Feasibility of harvesting, holding and culturing *Donax spp.* for resource enhancement aquaculture

Quarterly Report
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Objective A: Develop and document efficiency of various mass-harvesting techniques.

The project began with developing a device for harvesting the *Donax* from the beach. Ideas for a harvesting device originated from a simple mole crab (Emerita spp.) rake and a commercial hard clam harvester. Both devices had basic qualities that can be used for mass and test harvesting of *Donax* spp. We found through modifications and extensive testing of each device that we needed to combine both ideas in order to minimize labor and maximize efficiency.

A mole crab rake is a simple aluminum device with a triangular basket with an opening of 38.5 cm wide, 15 cm high and 20 cm deep. The device also has a single tube extension protruding out from the basket used as a pulling handle. The basket is outlined in an aluminum diamond shaped mesh. Modifications to the basket included insertion of harvest test meshes, 7 mm and 5 mm, and skids mounted to the sides of the mole crab rake to control depth of the dig.

The mole crab rake device was modified and constructed primarily to investigate and find the best mesh size for harvesting *Donax* spp. until commercial hard clam harvester could be modified. Two rakes were constructed; one with 7mm mesh (Harvester 1) and the other with 7mm mesh (Harvester 2). The small baskets of the modified mole crab rake only allowed for short distance pulls. However, the weight and scale of the harvester allowed for easy operation during the mesh tests.

Mole crab rake in use (left) and showing *Donax* catch and by-catch (right)

Three harvesting tests were completed using the two different size meshes, 5 mm and 7 mm. These tests were conducted on November 2, 2004, November 5, 2004, and December 10, 2004. Additional tests are underway to supplement this data. Data taken from each test included *Donax* volume, length, and total count of *Donax* spp. as well as the volume of by-catch. Hypothesis test were conducted to determine if there is a statistically significant difference in the average *Donax* spp. volume, average by-catch volume, average *Donax* spp. number harvested by each pull, and the average *Donax* spp. length using this data and SPSS to do the analysis. A T-test comparing means was conducted for each set of data, Test 1 (November 2, 2004), Test 2 (November 5, 2004) and Test 3 (December 10, 2004). A T-test of equality of means was also conducted on the data from Test 1 and 2 combined. Test 3 data was not included because the pull length was shorter amount of material collected from that location, Bogue Inlet Pier, was more than the modified mole crab rake could hold. The shorter pull length may adversely affect the results of the test (i.e. differences may be the result of the different pull length and not the mesh size). A great deal of effort was taken to ensure that any differences would be the result of the different mesh sizes only. The modified mole crab rakes where pulled at the same time in the same place for the same distance side by side. A coin was flipped to determine which harvester, mole crab rake, would be on the left and right. After the first pull was complete we alternated the harvesters so that the sample being taken with each harvester was randomized.

The results showed that the average differences in *Donax* spp. volume and by-catch volume were not statistically significant based on a .05 level of significance. However, generally the 5 mm mesh harvested more *Donax* spp. volume and more by-catch volume than the 7 mm mesh. The average difference in the volume of *Donax* spp. appears to be more than the average difference in the volume of by-catch. This indicates that the 5 mm mesh may be more efficient in harvesting *Donax* spp. (see attached hypothesis test).

The results also showed that the difference in the average number of *Donax* spp. harvested with 5 mm and 7 mm meshes was not statistically significant, but that generally the average number of *Donax* spp. harvested with the 5 mm mesh was more than the average number harvested with the 7 mm mesh (see attached hypothesis test). This is another indication that the 5 mm mesh may work better for harvesting *Donax* spp. from the beaches of Bogue Banks.

When a T-test was conducted on the difference in the average length of *Donax* spp. harvested with the two different mesh sizes we found, as one may suspect, that the average length of *Donax* spp. that were harvested with the smaller mesh, 5 mm, was statistically significantly different, smaller, than the average length of *Donax* spp. harvested with the larger mesh, 7 mm for test 1 and 3. Therefore, if one wishes to harvest *Donax* spp. of a specific length, then one can select the desired mesh size to harvest a specified *Donax* spp. length. This information may become helpful as the project progresses.

