ASSESSMENT OF BIOCONTROL AGENT POPULATIONS AT 15 RELEASE SITES IN THE COLUMBIA RIVER ESTUARY

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1 Introduction

This project was initiated in 2006 with the goal of monitoring *Galerucella pusilla* beetles introduced as biocontrol agents for purple loosestrife (*Lythrum salicaria*) at 15 U. S. Army Corps of Engineers (USACE) release sites in the Lower Columbia River Estuary (LCRE). The concern over purple loosestrife in the Columbia River, and in many other ecosystems, stems from the fact that this plant can affect individual organisms, entire communities, and processes within aquatic ecosystems. An excellent review of documented impacts prior to 2001 can be found in Blossey et al. (2001). Subsequent studies have continued to find effects. Many have focused on birds and found that *Lythrum* negatively affects populations of the Black Tern, Least Bittern, Pied-billed Grebe, Virginia Rail, Sora, and the long-billed Marsh-wren (references in Blossey 2001). Other studies have focused on invertebrate species and communities. Recent studies in the Pacific Northwest document several aquatic-plant feeding moth species that were found at reference sites but not at sites dominated by loosestrife; including the noctuid moths *Archanara alameda, A. oblonga, A. subflava,* and *Bellura obliqua* which specialize on cattails (*Typha* spp.), rushes (*Juncus* spp.) and sedges (*Carex* spp.) (Schooler et al. in press). Preliminary analysis of ongoing studies in the LCRE examining the effects of *Lythrum* on invertebrate communities using fallout traps suggests that there tend to be more but smaller invertebrates in *Lythrum* dominated sites (Yeates and Garono, unpublished data). A number of studies have found that *Lythrum* reduces the diversity of wetland plant communities and particular species such as *Typha* spp. and the native *Lythrum alatum* (Blossey et al. 2001, Schooler et al. 2006). *Lythrum* can also effect ecosystem processes such as changing litter decomposition rates and timing, porewater chemistry (reduced phosphorus), and increased evapotranspiration rates (references in Blossey et al. 2001).

With this knowledge of impacts on communities and ecosystem processes, we continued to monitor biocontrol agent populations at USACE release sites in 2007. We also expanded the scope of the study to include characterizing ecological factors associated with observed patterns in the abundance and distribution of these control agents in the LCRE. In addition, with a second year of data we have the opportunity to see if initial changes are occurring in biocontrol agent and purple loosestrife abundance or distribution. To do this we need to make equitable comparisons. We used growing degree days to make some of these comparisons, which will be described in more detail in the methods. This report summarizes our 2007 study results and observations, as well as making some comparisons to 2006 findings and discussing needs and suggestions for work in future years.

1.1 Goals of 2007 Study

The goals of the 2007 study were to (1) determine if biocontrol agent populations were successfully establishing at 15 USACE release sites; (2) evaluate whether effective biocontrol was beginning to occur; and, (3) develop an understanding of which environmental factors may be related to goals 1 and 2. These goals are incorporated into five overall study elements. The five work elements are described in the next section.

We collected data in 2007 to address these questions. Data collected under Element I will allow us to address questions pertaining to the population trends of biocontrol agents and densities of *Lythrum*. Data collected under Elements II-V will allow us to address the affect of key environmental factors on the abundance and distribution of biocontrol agents. We expect to use data collected to develop spatially explicit habitat suitability models.

1.1.1 Is the biological control of *Lythrum* progressing favorably? Key Questions Pertaining to Populations of Biocontrol Agents and *Lythrum*

- Is loosestrife continuing to spread and increase in density in the absence of the biological control agents? Has the stem density of *Lythrum* changed at the 15 sites from 2006 to 2007?
- Are biocontrol agents persisting and creating self-perpetuating populations at the 15 sites?
- Are biocontrol agent populations increasing?
- Are biocontrol agents spreading from the point of release and how quickly are they moving? What factors are related to their movement?
- Is biocontrol agent damage to *Lythrum* increasing?
- Are biocontrol agents affecting *Lythrum* populations?
- Are there other measures of impact of biocontrol agents: plant height and seed production?

- Is the *Lythrum* being controlled by the beetles?
- Is the plant community moving toward the "desired" state (i.e. composition and diversity)?
- When and how will we consider biocontrol to be successful?
- Does biological control re-connect the food web? i.e. Do salmon use the biocontrol agents as an energy source?

1.1.2 What might be limiting the progress of biological control? Key Questions Pertaining to Environmental Factors Affecting Populations of Biocontrol Agents

- What factors are related to persistence, spread, and damage of the biological control agents? Hydrologic disturbance or distance upriver? Vegetation cover surrounding the sites? Elevation or topographic variation surrounding the release sites? Exposure to unfavorable conditions (i.e. islands vs. dike vs. mainland)?
- Are the release locations at the sites optimal for beetle establishment?
- Other issues: Are other invasive plants present at release sites such that selectively controlling one will lead to increasing abundance of others, therefore not increasing plant diversity?

1.2 Elements of 2007 Grant

1.2.1 Element I: Assessment of Biocontrol Populations and Leaf Damage at 15 Release Sites (April-September)

We visited each of the 15 USACE release sites on three occasions - April, July and August of 2007. Timing of visits was coordinated with favorable tides and the timing of life history stages in *Galerucella* populations in the Columbia River Estuary. Based on our previous experience, we expected to observe breeding adults in April, larvae and eggs in July, and maximum plant damage from all life stages in August at the end of the growing season. Making three trips to each site in 2007, rather than two trips as in 2006, increased the likelihood of detecting adults (April) and larvae (July) while providing for observations of end-of-season damage to *Lythrum* (August). Moreover, multiple trips allowed us to observe patterns in the phenology of both the host plants and the beetles.

We followed 2006 sampling protocols developed for this project. As before, we recorded the position of each $1m^2$ quadrat using submeter global positioning system (GPS) to measure the spread of the beetle populations away from the release point. Dispersal is measured by annually delineating and mapping population dispersion of the beetle population from the point of release. Late in the season, we measured the level of beetle damage to purple loosestrife in each of the $1m^2$ quadrats. The degree of resource utilization is determined by measuring the percent of total leaf area that is damaged by *Galerucella* beetle adults and larvae. Although chiefly concerned with *Galerucella pusilla*, the biocontrol agent species released by USACE, we also recorded the presence of feeding damage due to *Hylobius transversovittatus* adults or if seed capsules were infested by *Nanophyes marmoratus*. We also made a digital photographic record of each $1m^2$ quadrat. Additional photos of biological control agents, plant damage, plant densities, field equipment, general site, and habitat conditions were also taken.

1.2.2 Element II: Site Characterization

We believe that site elevation, along with tidal inundation patterns and surrounding vegetation, will influence the establishment and control effectiveness of biocontrol agent populations. We speculate that with tidal inundation, rising water may startle and dislodge beetles from host plants and that dislodged beetles would be lost from the release site thereby reducing biocontrol agent populations. We also expect beetles to take refuge on shrubby or woody vegetation and in hollow stems during high tides, both summer and winter. Therefore, we expect biocontrol agent establishment and control effectiveness to increase with access to higher elevation areas and woody vegetation.

We explored the response of the beetles to flooding in a series of laboratory and field experiments described later. To examine the influences of higher elevation and woody vegetation, we collected detailed information on elevation, vegetation, and inundation patterns at each release site. Relationships between these site variables and patterns in beetle distribution and damage were examined using a geographic information system (GIS). In addition to elevation and vegetation, we acknowledge that other factors may also come into play in the LCRE (e.g., wind, water currents, and temperature).

Elevation (Element II A & C): In 2007, we acquired and evaluated available LiDAR data from the Puget Sound LiDAR Consortium (http://pugetsoundlidar.ess.washington.edu/). LiDAR, which stands for Light Detection and Ranging, is a remote sensing technology that measures properties of scattered light to measure distance to and/or other information of a distant target. We used LiDAR data to describe the elevation in proximity to the 15 release sites. These data were compared to Real Time Kinematic (RTK) GPS data collected by our field teams in 2006.

Vegetation (Element II B): We acquired existing Color Infrared (CIR) Photographs and added them to our project's spatial database. Using our knowledge of the field sites and numerous ground truth points, we classified the photos to describe the distribution of shrub and forest cover in existing imagery.

Inundation (Element II D & E): In 2006, we compiled tidal data from existing tide tables and tidal prediction software. This approach was limited by the lack of site specific information for each of the 15 release sites. To add to our knowledge of tidal inundation patterns at these release sites, we deployed instruments that record water depth (pressure transducers). Pressure transducers remain on station for collection of fall/winter data to be analyzed at a future date. This information will be used to plan future releases. We recorded barometrically corrected water depth at 15 minute intervals for the summer of 2007 (April-September) at each release site and retrieved the logged data periodically throughout the summer. Tidal data will be used along with elevation data (Element II A & C) and vegetation (Element II B) to predict suitable release sites (Element IV).

1.2.3 Element III: Remotely Sensed Land Cover

This element was not funded in 2007. Instead, we evaluated several available imagery data sets including the CIR photographs described above, along with exploring options to collect new imagery during 2008.

1.2.4 Element IV: Model Optimal Release Sites (April-November)

We have observed that release of biocontrol agents does not always result in the successful establishment of their populations. Based, in part, on observations made during our work in the LCRE from 2000-present, we believe that refuge from tidal inundation may be an important factor in allowing populations to become established and sustain themselves (described in Section 1.2.2 Element II). With the availability of new LiDAR data, we started to gather data to construct a spatial model to evaluate the environmental factors that may lead to successful biocontrol agent population establishment.

We are using a GIS to evaluate these factors. The 15 USACE release sites are arranged along an elevation gradient. In 2007, we began to examine the relationship between biocontrol agent presence and elevation, inundation, and land cover. If patterns in biocontrol agents/ control can be explained by certain site characteristics, this information can be used to select future release sites where success may be more likely. Once optimal release sites are selected, releases can be made and we can examine the success of biocontrol agent populations over time. We plan to seek additional funds to make more releases in 2008 at these optimum sites.

1.2.5 Element V: Tolerance of *Galerucella pusilla* to inundation (April-November)

The purpose of this work element is to measure how individuals in each life stage of *G. pusilla* respond to rapidly rising water, referred to as flee response, and submersion. Our attempt in these studies was to mimic the tidal inundation beetles experience in the Columbia River. We conducted lab and field experiments to better understand how insect behavior (adult movement) and demography (survival of eggs, larvae, and adults) change under submersion and inundation (flee) treatments.

2 Methods

The methods we used during 2007 sampling are very similar to those used in 2006. Please see Garono et al. (2006) for details. We highlight and discuss any differences or additions to 2006 sampling below.

2.1 Insect and Plant Field Surveys

We collected field data during three periods in 2007: April 26-30, July 11-16, and August 7-13. We used the methods given in Garono et al. (2006) to assess beetle populations and plant damage with the following modification to damage assessment. If *Galerucella* damage was present within the quadrat, an overall damage estimate was made by averaging the characteristic feeding damage across all leaves and all plants within the quadrat (as in the previous year). In addition, we assessed the position of the damage on the *Lythrum* plant (bottom, middle, and top third of the plant) by visually dividing plants into three equal length portions and estimating the average damage in those portions of the plant.

We measured the number of both living and dead *Lythrum* stems, along with the height of five haphazardly selected live stems. The number of dead stems can be used, along with many other measures, to determine whether *Lythrum* populations at a site are increasing or decreasing from the previous year. We recognize that the number of dead stems may also be related to the amount of disturbance (e.g., sites with lots of tidal action may have few dead stems due to removal by wave action), and that dead stems may remain for more than one season. The number of stems (living and dead) and the height of the *Lythrum* plants are expected to decrease if biocontrol is effective.

2.2 Sites and Site Characterizations

2.2.1 Release Sites

The 15 release sites were selected by the USACE in 2005. Location and characteristics of each release site are given in Garono et al. (2006) appendices A-0. At each site we sampled 50 $1m^2$ quadrats during each sampling event. Transects extended 20m to 100m from the release stake, depending on terrain. Since sites were sampled during low tide, we often extended transects onto mud and sand flats. By sampling these unvegetated areas over time, we expect to be able to detect the spread of *Lythrum* , if it encroaches into these areas.

2.2.2 Tidal Gauges

To monitor tidal water levels at each release site we deployed ventless HOBO U20 Water Level Loggers (U20-001-01-Ti). The water level loggers record absolute pressure with a ceramic pressure sensor housed in a titanium container at water depths ranging from 0-30ft. Using software supplied with the gauges, we converted absolute pressure values to water depth. To account for differences in pressure due to changing barometric pressure, we deployed reference water level loggers that were suspended above the release stake so that they never went under water, as recommended by the manufacturer.

To maintain temperature and protect from damage, Hobo loggers were enclosed in a white PVC tube capped at the bottom end. PVC tubes were installed at each site so that they extended ~5 cm above the substrate. Hobo loggers were fastened in the PVC tube with wire so that the top of the logger hung 5 cm below the top of the PVC tube (i.e., at the level of the substrate). The top of the PVC tube was covered with screen to keep out debris. Tubes were secured to a nearby stake or tree with wire and a bolt.

Generally, we deployed a reference barometric gauge within 5-6 km of the USACE release sites. A total of four barometric reference loggers were deployed in addition to the loggers at each of the 15 release sites (Figure 1). Pressure was measured and recorded every 15min. Data were periodically retrieved in the field using a HOBO Waterproof Shuttle (U-DTW-1) with a U20 Coupler2-B that communicates with an optical interface allowing for fast data transfer. After data transfers, loggers were immediately re-installed at each site. Loggers were deployed in early April and set to begin logging on April 12, 2007.

In the laboratory, tidal gauge data was transferred from the HOBO Shuttle to a computer. Barometric corrections were made as the data were downloaded using the Hoboware Pro (ver 2.3.1) software purchased with the data loggers. Hoboware Pro software automatically converts absolute pressure measurements into water depth. For this study we used the following software settings: fluid density was derived from temperature, assuming freshwater, and the barometric compensation was derived from a dedicated atmospheric logger, as described above. Data files were saved as a comma separated value file and summaries were created using Excel. We also calculated the maximum vertical rate of water change from the logged data using a Python software program developed specifically for this project.

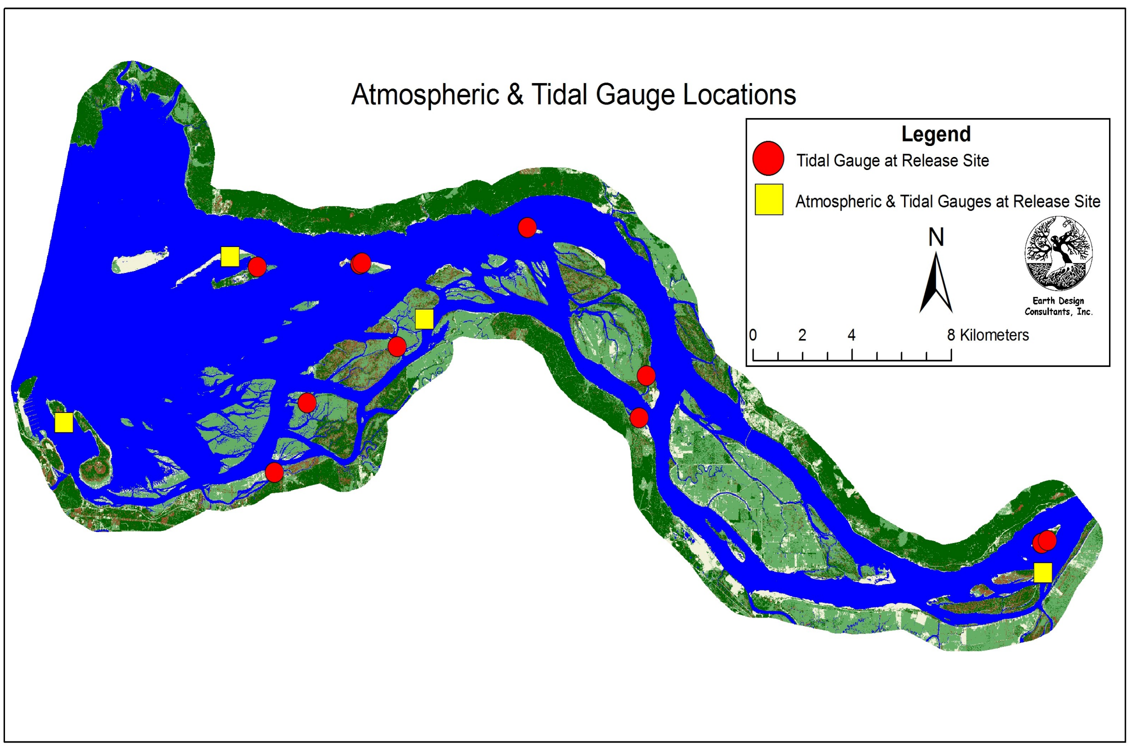


Figure 1. The study area in the Columbia River Estuary. Shown are the locations of (red) tidal gauges only and (yellow) tidal and barometric correction gauges. Land cover image was created by classifying CIR photos the floodplain boundary was derived from Landsat 7 ETM+. See text for details.

2.2.3 Water Quality

We used Eureka Environmental Manta multi-parameter water quality probes to measure depth, dissolved oxygen concentration, pH, salinity, and temperature at several locations within the study area. We placed probes near Karlson Island (N 46.190, W -123.612) in the west and Wallace Island (N 46.147, W -123.237) in the east (Figure 2). The probes were calibrated prior to deployment using standard solutions, set with fresh batteries, and set to log every 30 minutes with a two minute warm-up prior to logging. The probes were deployed for a continuous logging period during the following intervals: April 26-30, July 9-16, and August 8-28. Between each logging period, the probes were taken back to the lab, data was retrieved and transferred to a lab computer, and probes were cleaned.

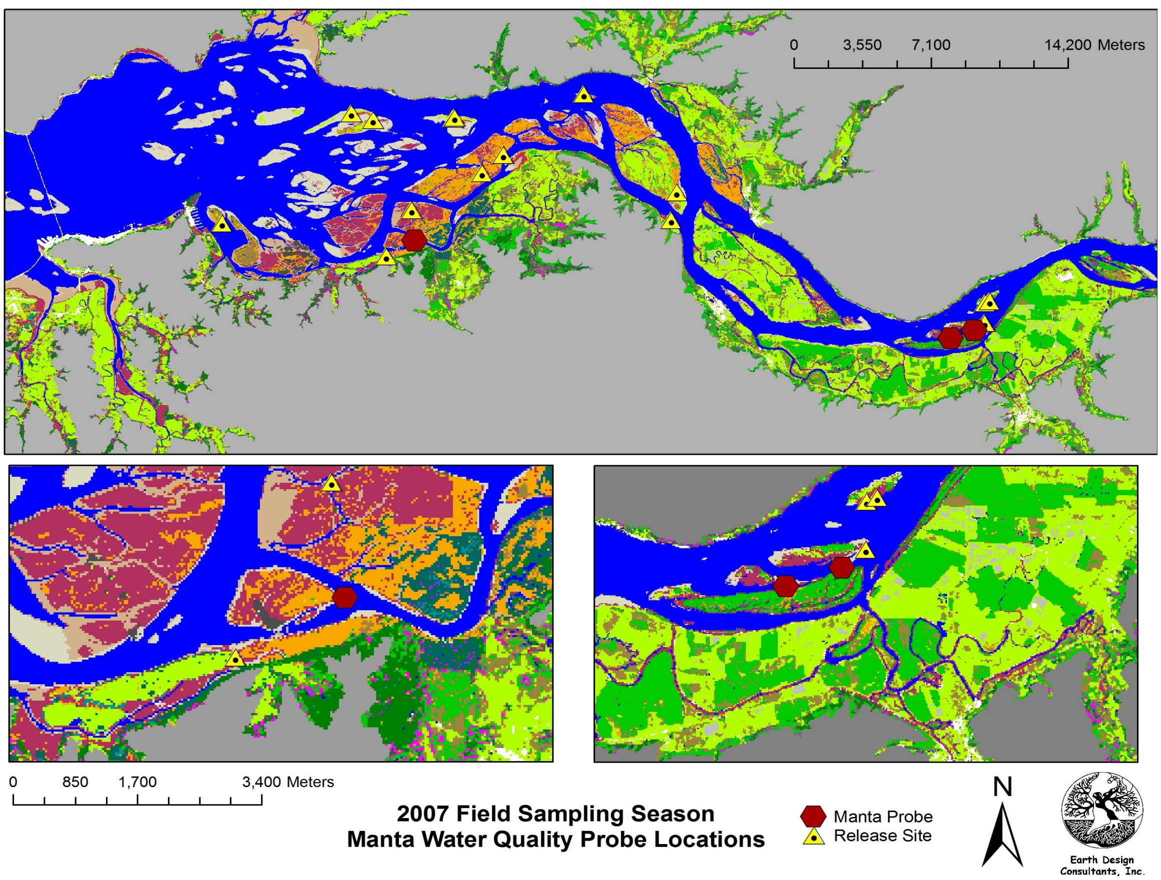


Figure 2. Locations of Manta recording water quality probes (red dots) and USACE release sites (yellow). Background image is Landsat 7 ETM+ imagery. See text for details.

2.2.4 Land Cover

We acquired digital Color Infrared (CIR) photos from 14 August 2003 for the study area from the USACE. In the three cases where photos were not available we supplemented the 2003 photos with photographs from May 20, 2001. We delineated the entire study area using the edge of the main channel depicted in the Landsat 7 ETM+ data (Figure 1: Garono et al. 2003a). We ran a proximity analysis on the resulting shapefile, using GIS, to add a 1000 m river buffer area beyond the edge of the main channel. Due to the size of the resulting image, multiple areas of interest (AOI) were made by delineating areas on the digital photos and selecting the best quality portions of each photo. Whenever possible we placed cutlines for the photo mosaic between islands. Photos subsets were then extracted for further processing

We classified CIR photos from the delineated AOI. We tested our initial classification procedure using one photo from May 20, 2001 (No. 1-948). From this initial test, we determined that mapping the study area for the desired vegetation classes would be possible but considerable hand editing would be needed to separate vegetation cover classes.

In addition to information from the CIR digital photographs, we added LiDAR digital elevation data (described above) from the Puget Sound LiDAR Consortium (http:// pugetsoundlidar. ess.washington.edu) to our land cover classification. The LiDAR images were captured between January 10 and February 20, 2005. We found the addition of LiDAR data, specifically the difference between Top Surface and Bare Earth values for vegetation height, to be very useful in separating cover classes. Bare Earth DEM values are the elevation at ground level (extrapolated) and Top Surface values are the original elevation from the LiDAR which come from the top of vegetation or any other object above the ground. LiDAR DEMs covering the study area were imported to ESRI GRID format and steps were taken to ensure that all DEMs were in the same projection with the same units because a number of images were found to have the wrong State Plane zone number. We then created mosaics of both the Bare Earth and Top Surface DEMs to cover the study area. The difference between the Bare Earth DEM and Top Surface DEM was modeled (Top Surface DEM minus Bare Earth DEM) to provide a measurement of vegetation height. This vegetation height was then used in the land cover classification to provide separation between the vegetation cover classes – i.e., herbaceous, scrub/shrub, and trees.

For the classification we performed the following steps on each aerial CIR photo using ERDAS Imagine Software. First, an unsupervised classification procedure (ISODATA), was run to produce 100 spectral signatures. We then ran another procedure (Maxclas) which categorized the photo according to the ISODATA spectral signatures. The resulting 100 spectral class image was then grouped into land cover classes by visual inspection of the ISODATA results using the grouping tool. Each spectral class was coded to a cover class and saved in an Excel spreadsheet. The cover classes were: 1 = water, 2 = bare, and 3 = vegetation. This was repeated for each photo and edits were made where needed. Cover class 3 (vegetation) was then modeled with the DEM difference image to produce more detailed vegetation classes. Vegetation less than 1m in height was classified as herbaceous, 1 – 3 m was classified as scrub/shrub, and greater than 3 m was classified as trees. This produced five cover classes of 1=water, 2=bare, 3=herbaceous, 4=shrub/scrub, and 5=trees. Land cover for the individual photos was then compared to the original photos and manual edits were made where necessary. Several issues are worth noting. The photographs were taken in 2001/03 and the LiDAR data were collected in 2005. For example, we noted large-scale changes in poplar tree plantations. Many trees appeared to have been cut after the August 2003 photo date and prior to the LiDAR acquisition. These apparent changes were left as is and not edited to conform to the photos, which are older than the LiDAR images. In addition, during 2006/07 field teams noticed vegetated areas in the CIR photos which were shown as bare substrate. Nevertheless, we produced a land cover mosaic for the entire study area from the best available data by merging the individual classified images. The mosaic was also reprojected to the OR Lambert Conformal Conic projection (Figure 1).

We then assessed the accuracy of our land cover classification. We used 171 ground truth points collected by field teams. At each point, we recorded the exact location using submeter GPS and the major land cover class. Ground truth points were distributed throughout the study area and selected to give a good representation of the land cover classes. Seven of the points were found to lie outside the actual study area and were removed from the assessment. Twenty-seven of the points were found to be underwater due to tide height during the time the aerial photos were taken and also removed from the assessment. From the remaining available ground truth points, accuracy of the land cover classification was estimated to be approximately 72.6% overall.

2.3 Response and Tolerance to Submergence

To test the response and tolerance of biocontrol agents to tidal submersion. We subjected various beetle life stages to different lengths of time under water. We collected *G. pusilla* larvae and egg masses from Horseshoe Lake near St. Paul, Oregon and sorted the larvae by instar stage (three stages) into separate containers. Five individuals of each instar were randomly assigned to a treatment of 0h, 1h, 2h, 4h, 8h, 16h, or 32h and placed into a 70mL vial capped with No-See-Um® netting. In the case of eggs, we considered each egg mass as one experimental unit and placed leaf tissue containing five masses into each treatment vial. We bound the individual vials of each life stage together with a rubber band and pre-filled the vials with de-chlorinated tap water (equilibrated to room temperature) approximately ¾ full. Each bundle was topped off with water, capped, and placed in de-chlorinated tap water bath maintained between 15.0-15.6 ºC. We tapped the netting to dislodge any residual air bubbles and allowed the vials to rest undisturbed the duration of the treatment. The entire experiment was replicated four times.

At the end of each trial we lifted the vials from the water bath. Working with one life stage at a time, we drained the vial and placed the organisms onto filter paper in a pre-labeled 8oz. polypropylene container. Filters were allowed to dry at room temperature. Larvae showing movement were transferred to a 32oz. polypropylene container containing *Lythrum* plant material. We drew a circle around each motionless larvae remaining on the filter paper and transferred them to the larger container if they moved beyond the circle within 24 hours. If they failed to move, we considered them to be dead. Leaves containing egg masses were placed in an 8oz. container with a moist cotton ball. We counted those masses containing eggs that hatched within seven days as having survived, those that did not as failing to survive.

To examine beetle response to rapidly rising water, referred to as "flee response," we conducted both laboratory and field experiments. We conducted the submersion experiment with *G. pusilla* adults in the Columbia River at the John Day River Boat Ramp outside of Astoria, OR, for periods reflective of natural tidal cycle durations in the estuary. We recorded beetles that resumed movement within 24 hours of removal from the water as surviving, those that did not as not surviving. Adult beetles were collected from the Salem, OR airport site two days prior to the experiment. Beetles were kept cool and supplied with purple loosestrife plants during transport. We placed five beetles at random into 70ml vials. The vials were covered with No-See-Um® which was held in place by the ring-lid. Each vial was assigned to a treatment time of 1h, 2h, 4h, 8h, 18h, 32h, and Control for a total of 28 vials and 140 individuals. Twenty to twenty-one individuals total were assigned to each treatment. Four replicates were run for each treatment time and control. During the slack tide we wedged the racks between rocks of rip-rap along the elevation gradient of the shoreline at the John Day County Park in Astoria, OR. As the tide rose the racks became fully submerged. The site was selected for its considerable gradient and ample shade provided to the beetles by *Salix* and *Pseudotsuga* trees. At the termination of each treatment period, we pulled the appropriate rack from the water, drained each vial, and placed the beetles on filter paper in 8oz polypropylene containers with air holes in the lids and provided with purple loosestrife plant material. These containers were kept in the shade in a cooler while at the site until placed at room temperature. We recorded whether each beetle resumed movement within a 24h period.

In the laboratory, we filled a 14 gal. Rubbermaid® tote with de-chlorinated tap water and maintained the water temperature at 15C° with ice. By removing one end wall, adding glass to the top, and standing it on end we converted a 20 in X 10 in X 12 in tote to a 12 in X 10 in X 20 in aquarium. We wedged a purple loosestrife plant stem (upon which to place *G. pusilla* larvae) into a small necked glass bottle and placed it in the aquarium. We placed three larvae at a time on the upper surface of the highest leaf and allowed them to rest undisturbed for 15 minutes. At the end of the 15 minutes we gravity fed the water from the tote to the aquarium through a ½ in (I.D) vinyl tube. We controlled the rate of flow by constricting the tube with an adjustable clamp, the final rate being approximately 2 in min^{-1} (0.05 m min^{-1}). While the loosestrife plant was approximately 14 in tall, the aquarium was allowed to fill to a depth of approximately 16-17 in. Throughout the filling time we recorded any change in position on the plant, activity and location of each beetle relative to the surface of the water. We used a formula in Excel that recorded the time the change occurred and terminated the run when the water reached 16-17 in. This experiment was performed on 21 individuals. Since actual rates of vertical water velocity increase were not available from our tidal gauges, we decided to postpone additional experimentation until a later time.

Finally, we observed the response of adult beetles to incoming tides at the Eureka Bar Upstream release site during April 2007. Beetles were placed in paper ketchup cups around base of *Lythrum* plants and their responses to incoming tide were observed. A total of eight adult *G. pusilla* beetles were observed for approximately 140 minutes. Notes were made about the beetle's ability to float on the water surface, to extricate themselves from the water onto herbaceous and woody plant material, and to remain submerged.

2.4 Geographic Information System (GIS) and Statistical Methods

Spatial data were stored and processed using ARGIS ver. 9.2 and Arcview 3.3 as described in Garono et al. (2006). We used JMP 5.1 for statistical summaries and comparison. Microsoft Excel was often used for simpler summaries and calculations, along with general database storage.

2.5 Growing Degree Day Calculation

Growing degree days are often used by farmers and gardeners to predict when plants will reach maturity or insects of interests will emerge. For this project, we used growing degree days as a tool to make equitable comparisons of data collected in different years and on different dates. Our field sampling did not occur on the exact same dates in 2006 and 2007. Even if it had, weather varies from year to year and affects both plant and insect growth. Growing degree days is a way to quantify this difference.

Growing degree days can be calculated by selecting a base temperature or lower temperature threshold at which organism growth or activity is affected. We chose 10ºC as a base temperature because it is a common lower threshold for many crops, as well as the value selected by Katovich et al. (2003) studying *Lythrum* emergence. Growing degree days are calculated by subtracting the lower threshold temperature (10ºC) from a daily temperature. The exact value used for daily temperature can vary greatly depending on what method is used. We chose a simple average, which divides the sum of the daily maximum and minimum temperature by two. Each day of the year, the growing degree days are added to the previous total, therefore the cumulative value increases throughout the calendar year. Negative values are ignored and not subtracted from the cumulative total. We calculated the growing degree days for the release sites using a degree day calculator for the Pacific Northwest, hosted by the Integrated Plant Protection Center at Oregon State University, located online at http://pnwpest.org/cgi-bin/ddmodel.pl?spp=aaa. We chose the following settings: 10ºC lower threshold, no upper threshold, simple average/growing degree days for calculation method, and Astoria Regional Airport KAST (46.1567, -123.8822) for weather data. Degree days were calculated for both 2006 and 2007 automatically by the online calculator. We located the cumulative growing degree days for all days of field sampling and used these values in our comparisons.

3 Results and Discussion

3.1 Plant and Insect Surveys

3.1.1 Site Surveys in 2006/07

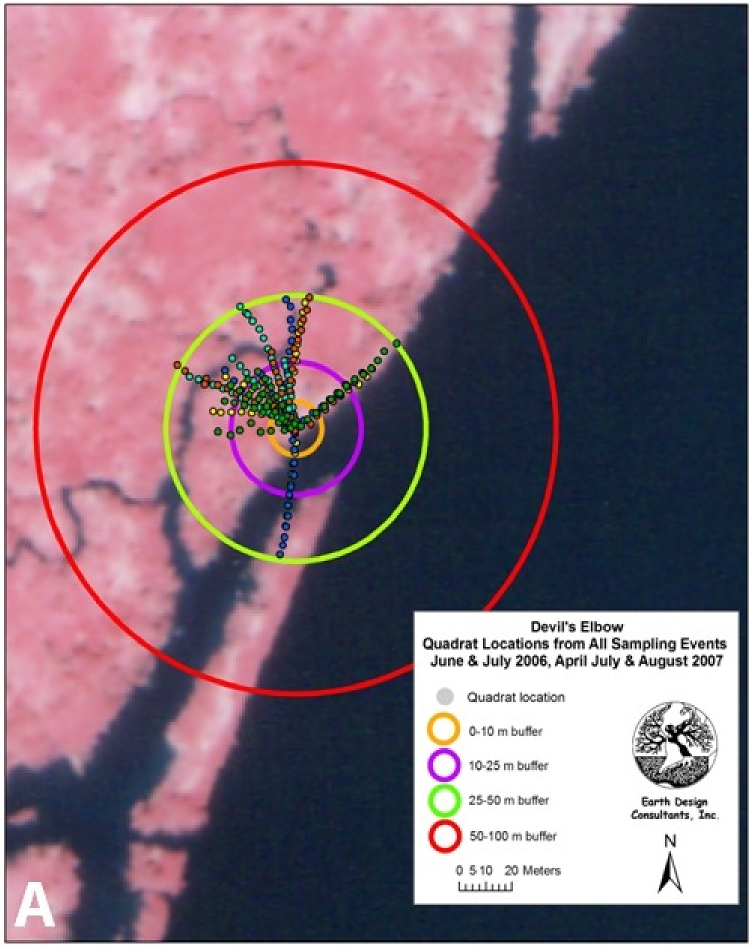
In 2005, the USACE released 1,000 *Galerucella pusilla* individuals at one point (marked by a metal stake) within each of 15 release sites. Sites vary in position along the river, elevation, land cover, sediment characteristics, exposure to wind and wave disturbance, in addition to other variables. This created the opportunity for our team to conduct a 'natural experiment' to determine which, if any, of these environmental factors are related to the abundance and distribution of biocontrol agent populations at these 15 sites. We recognized that hypotheses generated by this work will need to be tested under more rigorous (i.e., experimental manipulation) conditions.

We measured quadrats located along haphazardly placed transects. Each quadrat surveyed represents specific environmental conditions under which we are measuring biocontrol agent populations and plant damage. This method attempts to avoid surveying the same areas within a site during consecutive sampling events. Figures 3 a-n show the position of each quadrat surveyed at each site for 2006/ 07. We have intentionally measured areas within shrubby and forested areas, and tidal flats to try to determine (1) which environmental conditions, if any, that are not favorable to *Lythrum* and (2) document the year-to-year change in the distribution of *Lythrum*.

3.1.2 Abundance and Distribution of *Lythrum*

Lythrum is present at all release sites but its abundance and distribution differs dramatically between sites and throughout the estuary (Figures 4a-n). The Eureka Bar Downstream site has the greatest density of *Lythrum* based on the average number of live stems sampled per quadrat (mean 24 living stems, s.d. 19). The density of *Lythrum* stems is

Figure 3 a-n. Location of all 1m² quadrats sampled in 2006 and 2007 for each site. Colored circles represent 10, 25 and 50m buffered areas from release stake. Quadrats are colored by sampling event. Background images are CIR photographs. See text for details.


