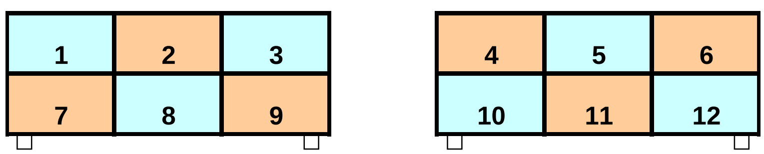


Fig.3. Oyster rack design – blue represents triploid bag arrangement and diploids are orange

5. Stocked at ~500/bag using volumetric measures at initial deployment reduced to 150/bag in spring of second year using hand counting
   - 2009 – reduced to 150/bag in second year using hand counting
   - 2010 – reduced to 150/bag in second year using hand counting

6. Growers removed fouling, tended bags throughout study

7. Growers were allowed to move oysters up to 12 mm bags only when all bags were ready

8. All Research Farm sites were sampled in spring and fall of each year (2008-2010)
   - Oysters sampled for condition index, disease, and ploidy verification
     - Oysters hand counted to obtain total live vs. dead
     - 5 oysters removed from each bag for ploidy verification
     - 10 oysters from each bag for condition index
     - 5 oysters from each diploid bag for pooled disease testing
     - 5 oysters from each triploid bag for pooled disease testing
   - Subset of 6 sites were sampled on a monthly basis in 2009 and 2010

Oysters were shipped to VIMS (Virginia Institute of Marine Science) to verify the ploidy of each bag to ensure that subsequent analysis of oysters correctly identified animals as diploid or triploid. Any bags with conflicting ploidy results were not included in any analysis due to their contamination (see ploidy result tables). Animals were also submitted to the Microtechnologies Testing Lab in Maine for disease analysis in addition to oysters shipped to Bill Walton at the Auburn University Shellfish Laboratory on Dauphin Island, AL for further analysis of condition index.

A grower survey was developed to elicit feedback from the industry on their experiences with growing triploid oysters. This information provides a better understanding of the performance of triploid oysters for local shellfish farmers.

Results

Note that for all the following graphs of data, all error bars shown are the standard error of the mean. The following abbreviations are used for sites:

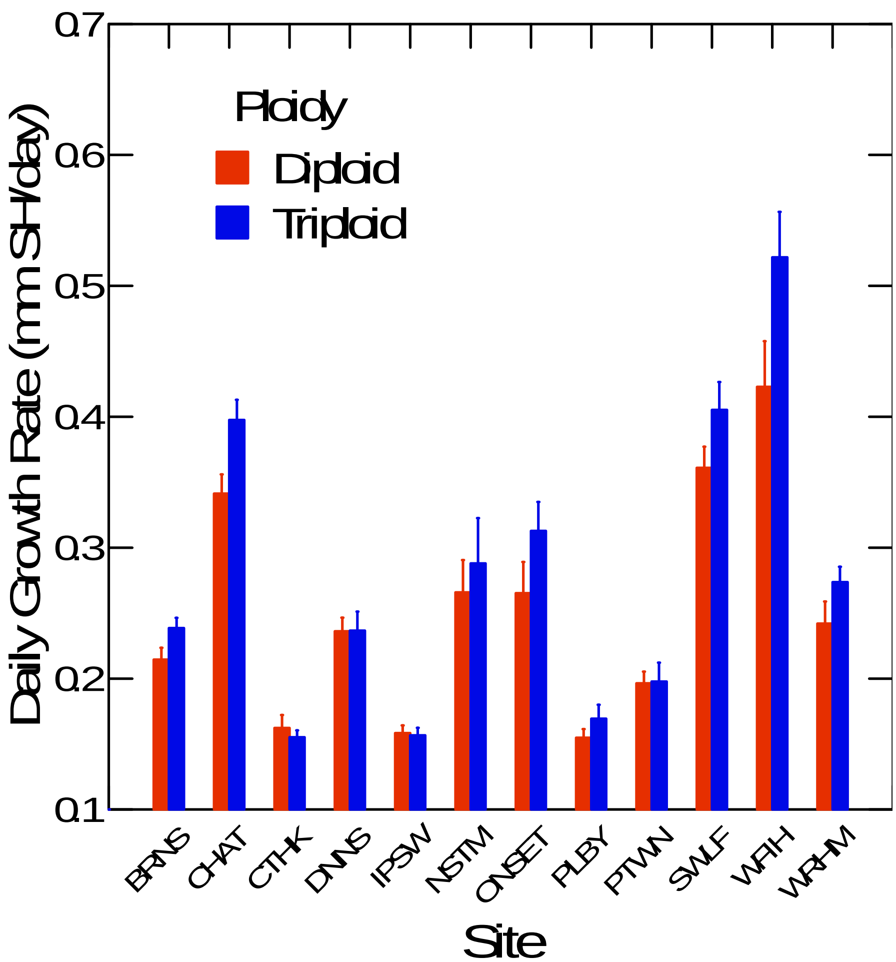
Site	**Abbreviation**
Barnstable Harbor	BRNS
Oyster River, Chatham	CHAT
Cuttyhunk	CTHK
Cape Cod Bay, Dennis	DNNS
Ipswich	IPSW
Nauset Marsh, Eastham	NSTM
Onset	ONSET
Pleasant Bay	PLBY
Provincetown Harbor	PTWN
South Wellfleet	SWLF
Inner Harbor, Wellfleet	WFIH
Wareham	WRHM

All analyses were performed using ANOVA, with Ploidy (Diploid, Triploid), Rack Level (Bottom, Top), Year Class (2008, 2009) and Site as the single factors. For purposes of analyses, only sites with sufficient replication were used; some sites were excluded due to loss of replicates or elimination of replicates from consideration due to flow cytometry results that indicated bags had been mixed. All data, however, are shown on the graphs for the sake of completeness.

First Year Performance

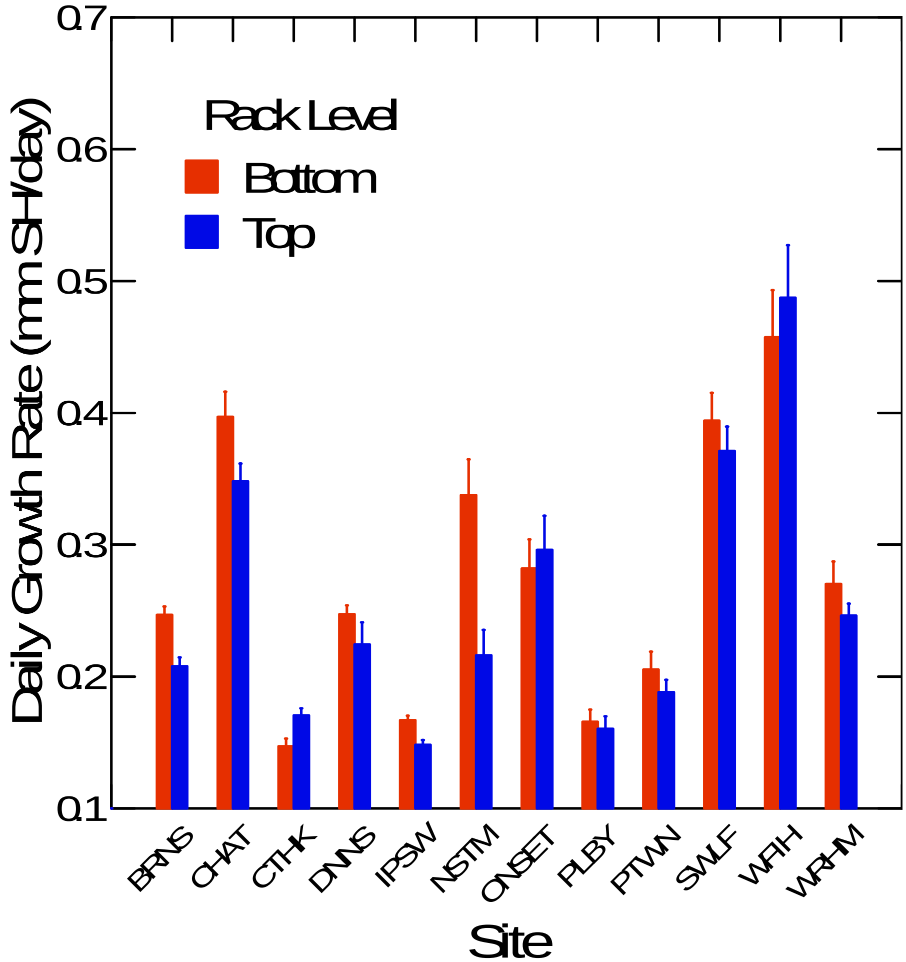
In terms of average daily growth rate (measured in mm of shell height, or SH, added per day between initial field deployment and the final measurements in the first year, taken in late October or early November, after the bulk of the first growing season), ploidy significantly interacted with site. CHAT, CTHK, IPSW and PLBY had to be excluded from this analysis. Within any of the compared sites, triploids grew significantly faster at ONSET & WFIH (Tukey HSD, $p \leq 0.001$), with no significant differences between ploidies at any of the other compared sites. Note the very high growth rates at some sites (Fig.4).

Fig. 4. Effect of Ploidy by Site on Daily Growth Rate



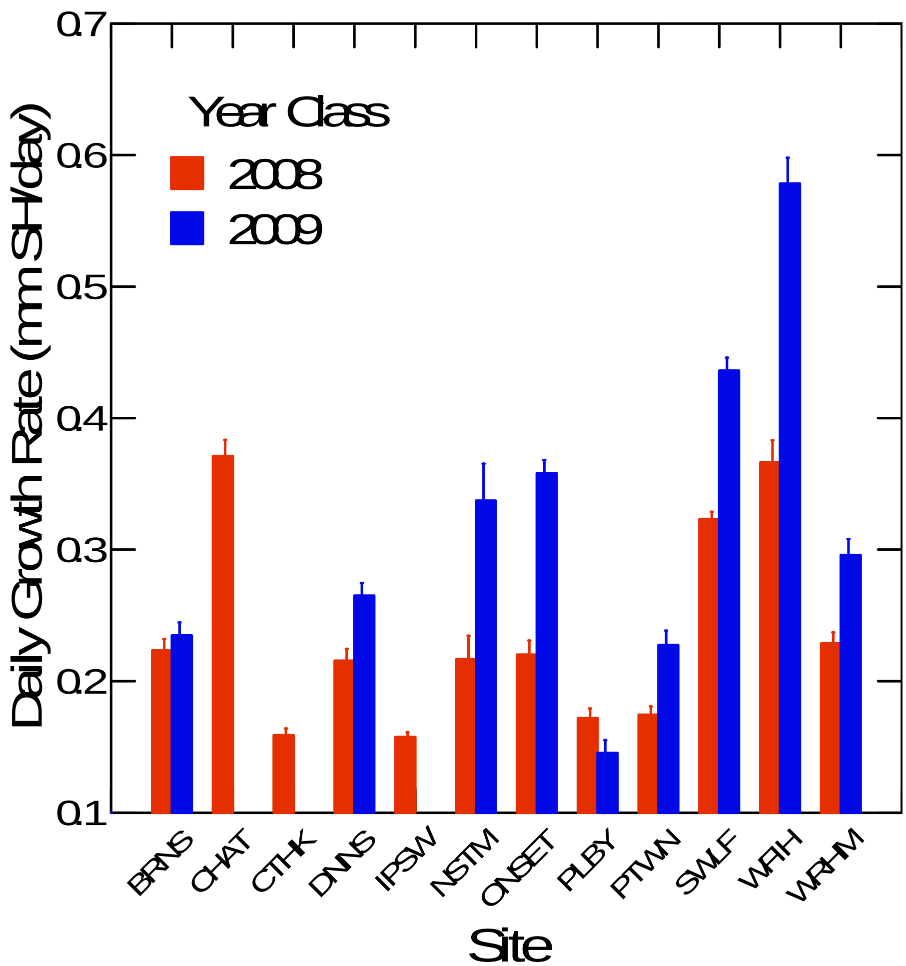
There were also significant interactions between rack level and site, and year class and site (Fig.5). For rack level, among compared sites, oysters grew faster in the first year on the bottom rack level at both BRNS and NSTM (Tukey HSD, $p \leq 0.03$) with no significant differences between rack levels at any other site.

Fig. 5. Effect of Rack Level by Site on Daily Growth Rate



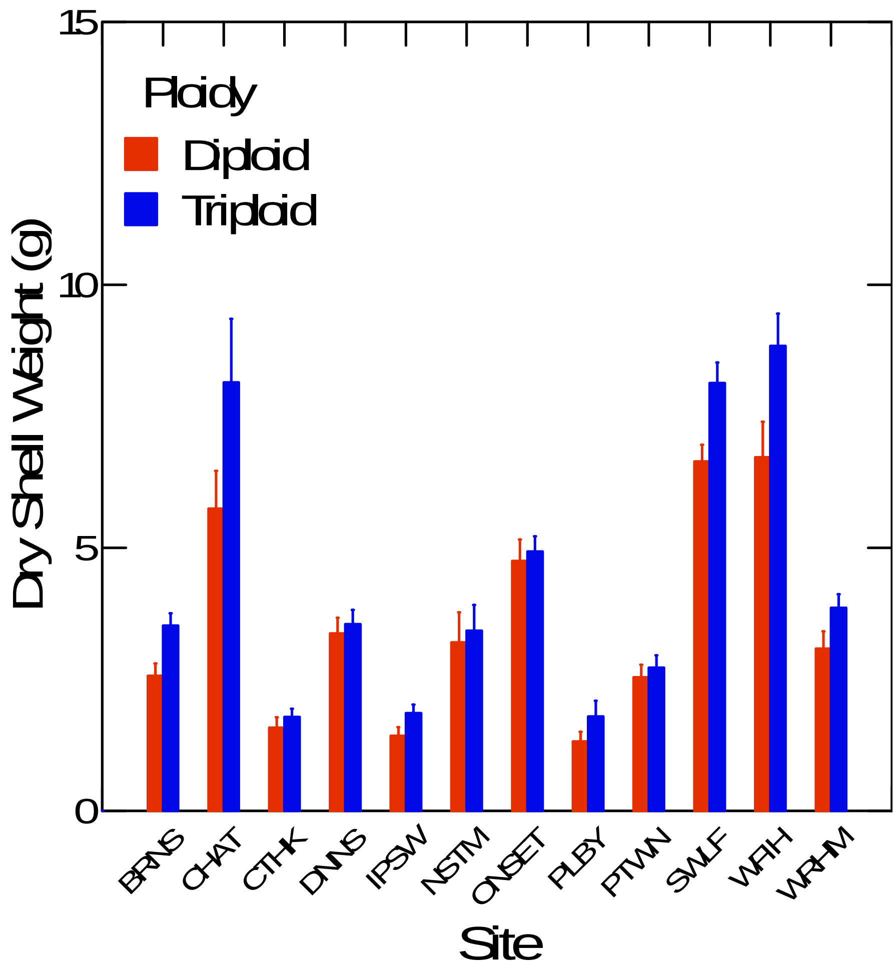
In regards to year class, the 2009 year class grew faster than the 2008 year class (Fig.6); this difference was statistically significant at NSTM, ONSET, WFIH and WRHM (Tukey HSD, $p < 0.001$).

