Oyster Condition Assessment Protocol

Developed by

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GOAL: The goal of this document is to assess and monitor oyster reefs using standardized protocols across states, counties and water bodies. All protocols listed below are designed for intertidal reefs. Below is a step-by-step list of agreed-upon protocols to use on every monitored reef. Copies of data sheets are provided on last pages of this document.

 SITE SELECTION: Site selection for application of oyster condition assessment should be based on the program monitoring goals and objectives. For example, the Northern Coastal Basin (NCB) program places importance on selecting random reefs within surface water geographic areas of interest.

In the NCB, reefs were randomly selected for condition assessment in the GIS environment. The NCB was divided into ten geographic areas of interest (AOI) based on surface water connectivity. The number of reefs to be evaluated per AOI was based on the proportion of reefs in an area compared to reefs mapped in the entire NCB footprint and the funding available for sampling. The numbers of reefs sampled for condition from previous years were also taken into account. In this example, an AOI would have between three to ten reefs identified for evaluation. Additionally, back-up reefs were provided in the event that reefs could not be accessed or did not meet minimum size requirements.

Each selected reef has a unique identifying code called the Sample ID, beginning with the alpha code per AOI and a unique number. Each AOI has an ordered list of reefs. Per geographic area, an effort should be made to sample reefs from the selected list of reefs. For example, if you are to monitor five reefs, the optimal is to monitor reefs numbered 1-5. If a reef cannot be sampled due to inaccessibility or the reef does not meet the minimum size requirement (5 meters in length), then search within 200 m of the reef for another appropriate reef, beginning north and searching clockwise. If no reef can be sampled within the 200 meters, proceed to the next closest (by distance) numbered reef from the list of back-up reefs. This method is intended to optimize sampling efforts while in the field that same day. After a back-up reef is sampled, return to the original list of selected reefs.

Site planning/reconnaissance for reef access and back-up sampling areas is encouraged if difficult field conditions are anticipated. Google Earth and Bing's Bird's Eye are good tools for office reconnaissance. It may be useful to draw 200-m buffers around planned reefs and print aerial images for the field.

- 2) SITE INFORMATION: Record general field information on data sheet A (e.g., crew, date, time, waterbody/region, county, nearest city, nearest landmarks if any, boat ramp used, reef number from GIS points, latitude, longitude) on data sheet. Record reef type: patch (independent from shoreline), fringing (parallel and adjacent to shoreline) or string reef (perpendicular and adjacent to the shoreline) (Appendix 1).
- 3) **TRANSECT**: Lay out a 30-meter transect tape to include the observed, densest area of live oysters on reef. Even if the dense growth is less than 30 meters long, lay out the 30 meter tape to include both dense and less dense areas. Lay out the transect tape in a straight line or a line that curves with the shape of the reef. If the densest portion of the reef is longer than 30 meters, select any 30 meter length within. If the reef is not large enough to lay out a 30-m line, then deploy the line to extent possible and record length on data sheet A. The minimum length for a line should be 5 meters (see Site Selection above). Put down at least one quadrat on the side of the reef to be monitored immediately to ensure no one steps on that side of the transect line (Appendix 2).
- 4) MAP & PHOTOGRAPH: On data sheet A, hand-draw a map of reef perimeter, relative location of transect line, and quadrat layout on the data sheet. Include in the map an arrow for north (use compass if necessary), nearby shorelines, and any obvious landmarks (Appendix 3a). Use "Notes" section to provide additional information if warranted. Take photograph of site that includes the transect line and quadrats. Include reef number in corner of photograph and/or record camera number/image number on data sheet A (Appendix 3b).
- 5) REEF HEIGHT & SLOPE: Measure the maximum reef height from the highest point on reef to the lowest edge of reef (oyster perimeter), where live oysters/shell is less than 10% cover and the benthos is sediment or dominated by other taxa such as seagrass. See Appendix 4 for example of what constitutes 10% cover. To calculate reef height, there are two alternative methods. Data for either method should be included in data sheet A. *Method 1:* If a laser level is available, place laser level on tripod at the highest reef point and ensure it is level. Place stadia rod at lowest point with numbers facing the laser level. Record the height on stadia rod and subtract height of level on tripod (Appendix 5). *Method 2:* Using string and a string (line) level, run the string straight out from the highest point on reef a stadia rod or marked PVC pipe positioned at the reef perimeter. Place the string level on the line and adjust the vertical position of the string until level. Measure the height of the reef by measuring the distance from the string to the base of the pole (Appendix 6).

