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Ecological Consultants

Memorandum

3 March 2015

To: Greg Dale, Southwest Operations Manager, Coast Seafoods Company

From: Neil Kalson, Fisheries Ecologist, and Ken Lindke, Quantitative Ecologist, H. T. Harvey & Associates

Subject: 1.5-Foot-Elevation Oyster Culture Feasibility Study—Final Report

Summary

This report presents the methods, results, and conclusions of a study carried out by H. T. Harvey & Associates (HTH), on behalf of Coast Seafoods Company, to characterize the commercial feasibility of cultivating oysters in Humboldt Bay at tidal elevations above (i.e., separate from) those suitable for eelgrass (*Zostera marina*) habitat. The study's results indicate that there was no significant difference in oyster growth, biofouling, or quality between the higher- and lower-elevation study plots. However, for one type of oyster, numbers and total weight per oyster cluster were significantly lower in the higher-elevation study plots. For another type of oyster, the total weight per cluster was significantly lower in the higher plots.

Background

On 11 June 2006, the California Coastal Commission (CCC) approved Coast Seafoods Company's Coastal Development Permit E-06-003 (Permit) to continue oyster culture operations in the coastal zone of northern Humboldt Bay. Permitted activities include "planting, growing and harvesting off-bottom oyster culture on approximately 255 acres; completing conversion (from bottom culture) and planting, growing and harvesting off-bottom oyster culture on approximately 45 acres; and operating a nursery area, floating upwelling system (FLUPSY), and wet storage floats" (CCC 2006).

Special Condition #5 of the Permit requires that Coast Seafoods Company "evaluate the feasibility of cultivating oysters at depths typically unsuitable for eelgrass (*Zostera marina*) growth (i.e., 1.5 feet above mean lower low water (MLLW)) in Humboldt Bay." Current commercial harvest plans in northern Humboldt Bay rely on planting and harvesting oysters at elevations at which eelgrass also grows; however, it is the National Marine Fisheries Service's [NMFS's] policy to recommend that there be no net loss of eelgrass habitat function (National Oceanic and Atmospheric Administration [NOAA] 2014). Eelgrass beds, and shallows

that may support eelgrass, are considered special aquatic sites under the Clean Water Act Section 404(b)(1) guidelines (Title 40, Code of Federal Regulations, Part 230.43). Eelgrass also is considered an essential fish habitat area of particular concern under the Magnuson-Stevens Fishery Conservation and Management Act for some fish species that are managed under the Pacific Coast Groundfish Fishery Management Plan (Pacific Fishery Management Council [PFMC]) 2008).

Cultivation of oysters is technically possible at a wide range of elevations in Humboldt Bay, but some locations and elevations are preferred because they produce consistently high-quality oysters. Although it is accepted that oysters can be grown at higher elevations (e.g., 1.5 feet above MLLW), the extent to which oysters grown at such elevations would meet commercial expectations for growth, biofouling, survival, and quality has not been documented.

HTH (2011) developed a study plan titled *Coast Seafoods Company +1.5' Elevation Oyster Culture Feasibility Study* to evaluate the feasibility of cultivation options and thus help Coast Seafoods Company fulfill the conditions of Special Condition #5. CCC approved the study plan on 7 June 2011. In the study plan, we posed four research questions to address the feasibility of culturing oysters 1.5 feet above MLLW:

1. Is there a difference in oyster growth rates when oysters are grown 1.5 feet above MLLW versus 1.5 feet below MLLW?

2. Is there a difference in the amount of oyster biofouling when oysters are grown 1.5 feet above MLLW versus below 1.5 feet MLLW?

3. Is there a difference in oyster quality (measured as the ratio of tissue weight to tissue volume) when oysters are grown 1.5 feet above MLLW versus 1.5 feet below MLLW?

