

**Spotted Seatrout Growth as a Bioindicator of Priority
Environmental Stressors in the Charlotte Harbor Estuarine
Ecosystem**

**A Final Report on a Contract Agreement Between the Charlotte Harbor National Estuary
Program and the Sanibel-Captiva Conservation Foundation**

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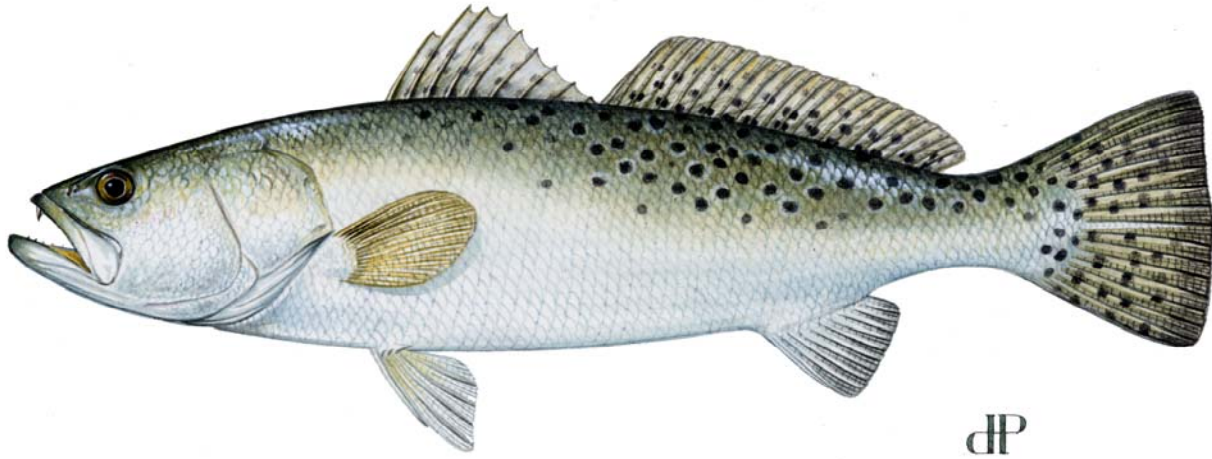
**Spotted Seatrout Growth as a Bioindicator of the Priority Environmental Stressors in the
Charlotte Harbor Estuarine Ecosystem**

**A Final Report on a Contract Agreement Between the Southwest Florida Regional
Planning Council and the Sanibel-Captiva Conservation Foundation**

Introduction

The Marine Laboratory at the Sanibel-Captiva Conservation Foundation entered into a Contract Agreement with the Southwest Florida Regional Planning Council and its agent, the Charlotte Harbor National Estuary program, to conduct research on Spotted Seatrout growth in the Charlotte Harbor area. The research is being conducted and continues according to the Scope of Work provided in the proposal.

The spotted seatrout (*Cynoscion nebulosus* - a fish species in the croaker and drum family Sciaenidae) is one of the preferred food and game fishes within its native distribution that includes estuaries along the southeastern portion of the USA, including the entire Gulf of Mexico. It is found exclusively within estuaries and has a strong affinity for seagrasses where it feeds on fishes and small crustaceans (see Bortone 2003a for a complete compilation of its life history).



SPOTTED SEATROUT

Cynoscion nebulosus

Northern American estuaries (and Charlotte Harbor is not exception) have become areas of rapid human population growth and development. Most of the development has resulted in stress to its local waters through hydrologic alterations, water quality degradation, and habitat loss (Kennish, 1992). Environmental managers must be vigilant to assure that estuarine and coastal waters so not become degraded to the point where the normal biological function of these estuarine ecosystems becomes impaired. Similarly, estuarine restoration efforts should have target goals that seek to reestablish the biological function of disturbed systems to approach or, hopefully, regain normal, undisturbed levels of biological integrity (Bortone, 2003b; Bortone, 2005).

After careful evaluation of several indigenous species, it became apparent that the spotted seatrout may be particularly valuable in serving as a sentinel to detect environmental stress to estuarine ecosystems along the warm temperate coast of North America (Bortone and Wilzbach,

1997a; Bortone, 2003b). The spotted seatrout is an exceptional fish species in that it spends its entire life within the confines of a single estuary. It is unlike most other estuarine fishes that migrate to sea (catadromous) or upstream (anadromous) during some part of their life history. In addition, the spotted seatrout is a long-lived species (often up to 10 years), thus it is subjected to the conditions of a single estuary for an extended period and therefore can serve as a time-series monitor of estuarine conditions. It feeds on fishes and crustaceans found among seagrasses and thus serves as an important trophic link with the estuary (Bortone, 2000). Lastly, it attracts considerable attention from the general public as it is an important sport and food fish with significant landings throughout its range but especially in Lee County, Florida (Bortone and Wilzbach, 1997b). Moreover, the spotted seatrout has the attention of the scientific community as evidenced by the substantial database of life history information on the species (Johnson and Seaman, 1986; Bortone et al., 1997).

The overall problem of issue addressed by this project was to develop an estuarine bioindicator that is capable of discerning the environmental stressors and their degree of impact on the overall environmental condition of the Charlotte Harbor estuarine ecosystem. Establishing a fully documented biological indicator would allow those concerned with the biological integrity of these estuarine waters to evaluate trends and determine the stressors that impact the functional attributes of estuarine ecosystems.

Three priority problems were addressed by this proposal. Growth in spotted seatrout is influenced by the total suite of environmental factors: salinity (hydrologic alterations), oxygen and nutrient conditions (water quality) and submerged aquatic vegetation (fish habitat loss). Each

of these features plays a significant role in providing conditions that impact growth in spotted seatrout. This study will permit us to determine the degree that each of these influences spotted seatrout growth and also establishes the database by which we can compare future modifications to the habitat features that provides essential habitat for this commercially recreationally important species.

The basic biology of the spotted seatrout (they are also known as specks, trout, and speckled trout) has been recently summarized in a volume dedicated to establishing its life history parameters as potential metrics to assess the environmental conditions within the estuary in which it resides (Bortone, 2003c). A host of biological characters are potential available to serve this purpose. For example, reproductive condition (Brown-Peterson, 2003), genetic differentiation (Gold et al., 2003), and parasite infestation (Blaylock and Overstreet, 2003), among others, are biological features that could prove useful in providing characters that would allow an assessment of environmental stressors. However, growth is reflective of a metabolic component of this species that is intimately interwoven with its environment that includes water conditions, habitat association, and salinity regime (Murphy and McMichael, 2003).

Bedee et al., (2003) and DeVries et al. (2003) clearly demonstrated the utility of using growth features of spotted seatrout to compare different estuaries. This study takes their concept a step further and makes use of comparative growth rates among spotted seatrout to monitor potential environmental stressors via time and space components in the Charlotte Harbor estuarine ecosystem.

Materials and Methods

Materials - The following materials and equipment were used during the preparation and analysis of specimens used in this study:

- Buehler® Isomet slow speed saw
- MTI Corporation Diamond EP Blades (4 inches x 0.5 millimeters)
- Fisherbrand® frosted microscope slides
- Lerner Laboratories Flo-texx®
- Crystal Bond® (thermoplastic cement)
- Leica Wild® M3Z dissecting microscope
- Olympus Americana Mirofire® model S99809 camera
- Pictureframe™ 2.0
- Image-Pro® Express (version used 4.5.1.3)
- 1-mm Stage Micrometer

About 400 spotted seatrout were captured using hook and line in the area of southern Pine Island Sound (Figure 1). Between April and July 2003. Upon capture fish were placed on ice and returned to the laboratory where they were labeled by date and field collection number and frozen. Later fish were thawed and measured for Fork Length to the nearest mm. Otoliths were removed from thawed fish. The method of otolith preparation and analysis is presented below.

Methods

1. Otolith removal

- a. Measure fork length (millimeters) of fish
 - b. Determine sex by examining gonads
 - c. Peel isthmus and brachioistegal membranes from base of cranial cavity
 - d. Score cranial cavity using knife and break using fingers
 - e. Remove exposed otoliths, rinse in fresh water bath, seal in envelope labeled with information (species, date caught, FL, OL, Sex, fish number)
2. Otolith mounting- there were two mounting procedures
- a. Using Flo-Texx®
 - i. Mount otolith convex side down onto slide using 3-4 drops of Flo-Texx
 - ii. Lay on flat surface to dry overnight
 - b. Using Crystal Bond®
 - i. Heat crystal bond in glass Petri dish at very low heat until highly viscous liquid
 - ii. Dip concave side otolith into liquid to coat underside
 - iii. Press to slide and hold for about 10 seconds
 - iv. Allow to cure overnight
3. Otolith sectioning
- a. Mark otolith slightly to the right of the core region with pencil
 - b. Insert mounted otolith slide into pivotal arm of saw aligning blade with mark for cutting
 - c. Check weight on pivotal arm should be between 25 and 30 grams
 - d. Start saw allow to reach full speed (between 5 and 6) before lowering slide onto the spinning blade

- e. Allow blade to cut through otolith and begin cutting through slide
- f. Raise arm and adjust spindle (one revolution plus four dash marks) ensuring the next cut will begin closer/in core region and lower arm to spinning blade
- g. When second cut is finished the section will either fall into catch basin, or remain attached to the slide
- h. Repeat f and g until finish cutting through core region
- i. Lift arm, stop saw, remove slide from pivotal arm

4. Section Mounting

- a. Using thin forceps remove any sections still attached to slide, and any fallen sections from catch basin
- b. Wash sections in small container fresh water to remove any dust/film
- c. Dry sections using Kim-Wipe®
- d. On fresh labeled (all information) slide make a separate pool (2 drops per pool) of Flo-Texx for each section
- e. Using forceps place dry sections on surface of pool and gently push through pool to slides surface allowing Flo-Texx to close in over top of section to completely cover section
- f. Using forceps push any remaining bubbles over section to the side out of future picture
- g. Allow newly mounted sections to dry over night

5. Otolith photography

- a. Install Pictureframe™ 2.0 software

- b. Attach Leica Wild M3Z dissecting microscope with Olympus Americana Microfire camera to computer
 - c. Place mounted otolith section slide under microscope and view on computer through the Picture Frame program
 - d. Adjust slide to find the best section on the slide
 - e. Set microscope magnification to 16x
 - f. Adjust exposure to optimum view of otolith section and photograph
6. Otolith reading using Image-Pro® Express

Calibration:

- a. Take a picture of a 1mm Stage Micrometer at 16x magnification using the above mentioned procedure
- b. Open the picture in Image-Pro® Express
- c. Click on Measure then Calibration then Spatial
- d. Click new then give the calibration a name and select the units
- e. In the Pixels/unit box, click Image
- f. Position the line so it extends the length of the micrometer and click OK
- g. Now click OK to complete the process

Measurement template creation:

- a. Click Measure then Measurements
- b. Use the line tool to draw a vertical line
- c. Click on the feature tab and name the line “Radius”

- d. Use the line tool and make a dot (click and release; don't move the mouse) at the bottom of the line
- e. Label the dot "Core"
- f. Place a dot in the middle of the line and at the end
- g. Label them "growth ring 1 (GR1)" and "Edge" respectfully
- h. In the Feature tab use the measure tool and measure from core to GR1, (1st year growth) from GR1 to Edge (marginal increment) and from Core to Edge (radius)
- i. Label measurements accordingly
- j. Save measurements titled "year 1"
- k. Repeat process adding another dot for each additional year and save as "year (2,3,4,...)"

Otolith measurements:

- a. Open the otolith picture
- b. Use the line tool and draw a line from one side to the other side of the sulcus at the outer edge
- c. Under the Import/Output tab open the measurement template for the appropriate age fish making sure the Load As Template box is checked
- d. Draw the Radius line thru the otolith core to the L of the label for the line you Previously made
- e. Put the Core dot in the center of the core on the Radius line (click and release; don't move the mouse)
- f. Follow the same procedure and mark the growth rings and the edge

Export measurements to Excel:

- a. Under the Import/Output tab select DDE to Excel
- b. In DDE options set position data set at to Row:1 Column: 1
select increment position for next data set by: to Row: 0 Column: 0
- c. The measurement that is needed is labeled Ctr-to-Ctr Dist.
- d. The measurements can now be copied into Access using Office Clipboard

Since only one fish was recorded at Age 5 it was excluded from the analysis. The size at capture, otolith radius and annular radii for each fish were used to back-calculate the size at annulus formation. The equation used was:

$$L_i = a + (L_c - a) \times (O_i/O_c)$$

where L_i = the fork length (mm) of the fish when it became age i , a = the y-axis (Fork length) intercept of the relationship between otolith radius (abscissa) and Fork Length (ordinate), L_c = size of fish (Fork Length in mm) at capture, O_i = distance in mm from central core of otolith to the distal edge of the annulus at age i , and O_c = otolith radius in mm.

Analysis for Environmental Variables

A time series analysis was conducted using the back-calculated size at year one for each year class versus several environmental variables and the SPSS statistical program package (version 8.0). Time series analyses were conducted to be able to relate time-lagged associations in environmental conditions of the estuary with growth as this was one of the major objectives of this study was to relate differences in annual growth to environmental features of the estuary.

Seagrass density data were obtained from the Florida Department of Environmental Protection's Burnt Store Road facility. The data used were Braun-Blanquet measures of percent cover and served as an indication of seagrass density. Salinity (as measured through conductivity) was used as a relative indicator of water quality conditions. Conductivity data were obtained from the South Florida Water Management District's water quality monitoring probe near the Sanibel Causeway at the entrance of San Carlos Bay.

Results

Although more than 400 fish were captured during this study, a total of 296 were aged (162 male and 134 female). Nearly 100 fish could not be reliably aged for a variety of reasons. Sometimes both otoliths were damaged during extraction, sectioning, or mounting. Some fish bore otoliths from which no age could reliably be determined. Each otolith was read at least twice by two investigators. Otoliths that were problematic were read a third time by three investigators. If agreement was not attained then that fish was excluded from ageing. Examples of the various age groups (ages) are presented in Figure 2. The appendix includes all data used in the preparation of this report.

Table 1 presents a summary of the number and size of spotted seatrout collected during the course of this study. The smallest male was 217 mm FL (Fork Length) and the largest was 420 mm. The smallest female was 228 mm long and the largest was 557 mm. The average size of males was smaller than the average female; 293.75 mm versus 321.36 mm, respectively. A

histogram of the length-frequency distribution of all fish (Figure 3) and males and females are separately presented in Figure 4.

The relationship between the otolith radius and length is presented in Figure 5. A regression line was calculated separately for each sex. While both lines were showed a positive correlation, the lines were statistically significant from each other both with regard to slope and y-axis intercept. Consequently, the analyses were conducted separately, using the respective slope and y-intercept information for each sex.

All fish used in the analysis were captured after March 2003. The literature indicates that spotted seatrout generally deposit the annulus in the early spring of each year. The bar chart (Figure 6) indicates that the marginal increment increased from April. This demonstrates that all fish examined here had already deposited the annulus for 2003 prior to being collected for this study.