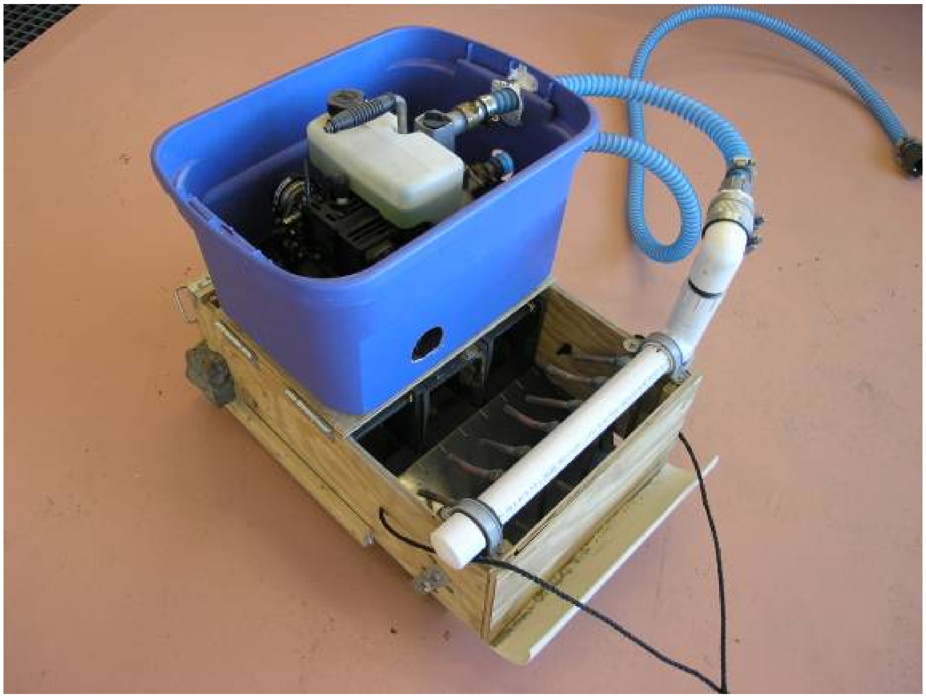
As a result of the lack of statistical differences experienced with the modified mole crab rake for harvesting *Donax* spp. a third smaller mesh (3 mm) will be incorporated into the next harvesting device. For mass harvesting of *Donax* spp. a mechanical hard clam harvester was considered.

A mechanical hard clam harvester is a device used by hard clam farmers to harvest bedded clams. The device is equipped with a five horsepower internal combustion engine used to pump water for loosening up the substrate, filtering out the substrate, and moving the clams from the cutting blade to a basket. The harvester can be used submerged or ebbed out as well as pulled manually or automatically by boat or other dry land vehicle. The dimensions of the harvester are 60 cm wide, 95 cm long and 39 cm height.

Commercial mechanical aquaculture hard clam harvester

The mechanical harvester was modified to harvest the small *Donax* spp. Small 3 mm mesh was mounted on the cutting blade to replace the 1" mesh used for separating hard clams. The basket was outfitted with the three test meshes, 3 mm, 5 mm, and 7 mm, and an adjustable ski used to control the depths of the harvesting pulls. This harvester can be adjusted, raised and lowered, to specified depths. The original positions of the primary spray nozzles were to loosen top layer of substrate in order for a deeper dig to harvest the hard clams. These nozzles were repositioned to filter sand and move the clams along the cutting blade.

Test harvesting took place along a sandy shoreline in Bogue Sound. Pulls were completed in both submerged and ebbed out conditions at approximately one meter in length. The mechanical harvester proved to be difficult to pull, even at the shallowest depth, because of its weight. The cut substrate was not efficiently conveyed up and over the long steep angled blade apparatus. Adjustments were made to the spray nozzles in order to concentrate the water pressure on the blade and improve the angle of spray. However, these changes did not improve the movement of the cut substrate back into the basket. Although the harvester proved to be too difficult to use, the ideas of water spray nozzles, three test meshes, and depth control consolidated into one device were applied on the first prototype *Donax* test harvester.



Prototype mechanical *Donax* harvester

The pros and cons of each device were considered during the design and construction of a harvester specifically for *Donax* spp. A prototype was constructed using lightweight plywood and plastic. The device is 38.5 cm wide, 64 cm long, and 18 cm height and is equipped with three test mesh boxes, adjustable cutting blade, and an internal combustion water pump equipped with spray nozzles. Preliminary testing of the device has shown a better harvesting efficacy.