4. Is there a difference in oyster survival when oysters are grown 1.5 feet above MLLW versus 1.5 feet below MLLW?

Each of these questions addresses a critical component of commercially viable oyster culture. Growth rates directly correspond with harvest rates and production schedules. Biofouling organisms colonizing oyster clusters can affect growth rates and survival by competing with oysters for food, or may suffocate oysters. Oyster quality can affect the marketable yield from an oyster bed. Survival (related to productivity) also directly affects yield, and thus economic feasibility.
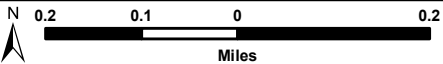
Methods

Planning Test and Control Plots

In 2012, three quarter-acre oyster beds were planted at industry-standard elevations (0.5 feet–1.0 feet above MLLW)—these served as the study's *control plots*. Three quarter-acre beds were planted at 1.5 feet–2.0 feet above MLLW to serve as *test plots*. These six plots had been randomly selected from an initial pool of 12 potential plots identified as having the correct characteristics for the study (Figure 1).

Project Vicinity

Eureka, CA

Study Area

Image Source: NAIP 2012

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Figure 1: Study Area
Coast Seafoods Company 1.5' Elevation Oyster Culture Feasibility Study
Northern Humboldt Bay, California (3225-02)
March 2015

In each plot, 20 longlines of Pacific oysters, (*Crassostrea gigas*), alternating with 20 longlines of Kumamoto oysters (*Crassostrea sikamea*) were planted by Coast Seafoods Company staff, using industry-standard methods. Each longline contained approximately 100 shell *cultch* (i.e., oyster shells with juvenile oysters [*spat*] attached). Each cultch had a similar number of spat attached, as determined by counting random samples and calculating the average spat count for each cultch. As spat grow on cultch, they become clusters of marketable-size oysters.

Modifications to the Study Plan

Although the study plan stated that we would estimate survival rates by monitoring the number of oysters on individual cultch/clusters over the study period, final results indicated that these data were inappropriate for analysis of survival. The data were not usable because there were several clusters that had more oysters present at the end of the study than had been counted at the beginning of the study. This circumstance resulted in estimates of survival greater than one, which is invalid. When clusters are set on lines, oysters are very small and can be difficult to see. Thus, undercounting at the beginning of the study is the most likely explanation for the spurious results; natural recruitment of oysters was considered as a possible explanation, but natural recruitment is unlikely or uncommon in Humboldt Bay (Dale pers. comm.). As a substitute for survival estimates, we used the total number of oysters present on individual clusters at the end of the study as a measure of productivity. This metric is expected to provide information similar to survival rates because the number of oysters per cluster should be strongly and positively related to survival. Also, the total number of oysters per cluster is important for evaluating production, because oysters may be sold individually.

One additional deviation from the study plan was to subsample some of the clusters for weighing and measuring individual oysters, in order to work within time constraints. Subsampling was not done systematically, but rather occurred on the rare occasion (5 out of 129 clusters) that HTH staff measuring oysters could not keep up with the speed at which Coast Seafoods personnel were processing clusters. We believe that the subsampling had no appreciable effect on the results of this study because it occurred so rarely, and most of the oysters were measured in each case.

One additional metric was not originally considered in the study plan, but was incorporated later into the study: total tissue weight per cluster. This, in addition to individual oyster weight, was measured to provide another indicator of productivity. Total weight per cluster is an important metric for commercial oyster production because oysters may be sold by weight as well as individually.

Sampling Methods

Sample Collection

Commercially grown oysters are typically harvested 18 to 30 months after planting. Accordingly, oysters were harvested after 24 months from the study plots. Ten to 17 longlines were randomly selected from each of the six study plots, and one to four clusters were randomly sampled from each of these longlines (Table 1). Each cluster was labeled and transported to Coast Seafoods Company's plant for processing.

Table 1.   Numbers of Longlines and Clusters Sampled

Plot	Number of Longlines Sampled per Plot	Total Number of Clusters Sampled per Plot
Control-1	17	22
Control-2	17	22
Control-3	11	21
Test-1	10	21
Test-2	12	21
Test-3	16	22

Sample Processing

The following methods were used to measure oyster growth, biofouling, quality, and productivity:

- **Growth** was evaluated by weighing individual oysters without their shells.

- **Biofouling** was measured by visually estimating the percent of an individual cluster that was covered with biofouling organisms. Individual oysters were then separated from their cluster, and all oysters were shucked for the growth and quality measurements.