Fig. 6. Effect of Year Class by Site on Daily Growth Rate



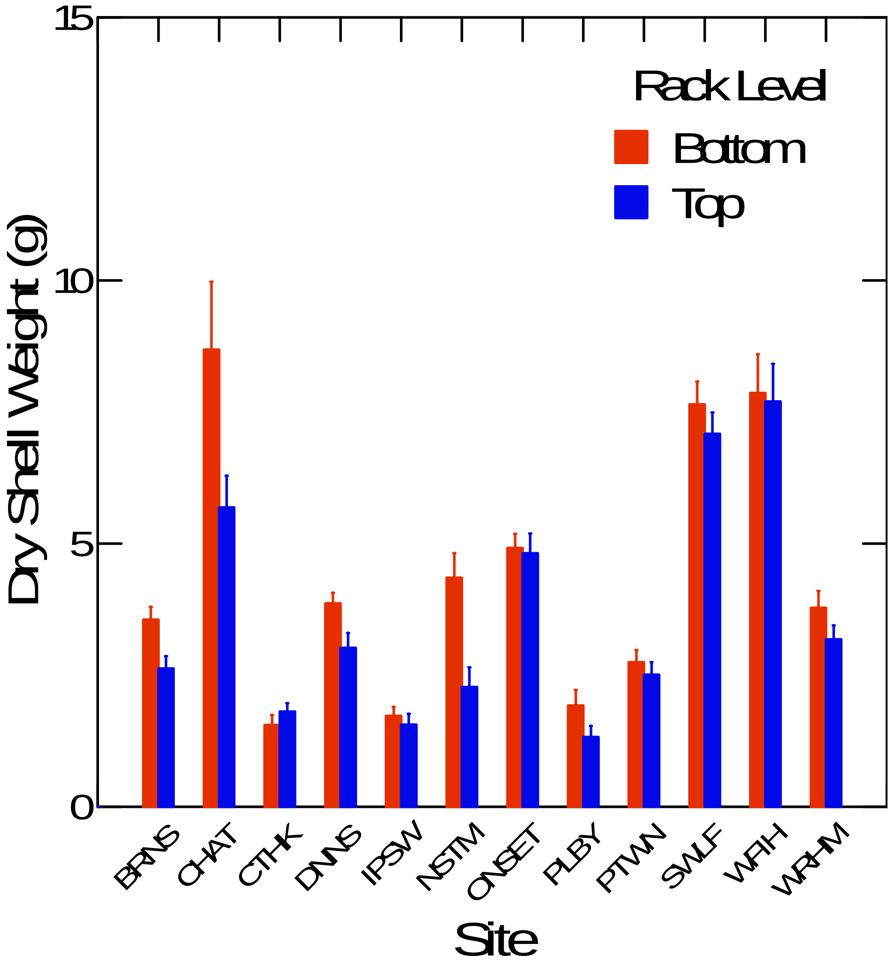
In addition to shell height, dry shell weight and dry tissue weight (Fig.7) were compared (with ONSET now excluded from further statistical analyses). For dry shell weight, triploids had significantly heavier shells than diploids at SWLF and WFIH (Tukey HSD, $p \leq 0.02$).

Fig. 7. Effect of Ploidy by Site on Dry Shell Weight



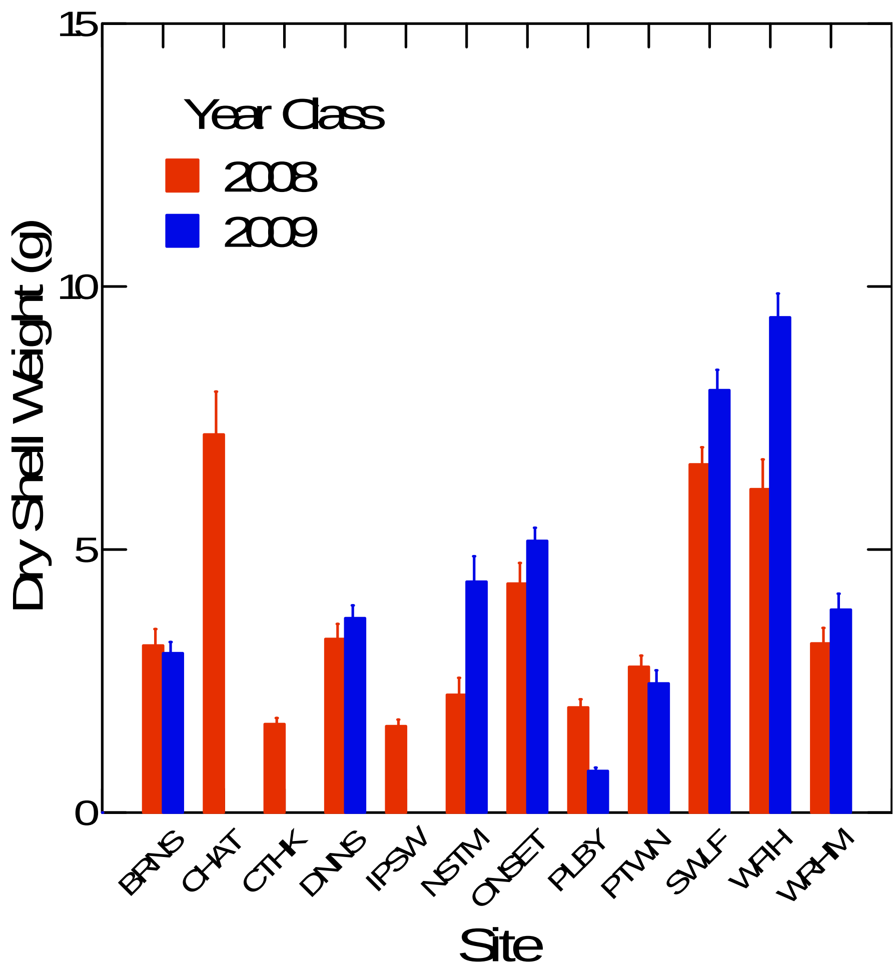
For dry shell weight, there were significant interactions between rack level and site and year class and site (Fig.8). Among sites, oysters grown on the bottom rack level had significantly heavier shells than those grown on the top level only at NSTM (Tukey HSD, $p < 0.001$) with no signficant differences between levels at the other sites.

Fig. 8. Effect of Rack Level by Site on Dry Shell Weight



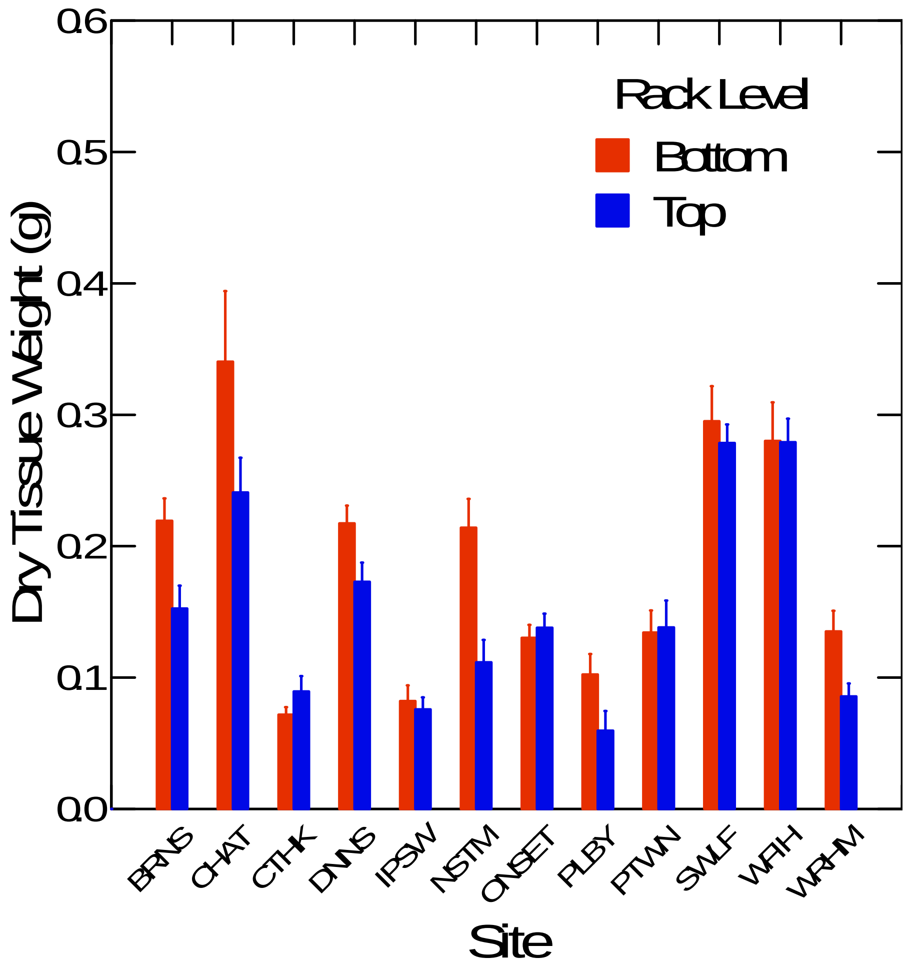
Among sites, oysters from the 2009 year class had significantly heavier shells than those from the 2008 year class at NSTM, SWLF & WFIH (Tukey HSD, $p < 0.04$) with no signficant differences between year classes at the other sites (Fig.9).

Fig. 9. Effect of Year Class by Site on Dry Shell Weight



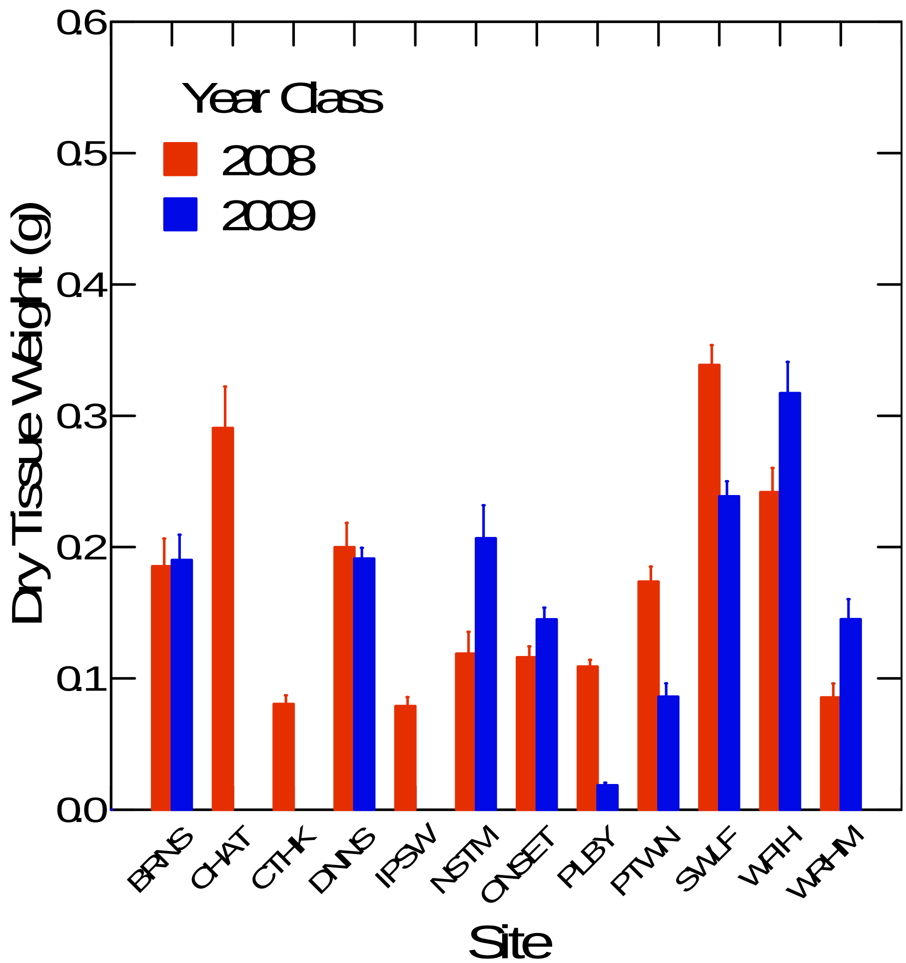
For dry tissue weight, triploids had significantly heavier dry tissue weights than diploids across all sites (ANOVA, $p = 0.02$). For dry tissue weight, there was a significant interaction between rack level and site (Fig. 10). As was the case with dry shell weight, oysters grown on the bottom rack level had significantly heavier tissues than those grown on the top level only at NSTM (Tukey HSD, $p < 0.001$) with no signficant differences between levels at the other sites.

Fig. 10. Effect of Rack Level by Site on Dry Tissue Weight



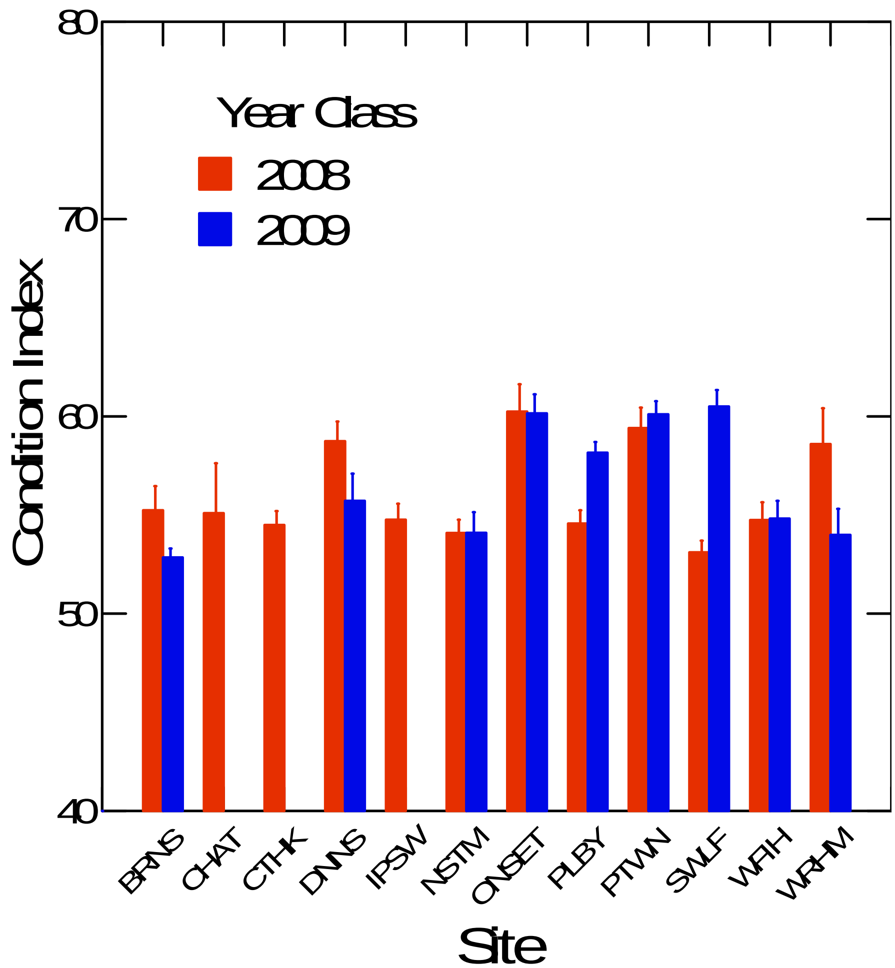
In addition, year class signficantly interacted with site, with oysters from the 2009 year class being significantly heavier than the 2008 year class at NSTM & WFIH while the 2008 year class were significantly heavier than the 2009 year class at PWTN & SWLF (Tukey HSD, $p \leq 0.01$), with no significant differences between year classes at the other compared sites (Fig.11).

Fig. 11. Effect of Year Class by Site on Dry Tissue Weight



For condition index, there was no significant effect of ploidy. There was however a significant year class by site interaction (Fig.12). Among the compared sites, the condition index was significantly different between years only at SWLF (Tukey HSD, $p < 0.01$), where the 2009 year class had a higher condition index than the 2008 year class.