Harvest testing will resume during the month of April with the new harvester and techniques. Harvest testing will consist of two tests per season with each test generating a total of 18 pulls, 6 different arrangements of the three different mesh size boxes at three different depths, three meters in length.

Objective B: Compare and refine feasibility of two long-term holding methods.

The renovation construction of the Carteret Community College aquaculture Technology Program facility has taken much longer than originally anticipated, which has delayed the startup of holding system development and testing. However, prototype raceways and upwellers have been constructed for comparative and observation purposes. For each of these holding systems different techniques will be used to determine which system is best for holding *Donax* spp. and which technique works best. Some techniques that will be tested are varying densities of clams and varying the flow rates of the water.

Test raceways have been constructed and hard clams, *Mercenaria mercenaria*, were used to simulate the *Donax variabilis* within this system. One meter raceways were constructed out of residential plastic gutter and were supplied with water from a 600 gallon head tank. The flow rate is controlled by individual ball valves on each supply line going to each raceway. Water velocities are controlled by varying the widths of rectangular cut-outs of the end caps on the drain end of the raceways.

Results showed that accumulation of sediments and *Donax* feces was a function of velocity but cannot be avoided completely. With prototype nurseries the collection of unwanted material inside the raceway required biweekly maintenance in order to keep each raceway clean.

This system design will accommodate all tests needed for the project requirements and will attempt to eliminate much routine cleaning and maintenance. This small scale system can be easily scaled up for mass holding using conventional raceways.

Prototype *Donax* holding upwellers (left) and raceways (right)

Both passive and active upwellers have been constructed and observed while holding various volumes of *Donax variabilis*. Preliminary testing using the two different style upwellers began in November 2004 using *Donax* harvested in the vicinity of the Sheraton Pier. Observations of the upwellers were used to gain insight into questions of efficiency, routine maintenance, flow rates, clam densities, and complexity of the systems. These questions needed to be addressed before the construction of a complete system for the holding tests.

The active upweller consists of a 60 cm length of clear PVC pipe (to observe sedimentation and effect of velocity on clam behavior), reducer couplings, flow rate control valve, and a rubber T-eliminator. The 60 cm length of PVC pipe, with a small mesh screen attached to one end, served as the holding chamber for the clams. The upweller was then connected perpendicular to a 3” diameter supply pipe to allow the upweller to stand vertically. The upwellers were placed in series along the 3” pipe so that an equal water pressure was supplied to each. The water supply was pumped from Bogue Sound using a 1-½ horsepower water pump to a 600 gallon head tank that served as the reservoir to ensure constant water pressure for the upwellers.

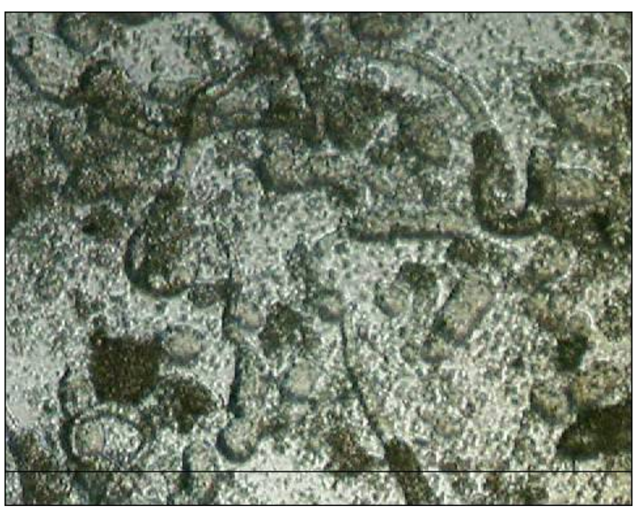
Several observations were made with the active upweller system to guide the construction of test systems. Holding chambers were fitted with mesh that was small enough to hold the smallest harvested *Donax* clams yet some clogging was experienced on the small mesh. The active upweller system design has proven simple to clean by draining the upwellers and flushing out sedimentary material. Several diameters of transparent PVC pipe were used for the holding chambers to allow observation of the effect of flow rates and velocity of water on waste removal and sediment clearing.