Devil's Elbow
Quadrat Locations from All Sampling Events
June & July 2006, April July & August 2007
Quadrat location
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
0 5 10 20 Meters
Earth Design Consultants, Inc.
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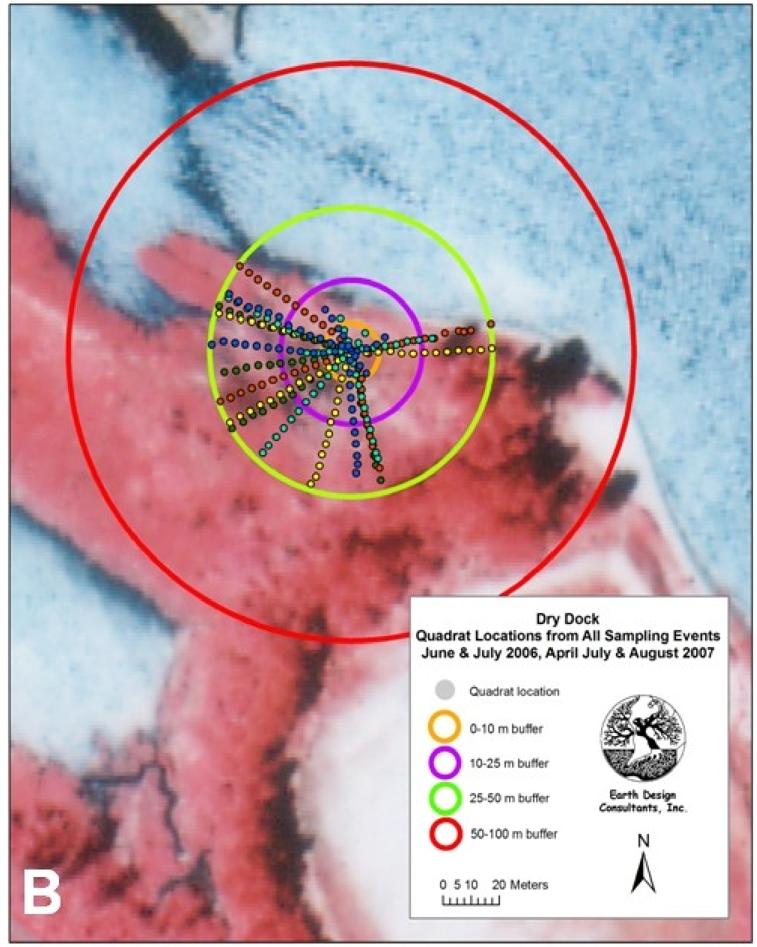
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Earth Design Consultants, Inc.
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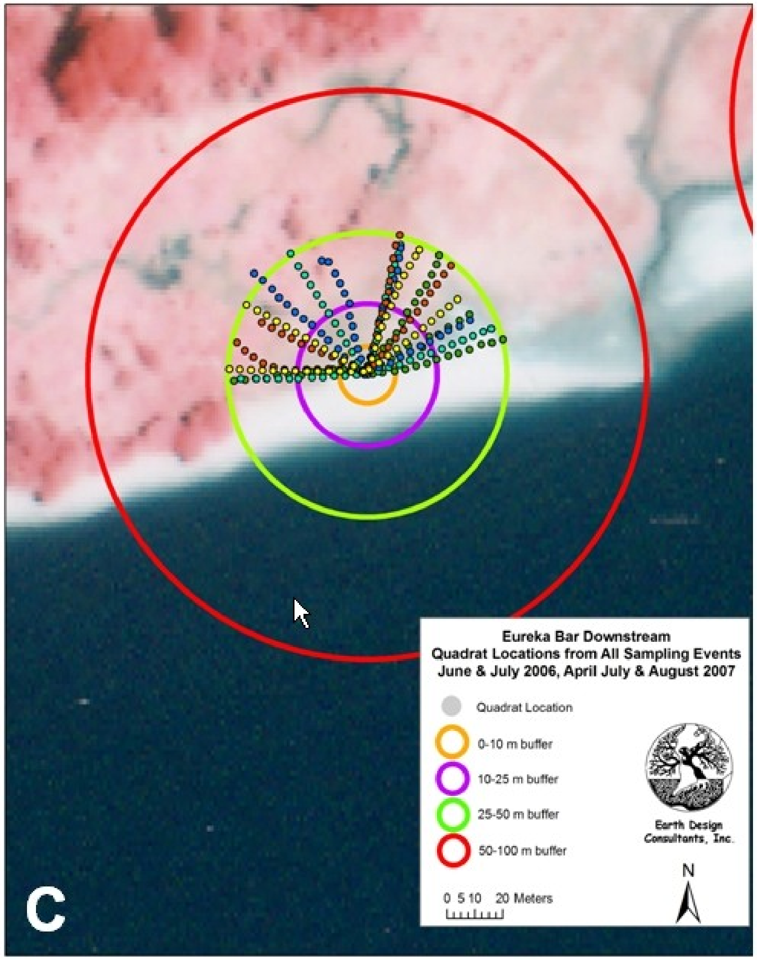
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Quadrat Locations from All Sampling Events
June & July 2006, April July & August 2007
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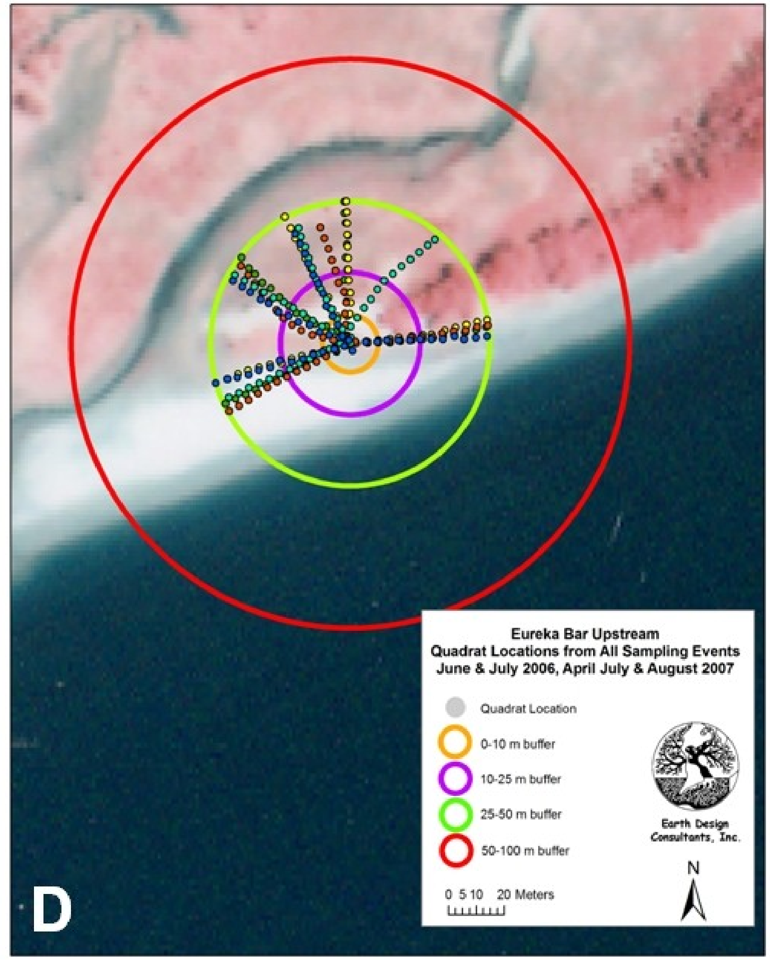
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June & July 2006, April July & August 2007
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Earth Design Consultants, Inc.
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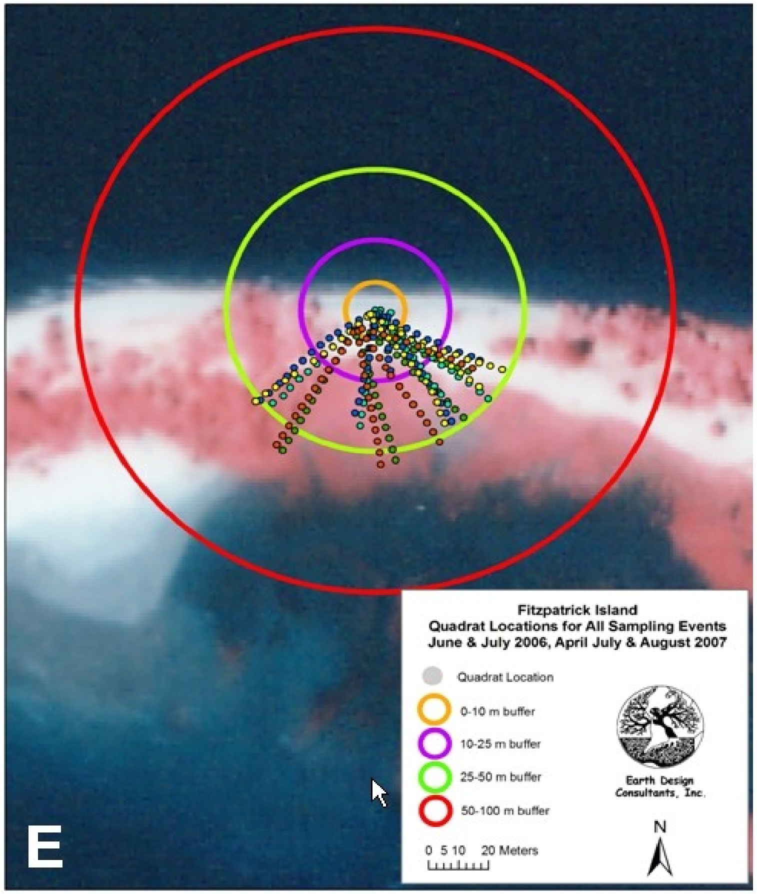
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Quadrat Locations for All Sampling Events
June & July 2006, April July & August 2007
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Earth Design Consultants, Inc.
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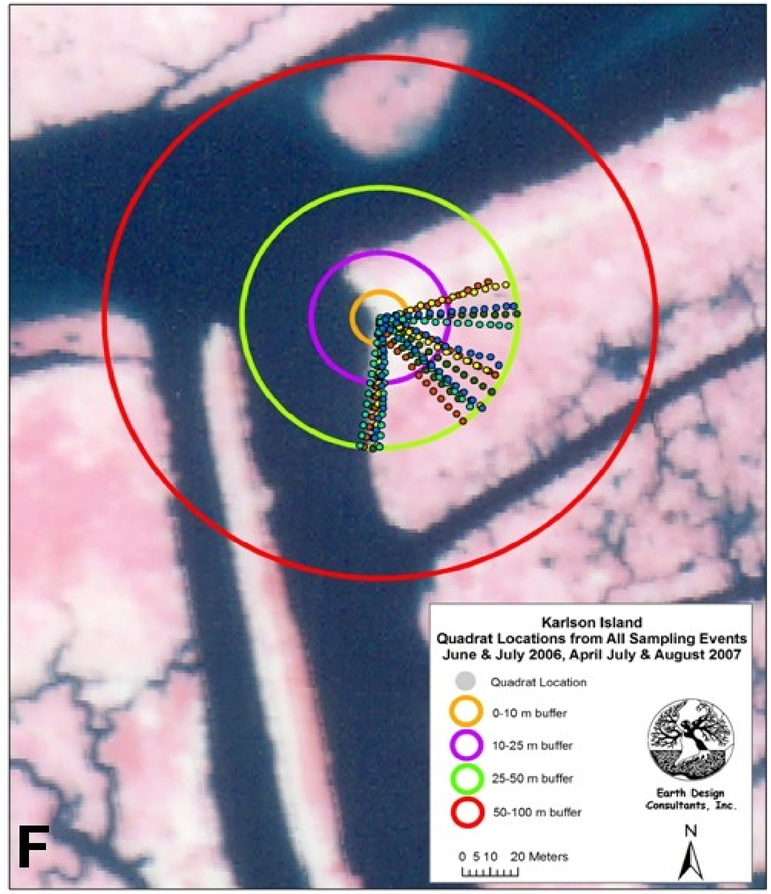
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Quadrat Locations from All Sampling Events
June & July 2006, April July & August 2007
Quadrat Location
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Earth Design Consultants, Inc.
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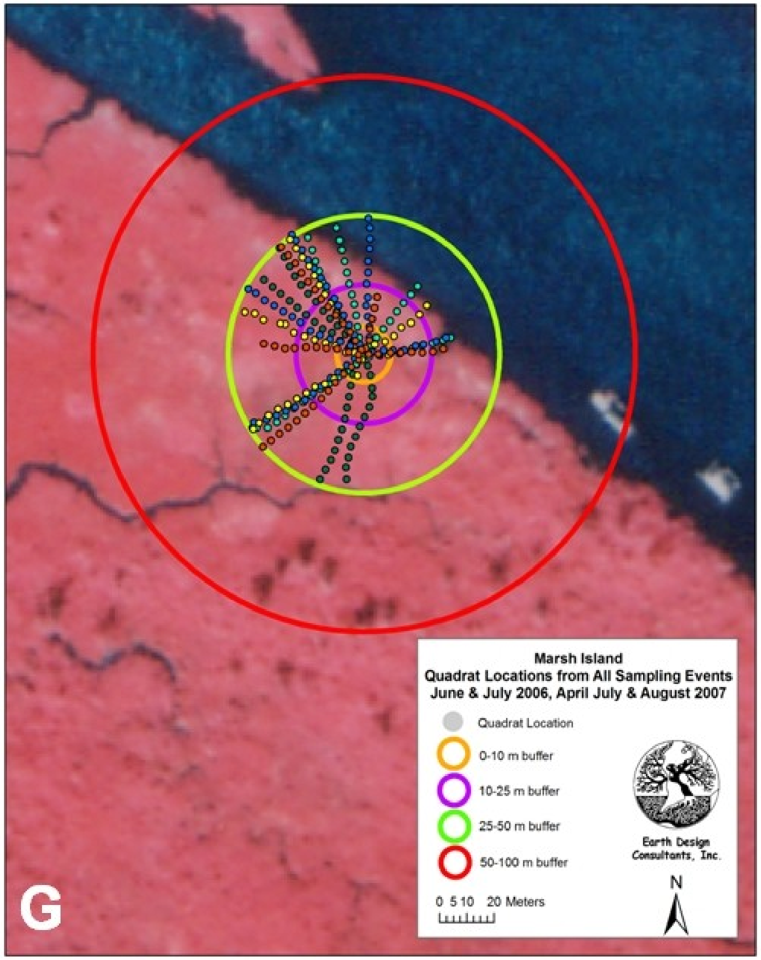
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Quadrat Locations from All Sampling Events
June & July 2006, April July & August 2007
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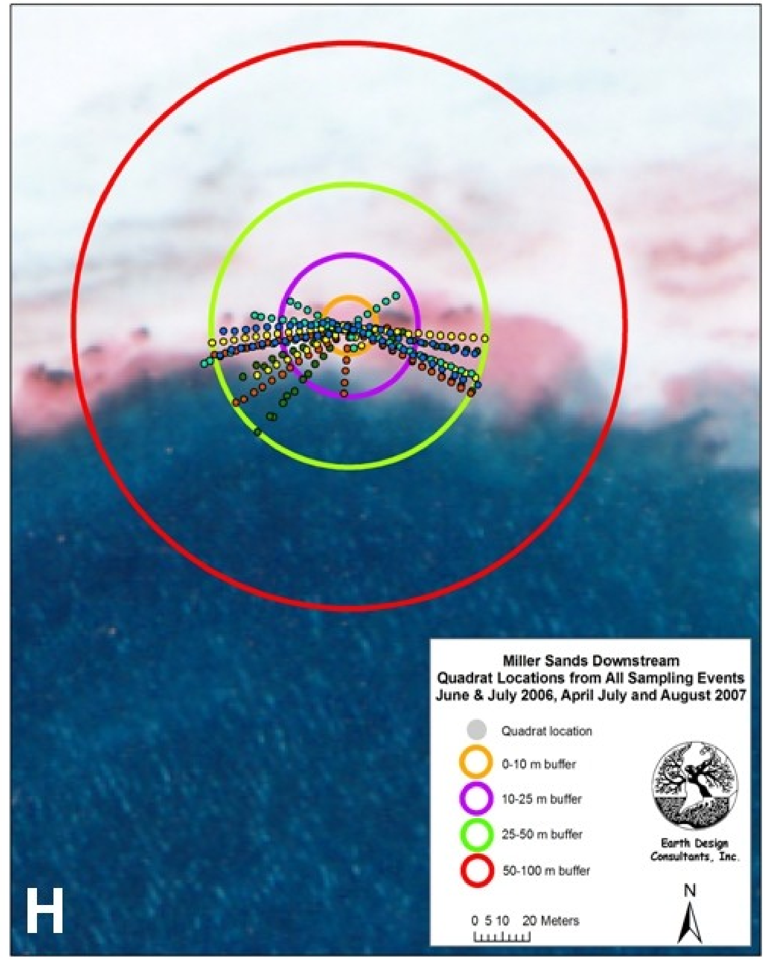
Miller Sands Downstream
Quadrat Locations from All Sampling Events
June & July 2006, April July and August 2007
Quadrat location
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25-50 m buffer
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0 5 10 20 Meters
Earth Design Consultants, Inc.
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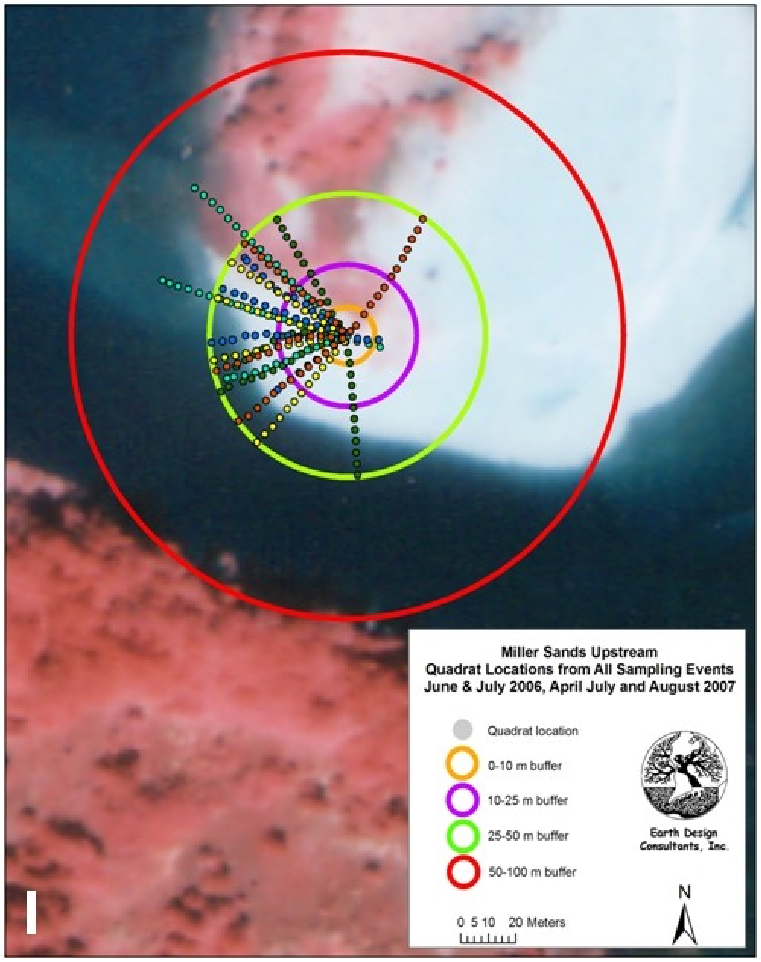
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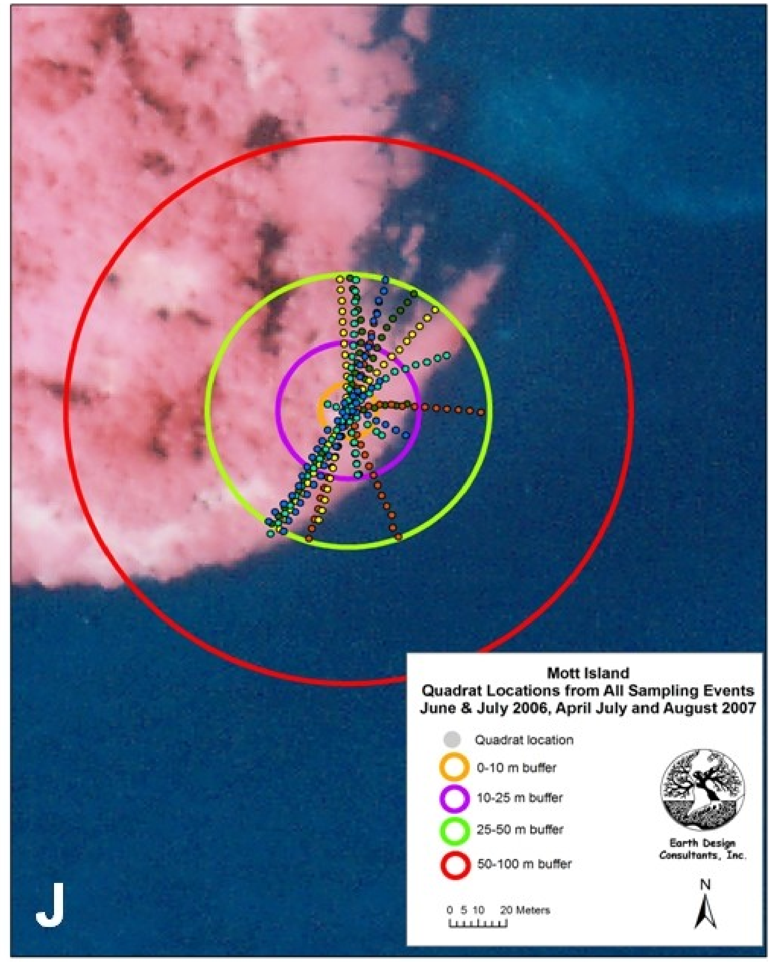
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Quadrat Locations from All Sampling Events
June & July 2006, April July and August 2007
Quadrat location
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Earth Design Consultants, Inc.
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0 5 10 20 Meters
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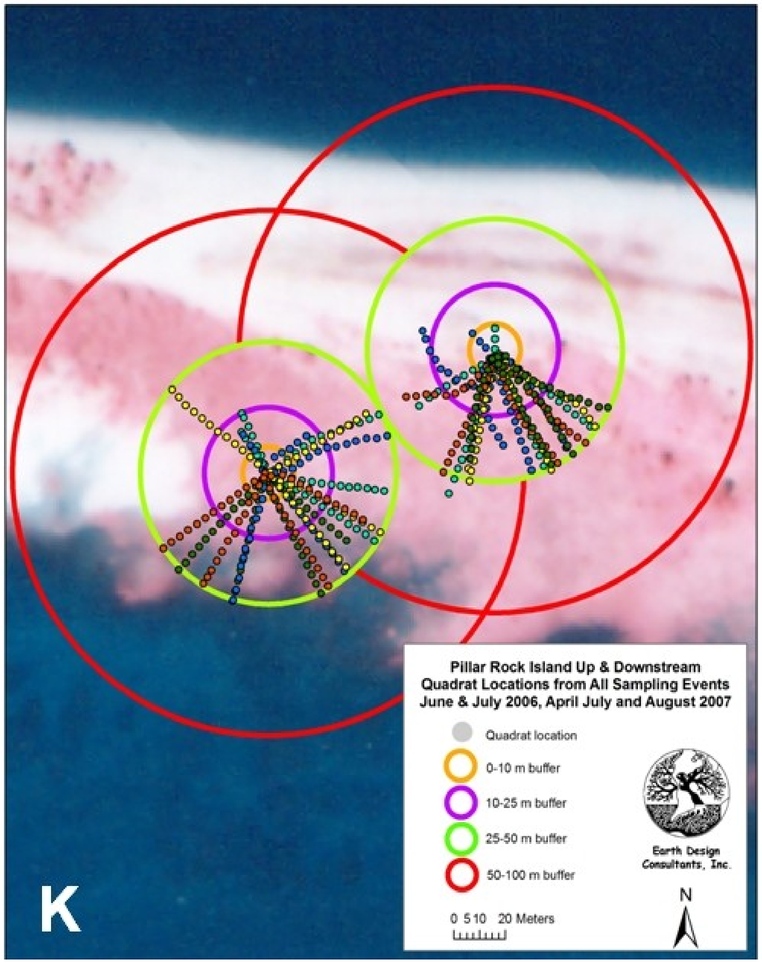
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June & July 2006, April July and August 2007
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Earth Design Consultants, Inc.
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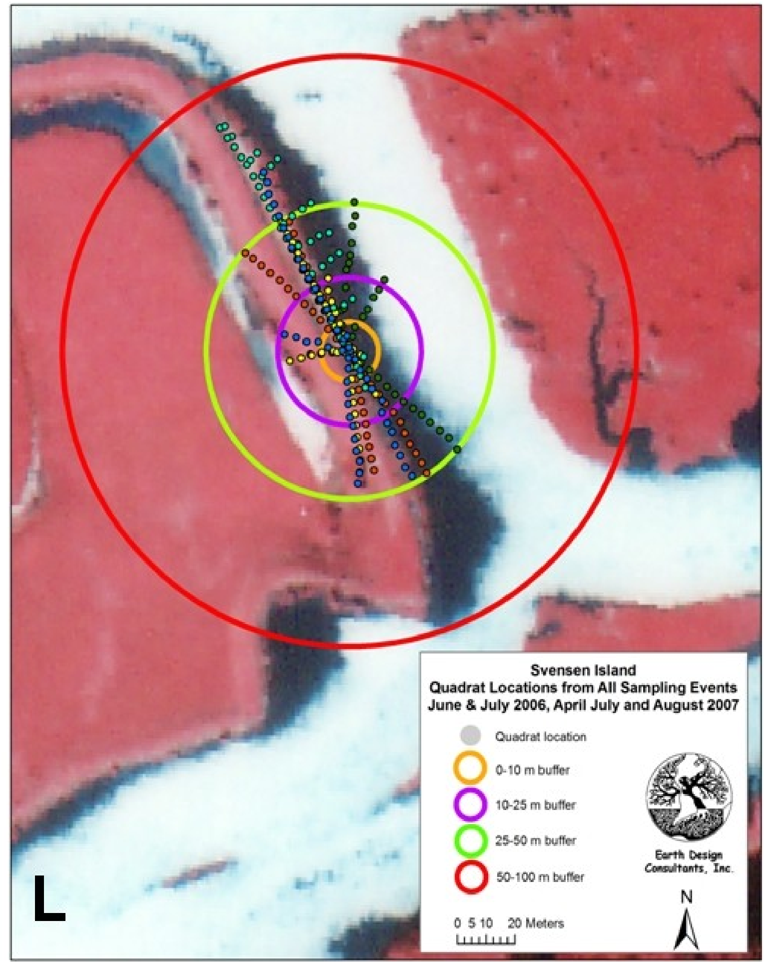
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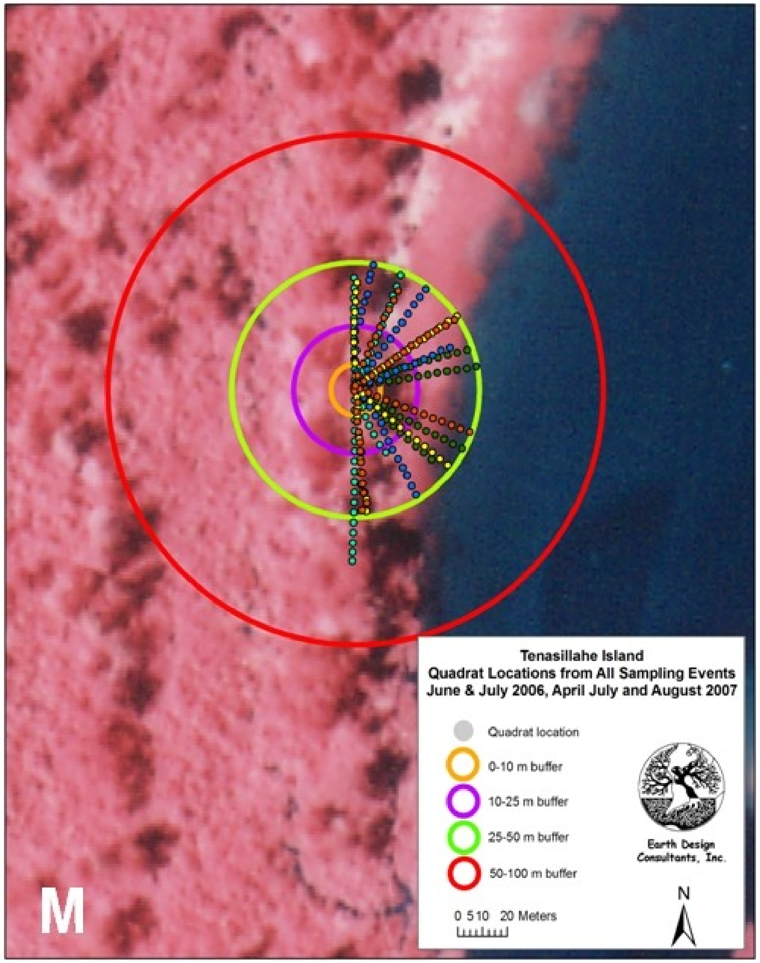
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June & July 2006, April July and August 2007
Quadrat location
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Earth Design Consultants, Inc.
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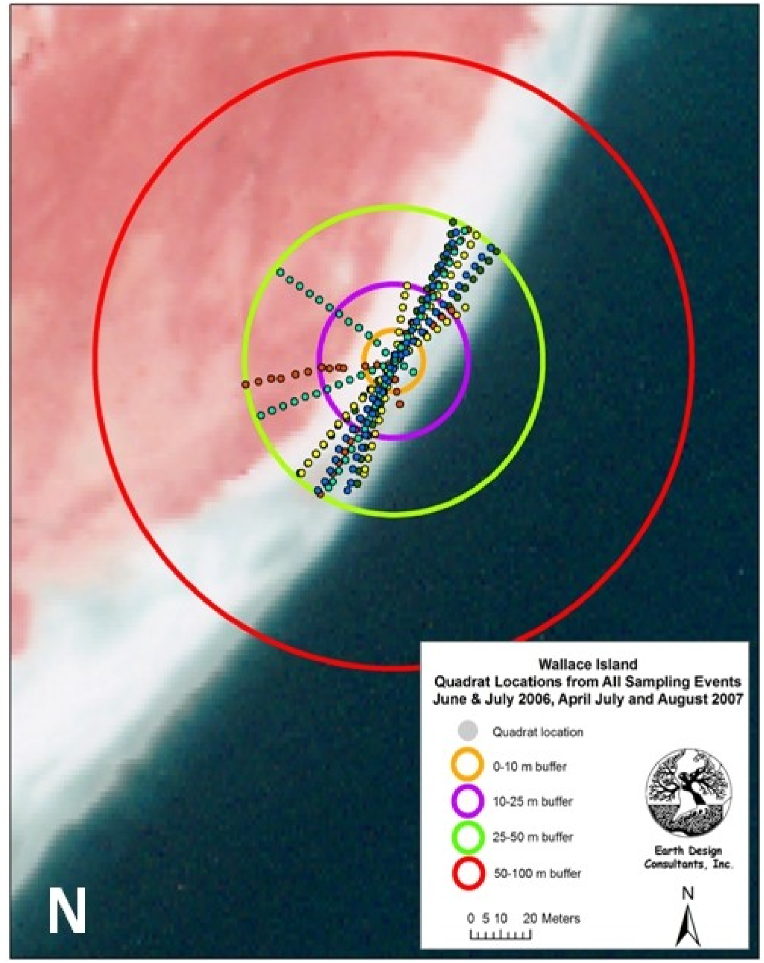
Wallace Island
Quadrat Locations from All Sampling Events
June & July 2006, April July and August 2007
Quadrat location
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25-50 m buffer
50-100 m buffer
0 5 10 20 Meters
Earth Design Consultants, Inc.
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N

also very high at the Eureka Bar Upstream, Pillar Island Upstream and Downstream, and Miller Sands Upstream and Downstream sites. Devil's Elbow (Figure 4 a) has the lowest density of *Lythrum* stems with an average of one living stem per quadrat (s.d. 3). The stem density at Dry Dock and Svensen is also quite low, averaging 3 stems per quadrat. The maximum number of living stems in a single quadrat, 132, was measured at Miller Sands Upstream (Figure 4 j).

We are interested in whether the density of live stems has changed from 2006 to 2007. We compared the average number of live stems per quadrat across all sites by sampling event and we found there to be no statistically significant difference (Tukey Kramer HSD alpha=0.05, q*=2.7, ANOVA F=2.24, p>0.06, df=4, 3789). Differences are also not biologically significant because we see the mean number of stems per quadrat differing by only 1-2 stems. We recommend that random samples be taken at each site to specifically address this question.

When looking at changes in live stem density over sampling events at an individual site basis, significant differences exist at Fitzpatrick, Karlson, and both Miller Sands sites. The other sites do not have statistically significant differences between sampling events based on ANOVA's with a p=0.05. At these four sites, our quadrats contained a greater number of living stems at the earlier sampling events than later events (Table 1). One possible explanation for this may be that earlier in the year, the stems are much shorter and easier to see. Later in the year, stem lengths increase dramatically which may interfere with our ability to accurately quantify the total number of stems and hinder our ability to see the shorter stems hidden in a patch of *Lythrum*. We acknowledge that the two year length of this study may not be long enough to see a trend. We present this information so that future comparisons can be made.

Table 1: Comparison of the mean number of living* Lythrum *stems for each sampling event in 2006 and 2007. Shown are comparisons for Miller Sands Upstream (MSU), Miller Sands Downstream (MSD), Karlson Island (KA), and Fitzpatrick Island (FZ). These sites had statistically significant differences in the number of live stems.

	MSU		MSD		KA		FZ	
	Levels	**Mean**	**Levels**	**Mean**	**Levels**	**Mean**	**Levels**	**Mean**
June 2006	A	20.698	A	17.580	A	7.333	A	6.960
July 2006	BC	13.280	AB	13.327	AB	3.760	BC	4.100
April 2007	AB	14.692	AB	14.780	AB	4.500	AB	6.588
July 2007	C	7.442	B	8.300	B	1.700	C	2.560
August 2007	BC	9.020	B	9.280	B	3.480	C	3.660
	F Ratio	**Prob > F**	**F Ratio**	**Prob > F**	**F Ratio**	**Prob > F**	**F Ratio**	**Prob > F**
	4.3464	0.002	2.4948	0.0435	2.4308	0.0482	3.8779	0.0045
		DF		**DF**		**DF**		**DF**
		4, 253		4, 247		4, 246		4, 246

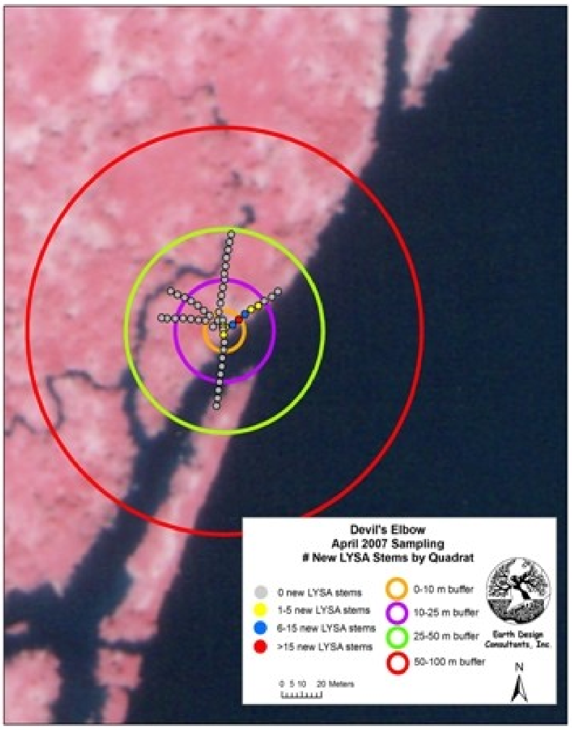
We were also specifically interested in comparing the number of live stems sampled between the July 2006 and July 2007 events because of their similar timing within the calendar year and degree days. We also wanted to compare July 2007 vs. August 2007. When all sites are compared together, there is not a statistically significant difference between the average number of live stems per quadrat in July 2007 compared to July 2006 (ANOVA F=0.02, p=0.89, DF=1, 1513), or July 2007 compared to August 2007 (F=1.99, p=0.15, DF=1, 1506). We compared these same sampling event differences on an individual site basis. The average number of live stems on Tenasillahe was greater in July 2007 than in August 2007 (F=4.0679, p=0.0464, DF=1, 98; 5.1 vs. 2.0 stems). The Fitzpatrick site had a higher number of live stems sampled in July 2006 compared to July 2007 (F=4.3459, p=0.0397, DF=1, 99; 6.6 stems vs. 3.7 stems). The opposite was true at Wallace Island where a larger number of live stems was sampled in July 2007 compared to July 2006 (F=8.4148, p=0.0046, DF=1, 99; 10.0 stems vs. 3.8 stems).

We also were interested in whether densities of loosestrife have changed within the buffered (0-10m, 10-25m, 25-50m, 50-100m) areas around the release stakes (see Figures 4 a-n). With this comparison, we can begin to track whether loosestrife is spreading or increasing in certain areas. Thus far, we have found no difference in the number of living stems per quadrat between the four buffers when comparing all sites in both July 2006 vs.

July 2007 and August 2007 vs. July 2007. We have extended our sampling transects into areas without loosestrife, such as mudflats and low elevation marshes to help us track the spread of loosestrife at the study sites. We hope to evaluate this in the future with more current and accurate land cover data and/or imagery.

Phenology of the plants is another important component to complete the understanding of our results. The condition of the plants, such as whether or not they are flowering or their height, is related to the weather and climate. The condition of the plant is related to the life stage and abundance of biocontrol agents. We used growing degree days as a way to quantify the weather during the different sampling events (see Section 2.5). As one would expect, cumulative growing degree days increases throughout the year (Figure 5). We see that temperatures were generally warmer during 2006 compared to 2007, evidenced by the larger cumulative growing degree day value (Figure 5). Looking at our data on average *Lythrum* stem length, we see a trend similar to that of growing degree days. Generally, the length of living *Lythrum* stems is highest at the end of season (July 2006 or August 2007) (Figure 6). It is also interesting to see the dramatic difference in stem length between the April and July 2007 sampling events (Figure 6). We also see little growth between July and August 2007 at most sites (Figure 6). Trends in the frequency of flowering stems are similar for the majority of study sites (Figure 7). Some sites, such as Tenasillahe and Fitzpatrick, show a decrease in the number of quadrats with flowering stems. This may be due to the fact that in August, some plants are finished flowering or have dried flower heads, or simply may be an artifact of sampling design.

Figure 4 a - o: Locations of quadrats at each of the 15 USACE release sites showing three density classes of *Lythrum*. Gray= 0, yellow= 1 to 5, blue = 6 to 15, and red > 15 stems. Circles represent different distances from release stakes (0-10m, 10-25m, 25-50m and 50-100m). Background images are CIR photographs. See text for details.