Use a metric transect tape to measure the distance from the level to the stadia rod/PVC pole so that slope can be calculated. Record data in cm on data sheet.

6) RANDOM NUMBERS: Come to reef prepared with a list of random whole numbers from 1 – 29. Each reef will have its own set of 29 numbers that are not in sequential order, but rather listed in the order in which they are generated. See Appendix 7 for a good random number

- generating tool. The first 5 numbers on the list will be the locations of the 5 monitoring quadrats on the reef if the transect is 30 m in length. If the transect is less than 30 m, use the first 5 numbers on the list that fall within the range of the transect length. Space is provided at bottom of data sheet A to list these values.
- 7) **PERCENT COVER**: Percent cover quadrats are 1 m X 1 m and each side has 10 lines made of nylon string, each spaced 0.1 m apart (8 string lines + 2 PVC edges = 10 lines), so that there are 100 intersecting points within each quadrat (Appendix 8). Deploy each quadrat so that the PVC touches the random number on transect tape in the lower left (or right) corner reading the tape from lowest to highest number. It is important to always be consistent on a reef using either the lower left or right. Use a pencil/rod to accurately record what is contacting each string intersection (point-intercept method) (Appendix 9). For each point-intercept, make hash marks on the data sheet (data sheet B) to record for the number of points contacting live oysters, oyster shell, sediment and other (list other). For the sediment category, the rod must extend down a minimum of 20 mm without contacting shell to be classified as sediment. The total number of points must = 100. In cases where there is a dead oyster and live oyster stacked so both are contacted by the point, record as a live oyster. A space for totaling up the tally marks is provided for each category on data sheet B.
- 8) **NUMBER OF CLUSTERS**: Within the 1 m X 1 m quadrat, record the total number of oyster clusters that contain a minimum of 5 adult (i.e. visible) live oysters. This will include clusters touching the edges of the quadrat. Record this value on data sheet B.
- 9) **CROWN CONCH & OTHER MOLLUSCKS**: Within the 1 m X 1 m quadrat, record the total number of crown conch shells visible on data sheet B. For each individual, record the shell length from tip to maximum extent of the aperture in mm using digital calipers (Appendix 10). Additionally record if shell contains a conch, hermit crab or appears to be empty on data sheet B. Collect the same data for any other large mollusks in quadrat (tulip shells, horse conch, whelks, etc.). Note also the number of egg cases for mollusks within each quadrat on data sheet B. Note that photographs of organisms and egg cases that will likely be encountered are included in Appendix 22.
- 10) OYSTER REEF THICKNESS (HIGHEST & AVERAGE): Within the 1 m X 1 m quadrat, deploy the 0.25 m X 0.25 m quadrat in the lower left corner. To determine the maximum thickness of the oysters/clusters above the benthos in mm, use a very thin, non-flexible rod and ruler/meter stick (Appendix 11). Place the rod adjacent to highest oyster/cluster to get the thickness. Remove rod while holding that point with your finger and record distance with ruler/meter stick (Appendix 12). Note that the highest point might not be live oyster. Record this value on data sheet B. Next, determine the average thickness of the oyster reef. To get the average thickness of oyster reef, repeat the measurement process described above, but instead of looking for the highest point, close your eyes and place the rod into the quadrat and measure the height whatever the rod contacts. If the rod does not contact oyster and instead contacts the benthos (mud, sand, sediment) record the height at "0". Repeat for a

total of 5 heights. These 5 numbers will later be averaged to obtain an average reef thickness. Record these 5 numbers on data sheet B.

11) **OYSTER BURIAL DEPTH**: Carefully remove all oysters/clusters from within the 0.25 m X 0.25 m quadrat. For any individuals/clusters touching the edges of quadrat, include only those individuals that lie directly under the quadrat edges. While removing all oysters, record whether individuals/clusters were buried into the sediment or not on data sheet B. For all that are extracted from sediment, record the depth in mm that the oysters/cluster (live or dead cluster, live single oyster) that was above the sediment line and buried beneath the sediment as evident from the mud/sediment line (Appendix 13). Excavate down to 15 cm and collect all oysters/clusters that have a top edge that is no more than 15 centimeters below the sediment line. Use abbreviations from the legend on data sheet B: DC = dead cluster; LC = live cluster; LO = live, single oyster; A = height above the sediment line; B = height below the sediment line.