- **Quality** was defined as the ratio of tissue weight to tissue volume. The volume of tissue of individual oysters was determined by submersing the tissue in a water-filled graduated cylinder sized appropriately to the volume of the oyster meat, then measuring the volume of water that was displaced (in milliliters). The oyster tissue was then drained in a sieve and weighed to the nearest 0.1 gram.

- **Productivity** was measured as the total number of oysters per cluster and the total weight per cluster. Only live oysters were counted to find the total number of oysters per cluster. Total weight per cluster was defined as the sum of the weights (without shells) of all oysters on a cluster. For 5 of the 129 clusters that were sampled, not all oysters in the cluster were processed, owing to time constraints. Instead, they were randomly subsampled. For these five clusters, the total weight per cluster was calculated as the weight of all the processed oysters in the cluster, plus the mean weight of the processed oysters in that cluster multiplied by the number of unprocessed oysters.

Statistical Analysis

We used generalized linear mixed-effects models (GLMMs), the second-order bias adjusted version of Akaike's Information Criterion (AICc) (Burnham and Anderson 2002), and the likelihood ratio test (LRT) to determine if there were significant differences in oyster growth, biofouling, quality, total oysters per cluster, and total weight per cluster between control plots and test plots. Mixed-effects models allow us to define experimental blocking variables (e.g., plot and/or cluster) as random variables, which avoids

pseudoreplication (Zuur et al. 2009). When pseudoreplication is unaccounted for, standard errors are underestimated, and predictive power is overestimated.

Data collected on individual oysters and clusters at the end of the 2-year study were used as dependent variables in model development. Prior to model fitting, quantile-quantile plots for each dependent variable were examined for each species of oyster (Kumamoto and Pacific). When data were not normally distributed, we applied data transformations to see if approximation to normality improved. When normality improved, the transformation that resulted in the closest approximation to normality was used in subsequent modeling. In a few cases, we considered error distributions other than the normal distribution. For example, a GLMM with gamma error was used for evaluating the quality of Kumamoto oysters because it fit the observed data better than a GLMM with normal error. Details of the transformation and model type (e.g., gamma) used for each dependent variable and oyster species can be found in Tables 2 and 3.

For each dependent variable and species, we defined a model with test/control as an independent variable and either plot number (for biofouling, total oysters per cluster, and total weight per cluster), or plot number and cluster number (for growth and quality) as random effects. This model was compared with a null model that excluded the test/control variable. Differences in AICc values between these two models, and p-values obtained from the LRT, were used to assess whether the test/control variable was significant, and thus whether there was a significant difference in the dependent variable between test and control plots. The model with the lowest AICc value fits the data best, so if the null model has a lower AICc score, then the test/control variable does not improve the fit, and we conclude that there is no difference between test and control plots. For the test/control variable to be considered important, the model that includes this variable must have the lower AICc value, and the null model should have an AICc value that is at least 1.5 lower (Burnham and Anderson 2002). This is not a definitive cut-off as in traditional hypothesis testing, so p-values from the LRT help us to interpret our results. P-values less than 0.05 indicate that there is a significant difference between test and control plots at the 95% confidence level.

Finally, model residuals for each top model were examined to ensure that model assumptions were adequately met. Plots of model residuals versus fitted values were examined to evaluate the assumption of independence for all models, and normal quantile-quantile plots of residuals were examined for models that assumed normally distributed error. All models were fit via maximum likelihood using either the function *glmer()* or *glmer.nb()* (for the number of oysters per cluster only) in package *lme4* (Bates et al. 2014) in the statistical computing environment R (R Core Team 2014).

Additional model assessment was necessary to analyze the number of oysters per cluster. A poisson mixed-effects model was initially used because it is the preferred model for count data. However, we found (using methods from Bolker et al. 2009) that the poisson mixed-effects model was overdispersed, so we instead used a negative-binomial GLMM. This is the preferred model for overdispersed count data (Bolker 2008), and it eliminated overdispersion for both species of oyster.

Results

Kumamoto Oysters

Individual oyster growth ($p=0.914$) and quality ($p=0.440$), and percent biofouling per cluster ($p=0.463$) did not differ significantly between test and control plots for Kumamoto oysters. AICc values differed between the test/control model and the null model by 2.07 for growth, 1.71 for quality, and 1.49 for percent biofouling. The null models had lower AICc values in all cases (Table 2). Also, there was little difference in mean growth, quality, or percent biofouling between test and control plots (Figure 2).