Fig. 12. Effect of Year Class by Site on Condition Index



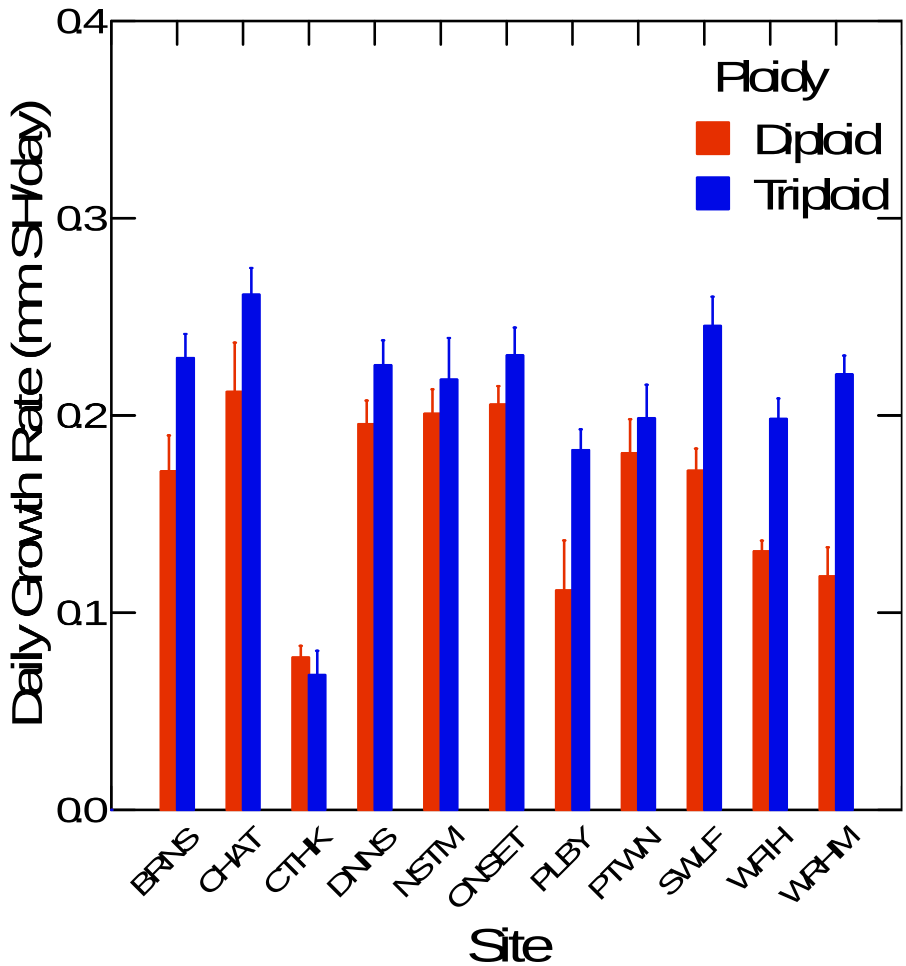
In summary of first year performance, *at some sites* triploids performed well, either growing relatively quickly (in terms of shell height) or adding shell weight. Triploids also consistently had higher dry tissue weights (at statistically compared sites). The site-specific nature of some of these benefits and the absolute magnitude of the benefits need to be considered by each grower to determine if these benefits warrant the added expense of triploid seed.

Additionally, not surprisingly, the rack level had very strong effects at certain sites. These effects can dominate the results. Similarly, there were significant differences among the year classes, although these were not consistent across sites, making any practical conclusions difficult.

Second Year Performance

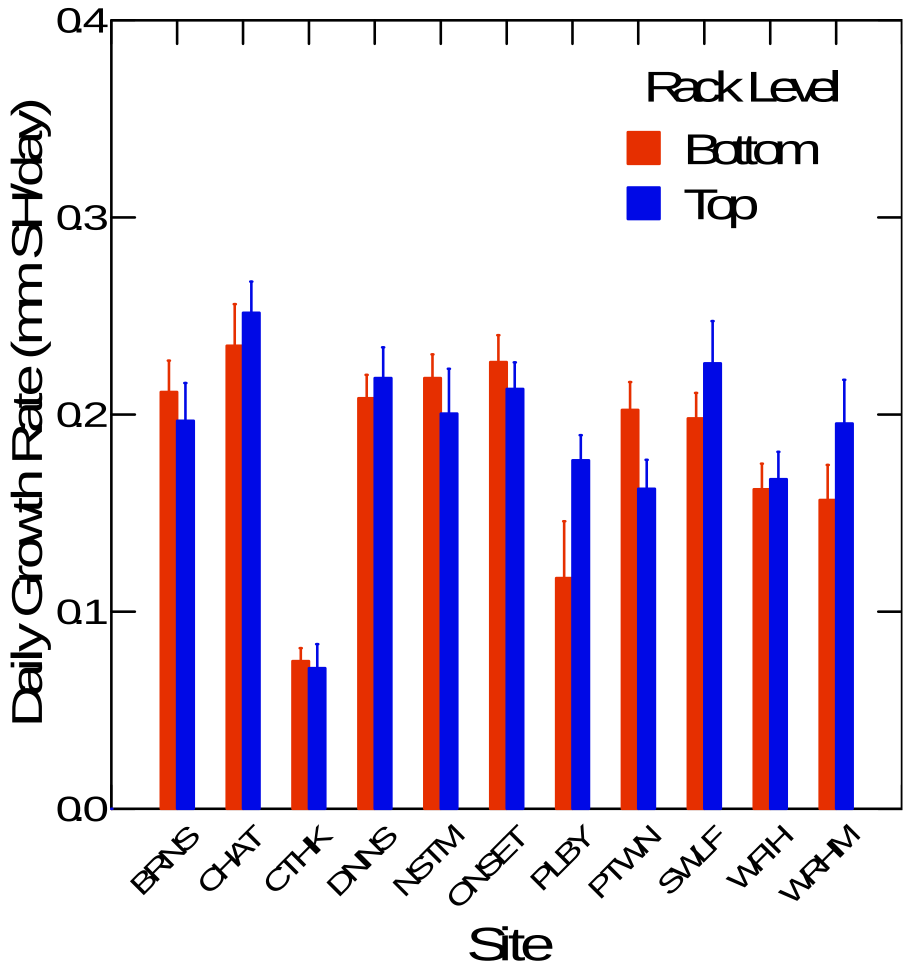
In terms of average daily growth rate across the second growing season (measured in mm of shell height, or SH, added per day between the start of the growing seasons and the final measurements in the second year, taken in late October or early November, after the bulk of the last growing season), ploidy significantly interacted with site (Fig.13). CHAT, CTHK, IPSW, PLBY, ONSET & PTWN had to be excluded from this analysis, leaving BRNS, DNNS, NSTM, SWLF, WFIH & WRHM. Within any of the compared sites, triploids grew significantly faster at SWLF, WFIH & WRHM (Tukey HSD, $p \leq 0.001$), with no significant differences between ploidies at the other three compared sites. Note that the values are lower than those observed during the first season as would be expected.

Fig. 13. Effect of Ploidy by Site on Daily Growth Rate



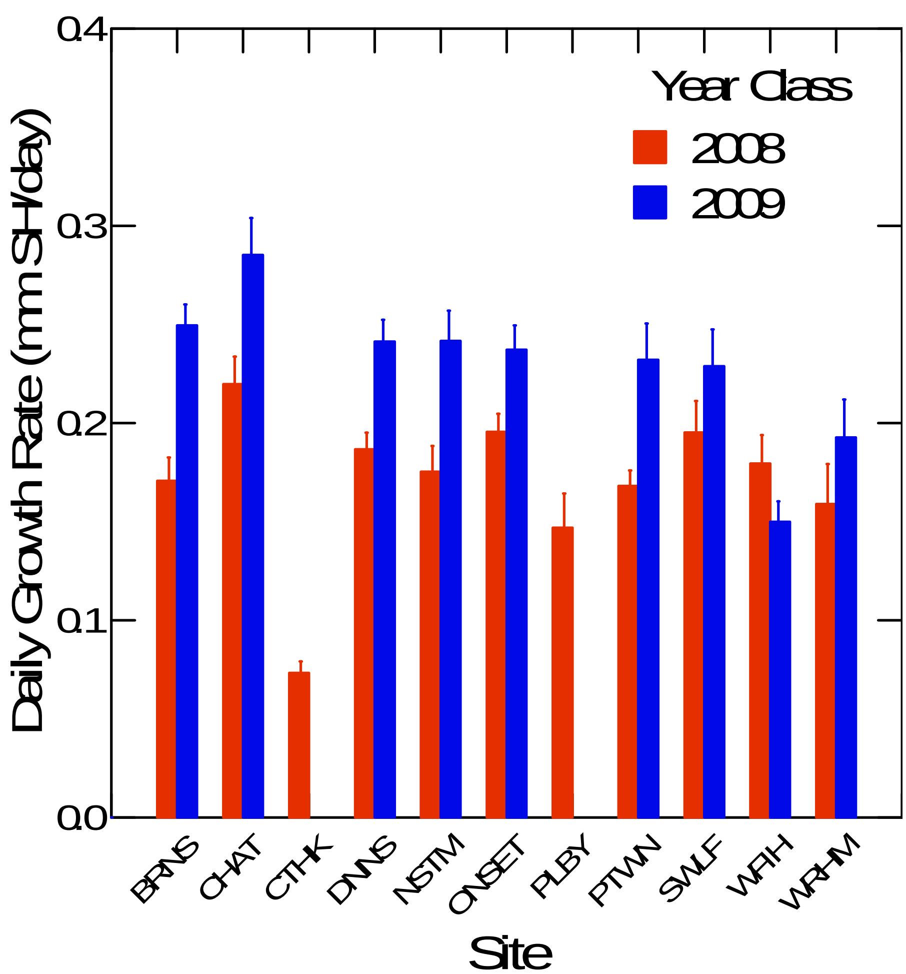
There were also significant interactions between rack level and site, and year class and site. Among compared sites, there were no significant differences between rack levels at any given site compared statistically.

Fig. 14. Effect of Rack Level by Site on Daily Growth Rate



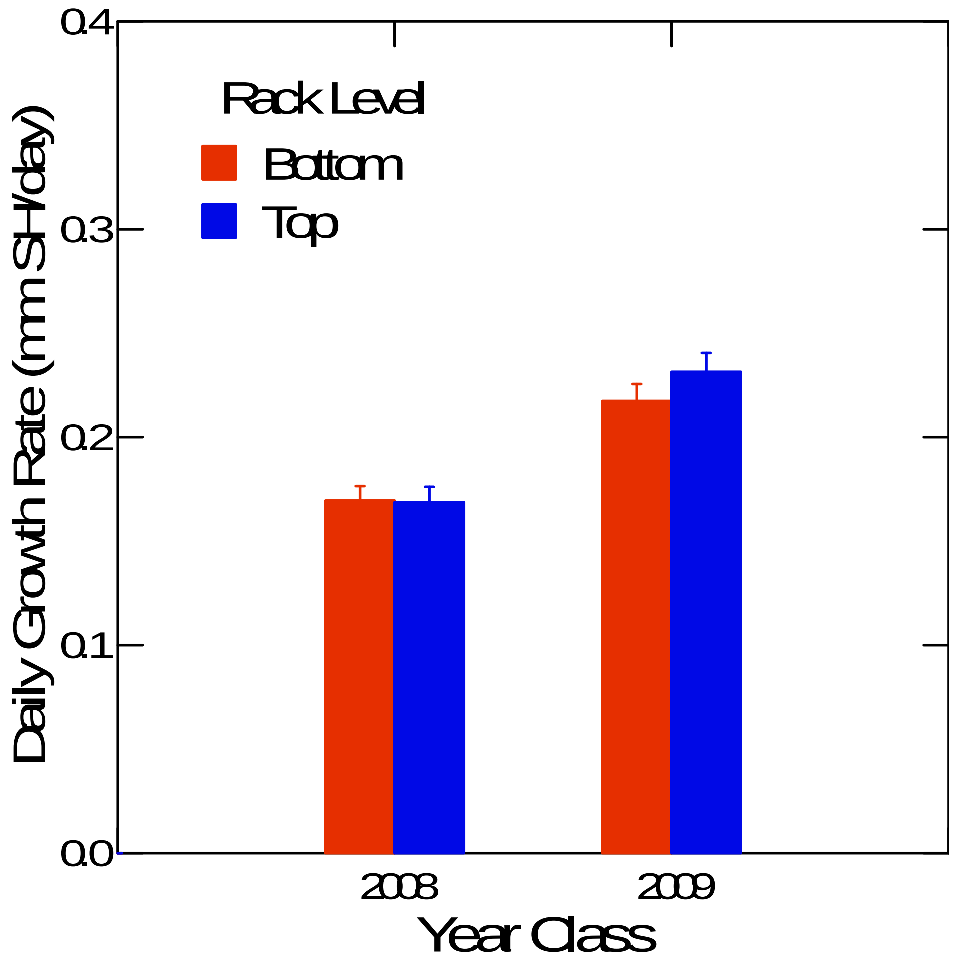
In regards to year class, the 2009 year class generally grew faster than the 2008 year class in their second year (Fig.15), though not at WFIH; this difference was statistically significant at BRNS, DNNS, & NSTM (Tukey HSD, $p \leq 0.009$).

Fig. 15. Effect of Year Class by Site on Daily Growth Rate



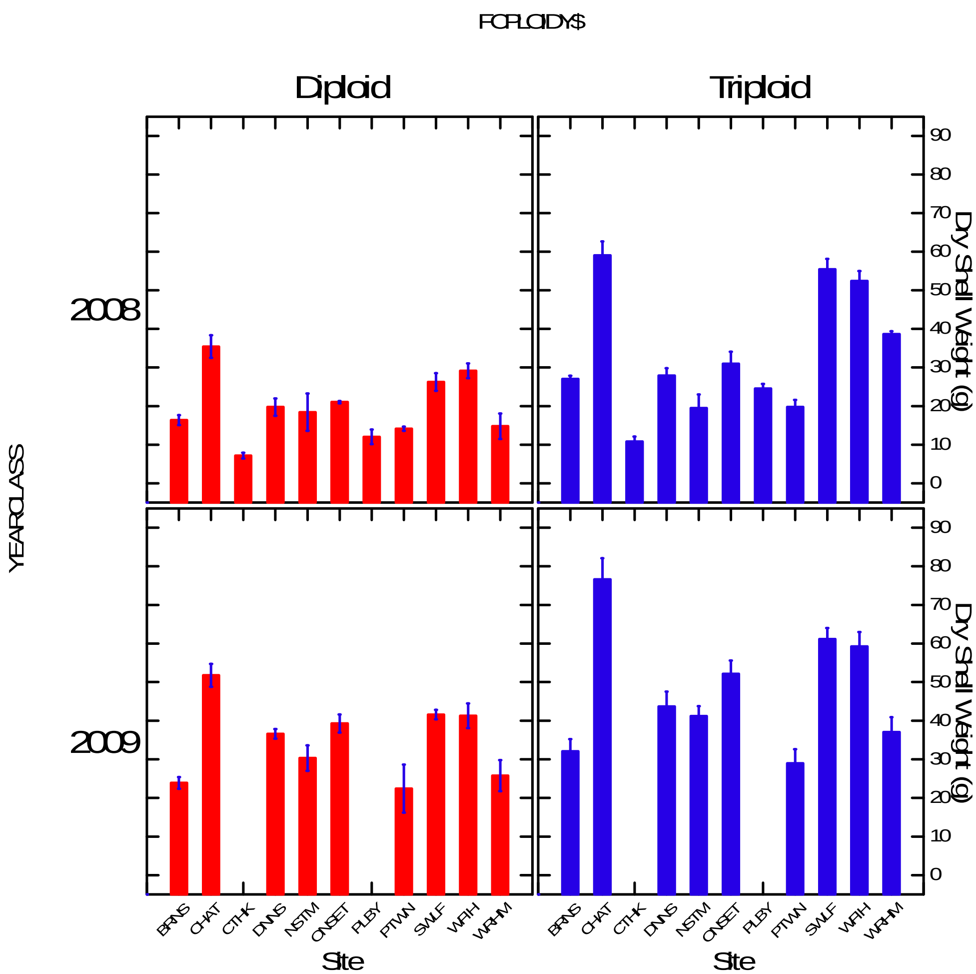
There was also a significant interaction between rack level and year class (Fig.16); while there was no difference in the growth rates between rack levels in the 2008 year class, there was a significant difference between the rack levels in the 2009 year class (Tukey HSD, $p = 0.01$).