Objective C: Test and improve techniques for aquaculture propagation of *Donax*.

Gonad investigations started in December 2004. *Donax* gonad indexing will provide information suggesting how and when the clams spawn. Qualitative monthly size samplings are concurrently conducted to compare with gonad index for the purpose of backtracking determination of the *Donax* spawning season. Conditions such as time of year, water temperature, shell pattern, shell length and stage of gonad maturity are being recorded so that these conditions can be replicated in a lab environment for spawning purposes. We examined approximately 50 *Donax* spp. monthly that are taken from their natural environment. The *Donax* examined for gonad indexing are selected randomly from those harvested using cluster sampling. Each month data will be collected, analyzed and compared to other months in terms of gonad maturation to determine if there is a difference in the proportion of *Donax* that are ready to spawn as well as other stages of maturation. The Gonad indexing is as follows…

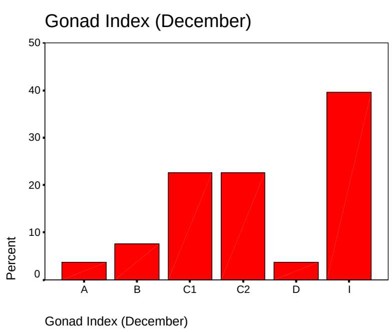
I: Immature (i.e. sex not distinguishable); no gonad seen
A: First signs of gonad maturation; sex starting to be distinguishable
B: Gonad differentiation well advanced; sex easily differentiable
C1: Male with spermatids or female with largely pedunculate
C2: Male with sperm ducts or female with eggs largely non-pedunculate
D: Gonads almost empty, but sex still distinguishable; immediate post-spawn

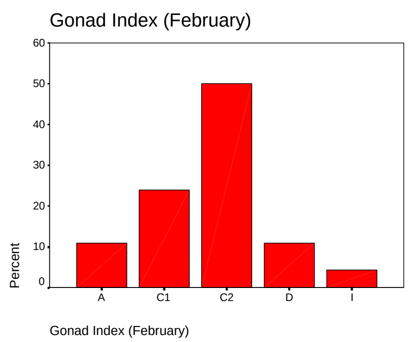
The *Donax* gonad yields a distinct coloration that determines its sex. A mature female has a noticeable pink to reddish gonad coloration and a mature male gonad is a milky color. During dissection to view the gonads of *Donax* clams an interesting phenomenon was observed. In one of the nearly 200 clams dissected the gonad was a dull orange color. A section of the gonad revealed active sperm with parasites within the gonad tissue. Images were taken of the parasite and are currently being analyzed by veterinarians at NCSU.

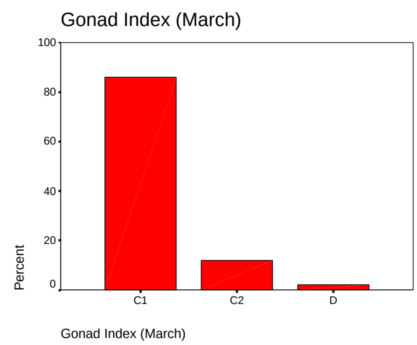


Video of unknown *Donax* gonad parasite (double click picture for video)

Gonad data collected for December, February, and March shows that a significant percentage of the *Donax* spp. have ripened gonads and are ready to spawn (index C2) for each month. For the months of December, February, and March we have 23%, 50% and 12% respectively of our samples were ready to spawn (C2). We also found that 23%, 24% and 86% were almost ready to spawn (index C1) for the months of December, February, and March respectively. Combining these percentages we have 46%, 73%, and 98% of our samples from December, February, and March respectively were ready (C2) or almost ready (C1) to spawn. Therefore, there is evidence that *Donax* spp. may trickle spawn meaning that the gonad is constantly releasing gametes. Additional data is required in order to make inferences concerning length and maturity, shell pattern and maturity, sex and length, and shell pattern and sex. Below are bar charts of the Gonad indexes for December, February, and March.