The number of live oysters per cluster ($p=0.009$) and the total weight per cluster ($p=0.014$) were significantly greater at control plots than at test plots at the 95% confidence level (Table 2). The AICc value for the test/control model was 4.58 lower than the null model for total oysters per cluster, and 3.82 lower than the null model for total weight per cluster. Mean total weight per cluster at control plots was 20.1 grams greater than at test plots—cluster weight in test plots thus averaged 51% of the cluster weight in control plots (Figure 2, panel E). The mean number of oysters per cluster in control plots was 5.2, versus 2.7 in the test plots; in other words, the average number of oysters in the test plots was 52% of the average number in the control plots (Figure 2, panel D). Residual analysis did not reveal any substantial violations of model assumptions, and there was no overdispersion for the negative-binomial model of the number of oysters per cluster.

Pacific Oysters

Individual oyster growth ($p=0.191$) and quality ($p=0.588$), percent biofouling ($p=0.457$), and the total number of oysters per cluster ($p=0.078$) did not differ significantly between test and control plots for Pacific oysters. AICc values differed between the test/control model and null model by 0.38 for growth, 1.81 for quality, and 1.75 for percent biofouling, with the null model having a lower AICc value in all cases. The AICc value for the test/control model was 0.81 lower than for the null model for the total number of oysters per cluster (Table 3). The mean values for control and test plots were very similar, and mean values of oyster quality were identical to the first decimal place (Figure 3, panel C).

The total weight per cluster was significantly greater at control plots at the 95% confidence level ($p=0.039$), and the AICc value was 1.99 lower for the test/control model. Mean total weight per cluster at control plots was 65.6 grams greater than at test plots; in other words, the cluster weight in test plots averaged about 65% of the average cluster weight in control plots (Figure 3, panel E). Residual analysis did not reveal any substantial violations of model assumptions, and there was no overdispersion for the negative-binomial model of the number of oysters per cluster.

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Table 2. Modeling Results for Kumamoto Oysters Grown at High (Test) and Low (Control) Elevation Plots in Humboldt Bay, California

Attribute	Measured Variable	Transformation	Error Distribution	Random Effects	Fixed Effect	Fixed Effect Coefficient (SE)	AICc	P-value
Growth	Individual oyster weight (grams)	n/a	Normal	Plot, cluster	Test/control	-0.042 (0.388)	1239.88	0.914
					Null	n/a	1237.81	
Biofouling	Percent biofouling per cluster	Arcsine-square root	Normal	Plot	Test/control	-0.036 (0.049)	-14.47	0.463
					Null	n/a	-16.18	
Quality	Individual oyster weight/volume (grams/milliliters)	n/a	Gamma	Plot, cluster	Test/control	0.009 (0.012)	-429.40	0.44
					Null	n/a	-430.89	
Total oysters per cluster	Total oysters per cluster	Log	Negative-binomial	Plot	Test/control	-0.648 (0.188)	314.02	0.009
					Null	n/a	318.60	
Total weight per cluster	Sum of individual weights for all oysters in cluster (grams)	n/a	Normal	Plot	Test/control	-20.168 (6.295)	647.49	0.014
					Null	n/a	651.31	