Figure 7 depicts the relative total mortality observed among fish captured during this study. The decline of fish at each subsequent age is indicative of total mortality. Males show a classic and typical decline where the most abundant year class is age group 1 and the decline asymptotically approached the abscissa. A slightly different profile was observed among females. Age group 2 is proportionately more abundant (or concomitantly, age group 1 was slightly underrepresented among the data).

Figure 8 depicts the size at age at capture (above) and size at age at annulus formation (below). There is little difference in the patterns among groups because a linear conversion equation was

used to derive the back-calculated size at age from the size at capture. The differences observed between males and females continue in this figure as females display a faster growth rate than males.

Figure 9 is a pair wise bar-chart for size for each year class at age one. The data for this figure were derived from the back-calculated size at age one for each of the four year classes. Again, females tended to attain a larger size at annulus formation than males for each year class. Interestingly, age 1 fish for the most recent year class (2002) was the longest. Size of fish at age 1 for prior year classes were generally less. This may indicate Rosa Lee's phenomenon where back-calculated fish tend to be shorter with time. However, it should be noted that females for year class 1999 and 2002 were statistically identical. Summarizing the results of a One-Way Analysis of Variance for size at age 1 for each year class (sexes analyzed separately) indicates that year-class 2002 was different from year-class 2001 and 2000 for females. Year-class 2002 was different from year-class 2001 and 2002 for males as well. Year-class 2000 was the shortest at age-1 annulus formation than all other year classes but this was not statistically significant in all cases.

Environmental Associations

Seagrass density for each of three species of seagrass from the lower portion of Pine Island Sound and San Carlos Bay was used as a measure habitat conditions. The results of the time-series analysis is presented graphically in Figures 10-24. There was a positive association of male growth with female growth with no time lag (Figure 10). This was to be expected as the trends in female and male growth were similar (Figure 9). During their first year, female growth

was positively associated with the density of the seagrass *Halodule* from the preceding year (Figure 11), positively with *Syringodium* density from the preceding year and negatively with the same year (Figure 12), and negatively with *Thalassia* density during the corresponding year of the year class (Figure 13).

Conductivity was the measurement recorded as an indication of salinity that also served as measure of water quality. During the first year of growth for each year class, female growth was positively associated with conductivity from the preceding year (Figure 14). There was little evidence that minimum salinity was related to female growth during their first year (Figure 15), however, the maximum salinity was positively associated with growth of females during their first year for each year class (Figure 16). The variance of water conditions, as measured by the annual variance of conductivity, was somewhat associated with female growth during the concurrent year for each year class (Figure 17).

Growth among males during the first year of life for each year class showed similar patterns to those described for females. A positive association was determined *Halodule* and *Syringodium* density from the preceding year (Figures 18 and 19), while a negative association of *Thalassia* density occurred with growth from two years preceding the first year of growth for males (Figure 20). The initial year of growth among males was positively associated with higher average salinity that preceded male growth by a year (Figure 21). There was a low level of positive association male growth with concurrent year minimum salinities (Figure 22) and a positive association of growth with preceding maximum salinities (Figure 23). Interestingly, a high

variance in salinity preceded a low level of growth among males during their first year of growth for each year class (Figure 24).

Discussion

The relationship between otolith size and fish size indicates the potential utility that aging otoliths can have on determining age and growth for the spotted seatrout in areas waters. Similarly the observed monthly increase in marginal increment after April indicates that the annulus is generally deposited in early spring for this species. This observation also helps validate the use of otoliths in determining age for the spotted seatrout in this area.

The observed differences among males and females, with females generally growing faster and attaining a larger size at an equivalent age, were observed here. Differences between sexes demanded that each sex be treated separately in all analyses. While outwardly cumbersome, it allows the advantage in offering a test by congruence of any observed trends detected for one sex.

Comparisons of size at a similar age are facilitated by using back-calculated size at annulus formation data. Assuming some feature such as Rosa Lee's phenomenon was not acting to invalidate comparisons, this technique allows ready comparison among previous year classes. One of the objectives of this study was to be able to relate environment features with growth parameters. In this study it is inviting to make the assumption that there may have been environmental factors affecting the slower growth (size at age) observed for both males and

females for year-class 2000. This year was environmentally significant in the Caloosahatchee River/Estuary in that extreme conditions of salinity, caused by excessive releases of freshwater from Lake Okeechobee followed by a complete reduction in releases in water from the Lake. It is tempting to speculate that the observed differences in growth among spotted seatrout were caused by the irregular salinity regime. As observed, it is but a single observation but the beauty of initiating such a database on spotted seatrout growth is that we now have a basis upon which to compare future potential responses of fish growth on extreme salinity conditions.

The time-series analysis indicated some interesting associations between environmental conditions, as measured by seagrass density and salinity, and growth during the first year of life for both males and females for each year class. Faster growth was preceded by higher densities of *Halodule* and *Syringodium* and higher average salinities. One should interpret these findings with some caution as they association factors are not necessarily causative. As with any time-series analysis there should be many replicates of the natural cycles and responses in order to make statements with regard to causation. Nevertheless, the model presented here does indicate that with more data, it may be possible to speculate on subsequent year-class growth based on seagrass density information or salinities.

Clearly, there is an indication that higher salinities favor faster growth among seagrasses during their first year of life. The inevitable implication for management is that increasing the average salinity of the estuary through reductions in discharges may lead to higher growth rates among spotted seatrout. However, more evaluation is needed before this could become an acceptable management action.

Previous studies on spotted seatrout have also found that females grow faster than males (Mercer, 1984) although the difference may not be significant during the first year of growth (Murphy and McMichael, 2003).

Murphy and McMichael (2003) summarized the growth of spotted seatrout through out their range for which there are data. Comparisons with other studies need to be done with caution as sometimes size at age data are reported as Standard Length as opposed to Total Length and Fork Length (these are equivalent in spotted seatrout) and authors do not always indicate which measure for length was used. With this caveat, an inspection of back-calculated lengths at age (Murphy and McMichael, 2003) indicates that spotted seatrout from Lower Pine Island Sound in the Charlotte Harbor system seem to grow faster than other spotted seatrout along the eastern Gulf of Mexico. For example, Moffet (1961) indicated that spotted seatrout for ages 1-4 attained size at annuli among males of 156, 245, 302, and 364 mm, respectively. This is considerably lower growth than reported here. However, data presented in Murphy and Taylor (1994) indicated reported back-calculated sizes at age similar in Charlotte Harbor (237, 305, 345, and 384) similar to those for males reported herein for the first four years of age (278, 310, 337, and 369).

Similar trends in growth were noted to occur for females as well (Murphy and McMichael, 2003). Moffett (1961) reported females at age of annuli 1-4 as 160, 248, 313, and 377, respectively, for Fort Myers whereas Murphy and Taylor (1994) reported sizes as 242, 357, 434, and 495 mm TL in Charlotte Harbor. Here female spotted seatrout were observed to be 288, 388, 371, and 457 mm TL for their back-calculated size at annulus formation. The present study

results are more similar the growth observed for both sexes by Murphy and Taylor (1994) but much faster than that observed by Moffett (1961) for Fort Myers. These differences could be due to methodological differences in aging fish, differences in back-calculation protocols, or real differences in growth rates taken over different time periods.

The final posed hypotheses begs the question: Are differences in growth reflective of differences in estuarine condition over time? This is the very premise of the current research effort. With only a few years of data (both with regard to fish growth and environmental data) with which to conduct the analyses, the hypothesis that environmental differences in the estuary can be observed in the growth rate among an estuarine resident fish like the spotted seatrout is not rejected. Continuation of the relationship between growth and the environment is warranted. If this hypotheses continues to stand after further examination, then growth rates of spotted seatrout can serve as a valuable indicator of estuary conditions. Furthermore, knowledge of this relationship could also provide environmental managers a gauge to determine the effects of efforts to restore estuaries.

Conclusions

The data on age and growth offered in these study results are important. They help form the basis for future comparisons of differences noted in age and growth in this ecologically and economically important species in being able to recognize and assess the status and trends both with and between estuaries in areas which they inhabit. The data presented here lend credence to the idea that some features or factors in the estuary are associated with growth. With a longer-

term data base it may one day be possible to predict fish growth in the estuary through evaluation of a few pertinent environmental features. Eventually it may even be possible to affect growth in some fish species through manipulation of controllable environmental features, such as salinity. Longer term trends in age and growth differences (actually similarities!) may allow us to detect long-term, global-scale phenomena related to factors related to global climate change. One such rule is the Moran Effect (Hudson and Cattadori, 1999; Ripa, 2000; Koenig, 2002; Stenseth et al., 2002) that implies common biological trends may be detected across a wide spatial scale if subjected to a generally widespread stressor such as climate change. While such a feature was not detected here, the baseline information is accumulating through data gathered by studies such as this to be able to make such determinations.

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Table 1. Size summary (Fork Length in mm) of spotted seatrout used in the age/growth analysis.

	Mean	Std. Dev.	Min.	Max	Number
Males	293.75	36.37	217	420	162
Females	321.36	51.08	228	557	134
Total	306.25	45.69	217	557	296

Table 2. Summary of Fork Length (mm) statistics (actual and back-calculated) by age for male and female spotted seatrout captured during this study.

Sex	Age	Actual					Back-calculated				
		Mean	Min.	Max.	SD	No.	Mean	Min.	Max.	SD	No.
Males	1	278.43	217	336	22.09	113	251.11	194	323	24.48	162
	2	310.25	265	350	21.15	28	293.72	251	338	19.97	49
	3	337.00	307	387	25.10	10	330.91	297	379	25.17	21
	4	368.60	252	420	51.73	11	373.30	328	413	34.10	11
Females	1	288.38	228	333	22.91	68	260.14	201	323	23.02	134
	2	338.43	269	428	29.34	48	324.06	245	418	29.54	66
	3	371.00	291	438	42.45	12	354.39	189	460	72.76	18
	4	457.33	379	557	66.33	6	443.23	364	539	67.41	6

Table 3. Age-Length (FL in mm) key for spotted seatrout captured in 2003 relative to sex

Class Size FL (mm)	Age group				Total Female	Age group				Total Male
	1	2	3	4		1	2	3	4	
200						1				1
220										
240	1				1	2				2
260	8				8	17				17
280	13	1			14	40	3			43
300	28	4	1		33	32	5			37
320	13	8	1		22	18	11	3		32
340	5	12			17	3	6	3	1	13
360		13	4		17		3	3	4	10
380		7		1	8			1	1	2
400		1	3	1	5					
420			3		3				4	4
440		1			1					
460				2	2					
480										
500				1	1					
520										
540										
560				1	1					
Totals	68	48	12	6	134	113	28	10	11	162

Figure Legends

Figure 1 – Map indicating the general area of sampling for spotted seatrout used in this study. No fish were captured outside the designated study area.

Figure 2 – Examples of otoliths from spotted seatrout representing various ages: A) Age 1, 292-mm FL female; B) Age 2, 341-mm female; C) Age 3, 391-mm female; D) Age 4, 457-mm female.

Figure 3 - Length-frequency histogram of all spotted seatrout according to size (Fork Length in mm) at capture.

Figure 4 - Length-frequency histogram of all males (above) and females (below) examined during this study.

Figure 5 – Scatter diagram of otolith radii and fork lengths (both in mm) for male (dots/solid line) and females (circles/dashed line).

Figure 6 – Bar graph of marginal increment, by month, of all fish examined.

Figure 7 – Connected scatter plot of the age group percent frequency for male and female spotted seatrout.

Figure 8 – Age group versus fork length (mm) for males (solid dots) and females (open circles) for fish at capture (above) and fish at annulus formation (below).

Figure 9 – Bar graph for back-calculated size at age 1 for males (black) and females (shaded) for each year class.

Figure 10 – Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of male growth versus female growth.

Figure 11 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of female growth versus *Halodule* density.

Figure 12 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of female growth versus *Syringodium* density.

Figure 13 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of female growth versus *Thalassia* density.

Figure 14 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of female growth versus average annual conductivity.

Figure 15 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of female growth versus the minimum conductivity.

Figure 16 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of female growth versus the maximum conductivity.

Figure 17 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of female growth versus the annual standard deviation of conductivity.

Figure 18 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of male growth versus *Halodule* density.

Figure 19 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of male growth versus *Syringodium* density.

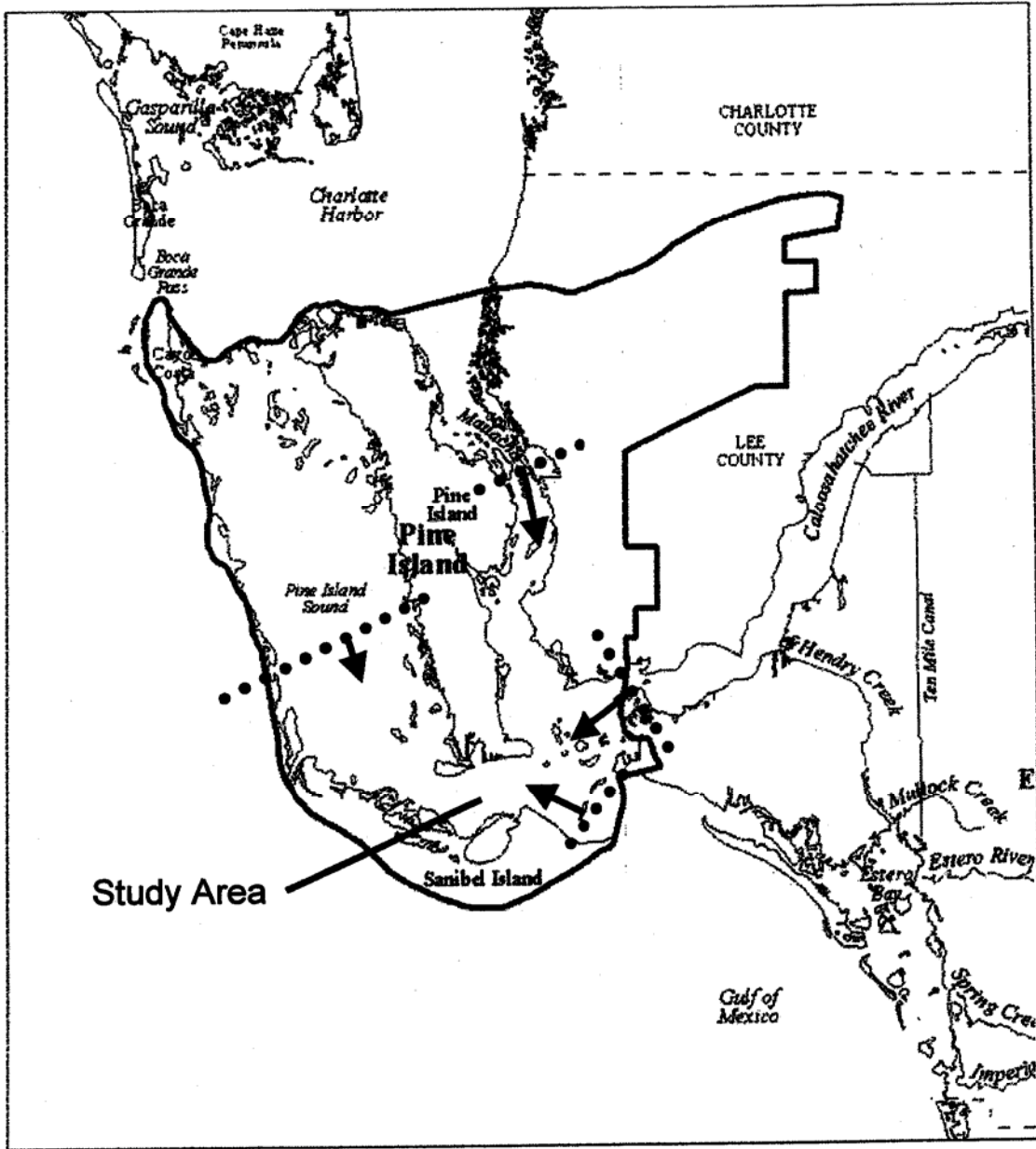
Figure 20 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of male growth versus *Thalassia* density.