Place all oysters/clusters in a basket with holes (Appendix 14). When done recording burial depths, rinse all individuals by swishing the basket in the water to get as much mud off the shells as possible (Appendix 15). An additional rinse with a battery-operated pump/hose system (Appendix 16) is recommended to remove mud in between and inside shells so that small oysters can be observed.

- 12) **OYSTER DENSITY & SHELL HEIGHT**: Count the number of all live oysters within/touching the edges of the quadrat. Reach into the basket without looking and individually select individuals and record shell height for each using calipers until 50 individuals have been measured. Measure from the umbo to the farthest growing edge in mm (Appendix 17). Count all additional live individuals that were not measured for shell length and record total number on data sheet B so we can determine the total number of live oysters in the quadrat (Tally of additional oysters section). Remember to look inside articulated shells of previously live oysters for juvenile oysters (spat) (Appendix 18). If spat are encountered within the first 50, make sure to include their shell lengths. It may be necessary to break apart clusters and rinse multiple times to ensure all oysters are counted.
- 13) **ASSOCIATED FAUNA**: In each 0.25 m x 0.25 m quadrat, record the total number of sessile invertebrates by taxa (barnacles, mussels, solitary and clonal ascidians, etc.) encountered in a tally for the quadrat on data sheet B (see Appendix 22 for photographs of commonly-encountered species). From the basket, record the tally number of motile invertebrates (porcelain crabs, quahog clams, oyster drills, and other species) to the lowest taxon level possible) on data sheet B. For mussels, clams and oyster drills, record each shell length in mm in space provided on data sheet B for the first ten individuals of each species. For porcelain crabs, record carapace width in mm for first 10 crabs removed from basket (Appendix 19). Make special note and collect known invasive species on oyster reefs: pink barnacles, green mussels, black charru mussel (no ribs). Place invasive mussels and barnacles in alcohol and label jar by location and submit to Dr. Walters for DNA extractions (Appendix 20).

14) **FINISH:** When complete, return all oysters live to original position on reef, including reburying oysters as appropriate (Appendix 21).

MATERIALS FOR THE FIELD:

 Data sheets on water resistant/proof paper
 Clipboards and metal clips/rubber bands to hold paper in place
 Pencils, extra lead or sharpener, Sharpies
 Camera and paper/white board to write reef number for photograph
 Compass
 Maps of reef locations
 GPS with reef locations pre-loaded, extra batteries
 Method 1: Laser level, tripod, 100-m transect tape, stadia rod, spare batteries for laser level
 Method 2: braided string, PVC pipe, string (line), string level, meter stick, 100-m transect tape
 30-m metric transect tape to use for transect line
 Sets of 5 random numbers from $1-29$ (plus spare number sets)
 1 X 1 m grid quadrats
 Pencils or rods to use to hit benthos for point-intercept method
 0.25 m x 0.25 m PVC quadrats
 Thin rods for reef thickness (e.g. purchased flags on wire stakes)
 Meter sticks/rulers in metric
 Bucket and brushes to clean clusters, if needed, to observe spat
 Plastic baskets to hold and rinse oysters
 Digital or dial calipers (if using dial calipers, train crew ahead of time in their use)
 Fresh water rinse and towels/paper towels to clean calipers
 Caliper batteries
 Hammer and chisel in case oysters need to be dislodged from cluster
 Saltwater fishing license to enable collection of invasive mussels, barnacles; any other required
permits
 Glass/plastic containers/Ziploc bags filled with ethyl/isopropyl alcohol
 Label tape for containers
 Work Gloves
 Boots/hard-soled sneakers
Hydrogen peroxide/first aid kit/sunscreen/extra water/extra hoat gas

Appendix 1





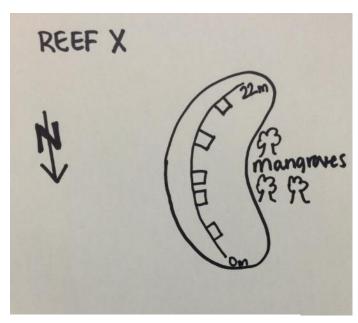




Laying transect line on reef



Five 1 X 1 meter square quadrats on one side of the transect tape



3A. Hand drawn map including arrow for north and any obvious landmarks



3B. Reef number in corner of photograph and/or record camera number/image number on data sheet A