Note: All models are generalized linear mixed-effects models, fit using maximum likelihood; p-values are based on the likelihood ratio test.
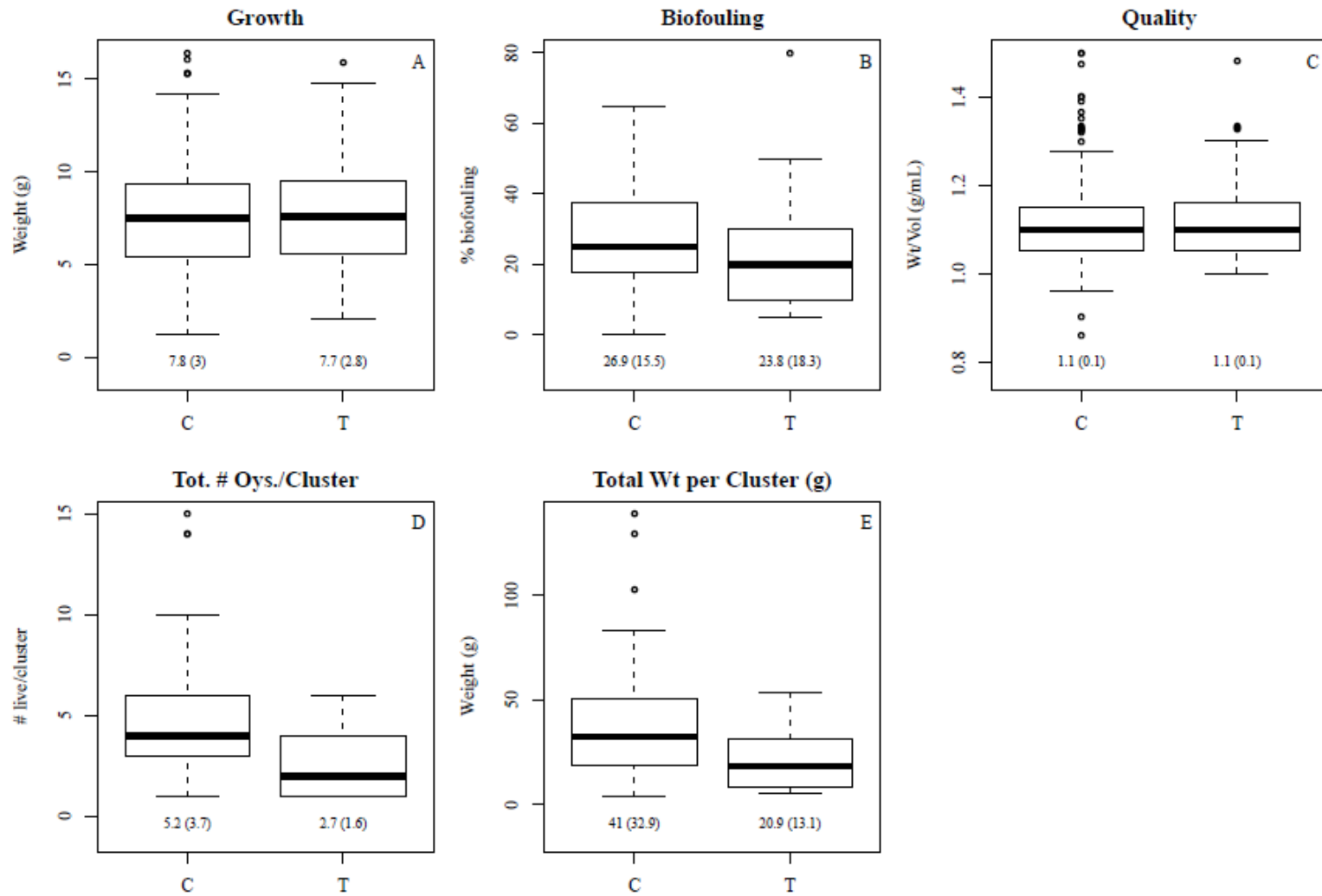
Figure 2. Box and Whisker Plots of Growth (A), Biofouling (B), Quality (C), the Number of Oysters per Cluster (D), and Total Tissue Weight per Cluster (E), for Kumamoto Oysters Grown in Humboldt Bay, California

C indicates control plots and *T* indicates test plots. Means are presented below each boxplot with standard deviations in parentheses. Thick horizontal bars represent medians, lower and upper edges of boxes represent first and third quartiles, whiskers extend to the smallest and largest observed values within 1.5 times the interquartile range, and open circles represent outliers.