Figure 21 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of male growth versus average annual conductivity.

Figure 22 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of male growth versus the minimum conductivity.

Figure 23 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of male growth versus the maximum conductivity.

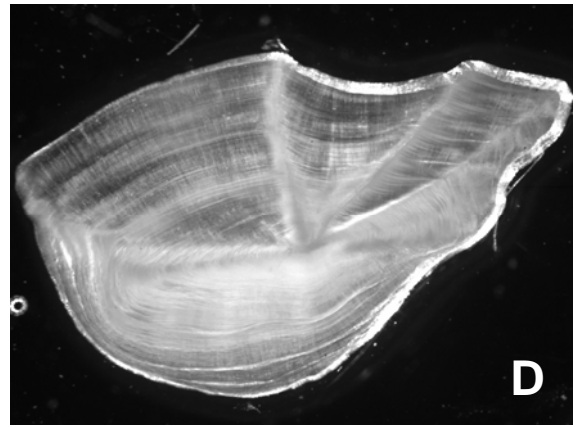
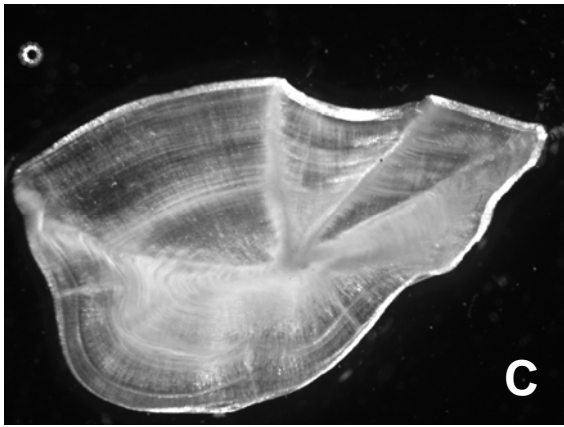
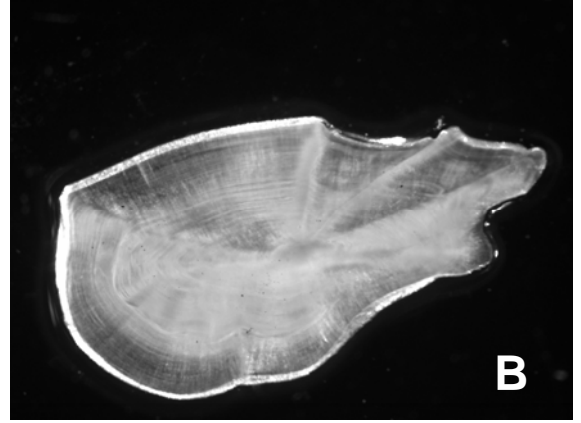
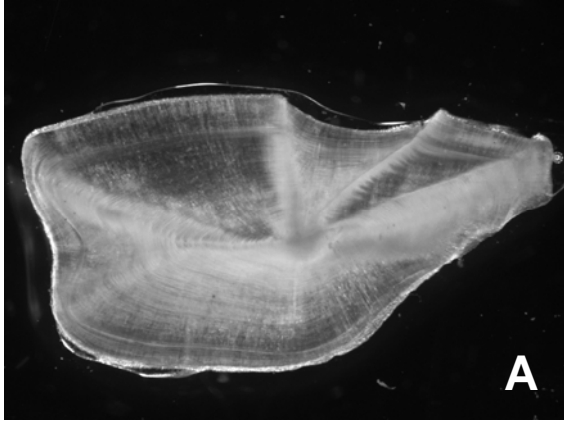
Figure 24 - Bar graph indicating the correlation coefficients for lagged time units (years) for first year of each year class of male growth versus the annual standard deviation of conductivity.

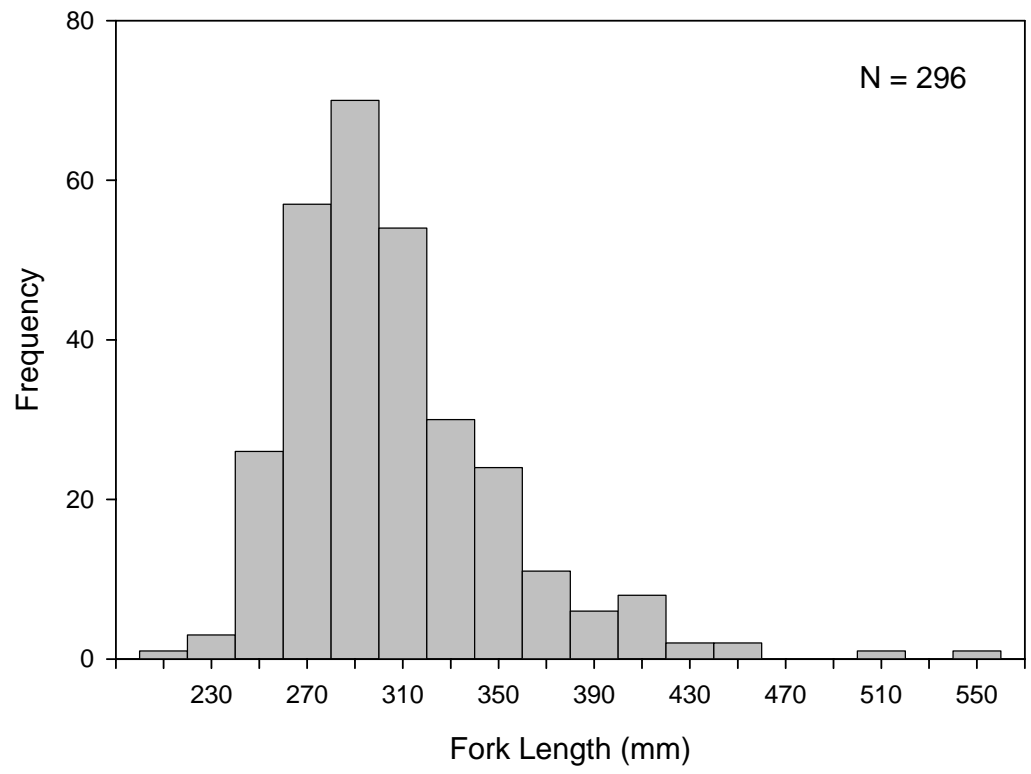


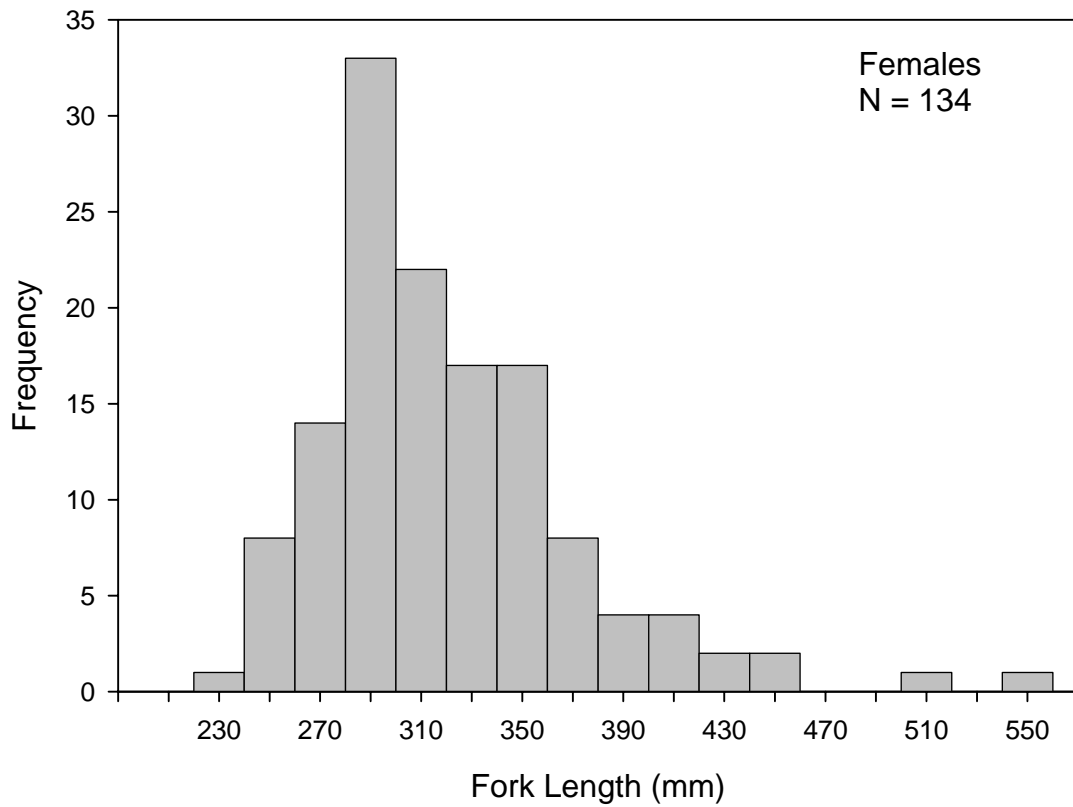
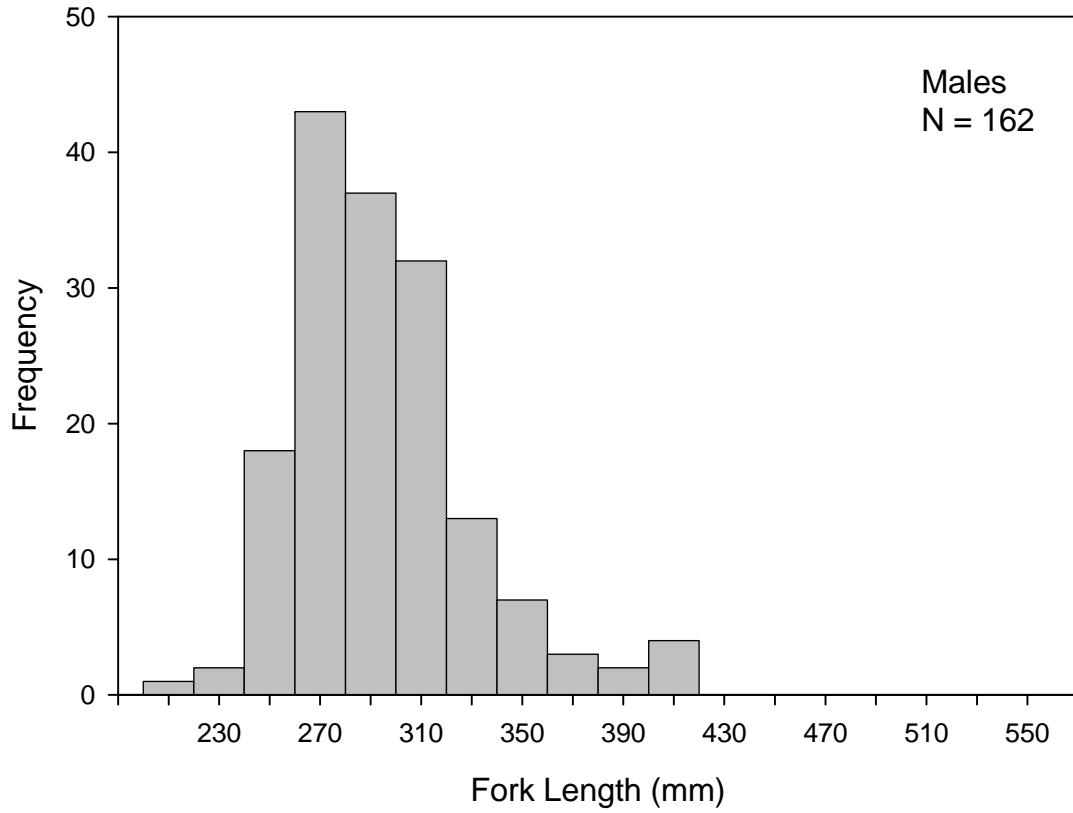
LOCATION
Pine Island Basin