Devil's Elbow
April 2007 Sampling
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6-15 new LYSA stems
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10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
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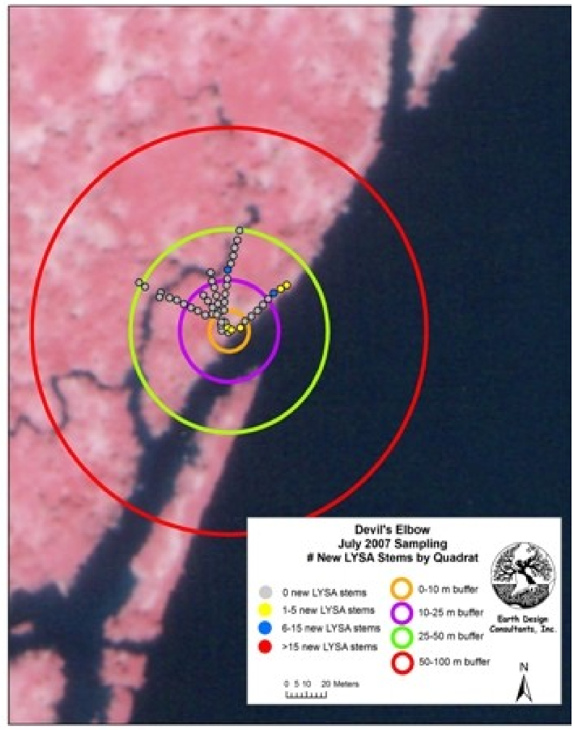
Devil's Elbow
July 2007 Sampling
New LYSA Stems by Quadrat
0 new LYSA stems
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0 5 10 20 Meters
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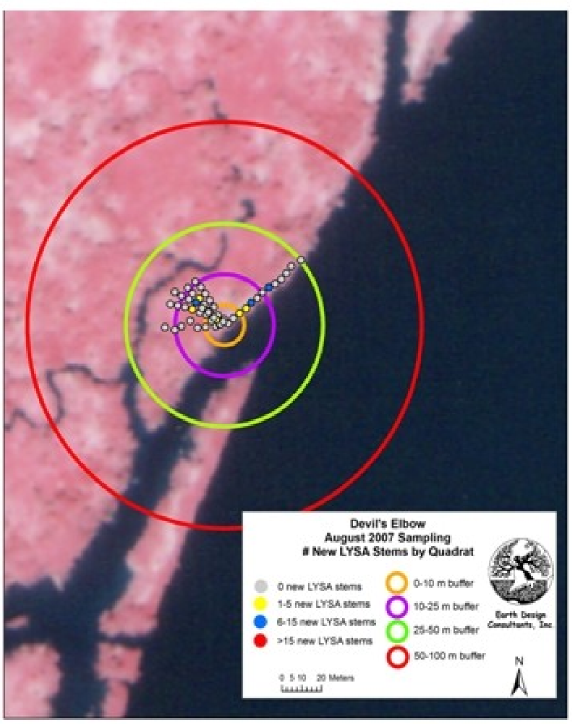
Devil's Elbow
August 2007 Sampling
New LYSA Stems by Quadrat
0 new LYSA stems
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Earth Design Consultants, Inc.
0 5 10 20 Meters
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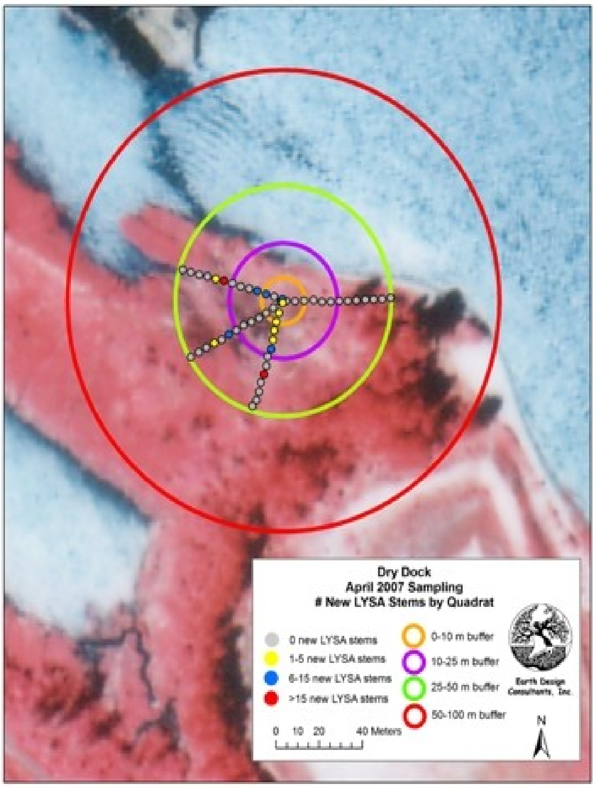
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April 2007 Sampling
New LYSA Stems by Quadrat
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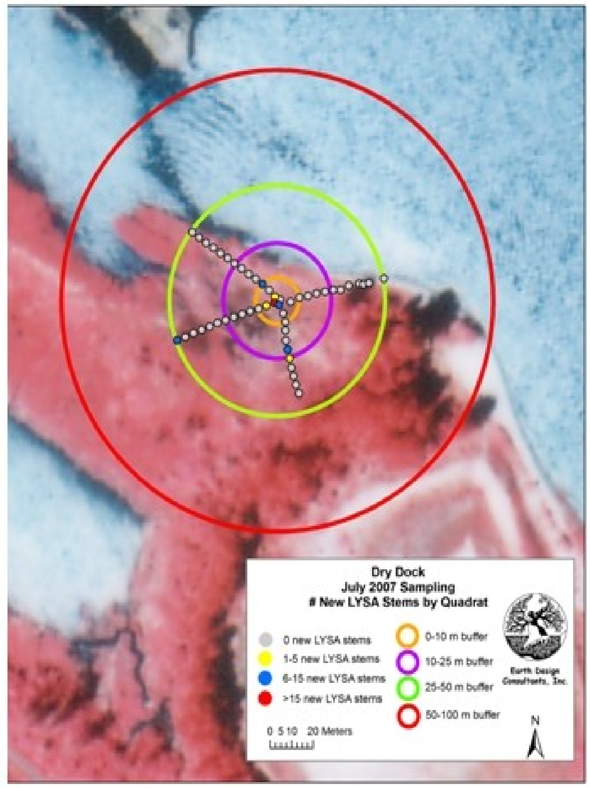
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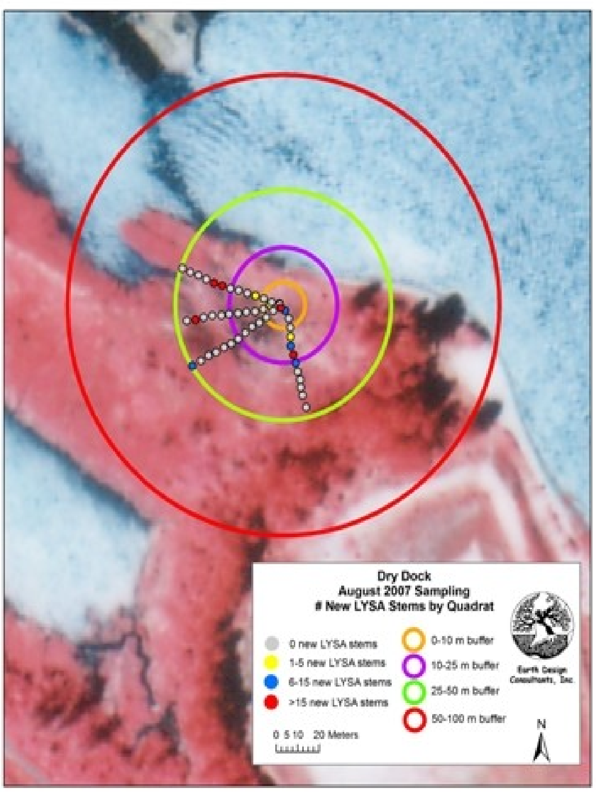
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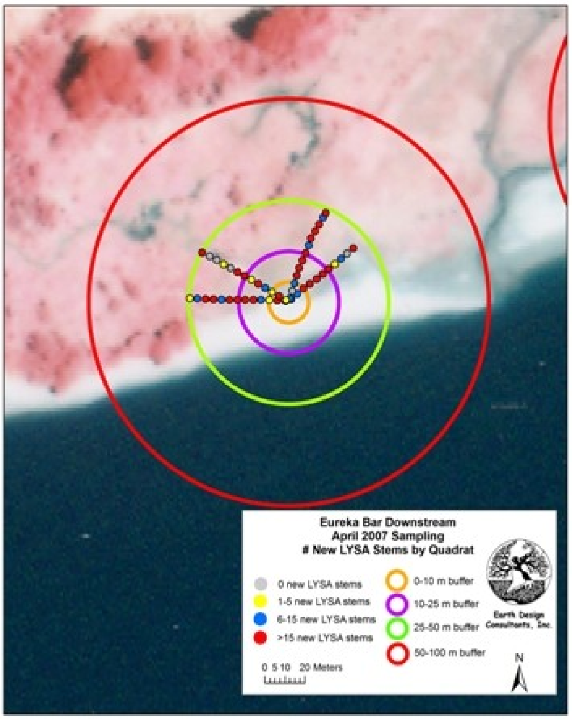
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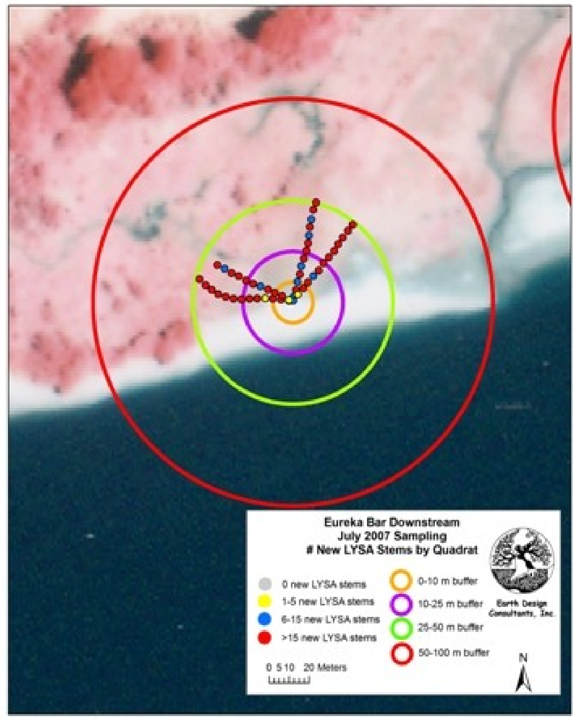
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July 2007 Sampling
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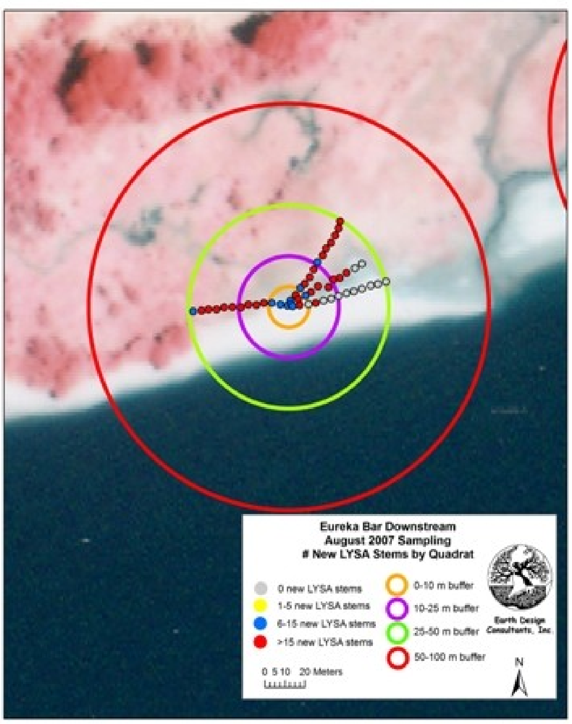
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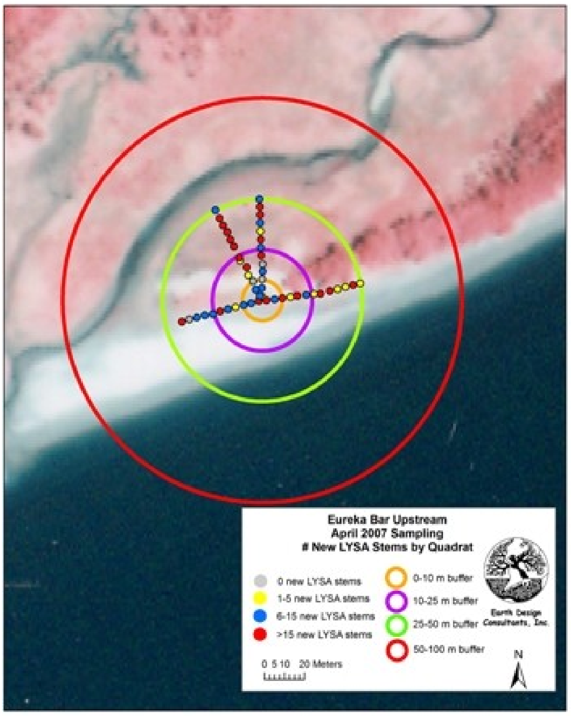
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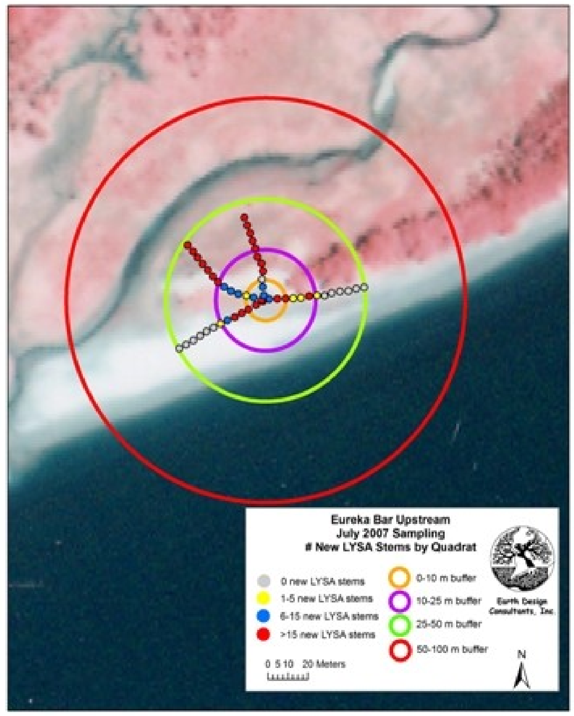
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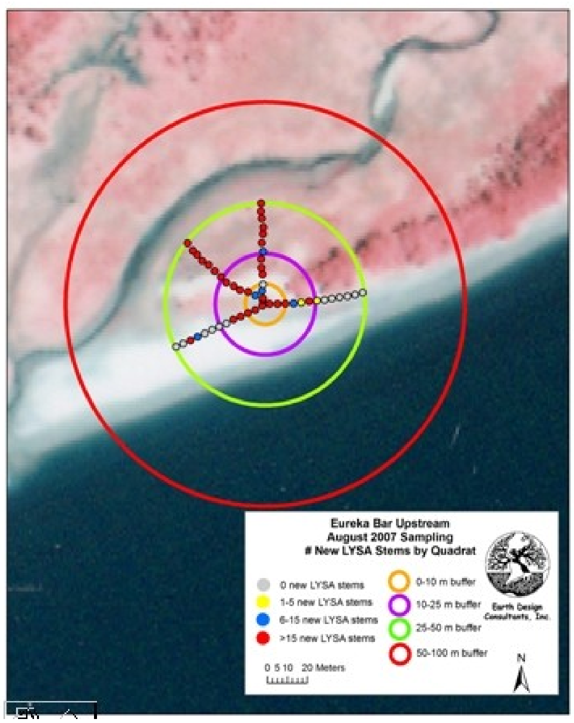
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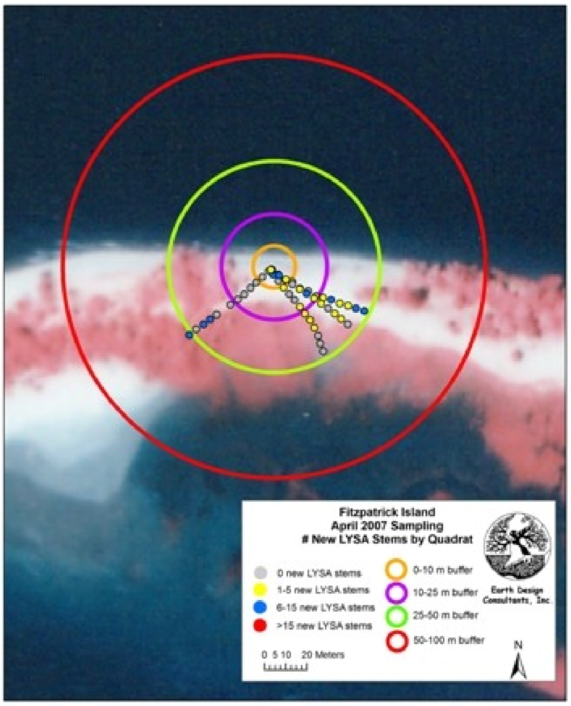
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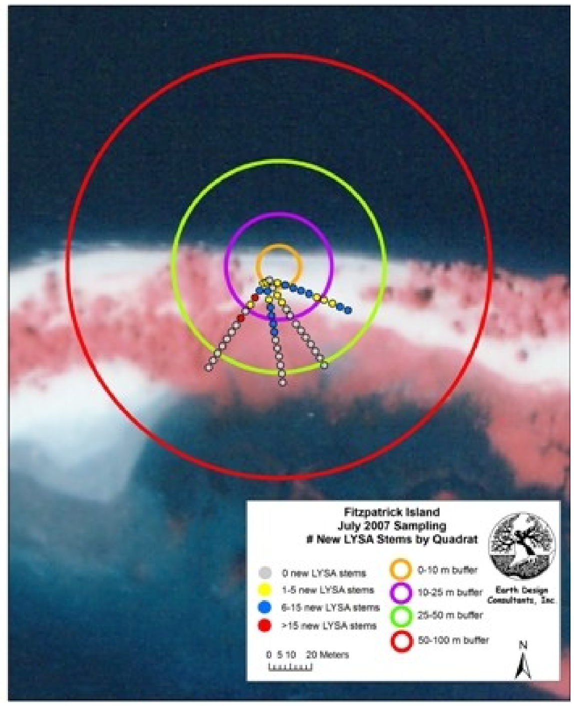
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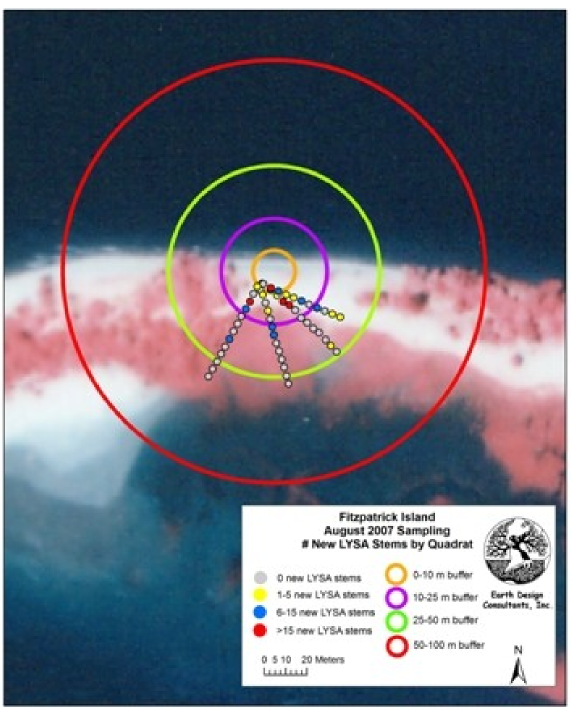
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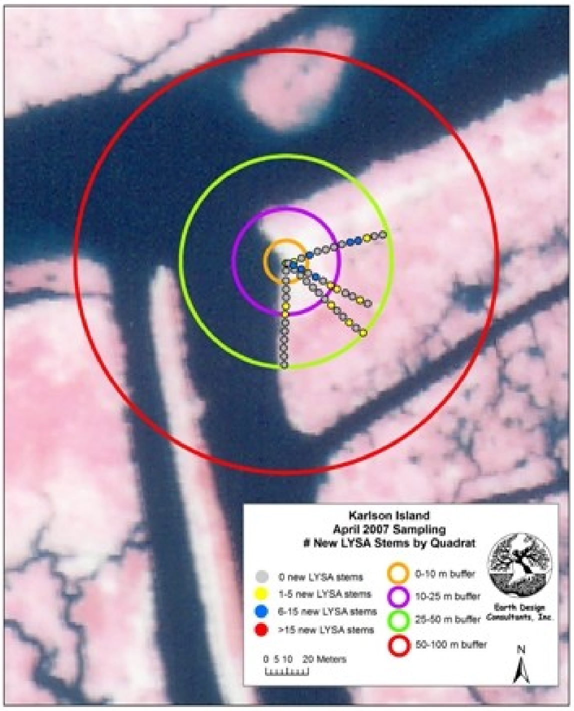
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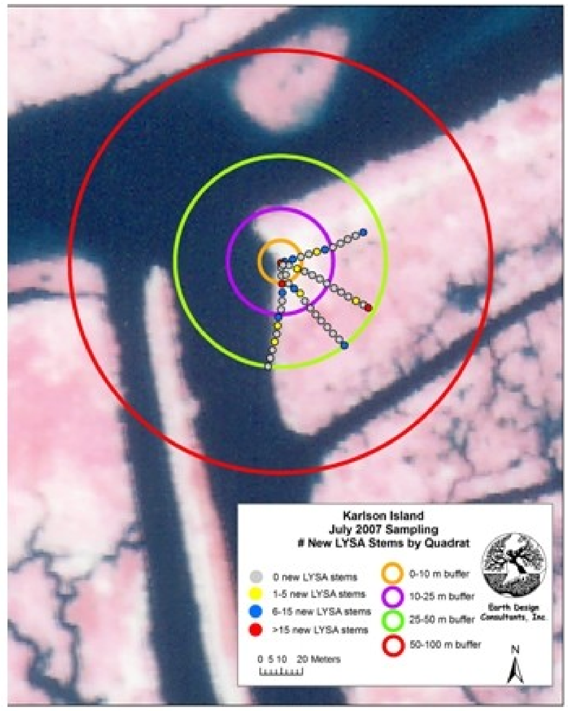
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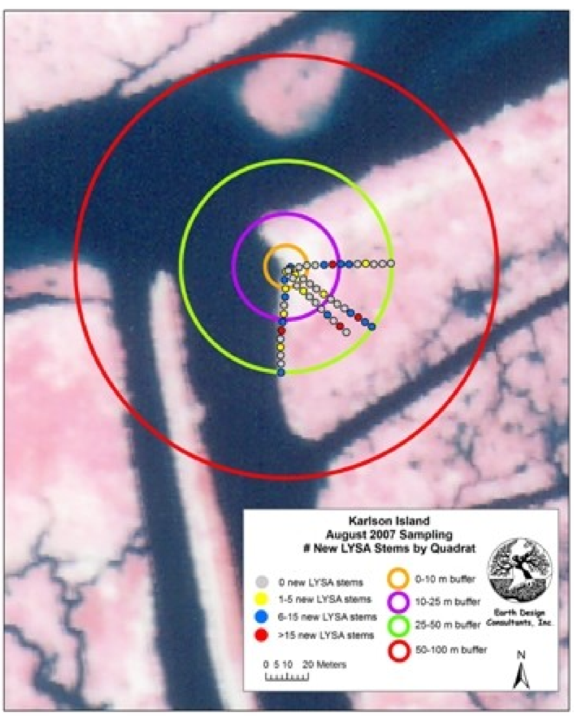
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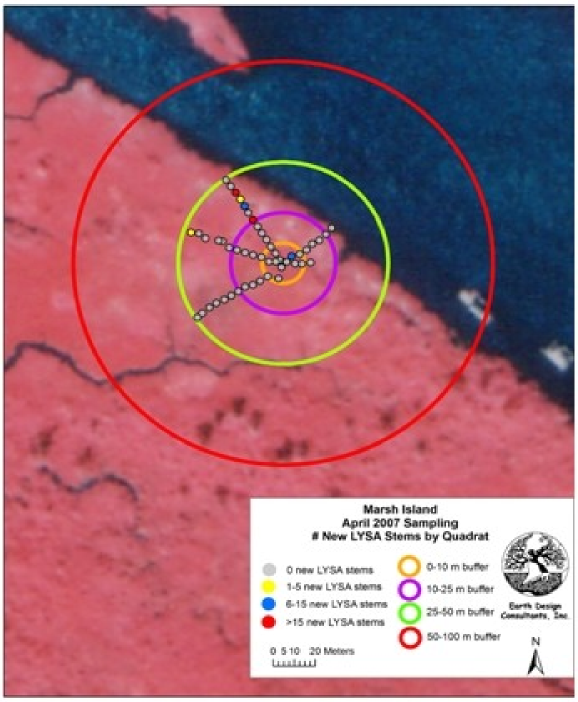
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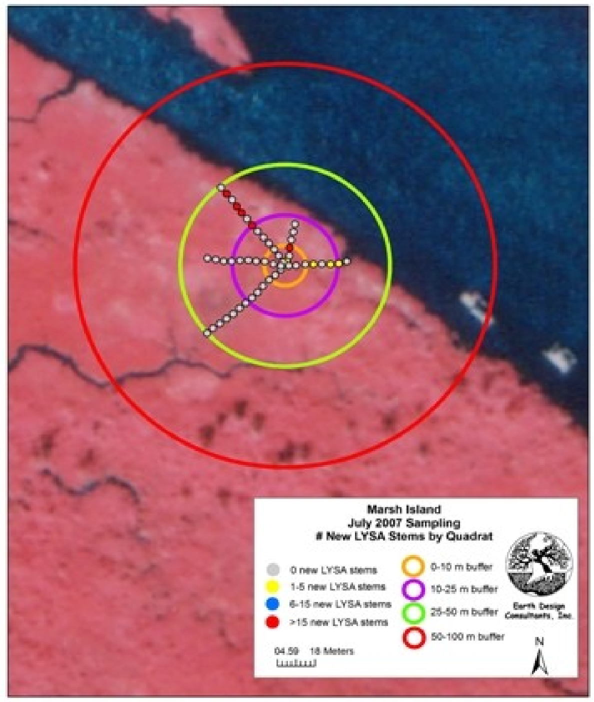
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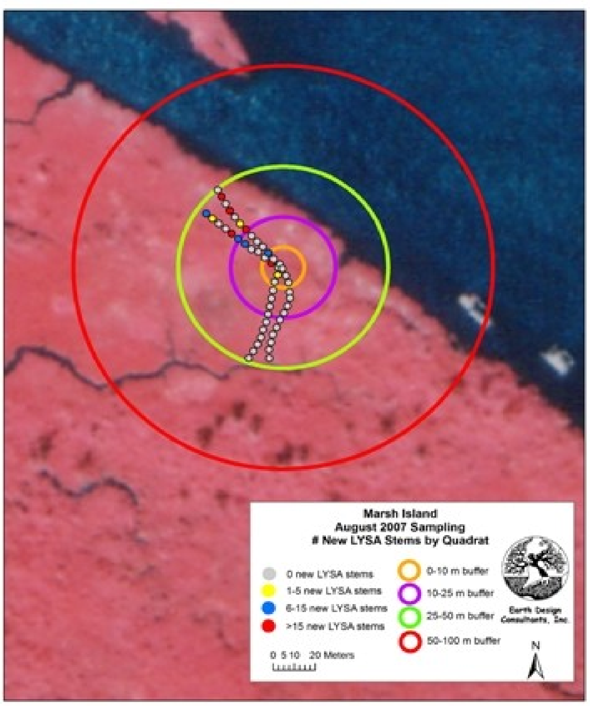
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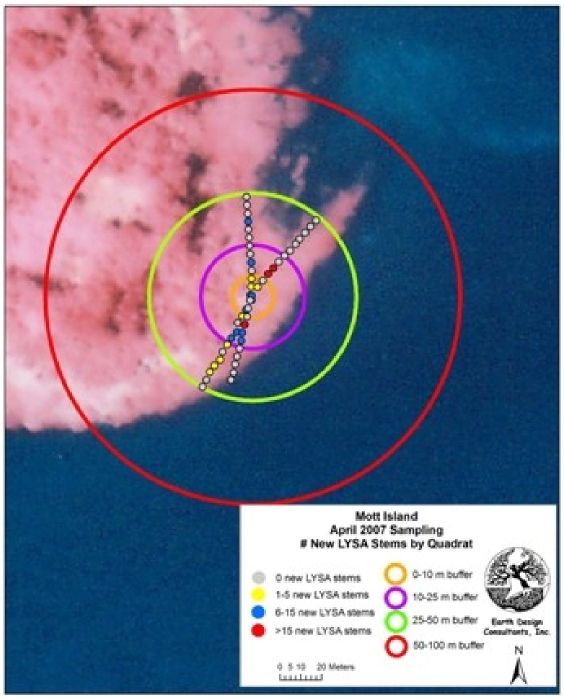
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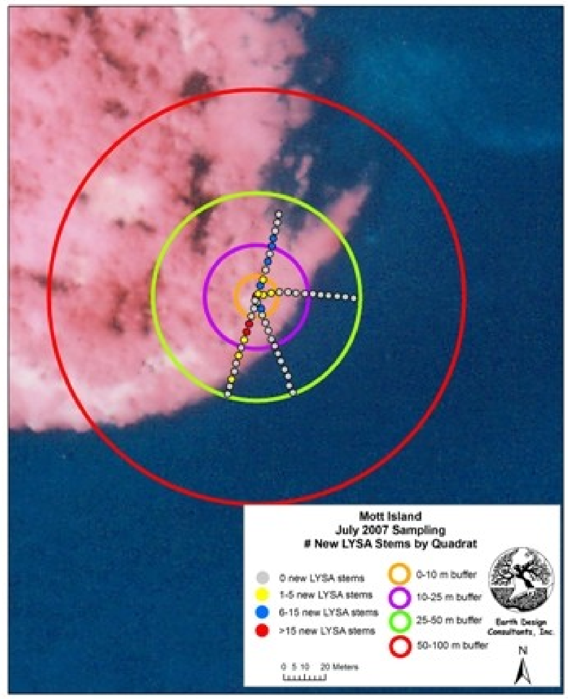
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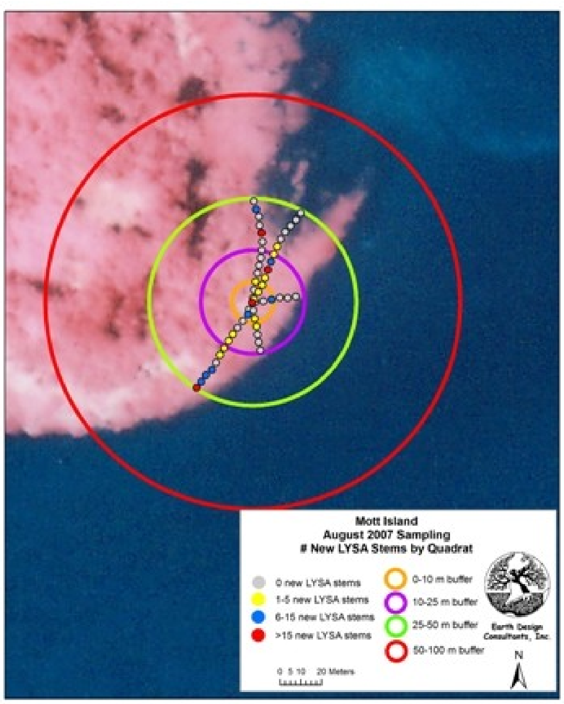
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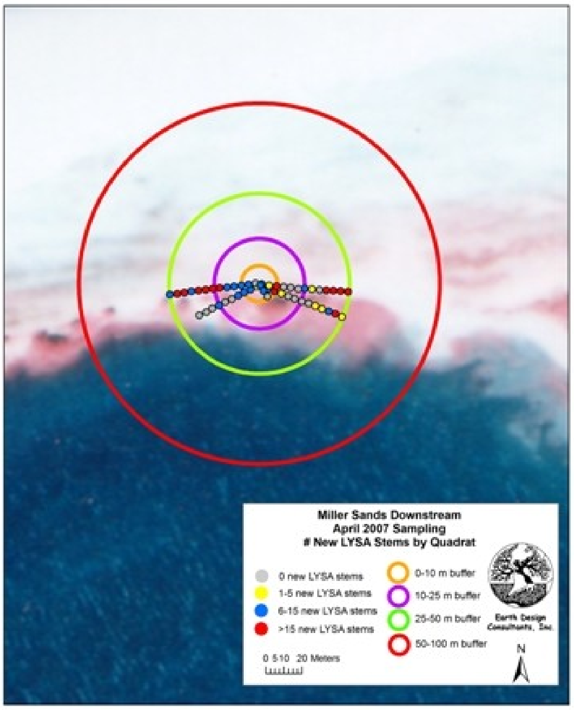
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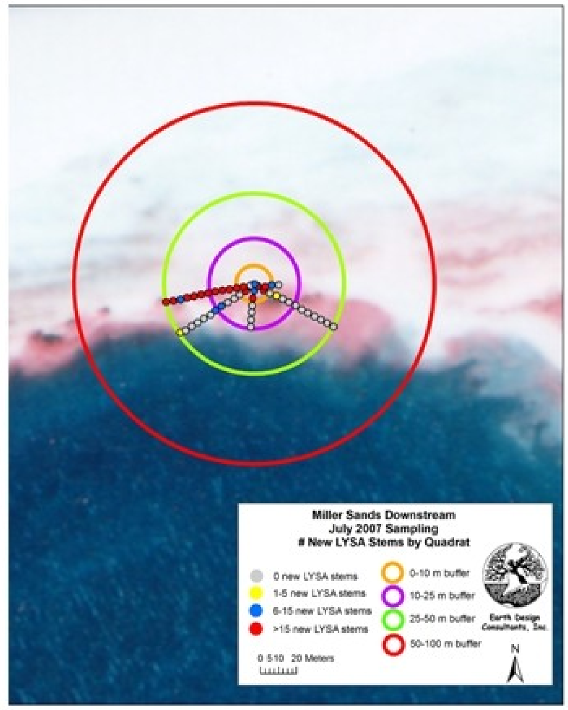
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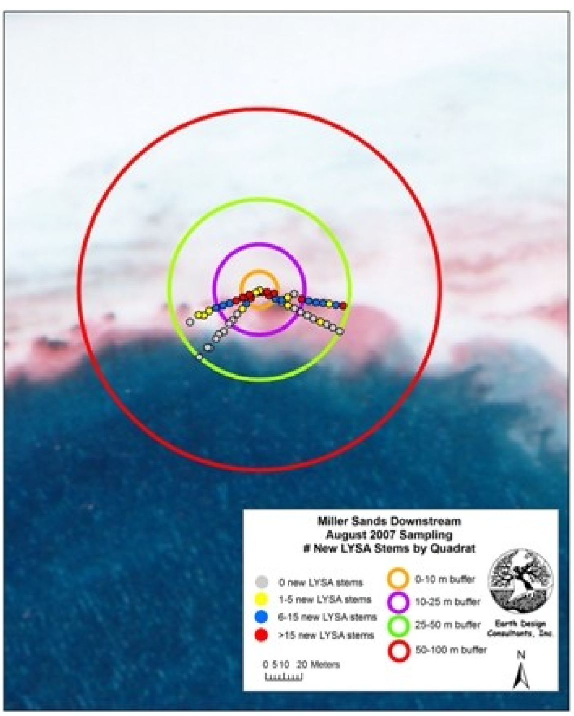
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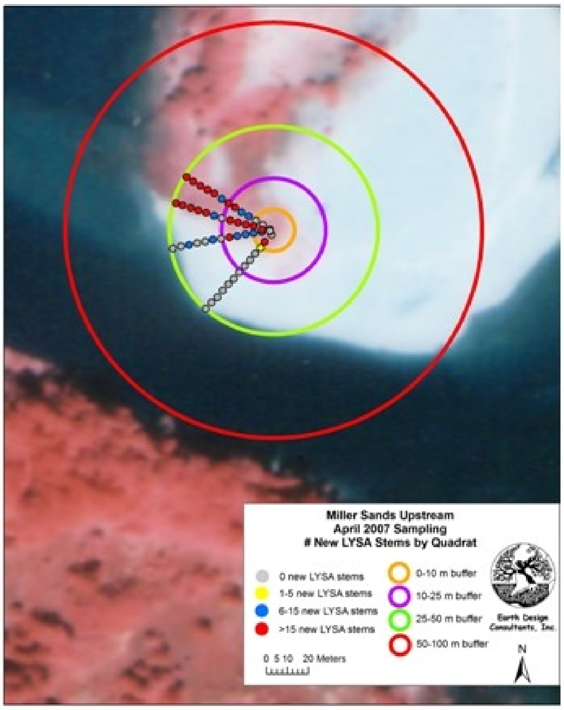
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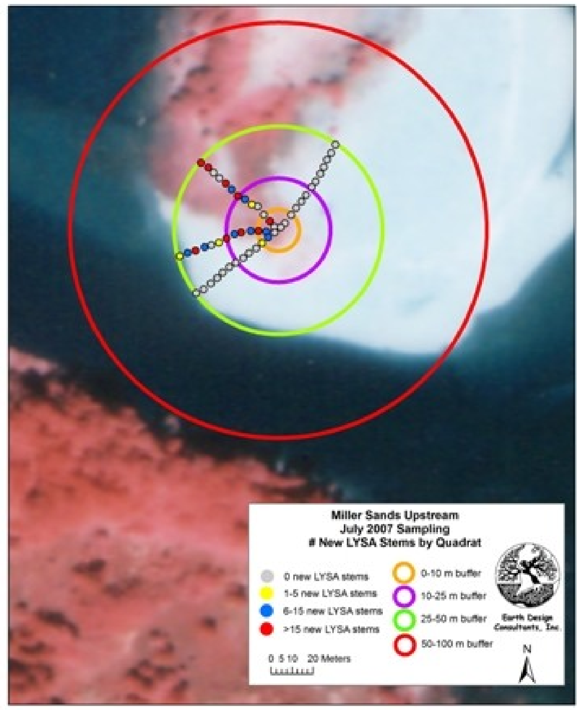
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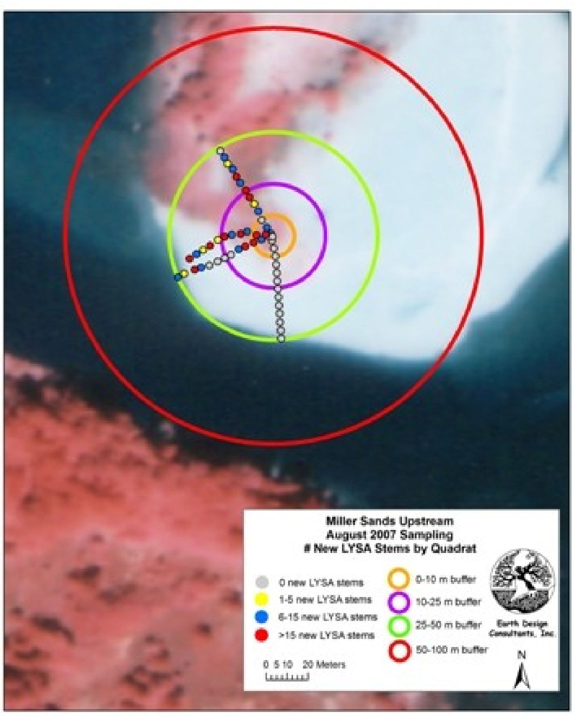
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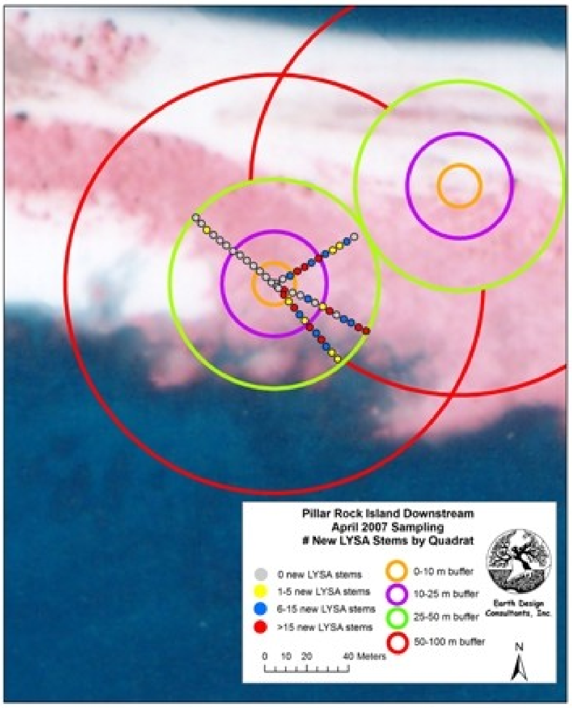
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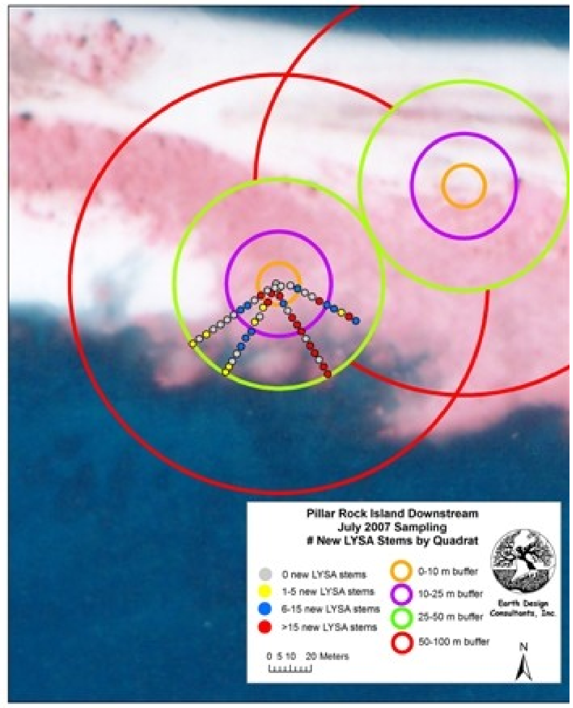
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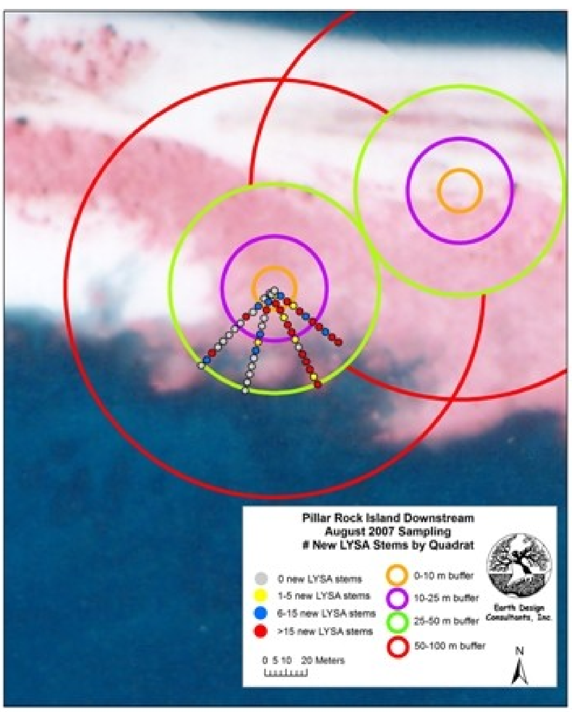
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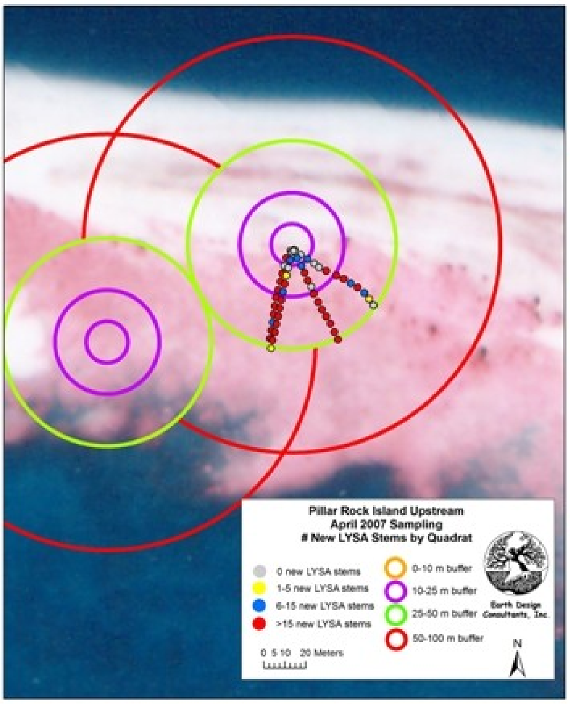
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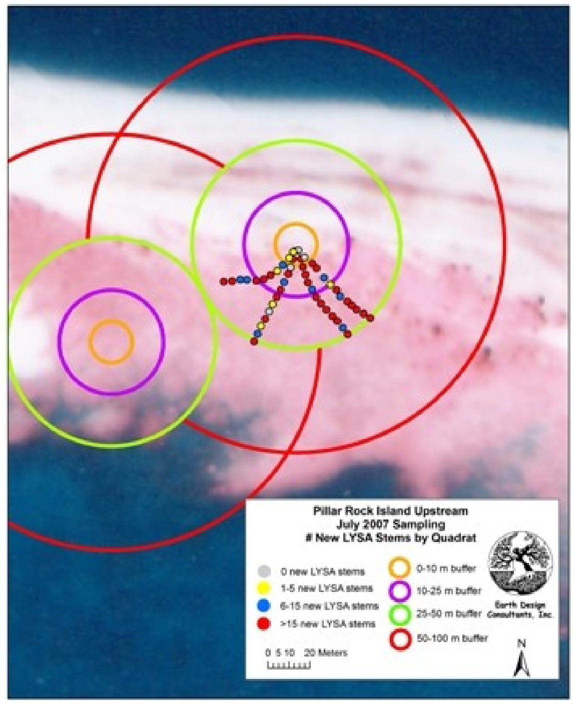
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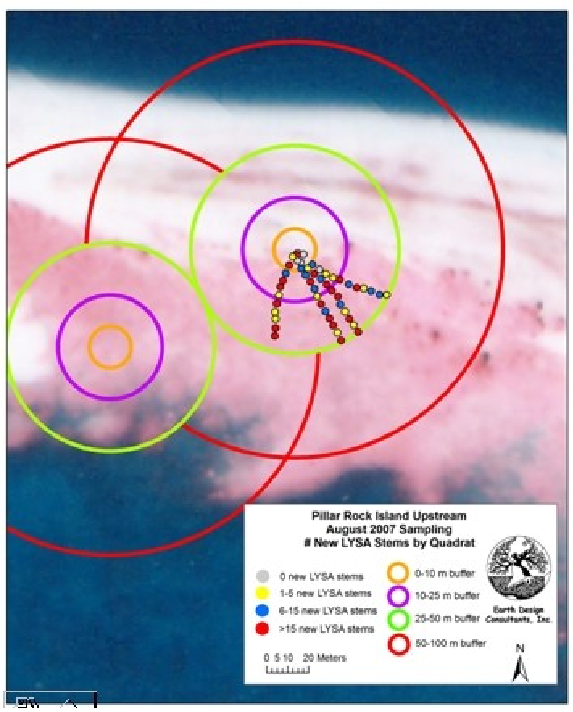
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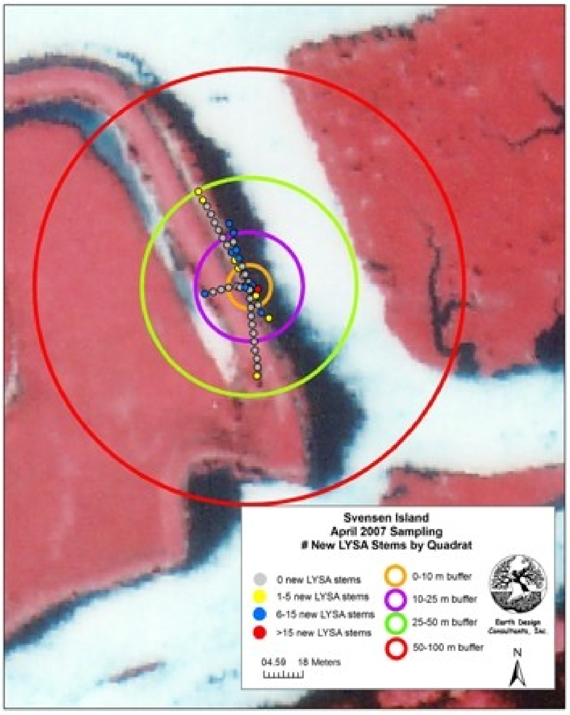
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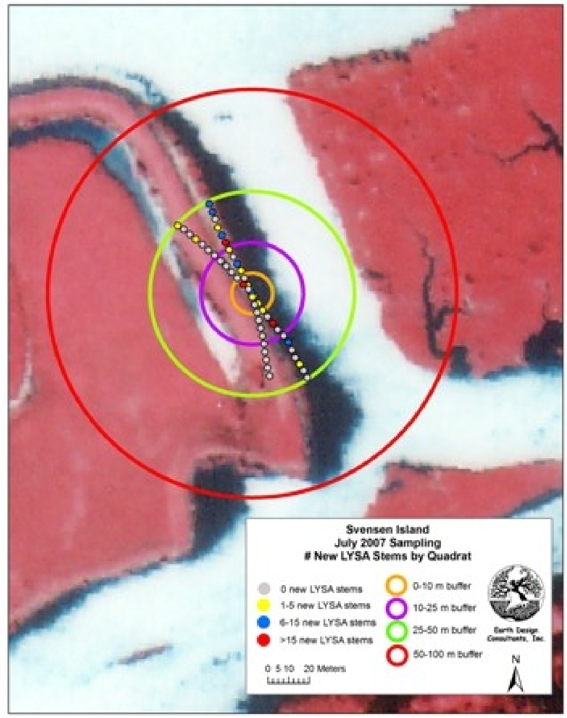
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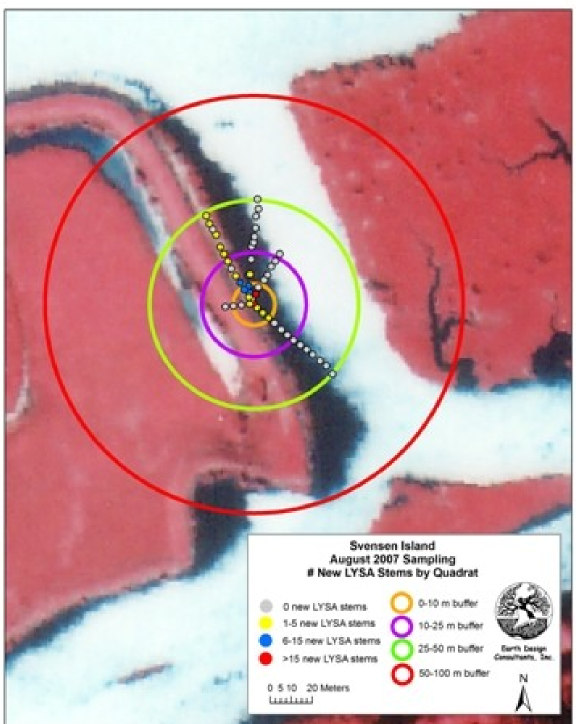
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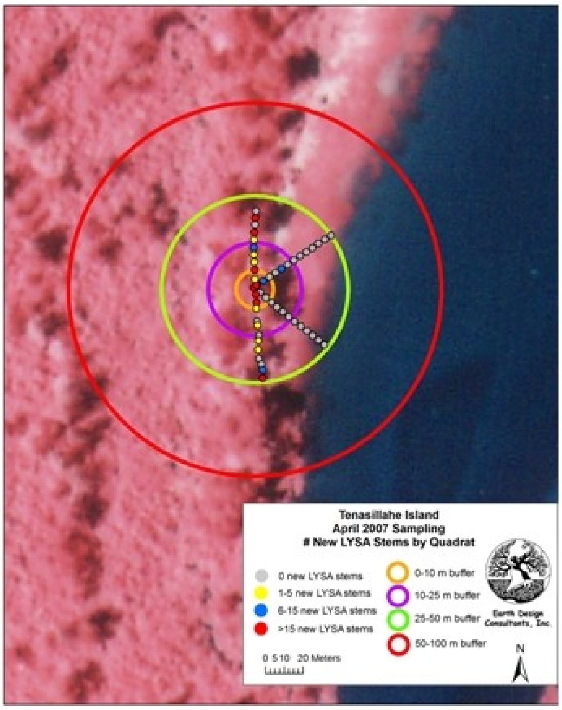
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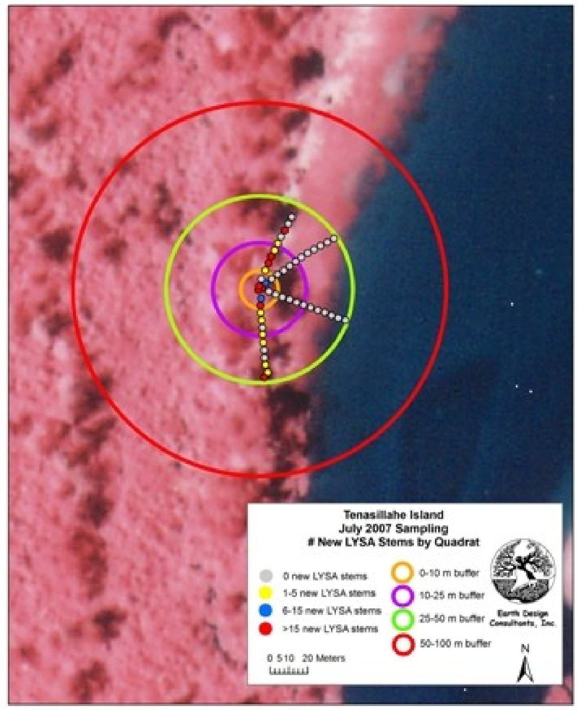
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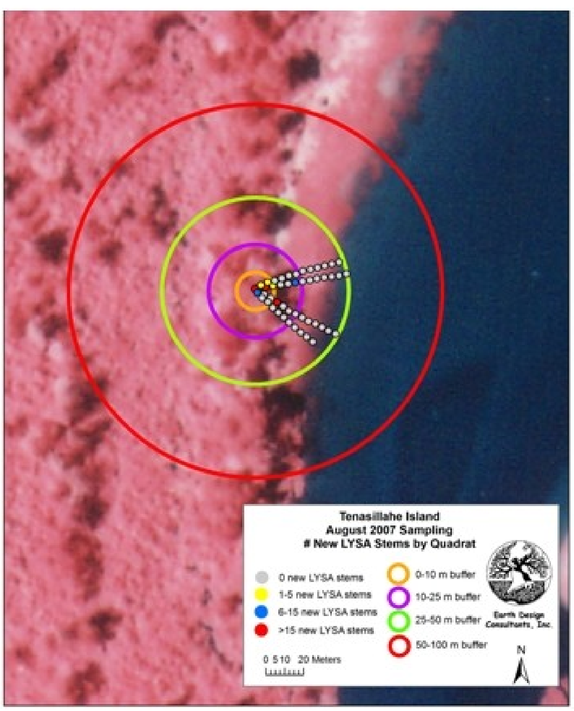
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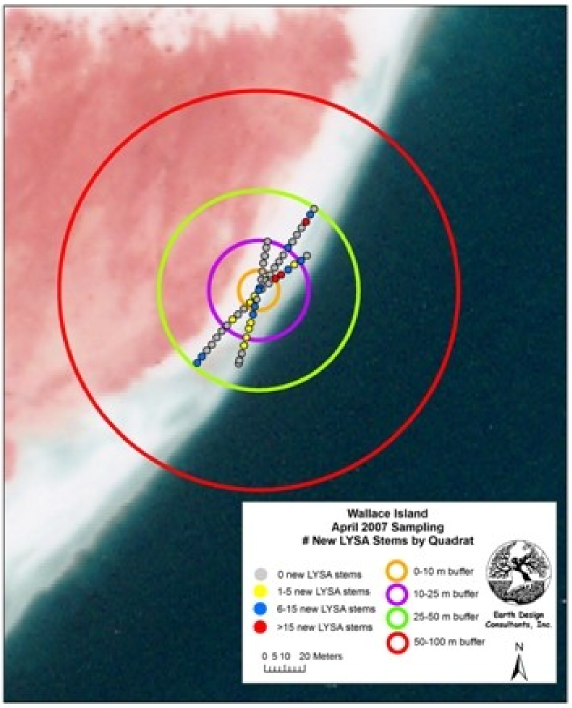
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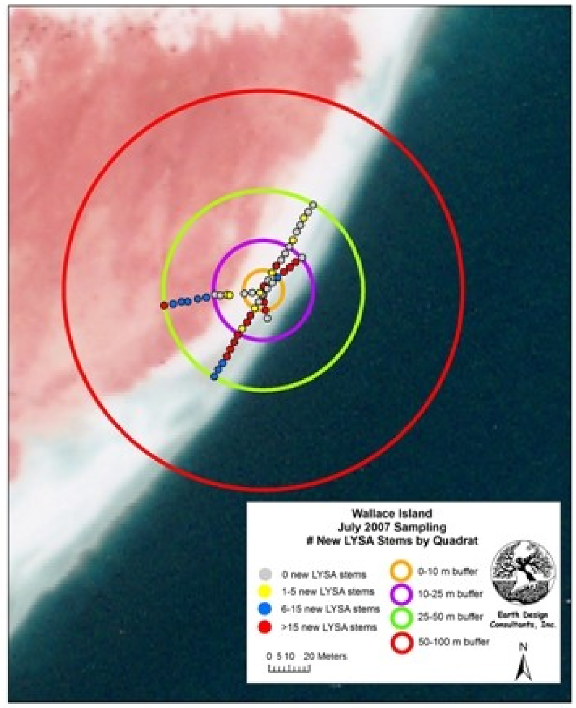
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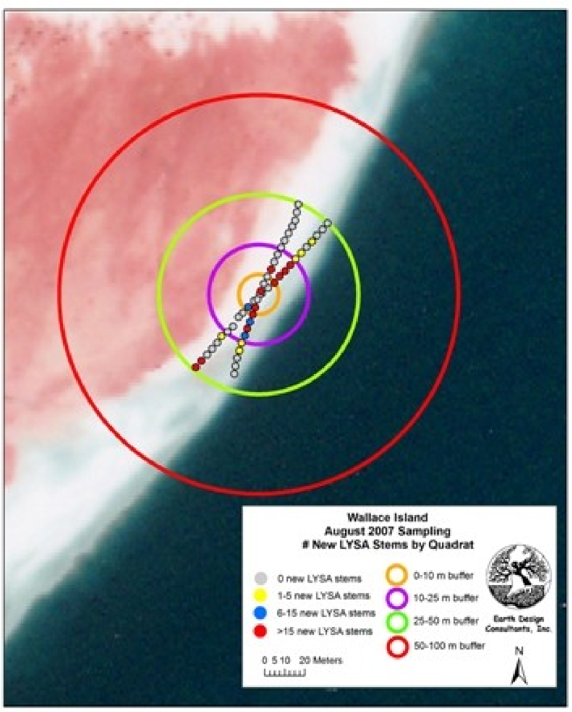
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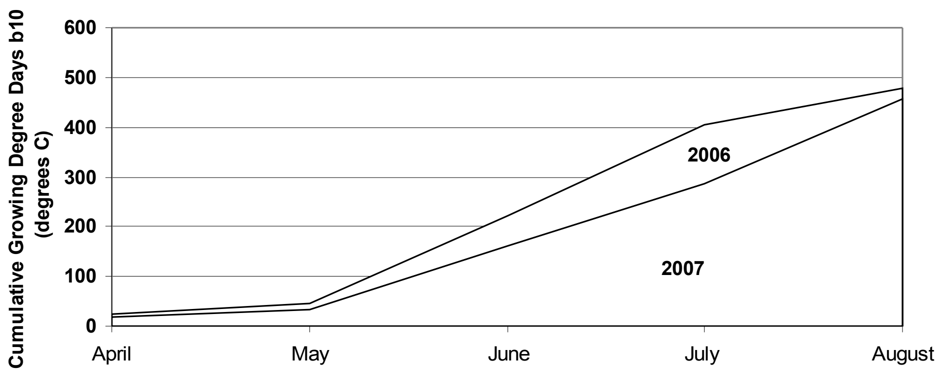


Figure 5: Degree days for our 2006 and 2007 ssampling events. Later sampling events, such as August 2007, have a higher growing degree day value than earlier dates. Degree days are based on Astoria Airport weather station records, the cumulative growing degree days (base 10ºC) for 2006 and 2007.