Table 3. Modeling Results for Pacific Oysters Grown at High (Test) and Low (Control) Elevation Plots in Humboldt Bay, California

Attribute	Measured Variable	Transformation	Error Distribution	Random Effects	Fixed Effect	Fixed Effect Coefficient (SE)	AICc	*P*-value
Growth	Individual oyster weight (grams)	n/a	Normal	Plot, cluster	Test/control	-4.701 (3.283)	1771.59	0.191
					Null	n/a	1771.21	
Biofouling	Percent biofouling per cluster	Arcsine-square root	Normal	Plot	Test/control	-0.053 (0.071)	23.80	0.457
					Null	n/a	22.05	
Quality	Individual oyster weight/volume (grams/milliliters)	n/a	Log-normal	Plot, cluster	Test/control	-0.012 (0.021)	-365.96	0.588
					Null	n/a	-367.77	
Total oysters per cluster	Total oysters per cluster	Log	Negative-binomial	Plot	Test/control	1.323e-6 (0.162)	265.08	0.078
					Null		265.89	
Total weight per cluster	Sum of individual weights for all oysters in cluster (grams)	n/a	Normal	Plot	Test/control	-65.62 (31.15)	754.13	0.039
					Null	n/a	756.12	

Note: All models are generalized linear mixed-effects models, fit using maximum likelihood; p-values are based on the likelihood ratio test.
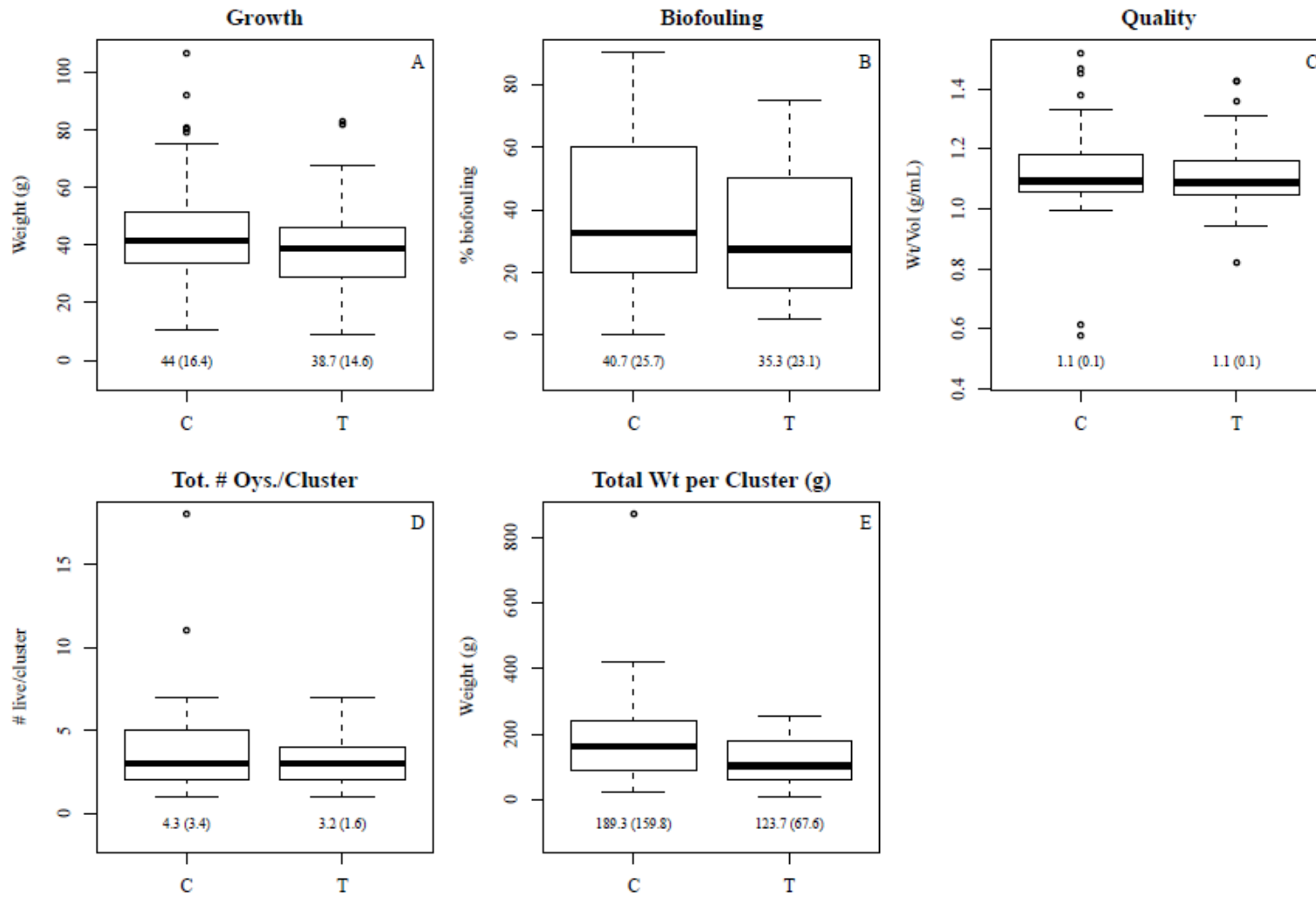
Figure 3. Box and Whisker Plots of Growth (A), Biofouling (B), Quality (C), the Number of Oysters per Cluster (D), and Total Tissue Weight per Cluster (E) for Pacific Oysters Grown in Humboldt Bay, California