Fig. 16. Effect of Rack Level by Year Class on Daily Growth Rate



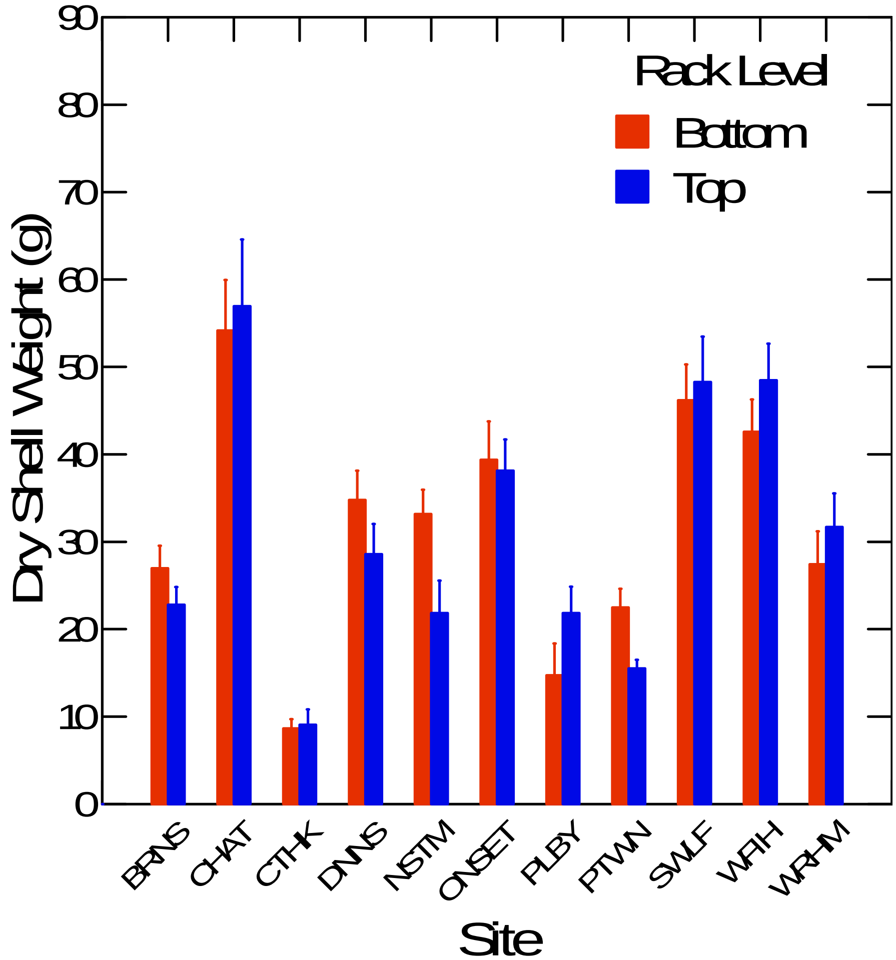
For dry shell weight, there was a complicated three way interaction among ploidy, year class and site (Fig.17). At both SWLF & WFIH, triploids had heavier shells than diploids in both year classes (Tukey HSD, $p < 0.001$), while there was no difference between the ploidies in either year class at DNNS. However, at both BRNS & WRHM, triploids had heavier shells in the 2008 year class but there was no difference in 2009 (Tukey HSD, $p \leq 0.03$). Conversely, at NSTM there was no difference between ploidies in 2008 but triploids were heavier in the 2009 year class (Tukey HSD, $p \leq 0.03$).

Fig. 17. Interactions between Ploidy, Year Class, and Site



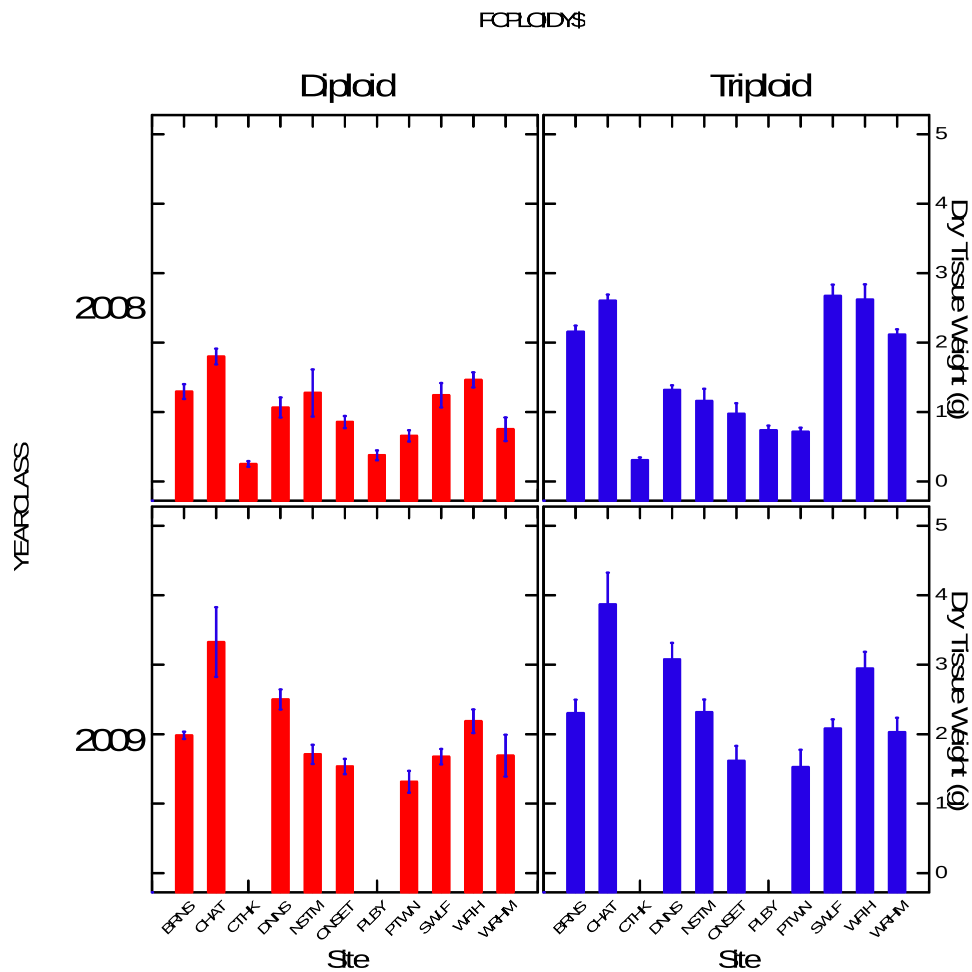
There was also a significant interaction between rack level and site in second year performance (Fig.18), with heavier oysters on the bottom level at NSTM (Tukey HSD, $p < 0.001$).

Fig. 18. Interaction between Rack Level and Site



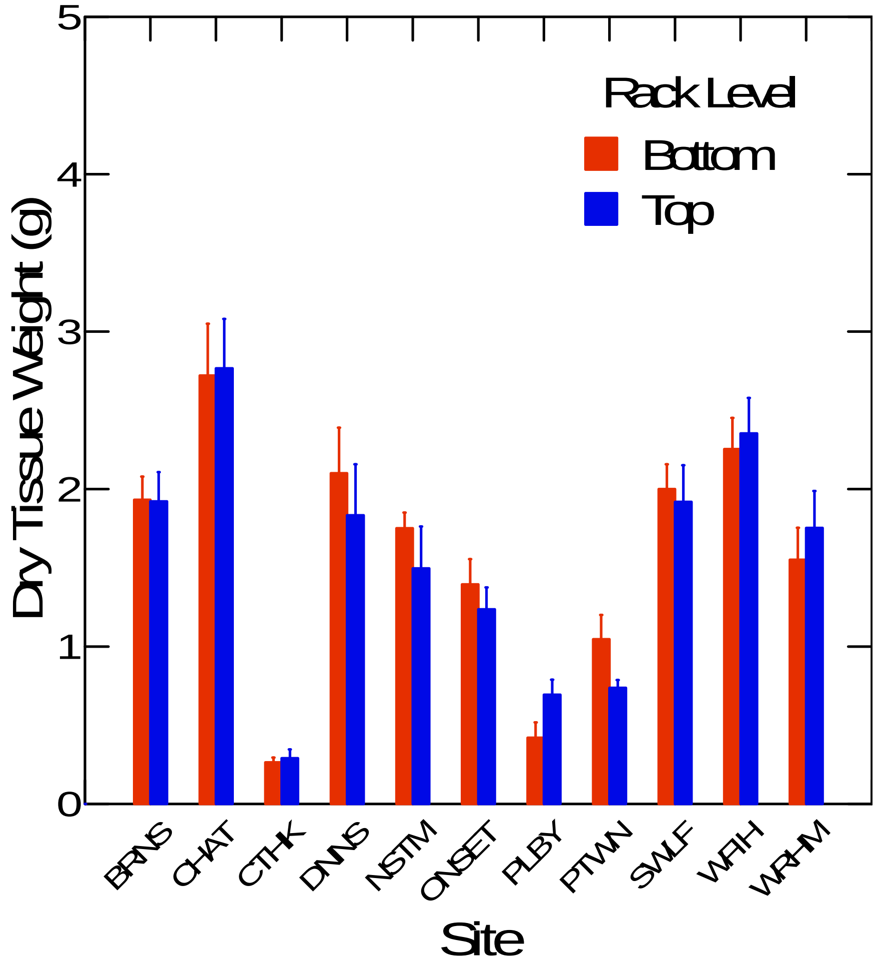
For dry tissue weight, there was a very strong effect of year class upon both ploidy and site (a three way interaction among these factors)(Fig.19). In the 2008 year class, dry tissue weights were greater for triploids at every compared site than diploids (Tukey HSD, $p \leq 0.01$), with the exception of NSTM where there was no difference between the ploidies. In the 2009 year class, however, the only significant difference between ploidies was observed at WFIH where triploids had significantly greater dry tissue weights (Tukey HSD, $p = 0.05$).

Fig. 19. Effect of Year Class on Ploidy and Site



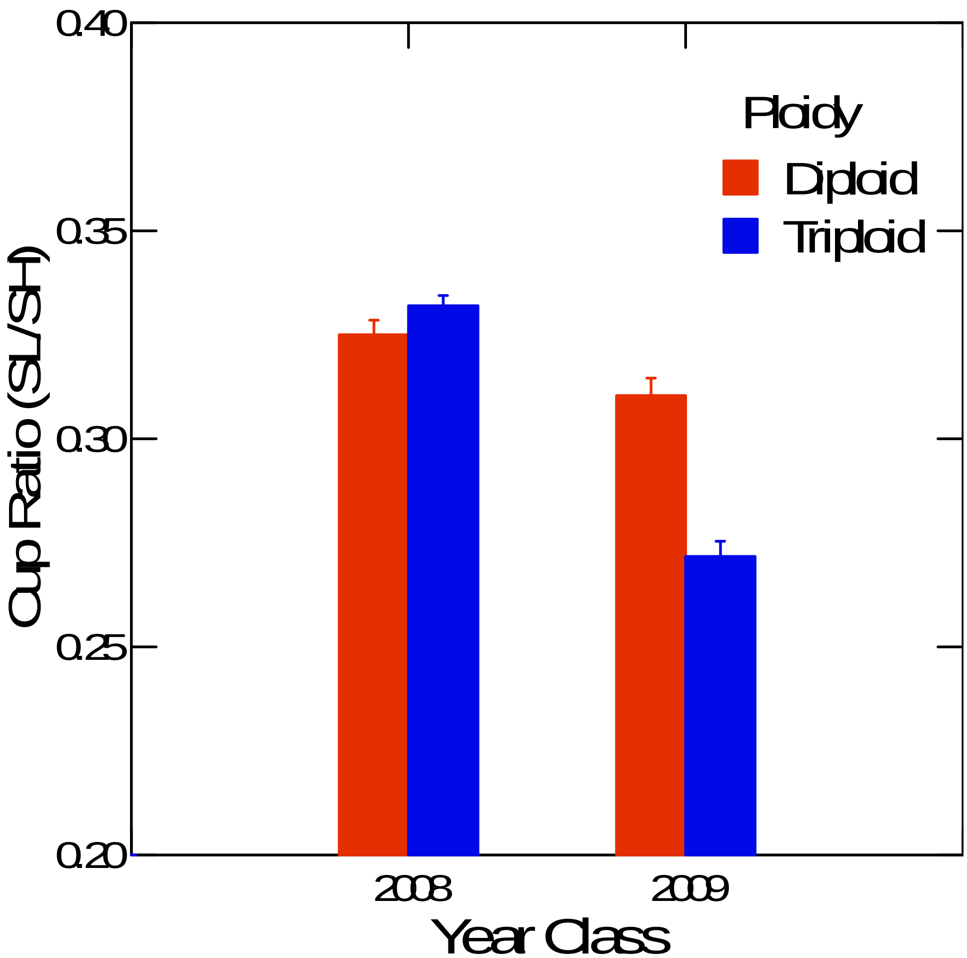
As with dry shell weight, there was a significant interaction between rack level and site for dry tissue weight (Fig.20).

Fig. 20. Effect of Rack Level by Site on Dry Tissue Weight



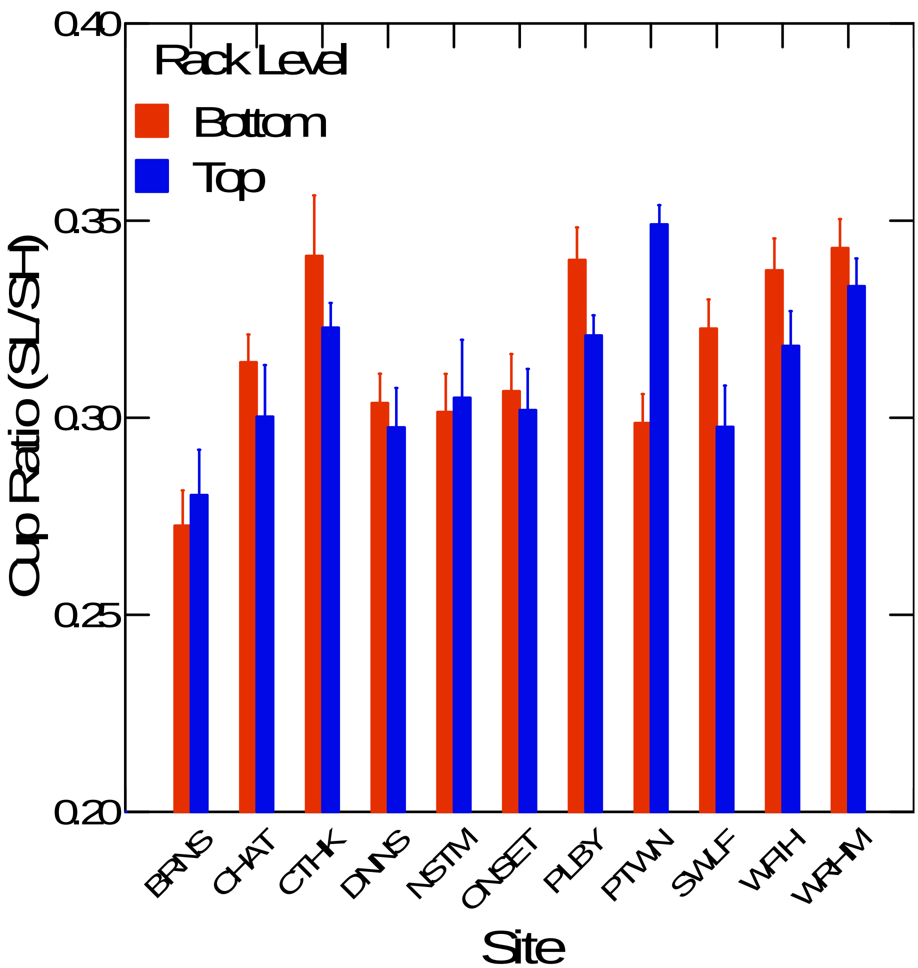
To gauge whether ploidy affected the shapes of cultured oysters, both cup (the ratio of shell width to shell height) and fan (the ratio of shell length to shell height) were measured at the conclusion of the second year (Fig.21). There was a significant interaction between ploidy and year class, with no difference in the cup ratio in 2008 but with triploids having a significantly smaller cup ratio in 2009 (Tukey HSD, $p < 0.001$).