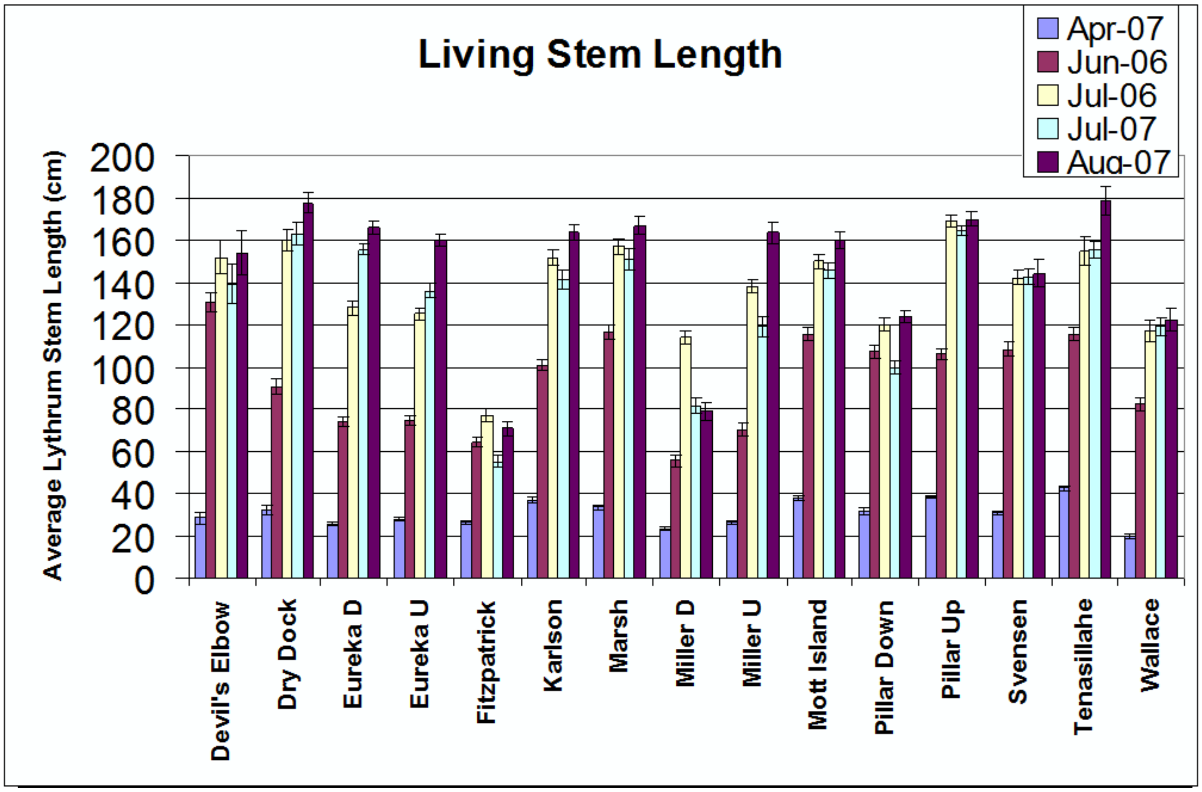


Figure 6: Average stem length (cm) of living *Lythrum* plants. We haphazardly selected five plants from each quadrat. Shown are average stem length for each site for each sampling event. Bars are SE.

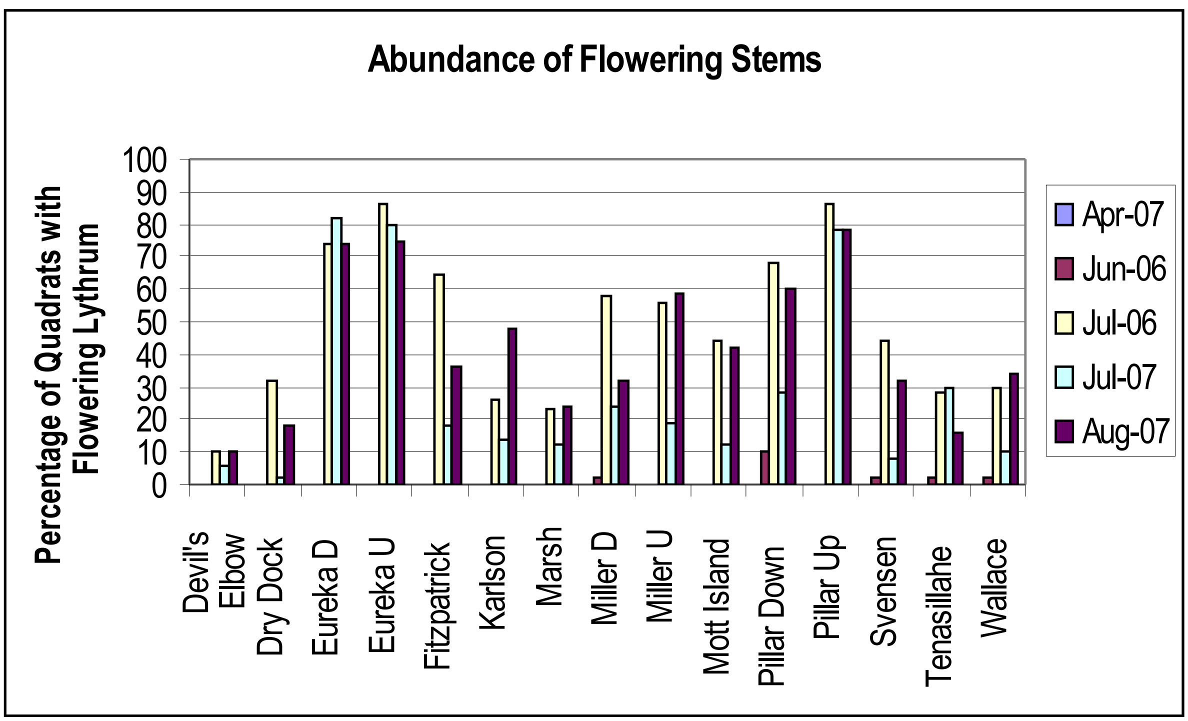


Figure 7: Percentage of total number of quadrats with flowing *Lythrum* stems. Shown are each sampling event for 2006 and 2007.

3.1.3 Presence of Biocontrol Agents

We observed evidence of *Galerucella* biocontrol agents at 13 of 15 sites in 2007 with none observed at Miller Sands Downstream and Pillar Island Downstream (Table 2 a). The presence of other species of biocontrol agents are given in Table 2 b. These observations are similar to those from 2006 where we observed *Galerucella* at all the same sites. Although it is too early to tell, these results strongly suggest that the biocontrol agent populations are becoming established at most sites. In order for a population to be considered established it must be (1) increasing, criterion of population biologists, and (2) recoverable from the release site for three consecutive years, criterion used by Eric Coombs, Oregon Department of Agriculture Entomologist. In this study, we consider evidence of biocontrol agent presence to be observations of eggs, larvae, or adults of the introduced *Galerucella* species on *Lythrum* plants located within the quadrats.

Biocontrol populations can be assessed by measuring the number of individuals per unit area or the frequency of encounter. Since *Galerucella* individuals are known to drop off their host plants when disturbed, thereby affecting beetle densities, we present here the frequency at which biocontrol agents are encountered. Table 3 shows the number of quadrats with *Lythrum* where introduced *Galerucella* agents were observed, either as eggs, larvae or adult beetles in both 2006 and 2007. We can see from Table 3 that overall we observed biocontrol agents at all the sites at which they were detected in 2006; however, on single site basis, observations between sampling events were variable.

Interestingly, introduced *Galerucella* populations at all sites, except as otherwise noted below, appear to be increasing, evidenced by increasing number of quadrats with observations, or stable in frequency across sampling events. There are, however, two important caveats: (1) this is a comparison of multiple generations made over only two growing seasons, and (2) observations were made at different times in 2006 and 2007. We did observe that overall frequency (of any life stage) of *Galerucella* beetles are down in 2007 at Marsh and Mott Island sites. We observed a consistent number of biocontrol agents during at each sampling event in 2007 at the Pillar Upstream site but the overall frequency of encounters at this site is lower than in 2006. At the Miller Sands Upstream site we encountered *Galerucella* more often in April 2007 than in either sampling event of 2006 but we failed to observe any *Galerucella* during the July and August sample events in 2007.

It is also important to have a better understanding of beetle phenology to explain the abundance of biocontrol agents we see. Phenology is related to weather and climate. We are able to quantify climate using growing degree days. Our study sites are within the Oregon Coastal growing zone, although eastern sites of Wallace and Eureka Bar, lie at the far end of this growing zone (Oregon Climate Service, http://www.ocs.oregonstate. edu/index.html). This difference in location may cause some differences in climate, which could affect the beetle maturity (i.e. life stage) and activity. One of our goals in 2007 was to better match sampling events with life stages of *Galerucella.* We can see from Figure 4a-o and Tables 2a, b and Table 3 that generally presence of biocontrol agents was higher later in the year, when cumulative growing degree days is higher (Figure 5).

We have observed that biocontrol agents are not evenly distributed across each site nor are they distributed solely around the release site (Figures 8a-o). Movement of individual beetles is guided by a number of factors including presence of other *Galerucella*, presence of suitable host plants, and favorable environmental conditions. We expect that beetles themselves are in constant motion across each site. Superimposed on the guided movement of individual beetles is the movement of beetles due to wind and water. Thus, the distribution of beetles within a site is a combination of movement due to beetle behavior and movement due to the environment. We have noticed that at many study sites there are patches, pockets, or distinct areas where biocontrol agents appear in relatively high numbers. One of the limitations of our sampling method is that it was not designed to give a synoptic view of beetle distribution within each site. Therefore, we recommend that more systematic sampling of some of our sites be done to see what, if any, environmental gradients are related to biocontrol agent distribution. In 2008, we plan to select several of the release sites for more exhaustive sampling to account for the patchy distribution of the biocontrol agents.

Table 2a: Presence of biocontrol agents at each site. Shown are date of survey, biocontrol agent species and life stage. Also shown are sites exhibiting feeding damage.

	Site	Plant-Insect Survey Dates	*Galerucella* Egg masses			*Galerucella* Larvae			*G. pusilla* Adult			*G. calmariensis* Adult			*Galerucella* Damage		
			April	July	Aug	April	July	Aug	April	July	Aug	April	July	Aug	April	July	Aug
1	Devil's Elbow	4/30, 7/15, 8/12	No	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes
2	Dry Dock	4/29, 7/14, 8/8	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes	Yes
3	Eureka Bar Downstream	4/28, 7/16, 8/10	No	Yes	Yes	No	Yes	No	No	Yes	No	Yes	Yes	No	Yes	Yes	Yes
4	Eureka Bar Upstream	4/28, 7/12, 8/10 & 8/13	No	Yes	Yes	No	Yes	No	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes
5	Fitzpatrick Island	4/29, 7/13, 8/8	No	No	Yes	No	No	No	No	No	Yes	No	No	No	No	No	Yes
6	Karlson Island	4/26, 7/12, 8/13	No	No	Yes	Yes	Yes	No	No	No	Yes	No	No	No	Yes	Yes	Yes
7	Marsh Island	4/26, 7/14,	No	Yes	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes

	Table 2a: Presence of biocontrol agents at each site. Shown are date of survey, biocontrol agent species and life stage. Also shown are sites exhibiting feeding damage.																
	Site	Plant-Insect Survey Dates	*Galerucella* Egg masses			*Galerucella* Larvae			*G. pusilla* Adult			*G. calmariensis* Adult			*Galerucella* Damage		
		8/12															
8	Miller Sands Downstream	4/27, 7/11, 8/9	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
9	Miller Sands Upstream	4/27, 7/11, 8/9	No	No	No	No	No	No	Yes	No	No	Yes	No	No	Yes	No	No
10	Mott Island	4/27, 7/12, 8/9	No	Yes	Yes	No	No	Yes	No	No	No	No	No	No	Yes	Yes	Yes
11	Pillar Island Downstream	4/29, 7/15, 8/12	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
12	Pillar Island Upstream	4/27, 7/15, 8/12	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes
13	Svensen Island	4/26, 7/12, 8/13	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
14	Tenasillahe Island	4/29, 7/14, 8/8	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
15	Wallace Island	4/28, 7/13, 8/7	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes

	Table 2 b: Presence of additional biocontrol agents at release sites. Shown are sites biocontrol agents, and survey dates.							
	Site	**Plant-Insect Survey Dates**	***Hylobius* Adult**			***Nanophyes* Adult**		
			April	**July**	**Aug**	**April**	**July**	**Aug**
1	Devil's Elbow	4/30, 7/15, 8/12	No	No	No	No	No	Yes
2	Dry Dock	4/29, 7/14, 8/8	No	No	No	Yes	Yes	Yes
3	Eureka Bar Downstream	4/28, 7/16, 8/10	No	No	No	No	Yes	Yes
4	Eureka Bar Upstream	4/28, 7/12, 8/10 & 8/13	No	No	No	No	Yes	Yes
5	Fitzpatrick Island	4/29, 7/13, 8/8	No	No	No	No	No	Yes
6	Karlson Island	4/26, 7/12, 8/13	No	No	No	No	No	Yes
7	Marsh Island	4/26, 7/14, 8/12	No	No	No	No	No	No
8	Miller Sands Downstream	4/27, 7/11, 8/9	No	No	No	No	Yes	Yes
9	Miller Sands Upstream	4/27, 7/11, 8/9	No	No	No	Yes	Yes	No
10	Mott Island	4/27, 7/12, 8/9	No	No	No	No	No	No
11	Pillar Island Downstream	4/29, 7/15, 8/12	No	No	No	No	No	No
12	Pillar Island Upstream	4/27, 7/15, 8/12	No	Yes	No	No	No	No
13	Svensen Island	4/26, 7/12, 8/13	No	No	No	No	Yes	No
14	Tenasillahe Island	4/29, 7/14, 8/8	No	No	No	No	Yes	Yes
15	Wallace Island	4/28, 7/13, 8/7	No	No	No	No	Yes	Yes

Table 3: Biocontrol Agent Presence. Percentage of *Lythrum* infested quadrats (quads with *Lythrum*) with evidence of introduced *Galerucella*, either as presence of eggs, larvae, or adults, for 2006/07 sample events.					
Site	**June 2006**	**July 2006**	**April 2007**	**July 2007**	**August 2007**
Devils Elbow	**70**	**27**	**44**	**29**	**56**
Dry Dock	**20**	**67**	**47**	**88**	**92**
Eureka Bar Downstream	**4**	**0**	**2**	**12**	**29**
Eureka Bar Upstream	**13**	**0**	**2**	**41**	**38**
Fitzpatrick Island	**0**	**3**	**0**	**0**	**10**
Karlson Island	**26**	**10**	**6**	**12**	**8**
Marsh Island	**13**	**44**	**0**	**11**	**0**
Miller Sands Downstream	**0**	**0**	**0**	**0**	**0**
Miller Sands Upstream	**3**	**7**	**13**	**0**	**0**
Mott Island	**5**	**0**	**0**	**0**	**0**
Pillar Island Downstream	**0**	**0**	**0**	**0**	**0**
Pillar Island Upstream	**23**	**14**	**7**	**11**	**7**
Svensen	**58**	**57**	**50**	**65**	**75**
Tenasillahe	**18**	**39**	**50**	**50**	**67**
Wallace Island	**22**	**45**	**36**	**42**	**85**
All Sites	**15**	**15**	**13**	**21**	**24**

3.1.4 Growth and Spread of Control Agents

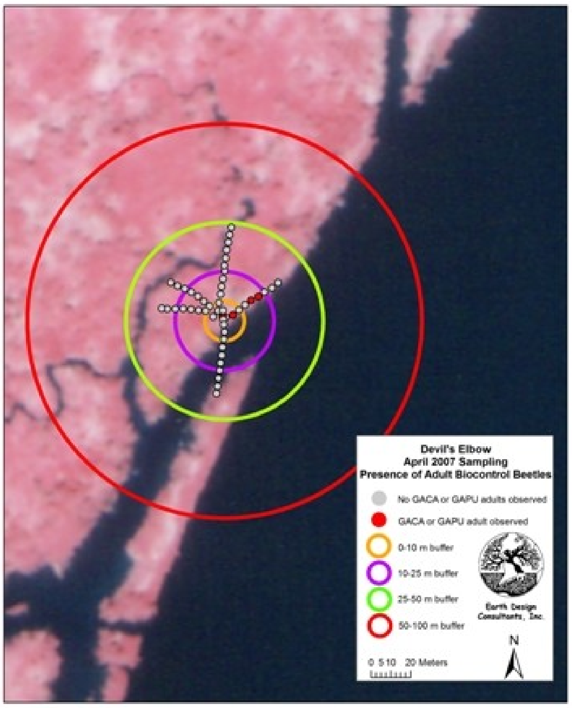
Figures 8 a-o show the locations of observations of adult introduced *Galerucella* beetles for April, July, and August 2007 sample events. From these site figures, we see that biocontrol agents are moving from the release points onto nearby host plants. However, the distance that beetles move away from the release points differs among sites. Table 4 displays the percentage of quadrats with *Lythrum* where introduced *Galerucella* species were observed either as eggs, larvae, or adults within each buffer around the release stake. At Svensen Island, we observed *Galerucella* biocontrol agents in at least 50% of the quadrats with *Lythrum* in 2006 located between 50 and 100m from the release stake (Table 4; Figure 8 m). There are also consistent observations of biocontrol agents in quadrats located between 25m and 50m from the release stake at many other sites including Dry Dock, Eureka Bar Downstream, Tenasillahe Island, and Wallace Island (Table 4; Figures 8 b,c,n, and o). Even though biocontrol agents are observed at some distance from the release stakes, the frequency

Table 4: Biocontrol Agent By Buffer. Percentage of quadrats with *Lythrum* where introduced *Galerucella* species were observed as either eggs, larvae, or adults. Results are presented by buffer, or distance away from release stake. Buffers cover the following distances: 0-10m from release stake (10m), 10-25m from stake (25m), 25-50m (50m), and 50-100m (100m). Cells with no data ("-") indicated that there were no quadrats located within that buffer.

	Buffer 10m					Buffer 25m				
Site	2006		2007			2006		2007		
	June	July	April	July	Aug.	June	July	April	July	Aug.
Devil's Elbow	75	67	33	0	33	50	33	67	-	80
Dry Dock	17	75	100	100	100	33	100	50	100	75
Eureka Bar Downstream	0	0	0	0	0	0	0	0	0	31
Eureka Bar Upstream	0	0	0	56	56	29	0	8	50	62
Fitzpatrick Island	0	10	0	0	0	0	0	0	0	18
Karlson Island	25	50	25	0	0	40	0	0	17	29
Marsh Island	25	33	0	0	0	25	80	-	0	0
Miller Sands Downstream	0	0	0	0	0	0	0	0	0	0
Miller Sands Upstream	20	0	60	0	0	0	10	10	0	0
Mott Island	0	0	0	0	0	0	0	0	0	0
Pillar Island Downstream	0	0	0	0	0	0	0	0	0	0
Pillar Island Upstream	80	57	50	60	50	23	12	9	14	0
Svensen Island	75	80	70	86	80	40	75	29	50	80
Tenasillahe Island	13	63	25	57	57	27	17	57	57	100
Wallace Island	0	100	83	40	75	50	40	11	45	100

Table 4: Continued										
Site	Buffer 50m					Buffer 100m				
	2006		2007			2006		2007		
	June	July	April	July	Aug.	June	July	April	July	Aug.
Devil's Elbow	100	0	-	50	0	-	-	-	-	-
Dry Dock	20	40	60	50	80	-	-	-	-	-
Eureka Bar Downstream	11	0	5	25	44	0	0	-	0	-
Eureka Bar Upstream	9	0	0	21	12	-	0	-	-	-
Fitzpatrick Island	0	0	0	0	0	-	-	0	-	0
Karlson Island	17	0	0	14	0	-	0	-	-	0
Marsh Island	0	25	0	25	0	-	-	-	-	-
Miller Sands Downstream	0	0	0	0	0	0	0	-	-	-
Miller Sands Upstream	0	8	0	0	0	0	-	-	-	-
Mott Island	17	0	0	0	0	0	-	-	-	-
Pillar Island Downstream	0	0	0	0	0	-	-	-	0	0
Pillar Island Upstream	10	0	0	0	4	0	-	0	0	-
Svensen Island	63	36	40	56	60	57	50	-	-	-
Tenasillahe Island	17	25	71	33	0	0	-	-	-	-
Wallace Island	13	43	29	43	75	-	-	-	0	-

Figure 8 a- o. Locations of quadrats with biocontrol agents present for each of the 15 USCAE release sites. Shown are quadrats from each sampling event (April, July and August 2007),Gray= No *Galerucella* adults observed, Red= *Galerucella* adults observed, Circles represent 0-10m, 10-25m, 25-50m and 50-100m buffer areas around release stakes. Background image are CIR photographs. See text for details.


Devil's Elbow
April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adults observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
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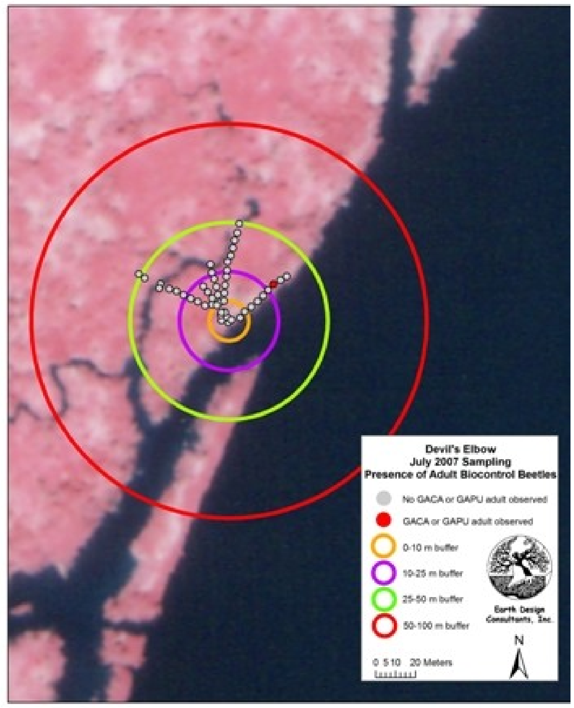
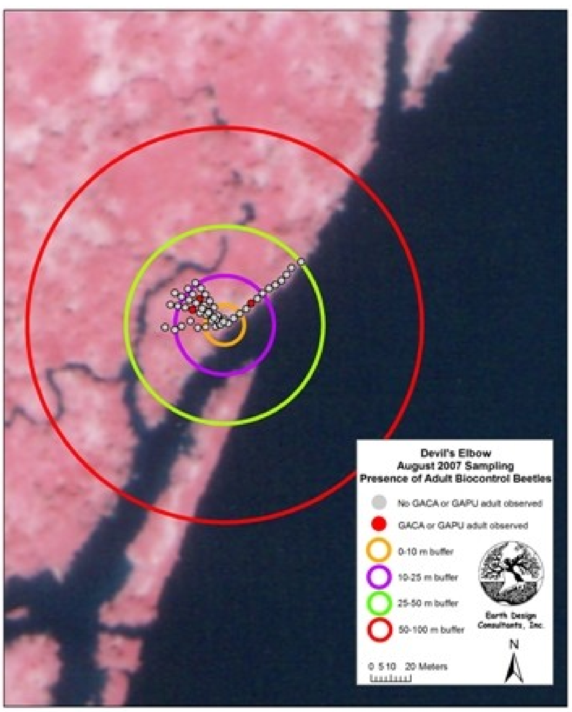

Devil's Elbow
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Devil's Elbow
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
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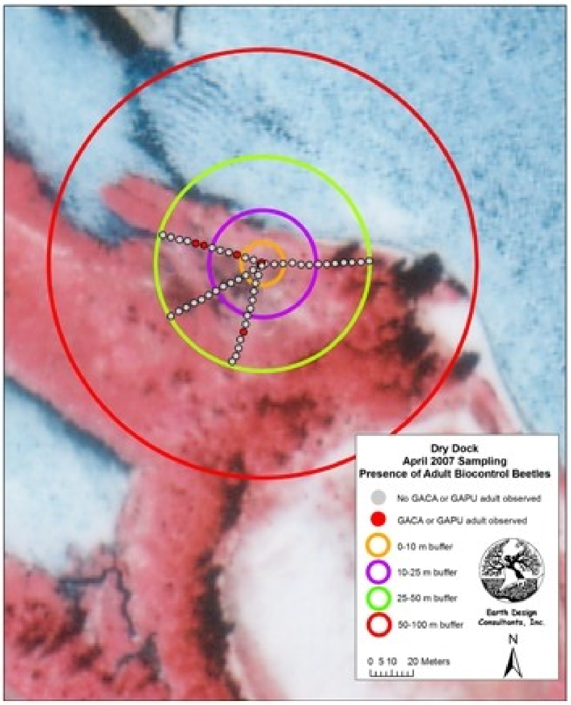
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

A



Dry Dock
April 2007 Sampling
Presence of Adult Biocontrol Beetles
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GACA or GAPU adult observed
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10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
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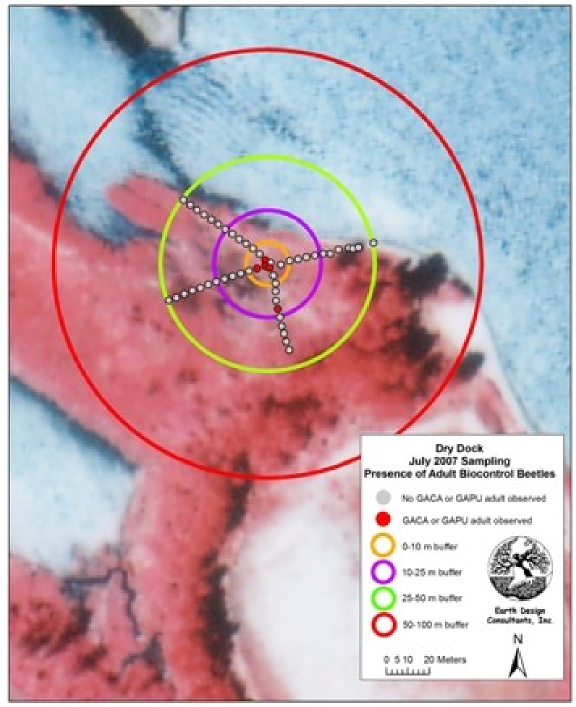
0 5 10 20 Meters



Dry Dock
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
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10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
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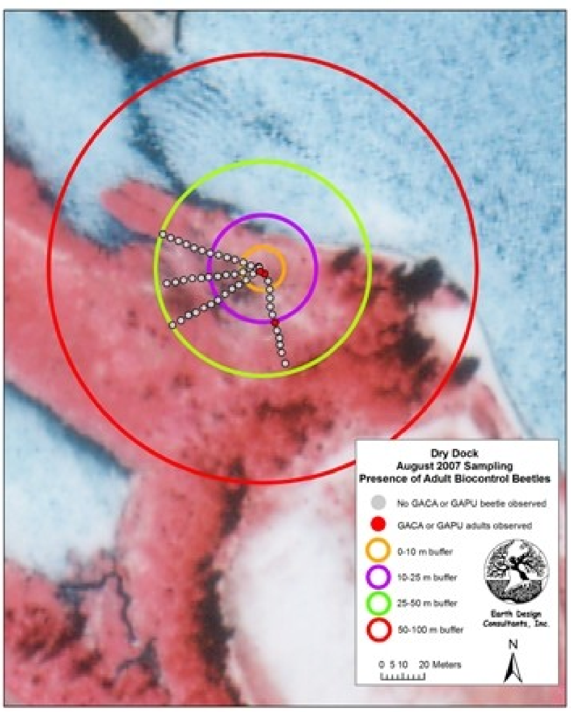

Dry Dock
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU beetle observed
GACA or GAPU adults observed
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25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
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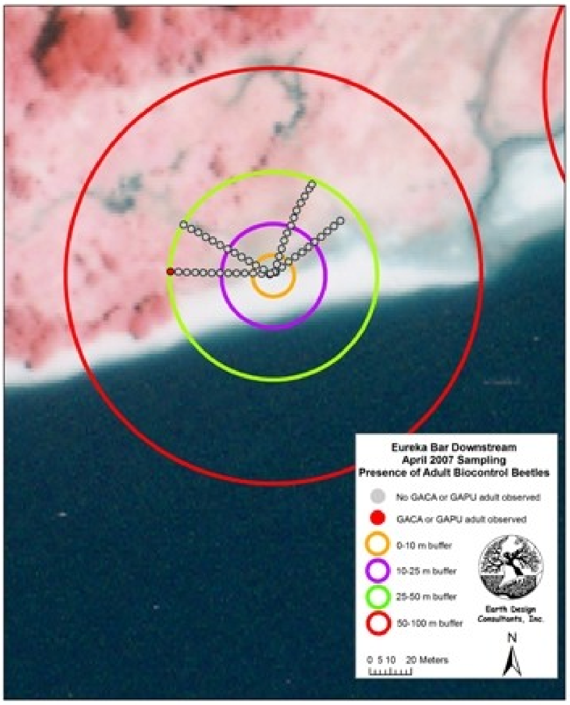
B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April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
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Earth Design Consultants, Inc.
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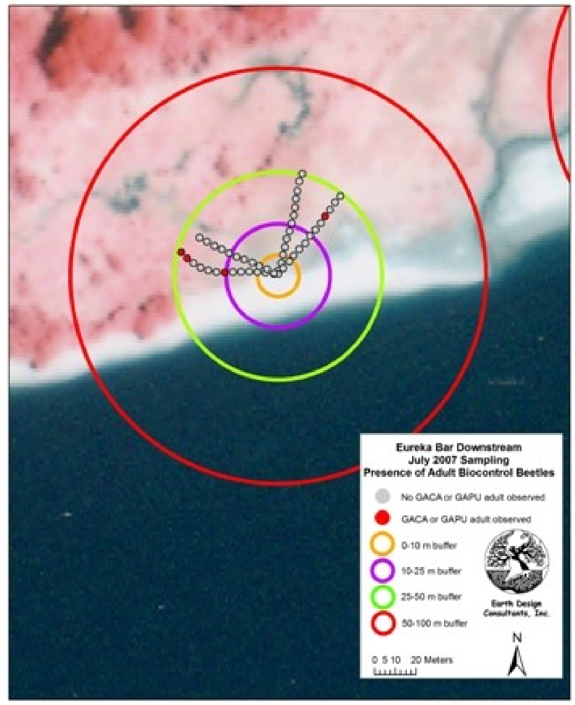
Eureka Bar Downstream
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
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Earth Design Consultants, Inc.
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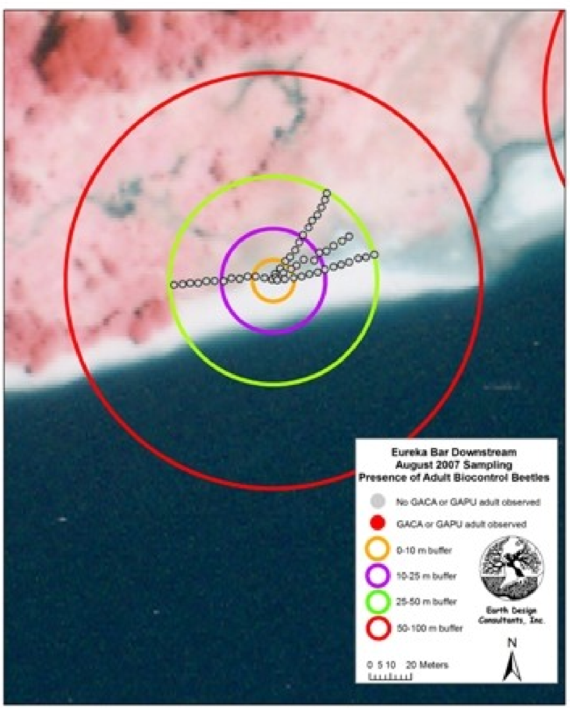
Eureka Bar Downstream
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
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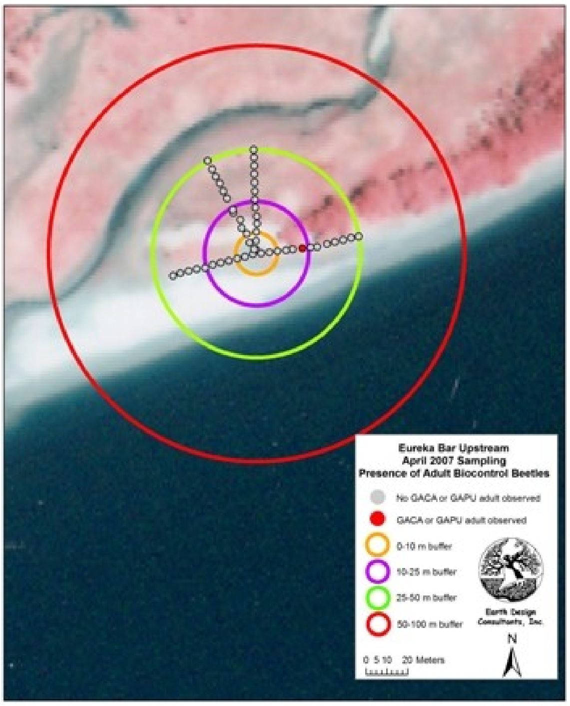
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

C


Eureka Bar Upstream
April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
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25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
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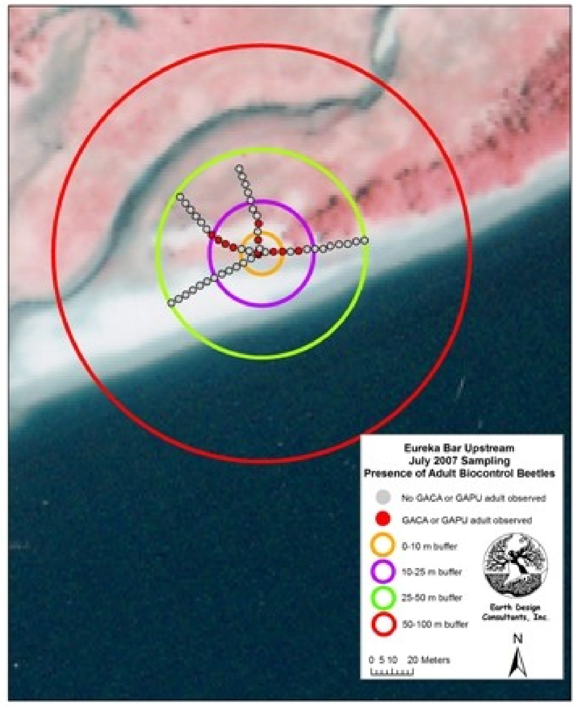
Eureka Bar Upstream
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
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25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
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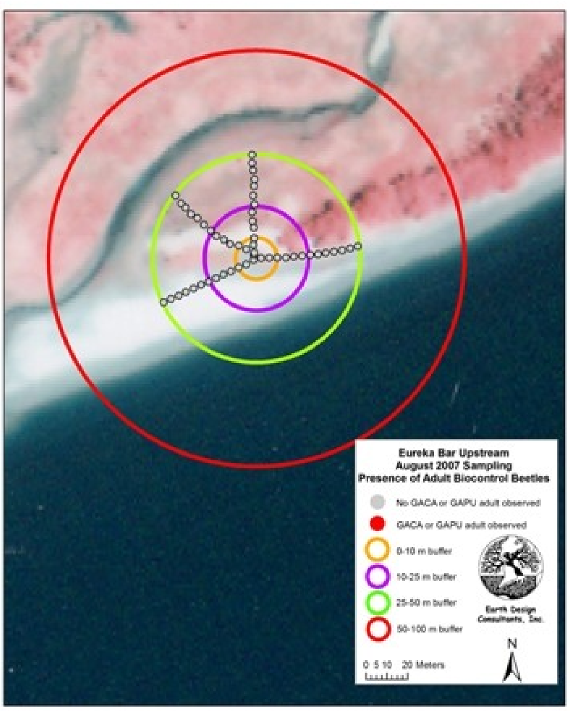
0 5 10 20 Meters


