A comparison of the growth and condition of the American oyster, *Crassostrea virginica*, on stabilized coal ash and oyster shell substrata

Presented to the faculty of Lycoming College
in partial fulfillment of the requirements
for Departmental Honors in Biology

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December 4, 1989

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Abstract

The growth and condition of the American oyster, Crassostrea virginica, on stabilized coal ash and oyster shell substrata was compared. Oysters from a benthic and a dock site on the Broadkill River, Lewes, Delaware were collected after two years growth. Growth was determined by height measurements, number of scars and boxes and wet and dry tissue and shell weights. Condition was determined by a condition index, organic, protein, and glycogen content. T-tests and regressions showed benthic coal ash oysters to have equal or better growth and condition than benthic shell or dock coal ash oysters. T-tests and regressions of organic and protein content indicated equal condition between groups. T-tests of glycogen content indicated better condition on the dock coal ash but equal condition between the benthic substrata. Therefore, stabilized coal ash does not appear to act as a stress on oysters and may actually enhance growth and condition.

Introduction

Crassostrea virginica (Gmelin), the American oyster, is a common edible species of the phylum Mollusca found on the Atlantic coast. The reproductive cycle of the oyster is characterized by a free floating larval stage which ends during spatting when the veliger larvae attach to substrata and become sessile. Oyster shells are the natural and preferred cultch or substratum. Aquaculturists, though, have experimented with alternate forms such as glass, plexiglass, slate, marble, and bamboo in order to find an inexpensive, plentiful, and less labor intensive substrata (Warren 1988).

Recently, the coal industry has offered coal waste as an alternative form of cultch. Flue gas desulfurization sludge, fly ash, and bottom ash are all produced as by products from the combustion of coal by power plants (Cross 1982). Currently, only ten to twelve percent of the annual 80 million tons of waste is recycled for uses such as roadbed fill and paving materials. The majority of coal waste is dumped in landfills at costs ranging from five to fifteen dollars per ton (Hardin 1989). Lime additives can stabilize the waste for environmentally sound oceanic disposal (Woodhead et al. 1981). Sea disposal, therefore, offers an attractive alternative to crowding and expensive landfills.

The University of Delaware, College of Marine Studies, has studied the feasibility of using coal ash waste to build underwater fish and oyster reefs through many research projects. Dinkins (1987) has shown stabilized coal waste to be a successful substratum for biological colonization. Garvey (1986) found some ash substrata to be very attractive cultch for oysters. Warren (1988), though, found stabilized coal ash substrata to function as a stress on oysters causing reduced shell and tissue growth and reduced condition.

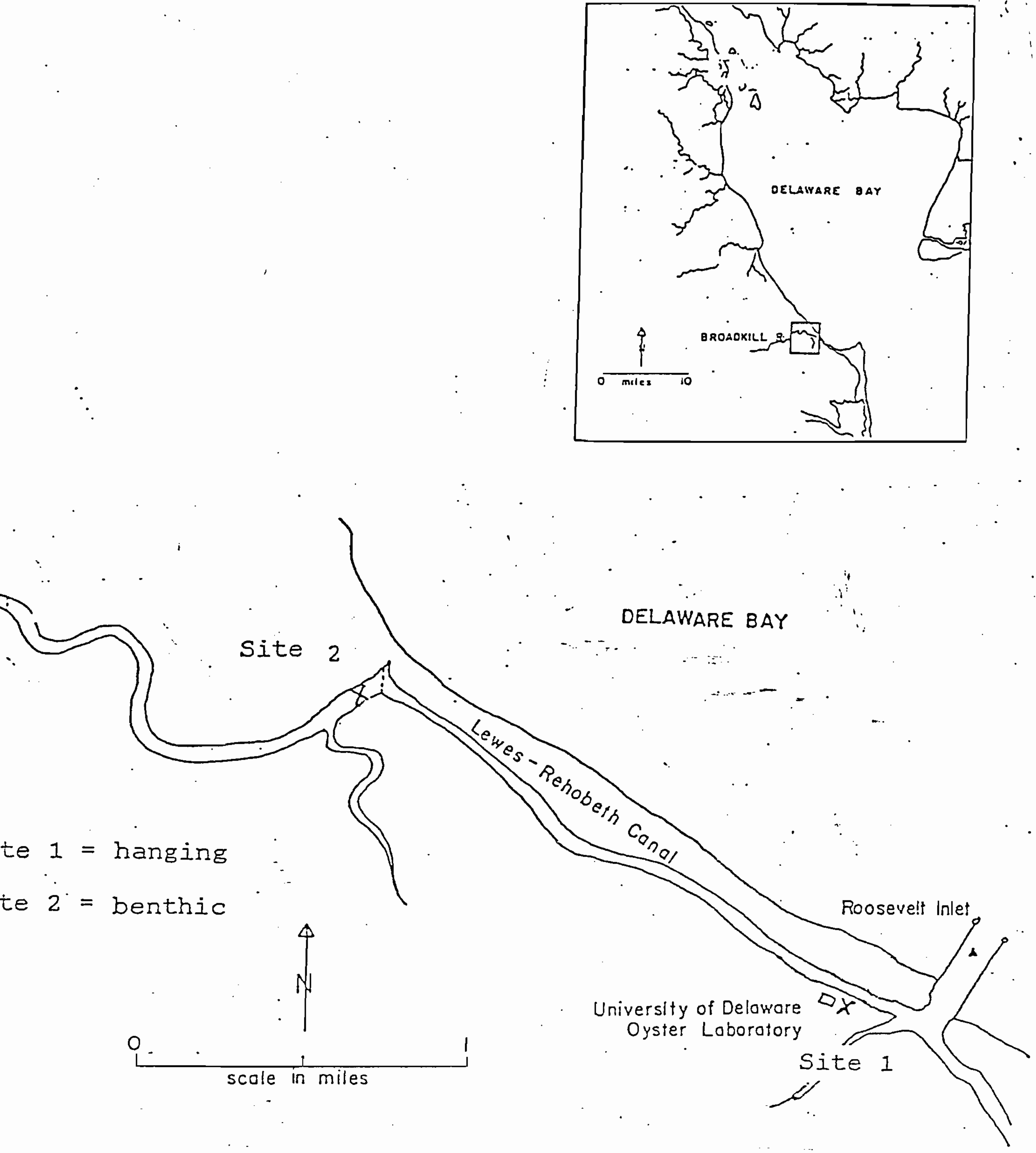
This study comparing the growth and condition of

Crassostrea virginica on coal ash pucks and oyster shells was begun during a marine sciences internship at the University of Delaware, College of Marine Studies, Lewes, DE. The project was completed as an honors independent study at Lycoming College, Williamsport, PA.

Materials and Methods

The American oyster, *Crassostrea virginica*, was collected from a benthic and a dock, hanging site where the oysters had been growing for two years. The benthic site was two miles upstream of Roosevelt Inlet, Lewes, Delaware on the Broadkill River (See Fig. 1). The experimental coal ash pucks (stabilized coal ash in the shape of hockey pucks) and control oyster shells were in polyethylene mesh sacks on the river's hard, sand bottom in approximately one meter depth of water at high tide.