Fig. 21. Effect of Ploidy by Site on Cup Ratio



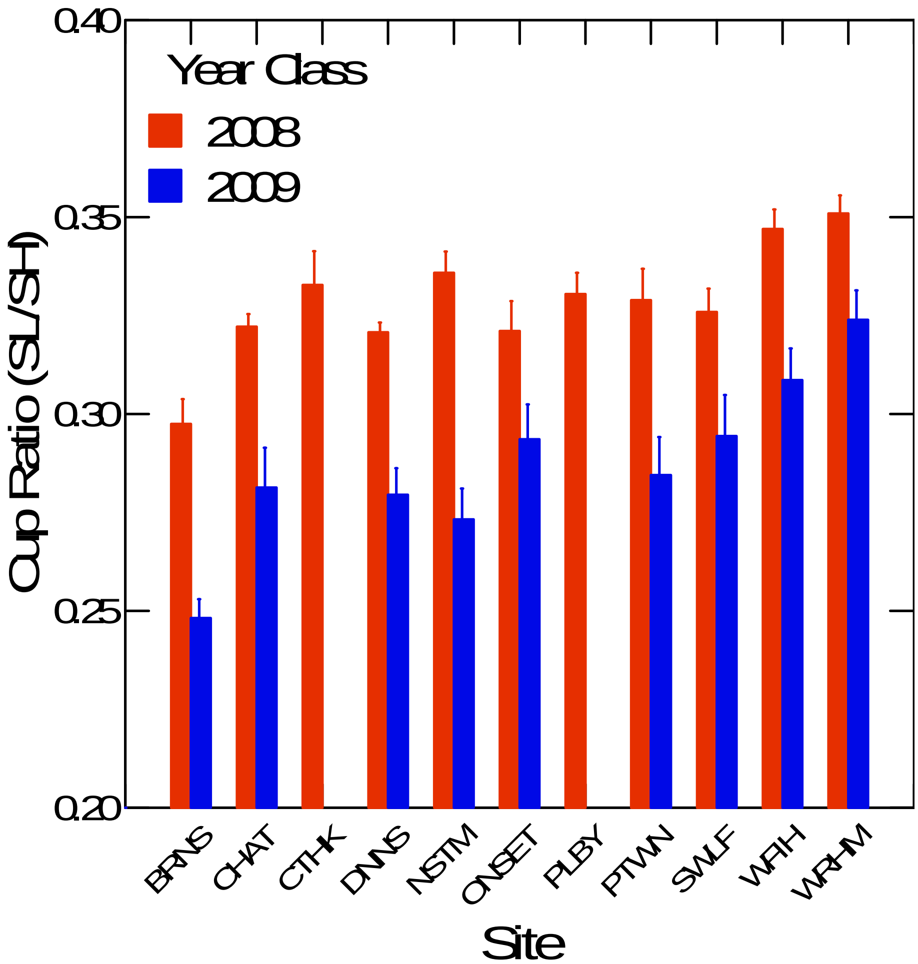
There were also significant interactions between rack level and site and year class and site; at SWLF, oysters raised on the bottom level were significantly more cupped than those raised on the top shelf ($p = 0.006$), with no significant differences between levels at any of the other compared sites (Fig.22).

Fig. 22. Effect of Rack Level by Site on Cup Ratio



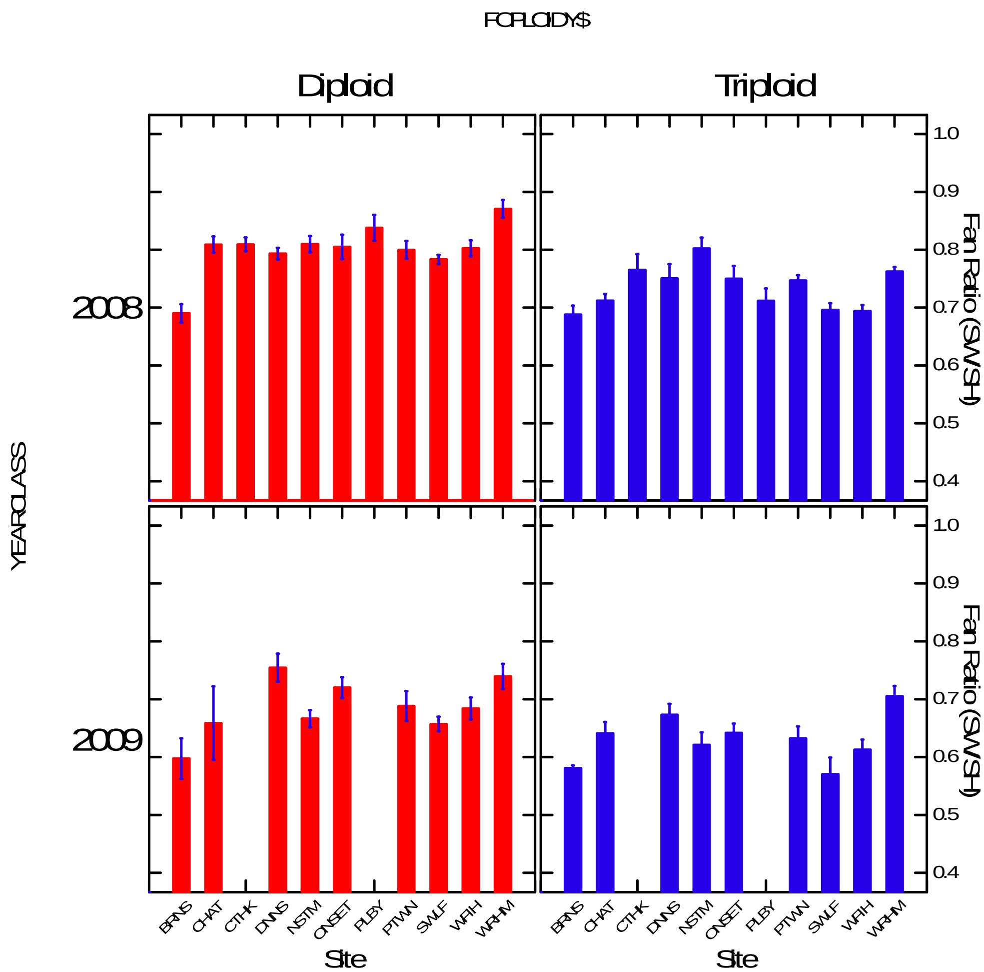
Cup was also affected by the interaction between year class and site (Fig.23). At any of the compared sites, oysters were significantly more cupped in the 2008 year class than the 2009 year class.

Fig. 23. Effect of Year Class by Site on Cup Ratio



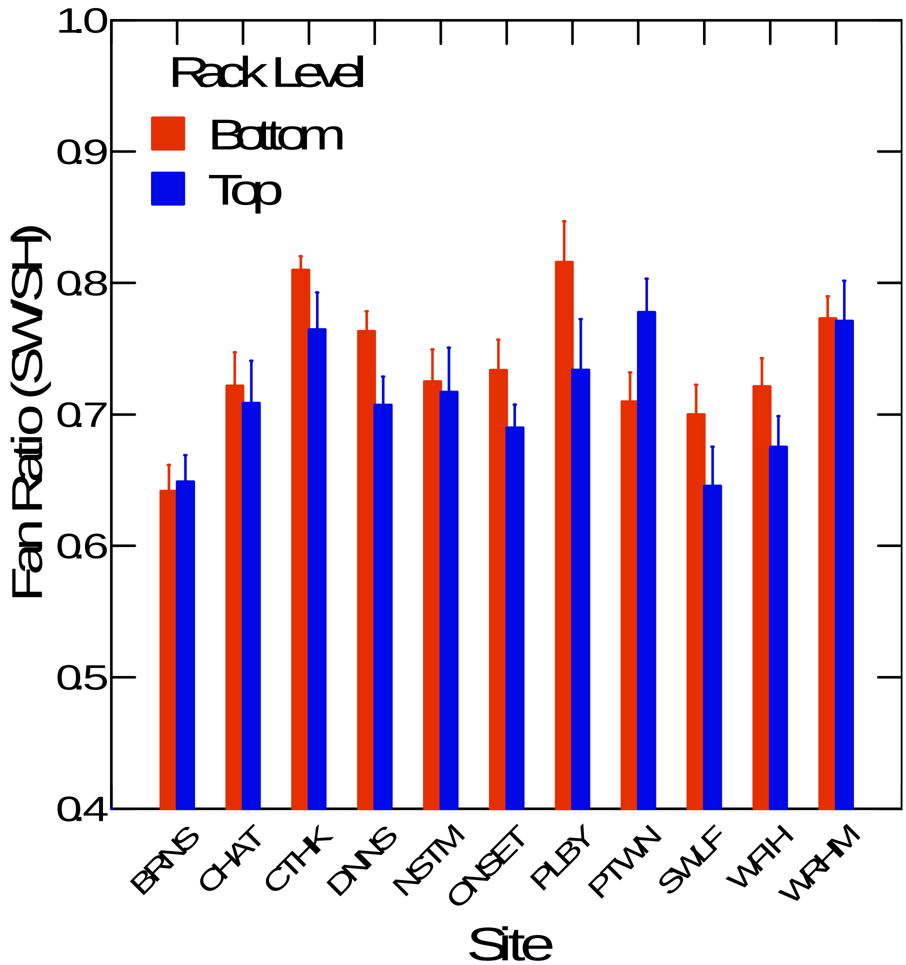
In terms of the fan ratio, there was again a complicated three-way interaction among ploidy, year class and site (Fig.24). While there were no differences between diploids and triploids in either year class for BRNS, DNNS and NSTM, the fan ratio of triploids was significantly lower than that of diploids in both year classes in SWLF and WFIH (Tukey HSD, $p \leq 0.02$). At WRHM, triploids had significantly lower fan ratios in the 2008 year class (Tukey HSD, $p < 0.001$) but did not significantly differ from diploids in the 2009 year class.

Fig. 24. Effect of Ploidy, Year Class and Site on Fan Ratio



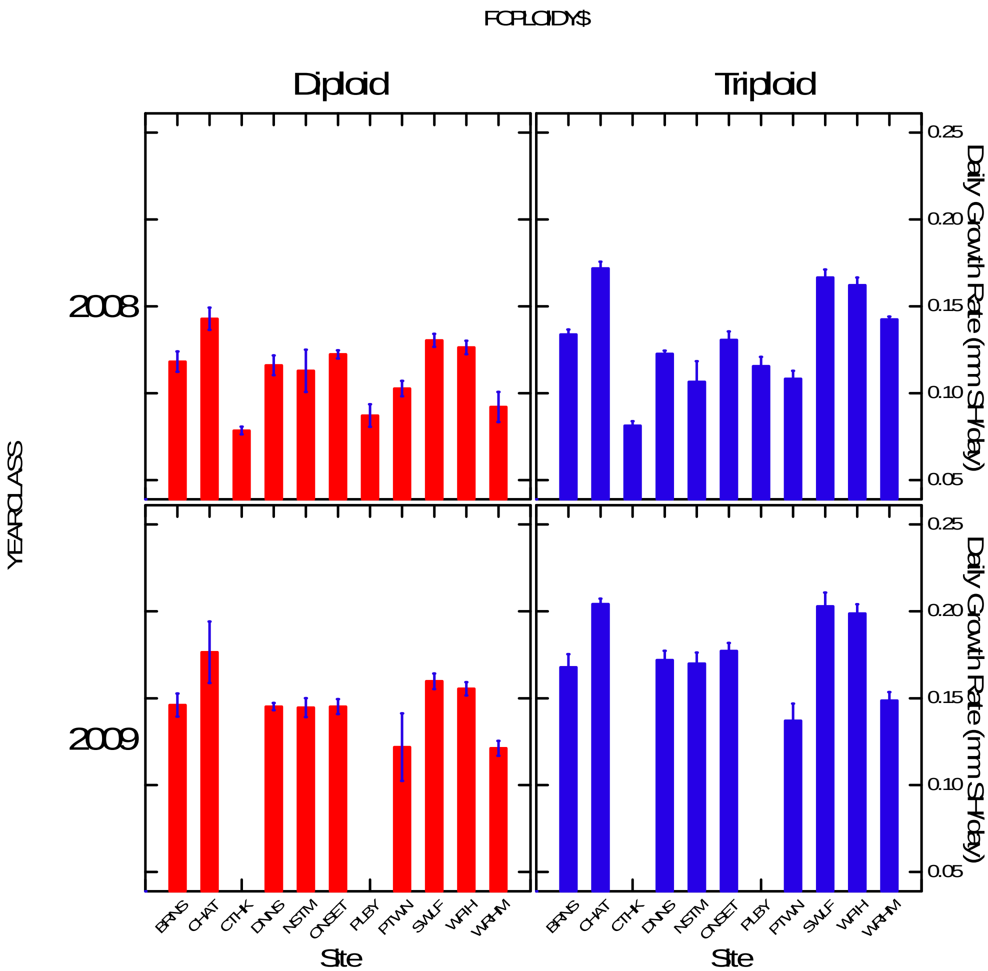
There was also a significant interaction of rack level and site upon fan (Fig.25). At both DNNS and WFIH, oysters raised in the bottom level had a higher fan ratio than those raised in the top rack level (Tukey HSD, $p \leq 0.03$).