Eureka Bar Upstream
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
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Earth Design Consultants, Inc.
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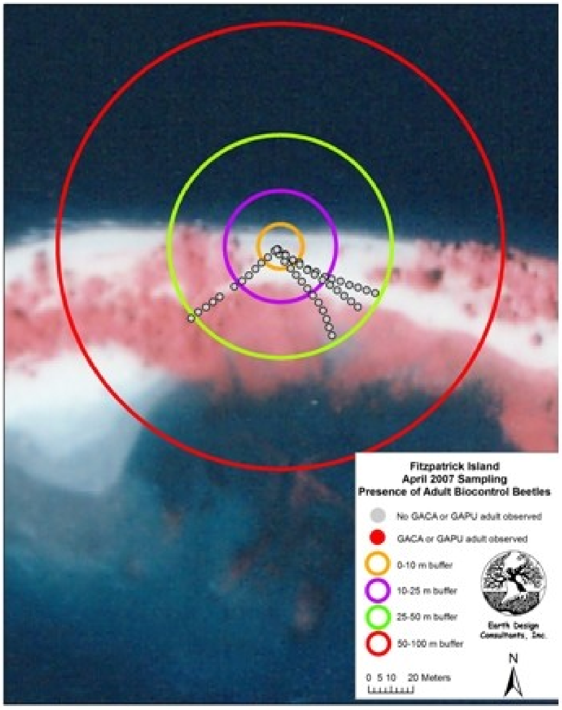
Fitzpatrick Island
April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
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Earth Design Consultants, Inc.
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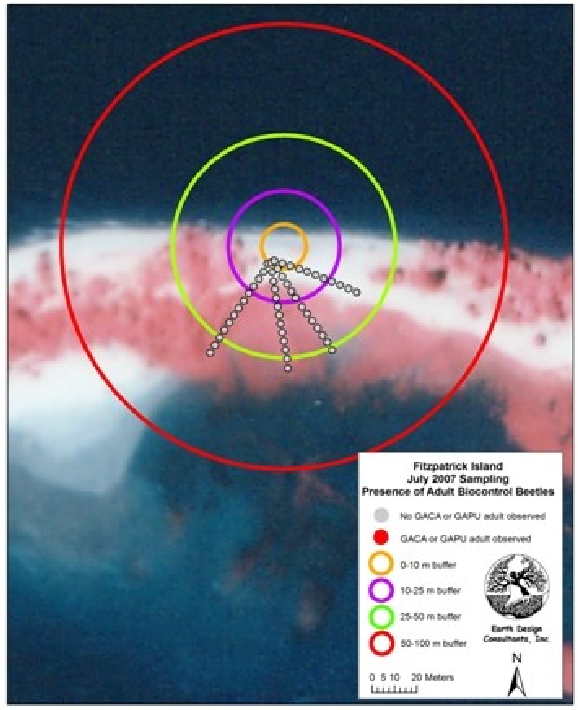
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Presence of Adult Biocontrol Beetles
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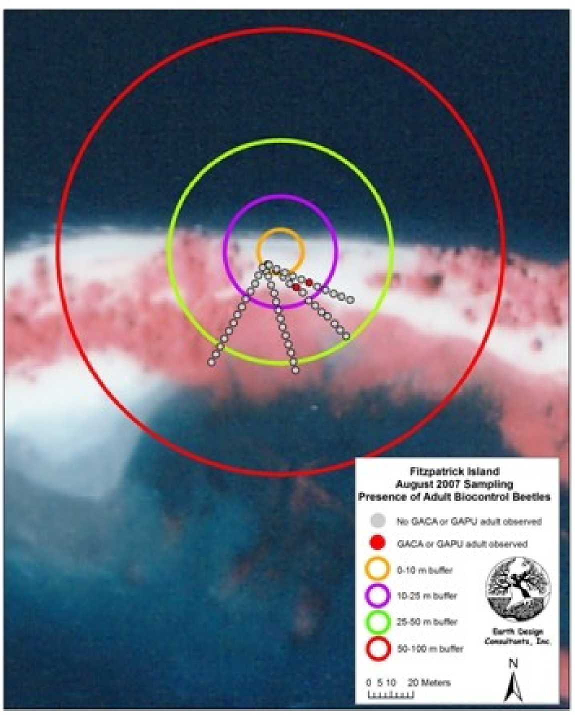
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August 2007 Sampling
Presence of Adult Biocontrol Beetles
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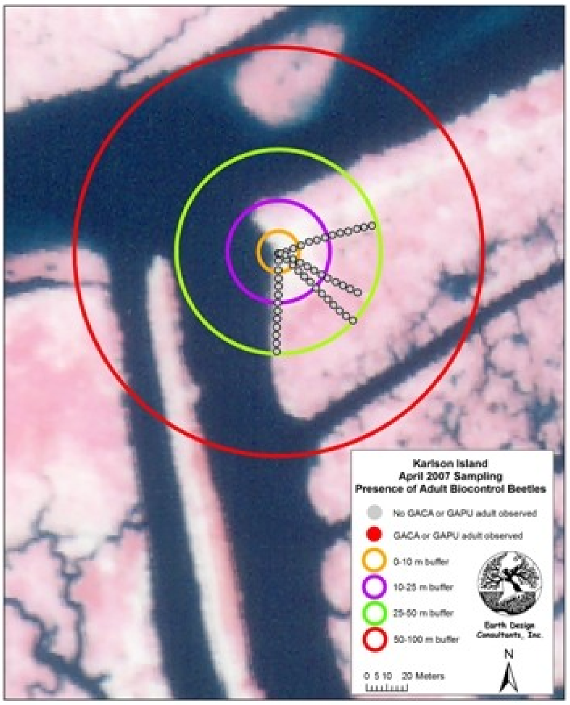
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Presence of Adult Biocontrol Beetles
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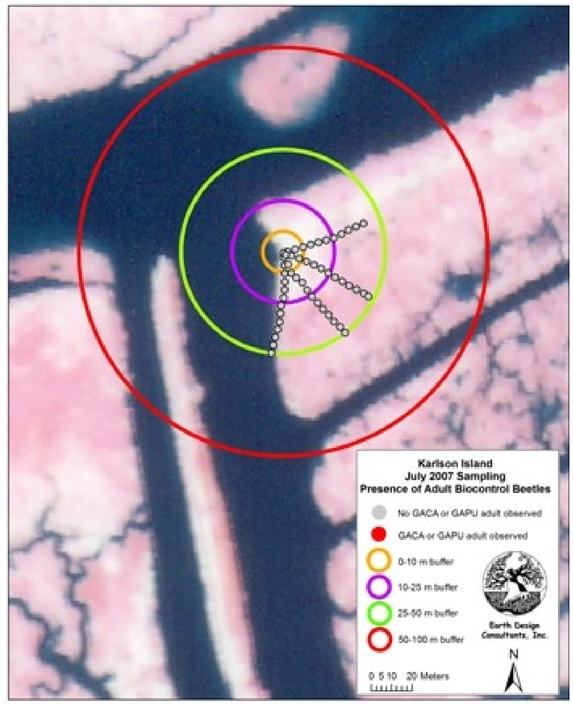
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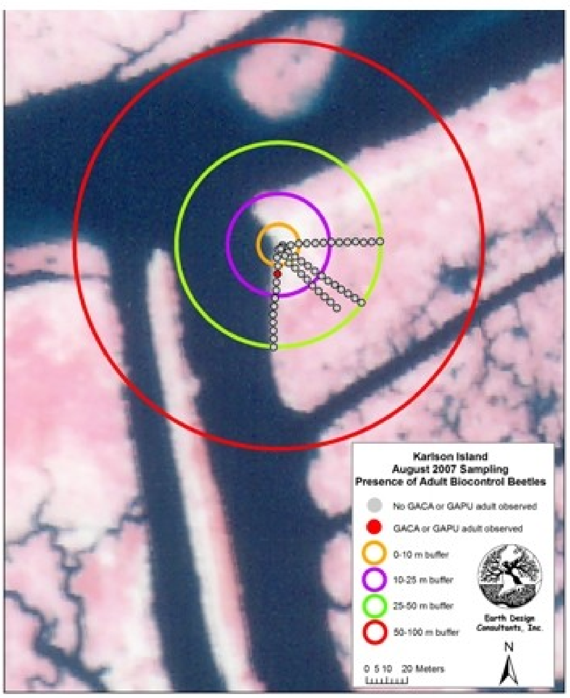
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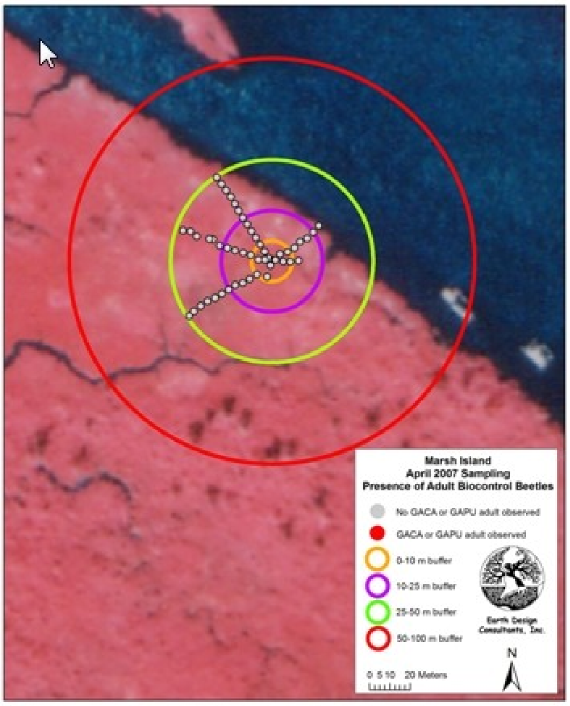
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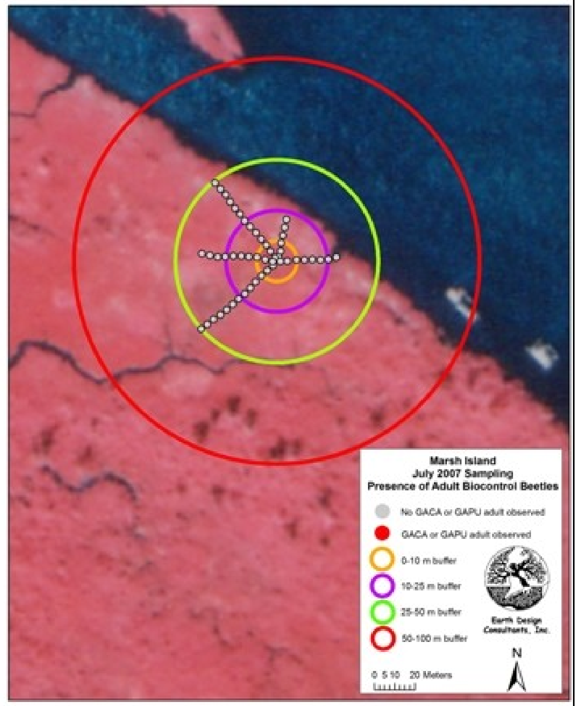
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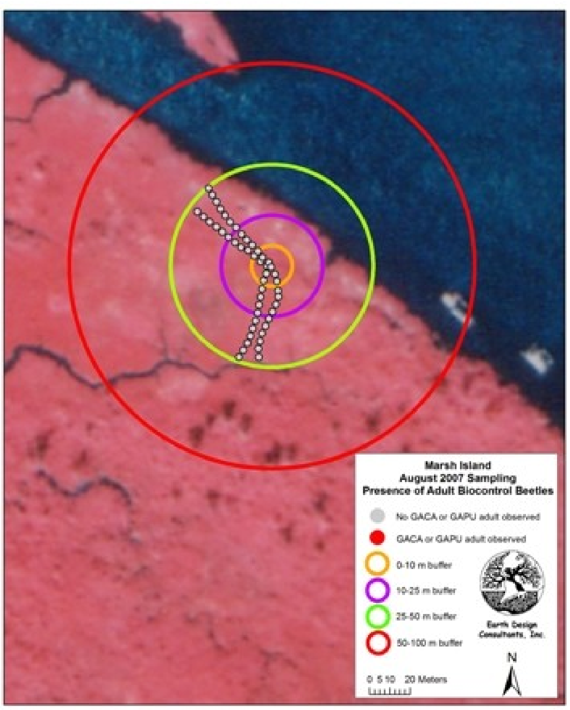
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Presence of Adult Biocontrol Beetles
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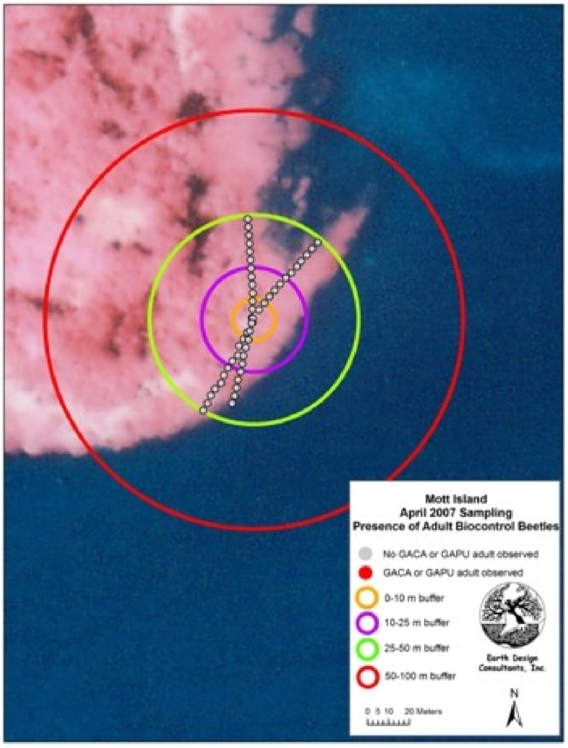
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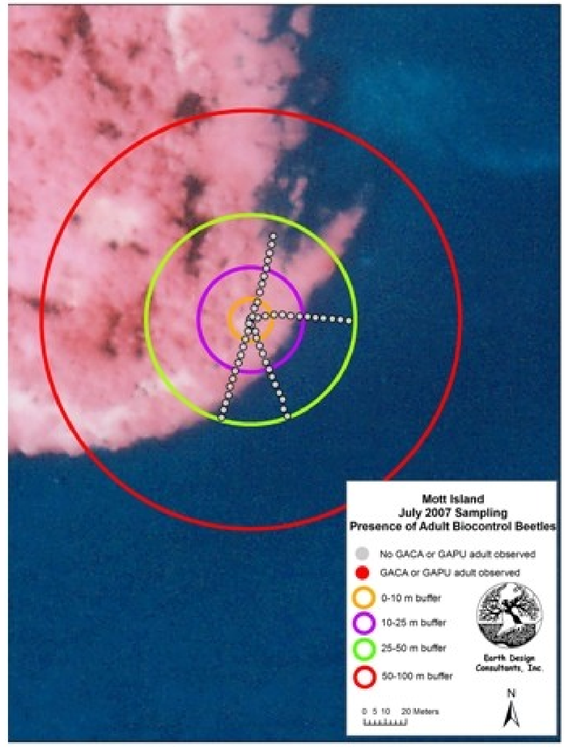
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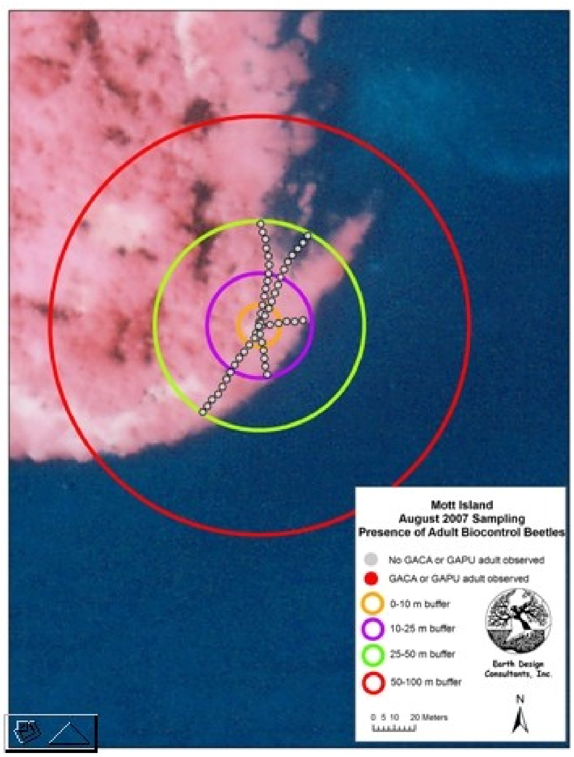
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0 5 10 20 Meters
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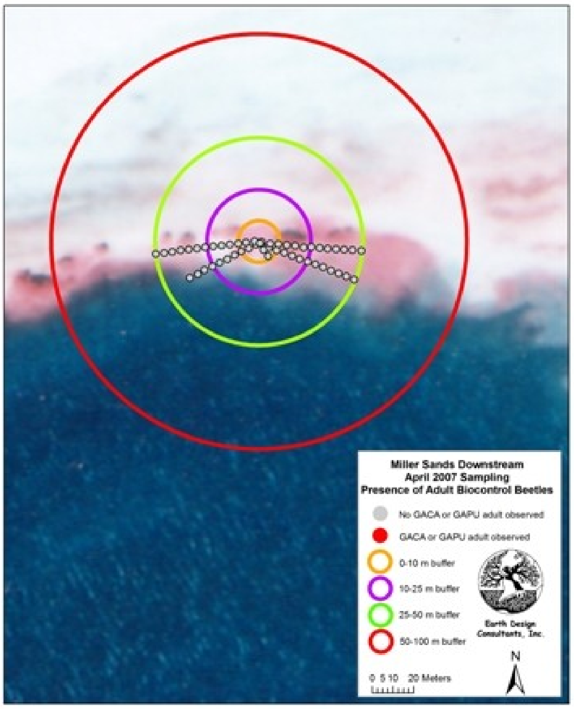
Miller Sands Downstream
April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
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Earth Design Consultants, Inc.
0 5 10 20 Meters
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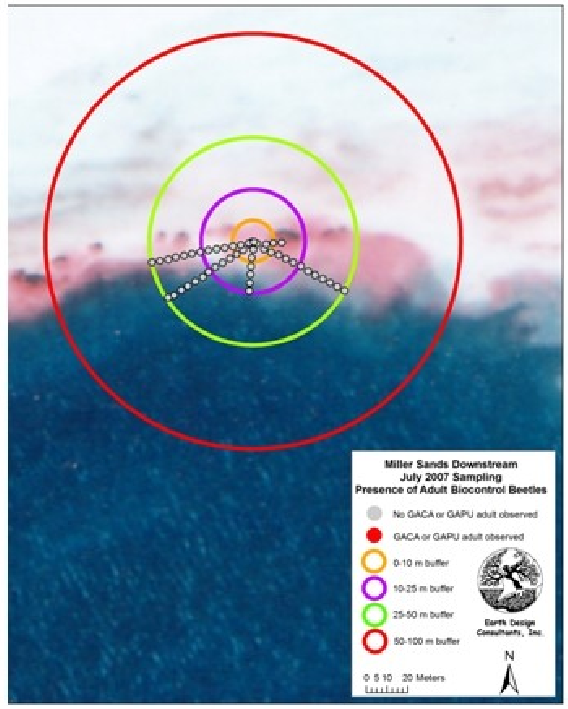
Miller Sands Downstream
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
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Earth Design Consultants, Inc.
0 5 10 20 Meters
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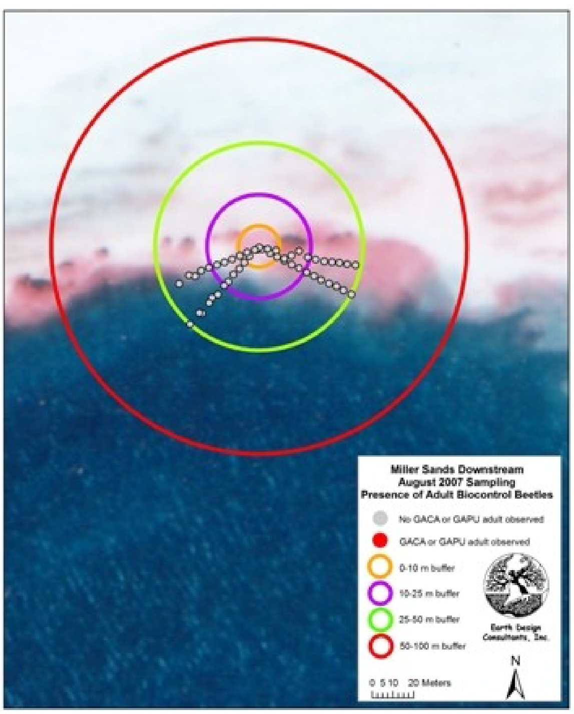
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August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
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25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N

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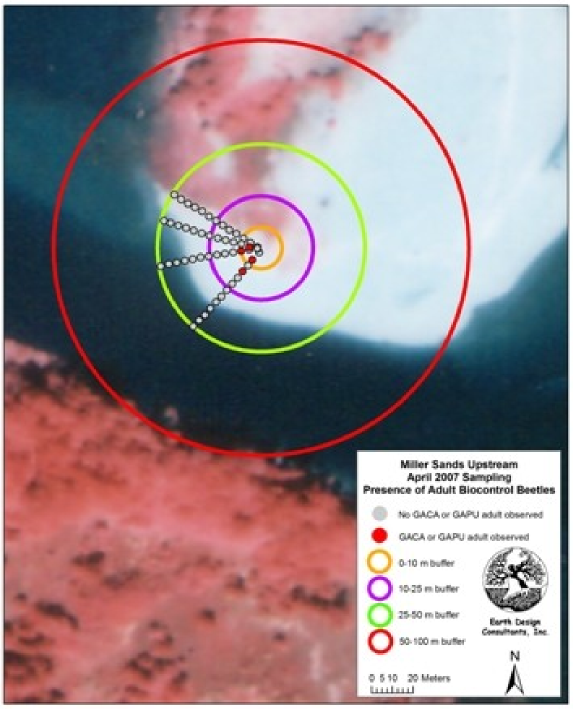
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April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
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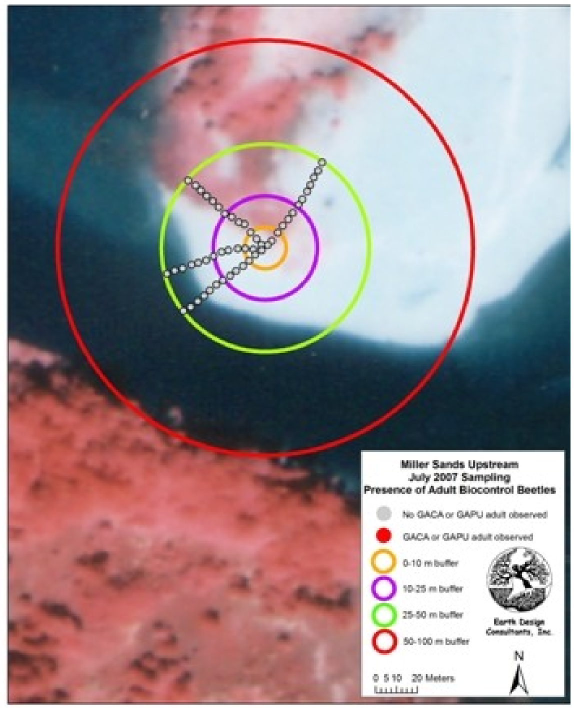
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Miller Sands Upstream
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
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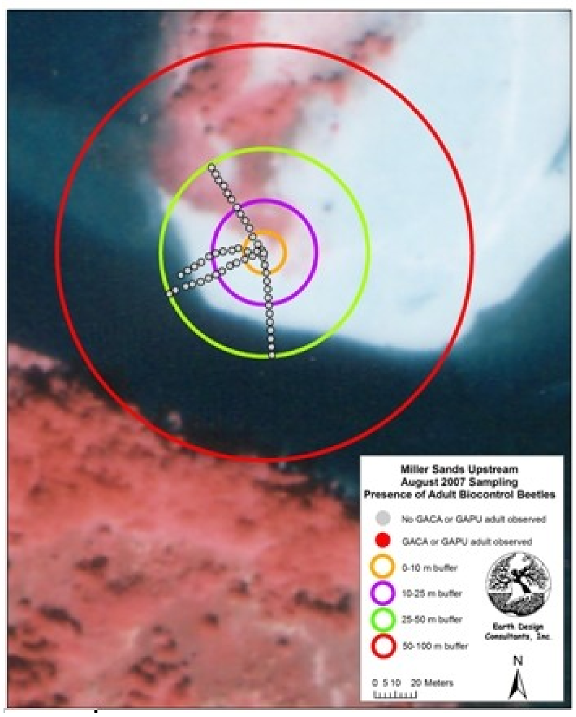
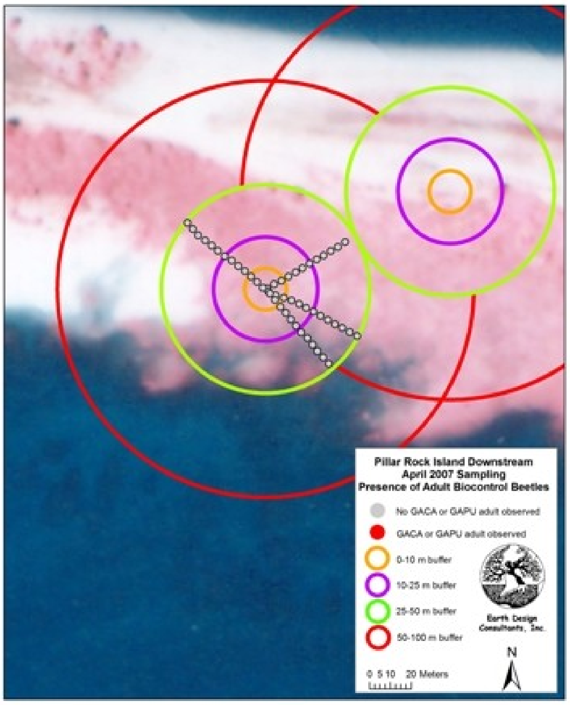

Miller Sands Upstream
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

J


Pillar Rock Island Downstream
April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
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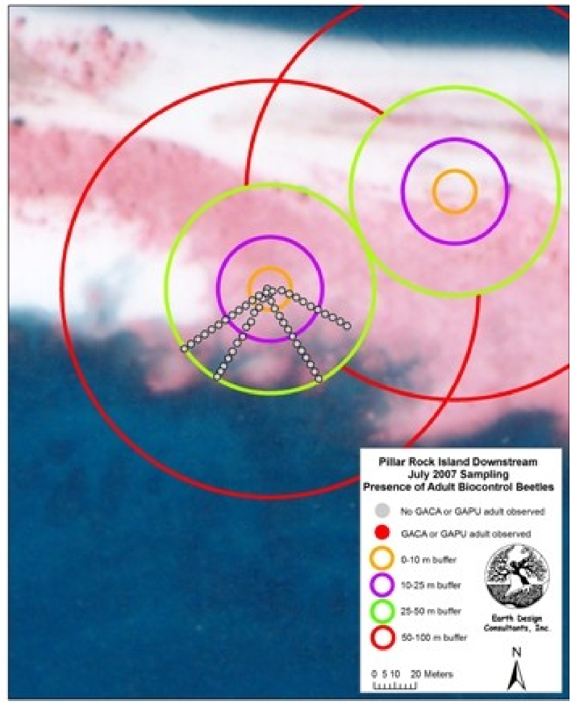
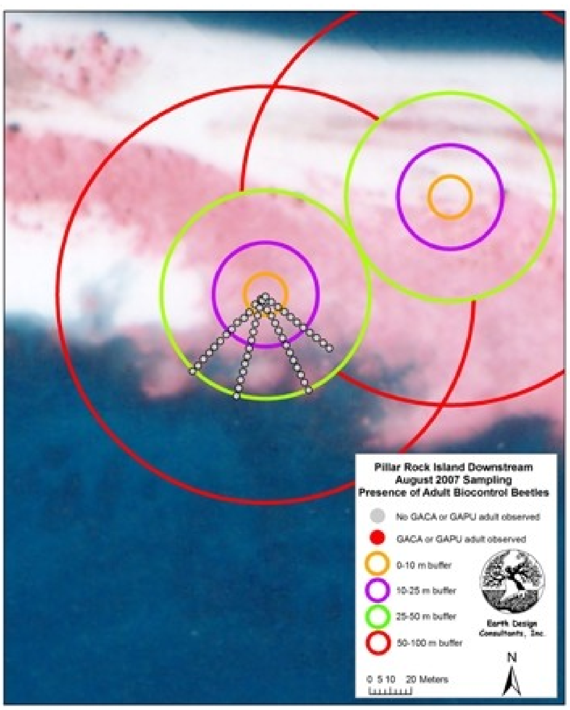
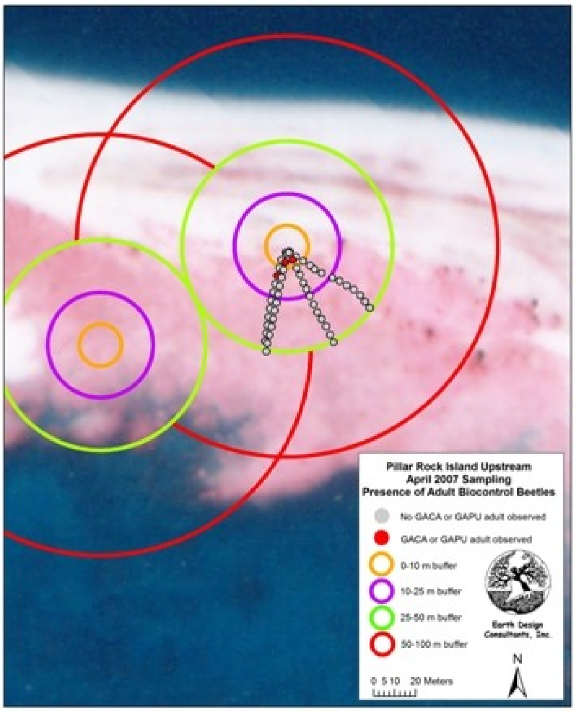

Pillar Rock Island Downstream
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N


Pillar Rock Island Downstream
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N

K


Pillar Rock Island Upstream
April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
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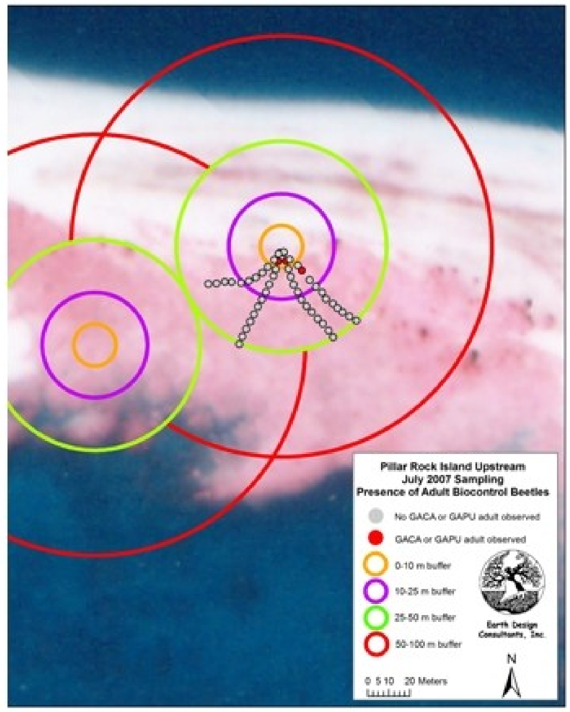
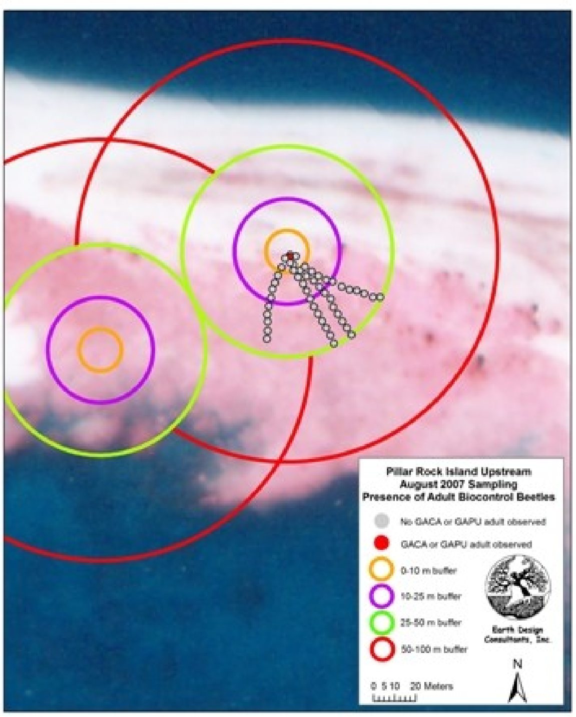
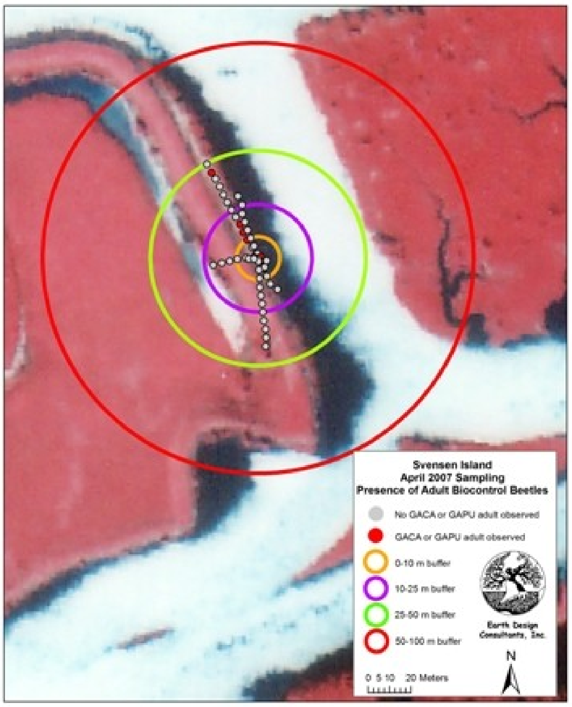
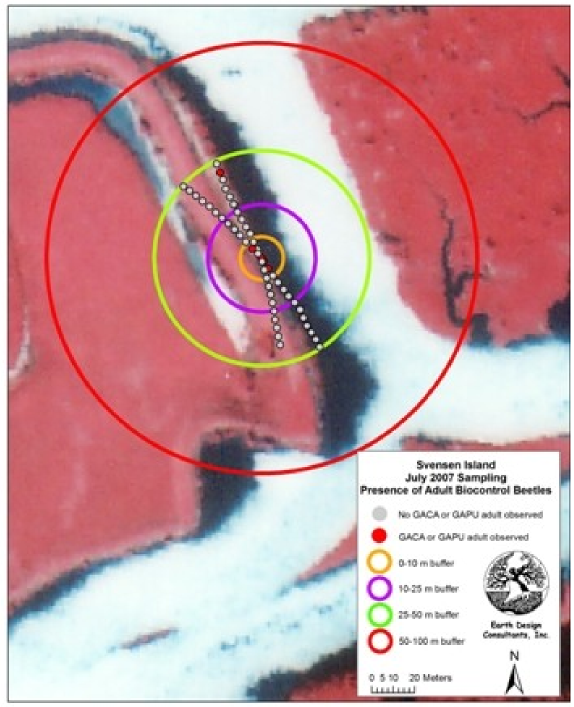
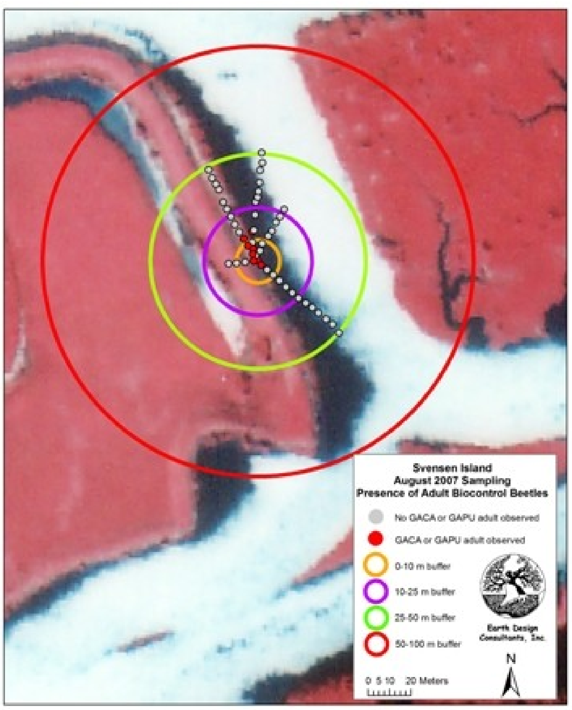

Pillar Rock Island Upstream
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Pillar Rock Island Upstream
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

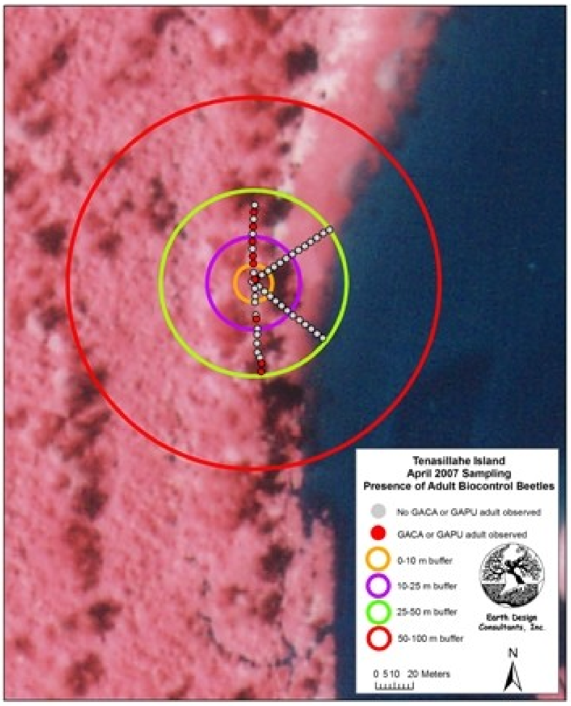
L

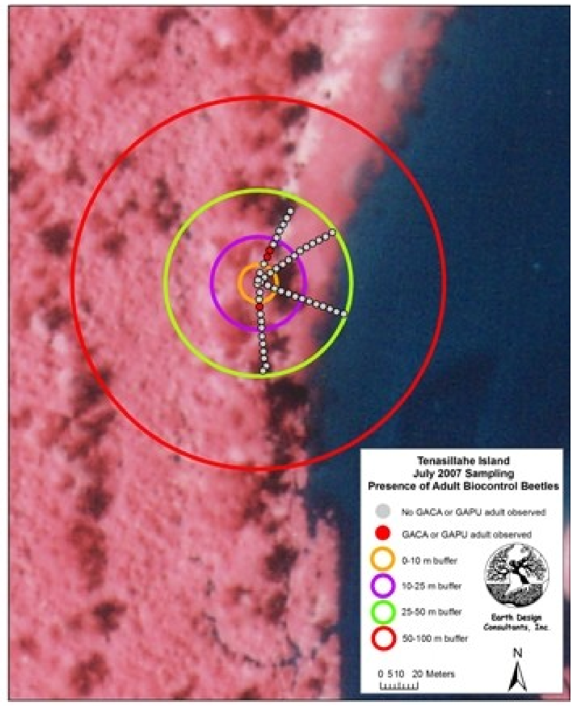

Svensen Island
April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N

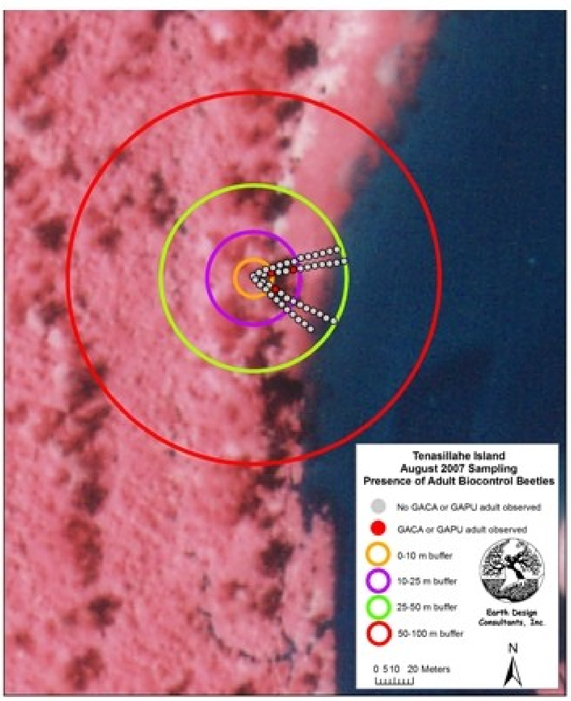

Svensen Island
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N


Svensen Island
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N

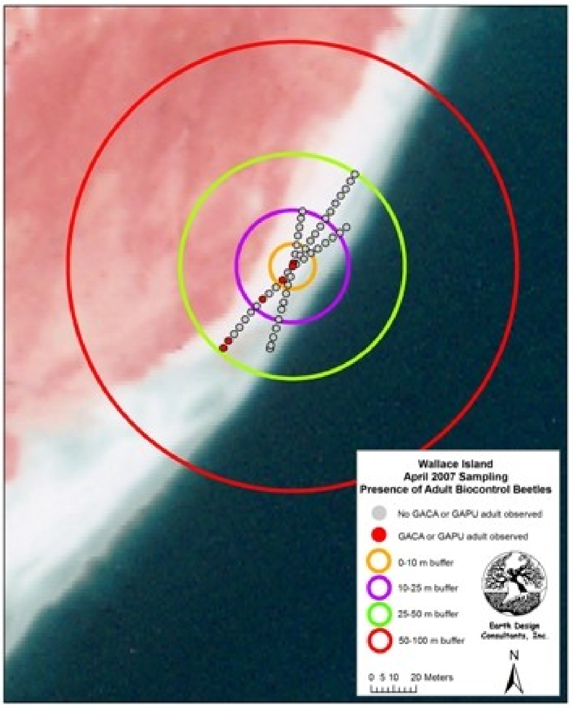
M

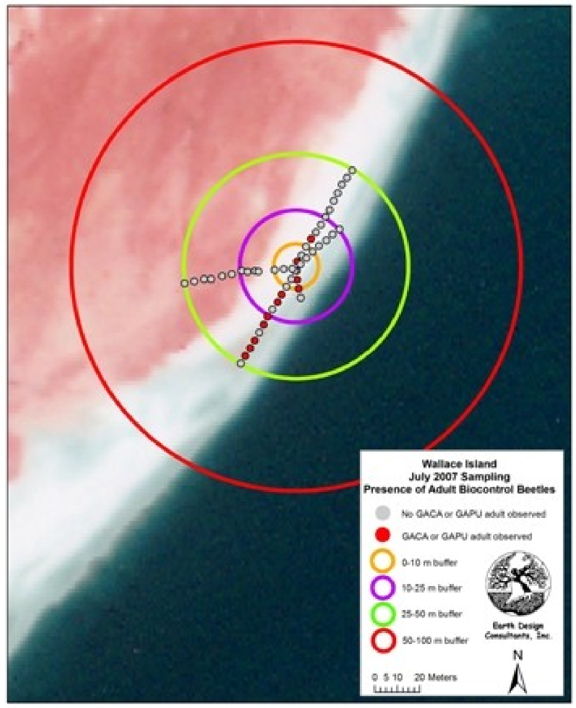

Tenasillahe Island
April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

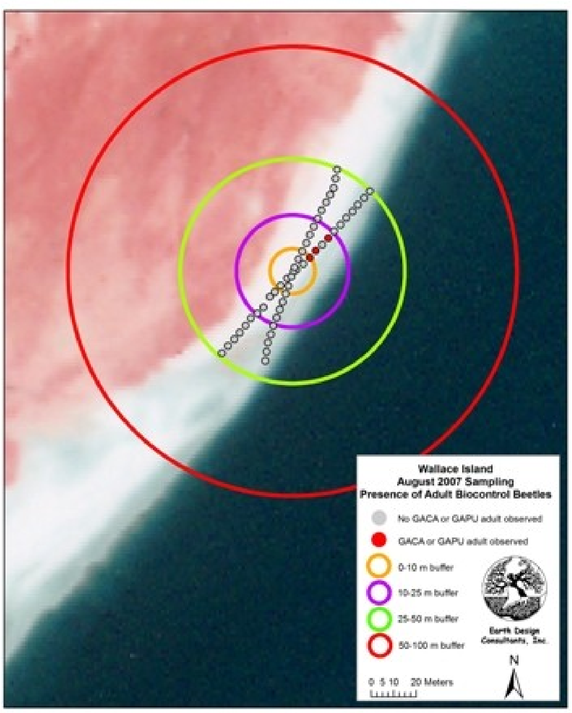

Tenasillahe Island
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Tenasillahe Island
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

N


Wallace Island
April 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Wallace Island
July 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Wallace Island
August 2007 Sampling
Presence of Adult Biocontrol Beetles
No GACA or GAPU adult observed
GACA or GAPU adult observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

O

of observation of agents is still higher closer to the release point. For example, as many as 100% of quadrats with *Lythrum* at the Dry Dock site had evidence of biocontrol agents in the 0-10m and 10-25m buffers (Table 3; Figure 8b).

It is still not clear what factors are affecting the distribution of biocontrol agents within a site from year to year. At several sites, the beetles appear to be moving away from the release stake. We recommend that a more systematic survey be completed at key sites to reveal distributional patterns within a site. Our current sampling method was not designed to measure these patterns. Moreover, as sampling continues during the next few years, we expect to develop a better understanding of the factors that affect the distribution and abundance of both biocontrol agents and *Lythrum* within the tidal marshes of the LCRE.

3.1.5 Beetle Feeding Damage

Along with presence of adult, larvae, and eggs of biocontrol agents, we also recorded damage on *Lythrum* plants within our quadrats. During the 2007 season, we observed damage on *Lythrum* at all sites but Miller Sands Downstream and Pillar Island Downstream (Table 2 a). We observed damage at Miller Sands Downstream and Pillar Island Downstream during 2006 but it can sometimes be difficult to determine if this damage is due solely to *Galerucella* when the beetles themselves are not present. Feeding damage due to *Galerucella* is typically recognizable and distinct to this biocontrol agent. However, some of the damage observed in 2006 may have been due to another herbivore or made by *Galerucella* individuals from the initial 2005 release. Figures 9 a-n show the distribution of quadrats sampled in 2007 with and without *Galerucella* feeding damage at each study site. As seen with the distribution of beetles (Figure 8), evidence of damage is also well-dispersed along transects and not solely concentrated around the stake. At several sites, we observed feeding damage at the ends of transects, around 50m from the release point.

The number of quadrats with *Galerucella* damage has not changed dramatically at any site from 2006 to 2007 (Table 5). This is not to say that the beetles are not feeding on plants but simply suggests that beetle populations may not yet be large enough to cause widespread damage to *Lythrum* populations or be resource limited. The largest increase in observations of *Galerucella* damage was at Eureka Bar Upstream site (Table 5). Interestingly, we observed damage at the Fitzpatrick Island site in August 2007 when we hadn't during previous sampling events (Table 5). We, however, recognize that there

Table 5. Percent of Quadrats with Feeding Damage. The first column within a sampling event gives the percentage of all quadrats sampled that contain damage from Galerucella. The second column presents the percentage of only Lythrum infested quadrats that had damage from Galerucella present.										
Site	**June 2006**		**July 2006**		**April 2007**		**July 2007**		**August 2007**	
Devils Elbow	**14**	**70**	**10**	**45**	**8**	**44**	**4**	**29**	**12**	**67**
Dry Dock	**22**	**55**	**30**	**83**	**12**	**40**	**16**	**100**	**24**	**92**
Eureka Bar Downstream	**6**	**7**	**0**	**0**	**2**	**2**	**14**	**14**	**16**	**21**
Eureka Bar Upstream	**22**	**23**	**10**	**11**	**2**	**2**	**56**	**76**	**38**	**51**
Fitzpatrick Island	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**14**
Karlson Island	**16**	**42**	**6**	**15**	**4**	**12**	**6**	**18**	**14**	**29**
Marsh Island	**13**	**47**	**16**	**63**	**0**	**0**	**10**	**56**	**4**	**17**
Miller Sands Downstream	**2**	**4**	**2**	**3**	**0**	**0**	**0**	**0**	**0**	**0**
Miller Sands Upstream	**11**	**15**	**10**	**17**	**6**	**10**	**0**	**0**	**0**	**0**
Mott Island	**4**	**10**	**10**	**21**	**2**	**5**	**6**	**20**	**6**	**13**
Pillar Island Downstream	**2**	**3**	**2**	**3**	**0**	**0**	**0**	**0**	**0**	**0**
Pillar Island Upstream	**38**	**48**	**28**	**33**	**10**	**12**	**16**	**18**	**32**	**36**
Svensen Island	**39**	**83**	**30**	**54**	**24**	**55**	**30**	**75**	**32**	**80**
Tenasillahe Island	**46**	**82**	**32**	**89**	**26**	**59**	**38**	**95**	**12**	**67**
Wallace Island	**30**	**50**	**24**	**60**	**16**	**36**	**34**	**55**	**34**	**85**
All Sites	**18**	**32**	**14**	**25**	**7**	**15**	**15**	**32**	**15**	**32**

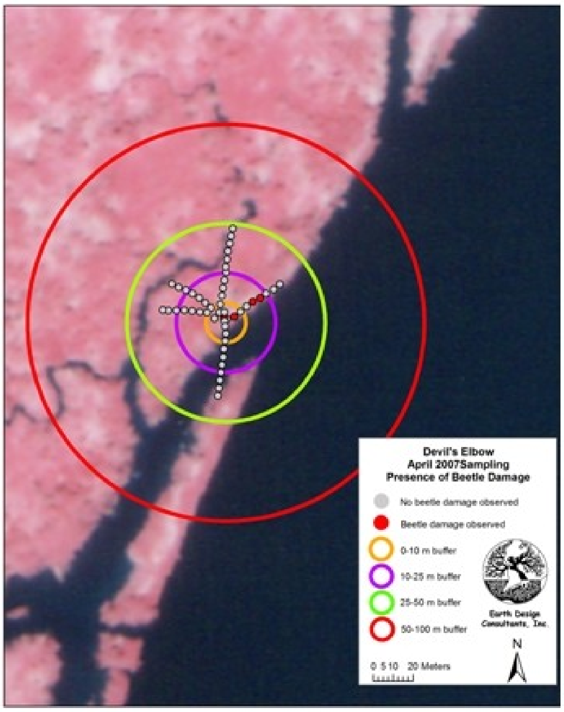
were other releases made on Fitzpatrick and the damage may agents from these earlier releases (Schooler and Garono 2002).

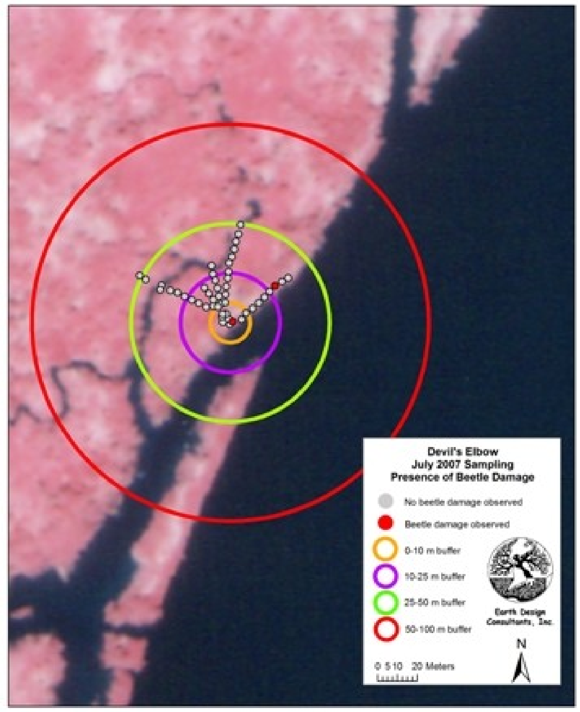
Besides simply presence or absence of damage due to *Galerucella*, we also quantified the amount of damage by estimating the average percent damage across all leaves on all plants within each quadrat. The average, range, and total percent damage for each site during each sampling event is presented in Table 6. As we mentioned before, no damage or biocontrol agents were observed at Miller Sands Downstream or Pillar Island Downstream sites during 2007 and very little, if any damage at these sites in 2006. The highest levels of damage were observed at Dry Dock and Svensen Island (Figures 9 b, m; Table 6). Levels were also quite high at Devil's Elbow, Eureka Bar Upstream, Tenasillahe Island, and Wallace Island (Table 6). As expected, damage levels were highest at the end of the summer during our August sampling event. We believe this is because by the end of the summer the plants reflect cumulative damage by both larvae and adult stages, and multiple generations of *Galerucella* beetles. Amounts of feeding damage observed in 2007 are generally similar to those in 2006, especially for the direct comparison between July samples. Slight fluctuations in the data are normal and can be affected by differences in weather and the influence of that on timing of life stages, as well as the exact location of transects and quadrats in relation to *Lythrum* and *Galerucella* populations at each site. When we examine the trends in damage alongside cumulative growing degree day (Figure 5), we don't see as clear of a pattern as with stem length (Figuer 6). At some sites damage is generally higher later in the season for reasons described above. For other sites, damage frequency does not appear to be as closely tied to the climate.