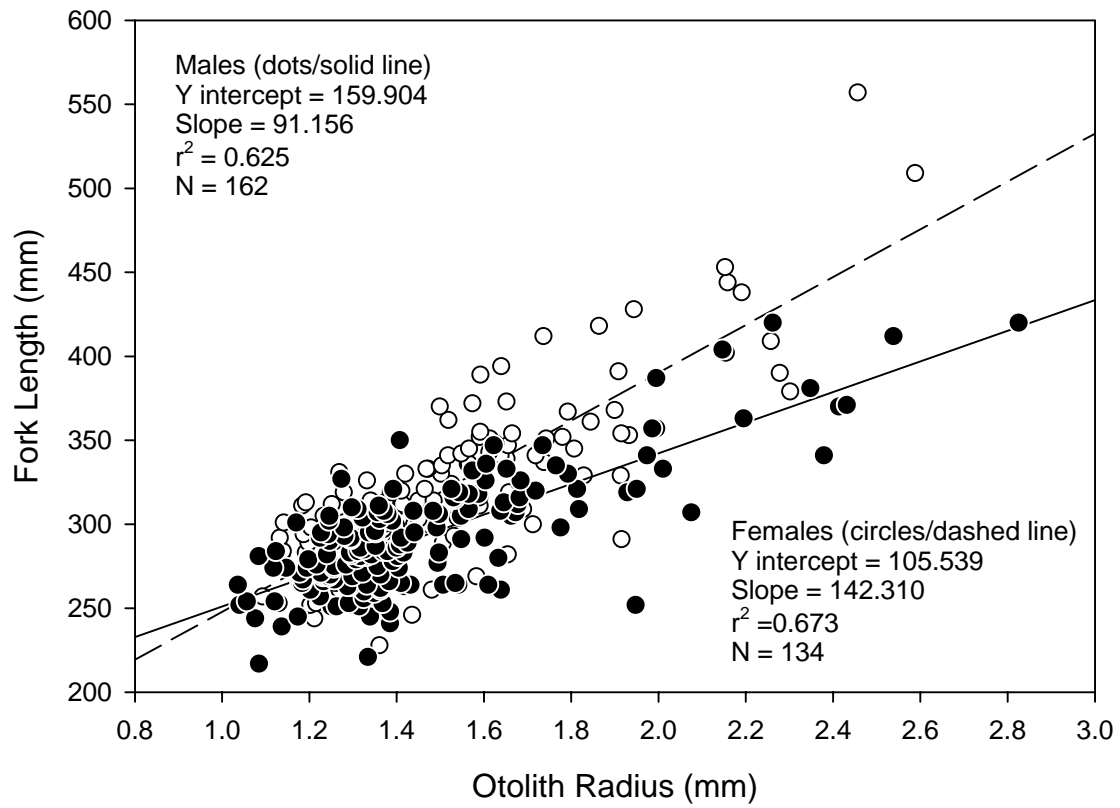


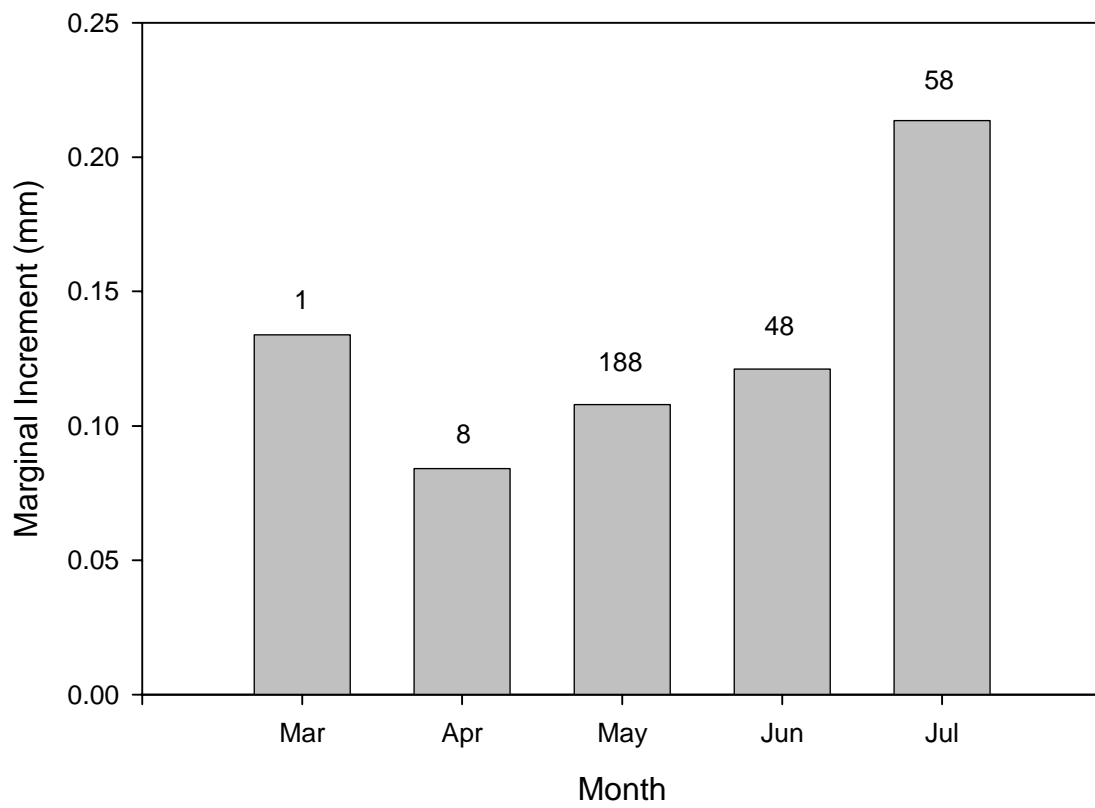
PBSJ 10320 80 01/09/99
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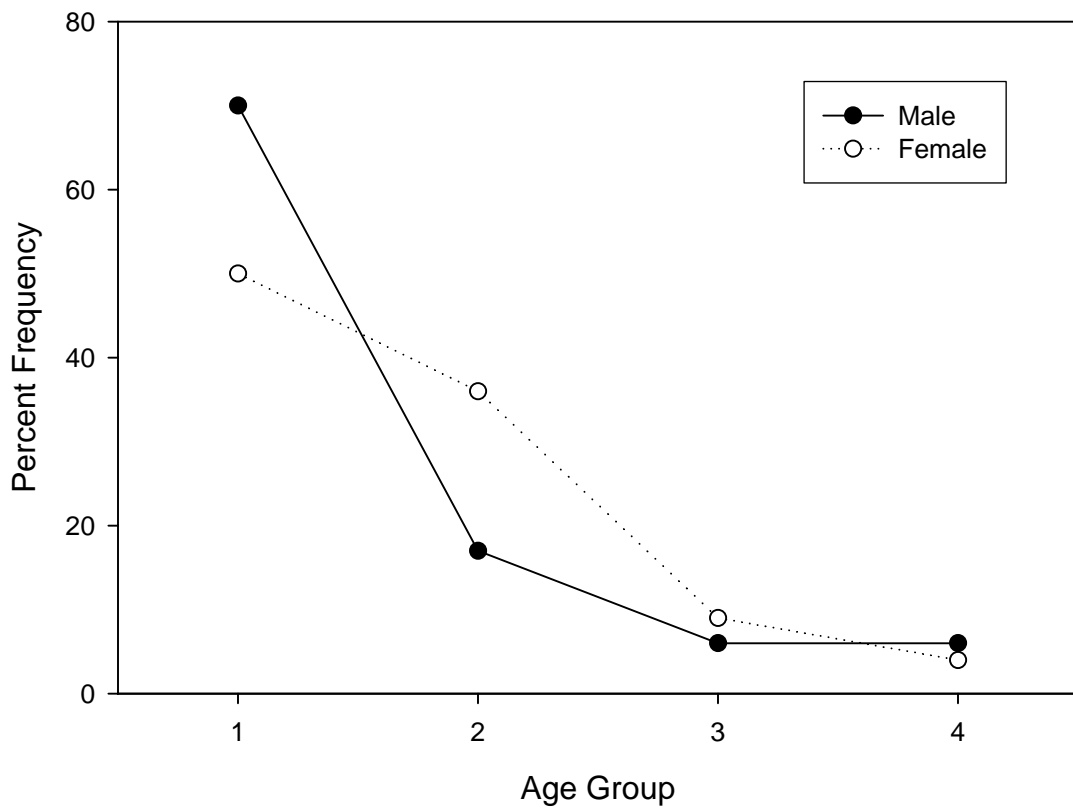


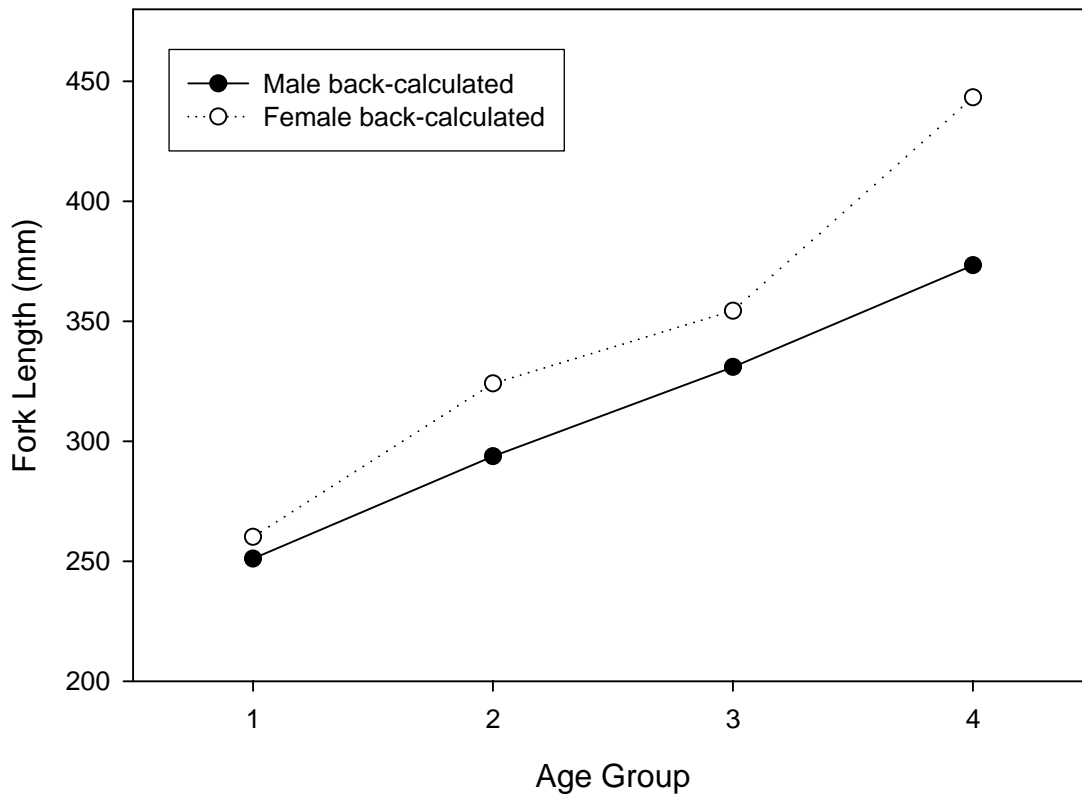
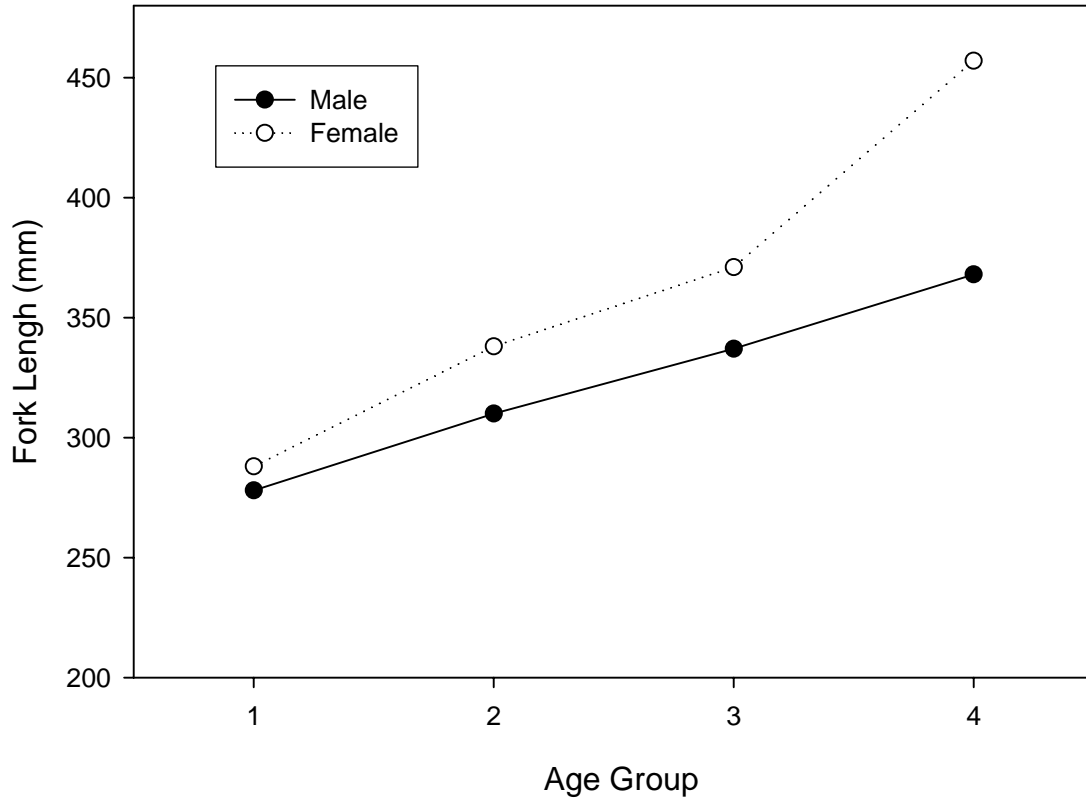