Fig. 25. Effect of Rack Level on Fan Ratio



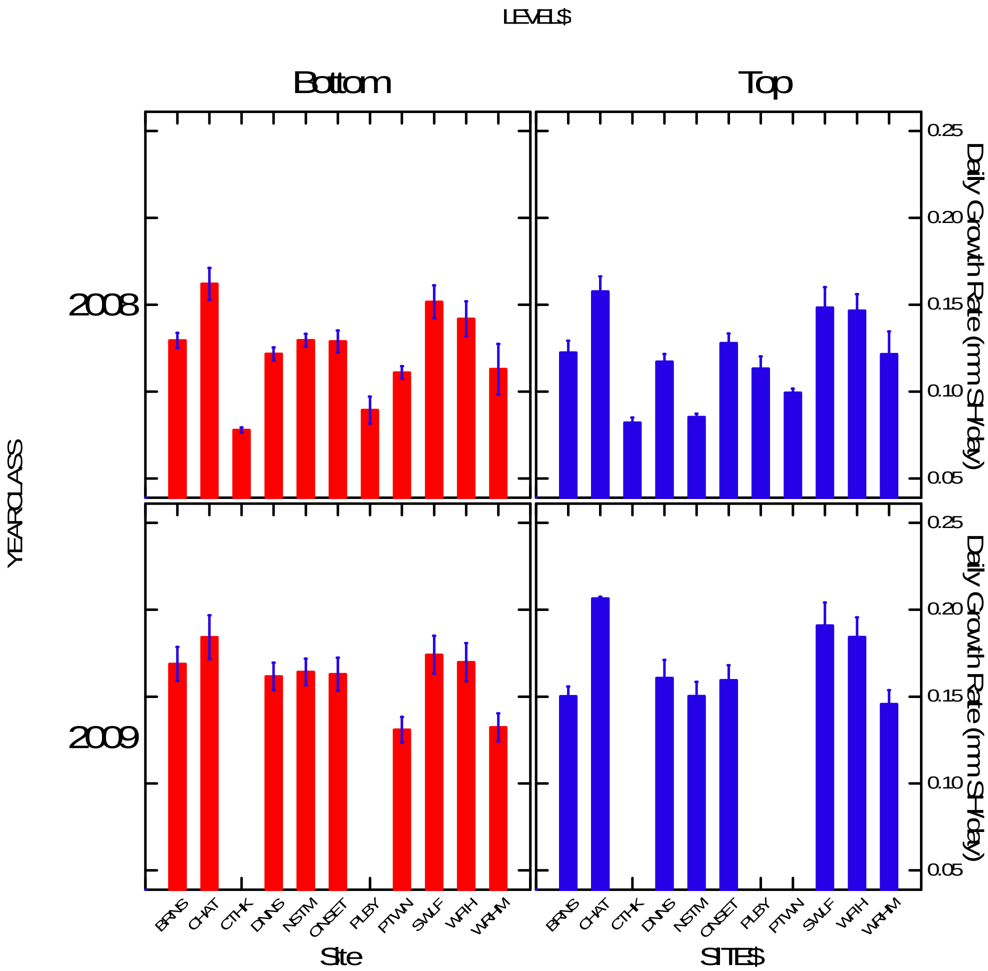
Finally, the overall daily growth rate from initial deployment to the final collection (in terms of shell height) was compared (Fig.26). These values, of course, were lower than either of the seasonal values. In both year classes, triploids grew faster than diploids at SWLF and WFIH (Tukey HSD, $p < 0.001$). Triploids also grew faster than diploids at WRHM in 2008 (Tukey HSD, $p < 0.001$) and not quite significantly in 2009 (Tukey HSD, $p = 0.09$). Triploids grew faster than diploids in the 2009 year class at both DNNS and NSTM (Tukey HSD, $p \leq 0.02$), with no difference observed in 2008. No difference was observed between ploidies in either year class at BRNS.

Fig. 26. Effect of Ploidy, Year Class, and Site on Daily Growth Rate



There was also a three-way interaction among rack level, year class and site (Fig.27). In terms of overall growth rate, the only significant difference between rack levels at any compared site was that 2008 year class oysters raised on the bottom rack level were larger than those raised on the top level at NSTM (Tukey HSD, $p < 0.001$). Otherwise, there were no signficant differences, including between rack levels in the 2009 year class at NSTM.

Fig. 27. Effect of Year Class, Rack Level, and Site on Daily Growth Rate



In terms of survival, in the final collection of the 2009 year class in late 2010, survival was significantly greater in the triploids than diploids (94% compared to 87%, p = 0.003 two-tailed t-test).

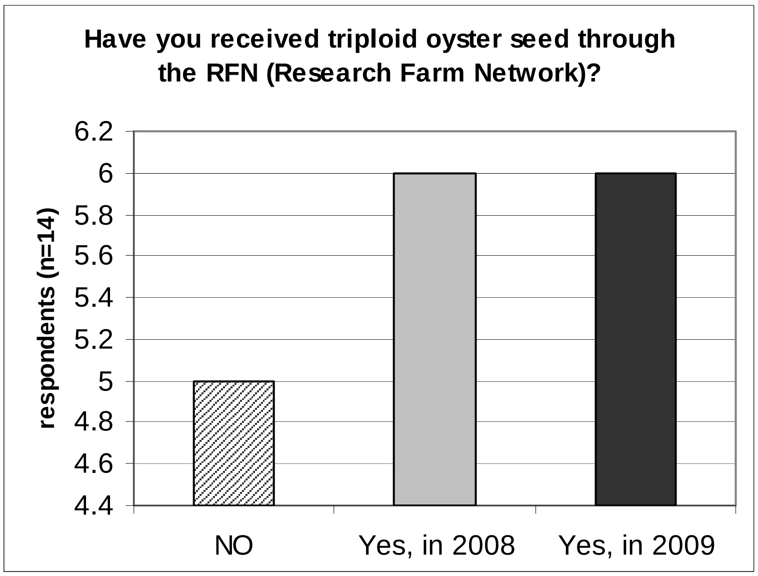
In summary of second year performance and overall performance, triploids had the potential to exhibit fast growth, with the potential for heavier shells and more tissue. These results were heavily affected by variation among sites and year classes, but it is worth noting that there were no cases where diploids statistically outperformed triploids in terms of growth.

There did appear to be some potential for costs in terms of shell shape with measures of both cup and fan being lower in some cases for triploids than diploids. Qualitatively, growers did not report any concerns about the quality of the triploid oysters produced, though this question may warrant further consideration with consumer tests.

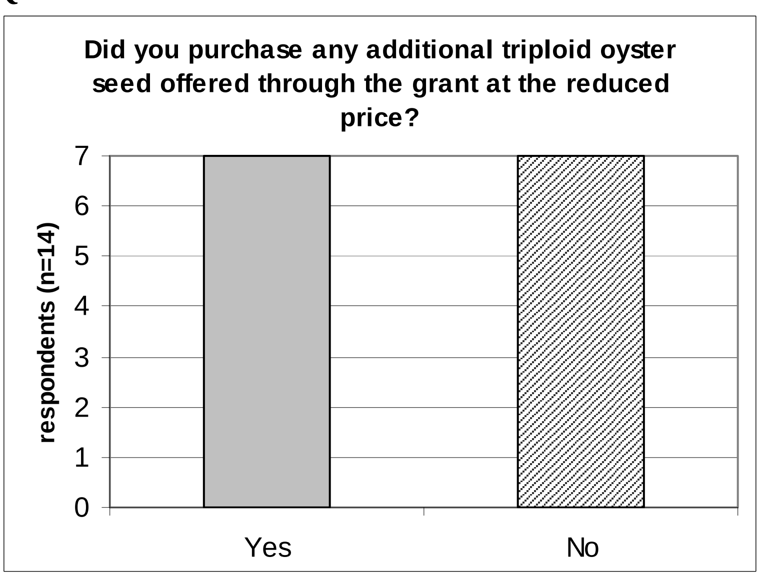
Survey Results

A survey link was sent via email to the industry on 4-11-11, asking for feedback on their experiences with triploid oysters. The survey was created through *Survey Monkey* and may be viewed at: http://www.surveymonkey.com/s/XQFB2X9. Forty (40) growers received this link and 14 completed the survey, for a response rate of 35%. Acceptable response rates vary by how surveys are administered (phone, written, mail, etc.), but 30% is considered average for online surveys.

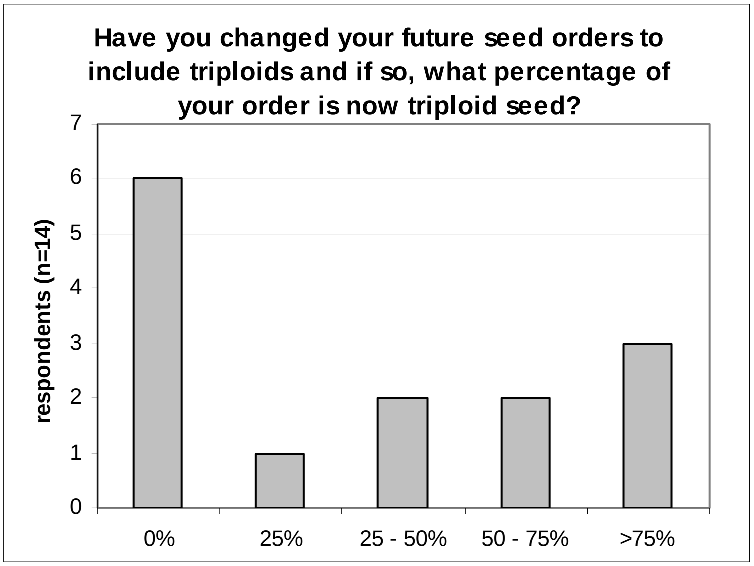
Question 1.



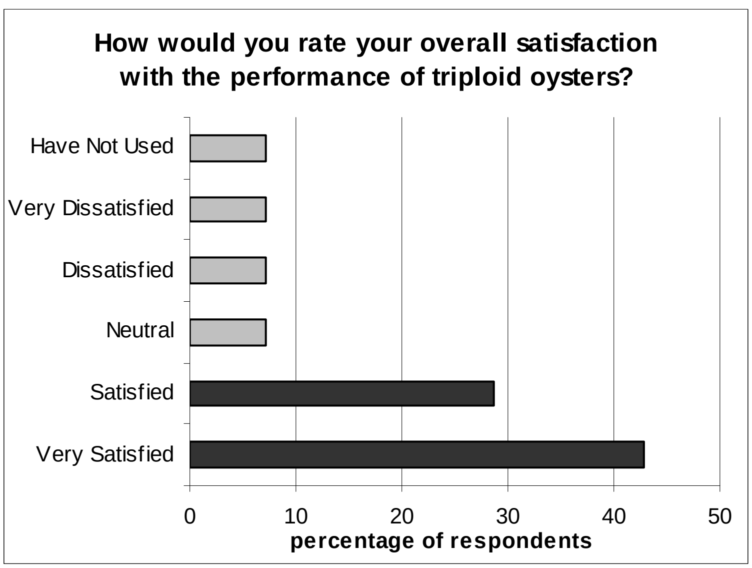
Question 2.



Question 3.

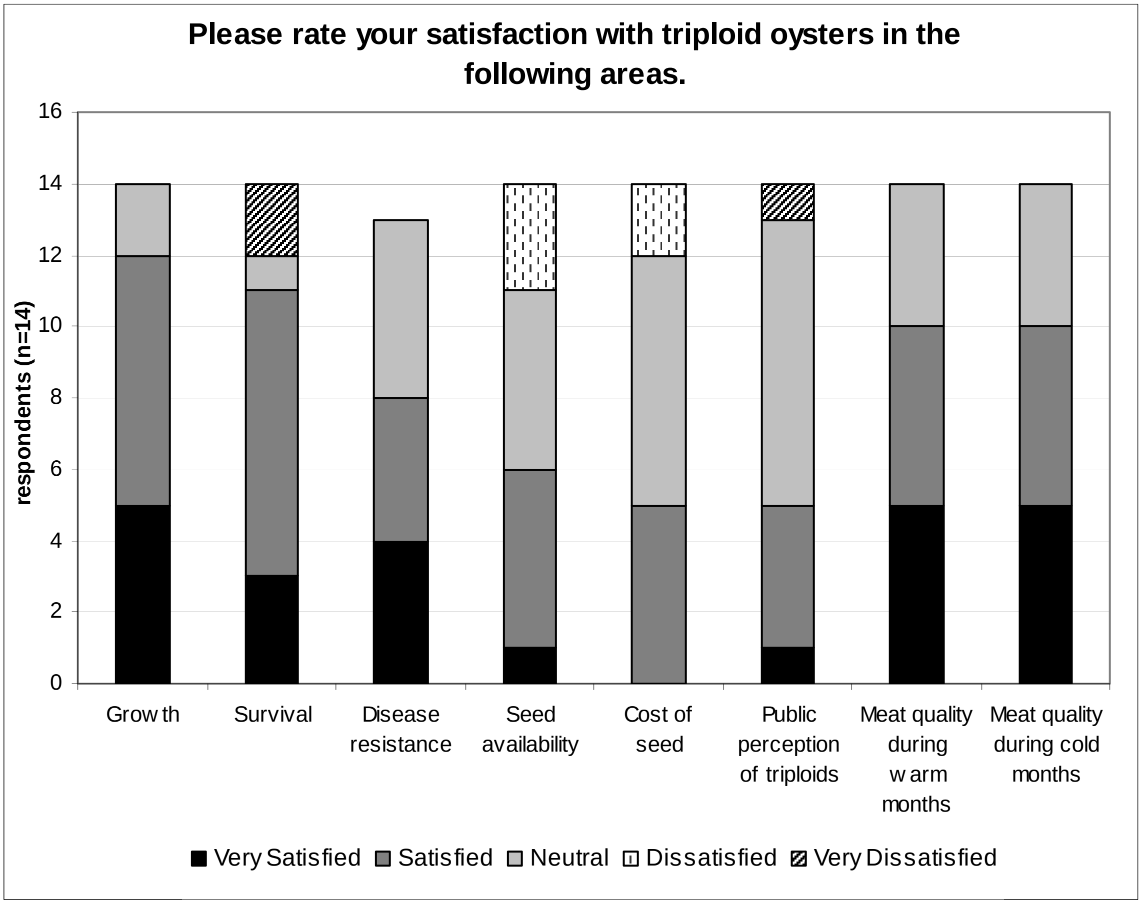


Question 4.



Note: 69.3% were very satisfied or satisfied with triploid oyster performance

Question 5.



Question 6.: Reason for neutral or dissatisfied response(s) in previous question #5:
answer: received the order late (by 1.5 months) and at a smaller size than ordered. Entire cost (broodstock tax) was not given upfront when order was made, 80% mortality by fall

answer: seed not readily available, cost add-ons are charged by hatcheries and public suspects genetic interference may be unnatural.

answer: few sources for triploid seed. I don't know what the public perception of triploids is but in my opinion I don't think they care. Most west coast oysters are triploids and I don't think the public is even aware of this.

answer: tetraploid providers are very unreliable

answer: I have very limited experience with growing oysters so I have nothing to compare this experience with.

answer: no real information, re: 'public perception of triploids'

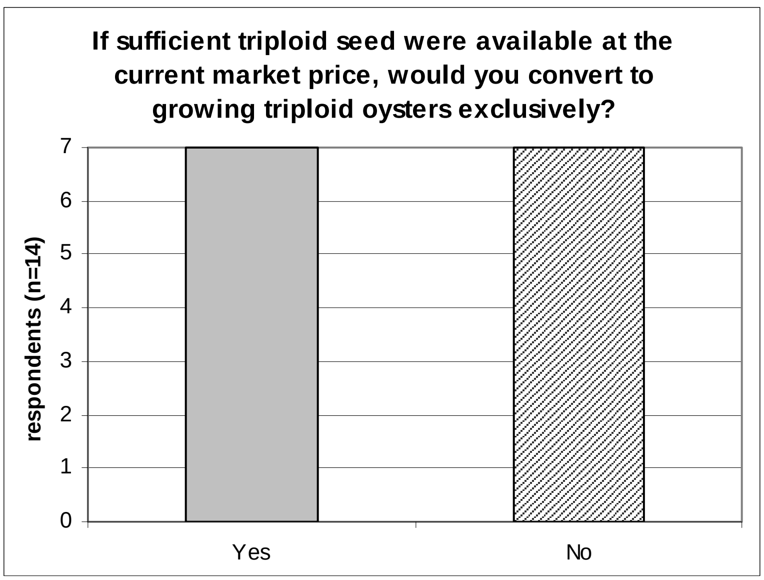
answer: not sure if Steve M from FI oyster does the triploids. I only buy from him, and I buy 25-40mm oysters. Does any group raise triploids to that size, if so, and the price was comparable, I would switch.
answer: not sure of difference - did not survey

answer: 25% survival – unsure of reason – looks like may have been weak seed from the beginning

answer: it is still to early for me to figure out how they are doing over all. This year 2011 should be the real indicator for me.

answer: did not use triploid oysters

Question 7.