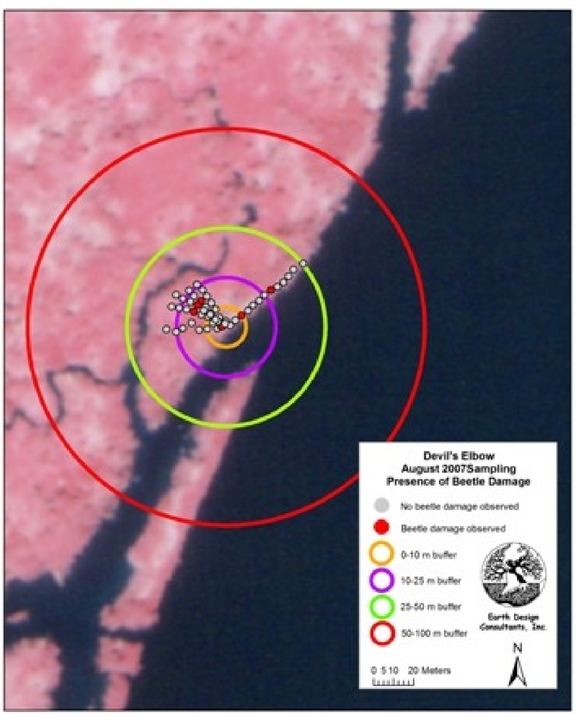
In addition to leaf damage, we recorded the presence of damage to the primary and secondary apical meristems of *Lythrum* plants within each quadrat. We refer to this damage as primary and secondary tip damage. Figures 10 a and b show the frequency of primary and secondary tip damage at each site for all three sampling events in 2007. Tip damage was rare in April and then increased throughout the summer. In fact, we observed primary tip damage at all sites in August. However, tip damage is simply recorded as present or absent on plants and may not be due to *Galerucella*. Tip damage can be caused by *Nanophyes* and by many other herbivores. Therefore, presence of tip damage should be considered along with the other measures indicating presence of *Galerucella*. Regardless of the causal agent, tip damage is of significant interest because it prevents flowering and seed production, therefore possibly reducing the colonization of new areas. We would expect observations of tip damage to increase through the summer, as we observed, because all life stages of *Galerucella* have been present, active, and feeding. This increase over the summer is similar to the trend we observed in leaf damage described above.

We recorded an additional field measure this year to begin to understand if damage from *Galerucella* is occurring evenly across a plant or if it's clustered near the top of the plant. To do this, we recorded the average percent damage across leaves in the bottom,

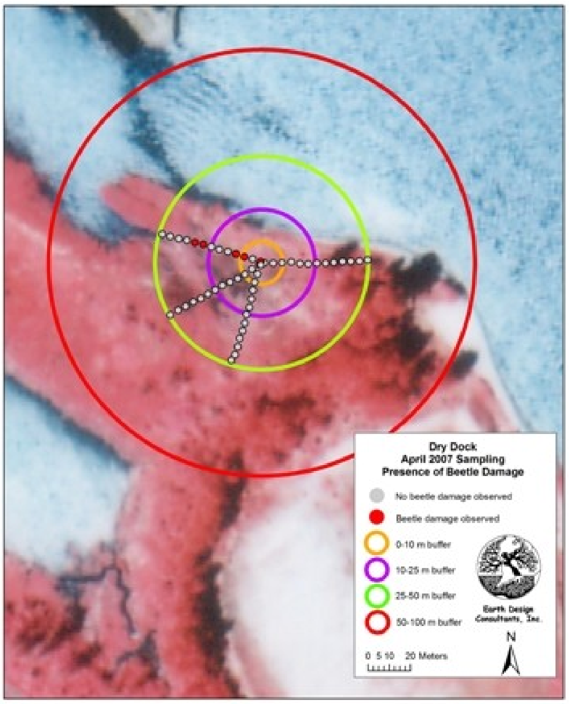
Figure 9 a-o. Location of quadrats from each of the 15 USACE release sites that have *Galerucella* feeding damage. Shown are quadrats from each sampling event (April, July and August 2007). Gray= No *Galerucella* adults observed, Red= *Galerucella* adults observed, Circles represent 0-10m, 10-25m, 25-50m and 50-100m buffer areas around release stakes. Background images are CIR photographs. See text for details.

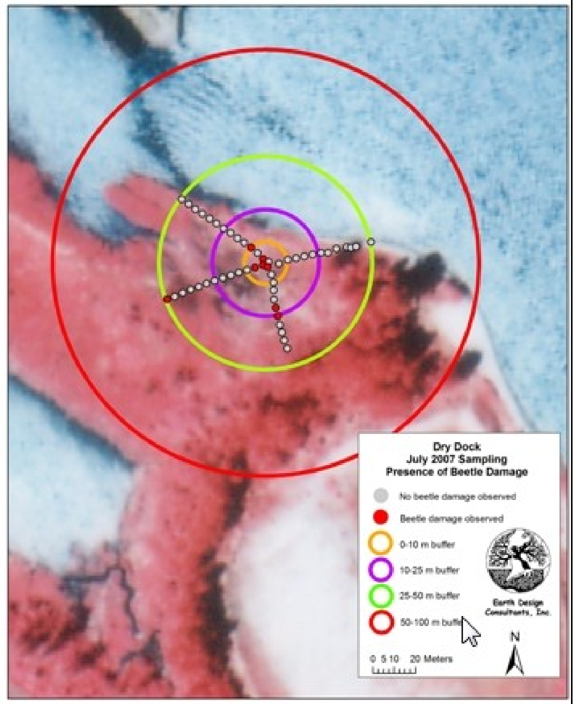

Devil's Elbow
April 2007Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

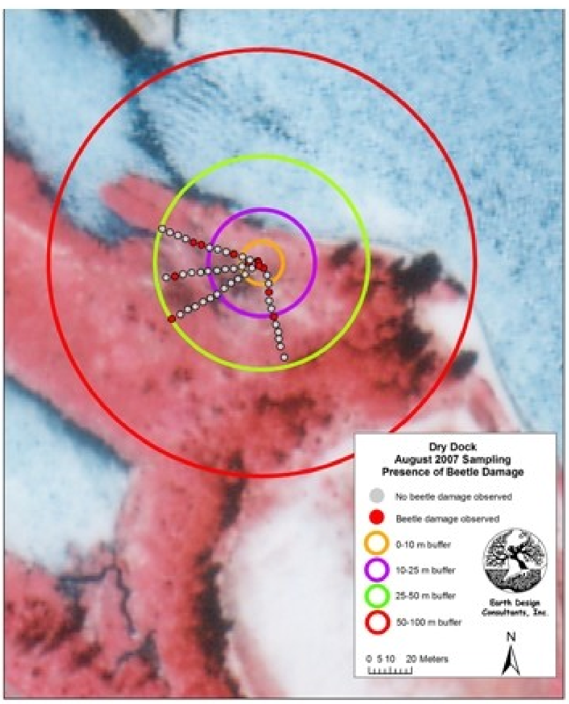

Devil's Elbow
July 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Devil's Elbow
August 2007Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

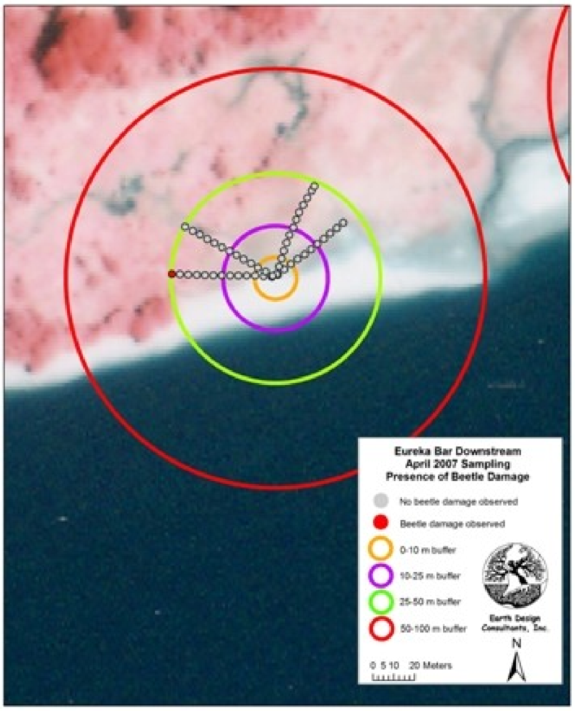
A

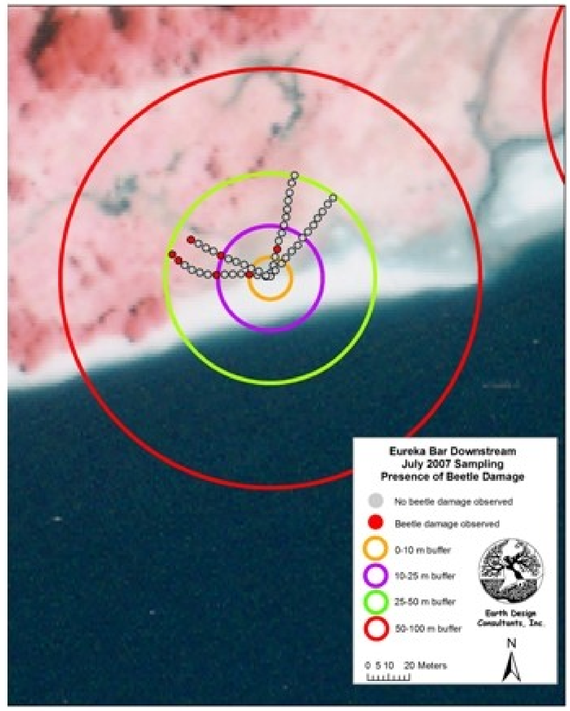

Dry Dock
April 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N

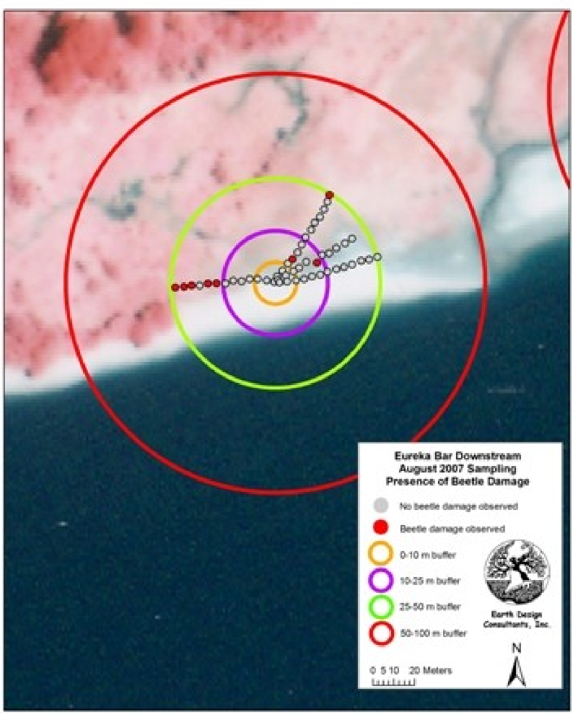

Dry Dock
July 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N


Dry Dock
August 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N

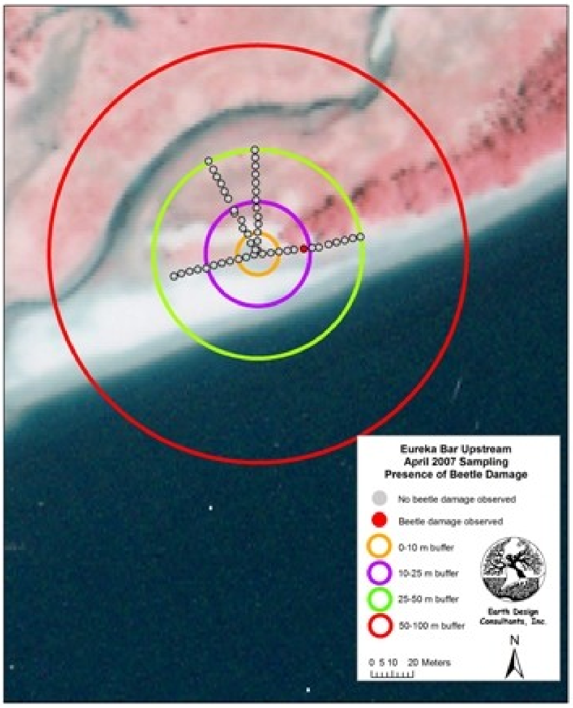
B

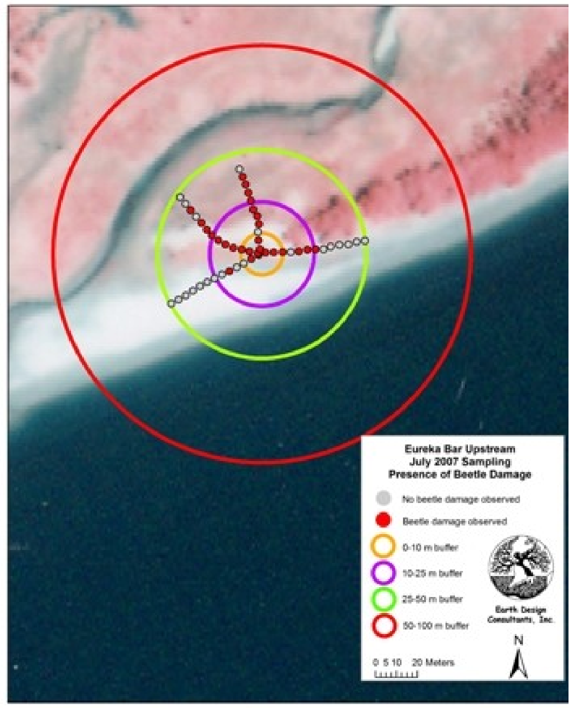

Eureka Bar Downstream
April 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

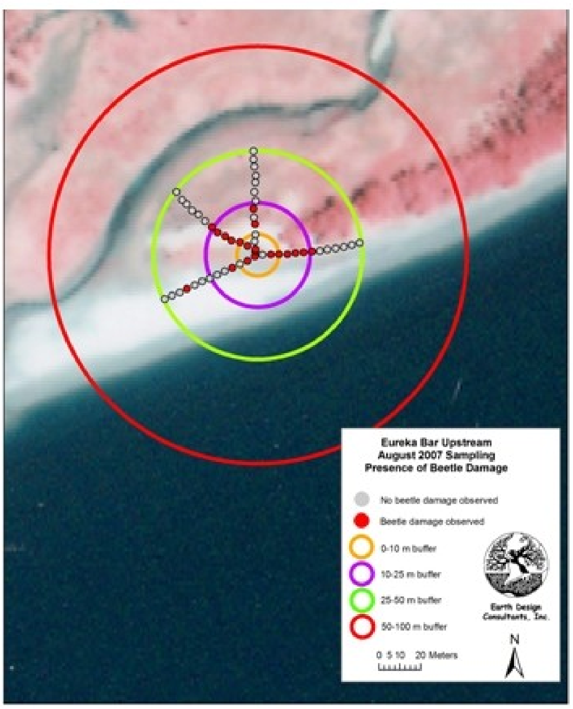

Eureka Bar Downstream
July 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Eureka Bar Downstream
August 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters

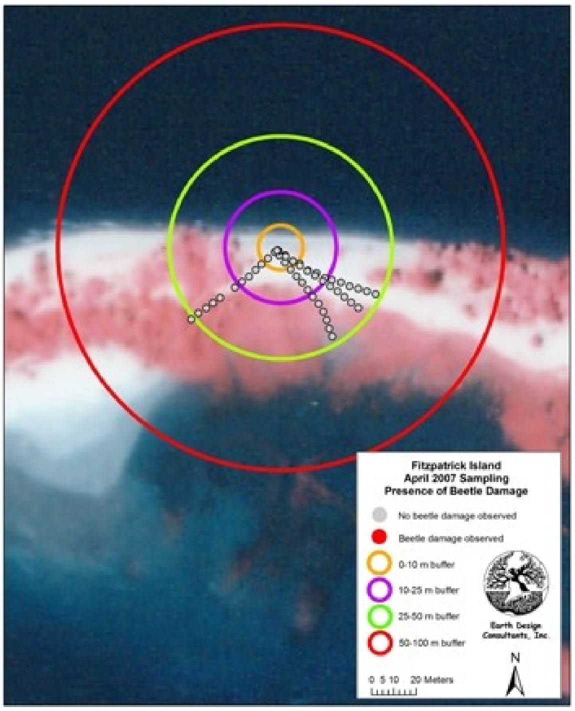
C

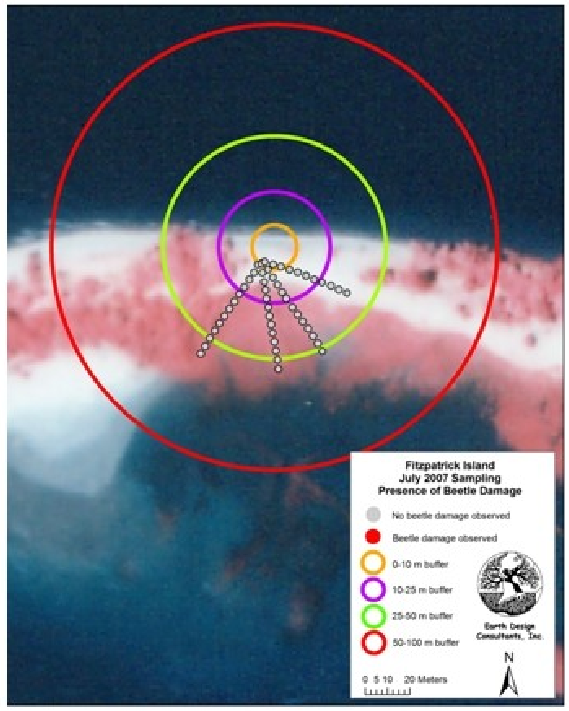

Eureka Bar Upstream
April 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N

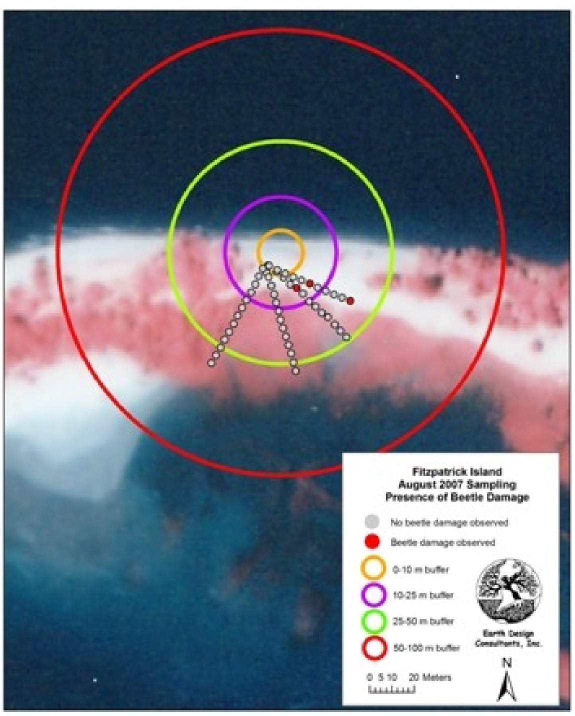

Eureka Bar Upstream
July 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N


Eureka Bar Upstream
August 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
0 5 10 20 Meters
N

D


Fitzpatrick Island
April 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Fitzpatrick Island
July 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
0-10 m buffer
10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
0 5 10 20 Meters


Fitzpatrick Island
August 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
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10-25 m buffer
25-50 m buffer
50-100 m buffer
Earth Design Consultants, Inc.
N
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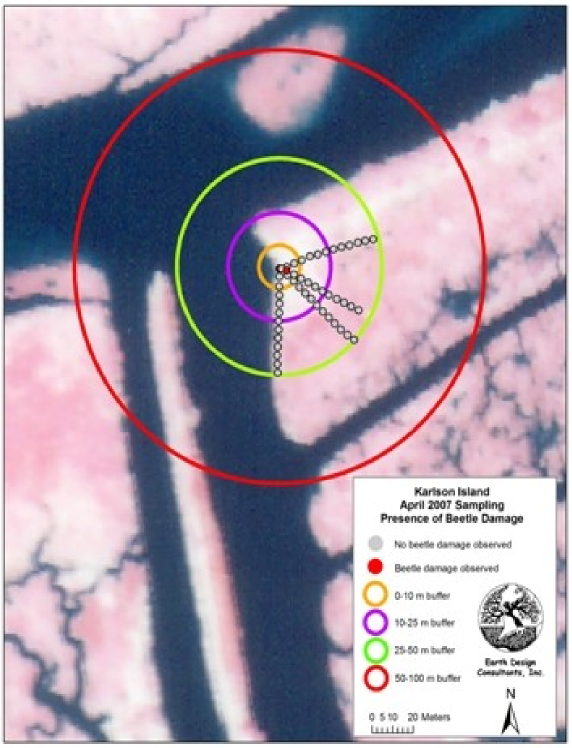
E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April 2007 Sampling
Presence of Beetle Damage
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Beetle damage observed
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Earth Design Consultants, Inc.
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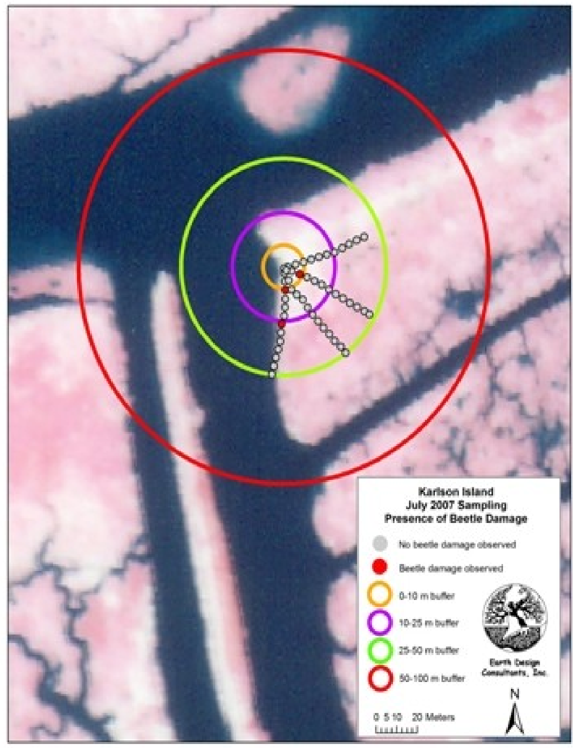
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July 2007 Sampling
Presence of Beetle Damage
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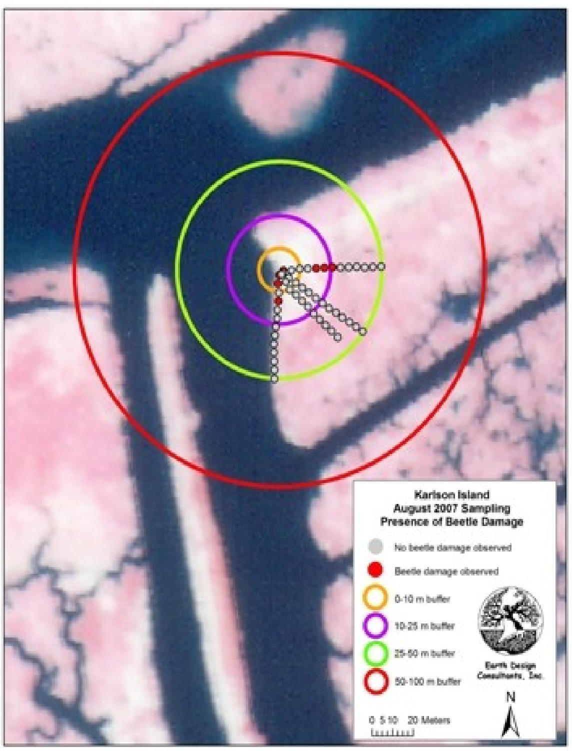
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Beetle damage observed
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Earth Design Consultants, Inc.
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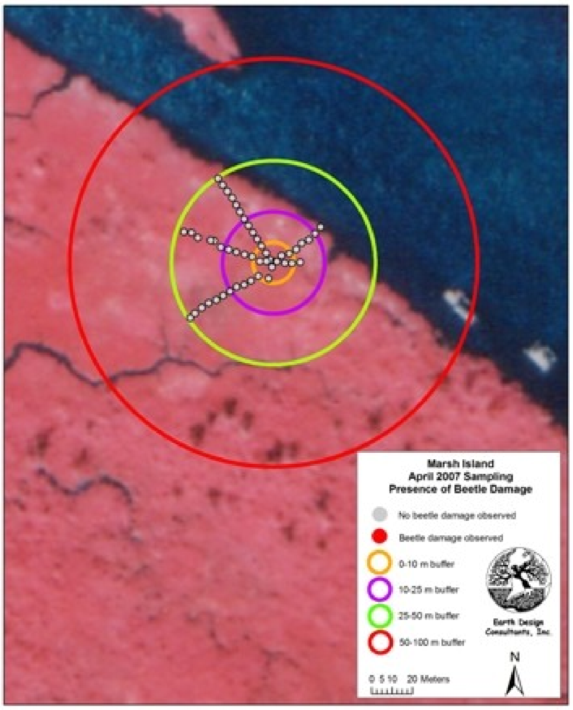
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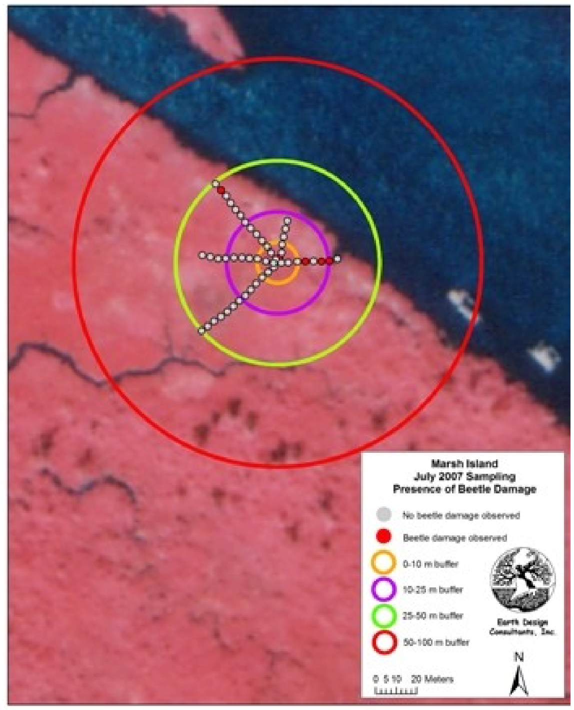
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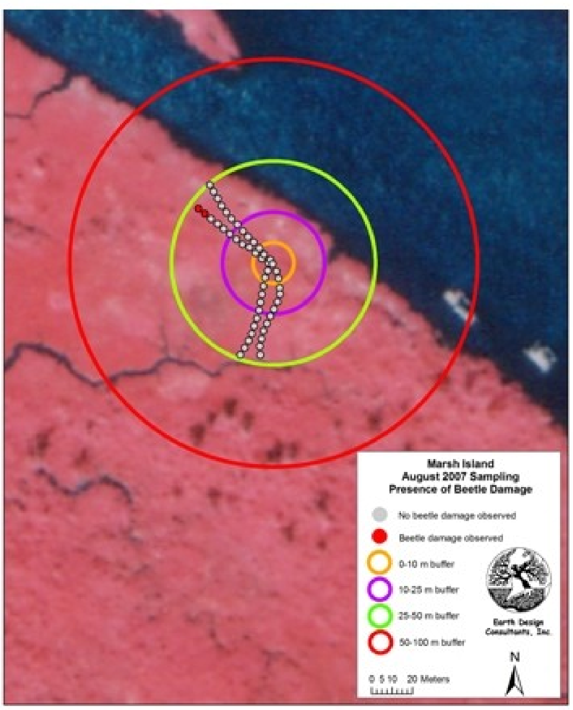
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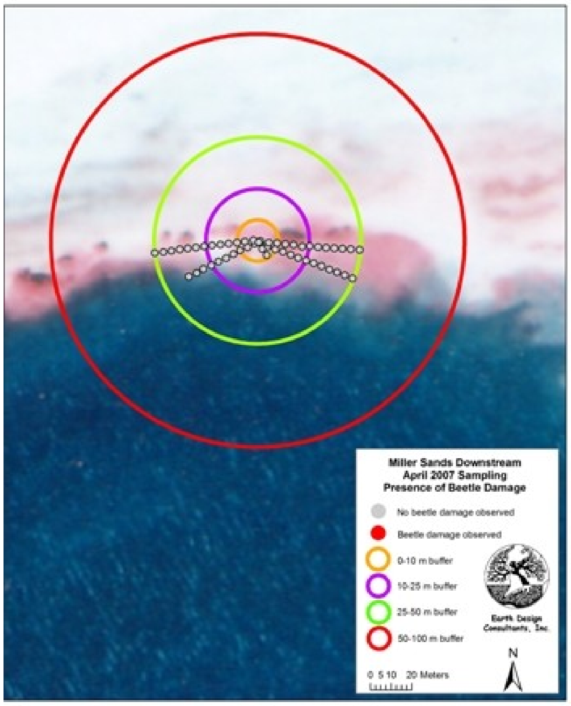
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April 2007 Sampling
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Beetle damage observed
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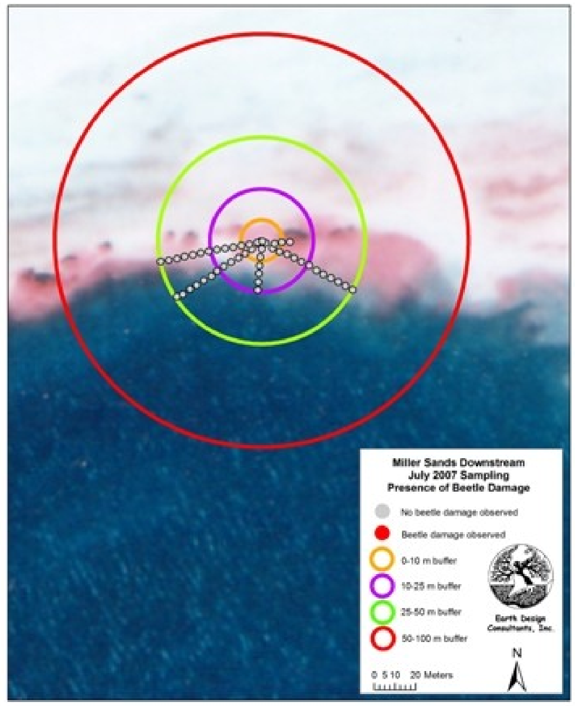
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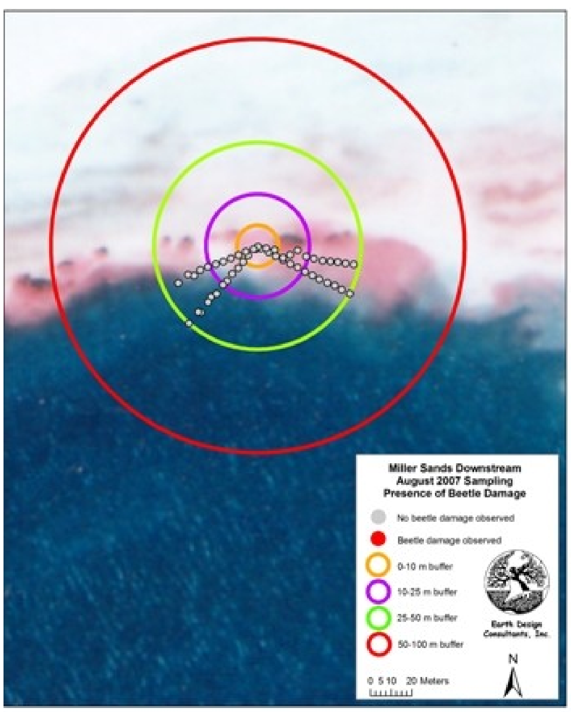
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Earth Design Consultants, Inc.
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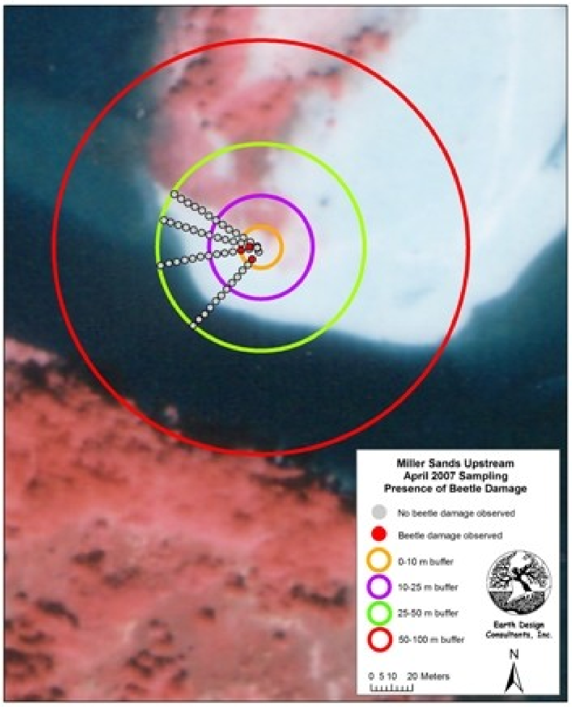
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Earth Design Consultants, Inc.
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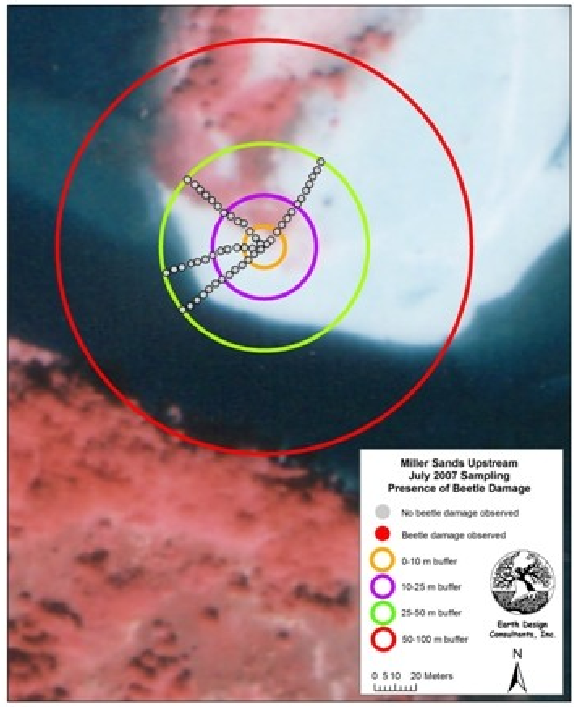
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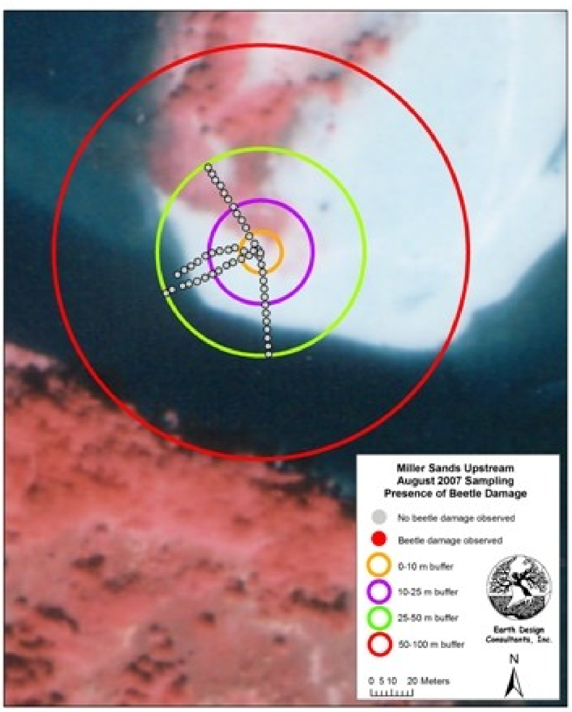
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Earth Design Consultants, Inc.
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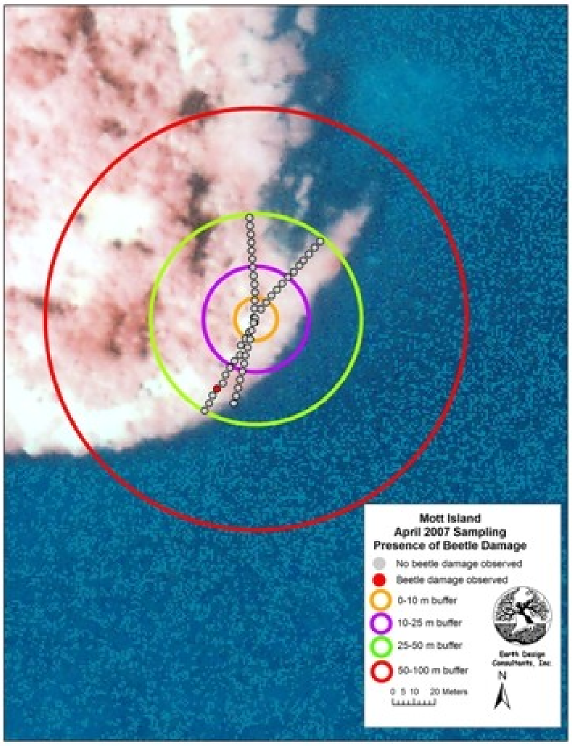
Mott Island
April 2007 Sampling
Presence of Beetle Damage
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Earth Design Consultants, Inc
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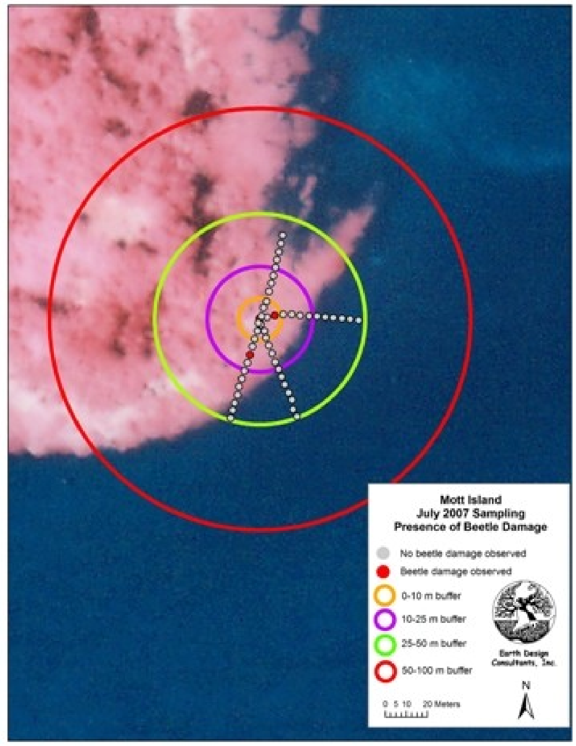
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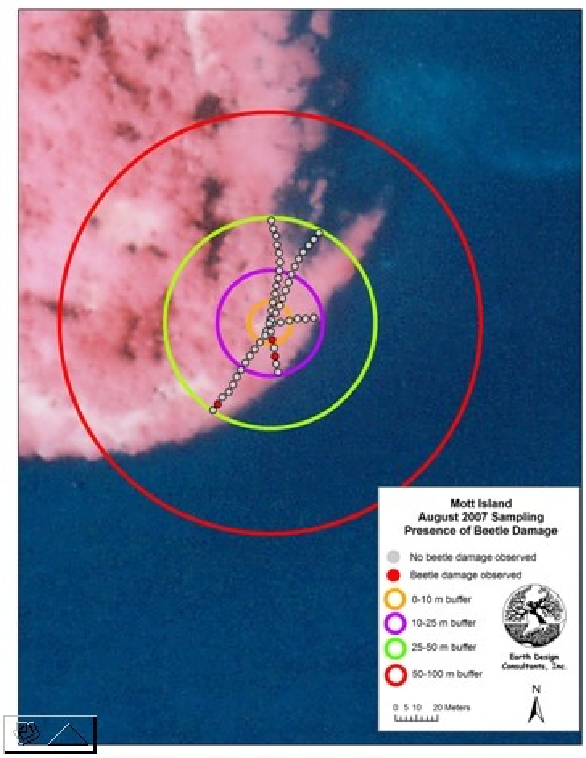
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Earth Design Consultants, Inc.
0 5 10 20 Meters
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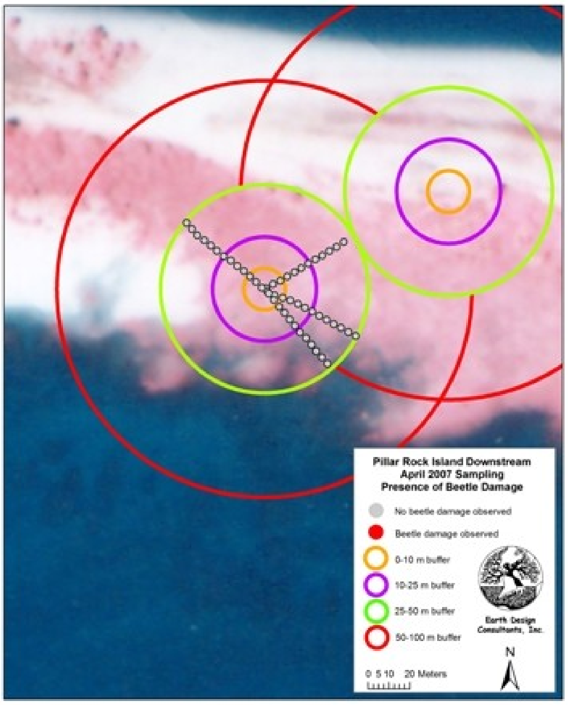
Pillar Rock Island Downstream
April 2007 Sampling
Presence of Beetle Damage
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Beetle damage observed
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Earth Design Consultants, Inc.
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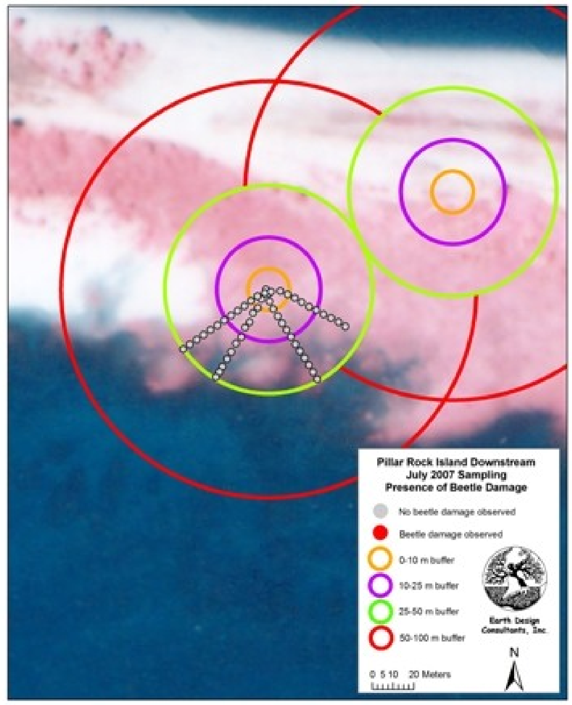
0 5 10 20 Meters