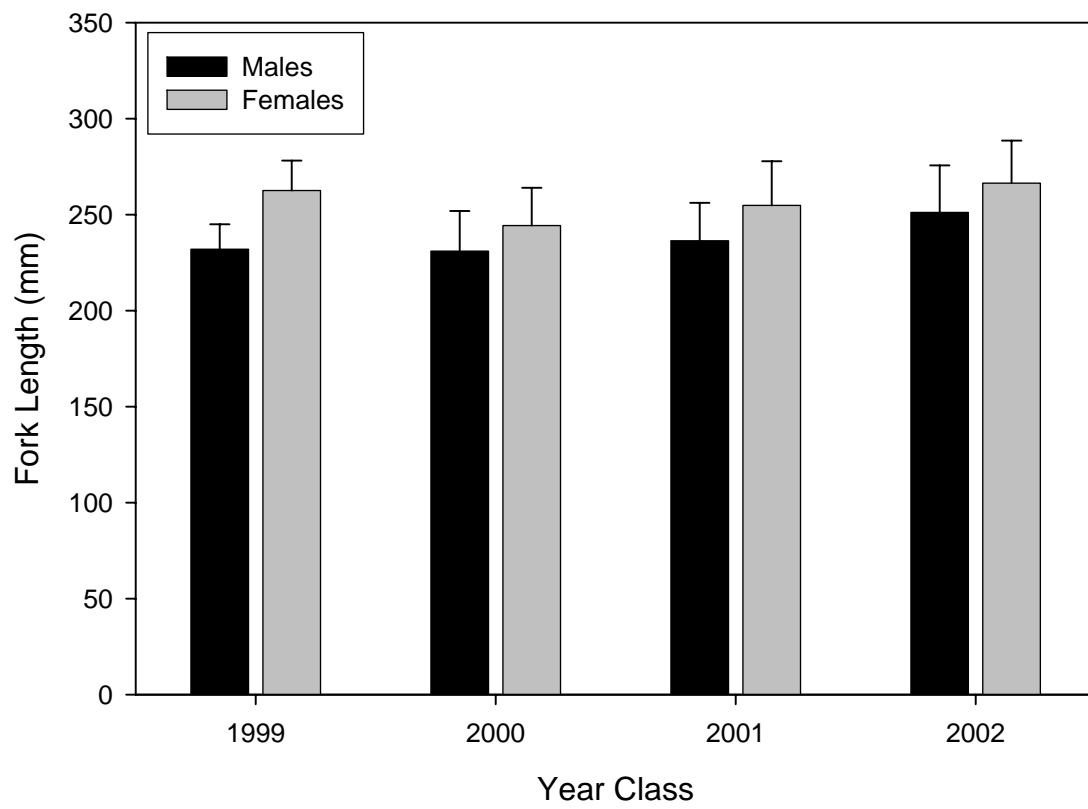


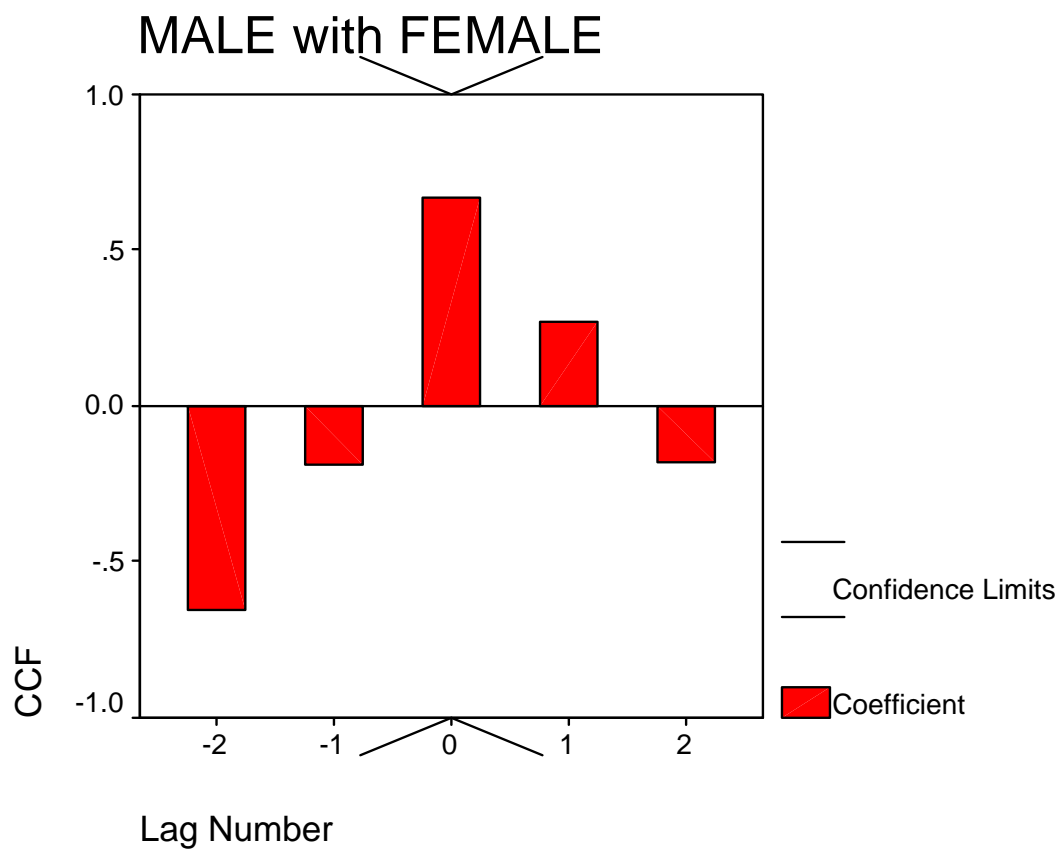




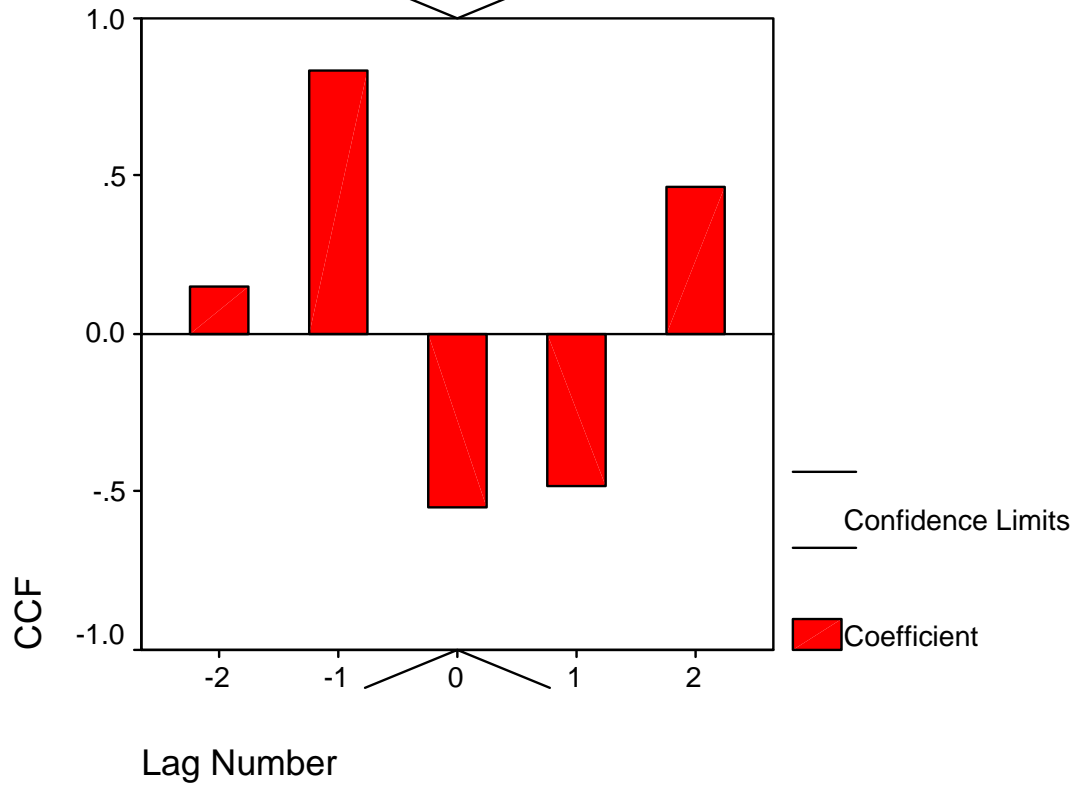




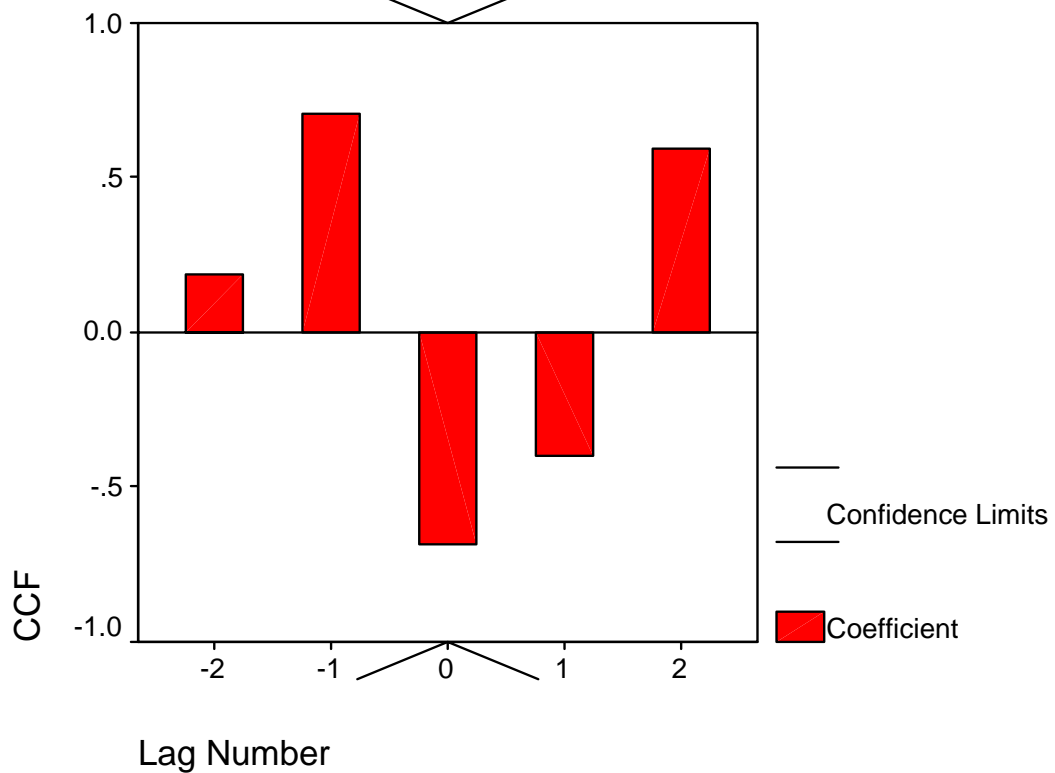




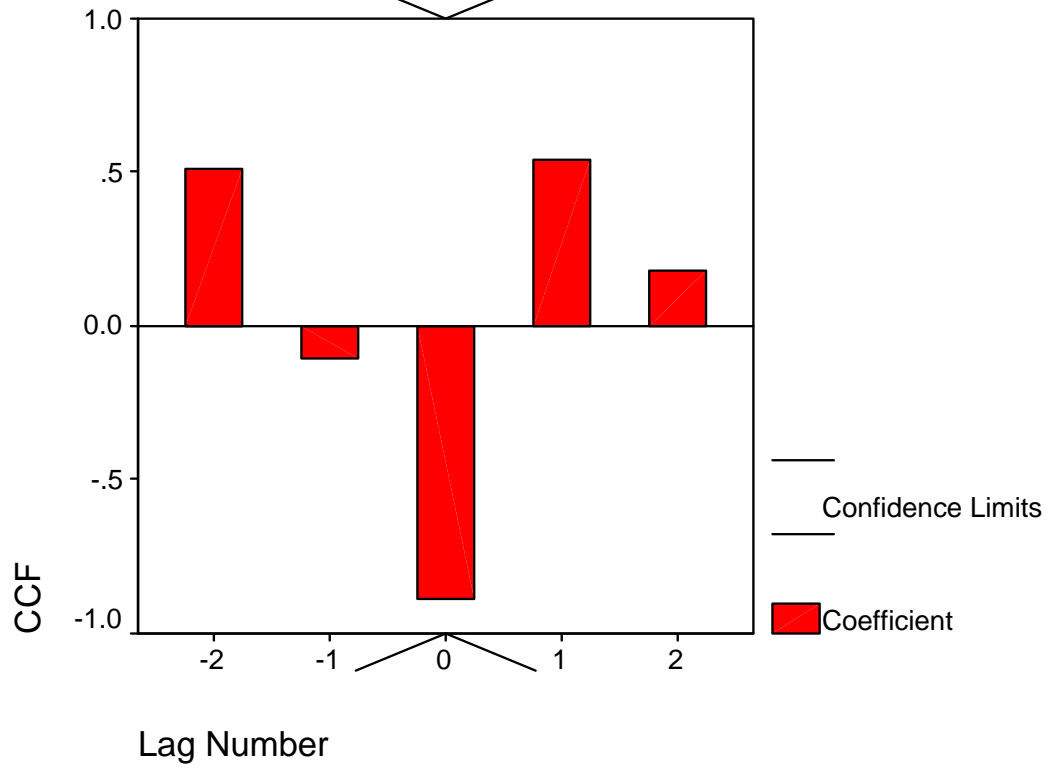
FEMALE with HALODULE



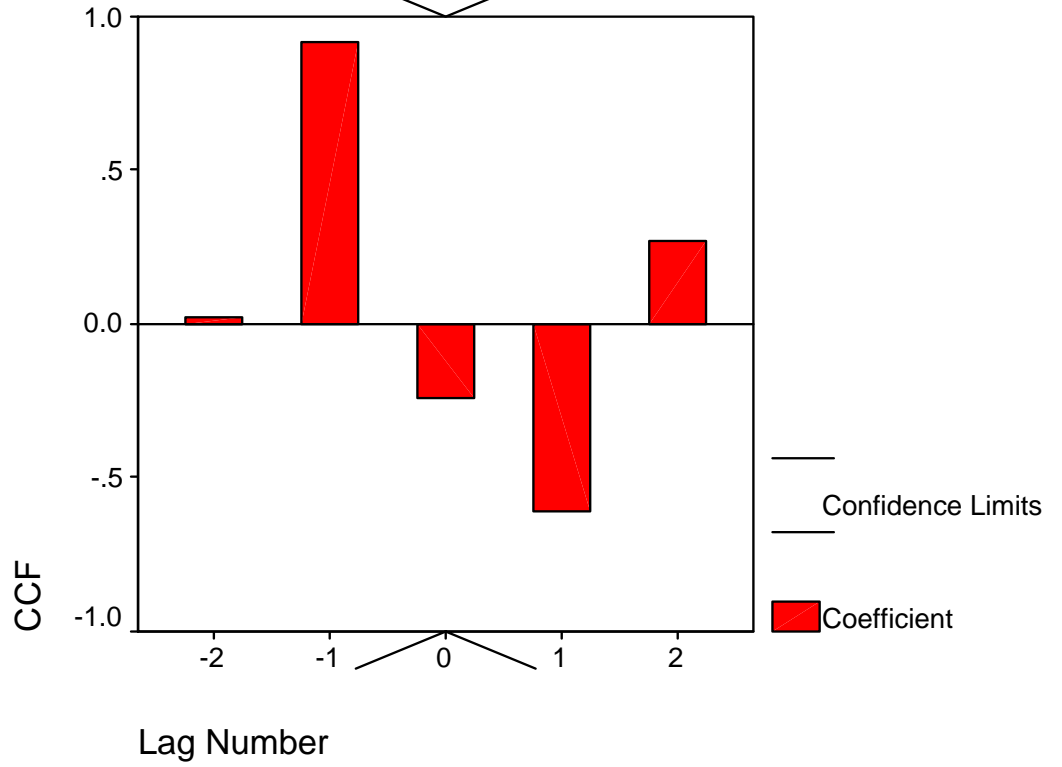
FEMALE with SYRINGOD



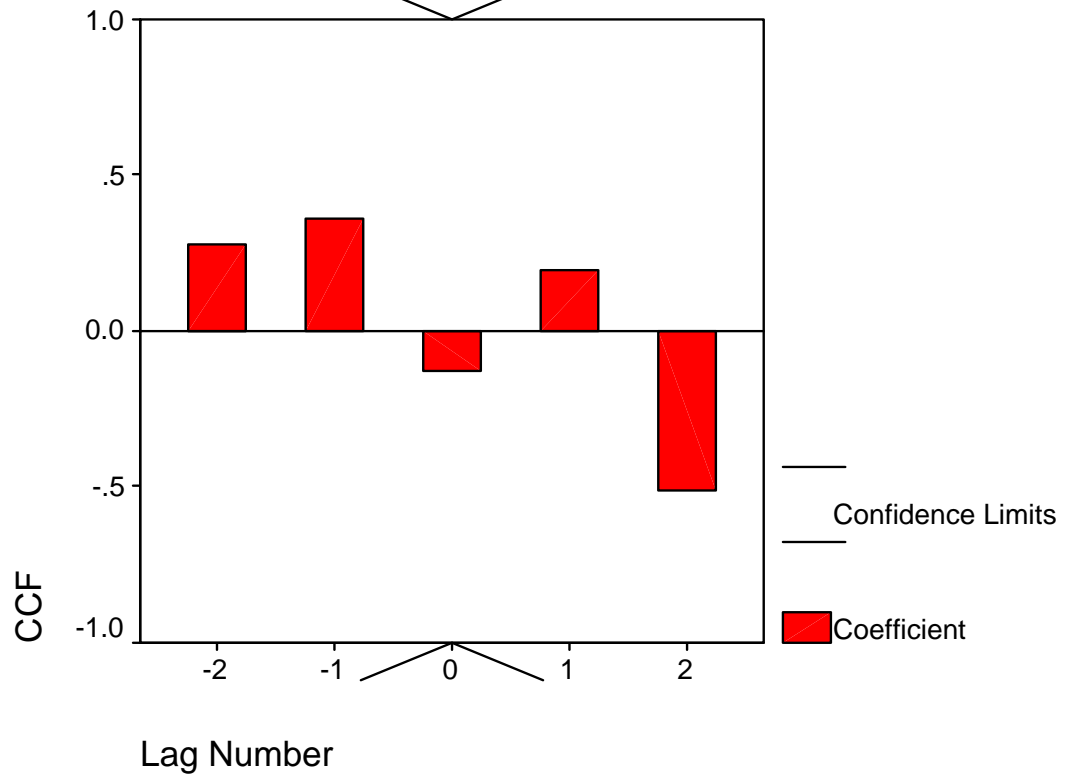
FEMALE with THALASS



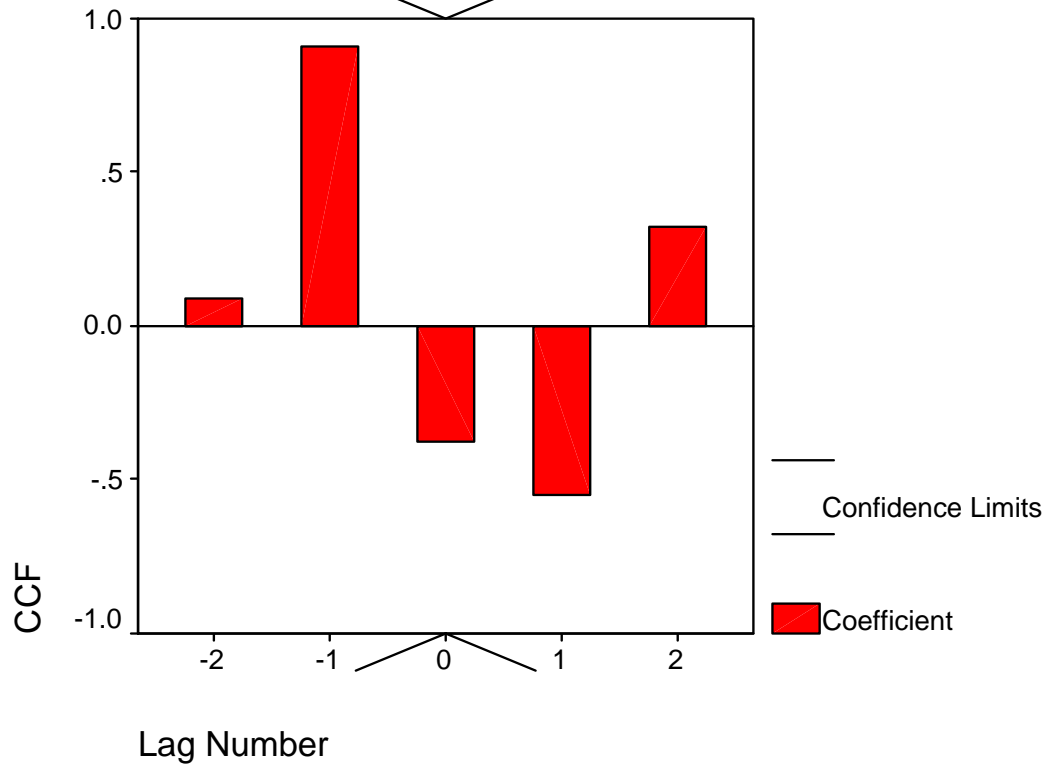
FEMALE with CONDAVE



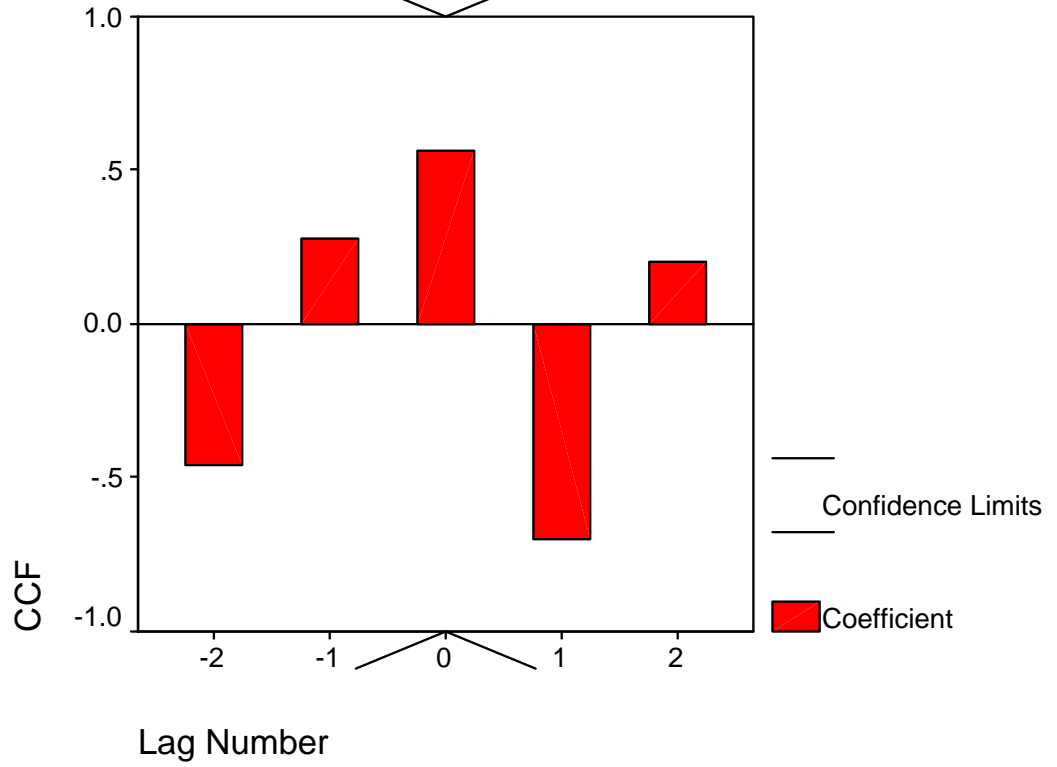
FEMALE with CONDMIN



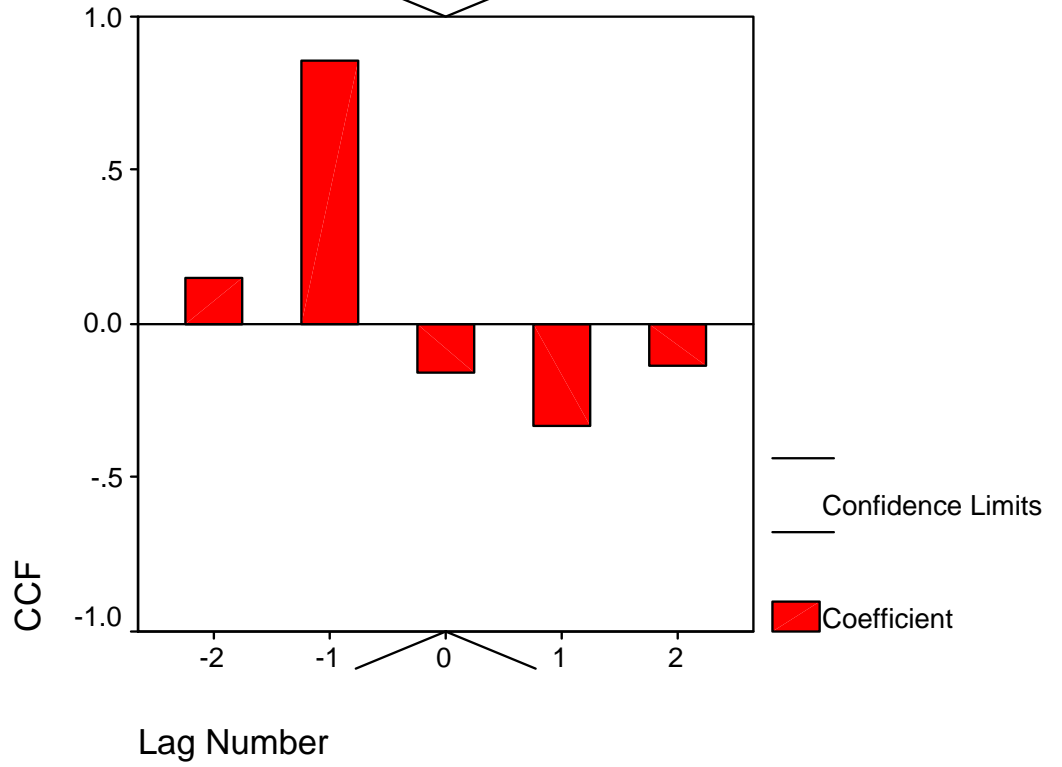
FEMALE with CONDMAX



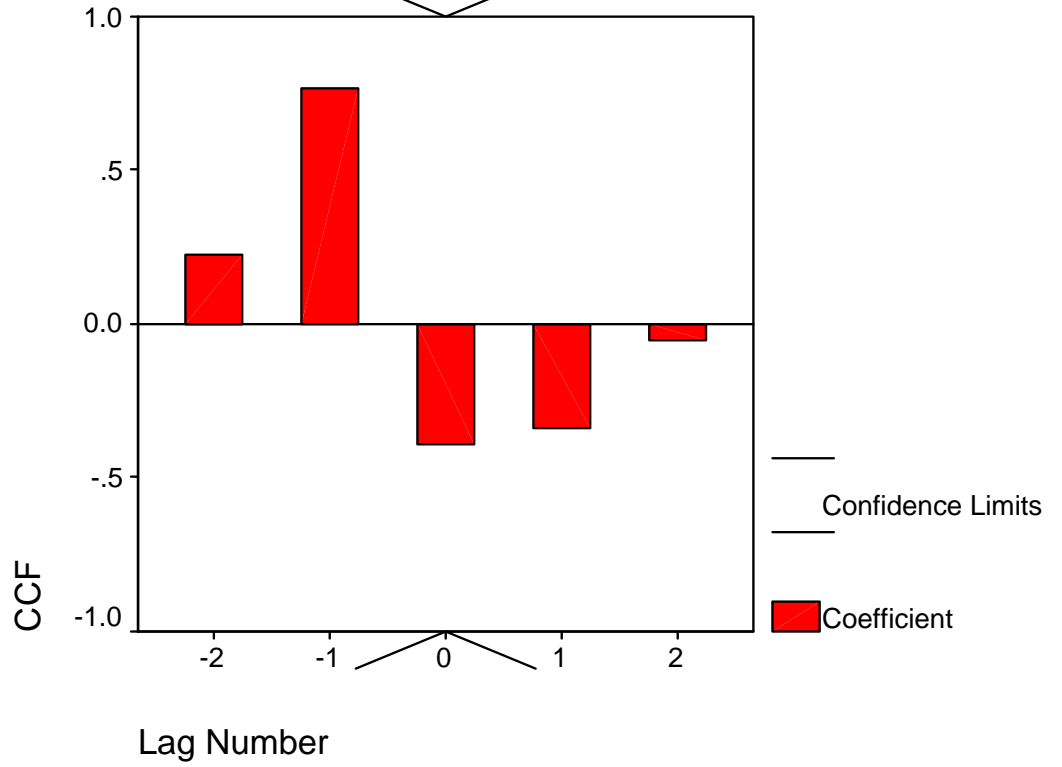
FEMALE with CONDSTD

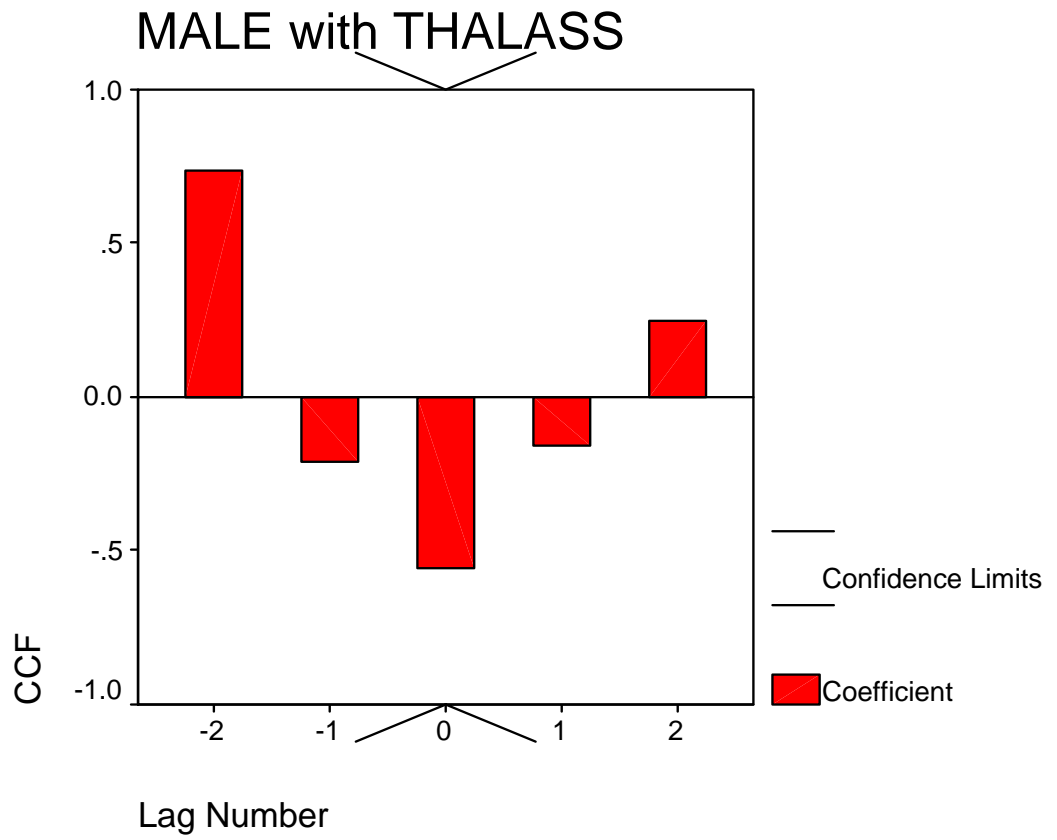


MALE with HALODULE

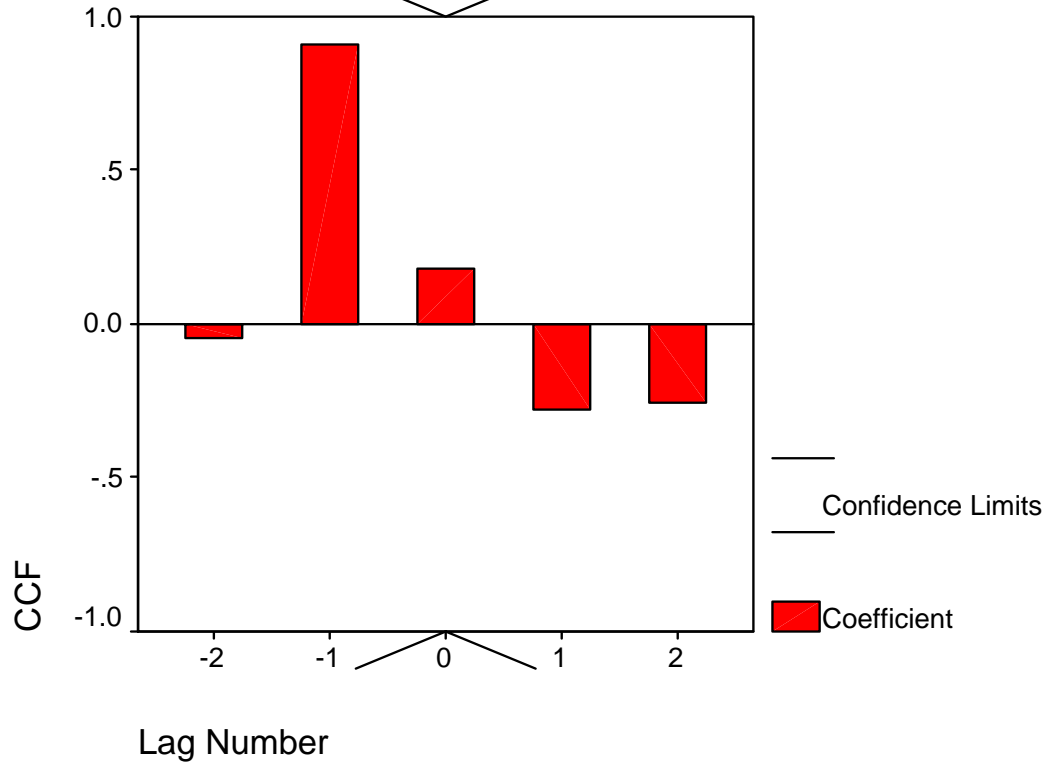


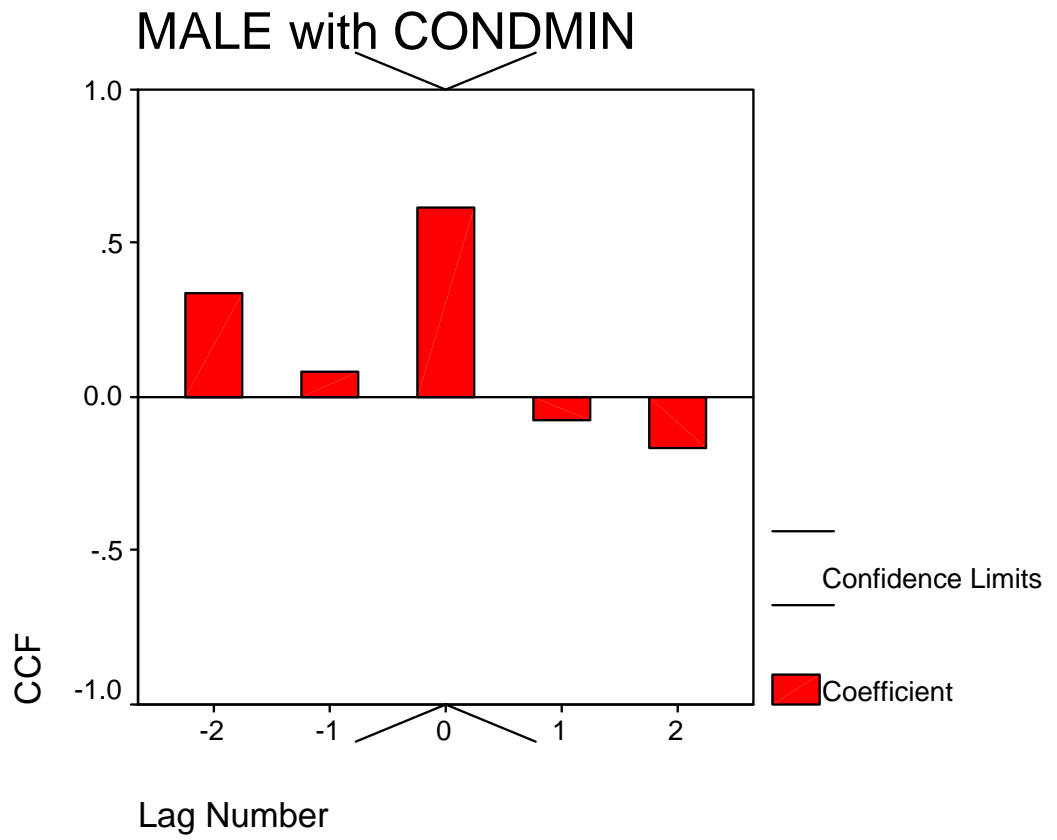
MALE with SYRINGOD



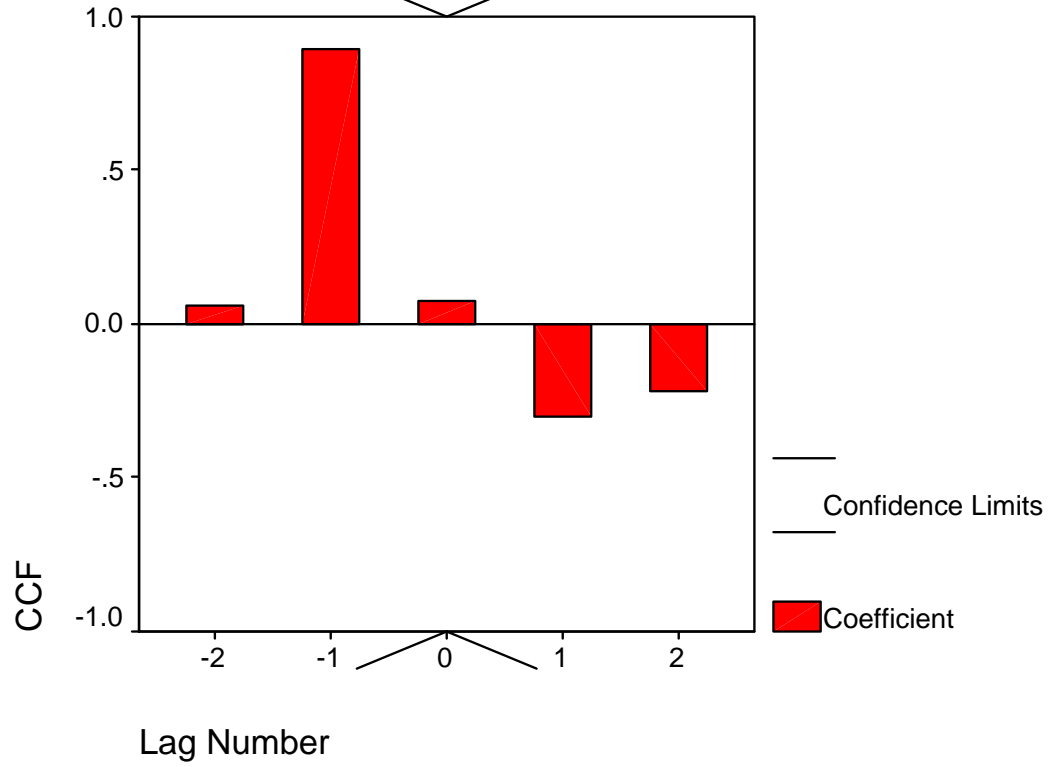


MALE with CONDAVE

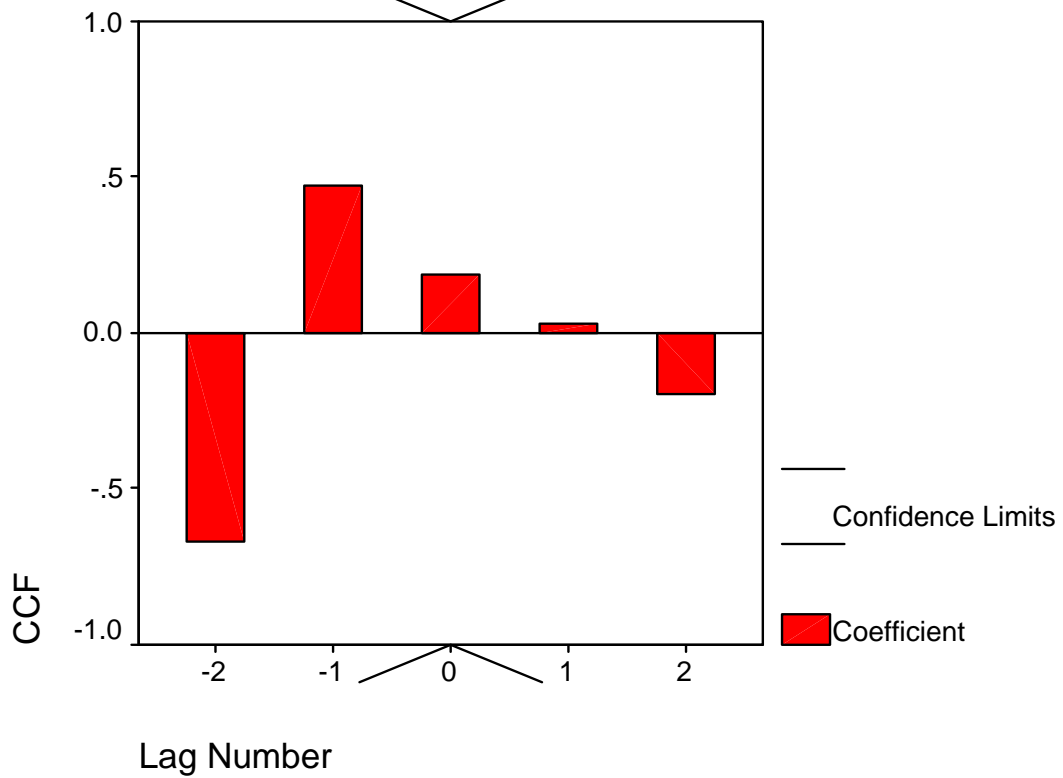




MALE with CONDMAX



MALE with CONDSTD



Appendix - Raw data of sex (female or male), OR (otolith radius in mm), Fork Length (mm), R1..5 (otolith radius to annulus in mm), MI (marginal increment in mm), and date collected.

Sex	OR (mm)	FL (mm)	R1 (mm)	R2 (mm)	R3 (mm)	R4 (mm)	R5 (mm)	MI	Date_Collected
Female	1.360	228	1.197					0.164	21-May-2003
Female	1.211	244	1.131					0.081	21-May-2003
Female	1.435	246	1.255					0.180	28-May-2003
Female	1.295	252	1.095					0.200	26-May-2003
Female	1.202	252	0.986					0.216	28-May-2003
Female	1.215	253	1.069					0.148	13-May-2003
Female	1.129	253	0.950					0.179	21-May-2003
Female	1.283	254	1.198					0.086	21-May-2003
Female	1.093	257	0.999					0.094	25-Apr-2003
Female	1.480	261	1.324					0.157	15-May-2003
Female	1.269	261	1.073					0.195	25-May-2003