Question 8.: General comments on your observed performance of triploid oysters:
answer: we received triploids from ARC. We had good growth through the summer. By fall 80% suddenly died. No known disease or management cause for mortality. The cause is still unknown – other farms in the area had the same experience. We suspect that we received the runts of the batch.

answer: rapid growth evident to us but mature oysters were misshaped in a higher percentage than we normally see(banana shells). We are not accustomed to growing out our oysters in the confinement of bags and felt this may have caused the shaping we experienced.

answer: it really all comes down to a good looking and good tasting oyster during the spawning months *answer:* nice size, shape and meat quality was superb. Many customers also commented they were very high quality and superb taste

answer: heh- I grow exclusively triploids and will continue to do so

answer: depth good and quality of meat good

answer: RFN site in East Dennis is similar to my site and performance of triploid was not significantly different than diploid. Seed that I use from Fishers Island produce market oysters in 16 months and have no problem with meat quality.

answer: the oysters grew so fast that some of the shells were a bit thin

Question 9.: What specifically would you like to see in the future for triploid oyster production?

answer: healthier seed.

answer: if we could, we'd prefer to remove trips from bags at 1 1/2" to 2" size and spread on bottom.

answer: drastically increased sales

answer: would like to see tetraploid research and production taking place locally (ARC?)

answer: market and customer perception of triploid oysters. Are they considered genetically modified organisms or .....?

answer: more, cheaper seed, but as Rutgers et al have a patent on the process, that's unlikely

answer: see above

answer: better availability of seed

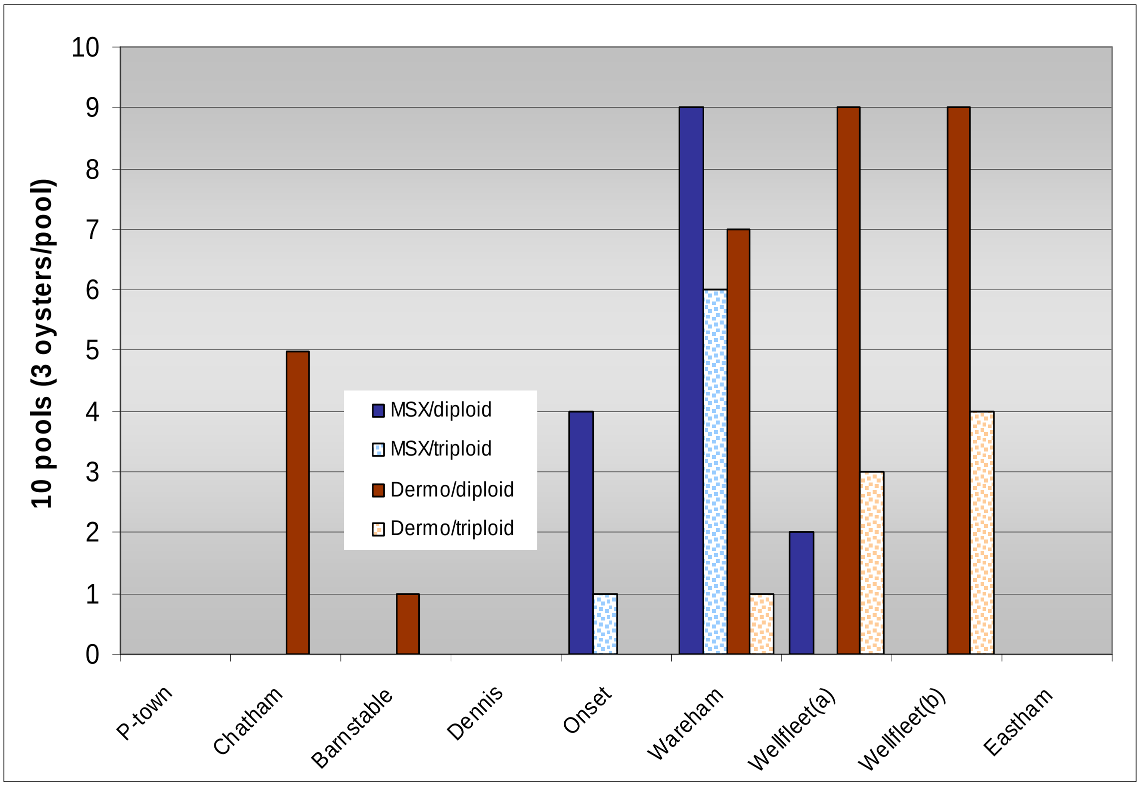
answer: if Fishers Island sold a triploid version I would try some but don't to take time and effort with another hatchery stock and do a study when have no problems with current stock.

answer: I would like to see triploids available at more hatcheries, so that the prices might come down

answer: early availability

Disease Results

T-test: Paired two sample for means – all sites (2010)

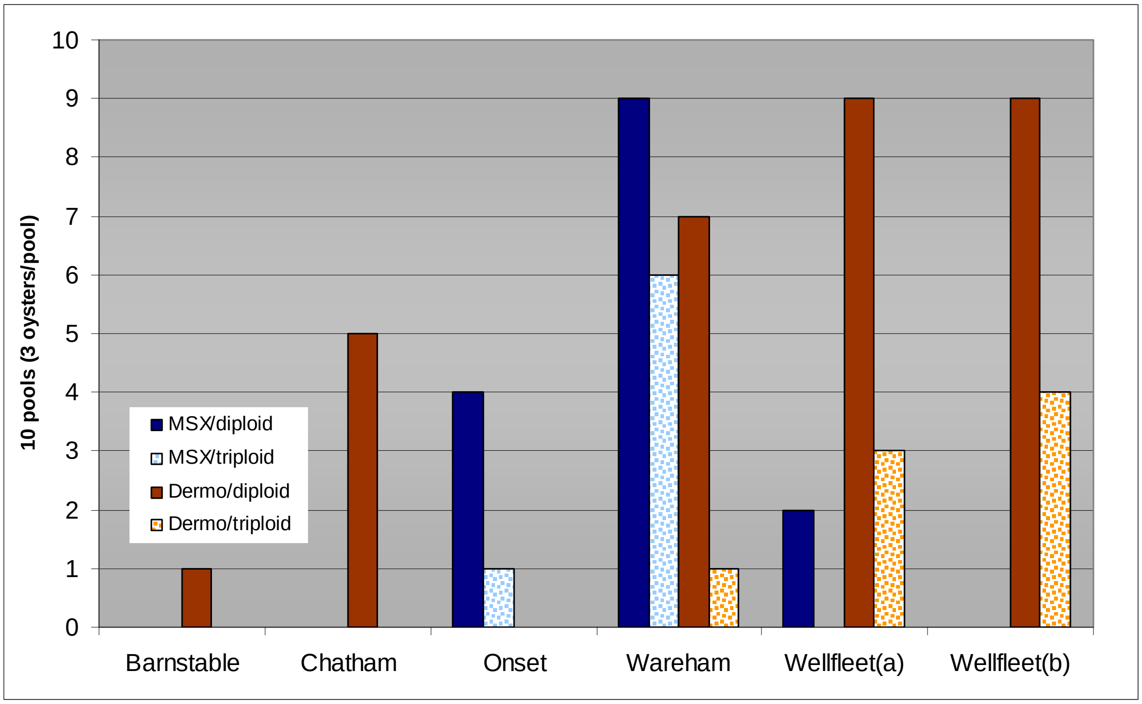


t-Test: Paired Two Sample for Means

	Dermo/diploid	***Dermo/triploid***
Mean	3.444	0.889
Variance	16.278	2.361
Observations	9	9
df	8	
P(T<=t) two-tail	**0.0268**	
	MSX/diploid	***MSX/triploid***
Mean	1.667	0.778
Variance	9.5	3.944
Observations	9	9
df	8	
P(T<=t) two-tail	**0.0864**	

These are results from all sites including those negative for disease. Dermo was found to have significantly higher means (p=0.0268) in diploid vs. triploid oyster pools and MSX approached significantly higher means in diploids (p=0.0864).

T-test: Paired two sample for means – disease positive sites only (2010)

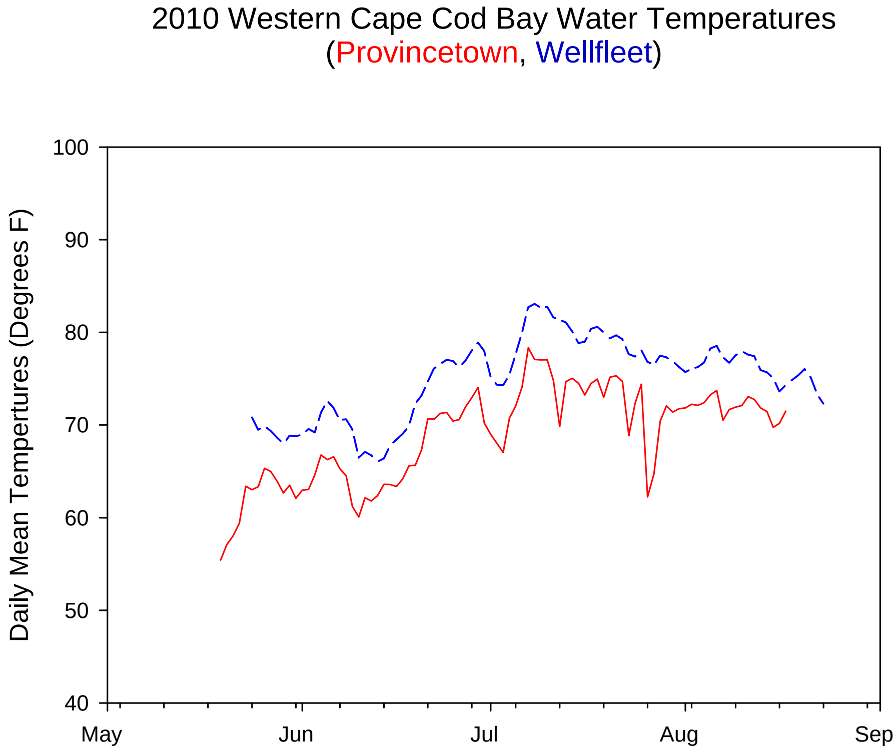


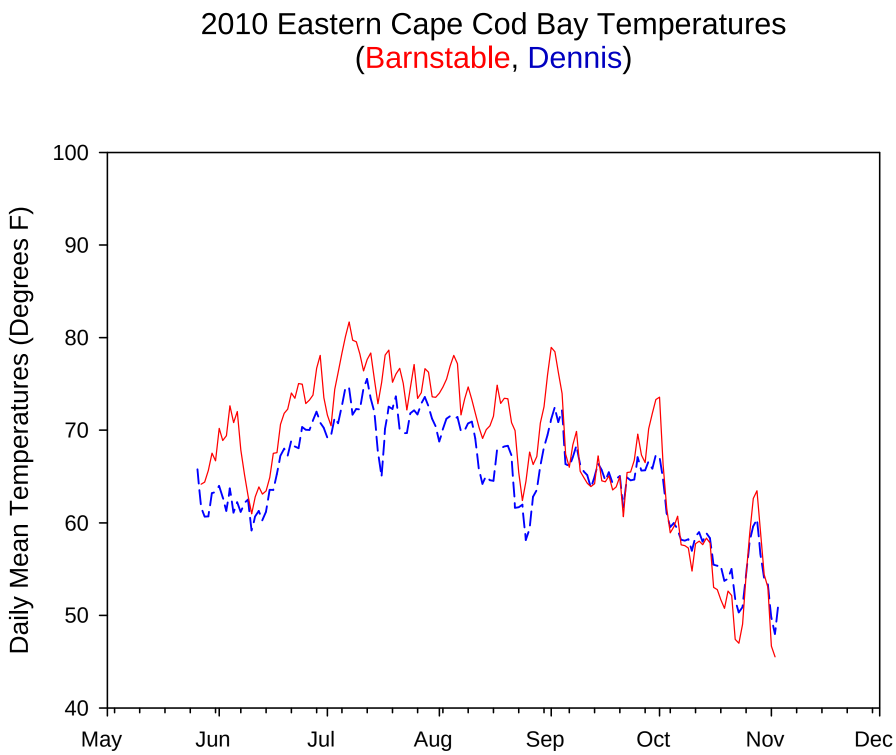
t-Test: Paired Two Sample for Means

	Dermo/diploid	*Dermo/triploid*
Mean	6.2	1.6
Variance	11.2	3.3
Observations	5	5
df	4	
P(T<=t) two-tail	**0.0077**	
	MSX/diploid	***MSX/triploid***
Mean	5	2.333
Variance	13	10.333
Observations	3	3
df	2	
P(T<=t) two-tail	**0.0153**	

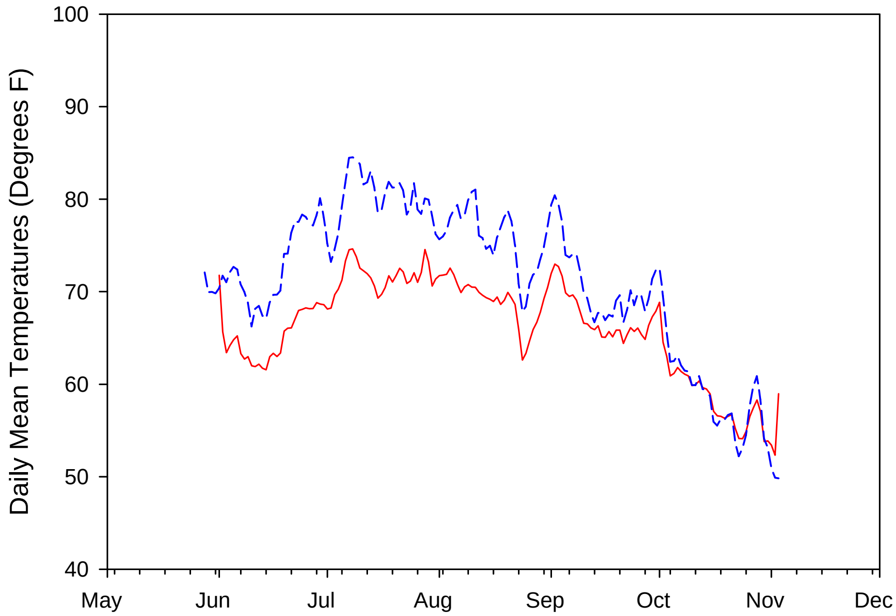
Sites negative for disease were removed and analysis was repeated on disease-positive sites only. Both Dermo and MSX had significantly higher means in pooled diploid vs. triploid oysters (p=0.0077 and p=0.0153, respectively).