6% to 10%: These are all 10% cover

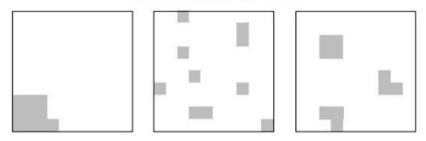
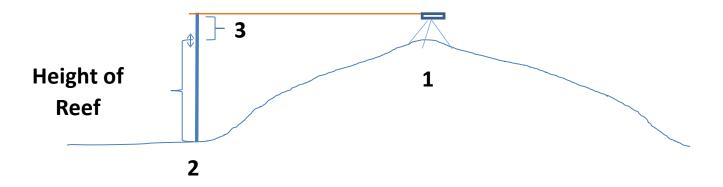


Diagram showing what 10% coverage looks like (Photo credit: Carlisle et al. 2006)



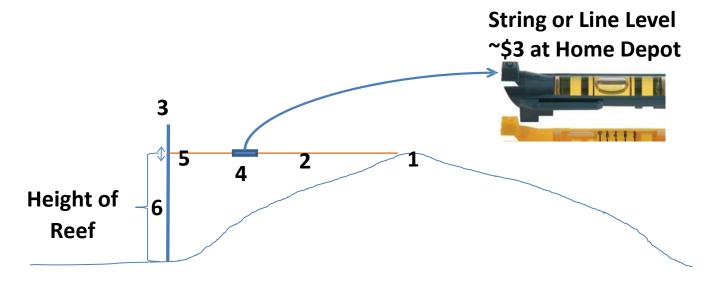
Measuring the height of the reef with laser level and pole

Diagram (below) showing laser level method for measuring reef height



- 1. Find the highest point of the reef and place tripod there with attached laser level
- 2. Place the stadia rod just off the edge of reef
- 3. Record the laser height on stadia rod and subtract height of level on tripod

Diagram showing string method for measuring reef height



- 1. Find the highest point of the reef
- 2. Run level line (string) to the perimeter
- 3. Place a pole just off the reef
- 4. Place the string level on the line
- 5. Adjust the vertical position of the string on the pole until level
- 6. Measure the pole base to string

Testimonials

Drawings

https://www.random.org/sequences/

Search RANDOM.ORG RANDOM.ORG Google™ Custom Search Search True Random Number Service Do you own an iOS or Android device? Check out our app! RANDOM. ORG Random Sequence Generator Do you own an iOS or Android device? Check out our app This form allows you to generate randomized sequences of integers. The randomness Random Sequence Generator purposes is better than the pseudo-random number algorithms typically used in comp Here is your sequence: Part 1: Sequence Boundaries 19 11 Smallest value 1 (limit -1,000,000,000) 10 Largest value 29 (limit +1,000,000,000) 28 15 Format in 1 column(s) 23 The length of the sequence (the largest minus the smallest value plus 1) can be no gre 16 29 Part 2: Go! 3 22 21 a little while to generate your sequence... 17 Get Sequence 24 Reset Form Switch to Advanced Mode ized sequence does not contain duplicates (the numbers are like raffle 12 Generator which generates the numbers independently of each other (like rolls of a die 13 Follow @RandomOrg 27 **8+1** 16k



1 m X 1 m point-intercept quadrat on transect.



Use a pencil/rod to accurately record what is contacting each string intersection (point-intercept method). In this case a flag attached to thin wire rod was used.



Record shell lengths of crown conch and other large mollusks from spire tip to maximum extent of the aperture in mm using digital calipers