Female	1.418	263	1.302					0.116	26-May-2003
Female	1.261	265	1.121					0.140	25-May-2003
Female	1.583	269	1.077	1.509				0.074	14-Jul-2003
Female	1.348	271	1.178					0.170	26-May-2003
Female	1.346	273	1.095					0.251	26-May-2003
Female	1.220	276	1.085					0.136	28-May-2003
Female	1.374	277	1.131					0.243	13-Jun-2003
Female	1.214	278	1.067					0.148	9-May-2003
Female	1.323	278	1.140					0.183	19-Jul-2003
Female	1.368	279	1.208					0.160	13-May-2003
Female	1.379	279	1.196					0.183	13-May-2003
Female	1.334	279	1.171					0.163	13-May-2003
Female	1.377	281	1.256					0.120	25-May-2003
Female	1.126	282	0.950					0.175	28-May-2003
Female	1.136	282	0.967					0.170	1-Jun-2003
Female	1.654	282	1.487					0.168	1-Jun-2003
Female	1.192	283	1.027					0.165	20-Jul-2003
Female	1.139	284	0.923					0.216	13-May-2003
Female	1.331	286	1.164					0.167	21-May-2003
Female	1.506	289	1.380					0.126	8-May-2003
Female	1.321	289	1.219					0.103	18-Jun-2003
Female	1.198	289	1.013					0.185	19-Jul-2003
Female	1.307	291	1.184					0.123	21-May-2003
Female	1.915	291	0.992	1.443	1.805			0.111	18-Jun-2003
Female	1.260	292	1.063					0.197	13-May-2003
Female	1.232	292	1.133					0.100	26-May-2003
Female	1.282	292	1.186					0.096	28-May-2003
Female	1.132	292	1.069					0.063	1-Jun-2003
Female	1.315	293	1.174					0.140	15-May-2003
Female	1.403	293	1.214					0.190	25-May-2003

Female	1.531	293	1.460			0.071	26-May-2003
Female	1.358	293	1.142			0.216	1-Jun-2003
Female	1.370	294	1.240			0.130	15-May-2003
Female	1.187	294	1.109			0.077	28-May-2003
Female	1.250	296	1.130			0.123	21-May-2003
Female	1.285	296	1.155			0.130	26-May-2003
Female	1.302	296	1.120			0.182	20-Jul-2003
Female	1.370	297	1.265			0.105	17-May-2003