The second site was a University of Delaware dock on the lower Broadkill River directly above Roosevelt Inlet (See Fig. 1). Envelope-like polyethylene mesh bags containing coal ash pucks hung from the dock. The bags were submerged at high tide but were hung above the waterline at low tide to discourage fouling. Despite this, heavy fouling by *Mytilus edulis*, the blue mussel, occurred. No control oyster shells were available at this site. Shell height of all live oysters was measured with a Manostat caliper to the nearest 0.1 mm. One hundred eighteen dock coal ash oysters and sixty-four benthic coal ash oysters were measured on the flat surfaces of the pucks. Forty-six dock



te 1 = hanging

te 2 = benthic

Fig. 1. Locations of C. virginica collection sites on the Broadkill River, Lewes, Delaware.

coal ash oysters and forty-one benthic coal ash oysters were measured on the rim. The rim and flat surfaces of the coal ash pucks were considered separately since previous literature considered only the flat surfaces of the puck (Garvey 1986, Price et al. 1988). A total of 87 benthic shell oysters were measured. Height was taken as the distance between the umbo and the ventral margin of the valve. The number of scars where oysters were previously attached was recorded as well as the number of boxes which are attached empty shells. Scars and boxes were unable to be counted on the oyster shell cultch as C. virginica did not remain attached to the shells. The oysters, still on pucks or shell, were then frozen at - 15^{O}C for later analysis.

A Statgraphics computer program was used throughout for statistical analysis. T-tests were performed between benthic coal ash and benthic shell ash means of height, number of boxes, and number of scars as well as benthic coal ash and dock coal ash means of the same parameters. Rim to flat surface coal ash puck comparisons were also done for all three factors.

Upon thawing, the upper valve and tissue was removed by cutting the adductor muscle with a scalpel. The left valve remained fused to the coal ash puck or was discarded. Thirty oysters from each substrata were used. Wet weights were recorded after blotting the tissues and the right valves. The tissues and valves were then baked in an oven for twenty-four hours at 60 degrees Celsius, and dry weights were found. T-tests were performed for both wet and dry mean weights of tissue and shell between benthic coal ash and benthic shell and also benthic coal ash and dock coal ash.

Dry weights were also used for a condition index of oysters upon each substratum. Instead of using the common dry shell weight to tissue weight ratio, the tissue weight to shell weight relationship was found by a simple regression of dry tissue weight on dry shell weight. T-tests then compared the regression coefficients of benthic coal ash and benthic shell and also benthic coal ash and dock coal ash. A regression gives a more accurate account due to the inability to use the left valve because of the fusion to the substratum (Warren 1988).

Condition of the oysters was also considered by the percentage of organics in the tissues and shells. The dried oyster tissue was homogenized then 50 mg of each oyster was place in pre-ashed test tubes. Shells were ashed in individual beakers. Both tissue and shells were ashed at 450°C in a muffle furnace for approximately 48 hours then weighed. The tissue ash weight was converted to a percentage from which individual oyster ash weight was calculated. The following formula was then used to calculate percent organic:

$$\frac{\text{Dry weight} - \text{Ash weight}}{\text{Dry weight}} \times 100$$

Regressions of tissue ash on tissue weight and shell ash on shell weight were done for each substratum. T-tests looked for significant differences in regression coefficients between benthic coal ash and benthic shell and also benthic coal ash and dock coal ash. T-tests of the percent organics of tissue and shell were also done between the same substrata.

Biochemical analysis was done as another indicator of condition. Twenty oyster meats from each group were removed and freeze-dried on a Virtis Freezemobile 24 for 24 - 48 hours. The lyophilized tissue was weighed, homogenized, and kept in a desiccator until needed. The tissue was then used for a protein and a glycogen assay. For the protein assay, ten oyster tissues from each substrata were digested and prepared for analysis according to procedures of Gaffney & Diehl (1986). A standard Bio-Rad protein assay was performed with the instructed volumes cut by fifty percent. Bovine Serum Albumin was used as the standard.

The glycogen assay was based on a procedure by Carr and Neff (1984). Modifications included grinding the tissue by hand in a homogenizer, using 0.1% amyloglucosidase solution, and centrifuging at 10,000g. Lacking a glucose analyzer, free glucose in the supernatant was measured by the glucose oxidase method (Sigma 1984). Oyster glycogen was used as the standard. Both protein and glycogen weights were read from a standard curve and converted to percentages of protein or glycogen per tissue weight. T-tests compared the mean percentages of protein and glycogen in benthic coal ash and benthic shell oyster tissue as well as benthic coal ash and dock coal ash oyster tissue.

Results

The mean height of *C. virginica* on coal ash pucks and shell from both sites is depicted in Fig. 2. The benthic site

Table 1. Mean number of scars and boxes on the flat surfaces and rims of coal ash pucks. * indicates a significant difference between the flat and rim of that substratum. ($\alpha = 0.05$, t-test)

	Benthic Coal		Dock Coal	
	flat	side	flat	side
Scars	* 7.50 ± 9.24	* 5.23 ± 6.57	15.74 ± 15.73	15.45± 11.09
Boxes	0.57± 1.10	0.59 ± 1.32	9.31 ± 9.19	8.96± 22.00

Table 2. Regression of dry meat weight on dry shell weight. * denotes a significant difference in slopes. ($\alpha = 0.05$, t-test)

Substratum	Regression Equation		SE of slope
Benthic Shell	$Y = 0.07 + 0.05x$		0.001
		*	
Benthic Coal	$Y = -0.10 + 0.09x$		0.011
		*	
Dock Coal	$Y = -0.01 + 0.20x$		0.015

SE = standard error

Table 3. Regression of tissue ash weight on tissue weight. ($\alpha = 0.05$, t-test)

Substratum	Regression Equation	SE of slope
Benthic Shell	$Y = 0.01 + 0.08x$	0.016
Benthic Coal	$Y = 0.01 + 0.07x$	0.009
Dock Coal	$Y = 0.00 + 0.08x$	0.003

SE = standard error

Table 4. Regression of shell ash weight on shell weight. ($\alpha = 0.05$, t-test)

Substratum	Regression Equation	SE of slope
Benthic Shell	$Y = -0.05 + 0.96x$	0.024
Benthic Coal	$Y = -0.01 + 0.95x$	0.015
Dock Coal	$Y = -0.01 + 0.94x$	0.005