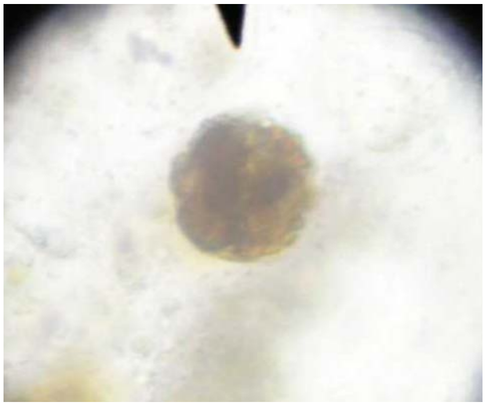




Note: In March all of the dissected *Donax* spp. were almost ready to spawn or more mature.

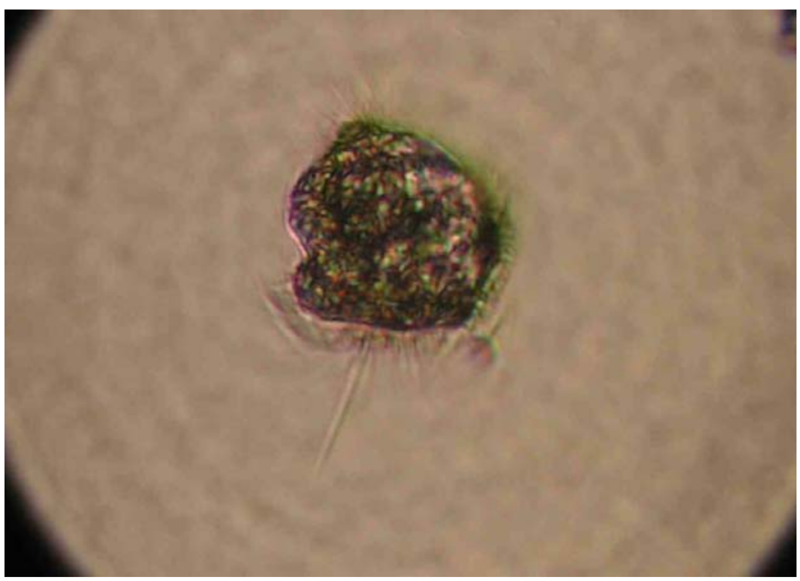
Hypothesis tests were conducted comparing the proportion of *Donax* spp. that were ready to spawn for December, February, and March. It was found that the proportion of *Donax* spp. that were ready to spawn (C2) in December was less than the proportion of *Donax* spp. that were ready to spawn (C2) in February and that the proportion of *Donax* spp. that were ready to spawn (C2) in February was greater than the proportion of *Donax* spp. that were ready to spawn (C2) in March (see attached hypothesis test). This is additional evidence that *Donax* spp. may be trickle spawners.

Several attempts to strip spawn *Donax* spp. with mature gonads have been successful with fertilization and cell division occurring in petri dishes and were observed for several hours under a compound scope.



First fertilized *Donax* egg resulting from strip spawn (left); Project specialist Matt Penny (at microscope) and helper Dan Santorelli (with calipers).

During the April examination of *Donax* gonads another interesting phenomenon was observed. Eggs that apparently had been previously fertilized, developing embryos and larvae began emerging from the ovary and several stages of development were observed. The larvae were transferred to container of filtered seawater and observed for several days. After five days the larvae were still in the trochophore stage of development and no veliger larvae had been observed. Observations are continuing.



Five-day old *Donax* spp. trochophore larvae (approximately 70 microns diameter)

Attachment:

Donax Hypothesis Tests

The first test that was accomplished was Nov. 2, 2004 where we were comparing mesh sizes 7 mm (Harvester 1) and 5 mm (Harvester 2). The following is a comparison between the two harvesters for average *Donax* volume, the average by-catch volume, number of *Donax* spp. harvested and the average length of *Donax* spp. harvested as well. The goal is to determine which mesh size is most efficient for harvesting *Donax* clams.