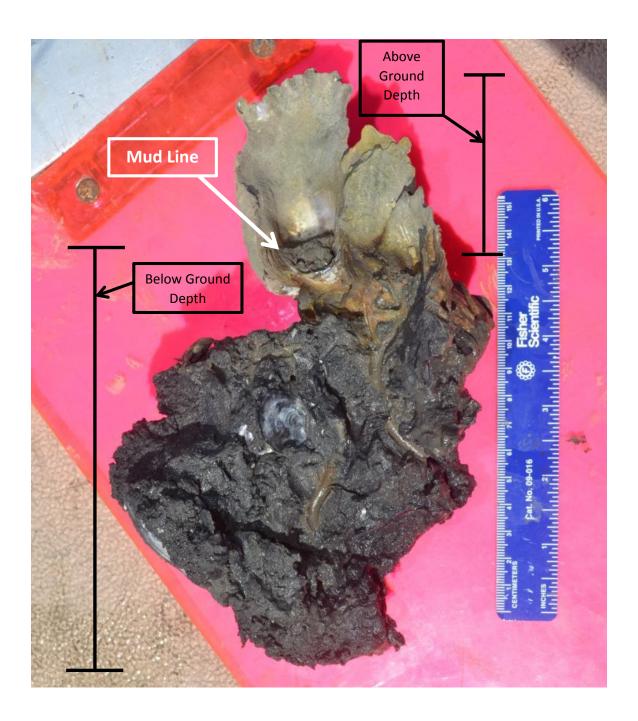


Using a very thin, non-flexible rod and ruler/meter stick to determine the maximum thickness of the oysters/clusters above the benthos in mm.





Remove rod while holding that point with your finger and record distance with ruler/meter stick

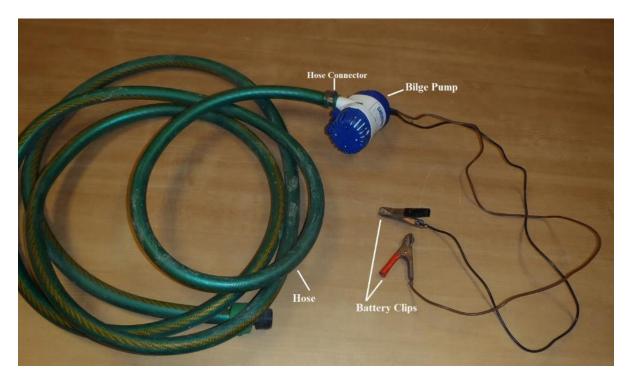




Collecting oysters from 0.25 m X 0.25 m quadrat into basket to be rinsed.



Rinsing the oysters in bucket to get the mud off.



A battery-operated pump/hose system used to remove mud in between and inside shells so that small oysters can be observed.





Obtaining shell length data for live oysters in oyster cluster with digital calipers.



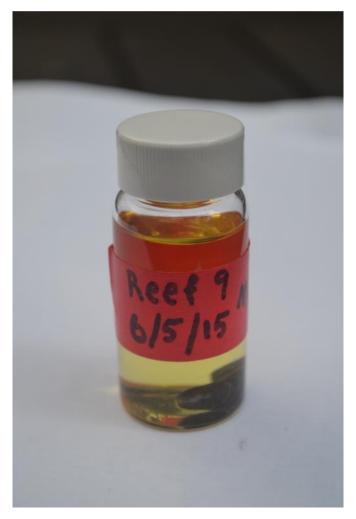


Oyster spat. Measure shell lengths of all visible spat with digital calipers.

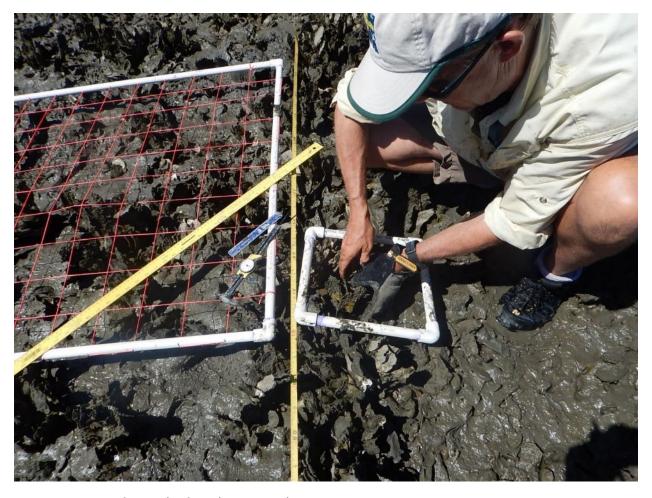


Carapace width of porcelain crabs. Measure with calipers.





Labels for alcohol-filled vials for collecting invasive mussels and barnacles should include: date, reef number and species name. These should be sent to Dr. Walters for genetic processing.



Returning oyster clusters back to their original positions.