SE = standard error

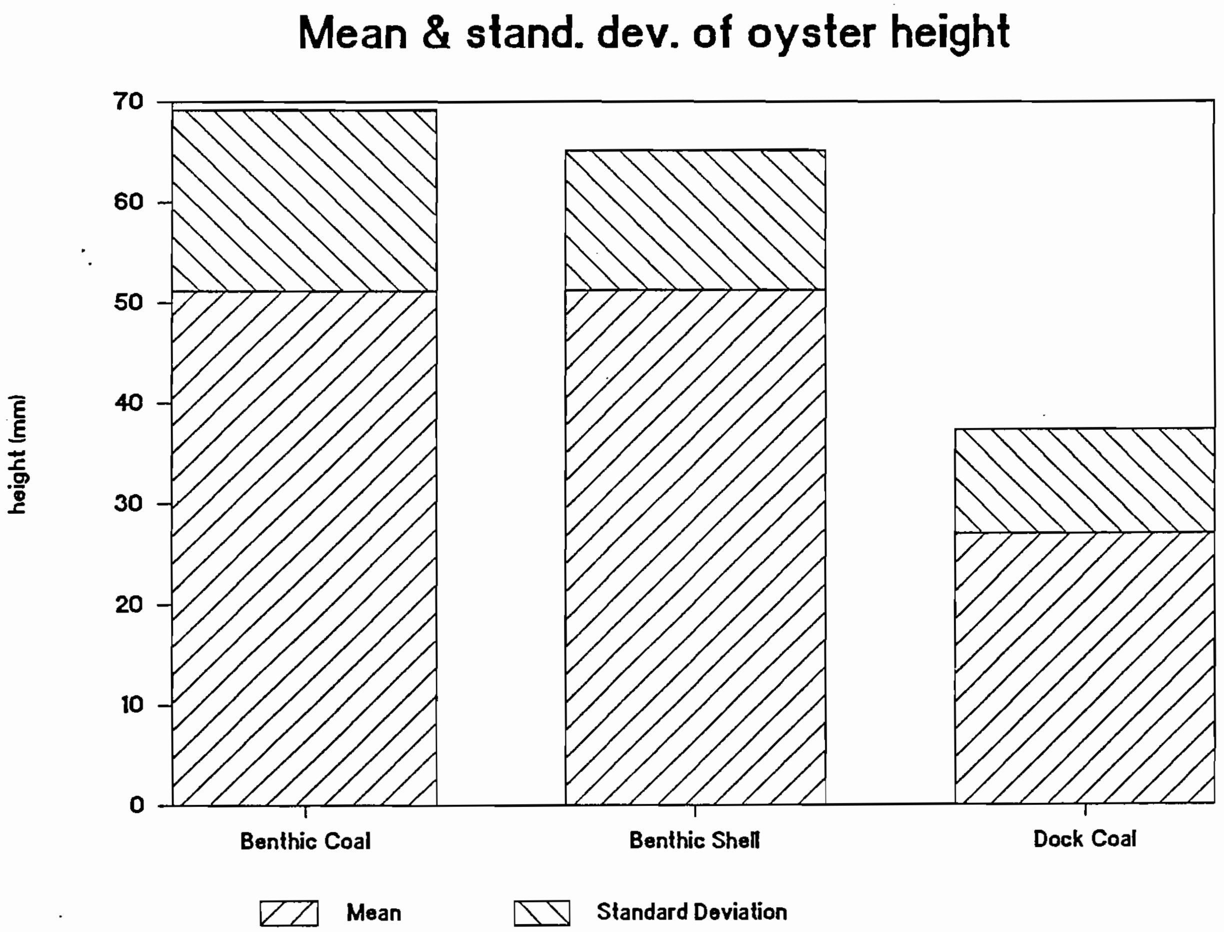


Fig. 2. Mean oyster heights and positive standard deviations of the mean on benthic coal ash, benthic shell, and dock coal ash.

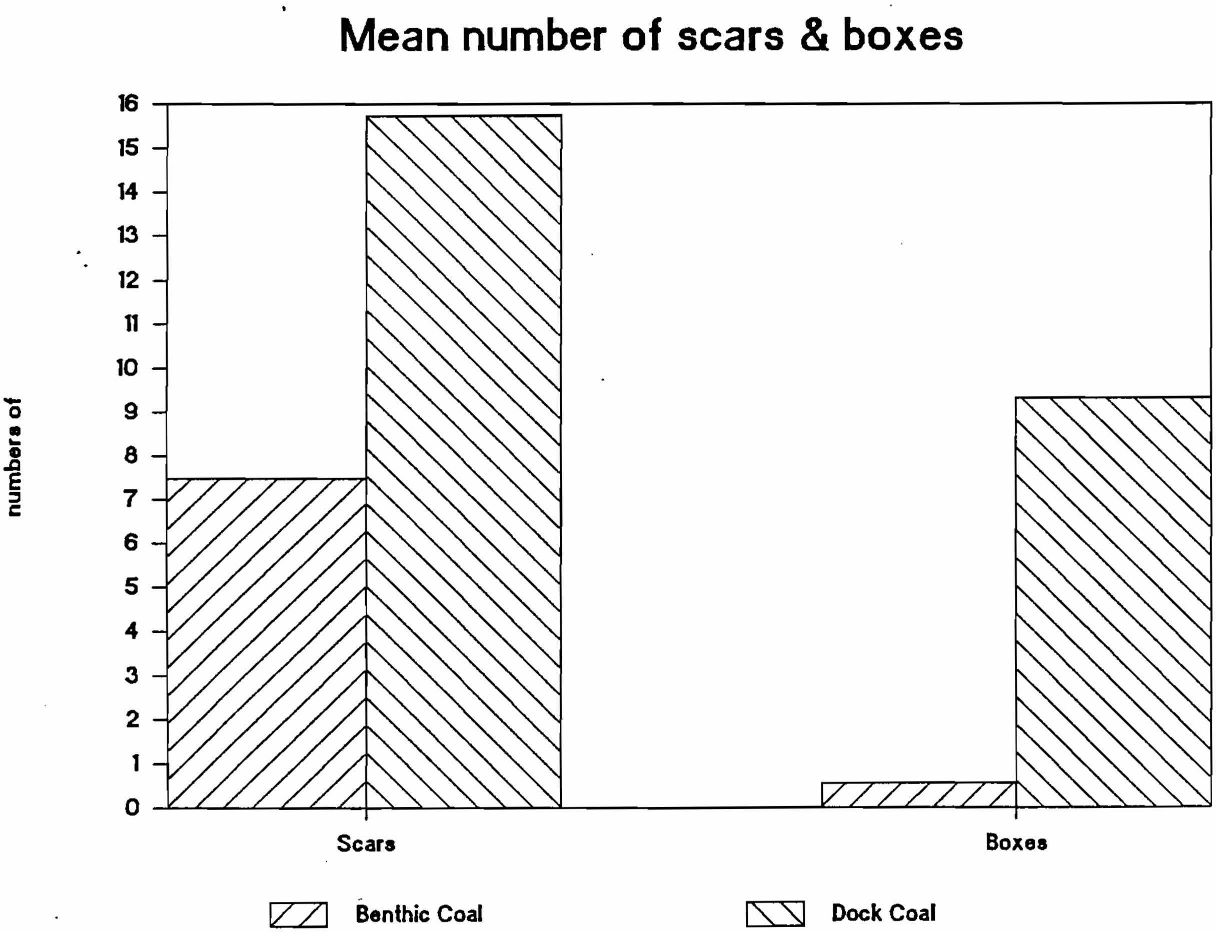


Fig. 3. Mean number of oyster scars and boxes on benthic coal ash and dock coal ash.