Temperature





2010 Buzzards Bay Water Temperatures
(Onset, Wareham)



Ploidy Results

Barnstable – Fall 2010 Ploidy Results

Bag Number	Putative ploidy	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Top	5	81	5/5 triploid
2	Diploid	Top	5	74	3/5 diploid, 2/5 triploid
3	Triploid	Top	5	80	5/5 triploid
4	Diploid	Top	5	70	5/5 diploid
5	Triploid	Top	5	84	5/5 triploid
6	Diploid	Top	5	77	3/5 diploid, 2/5 triploid
7	Diploid	Bottom	5	77	5/5 diploid
8	Triploid	Bottom	5	76	5/5 triploid
9	Diploid	Bottom	5	80	4/5 diploid, 1/5 triploid
10	Triploid	Bottom	5	91	5/5 triploid
11	Diploid	Bottom	5	76	5/5 diploid
12	Triploid	Bottom	5	100	5/5 triploid

Barnstable – Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Blue	Top	5	68	5/5 triploid
2	Diploid	Orange	Top	5	70	5/5 diploid
3	Triploid	Blue	Top	5	70	5/5 triploid
4	Diploid	Orange	Top	5	62	5/5 diploid
5	Triploid	Blue	Top	5	75	5/5 triploid
6	Diploid	Orange	Top	6	61	6/6 diploid
7	**Diploid**	Orange	Bottom	5	77	**5/5 triploid**
8	Triploid	Blue	Bottom	5	76	5/5 triploid
9	Diploid	Orange	Bottom	6	61	6/6 diploid
10	Triploid	Blue	Bottom	5	79	5/5 triploid
11	Diploid	Orange	Bottom	6	60	6/6 diploid
12	**Triploid**	Blue	Bottom	5	66	**5/5 diploid**

Chatham – Fall 2010 Ploidy Results

Bag Number	Putative ploidy	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Top	5	114	5/5 triploid
2	Diploid	Top	5	81	5/5 diploid
3	Triploid	Top	5	101	5/5 triploid
4	Diploid	Top	0	n/a	n/a
5	Triploid	Top	0	n/a	n/a
6	Diploid	Top	0	n/a	n/a
7	Diploid	Bottom	5	91	5/5 diploid
8	Triploid	Bottom	5	96	5/5 triploid
9	Diploid	Bottom	5	88	5/5 diploid
10	Triploid	Bottom	0	n/a	n/a
11	Diploid	Bottom	0	n/a	n/a
12	Triploid	Bottom	0	n/a	n/a

Chatham – Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Length (mm)	Actual ploidy
1	Triploid	Blue	Top	5	98	5/5 triploid
2	Diploid	Orange	Floated	5	60	5/5 diploid
3	Triploid	Blue	Top	5	87	5/5 triploid
4	Triploid	Floated		5	80	5/5 triploid
5	Triploid	Blue	Top	5	83	5/5 triploid
6	Diploid	Orange	Top	5	75	5/5 diploid
7	Diploid	Orange	Bottom	5	72	5/5 diploid
8	Triploid	Blue	Bottom	5	89	5/5 triploid
9	Diploid	Orange	Bottom	5	76	5/5 diploid
10	Triploid	Blue	Bottom	5	90	5/5 triploid
11	Diploid	Orange	Bottom	5	71	5/5 diploid
12	Triploid	Blue	Bottom	5	93	5/5 triploid

Cuttyhunk – Spring 2011 Ploidy Results

Bag Number	No. of Samples	Average Size (mm)	Actual ploidy
1	5	50	5/5 triploid
2	4	42	4/4 triploid
3	5	49	5/5 triploid
4	5	50	5/5 diploid
5	4	50	4/4 triploid
6	5	47	5/5 diploid
7	5	39	5/5 diploid
8	5	42	5/5 triploid
9	5	39	4/5 diploid, 1/5 triploid
10	5	37	5/5 diploid
11	5	46	5/5 diploid
12	5	49	5/5 triploid

Cuttyhunk – Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	**Triploid**	Blue	Top	6	46	**6/6 diploid**
2	**Diploid**	Orange	Top	Labeled "missing"		N/A
3	**Triploid**	Blue	Top	5	47	**5/5 diploid**
4	**Diploid**	Orange	Top	6	45	**6/6 triploid***
5	**Triploid**	Blue	Top	5	45	**5/5 diploid**
6	**Diploid**	Orange	Top	5	59	**5/5 triploid**
7	**Diploid**	Orange	Bottom	5	51	**5/5 triploid**
8	**Triploid**	Blue	Bottom	6	41	**5/5 diploid**
9	**Diploid**	Orange	Bottom	5	49	**5/5 triploid**
10	**Triploid**	Blue	Bottom	5	42	**5/5 diploid**
11	**Diploid**	Orange	Bottom	5	52	**5/5 triploid**
12	**Triploid**	Blue	Bottom	6	43	**5/5 diploid**

Dennis – Fall 2010 Ploidy Results

Bag Number	Putative ploidy	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Top	5	90	5/5 triploid
2	Diploid	Top	5	79	3/5 diploid, 2/5 triploid
3	Triploid	Top	5	89	5/5 triploid
4	Diploid	Top	5	80	4/5 diploid, 1/5 triploid
5	Triploid	Top	5	89	5/5 triploid
6	Diploid	Top	5	81	5/5 diploid
7	Diploid	Bottom	5	81	5/5 diploid
8	Triploid	Bottom	5	102	5/5 triploid
9	Diploid	Bottom	5	81	5/5 diploid
10	Triploid	Bottom	5	89	5/5 triploid
11	Diploid	Bottom	5	78	5/5 diploid
12	Triploid	Bottom	5	91	5/5 triploid

Dennis – Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Blue	Top	5	66	5/5 triploid
2	Diploid	Orange	Top	5	53	5/5 diploid
3	Triploid	Blue	Top	5	73	5/5 triploid
4	Diploid	Orange	Top	0	N/A	N/A
5	Triploid	Blue	Top	5	68	5/5 triploid
6	Diploid	Orange	Top	5	55	5/5 diploid
7	Diploid	Orange	Bottom	5	62	5/5 diploid
8	Triploid	Blue	Bottom	5	62	5/5 triploid
9	Diploid	Orange	Bottom	5	55	5/5 diploid
10	Triploid	Blue	Bottom	5	64	5/5 triploid
11	Diploid	Orange	Bottom	5	63	5/5 diploid
12	Triploid	Blue	Bottom	5	65	5/5 triploid

Eastham – Fall 2010 Ploidy Results

Bag Number	Putative ploidy	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Top	5	82	5/5 triploid
2	Diploid	Top	5	69	5/5 diploid
3	Triploid	Top	5	90	5/5 triploid
4	Diploid	Top	5	71	5/5 diploid
5	Triploid	Top	5	79	5/5 triploid
6	Diploid	Top	5	83	5/5 diploid
7	Diploid	Bottom	5	83	5/5 diploid
8	Triploid	Bottom	5	91	5/5 triploid
9	Diploid	Bottom	5	82	5/5 diploid
10	Triploid	Bottom	5	88	5/5 triploid
11	Diploid	Bottom	5	76	5/5 diploid
12	Triploid	Bottom	5	103	5/5 triploid

Eastham – Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Length (mm)	Actual ploidy
1	Triploid	Blue	Top	5	49	5/5 triploid
2	Diploid	Orange	Top	5	46	5/5 diploid
3	Triploid	Blue	Top	5	50	5/5 triploid
4	Diploid	Orange	Top	5	47	5/5 diploid
5	Triploid	Blue	Top	7	49	7/7 triploid
6	Diploid	Orange	Top	5	46	4/5 diploid, 1 triploid
7	Diploid	Orange	Bottom	6	62	5/6 diploid, **1 dead**
8	Triploid	Blue	Bottom	5	73	5/5 triploid
9	Diploid	Orange	Bottom	5	66	5/5 diploid
10	Triploid	Blue	Bottom	5	71	5/5 triploid
11	Diploid	Orange	Bottom	5	65	5/5 diploid
12	Triploid	Blue	Bottom	5	73	5/5 triploid

Onset – Fall 2010 Ploidy Results

Bag Number	Putative ploidy	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Top	5	95	5/5 triploid
2	Diploid	Top	5	76	5/5 diploid
3	Triploid	Top	5	98	5/5 triploid
4	Diploid	Top	5	77	5/5 diploid
5	Triploid	Top	5	98	5/5 triploid
6	Diploid	Top	5	82	5/5 diploid
7	Diploid	Bottom	5	80	5/5 diploid
8	Triploid	Bottom	5	94	5/5 triploid
9	Diploid	Bottom	5	78	5/5 diploid
10	Triploid	Bottom	5	97	5/5 triploid
11	Diploid	Bottom	5	79	5/5 diploid
12	Triploid	Bottom	5	87	5/5 triploid

Onset – Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Length (mm)	Actual ploidy
1	Triploid	Blue	Top	5	65	5/5 triploid
2	Diploid	Orange	Top	5	61	4/5 diploid, 1/5 triploid*
3	Triploid	Blue	Top	5	83	5/5 triploid
4	Diploid	Orange	Top	6	59	5/6 diploid, 1/6 triploid*
5	Triploid	Blue	Top	5	71	5/5 triploid
6	Diploid	Orange	Top	5	71	4/5 diploid, 1/5 triploid*
7	Diploid	Orange	Bottom	5	60	5/5 diploid
8	Triploid	Blue	Bottom	5	72	5/5 triploid
9	Diploid	Orange	Bottom	5	67	5/5 diploid
10	Triploid	Blue	Bottom	5	68	5/5 triploid
11	Diploid	Orange	Bottom	5	62	3/5 diploid, [2]/5 triploid*
12	Triploid	Blue	Bottom	5	84	5/5 triploid

Orleans– Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Length (mm)	Actual ploidy
1	Triploid	Blue	Top	5	64	5/5 triploid
2	Diploid	Orange	Top	5	49	5/5 diploid
3	Triploid	Blue	Top	5	67	5/5 triploid
4	Diploid	Orange	Top	6	50	6/6 diploid
5	Triploid	Blue	Top	5	63	5/5 triploid
6	Diploid	Orange	Top	5	43	4/5 diploid, 1/5 triploid*
7	Diploid	Orange	Bottom	5	46	5/5 diploid
8	Triploid	Blue	Bottom	5	54	5/5 triploid
9	Diploid	Orange	Bottom	4	45	4/4 diploid
10	Triploid	Blue	Bottom	0	N/A	N/A
11	Diploid	Orange	Bottom	5	36	2/5 diploid, **3/5 dead**
12	Triploid	Blue	Bottom	5	55	5/5 triploid

Provincetown – Fall 2010 Ploidy Results

Bag Number	Putative ploidy	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Top	0	n/a	n/a
2	Diploid	Top	0	n/a	n/a
3	Triploid	Top	0	n/a	n/a
4	Diploid	Top	5	65	4/5 diploid, 1/5 triploid
5	Triploid	Top	0	n/a	n/a
6	Diploid	Top	0	n/a	n/a
7	Diploid	Bottom	0	n/a	n/a
8	Triploid	Bottom	5	82	5/5 triploid
9	Diploid	Bottom	5	66	5/5 triploid
10	Triploid	Bottom	5	68	5/5 triploid
11	Diploid	Bottom	5	60	5/5 diploid
12	Triploid	Bottom	5	83	5/5 triploid

Provincetown – Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Length (mm)	Actual ploidy
1	Triploid	Blue	Top	5	48	4/5 triploid, 1/5 diploid*
2	Diploid	Orange	Top	7	47	7/7 diploid
3	Triploid	Blue	Top	5	60	5/5 triploid
4	Diploid	Orange	Top	6	50	5/6 diploid, [1]/6 triploid*
5	Triploid	Blue	Top	5	61	5/5 triploid
6	Diploid	Orange	Top	6	45	6/6 diploid
7	Diploid	Orange	Bottom	6	58	4/6 diploid, [2]/6 triploid*
8	Triploid	Blue	Bottom	5	68	5/5 triploid
9	Diploid	Orange	Bottom	6	58	6/6 diploid
10	Triploid	Blue	Bottom	5	62	5/5 triploid
11	Diploid	Orange	Bottom	6	54	6/6 diploid
12	Triploid	Blue	Bottom	5	68	5/5 triploid

Wareham – Fall 2010 Ploidy Results

Bag Number	Putative ploidy	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Top	5	77	5/5 triploid
2	Diploid	Top	5	60	4/5 diploid, 1/5 triploid
3	Triploid	Top	5	84	5/5 triploid
4	Diploid	Top	5	61	4/5 diploid, 1/5 triploid
5	Triploid	Top	5	78	5/5 triploid
6	Diploid	Top	5	62	5/5 diploid
7	Diploid	Bottom	5	62	5/5 diploid
8	Triploid	Bottom	5	80	5/5 triploid
9	Diploid	Bottom	5	70	5/5 diploid
10	Triploid	Bottom	5	89	5/5 triploid
11	Diploid	Bottom	5	66	5/5 diploid
12	Triploid	Bottom	5	72	5/5 triploid

Wareham – Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Blue	Top	5	70	5/5 triploid
2	Diploid	Orange	Top	5	53	5/5 diploid
3	Triploid	Blue	Top	5	84	5/5 triploid
4	Diploid	Orange	Top	2	43	2/2 diploid
5	Triploid	Blue	Top	5	74	5/5 triploid
6	Diploid	Orange	Top	5	60	5/5 diploid
7	Diploid	Orange	Bottom	2	42	2/2 diploid
8	Triploid	Blue	Bottom	5	71	5/5 triploid
9	Diploid	Orange	Bottom	4	54	4/4 diploid
10	Triploid	Blue	Bottom	5	71	5/5 triploid
11	Diploid	Orange	Bottom	4	47	4/4 diploid
12	Triploid	Blue	Bottom	5	72	5/5 triploid

Wellfleet (Inner Harbor) – Fall 2010 Ploidy Results

Bag Number	Putative ploidy	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Top	5	118	5/5 triploid
2	Diploid	Top	5	84	5/5 diploid
3	Triploid	Top	5	105	5/5 triploid
4	Diploid	Top	5	83	5/5 diploid
5	Triploid	Top	5	101	5/5 triploid
6	Diploid	Top	5	93	5/5 diploid
7	Diploid	Bottom	5	86	5/5 diploid
8	Triploid	Bottom	5	95	5/5 triploid
9	Diploid	Bottom	5	85	5/5 diploid
10	Triploid	Bottom	5	97	5/5 triploid
11	Diploid	Bottom	5	76	5/5 diploid
12	Triploid	Bottom	5	98	5/5 triploid

Wellfleet (Inner Harbor) – Fall 2009 Ploidy Results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Length (mm)	Actual ploidy
1	Triploid	Blue	Top	5	81	5/5 triploid
2	Diploid	Orange	Top	5	61	5/5 diploid
3	Triploid	Blue	Top	5	89	5/5 triploid
4	Diploid	Orange	Top	5	66	5/5 diploid
5	Triploid	Blue	Top	5	86	5/5 triploid
6	Diploid	Orange	Top	5	75	5/5 diploid
7	Diploid	Orange	Bottom	5	56	5/5 diploid
8	Triploid	Blue	Bottom	5	80	5/5 triploid
9	Diploid	Orange	Bottom	5	68	5/5 diploid
10	Triploid	Blue	Bottom	5	79	5/5 triploid
11	Diploid	Orange	Bottom	5	71	5/5 diploid
12	Triploid	Blue	Bottom	5	85	5/5 triploid

Wellfleet (South Wellfleet) – Fall 2010 Ploidy results

Bag Number	Putative ploidy	Level	No. of Samples	Average Size (mm)	Actual ploidy
1	Triploid	Top	5	102	5/5 triploid
2	Diploid	Top	5	86	5/5 diploid
3	Triploid	Top	5	111	5/5 triploid
4	Diploid	Top	5	81	5/5 diploid
5	Triploid	Top	5	92	5/5 triploid
6	Diploid	Top	5	94	5/5 diploid
7	Diploid	Bottom	5	86	5/5 diploid
8	Triploid	Bottom	5	103	5/5 triploid
9	Diploid	Bottom	5	82	4/5 diploid, 1/5 triploid
10	Triploid	Bottom	5	91	5/5 triploid
11	Diploid	Bottom	5	87	5/5 diploid
12	Triploid	Bottom	5	97	5/5 triploid

Wellfleet (South Wellfleet) – Fall 2009 Ploidy results

Bag Number	Putative ploidy	Color	Level	No. of Samples	Average Length (mm)	Actual ploidy
1	Triploid	Blue	Top	5	87	5/5 triploid
2	Diploid	Orange	Top	5	69	4/5 diploid, 1/5 triploid
3	Triploid	Blue	Top	5	88	5/5 triploid
4	Diploid	Orange	Top	5	61	5/5 diploid
5	Triploid	Blue	Top	5	83	5/5 triploid
6	Diploid	Orange	Top	5	65	5/5 diploid
7	Diploid	Orange	Bottom	5	69	5/5 diploid
8	Triploid	Blue	Bottom	5	91	5/5 triploid
9	Diploid	Orange	Bottom	5	68	5/5 diploid
10	Triploid	Blue	Bottom	5	93	5/5 triploid
11	Diploid	Orange	Bottom	5	64	5/5 diploid
12	Triploid	Blue	Bottom	5	95	5/5 triploid