Pillar Rock Island Downstream
July 2007 Sampling
Presence of Beetle Damage
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Beetle damage observed
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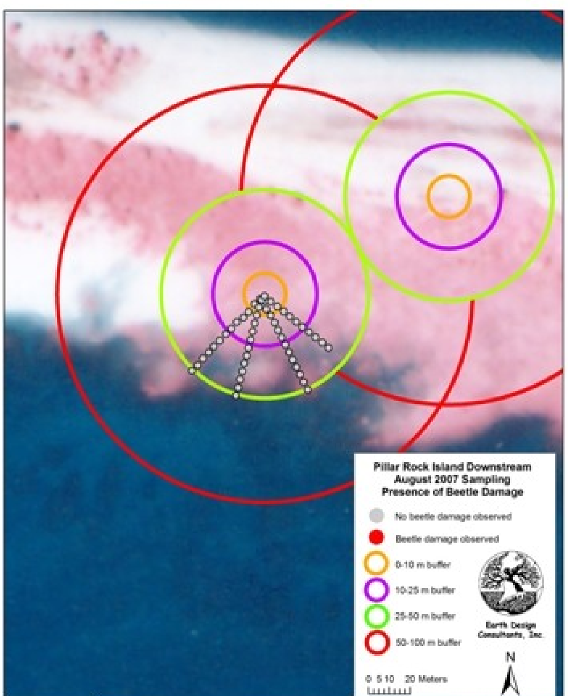
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Earth Design Consultants, Inc.
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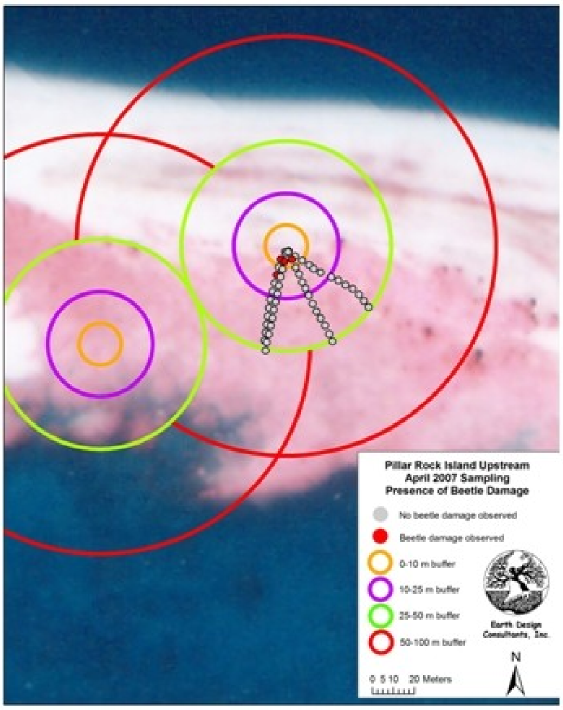
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April 2007 Sampling
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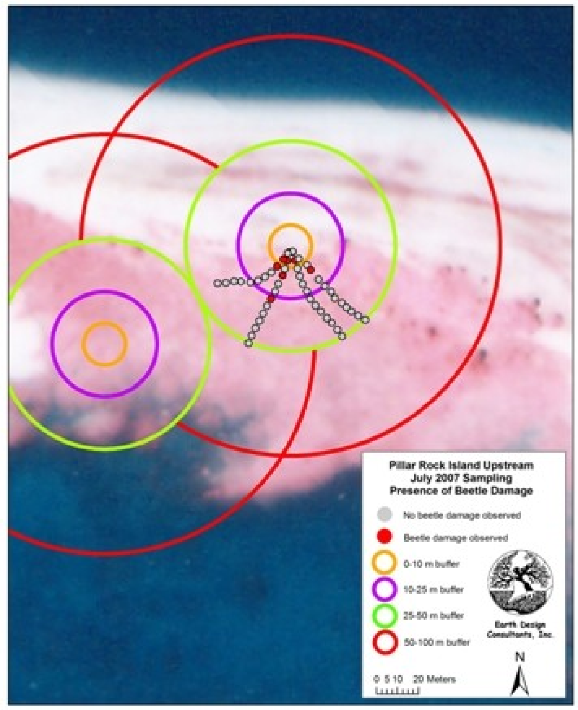
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Earth Design Consultants, Inc.
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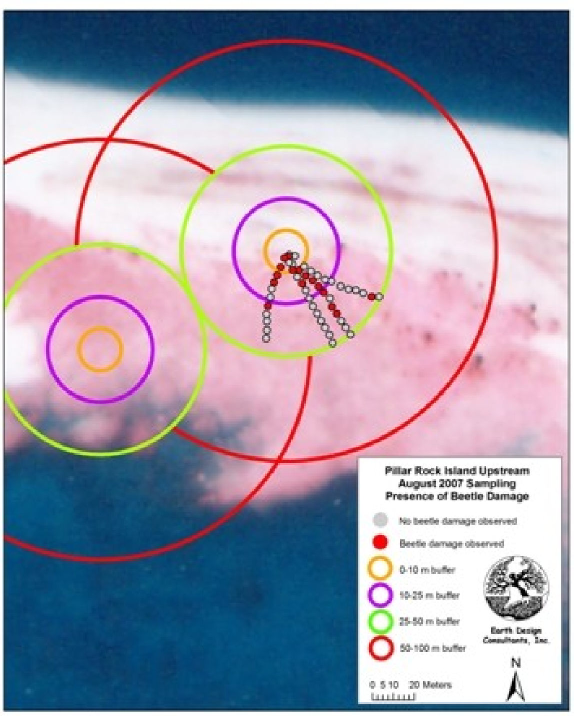
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Earth Design Consultants, Inc.
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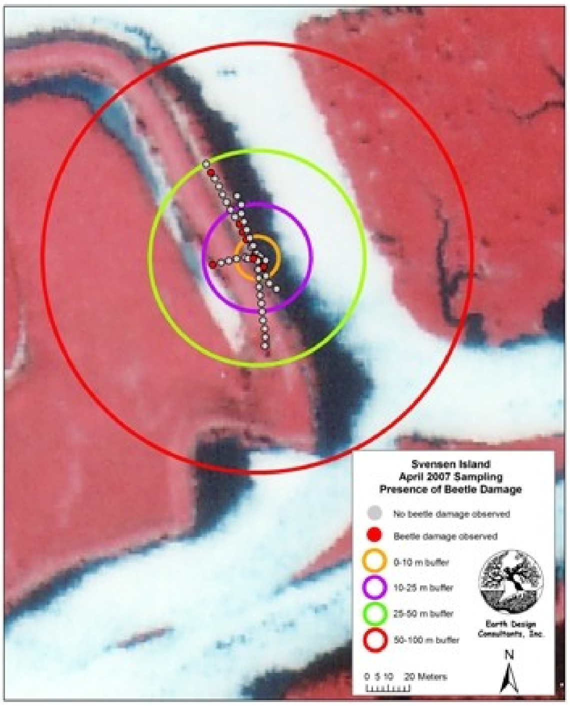
Svensen Island
April 2007 Sampling
Presence of Beetle Damage
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Beetle damage observed
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Earth Design Consultants, Inc.
0 5 10 20 Meters
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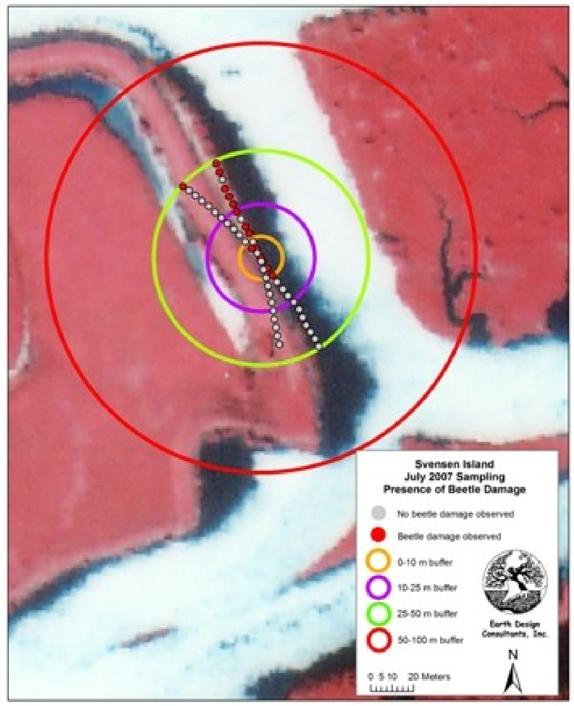
Svensen Island
July 2007 Sampling
Presence of Beetle Damage
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Beetle damage observed
0-10 m buffer
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Earth Design Consultants, Inc.
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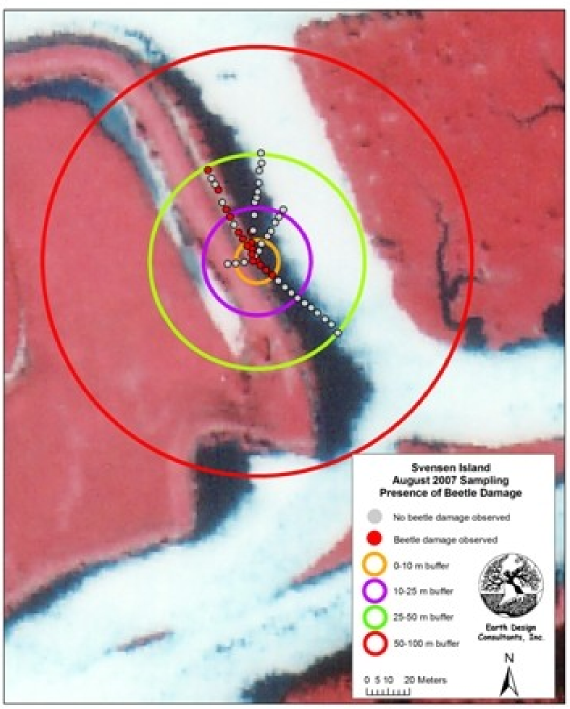
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August 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
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Earth Design Consultants, Inc.
0 5 10 20 Meters
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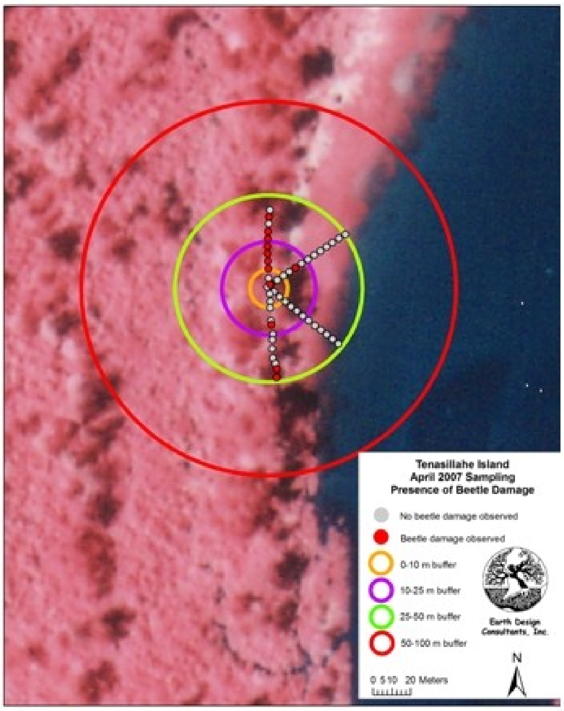
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April 2007 Sampling
Presence of Beetle Damage
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Earth Design Consultants, Inc.
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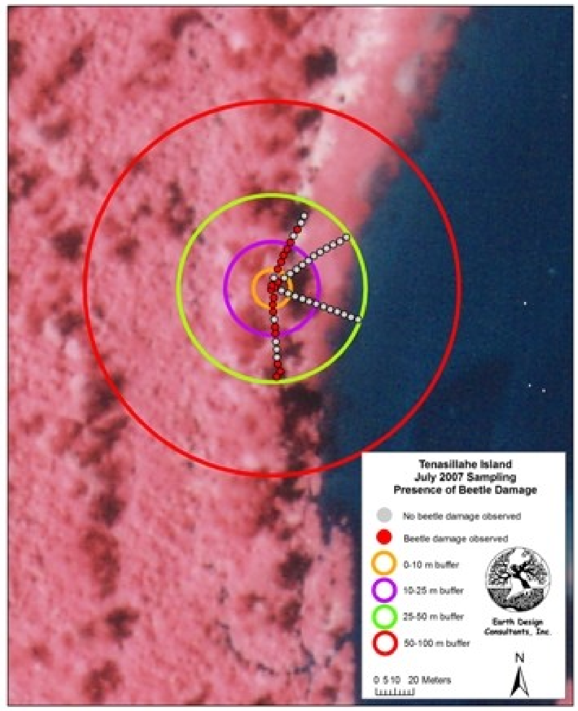
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Presence of Beetle Damage
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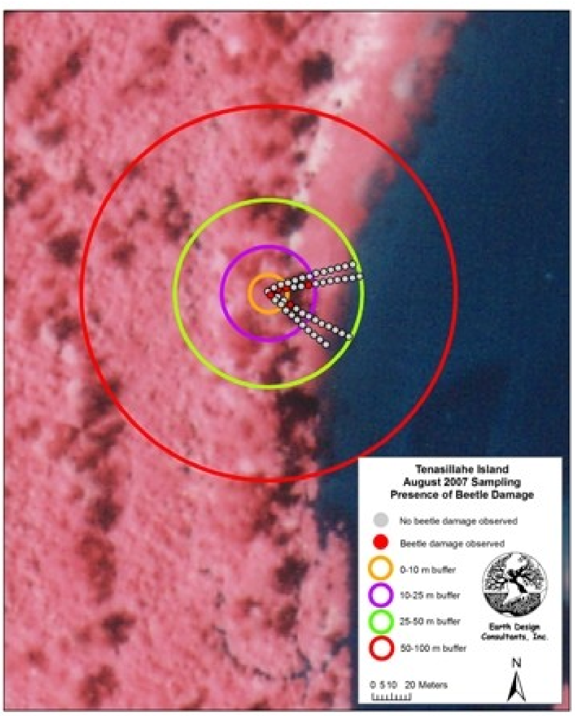
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Presence of Beetle Damage
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Earth Design Consultants, Inc.
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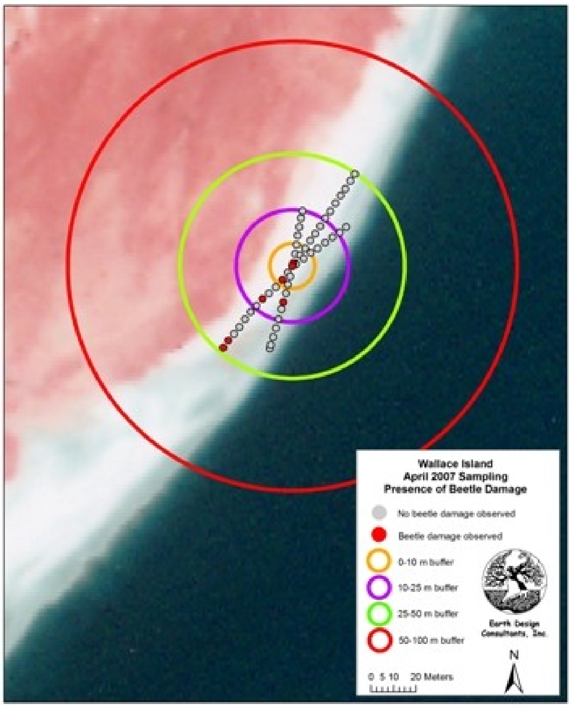
Wallace Island
April 2007 Sampling
Presence of Beetle Damage
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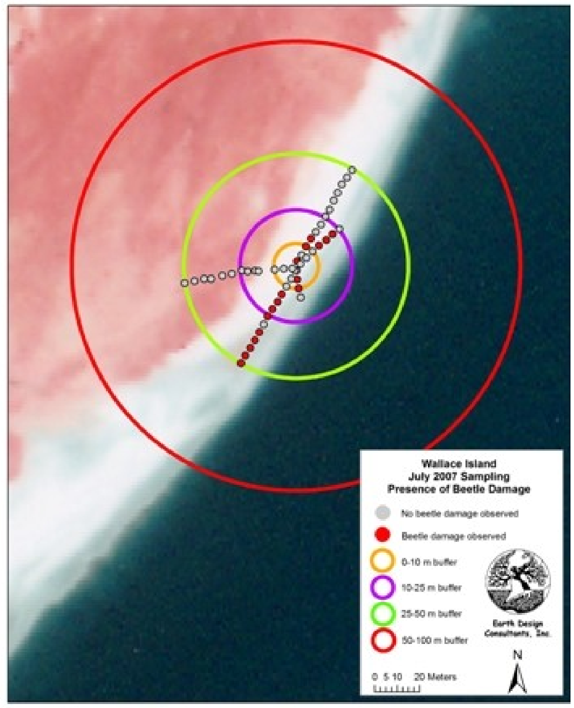
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July 2007 Sampling
Presence of Beetle Damage
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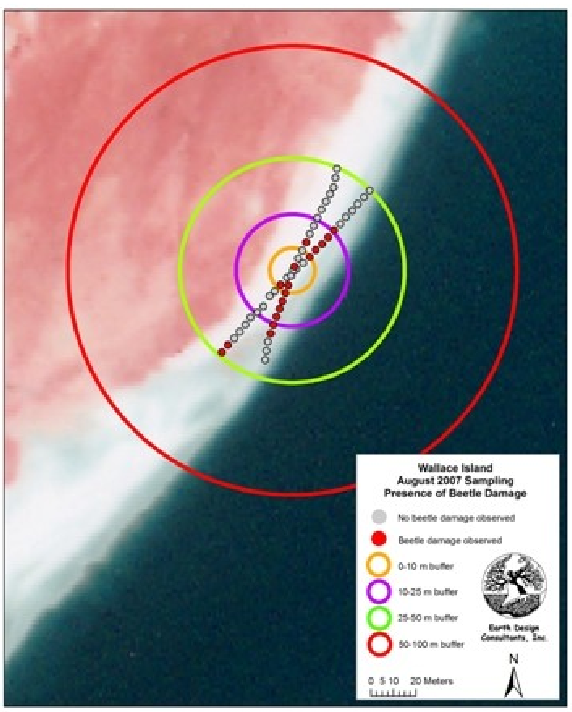
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August 2007 Sampling
Presence of Beetle Damage
No beetle damage observed
Beetle damage observed
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Earth Design Consultants, Inc.
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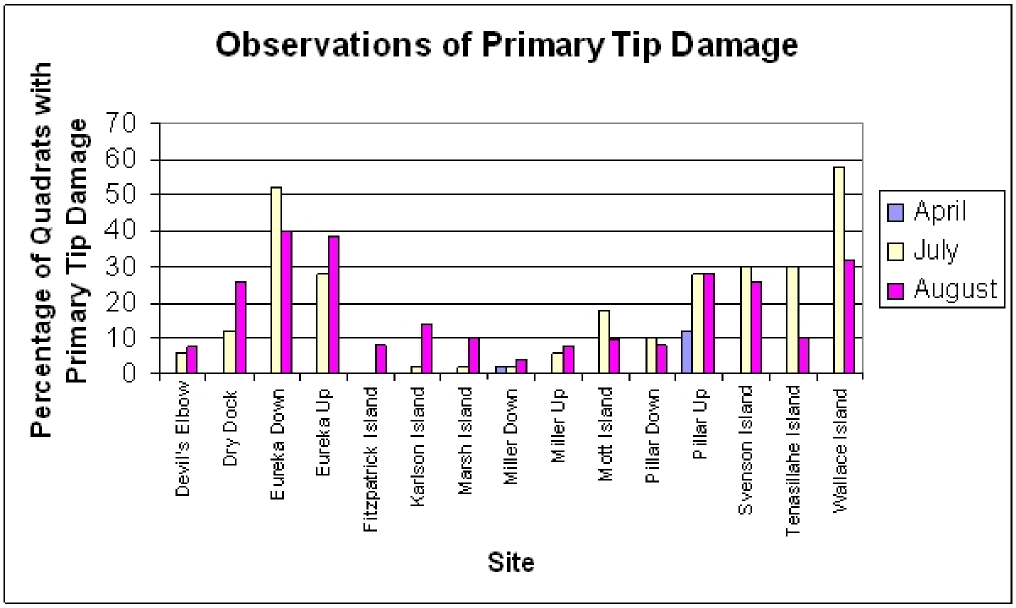


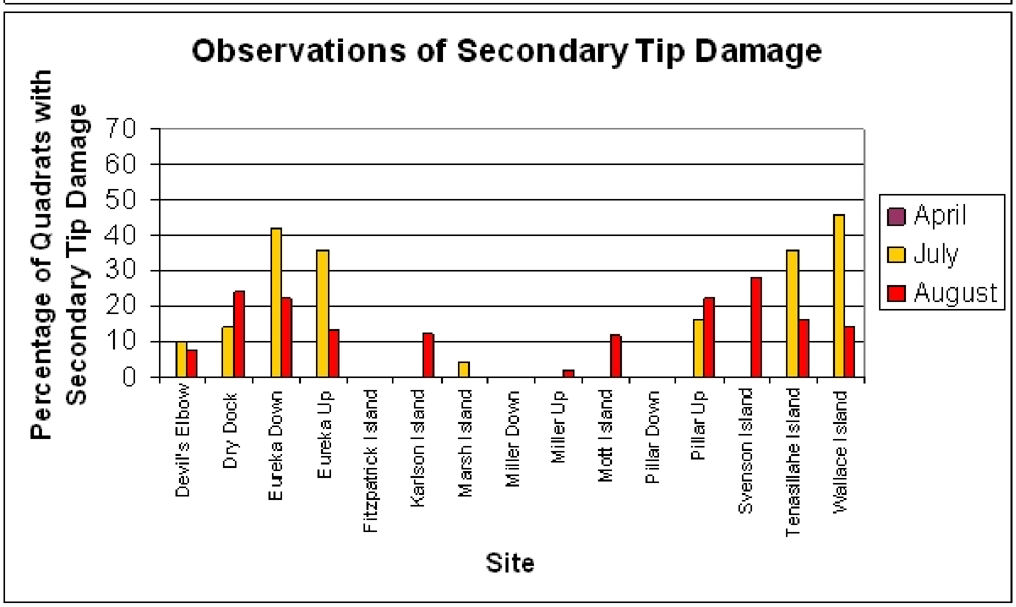


Figure 10: a and b. Observations of Primary and Secondary tip damage for each release site. Each sampling event is shown.

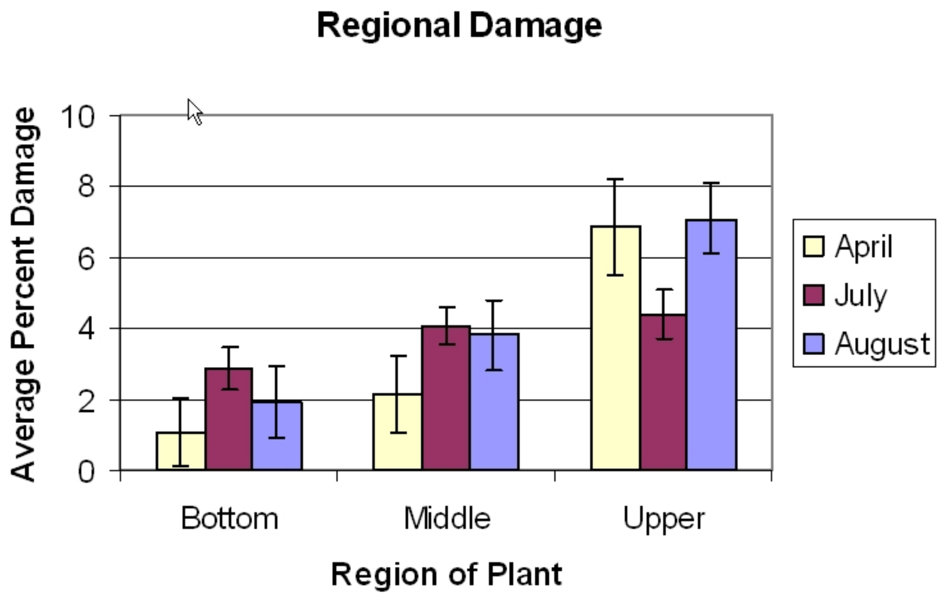


Figure 11: Average percent damage occurring in different position on the plant. During field sampling, plants were divided into thirds (bottom portion, middle portion, top portion) and the average percent damage for the three regions for plants in each quadrat. Error bars are SE.

Table 6. Summary of Percent Feeding Damage. Shown are the site, the sample month, the total number of quadrats sampled, the number of quadrats with feeding damage, the mean percent damage, the standard deviation, minimum and maximum and the sum of the percent damage. Averages are calculated across all quadrats sampled, not just those containing *Lythrum.*

Site	Month	Total Quads	Quads with damage	Mean % Damage	Std. Dev.	Min.	Max.	Sum of % Damage
Devil's Elbow	April	50	4	0.2	0.83	0	5	10
	July	50	2	0.06	0.31	0	2	3
	Aug.	51	6	1.16	4.16	0	20	59
Dry Dock	April	52	6	0.25	0.86	0	5	13
	July	50	7	0.86	2.7	0	15	43
	Aug.	50	12	2.44	5.24	0	20	122
Eureka Bar Downstream	April	50	2	0.02	0.14	0	1	1
	July	50	7	0.36	1.48	0	10	18
	Aug.	50	8	0.24	0.59	0	2	12
Eureka Bar Upstream	April	50	0	0.02	0.14	0	1	1

Table 6. Summary of Percent Feeding Damage. Shown are the site, the sample month, the total number of quadrats sampled, the number of quadrats with feeding damage, the mean percent damage, the standard deviation, minimum and maximum and the sum of the percent damage. Averages are calculated across all quadrats sampled, not just those containing *Lythrum*								
Site	**Month**	**Total Quads**	**Quads with damage**	**Mean % Damage**	**Std. Dev.**	**Min.**	**Max.**	**Sum of % Damage**
	July	**50**	**28**	**0.62**	**0.6**	**0**	**2**	**31**
	Aug.	**52**	**20**	**1.27**	**2.11**	**0**	**10**	**66**
Fitzpatrick Island	**April**	**50**	**0**	**0**	**0**	**0**	**0**	**0**
	July	**50**	**0**	**0**	**0**	**0**	**0**	**0**
	Aug.	**50**	**1**	**0.34**	**1.59**	**0**	**10**	**17**
Karlson Island	**April**	**50**	**2**	**0.04**	**0.2**	**0**	**1**	**2**
	July	**50**	**3**	**0.06**	**0.24**	**0**	**1**	**3**
	Aug.	**50**	**7**	**0.24**	**0.8**	**0**	**5**	**12**
Marsh Island	**April**	**50**	**0**	**0**	**0**	**0**	**0**	**0**
	July	**50**	**5**	**0.24**	**0.77**	**0**	**3**	**12**
	Aug.	**50**	**2**	**0.04**	**0.2**	**0**	**1**	**2**
Miller Sands Downstream	**April**	**52**	**0**	**0**	**0**	**0**	**0**	**0**
	July	**50**	**0**	**0**	**0**	**0**	**0**	**0**
	Aug.	**50**	**0**	**0**	**0**	**0**	**0**	**0**
Miller Sands Upstream	**April**	**52**	**3**	**0.06**	**0.24**	**0**	**1**	**3**
	July	**52**	**0**	**0**	**0**	**0**	**0**	**0**
	Aug.	**51**	**0**	**0**	**0**	**0**	**0**	**0**
Mott Island	**April**	**50**	**0**	**0.02**	**0.14**	**0**	**1**	**1**
	July	**50**	**3**	**0.14**	**0.73**	**0**	**5**	**7**
	Aug.	**52**	**3**	**0.08**	**0.33**	**0**	**2**	**4**
Pillar Island Downstream	**April**	**50**	**0**	**0**	**0**	**0**	**0**	**0**
	July	**50**	**0**	**0**	**0**	**0**	**0**	**0**
	Aug.	**50**	**0**	**0**	**0**	**0**	**0**	**0**
Pillar Island Upstream	**April**	**51**	**5**	**0.2**	**0.78**	**0**	**5**	**10**
	July	**50**	**8**	**0.3**	**0.79**	**0**	**3**	**15**
	Aug.	**50**	**16**	**0.6**	**1.59**	**0**	**10**	**30**
Svensen Island	**April**	**50**	**11**	**1.4**	**4.87**	**0**	**25**	**70**
	July	**50**	**15**	**2.14**	**4.34**	**0**	**20**	**107**
	Aug.	**50**	**16**	**3.16**	**5.77**	**0**	**20**	**158**
Tenasillahe Island	**April**	**50**	**11**	**0.78**	**2.87**	**0**	**20**	**39**
	July	**50**	**19**	**1.28**	**2.46**	**0**	**10**	**64**
	Aug.	**50**	**6**	**0.38**	**1.31**	**0**	**8**	**19**
Wallace Island	**April**	**51**	**8**	**0.27**	**0.78**	**0**	**4**	**14**
	July	**50**	**17**	**1.08**	**1.99**	**0**	**8**	**54**

Table 6. Summary of Percent Feeding Damage. Shown are the site, the sample month, the total number of quadrats sampled, the number of quadrats with feeding damage, the mean percent damage, the standard deviation, minimum and maximum and the sum of the percent damage. Averages are calculated across all quadrats sampled, not just those containing *Lythrum*

Site	Month	Total Quads	Quads with damage	Mean % Damage	Std. Dev.	Min.	Max.	Sum of % Damage
	Aug.	50	17	1.32	2.74	0	12	66
All Sites	April	758	51	0.22	1.55	0	25	164
	July	752	114	0.47	1.73	0	20	357
	Aug.	756	114	0.75	2.69	0	20	567

middle, and top portions of the plants in each quadrat. It does appear that damage from *Galerucella* is generally higher near the top of plant compared to the base or middle portions of the plant (Figure 11). Within each sampling event in 2007 (i.e. April, July, or August), the average percent damage increases from the lowest portion of the plant to the top. These data should be considered anecdotal because it was only the first year using this method and the three damage values were not recorded for every single case in which *Galerucella* damage was observed. <u>We recommend continuing this damage assessment. We also recommend developing a better (quantitative) measure of feeding damage.</u>

3.1.6 Other Invasive Plants

Other invasive species of concern are present at the 15 study sites including *Phalaris arundinaceae* (reed canarygrass), *Iris pseudacorus* (yellow iris), *Cirsium arvense* (Canada thistle), and *Senecio jacobaea* (tansy ragwort). We chose to examine these four plants because some are listed as noxious "B" weeds by Oregon Department of Agriculture (iris, ragwort, and thistle) and the fact that we encounter them often at the release sites. *Phalaris* is the most common invasive we see at the study sites, present in 35-42% of quadrats (Table 7 a,b). We encounter yellow iris at every study site in an average of 25% of our quadrats. *Senecio* and *Cirsium* are less frequently encountered, occurring in 1-3% of quadrats (Table 7 a,b). While *Phalaris* is more widespread, *Senecio* and *Cirsium* are present only at a few study sites. We have not observed *Cirsium* at Karlson, Marsh, Miller Downstream, Svensen, or Tenasillahe. At Devil's Elbow, Fitzpatrick, Mott, and Pillar Downstream we have only encountered it during one of four sampling events. *Senecio* is most abundant at Miller

Downstream, while *Phalaris* is not as common at this site. *Senecio* is also present, but rare, at Devil's Elbow, Fitzpatrick, Karlson, and Eureka Upstream. We have not encountered *Senecio* in quadrats at the other study sites. Iris is most abundant at Pillar Upstream site. We hope to examine in future years the relationship these other invasive species have with *Lythrum* abundance and site characteristics. We are especially interested in seeing if control of one weedy species, i.e. *Lythrum*, leads to the increase of or replacement by another weed species, such as yellow iris, and *Phalaris,* as reported by Schooler *(1998).*

3.2 Modeling Factors Associated with Successful Biocontrol

In 2007, we found a relatively large area where beetles seemed to be responsible for killing *Lythrum* plants (see Section 3.2.3); unfortunately this area was not one of the 15 USACE release sites, nor was the site known to be the site of a previous biocontrol agents release (Moore et al., in review). In order to understand what environmental factors may be related to control agent population establishment and successful biocontrol, we began to measure key environmental variables at and between the USACE release sites, and experiment with the beetle's response to inundation. Our initial work focuses on elevation, especially as how it relates to tidal inundation, vegetation and water quality (e.g., temperature, salinity, etc.).

3.2.1 Environment of the Release Sites

3.2.1.1 Comparison of Water Quality at Eastern and Western End of Study Area

Water flows onto the USACE release sites from multiple deep Columbia River channels during high tides. The western most release sites are within the brackish zone[1], the area where fresh and sea water mix in the LCRE (CORIE website). This mixing depends, for the most part, on river discharges and tidal patterns and is, therefore, quite variable. To make general comparisons in the upstream and downstream aquatic environments we deployed Manta recording water quality probes in channels near the USACE release sites at the eastern and western most extents of our study area.

[1]This area maps as brackish on CORIE but salt water may not be at the surface. See CORIE for details.

The probes were not deployed in the same exact location (Figure 2) nor at the same depth from month to month so the results should be compared carefully. Our intent was to document conditions at both ends of the study area during the time our teams were in the field. Ideally, water quality at each release site should be measured since it may ultimately affect the quality of the host plant and the environment into which the biocontrol agents must exist. For example, we observed that the hardware used to secure the tidal gauges to each release site were heavily oxidized at the Mott Island site and not at other sites.

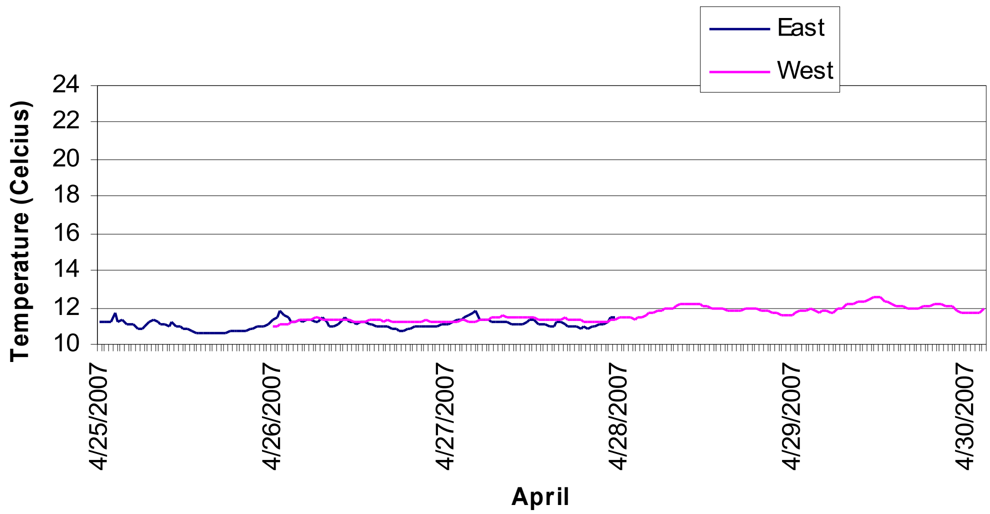
We found that channel water temperatures at the eastern sites were similar to those at the western sites but that the eastern sites tended to have higher daily peaks than the western sites (Figure 12 a). Values for pH tended to fluctuate less and to be lower for western sites than for eastern sites (Figure12 b). Dissolved oxygen concentration was consistently greater at the eastern sites than at the western sites (Figure 12 c). Interestingly, the specific conductivity was greater at the eastern sites than at western sites (Figure 12 d). Finally, the tidal propagation offset and magnitude of tidal exchange are shown in Figure 12 e.

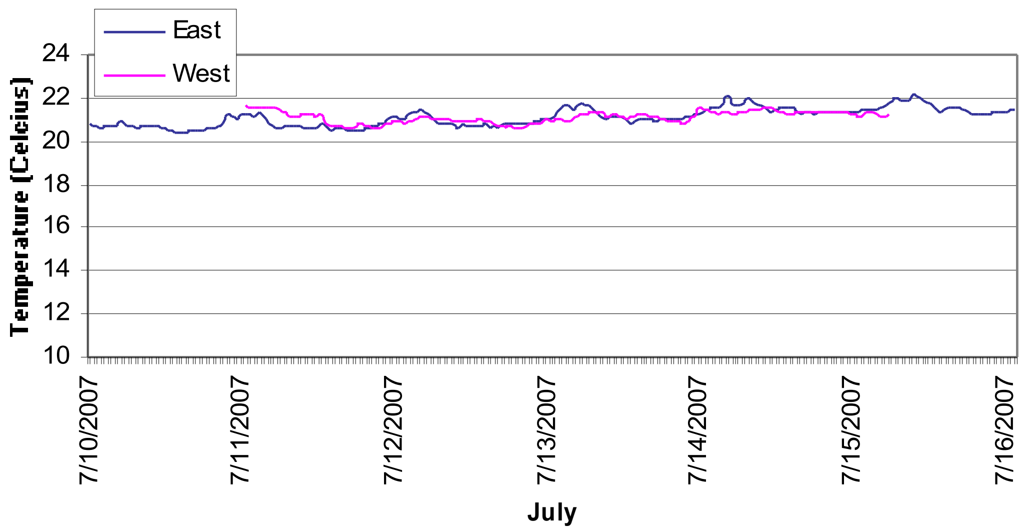
Table 7a Percentage of quadrats per site by sampling event with invasive plant species of concern present. Values for July 2006 - No June 2006 data presented because other plant species were not consistently recorded at this event. Plant codes are PhAr= *Phalaris arundineae* (reed canarygrass), IrPs= *Iris pseudacorus* (yellow iris), CiAr= *Cirsium arvense* (Canada thistle), SeJa= *Senecio jacobaea* (tansy ragwort), and LySa= *Lythrum salicaria* (purple loosestrife). Data for Lysa are presented for comparison purposes.					
Site	**PhAr**	**IrPs**	**CiAr**	**SeJa**	**LySa**
Devils Elbow	**4**	**48**	**2**	**4**	**22**
Dry Dock	**16**	**28**	**8**	**6**	**36**
Eureka Bar Downstream	**36**	**18**	**6**	**0**	**92**
Eureka Bar Upstream	**28**	**22**	**8**	**0**	**88**
Fitzpatrick Island	**39**	**8**	**4**	**2**	**75**
Karlson Island	**38**	**8**	**0**	**2**	**40**
Marsh Island	**11**	**15**	**0**	**0**	**26**
Miller Sands Downstream	**48**	**34**	**0**	**0**	**76**
Miller Sands Upstream	**48**	**44**	**0**	**0**	**58**
Mott Island	**46**	**32**	**2**	**0**	**48**
Pillar Island Downstream	**24**	**10**	**2**	**0**	**80**
Pillar Island Upstream	**46**	**18**	**2**	**0**	**84**
Svensen Island	**64**	**12**	**0**	**0**	**56**
Tenasillahe Island	**46**	**10**	**0**	**0**	**36**
Wallace	**39**	**4**	**2**	**0**	**39**
ALL	**35**	**21**	**2**	**1**	**57**

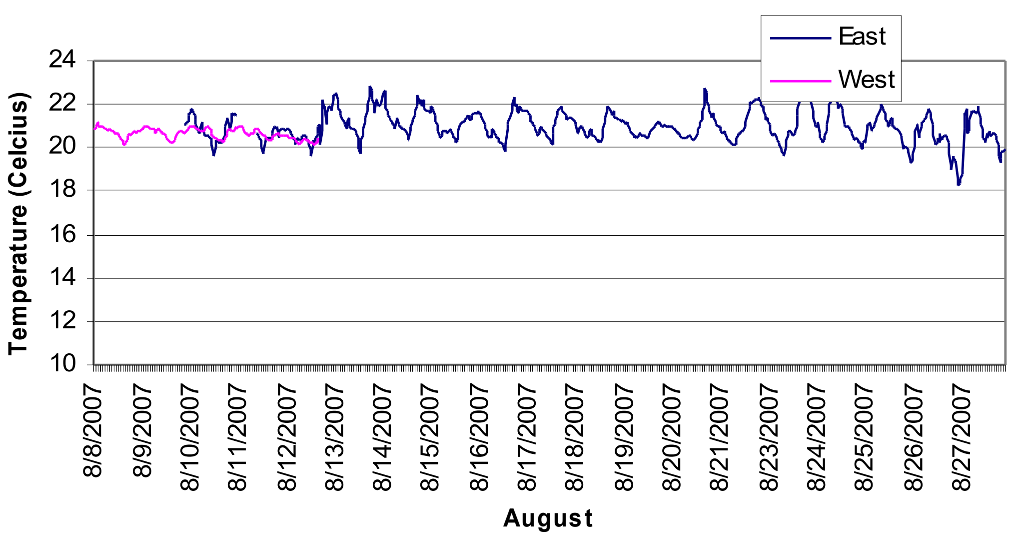
Table 7b Percentage of quadrats per site by sampling event with invasive plant species of concern present. Values for July 2007. Plant codes are PhAr= *Phalaris arundineae* (reed canarygrass), IrPs= I*ris pseudacorus* (yellow iris), CiAr= *Cirsium arvense* (Canada thistle), SeJa= *Senecio jacobaea* (tansy ragwort), and LySa= *Lythrum salicaria* (purple loosestrife).

	April					July					Aug.				
	PhAr	IrPs	CiAr	SeJa	LySa	PhAr	IrPs	CiAr	SeJa	LySa	PhAr	IrPs	CiAr	SeJa	LySa
Devils Elbow	68	10	0	0	18	64	22	0	0	14	61	29	0	0	18
Dry Dock	85	4	0	0	29	56	6	2	2	16	80	22	0	0	26
Eureka Bar Downstream	12	26	4	0	90	64	16	12	0	100	12	10	12	0	76
Eureka Bar Upstream	42	12	2	10	92	62	4	6	0	74	65	6	4	0	75
Fitzpatrick Island	0	18	0	0	50	0	4	0	0	48	0	4	0	0	42
Karlson Island	66	12	0	0	34	58	22	0	0	34	72	22	0	0	48
Marsh Island	52	12	0	0	14	62	30	0	0	18	42	34	0	0	24
Miller Sands Downstream	0	65	0	21	71	0	50	0	18	50	0	52	0	22	62
Miller Sands Upstream	0	40	2	2	58	0	33	19	4	40	2	45	18	4	59
Mott Island	84	4	0	0	38	40	6	0	0	30	73	12	0	0	46
Pillar Island Downstream	22	24	0	0	58	6	14	0	0	62	16	16	0	0	62
Pillar Island Upstream	49	73	2	0	80	56	80	0	0	90	70	74	0	0	88
Svensen Island	40	22	0	0	44	42	24	0	0	40	36	32	0	0	40
Tenasillahe Island	70	10	0	0	44	68	8	0	0	40	46	0	0	0	18
Wallace Island	37	2	0	0	43	48	0	2	0	62	60	0	0	0	40
ALL	42	22	1	2	51	42	21	3	2	48	42	24	2	2	48

Figure 12. Water quality at eastern and western ends of study area (see Figure 2). We measured temperature (ºC), pH, specific conductivity (mS cm^{-1}), percent saturation, and depth during our April, July and August sampling events. Manta probes were not referenced to same depth or placed in same locations each month. Data are provided to help characterize study area.