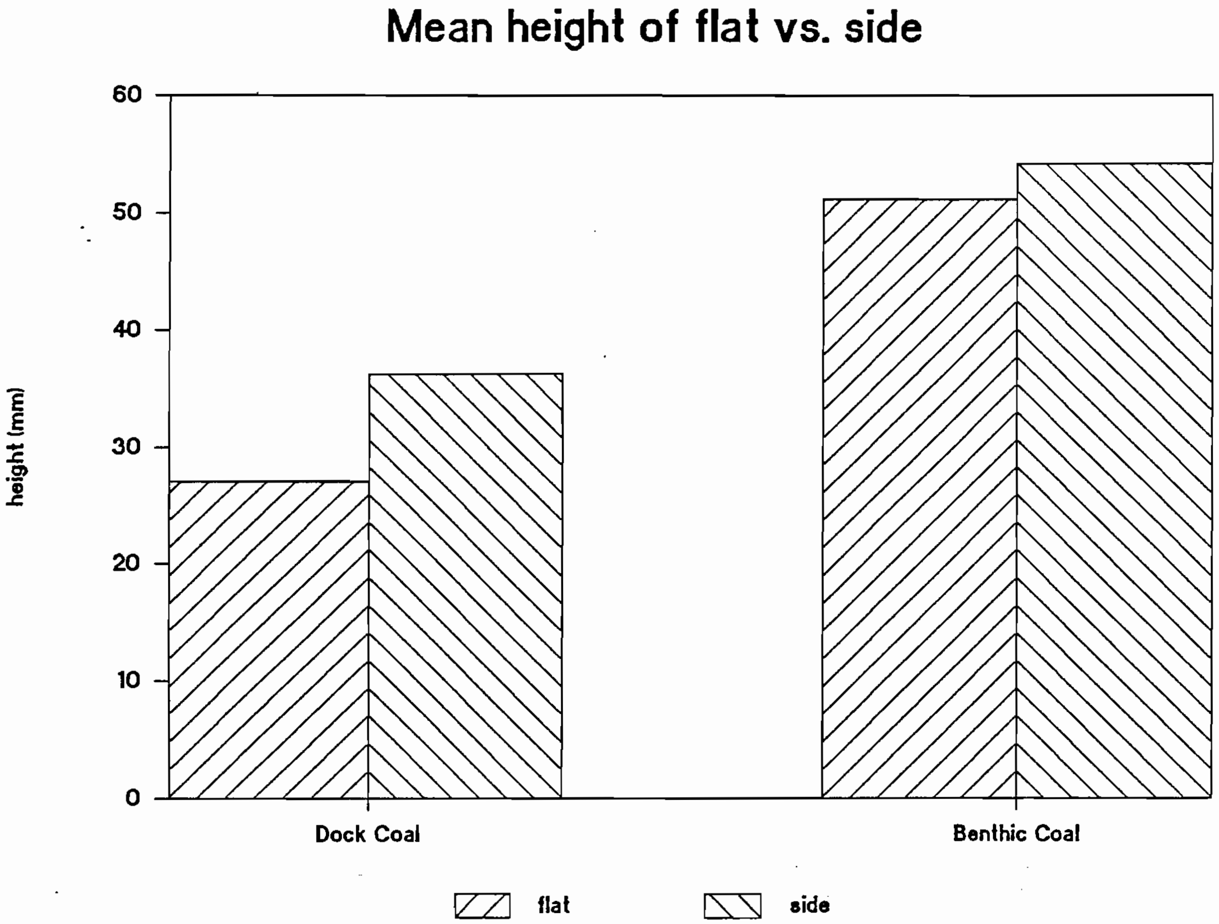


Fig. 4. Mean heights of oysters on the flat surfaces and rims of coal ash pucks form the benthic and dock sites.

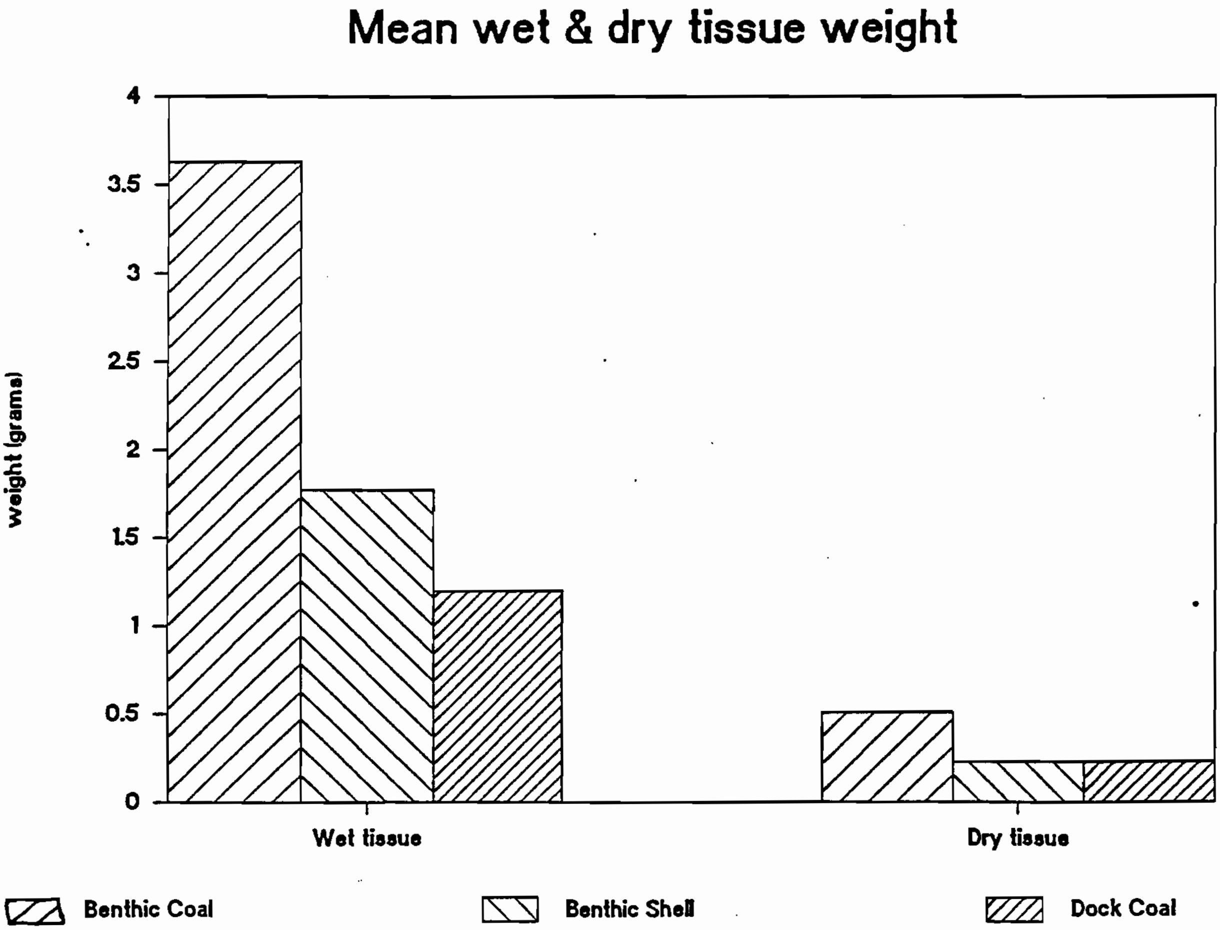


Fig. 5. Mean wet and dry tissue weights of oysters from benthic coal ash, benthic shell, and dock coal ash.