Female	1.204	298	1.131			0.073	17-May-2003
Female	1.399	299	0.843	1.353		0.046	17-May-2003
Female	1.712	300	1.242	1.648		0.064	15-May-2003
Female	1.472	300	1.025	1.424		0.000	13-Jun-2003
Female	1.300	300	1.102			0.199	19-Jul-2003
Female	1.440	300	0.846	1.317		0.123	19-Jul-2003
Female	1.371	300	1.161			0.209	20-Jul-2003
Female	1.141	301	1.040			0.101	13-May-2003
Female	1.450	303	1.278			0.172	28-May-2003
Female	1.539	304	1.080	1.407		0.132	14-Jun-2003
Female	1.546	304	1.382			0.163	14-Jun-2003
Female	1.232	305	1.128			0.105	15-May-2003
Female	1.244	305	1.111			0.386	28-May-2003
Female	1.690	309	1.121	1.616		0.074	14-Jun-2003
Female	1.266	310	1.220			0.046	17-May-2003
Female	1.590	311	1.033	1.506		0.084	15-May-2003
Female	1.183	311	1.035			0.148	20-Jul-2003
Female	1.413	312	0.933	1.368		0.045	28-Apr-2003
Female	1.251	312	1.150			0.101	15-May-2003
Female	1.373	313	1.261			0.112	17-May-2003
Female	1.192	313	1.040			0.152	28-May-2003
Female	1.450	314	0.900	1.413		0.037	13-May-2003
Female	1.485	314	0.852	1.214	1.448	0.037	21-May-2003
Female	1.340	314	1.130			0.210	19-Jul-2003
Female	1.589	316	0.821	1.468		0.122	26-May-2003
Female	1.278	319	1.184			0.094	21-May-2003
Female	1.657	319	1.009	1.568		0.089	25-May-2003
Female	1.386	319	1.210			0.176	11-Jul-2003
Female	1.410	320	0.893	1.338		0.000	15-May-2003
Female	1.464	321	1.029	1.363		0.101	6-Jun-2003
Female	1.525	324	0.903	1.457		0.069	15-May-2003
Female	1.680	325	1.096	1.618		0.063	25-May-2003
Female	1.332	326	1.100			0.232	21-Jul-2003
Female	1.829	329	1.020	1.758		0.071	13-Jun-2003
Female	1.913	329	1.057	1.779		0.134	13-Jun-2003
Female	1.501	330	1.050	1.332		0.169	14-Jun-2003
Female	1.419	330	1.196			0.223	19-Jul-2003
Female	1.268	331	1.072			0.197	20-Jul-2003