(Note: In order to reject the null hypothesis (H_o), the means are equal, the p-value must be less than .05)

$$H_o : \mu_{1DV} = \mu_{2DV}$$
$$H_a : \mu_{1DV} \neq \mu_{2DV}$$

The mean *Donax* volume for harvester 1 and 2 were 9.60 ml and 8.10 ml respectively, with standard deviations of 13.67 and 10.15 respectively. However the **p-value = .78** which is greater than .05 indicating that there was not a statistically significant difference in the volume of *Donax* harvested between the two mesh sizes.

$$H_o : \mu_{1BV} = \mu_{2BV}$$
$$H_a : \mu_{1BV} \neq \mu_{2BV}$$

The mean By-catch volume for harvester 1 and 2 were 11.03 ml and 7.30 ml respectively, with standard deviations of 12.05 and 7.33 respectively. Again, the **p-value = .42** which is greater than .05 indicating that there was not a statistically significant difference in the volume of By-catch harvested between the two mesh sizes.

$$H_o : \mu_{1DN} = \mu_{2DN}$$
$$H_a : \mu_{1DN} \neq \mu_{2DN}$$

The mean number of *Donax* harvested for harvester 1 and 2 were 32.20 and 50.50 respectively, with standard deviations of 35.26 and 58.58 respectively. The **p-value = .41** which is greater than .05 indicating that there was not a statistically significant difference in the number of *Donax* harvested between the two mesh sizes.

$$H_o : \mu_{1DL} = \mu_{2DL}$$
$$H_a : \mu_{1DL} \neq \mu_{2DL}$$

The mean length of *Donax* harvested for harvester 1 and 2 were 11.66 and 10.22 respectively, with standard deviations of .91 and 1.17 respectively. The p-value = .007

which is less than .05 indicating that there was a statistically significant difference in the average length of *Donax* harvested between the two mesh sizes.

On Nov. 5, 2004 a second harvesting test was conducted the same way with the same harvesters and the results follow.

$$H_o : \mu_{1DV} = \mu_{2DV}$$
$$H_a : \mu_{1DV} \neq \mu_{2DV}$$

The mean *Donax* volume for harvester 1 and 2 were 5.90 ml and 12.30 ml respectively, with standard deviations of 5.04 and 10.90 respectively. The **p-value = .12** which is greater than .05 indicating that there was not a statistically significant difference in the volume of *Donax* harvested between the two mesh sizes.

$$H_o : \mu_{1BV} = \mu_{2BV}$$
$$H_a : \mu_{1BV} \neq \mu_{2BV}$$

The mean By-catch volume for harvester 1 and 2 were 9.40 ml and 16.70 ml respectively, with standard deviations of 9.13 and 16.70 respectively. The **p-value = .34** which is greater than .05 indicating that there was not a statistically significant difference in the volume of By-catch harvested between the two mesh sizes.

$$H_o : \mu_{1DN} = \mu_{2DN}$$
$$H_a : \mu_{1DN} \neq \mu_{2DN}$$

The mean number of *Donax* harvested for harvester 1 and 2 were 32.70 and 84.40 respectively, with standard deviations of 30.22 and 77.13 respectively. The **p-value = .08** which is greater than .05 indicating that there was not a statistically significant difference in the number of *Donax* harvested between the two mesh sizes.

$$H_o : \mu_{1DL} = \mu_{2DL}$$
$$H_a : \mu_{1DL} \neq \mu_{2DL}$$

The mean length of *Donax* harvested for harvester 1 and 2 were 10.74 and 10.81 respectively, with standard deviations of 4.02 and 1.75 respectively. The **p-value = .96** which is greater than .05 indicating that there was not a statistically significant difference in the average length of *Donax* harvested between the two mesh sizes.

The following are the statistics for test 1 and 2 combined. We could not combine the results of test 3 because the length of the pull for harvesting was shorter so the volumes, numbers, etc. differences may be a result of the differences in the lengths of the pulls and not the mesh size.

$$H_o : \mu_{1DV} = \mu_{2DV}$$
$$H_a : \mu_{1DV} \neq \mu_{2DV}$$

The mean *Donax* volume for harvester 1 and 2 were 7.75 ml and 10.20 ml respectively, with standard deviations of 10.21 and 10.47 respectively. The **p-value = .46** which is greater than .05 indicating that there was not a statistically significant difference in the volume of *Donax* harvested between the two mesh sizes.

$$H_o : \mu_{1BV} = \mu_{2BV}$$
$$H_a : \mu_{1BV} \neq \mu_{2BV}$$

The mean By-catch volume for harvester 1 and 2 were 10.21 ml and 12.00 ml respectively, with standard deviations of 10.44 and 16.44 respectively. The **p-value = .68** which is greater than .05 indicating that there was not a statistically significant difference in the volume of By-catch harvested between the two mesh sizes.