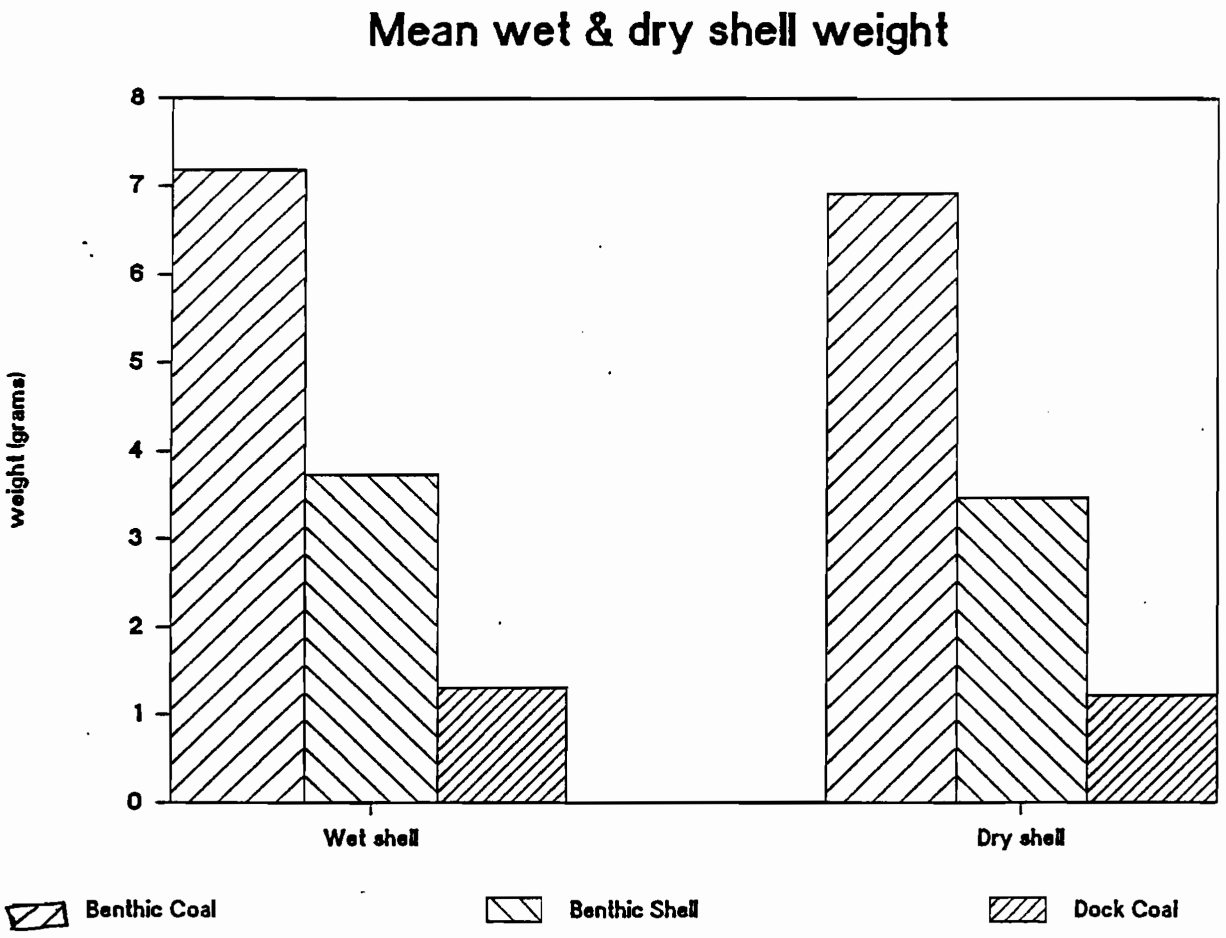


Fig. 6. Mean wet and dry shell weights of oysters from benthic coal ash, benthic shell, and dock coal ash.

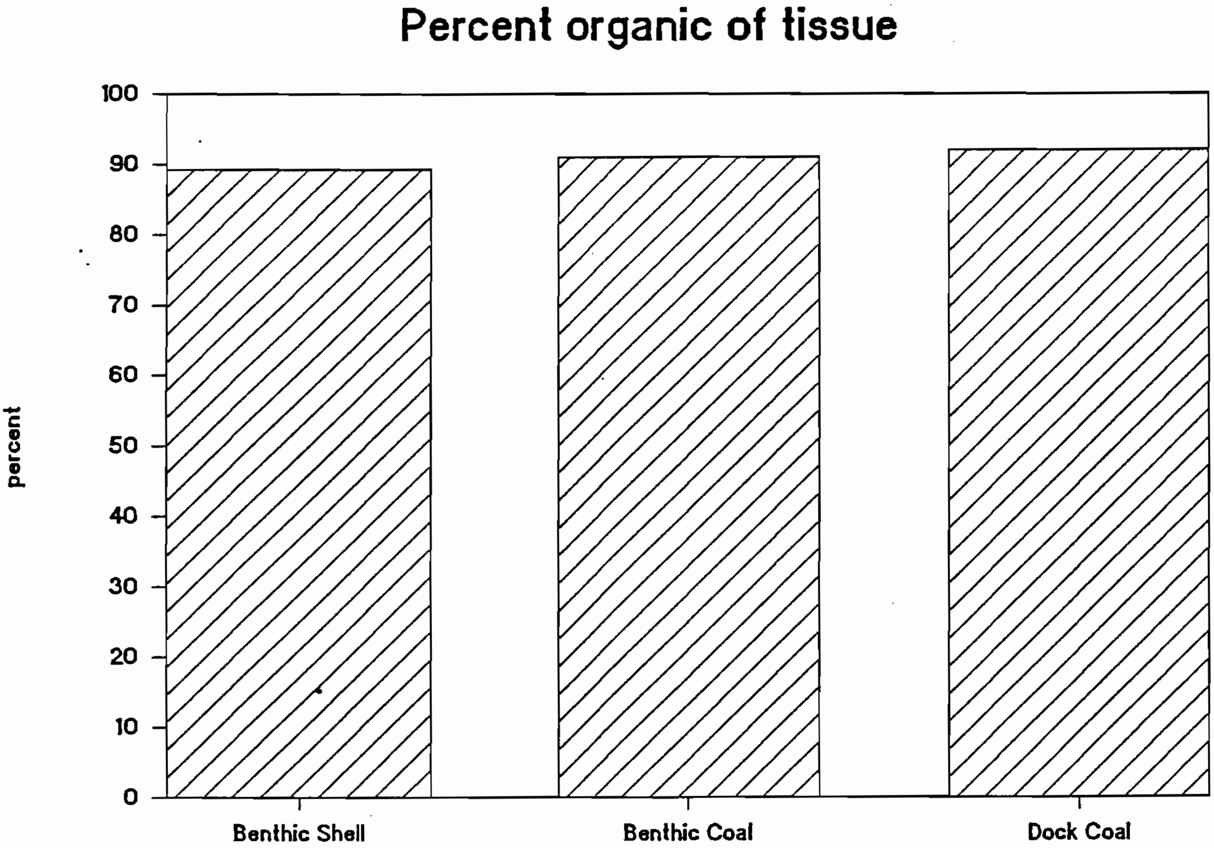


Fig. 7. Percent organics of oyster tissue from benthic coal ash, benthic shell, and dock coal ash.