C indicates control plots and *T* indicates test plots. Means are presented below each boxplot with standard deviations in parentheses. Thick horizontal bars represent medians, lower and upper edges of boxes represent first and third quartiles, whiskers extend to the smallest and largest observed values within 1.5 times the interquartile range, and open circles represent outliers.

Conclusion

This study demonstrated that cultivating oysters at elevations lower than 1.0 foot above MLLW produced more Kumamoto oysters by number and total weight, and more Pacific oysters by total weight, than cultivation 1.5 feet above MLLW. The difference observed in total weight per cluster was greater for Kumamoto oysters than for Pacific oysters. Specifically, the total weight per cluster of Kumamoto oysters in test plots was 51% of the total weight per cluster in control plots. For Pacific oysters, the total weight per cluster in test plots was 65% of the total weight per cluster in control plots. The mean total number of Kumamoto oysters per cluster in test plots was 52% of the mean total number in control plots.

Individual oyster weight did not differ between the two elevations for either species. For Kumamoto oysters, the difference in total weight per cluster can be attributed to the greater number of oysters per cluster at the low-elevation control plots. For Pacific oysters, the combination of a slightly greater average oyster weight and a slightly greater average number of oysters per cluster in the control plots (even though these effects were not statistically significant) is likely responsible for the significantly greater total weight per cluster observed in the control plots.

Other characteristics relevant to commercial cultivation (growth, biofouling, and quality) did not differ significantly between the test and control plots for either species.

References

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, and B. Dai. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. [online]: http://CRAN.R-project.org/package=lme4.

Bolker, B. M. 2008. Ecological Models and Data in R. Princeton University Press, Princeton, New Jersey.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology and Evolution 24:127–135.

Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Second edition. Springer, New York, New York.

[CCC] California Coastal Commission. 2006. Staff Report and Recommendation Coastal Development Permit Application. California Coastal Commission, San Francisco, California. CDP Application No. E-06-003. [online]: http://documents.coastal.ca.gov/reports/2006/5/Th5a-5-2006.pdf. Accessed 25 February 2015.

[HTH] H. T. Harvey & Associates. 2011. Coast Seafoods Company +1.5' Elevation Oyster Culture Feasibility Study. Coast Seafoods Company, Eureka, California.

[NOAA] National Oceanic and Atmospheric Administration. 2014. California Eelgrass Mitigation Policy and Implementing Guidelines. NOAA Fisheries, West Coast Region.

[PFMC] Pacific Fishery Management Council. 2008. Pacific Coast Groundfish Fishery Management Plan for the California, Oregon, and Washington Groundfish Fishery as Amended through Amendment 19 Including Amendment 15. NOAA Award No. NA05NMF441008. Pacific Fishery Management Council, Portland, Oregon.

R Core Team. 2014. R: A language and environment for statistical computing, v 3.1.1. R Foundation for Statistical Computing, Vienna, Austria. [online]: http://www.R-project.org/.

Zuur, A. F., E. N. Ieno, J. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York, New York.

Personal Communications

Dale, Greg. Southwest Operations Manager. Coast Seafoods Company. January 2015—Meeting with Adam Wagschal, Neil Kalson, and Ken Lindke of H. T. Harvey & Associates, regarding results of oyster feasibility study, project #3225-01.