$$H_o : \mu_{1DN} = \mu_{2DN}$$
$$H_a : \mu_{1DN} \neq \mu_{2DN}$$

The mean number of *Donax* harvested for harvester 1 and 2 were 32.45 and 66.95 respectively, with standard deviations of 31.96 and 68.76 respectively. The **p-value = .052** which is slightly greater than .05 indicating that there was not a statistically significant difference in the number of *Donax* harvested between the two mesh sizes.

$$H_o : \mu_{1DL} = \mu_{2DL}$$
$$H_a : \mu_{1DL} \neq \mu_{2DL}$$

The mean length of *Donax* harvested for harvester 1 and 2 were 11.20 and 10.52 respectively, with standard deviations of 2.88 and 1.48 respectively. The **p-value = .35**

which is greater than .05 indicating that there was not a statistically significant difference in the average length of *Donax* harvested between the two mesh sizes.

On Dec. 10, 2004 a third harvesting test was conducted the same way with the same harvesters except the length of the pulls were shortened to 70 cm from 81.28 cm and the results follow.

$$H_o : \mu_{1DV} = \mu_{2DV}$$
$$H_a : \mu_{1DV} \neq \mu_{2DV}$$

The mean *Donax* volume for harvester 1 and 2 were 4.65 ml and 7.10 ml respectively, with standard deviations of 4.06 and 9.62 respectively. The **p-value = .47** which is greater than .05 indicating that there was not a statistically significant difference in the volume of *Donax* harvested between the two mesh sizes.

$$H_o : \mu_{1BV} = \mu_{2BV}$$
$$H_a : \mu_{1BV} \neq \mu_{2BV}$$

The mean By-catch volume for harvester 1 and 2 were 1.25 ml and 1.50 ml respectively, with standard deviations of 1.25 and 1.50 respectively. The **p-value = .73** which is greater than .05 indicating that there was not a statistically significant difference in the volume of By-catch harvested between the two mesh sizes.

$$H_o : \mu_{1DN} = \mu_{2DN}$$
$$H_a : \mu_{1DN} \neq \mu_{2DN}$$

The mean number of *Donax* harvested for harvester 1 and 2 were 31.10 and 68.30 respectively, with standard deviations of 34.83 and 97016 respectively. The **p-value = .27** which is greater than .05 indicating that there was not a statistically significant difference in the number of *Donax* harvested between the two mesh sizes. This lack of significant difference is due in part to the large standard deviations.

$$H_o : \mu_{1DL} = \mu_{2DL}$$
$$H_a : \mu_{1DL} \neq \mu_{2DL}$$

The mean length of *Donax* harvested for harvester 1 and 2 were 10.52 and 9.39 respectively, with standard deviations of .61 and 1.24 respectively. The **p-value = .02**

which is less than .05 indicating that there was a statistically significant difference in the average length of *Donax* harvested between the two mesh sizes. This significant difference is in [art a result of the small standard deviations.

Gonad Hypothesis Test

for proportion of ready to spawn (C2)
for the months of December, February, and March

Note: All hypothesis tests are conducted with a .05 level of significance.

The proportion of *Donax* spp. that were ready to spawn (C2) in December was 23% and the proportion of *Donax* spp. that were ready to spawn (C2) in February was 50%. Testing that the Proportion in December is less than the proportion in February we have …

$$H_o : p_{D(c2)} \geq p_{F(C2)}$$

$$H_a : p_{D(C2)} < p_{F(C2)}$$

The **p-value = .002** which is less than .05 so there is sufficient statistical evidence that the proportion of *Donax* spp. that were ready to spawn in December is less than the proportion of *Donax* spp. that were ready to spawn in February.

The proportion of *Donax* spp. that were ready to spawn (C2) in February was 50% and the proportion of *Donax* spp. that were ready to spawn (C2) in March was 12%. Testing that the Proportion in February is greater than the proportion in March we have …

$$H_o : p_{F(c2)} \leq p_{M(C2)}$$

$$H_a : p_{F(C2)} > p_{M(C2)}$$

The **p-value = .00003** which is less than .05 so there is sufficient statistical evidence that the proportion of *Donax* spp. that were ready to spawn in February is greater than the proportion of *Donax* spp. that were ready to spawn in March.