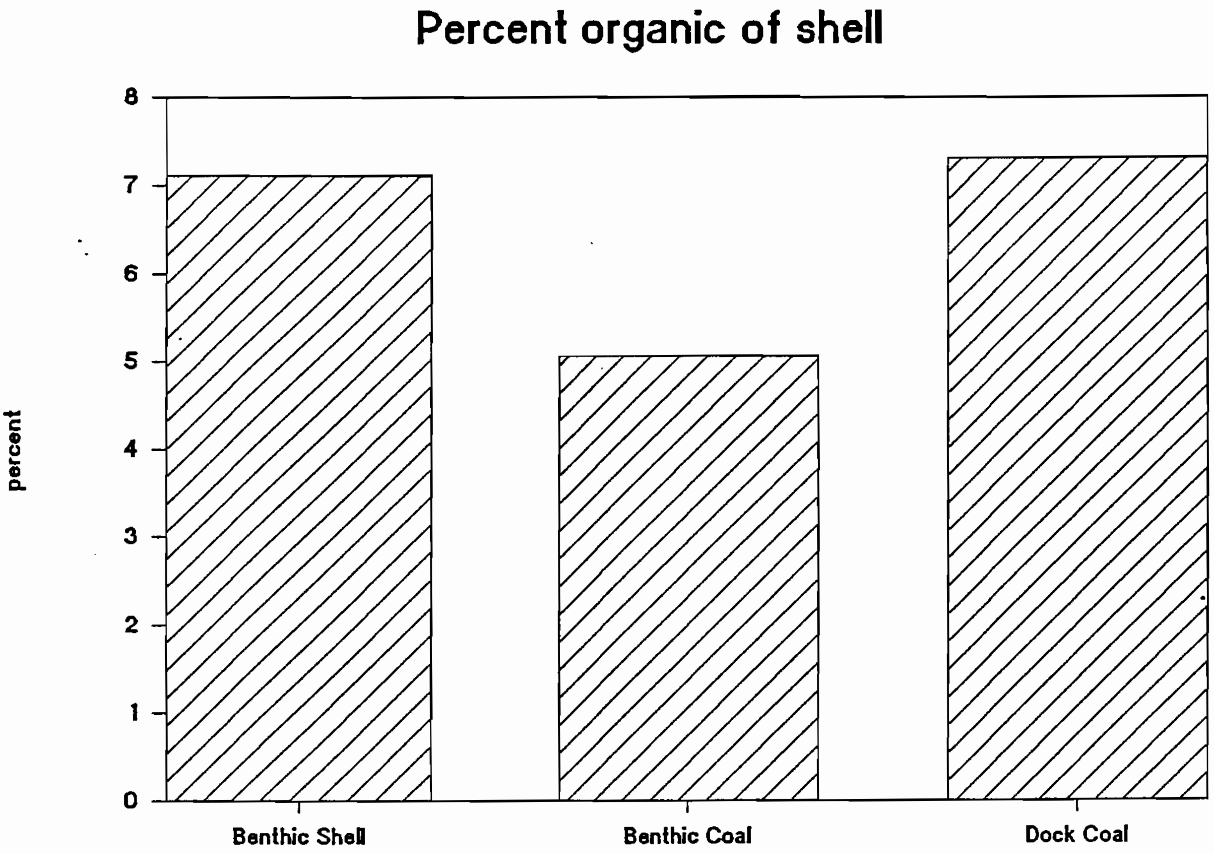


Fig. 8. Percent organics of oyster shells from benthic coal ash, benthic shell, and dock coal ash.

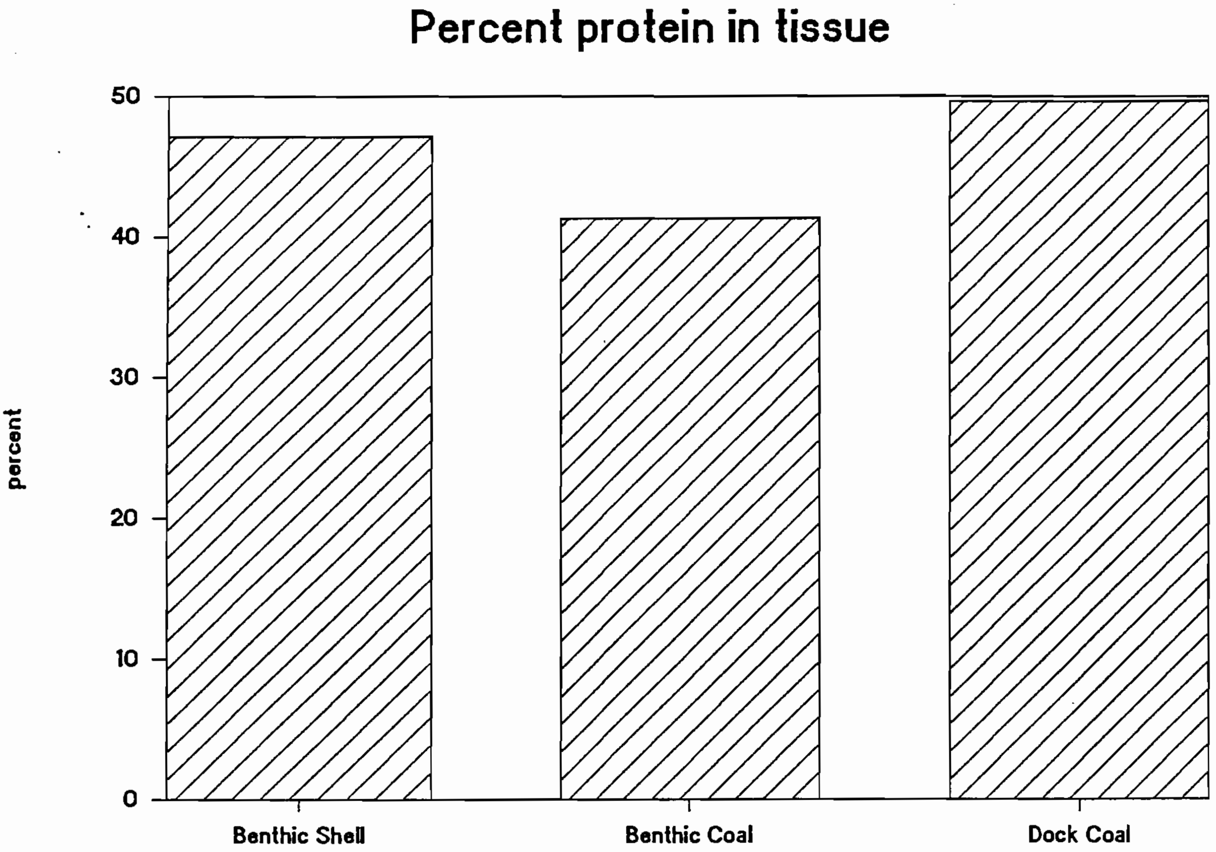


Fig. 9. Percent protein of oyster tissue from benthic coal ash, benthic shell, and dock coal ash.