Some Commonly Encountered Organisms on Florida's Oyster Reefs

(Photo credits: L. Walters unless specified)



Lined Hermit Crab (*Clibanarius vittatus*) in Crown Conch Shell (Photo Credit: Donna Campbell)



Florida Crown Conch: Melongena corona



Florida Horse Conch: *Triplofusus giganteus*



Crown conch egg case (Photo Credit: Paula Yespelkis)



Atlantic Oyster Drill: Urosalpinx cinera



Potentially non-native Green Porcelain Crab: Petrolisthes armatus

Revised 3/28/16



Non-native smooth, black mussel: Mytella charuanna



Non-native green mussel: *Perna viridis* (photo credit: Univ. of Florida)



Ribbed mussels in area include the scorched mussel *Brachidontes exustus* (shown here) and the Atlantic ribbed mussel *Geukensia demissa*. The latter grow to be significantly larger than the former. Individuals are lumped here as "ribbed mussels" as field samplers frequently encountered indivduals < 10 mm in length that were not distinguishable.



Common jingle shell: *Anomia simplex*



Mahogeny date mussel: *Lithophaga bisulcata*(photo credit: Smithsonian Institution in Fort Pierce)



Keyhole limpet: *Diodora* sp. (photo credit: 2FLA.com)





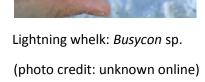
Southern quahog: *Mercenaria campechiensis* (photo credit: jaxshells.com)

Crested oyster: *Ostreola equestris* (photo credit: jaxshells.com)



Non-native pink baranacle: Megabalanus cocopoma

(photo credit: H. Sweat)





Red mangroves (*Rhizophora mangle*) growing on live oyster reefs.

Data Sheet A: Oyster Condition Index	Reef Number for Photograph
County: Date:	Camera #:
Nearest City: Start Time:	Image #:
Crew: End Time:	
Waterbody/ Region: Latitude:	
Decription of Area (Boat Ramp	
used, Nearest Landmark,	
Protected Area?):	
Type of Reef (circle one): Fringing Patch	String
Length of Transect Line (minimum = 5m):	
Reef Height (circle: cm/ft/inch)	
Height of laser/string on stadia rod/PVC:	
Height of level above tripod:	
Distance between highest point and stadia road/PVC:	
Map of Reef	
Include transect tape, location of quadrats, and N arrow	
	Notes:
Random numbers for quadrats:	

Data Sheet B: Oyster Conditioning Index	■ 0.25 X 0.25 m Quad	þ		
Reef Number: Date:	Reef Thickness	Random 1:	m 1:	Random 3:
Number on Transect Line:	Highest Point (mm):	Random 2:	m 2:	Random 4:
Observers:	■ Live Oyster			Random 5:
1 X 1 m Quad	Shell Lengths (mm)	nm)		
Point-Intercept (Tally): MUST = 100 total points	1 26	Tot	tal Number in Basket	Total Number in Basket (Tally) + Length for first 10
Live Oyster: Tally Total:	2 27	Whit	White/Striped Barnacles	Pink Barnacles *
	3 28			
	4 29			
	8 30	Black	Black, Ribbed Mussels	⇒ Length (mm)
Oyster Shell: Tally Total:	6 31			
	7 32			
	8 33	9	Green Mussels *	⇒ Length (mm)
	9 34			
Benthos (Mud, sand, etc): Tally Total:	10 35			
	11 36	Black	Black, Smooth Mussels *	⇒ Length (mm)
	12 37			
	13 38			
Other (describe): Tally Total:	14 39		Porcelain Crabs	⇒ Carapace Width (mm)
	15 40			
	16 41			
	17 42		Oyster Drills	⇒ Shell Length (mm)
Oyster Clusters (5+ visible oysters, Tally):	18 43			
	19 44			
	I 20 45	S	Solitary Ascidians	Clonal Ascidians
	21 46			
	22 47			
Mollusks	23 48		Other	Other
Organism Shell Length (mm) Content	24 49			
	I 25 50			
	Tally of additional Oysters:	ysters:	* = collect in alcohol	hol
	_	•		
	Burial Depth of Oysters/Clusters (mm)	ers/Clusters (mm)		
Tally Egg Cases By Species:				
Tally Red Mangroves:				
	_			
th Leg				
LC = live cluster B=below		_		