Female	1.589	332	1.182	1.496			0.093	9-May-2003
Female	1.472	333	1.250				0.222	17-May-2003
Female	1.468	333	1.321				0.147	26-May-2003
Female	1.503	335	0.868	1.396			0.107	13-May-2003
Female	1.563	336	1.120	1.539			0.023	15-May-2003
Female	1.566	336	0.751	1.456			0.110	25-May-2003
Female	1.737	337	1.139	1.663			0.075	28-Apr-2003
Female	1.652	339	1.139	1.576			0.076	13-May-2003
Female	1.718	341	0.956	1.610			0.108	26-May-2003
Female	1.638	341	1.030	1.577			0.062	14-Jun-2003
Female	1.518	341	1.016	1.441			0.077	18-Jun-2003
Female	1.548	342	0.965	1.459			0.090	13-May-2003
Female	1.613	343	1.169	1.572			0.041	26-May-2003
Female	1.806	345	1.085	1.662			0.144	17-May-2003
Female	1.566	345	1.036	1.421			0.145	19-Jul-2003
Female	1.655	347	1.118	1.536			0.119	14-Jun-2003
Female	1.626	348	1.205	1.550			0.076	26-May-2003
Female	1.742	351	0.865	1.437	1.704		0.039	13-May-2003
Female	1.614	351	1.014	1.509			0.105	26-May-2003
Female	1.780	352	1.024	1.672			0.108	25-May-2003
Female	1.590	352	0.906	1.460			0.130	14-Jun-2003
Female	1.932	353	1.121	1.577	1.866		0.066	26-May-2003
Female	1.915	354	1.188	1.564	1.843		0.000	15-May-2003
Female	1.664	354	1.017	1.540			0.124	30-May-2003
Female	1.592	355	0.817	1.208	1.494		0.097	19-Jul-2003
Female	1.844	361	1.262	1.791			0.053	13-May-2003
Female	1.518	362	1.040	1.478			0.041	26-May-2003
Female	1.792	367	0.939	1.598			0.194	19-Jul-2003
Female	1.900	368	1.226	1.778			0.122	14-Jun-2003
Female	1.499	370	0.955	1.442			0.057	13-May-2003
Female	1.573	372	1.054	1.493			0.080	6-Jun-2003
Female	1.652	373	0.874	1.467			0.185	28-May-2003
Female	2.301	379	1.195	1.539	0.700	2.175	0.127	19-Jul-2003
Female	1.592	389	1.009	1.479			0.113	20-Jul-2003
Female	2.278	390	1.137	1.799	2.166		0.111	17-May-2003
Female	1.908	391	0.976	1.499	1.852		0.057	13-Jun-2003
Female	1.639	394	0.902	1.277	1.599		0.010	15-May-2003
Female	2.154	402	1.044	1.416	0.695	2.009	0.146	19-Jul-2003
Female	2.257	409	1.102	1.746	2.133		0.124	14-Jun-2003
Female	1.736	412	0.784	1.322	1.665		0.071	18-Jun-2003
Female	1.863	418	1.227	1.725			0.138	19-Jul-2003
Female	1.943	428	1.311	1.888			0.055	13-May-2003
Female	2.191	438	1.108	1.708	2.068		0.123	6-Jun-2003
Female	2.158	444	1.022	1.404	1.747	2.055	0.103	6-Jun-2003
Female	2.152	453	0.980	1.375	1.720	2.111	0.041	20-Jul-2003
Female	2.588	509	1.186	1.770	2.179	2.530	0.058	30-May-2003

Female	2.457	557	0.832	1.475	1.933	2.360	0.097	20-Jul-2003
Male	1.085	217	0.993				0.091	2-May-2003
Male	1.334	221	1.241				0.093	26-May-2003
Male	1.136	239	0.805				0.331	21-May-2003
Male	1.385	241	1.252				0.133	25-May-2003
Male	1.076	244	0.762				0.314	20-Jul-2003
Male	1.339	245	1.279				0.061	26-May-2003
Male	1.173	245	0.898				0.275	28-May-2003
Male	1.385	248	1.165				0.220	11-Jul-2003
Male	1.250	251	1.120				0.130	21-May-2003
Male	1.314	251	1.153				0.161	25-May-2003
Male	1.262	251	1.120				0.142	26-May-2003
Male	1.947	252	0.832	1.331	1.642	1.928	0.022	15-May-2003
Male	1.040	252	0.871				0.169	20-Jul-2003
Male	1.367	253	1.178				0.189	15-May-2003
Male	1.290	253	1.178				0.189	25-May-2003
Male	1.056	254	1.007				0.050	26-May-2003
Male	1.120	254	1.044				0.076	26-May-2003
Male	1.324	256	1.182				0.143	25-May-2003
Male	1.224	257	1.110				0.114	26-May-2003
Male	1.349	259	0.925				0.423	20-Jul-2003
Male	1.325	260	1.214				0.111	25-May-2003
Male	1.639	261	1.530				0.109	15-May-2003
Male	1.362	261	1.245				0.117	21-May-2003
Male	1.202	261	1.017				0.185	13-Jun-2003
Male	1.362	262	1.214				0.149	25-May-2003
Male	1.292	263	1.091				0.201	25-May-2003
Male	1.287	263	1.081				0.191	20-Jul-2003
Male	1.432	264	1.370				0.061	15-May-2003
Male	1.610	264	1.483				0.127	21-May-2003
Male	1.332	264	1.227				0.105	25-May-2003
Male	1.036	264	0.960				0.077	28-May-2003
Male	1.541	264	1.274				0.268	11-Jul-2003
Male	1.506	264	1.206				0.300	20-Jul-2003
Male	1.179	265	1.047				0.132	2-May-2003
Male	1.410	265	1.348				0.062	26-May-2003
Male	1.183	265	1.171				0.012	28-May-2003
Male	1.535	265	0.910	1.412			0.123	13-Jun-2003
Male	1.232	266	1.152				0.080	13-May-2003
Male	1.383	266	1.204				0.179	26-May-2003
Male	1.185	267	1.085				0.100	25-May-2003
Male	1.257	267	0.987				0.271	20-Jul-2003
Male	1.298	269	1.166				0.133	15-May-2003
Male	1.231	269	1.131				0.100	21-May-2003
Male	1.238	269	1.207				0.031	26-May-2003
Male	1.245	269	1.042				0.203	14-Jun-2003

Male	1.248	270	1.116		0.132	28-May-2003
Male	1.362	270	1.214		0.149	28-May-2003
Male	1.178	271	1.139		0.039	13-May-2003
Male	1.322	271	1.245		0.077	25-May-2003
Male	1.226	271	1.056		0.170	26-May-2003
Male	1.146	274	1.070		0.077	26-May-2003
Male	1.117	274	1.053		0.064	26-May-2003
Male	1.256	274	1.126		0.130	28-May-2003
Male	1.194	274	1.030		0.164	28-May-2003
Male	1.405	274	1.113		0.292	20-Jul-2003
Male	1.356	275	1.292		0.064	25-Apr-2003
Male	1.257	275	1.128		0.130	13-May-2003
Male	1.216	276	1.111		0.106	25-Apr-2003
Male	1.284	276	1.134		0.150	1-Jun-2003
Male	1.494	277	0.997	1.454	0.040	15-May-2003
Male	1.391	278	1.227		0.164	26-May-2003
Male	1.307	279	1.181		0.126	13-May-2003
Male	1.197	279	1.155		0.043	13-May-2003
Male	1.633	280	1.061	1.527	0.105	15-May-2003
Male	1.402	281	1.291		0.111	26-May-2003
Male	1.331	281	1.177		0.154	28-May-2003
Male	1.083	281	1.016		0.068	28-May-2003
Male	1.316	281	1.143		0.173	19-Jul-2003
Male	1.240	282	1.096		0.145	13-May-2003
Male	1.296	282	1.188		0.109	15-May-2003
Male	1.342	282	1.106		0.236	19-Jul-2003
Male	1.497	283	1.364		0.132	28-May-2003
Male	1.293	283	1.210		0.083	28-May-2003
Male	1.415	283	0.915	1.357	0.058	28-May-2003
Male	1.369	284	1.248		0.121	17-May-2003
Male	1.312	284	1.164		0.148	26-May-2003
Male	1.123	284	1.083		0.040	19-Jul-2003
Male	1.379	284	1.169		0.210	20-Jul-2003
Male	1.325	285	1.273		0.052	15-May-2003
Male	1.400	286	1.297		0.102	28-Apr-2003
Male	1.316	286	1.236		0.080	2-May-2003
Male	1.352	287	1.192		0.160	20-Jul-2003
Male	1.407	288	0.968	1.370	0.037	13-Jun-2003
Male	1.425	289	1.261		1.261	19-Jul-2003
Male	1.246	290	1.151		0.095	17-May-2003
Male	1.295	291	1.200		0.095	13-May-2003
Male	1.548	291	1.027	1.501	0.047	28-May-2003
Male	1.294	292	1.164		0.130	15-May-2003
Male	1.410	292	1.336		0.074	26-May-2003
Male	1.224	292	0.979		0.245	20-Jul-2003
Male	1.602	292	1.016	1.555	0.047	20-Jul-2003

Male	1.280	293	1.178			0.102	25-May-2003
Male	1.243	294	1.196			0.050	25-May-2003
Male	1.441	295	1.237			0.204	15-May-2003
Male	1.336	295	1.210			0.127	21-May-2003
Male	1.227	295	1.150			0.077	26-May-2003
Male	1.351	295	1.190			0.162	30-May-2003
Male	1.350	296	1.225			0.125	9-May-2003
Male	1.775	298	1.245	1.653		0.122	25-Apr-2003
Male	1.279	298	1.016			0.263	20-Jul-2003
Male	1.492	298	1.199			0.292	20-Jul-2003
Male	1.170	301	1.019			0.151	26-May-2003
Male	1.390	302	1.317			0.072	20-Jul-2003
Male	1.245	302	1.062			0.183	21-Jul-2003
Male	1.361	303	0.873	1.308		0.053	28-May-2003
Male	1.321	304	1.168			0.154	13-Jun-2003
Male	1.246	305	1.018			0.228	18-Jun-2003
Male	1.662	305	0.978	1.507		0.155	19-Jul-2003
Male	1.547	305	1.313			0.234	19-Jul-2003
Male	1.497	306	1.082	1.444		0.053	25-May-2003
Male	1.379	306	1.288			0.090	28-May-2003
Male	1.356	307	1.266			0.090	15-May-2003
Male	2.075	307	1.014	1.646	1.992	0.083	25-May-2003
Male	1.672	307	1.209	1.538		0.134	14-Jun-2003
Male	1.371	308	1.234			0.136	13-May-2003
Male	1.637	308	1.043	1.560		0.077	15-May-2003
Male	1.483	308	1.059	1.407		0.076	25-May-2003
Male	1.438	308	1.285			0.154	1-Jun-2003
Male	1.818	309	1.050	1.438	1.737	0.080	13-May-2003
Male	1.310	309	1.229			0.081	15-May-2003
Male	1.565	309	1.337			0.228	19-Jul-2003
Male	1.297	310	1.183			0.114	21-May-2003
Male	1.359	311	1.293			0.066	25-May-2003
Male	1.677	312	0.993	1.593		0.084	14-Jun-2003
Male	1.646	313	1.180	1.586		0.059	26-May-2003
Male	1.533	315	1.379			0.154	20-Jul-2003
Male	1.530	316	1.406			0.124	13-Jun-2003
Male	1.682	316	1.104	1.536		0.146	19-Jul-2003
Male	1.587	318	1.367			0.220	19-Jul-2003
Male	1.565	318	1.031			0.534	20-Jul-2003
Male	1.928	319	1.287	1.856		0.072	14-Jun-2003
Male	1.543	319	0.898	1.373		0.170	19-Jul-2003
Male	1.718	320	0.976	1.449	1.695	0.023	15-May-2003
Male	1.392	321	0.982	1.318		0.074	21-May-2003
Male	1.950	321	1.099	1.588	1.886	0.064	30-May-2003
Male	1.813	321	1.322	1.646		0.167	11-Jul-2003
Male	1.525	321	1.371			0.154	11-Jul-2003

Male	1.684	326	1.081	1.621				0.029	15-May-2003
Male	1.604	326	0.999	1.419				0.185	18-Jun-2003
Male	1.273	327	1.175					0.098	15-May-2003
Male	1.793	330	1.212	1.627				0.165	19-Jul-2003
Male	1.573	332	0.830	1.216	1.527			0.046	21-May-2003
Male	1.652	333	0.919	1.297	1.587			0.065	28-Apr-2003
Male	2.010	333	1.124	1.445	1.712	1.970		0.041	6-Jun-2003
Male	1.765	335	1.300	1.706				0.058	13-May-2003
Male	1.605	336	1.517					0.088	9-May-2003
Male	2.379	341	1.355	1.709	2.013	2.305		0.074	21-May-2003
Male	1.973	341	1.426	1.860				0.114	26-May-2003
Male	1.735	347	1.006	1.380	1.644			0.090	15-May-2003
Male	1.622	347	0.814	1.542				0.081	18-Jun-2003
Male	1.407	350	1.007	1.340				0.068	13-Jun-2003
Male	1.995	357	1.235	1.622	1.946			0.048	4-May-2003
Male	1.986	357	1.185	1.637	1.959			0.028	1-Jun-2003
Male	2.195	363	1.026	1.419	1.794	2.098		0.097	6-Jun-2003
Male	2.413	370	1.286	1.704	2.036	2.315		0.098	15-May-2003
Male	2.431	371	1.182	1.636	1.993	2.329		0.102	11-Jul-2003
Male	2.348	381	1.069	1.464	1.752	2.006	2.297	0.051	25-May-2003
Male	1.995	387	1.171	1.592	1.941			0.054	11-May-2003
Male	2.147	404	1.020	1.428	1.814	2.200		0.031	15-May-2003
Male	2.538	412	0.932	1.609	2.080	2.434		0.105	13-Jun-2003
Male	2.262	420	1.083	1.544	1.906	2.215		0.047	26-May-2003
Male	2.825	420	1.325	1.859	2.276	2.701		0.124	18-Jun-2003