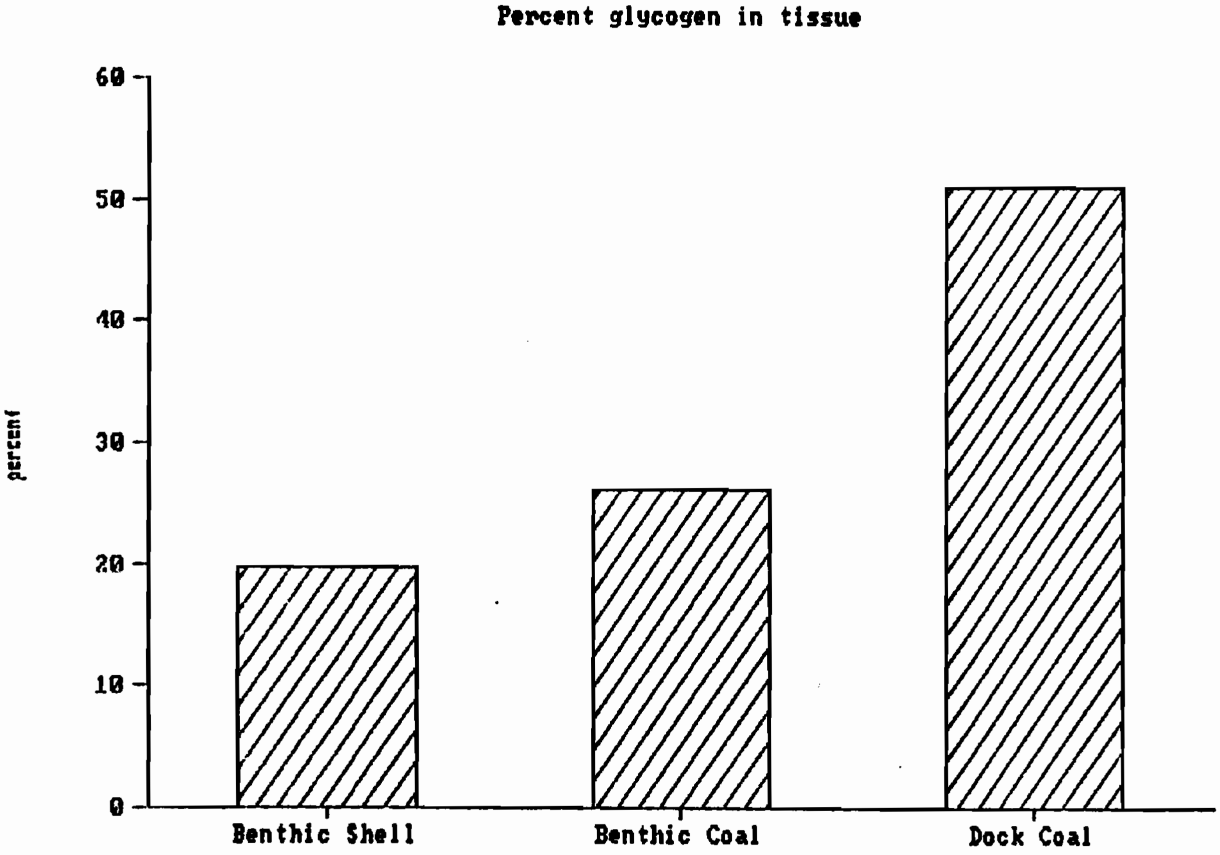


Fig. 10. Percent glycogen of oyster tissue from benthic coal ash, benthic shell, and dock coal ash.

showed little difference in height on coal ash and shell (coal ash mean = 51.18 ± 18.07, shell mean = 51.28 ± 13.92). The coal ash between sites, though, did display a significant difference (dock mean = 27.02 ± 10.25). The mean number of scars and boxes on coal ash pucks also differed significantly between sites as displayed by Fig. 3 (benthic scar mean = 7.49 ± 9.24, dock scar mean = 15.74 ± 15.73; benthic box mean = 0.57 ± 1.10, dock box mean = 9.31 ± 9.19).

A comparison of oyster height on flat surfaces and rims of coal ash pucks are seen in Fig. 4. A significant difference was found at the dock site (flat mean = 27.02 ± 10.25, rim mean = 36.28 ± 18.67) but not at the benthic site (flat mean = 51.18 ± 18.07, rim mean = 54.24 ± 17.71). Table 1 shows the comparison of number of scars and boxes on flat surfaces versus the rim of the pucks. Only benthic coal ash scars showed a significant difference (flat mean = 7.50 ± 9.24, rim mean = 5.23 ± 6.57).

The mean wet and dry tissue weights of oysters from the three groups is seen in Fig. 5. Significant differences were found here for both wet and dry meat between benthic coal ash and benthic shell as well as benthic coal ash and dock coal ash. Mean values for wet meat were: benthic coal ash = 3.63 ± 1.80, benthic shell = 1.77 ± 0.91, and dock coal ash = 1.20 ± 1.18. While dry weight values were: benthic coal ash = 0.51 ± 0.28, benthic shell = 0.23 ± 0.11, and dock coal ash = 0.23 ± 0.23. Benthic coal ash oysters had the highest weights while benthic shell and dock coal ash weights were somewhat similar.

Mean wet and dry shell weights followed a similar trend with significant differences between the same groups. Benthic coal ash was once again the highest (wet mean = 7.18 ± 2.73, dry mean = 6.92 ± 2.65). Benthic shell was of intermediate weight where wet mean = 3.73 ± 2.20 and dry mean = 3.47 ± 2.00. Dock coal ash values were lowest (wet mean = 1.31 ± 1.11, dry mean = 1.22 ± 1.08).

A condition index depicting the relationship of dry meat weight on dry shell weight was found by a simple regression. The regression equations and standard errors of the slopes are found in Table 2. Significant differences were found in the slopes of benthic shell and benthic coal ash and also benthic coal ash and dock coal ash where dock coal ash was highest and benthic shell was lowest. The slopes, though, indicate a moderate linear relationship.

Regression equations of tissue ash weight on tissue weight and shell ash weight on shell weight are shown in Tables 3 and 4 respectively. No significant differences were found between slopes of the groups on either table. The slopes of the shell regression showed a strong linear relationship, whereas the tissue regression was not as strong.

Fig. 7 depicts the percentage of organics in tissue from the three groups. All groups had high percentages (benthic shell = 89.23 ± 4.83%, benthic coal ash = 90.99 ± 2.93%, dock coal ash = 92.00 ± 2.02%). No significant differences were found between groups. The percentage of organics in the shells (Fig. 8) were low. Although benthic coal ash was lower than benthic shell, a significant difference was not found at that site (benthic shell

= 7.11 ± 6.52%, benthic coal ash = 5.06 ± 3.42%). Between benthic coal ash and dock coal ash, though, a significant difference was found (dock coal ash = 7.31 ± 2.50%).

Fig. 9 shows the percentage of protein in the tissue at each substrata. Protein accounted for almost half the organics in the tissue (benthic shell = 47.13 ± 24.80%, benthic coal ash = 41.3 ± 24.72%, dock coal ash = 49.63 ± 24.80%). No significant differences were found between the groups.

The percentage of glycogen in the tissue of oysters at each substrata is seen in Fig. 10. A significant difference in percentages was seen between dock coal ash oysters and benthic coal ash oysters (dock coal ash = 51.1± 11.8%, benthic coal ash = 26.2 ± 11.3%). Benthic coal ash oysters did not differ significantly from benthic shell oysters though (benthic shell = 19.8± 10.3%).

Discussion

Growth is an indication of the energy status of an organism which may vary under different environmental conditions. Therefore, measured growth is a useful index of stress. The similar mean heights of oysters on coal ash and shell at the benthic site indicates that stabilized coal ash does not reduce the growth of C. virginica (See Fig. 2). This is supported by the mean heights of the same oysters at three months of age where benthic coal ash oysters were actually significantly longer than benthic shell oysters (Price et al. 1988). Garvey (1986) also did not find coal ash to inhibit oyster growth but found rather an inverse relationship of density to growth. Warren (1988), though, did see smaller heights on coal ash than oyster shell.

The benthic coal ash oysters were significantly larger than those on dock coal (See Fig. 2). Lacking a dock control shell, however, the significance between sites cannot be determined, and all dock results are shown primarily as a comparison of populations at the two sites. At three months of age, the same pattern preceded with benthic coal ash oysters being longer than dock coal ash oysters (Price et al. 1988). Exposure of bivalves to the air can result in energy loss through respiration and excretion without a corresponding energy consumption from food, thereby, reducing growth (Bayne et al. 1985). This may explain the smaller heights found at the dock where the oysters hung above the waterline at low tide.

The dock coal ash population was also characterized by a significantly greater number of scars and boxes (See Fig. 3). This may indicate heavy mortality perhaps due to heavy fouling smothering the oysters as well as competition from those organisms. This offers another explanation of the smaller dock heights due to competition by fouling organisms creating a poor growing environment.

Only the flat surfaces of coal ash pucks had ben considered in previous literature (Garvey 1986, Price et al. 1988). A comparison of the rims and flat surfaces of the pucks were, therefore, examined to determine any missing trends. Only two differences were seen. Side height was greater and flat scars were greater on benthic coal (See Fig 4 & Table 1). Flat surfaces would be expected to have more scars due to a larger possible surface area for oysters to set on. The side effects for only one group may have been random.

Wet and dry tissue and shell weights are also indicators of growth. Once again the benthic coal ash was significantly higher in all four factors when compared to benthic shell and dock coal ash (See Fig. 5 & 6). Therefore, coal ash does not appear to impede growth. Warren (1988), again, reported a reduction on coal ash.

Condition indices are important in characterizing the apparent "health" of bivalve populations by summarizing the physiological activity of organisms under given environmental conditions. These indices are also useful in designating the quality of a marketed product (Lucas & Beninger 1985). A regression of dry meat weight on dry shell weight was used to determine condition. The use of dry weights eliminates the bias due to fluctuations in water content (Lucas and Beninger 1985). The significantly higher slope of benthic coal ash over benthic shell, indicates better condition of oysters on the benthic coal ash. Dock coal ash's higher slope indicated better condition than the benthic coal oysters with almost three times the amount of meat per shell weight read off the regression line (See Table 2). Muniz et al. (1986) reported condition indices consistently higher for smaller oysters as well as a corresponding decrease in condition with aging. Garvey (1986) also found better condition of oysters on coal ash rather than on oyster shells. Warren

(1988), though, found condition of oysters to be better on oyster shell than coal ash.

Organic content is another type of condition index. Oysters with high organic levels are in superior condition to those with low levels. Levels of ash and organics are inversely related. Therefore, oysters with high amounts of ash have low amounts of organics and a resulting poorer condition than those with low levels of ash (Warren 1988).

Regressions of tissue ash weight on tissue weight and shell ash weight on shell weight did not have any significant differences between slopes (See Tables 3 & 4). This is indicative of even condition levels. Warren (1988) reported lower tissue ash level (better condition) on coal ash rather than shell. His shell ash levels on coal ash were greater than shell at first but no difference was perceived after six weeks. Therefore, condition of oysters may be more sensitive as spat, but over time, coal ash substrata affects oyster condition less.

Percent organics of tissue and shell shows the partitioning of energy of the oysters. The organic content of the tissue showed approximately equal levels and thus, even energy content of tissue (Mann 1978) (See Fig. 7). Shell organic content was not statistically different at the benthic site and thus, spent nearly equal portions of energy synthesizing the shell (Mann 1978) (See Fig. 8). The dock coal ash, though, had a greater percent of organics in the shell than did benthic coal ash.

Price et al. (1975) reported C. virginica to have 72% organics in the tissue and 3% organics in the shell. Warren

(1988) found levels of 84% organics in the tissue and 3% organics in the shell. The present study showed slightly higher values with 91% percent organics in the tissue and 7% organics in the shell.

Biochemical analysis is another indicator of condition. The percent protein content of oyster tissue was found to be approximately 46%. No statistical differences were seen between the groups and thus, little difference in condition (See Fig 9). Giese (1969) reported a similar value of 49% protein in oysters. Warren's (1988) average value for protein was 37%. He also found no statistical difference among coal ash and oyster shell protein content.

Glycogen content is also a good indication of the metabolic state of a bivalve because glycogen is the principal energy reserve under unfavorable conditions and formations of gametes (Lucas & Beninger 1984). The percentage of glycogen per tissue weight was 51.1 ± 11.8% in dock coal ash oysters. This is significantly higher than benthic coal ash oysters at 26.2 ± 11.3%. Oysters that have spawned tend to have lower glycogen levels indicating a possible reason for the lower values (Soniat & Ray 1983). The benthic shell oysters were not significantly different from the benthic coal ash oysters with 19.8 ± 10.3% glycogen.

Muniz et al. (1986) reports percentage of glycogen content to average from 2.2 to 24.9% in Crassostrea gigas and Crassostrea brasiliana, the Pacific and Brazilian oysters. Gaffney & Diehl (1986) showed glycogen percentages ranging from 10 to 35% in another bivalve, Mytilus edulis, the blue mussel. Warren (1988) found an average of 8% glycogen without significant differences in coal ash and oyster shell grown oysters but reported a low percent recovery due to methodology. The benthic oysters appear to have more accurate glycogen contents in corresponding to literature values. These oysters show approximately equal condition.

Stabilized coal ash has not been shown to act as a stress on Crassostrea virginica after two years growth. Tissue and shell growth was actually greater on coal ash when compared to natural oyster shell cultch. While the condition of oysters on coal ash was fairly equal to that on the shell. In the future, aquaculturists may want to consider the use of coal ash as an alternative cultch. While coal burning power plants may find oceanic disposal of wastes an attractive dumping solution.

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