East
West
Temperature (Celcius)
24
22
20
18
16
14
12
10
4/25/2007
4/26/2007
4/27/2007
4/28/2007
4/29/2007
4/30/2007
April


East
West
Temperature (Celcius)
24
22
20
18
16
14
12
10
7/10/2007
7/11/2007
7/12/2007
7/13/2007
7/14/2007
7/15/2007
7/16/2007
July


East
West
Temperature (Celcius)
24
22
20
18
16
14
12
10
8/8/2007
8/9/2007
8/10/2007
8/11/2007
8/12/2007
8/13/2007
8/14/2007
8/15/2007
8/16/2007
8/17/2007
8/18/2007
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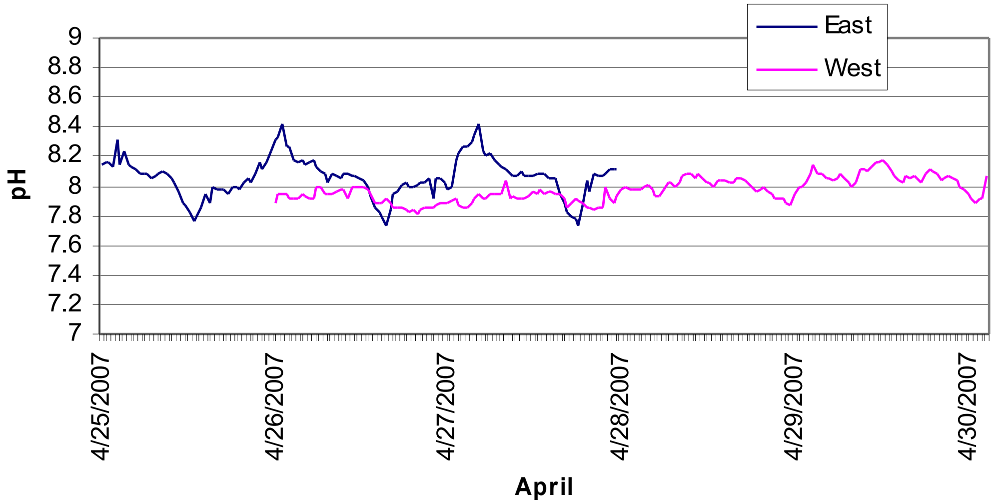
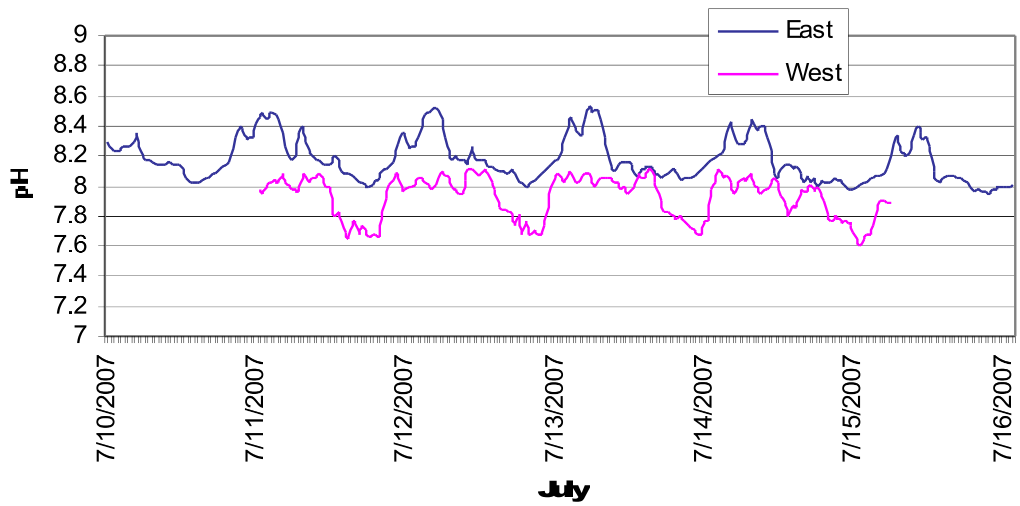
8/25/2007
8/26/2007
8/27/2007
August


East
West
9
8.8
8.6
8.4
8.2
8
7.8
7.6
7.4
7.2
7
pH
4/25/2007
4/26/2007
4/27/2007
4/28/2007
4/29/2007
4/30/2007
April


East
West
9
8.8
8.6
8.4
8.2
8
7.8
7.6
7.4
7.2
7
pH
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7/11/2007
7/12/2007
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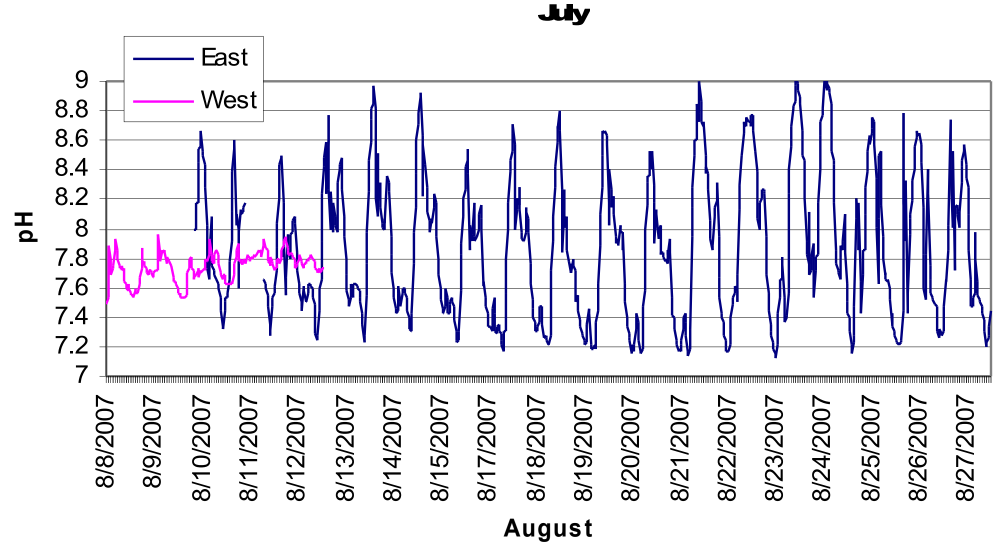
7/14/2007
7/15/2007
7/16/2007
July


East
West
9
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8.6
8.4
8.2
8
7.8
7.6
7.4
7.2
7
pH
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8/9/2007
8/10/2007
8/11/2007
8/12/2007
8/13/2007
8/14/2007
8/15/2007
8/17/2007
8/18/2007
8/19/2007
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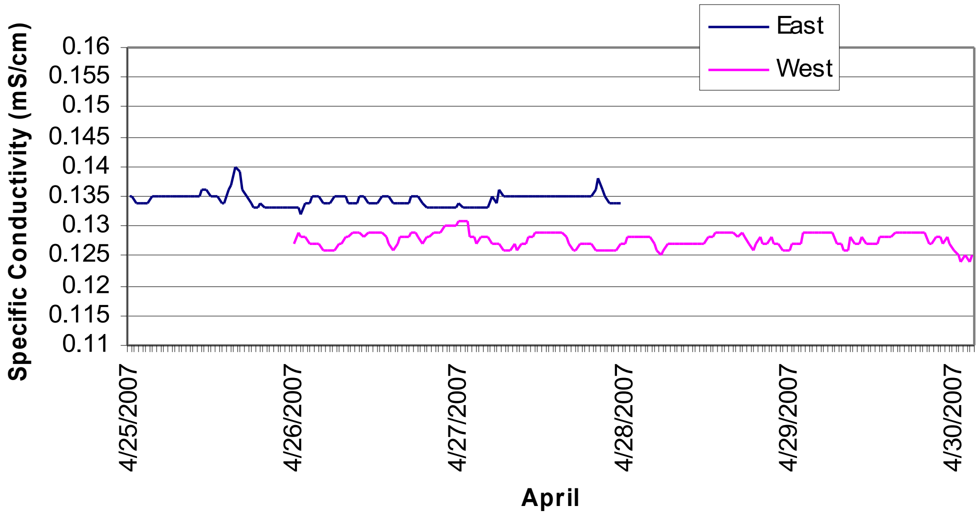
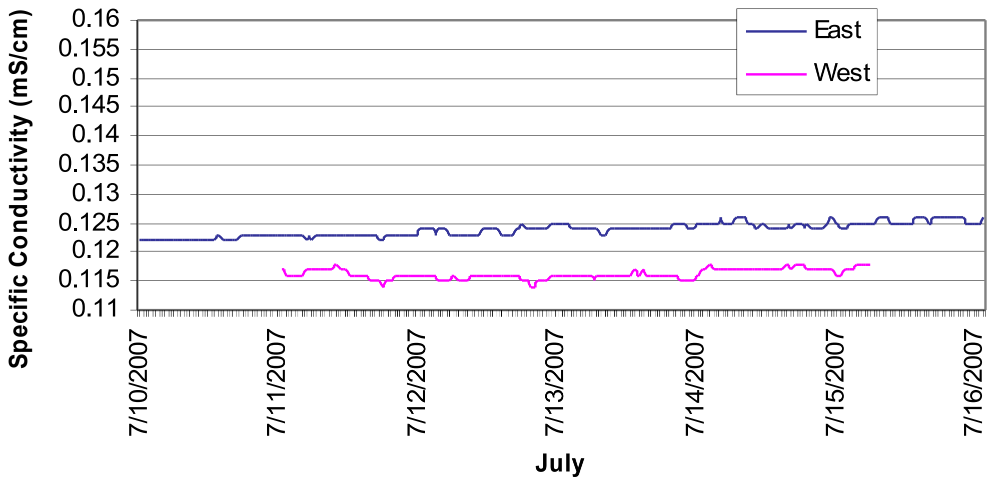
8/26/2007
8/27/2007
August


East
West
Specific Conductivity (mS/cm)
0.16
0.155
0.15
0.145
0.14
0.135
0.13
0.125
0.12
0.115
0.11
4/25/2007
4/26/2007
4/27/2007
4/28/2007
4/29/2007
4/30/2007
April


East
West
Specific Conductivity (mS/cm)
0.16
0.155
0.15
0.145
0.14
0.135
0.13
0.125
0.12
0.115
0.11
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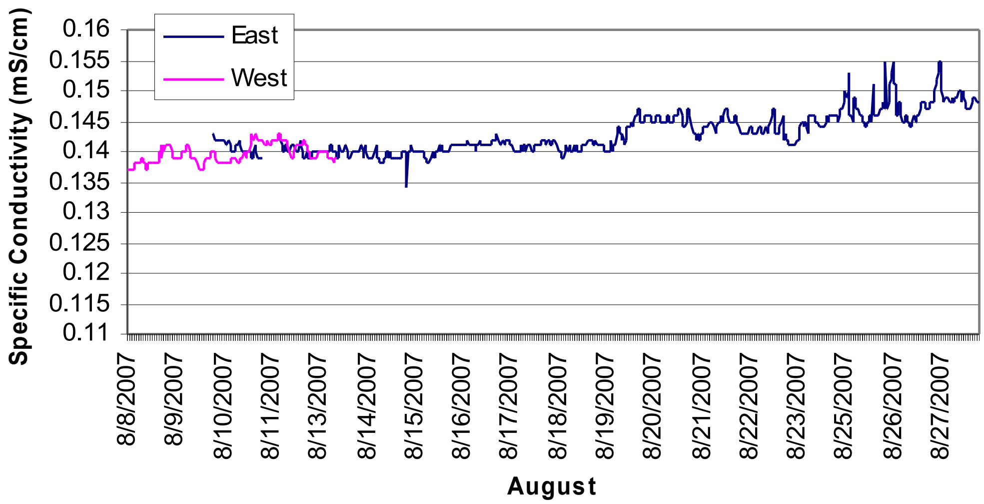
7/14/2007
7/15/2007
7/16/2007
July


East
West
Specific Conductivity (mS/cm)
0.16
0.155
0.15
0.145
0.14
0.135
0.13
0.125
0.12
0.115
0.11
8/8/2007
8/9/2007
8/10/2007
8/11/2007
8/13/2007
8/14/2007
8/15/2007
8/16/2007
8/17/2007
8/18/2007
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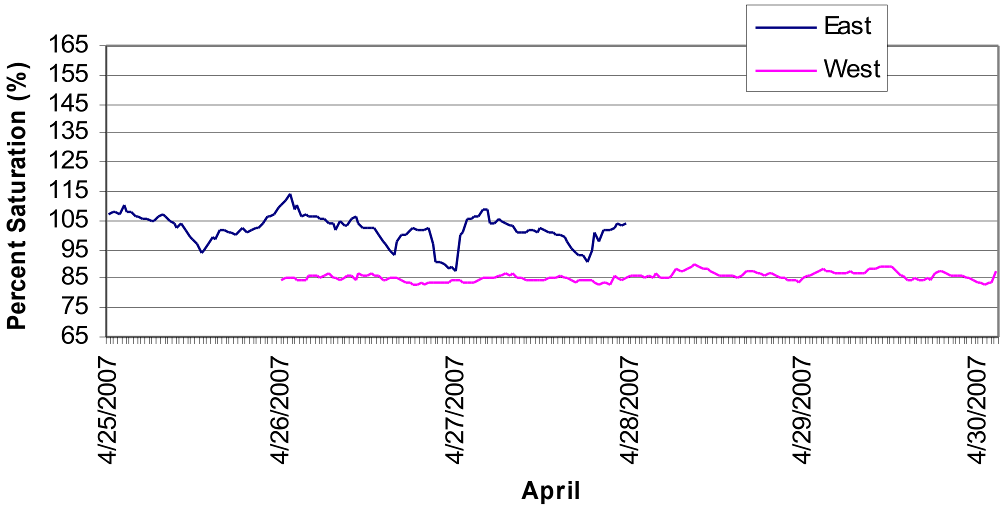
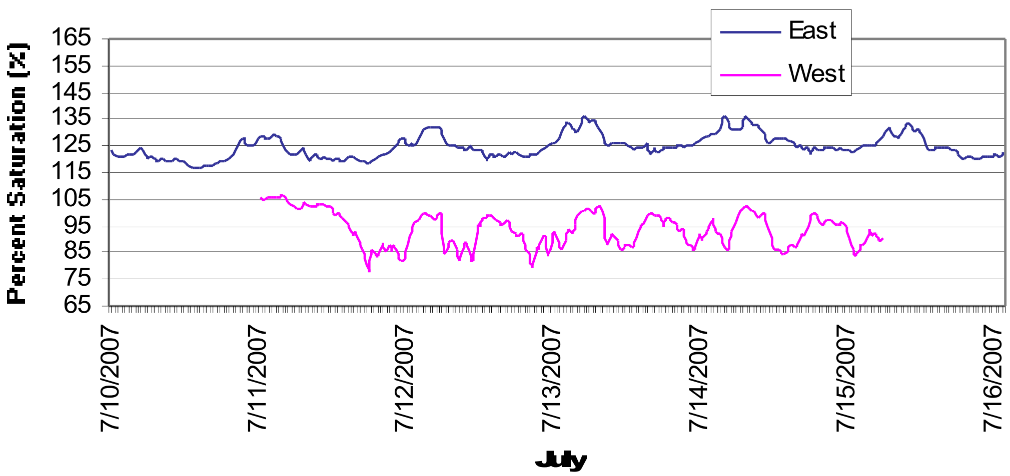
8/26/2007
8/27/2007
August


East
West
Percent Saturation (%)
165
155
145
135
125
115
105
95
85
75
65
4/25/2007
4/26/2007
4/27/2007
4/28/2007
4/29/2007
4/30/2007
April


East
West
Percent Saturation (%)
165
155
145
135
125
115
105
95
85
75
65
7/10/2007
7/11/2007
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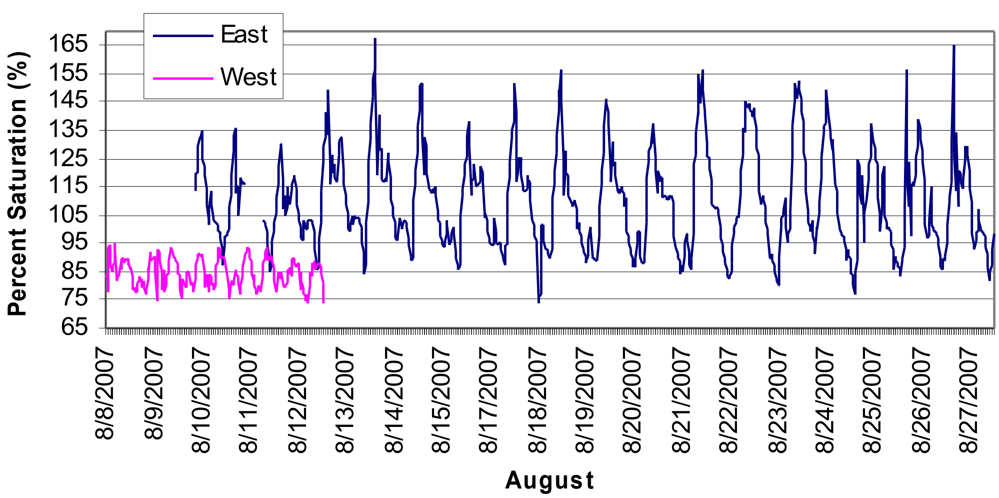
7/15/2007
7/16/2007
July


East
West
Percent Saturation (%)
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155
145
135
125
115
105
95
85
75
65
8/8/2007
8/9/2007
8/10/2007
8/11/2007
8/12/2007
8/13/2007
8/14/2007
8/15/2007
8/17/2007
8/18/2007
8/19/2007
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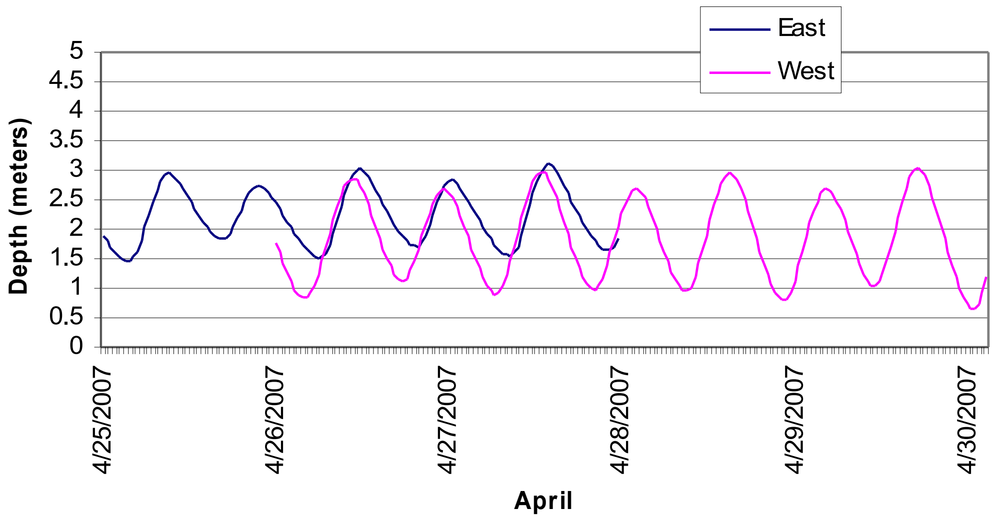
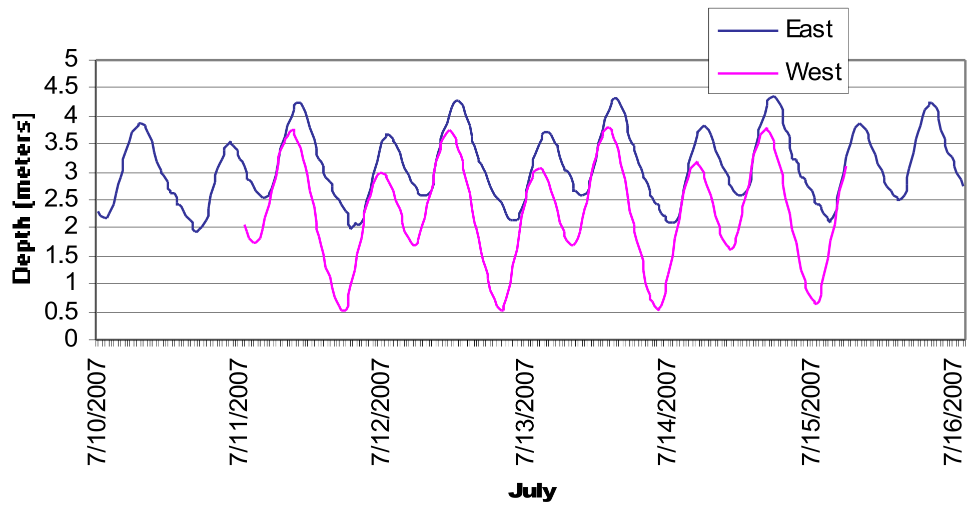
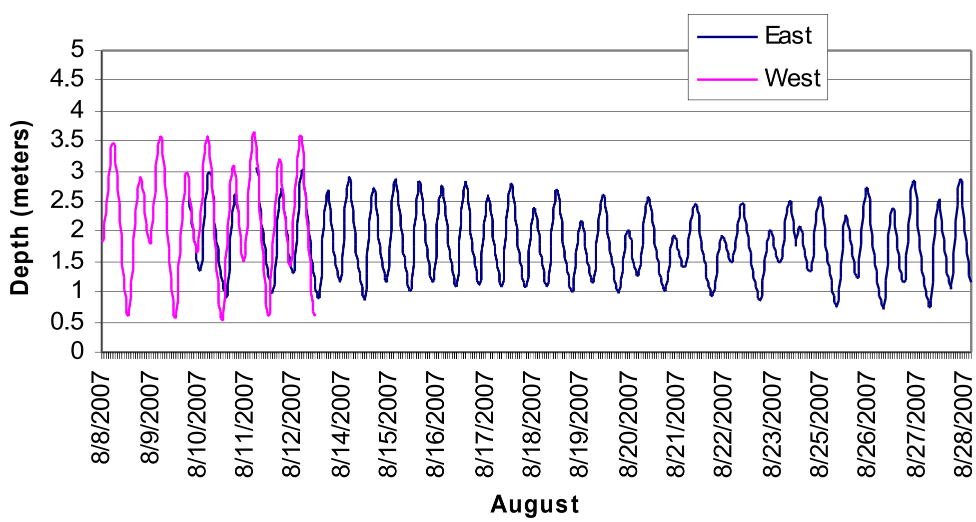
8/25/2007
8/26/2007
8/27/2007
August


East
West
Depth (meters)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
4/25/2007
4/26/2007
4/27/2007
4/28/2007
4/29/2007
4/30/2007
April


East
West
Depth [meters]
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
7/10/2007
7/11/2007
7/12/2007
7/13/2007
7/14/2007
7/15/2007
7/16/2007
July


East
West
Depth (meters)
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4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
8/8/2007
8/9/2007
8/10/2007
8/11/2007
8/12/2007
8/14/2007
8/15/2007
8/16/2007
8/17/2007
8/18/2007
8/19/2007
8/20/2007
8/21/2007
8/22/2007
8/23/2007
8/25/2007
8/26/2007
8/27/2007
8/28/2007
August

3.2.1.2 Land Cover

The final classification had an overall classification accuracy of 72.6% (water was underrepresented as a category for the accuracy assessment to focus on the other land cover classes). The total study area for which land cover classification was performed encompasses 37,649 hectares. The three major land cover classes are water, bare earth, and vegetation. The vegetation cover class was further divided into herbaceous, scrub/shrub, and forested subclasses. Herbaceous vegetation was the most abundant type of vegetation but forested areas were almost as common in the study area (Table 8). Following image classification it is common to use a moving window filter to remove 'salt and pepper' in the classified image to improve map accuracy: we have not applied a moving window filter to this data set. One of the limitations in developing this data layer was the time interval between the acquisition date of the photography and the collection of the field data that went into the classification. We would have expected a much better classification accuracy had the field work and the image acquisition been coincident. We plan to use this classified imagery in our modeling efforts unless a more up-to-date data layer becomes available.

Table 8. Area (ha) of five land cover classes for the study area. Classification was based on CIR photographs (see text for details). Also, shown are the area totals for vegetated and vegetated cover classes.

Class Code	Cover Class	Area (hectares)
1	Water	20,901
2	Bare	1,807
3	Herbaceous	7,263
4	Scrub/Shrub	1,290
5	Tree	6,388
Vegetated		14,941
Unvegetated		22,708
Total		37,649

3.2.1.3 Elevation

The elevation of the USACE release sites ranged from just over 2 m (Mott Island) to over 5 m (Eureka Bar Upstream) (Figure 13). Elevation is important because it is one of the major factors (in addition to site topography, distance from main channel, vegetation, etc.) affecting site inundation and vegetation patterns. In 2006, we collected elevation data using RTK GPS (page 11, Garono et al. 2006). In 2007, we acquired an existing LiDAR data set (described above) to describe the landscape settings of each of the release sites. LiDAR provides elevation data for exposed land areas at the time of data acquisition. In addition to elevations at the release sites, these data provide important topographic information for much of the study area.

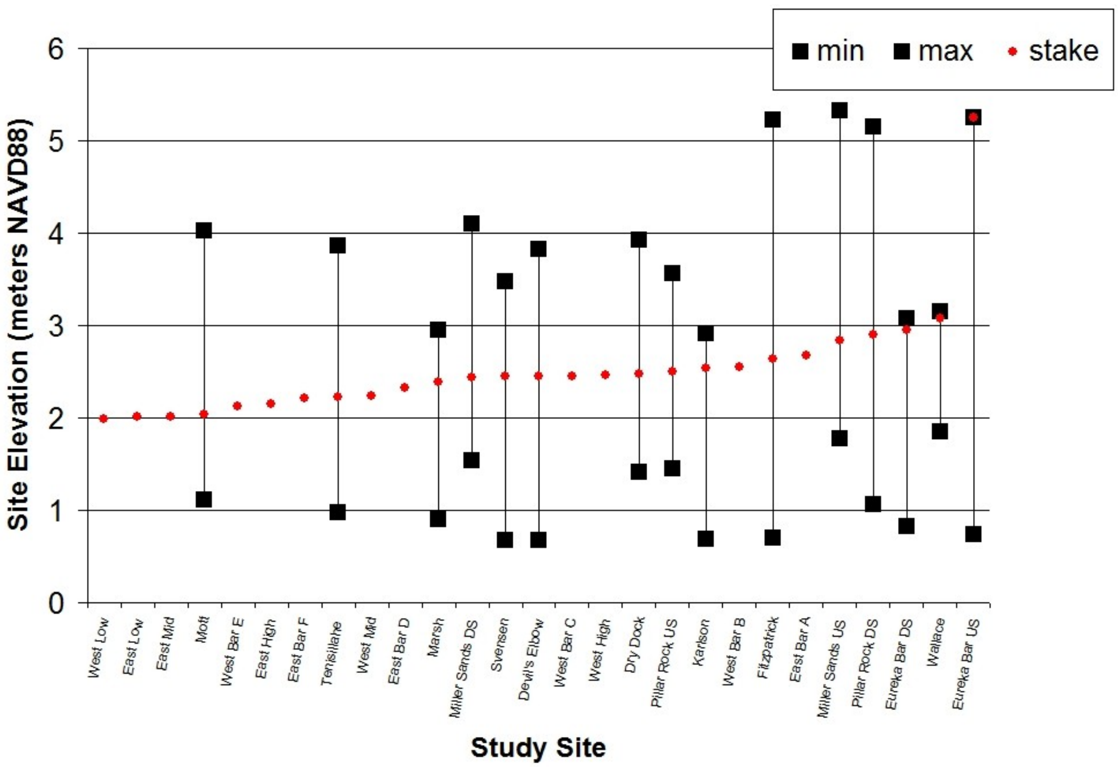


Figure 13. Elevations of 15 USCAE release site stakes (red) and min and max elevations of area surrounding release stakes determined by RTK GPS in 2006 by our field teams. Also shown are nearby release site elevations from LiDAR for our other 2002/07 release sites near Wallace and Russian Islands (West Low, East Low, East Mid, West Bar E, East High, East Bar F, West Mid, West Bar C, West High, and East Bar A)

We reviewed the LiDAR data to find numerous data gaps both over land and water areas. We checked with the LiDAR Consortium and found that multiple LiDAR vendors supplied the data and that there were areas where adjacent missions did not overlap (over land data gaps) and that LiDAR returns were poor over some water areas (over water data gaps) (Diana Martinez, personal communication November 2007). Nevertheless, these LiDAR data constitute an important data set that will add to our future modeling efforts.

The RTK data that we collected in 2006 are the most accurate; therefore, we compared the existing LiDAR and DEM in the areas for which we had RTK coverage. Generally, we found good agreement between the elevation data sets for release stake elevations (Table 9). We did find, however, that the LiDAR data may have underestimated the elevation of Devils Elbow by 2.2m and that the DEM data set may have overestimated the elevation of the Karlson Island Site by 2.1m and Svensen Island by 1m and underestimated the elevation of Mott Island by 1.2m (Table 9).

Discrepancies in reported elevation values can be understood by comparing the graininess of the actual data sets and the topographic complexity of each of the sites. The RTK data were collected at specific points, including the USACE release stakes, and probably represent the most accurate measurement of elevation. To be useful in computer models, elevation values are often interpolated from point data into computer modeled surfaces. The RTK TIN (Triangulated Irregular Network), LiDAR, and DEM data are examples of interpolated data sets. A single elevation value is mapped into each of the surface model cells and the cell sizes vary from a few square meters to well over 100 m^2. Therefore, a single elevation value in the computer model may actually represent a wide range of real-world elevations as in the case of the Svensen Island release site which is located on the side of a dike.

3.2.1.4 Inundation

Tidal inundation may be one of the most important disturbances to the biocontrol agent populations in the LCRE. Semidiurnal tides may flood Columbia River wetlands twice each day. Incoming tide waters may act to dislodge beetles from host plants directly or by startling the beetles causing them to drop. Water may also wash eggs from leaves and stems. Inundation patterns may also affect the distribution of *Lythrum*. We deployed tidal gauges at the 15 USACE release sites in April 2007 (Figure 1). We collected and analyzed data collected from April to July. Tidal gauges are still in place collecting data that will be used to characterize winter tides, which may impact overwintering biocontrol agent adults. Table 10 below summarizes inundation patterns for most of the release sites. We plan to summarize inundation duration in the future.

What is interesting is that actual water depth and vertical rate of water increase is quite variable among sites and that these parameters are not simply a function of site elevation. Mott Island, the lowest site, was inundated 90 times to a depth > 6cm while Eureka Bar Upstream, the highest site, was inundated 87 times. Tenasillahe Island was inundated 151 times and Pillar Island Downstream, 18 times. Maximum water depth ranged from 0.308m (Pillar Island Downstream) to 1.169m (Tenasillahe). Average flood tide vertical water velocity was also variable, ranging from 0.0069 m min^{-1} (Dry Dock) to 0.0115 m min^{-1} (Svensen Island). Maximum flood tide water vertical water velocities, possibly related to startle response in the beetles, were much less variable than the average values. In general, maximum flood water vertical velocities were approximately 0.0161 m min^{-1} for the early summer dates. We recommend using these flood velocity values in future flee response experiments.

3.2.2 Response Behavior of *Galerucella pusilla* to Submersion and Inundation

In an effort to control purple loosestrife (*Lythrum salicaria*) in the LCRE, practitioners first introduced biological control agents in 1997 and have done so 638 times since (Moore et al., in review). Of the four approved agents: *Galerucella pusilla, Galerucella calmariensis, Nanophyes marmoratus,* and *Hylobius transversovittatus*; it is *G. pusilla* that has been released the most frequently in the estuary (41.3%) and in the greatest numbers (66.5%). Regardless of the large quantities of beetles released, the population establishment of *G. pusilla* has been variable. One of the most significant and persistent disturbances the beetles experience is that of a semi-diurnal tidal exchange. In an attempt to identify variables which might account for the varied population levels, we designed several observational and experimental studies. We conducted immersion experiments that submerged *G. pusilla* adults in the Columbia River for periods of time reflective of natural tidal cycle durations in the estuary, recording movement within 24 hours of removal from the water as surviving. These were also conducted in the laboratory with eggs and larvae. We

Table 9. Comparison of USACE Release Site Stake Elevations using the RTK GPS, LiDAR and DEM data sets. RTK are from Garono et al. 2006; LiDAR are from the LiDAR Consortium; and DEM are from the Univ. of WA.				
Site	**RTK Elevation (m)**	**TIN Elevation (m)**	**LiDAR Elevation (m)**	**DEM (m)**
Dry Dock	**2.448**	**2.451**	**2.685**	**2.71**
Devils Elbow	**2.449**	**2.442**	**0.357**	**2.548**
Eureka Bar Downstream	**3.058**	**3.058**	**2.905**	**2.886**
Eureka Bar Upstream	**5.169**	**5.069**	**2.554**	**2.554**
Fitzpatrick Island	**2.69**	**2.692**	**2.752**	**3.157**
Karlson Island	**2.32**	**2.319**	**2.615**	**4.273**
Marsh Island	**2.426**	**2.434**	**2.594**	**2.502**
Mott Island	**2.018**	**2.028**	**2.14**	**0.887**
Miller Sands Downstream	**2.278**	**2.286**	**2.271**	**2.512**
Miller Sands Upstream	**3.337**	**3.28**	**3.091**	**3.267**
Pillar Island Downstream	**2.887**	**2.888**	**2.71**	**2.688**
Pillar Island Upstream	**2.335**	**2.33**	**2.475**	**2.637**
Svensen Island	**2.366**	**2.368**	**2.502**	**3.35**
Tenisillahe Island	**2.231**	**2.231**	**2.359**	**2.36**
Wallace Island	**2.889**	**2.885**	**2.957**	**3.03**

Table 10. Average, maximum and minimum flood depth and average, maximum, and minimum vertical flood velocity for each of the 15 USACE release sites. N= number of times water flooded release site to a depth greater than 6 cm. Values calculated from tidal gauges deployed at each site as part of this study. Data for Miller Sand sites were not available due to a gauge read error. See text for details.

Site	Elevation Rank (low to high) from RTK	N	Flood Depth (m)			Flood Max Velocity (m min^{-1})		
			Avg	Min	Max	Avg	Min	Max
Pillar Island Downstream	12	18	0.2039	0.0620	0.3080	0.0081	0.0019	0.0127
Fitzpatrick	10	30	0.2733	0.0640	0.5090	0.0078	0.0021	0.0148
Wallace	14	34	0.3717	0.1080	0.6230	0.0081	0.0023	0.0144
Eureka Downstream	13	39	0.3132	0.0850	0.6650	0.0082	0.0023	0.0138
Svensen	5	75	0.4293	0.0670	0.8930	0.0115	0.0026	0.0177
Devils Elbow	6	82	0.4132	0.0600	0.8760	0.0108	0.0022	0.0177
Karlson	9	86	0.4205	0.0600	0.9230	0.0104	0.0015	0.0175
Eureka Upstream	15	87	0.4187	0.0600	0.9750	0.0105	0.0021	0.0176
Marsh	3	92	0.4184	0.0600	0.9260	0.0110	0.0021	0.0178
Pillar Island Upstream	8	94	0.4387	0.1170	0.9360	0.0100	0.0058	0.0169
Dry Dock	7	110	0.3840	0.0620	0.9620	0.0069	0.0014	0.0131
Tenasillahe	2	151	0.4858	0.0750	1.1690	0.0094	0.0020	0.0173
Miller Sands Upstream	11		NA					
Miller Sands Downstream	4		NA					
Mott	1	90	0.4251	0.072	0.922	0.011007	0.0031	0.0175

also examined the beetle's ability to withstand the physical removal from the plant by the ebbing and flowing tidal water, referred to as the flee response studies.

3.2.2.1 Mortality After Submersion

We tested the response of *Galerucella* eggs and three larval instars to submersion in the laboratory and we tested adults in the field to assess whether the beetles were physiologically limited in their ability to establish viable populations at the sites due to physical disturbances experienced.

In the laboratory, we found that the egg life stage was most vulnerable to submersion. There was approximately 50% mortality after ~6-7 hrs of immersion (Figure 14). All four developmental stages receiving submersion treatments had survivorship equal to, or less than, those of the control groups.. One hundred percent of the individuals of the L1 and L3 larval stages survived submersion of 1h, 2h, and 4h. Larval stage L2 showed decreased survivability after 1h of immersion. Survivorship across all treatments was 40.7% for eggs, 90.3% for L1, 87.3% for L2, and 93.4% for L3 (Figure 14).

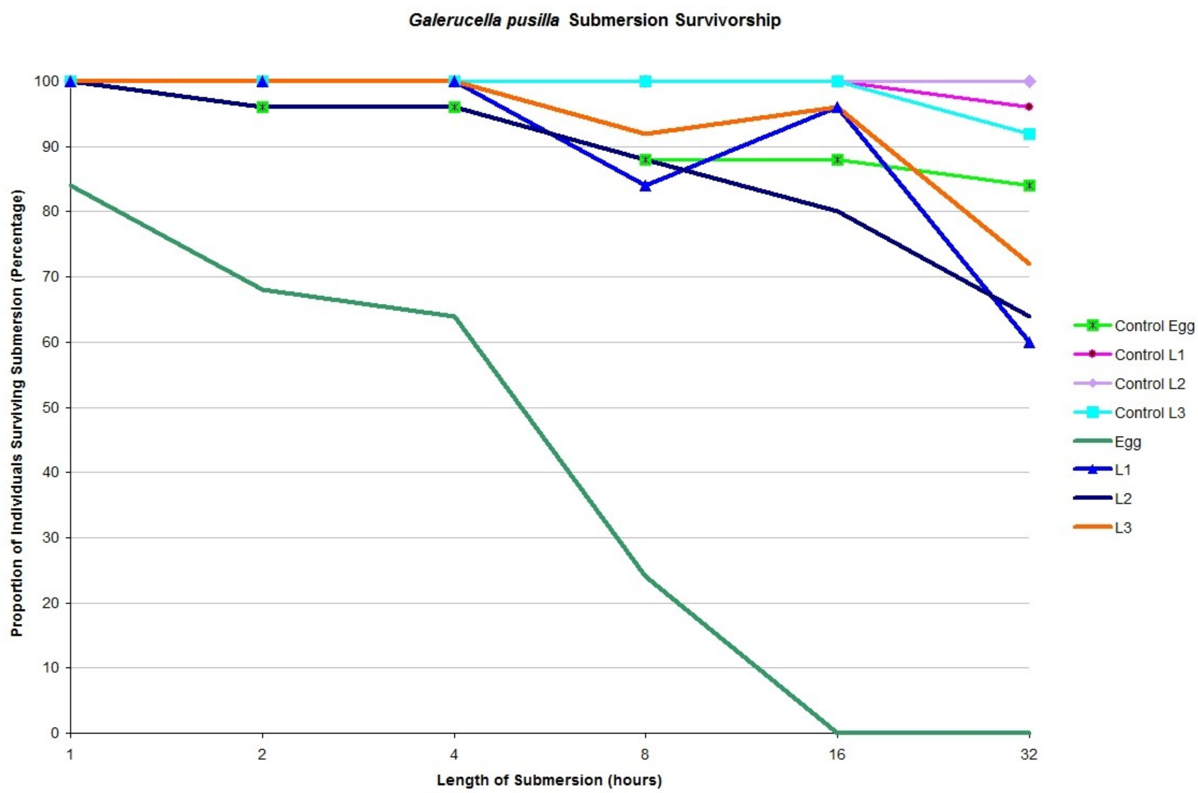


Figure 14: Survivorship of *Galerucella pusilla* after submersion in the laboratory. Shown are data for egg and three larval life stages, and control groups.

We repeated submersion experiences in the field on adult *G. pusilla* beetles and observed 100% survivorship after up to 8 hours of immersion, 70% at 18 hours, and 90% at 32 hours (Table 11). We had some difficulty in getting the vials to fill completely with water, even after "topping-off" with water poured from above and knocking the racks against large rocks to dislodge air bubbles. We also observed considerable disruption due to waves and boat wakes. We recommend repeating this experiment using vials opened at both ends at a more sheltered location. We also recommend completing our characterization of the flooding regime at each site to determine how tidal inundation patterns may be related to observed abundance and distribution of biocontrol agents at each site.

Table 11. Field Submersion Trials. Percent of *G. pusilla* adults that resumed movement within 24 hours of seven submersion treatments and control group conducted in the field. Shown are the percent surviving and the number of individuals in each trial ( ).							
Reps	**Ctrl**	**1h**	**2h**	**4h**	**8h**	**18h**	**32h**	
1	**100 (5)**	**100 (5)**	**100 (5)**	**100 (5)**	**100 (5)**	**100 (5)**	**100 (5)**	
2	**100 (5)**	**100 (5)**	**100 (5)**	**100 (5)**	**100 (6)**	**100 (5)**	**100 (5)**	
3	**100 (5)**	**100 (5)**	**100 (5)**	**100 (5)**	**100 (5)**	**17 (5)**	**100 (5)**	
4	**100 (5)**	**100 (6)**	**100 (5)**	**100 (5)**	**100 (5)**	**100 (5)**	**100 (5)**	
Total	**20**	**21**	**20**	**20**	**21**	**14**	**18**	
%	**100**	**100**	**100**	**100**	**100**	**70**	**90**	**87.3**

3.2.2.2 Flee Response

In the field, we observed that adult beetles that were able to position themselves face down at the shoot tips could remain on the plant during flooding tides and those positioned elsewhere on the host plant became dislodged. When in the leaf axils of loosestrife, *G. pusilla* adults were able to survive submergence periods of at least **45 minutes**. If dislodged, we observed that beetles were able to float but did not actively pull themselves from the water when they came into contact with plant material or wrack. It appeared beetles only actively pulled themselves onto plants/wrack when their movement was timed with the ebbing of the water. If timed correctly, the water would recede leaving the beetles exposed on the stem/wrack, and then they would begin to climb. If not timed correctly the beetles were observed bumping into stems/wrack but not able to climb out of the water.

Larvae behaved differently. Of the 21 larval individuals tested in the laboratory only two became dislodged, even though the time spent in the shoot tips by all larvae was only 0.5%. Larvae spent a greater percentage of time on upper (51.4%) compared to lower (24.2%) leaf surfaces, spending the remainder of the time on the stem or in the leaf axils. In the laboratory, once the larvae became submerged they continued to move for only 1-3 seconds and then ceased all noticeable movement. When they resurfaced during the draining of the aquarium, all larvae resumed normal movement.

We only measured the flee response of 21 larvae because of an error in calibrating the water velocity. The speed of inundation of 5 cm min^{-1} was approximately four to five times greater than we believe the beetles experience in the field. Although our field observations of vertical water velocity are not yet complete, the fastest velocity observed was 1.5 cm min^{-1} at Eureka Bar Upstream in early 2007. Once the tidal gauge data are processed, we should have actual measured values of vertical water velocity for each site. We were further hampered because L1 + L2 + adults were unavailable for additional laboratory testing in 2007. Nevertheless, the fact that only two beetles became dislodged at this highly accelerated rate seems to indicate that individuals in the L3 developmental stage would be able to tolerate the slower vertical velocities in the field. Even though this experiment was not entirely completed, the methodology, equipment, and computer programs are available for future studies. We highly recommend conducting these studies when all developmental stages are available during the summer of 2008 in order to understand the vulnerabilities of this biological control agent.

3.2.3 Working Towards a Habitat Suitability Model

During the past two years, we have observed that biocontrol agent populations seem to be responding to each of the 15 USACE sites differently. We have seen that during the past two years the beetles have been present at the same 13 release sites. We have observed that, in some cases, beetles are located well way from the release stakes. Although we have not tested the beetle distribution against a random pattern, beetles seem to be seeking out favorable microhabitats at some release sites. We have also observed that beetles are present at some sites where releases have been made in the past by other workers (not by USACE) and at sites where there have been no known releases (Moore et al., in review).

Of particular interest is an area in which the *Lythrum* was heavily affected by biocontrol agents (Figure 15; N 46.21305, W -123.43145). We observed leaves that were riddled with *Galerucella* feeding holes (Figure 16a-c). In this area, near the Tenasillahe release site, there were also *Galerucella* larvae and eggs present on many of the *Lythrum* plants. What is interesting is that the area was heavily affected by *Galerucella* but is not a known release site (see Figure 2). Moreover, other plant species appeared to be healthy so it was unlikely that this site was sprayed (Figure 17). Our observations at this site and at

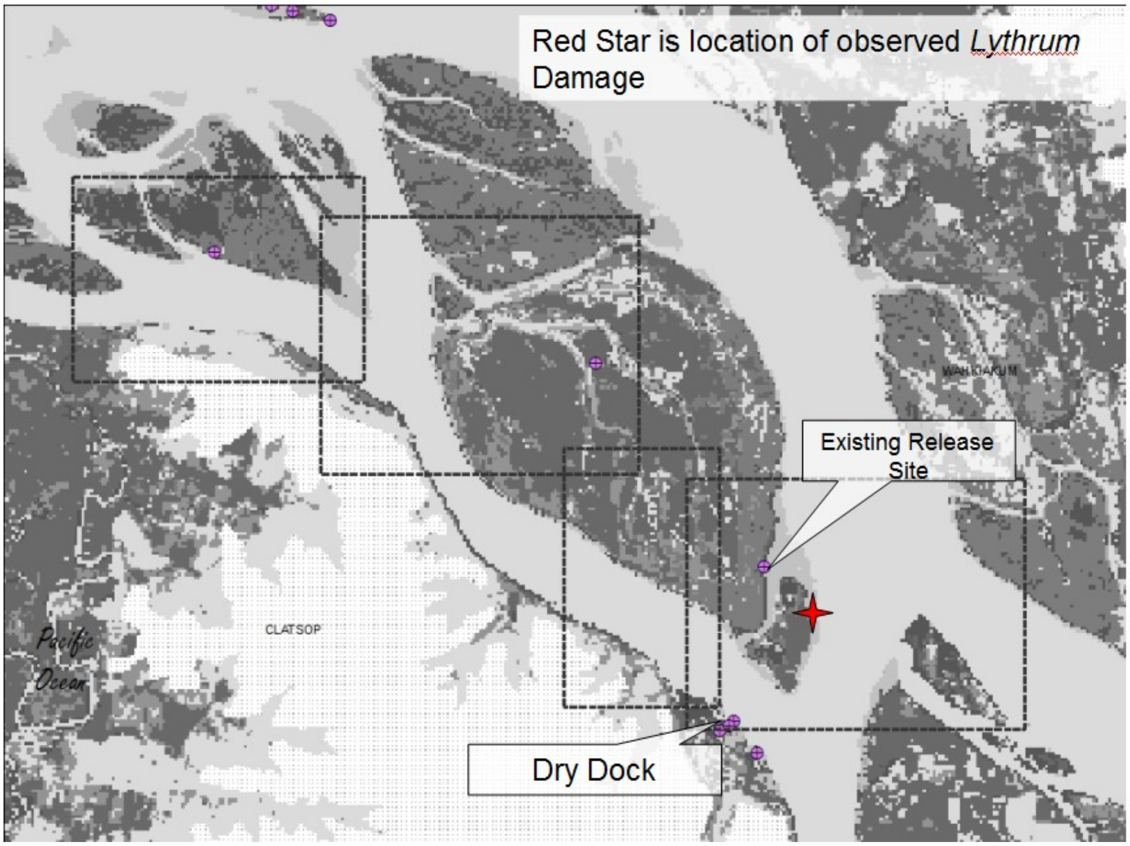


Figure 15. Location of an area of *Lythrum* heavily affected by biocontrol agents on the East side of Tenasillahe Island. Also shown are known biocontrol release sites (Moore et al., unpublished data).

Figures16 a, b, and c. Area of heavy *Lythrum* damage on Tenasillahe Island observed in September 2007.

Figure 17. Area of heavy *Lythrum* damage on Tenasillahe Island observed in September 2007. Note that only the *Lythrum* plants seem to be affected suggesting that the area was not sprayed with an herbicide.

other sites located between the 15 USACE release sites suggest that there may be specific environmental conditions that favor *Galerucella* populations.

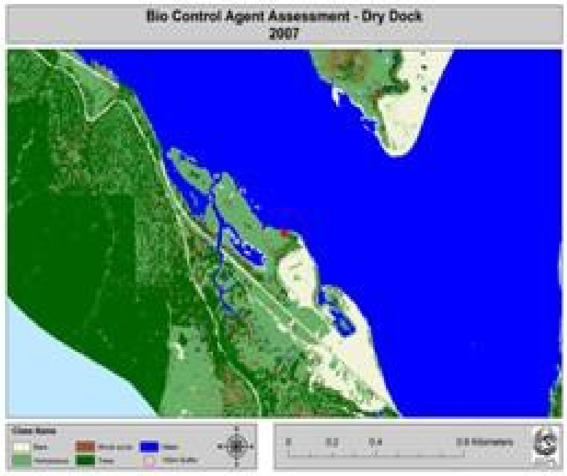
In order to evaluate the relationship between environmental conditions and beetle populations, we are building a spatial database. This database will be used to generate hypotheses that can be experimentally tested in the future. We are collecting information on periods of tidal inundation, wind/ wave exposure, land cover, and water velocity with the express goal of predicting where biocontrol agent populations are likely to successfully establish. As described above, we have developed a dataset describing the land cover (forest, shrub-scrub, herbaceous and bare) for the study area from existing color infrared photography. However, the classification accuracy of this spatial data product was only 72.6% and could have been better. Multispectral imagery is a much more data-rich type of imagery than photography. We have had much better luck classifying this type of imagery (e.g., classification accuracies ranging from 81-91%) (Table 3a-d in Garono et al. 2003b). In addition, we do not know the current distribution of *Lythrum* and were unable to detect *Lythrum* in the existing CIR photography. Since populations of biocontrol agents are closely linked to the presence of their host plants, the current distribution of *Lythrum* and current land cover are data gaps that we recommend filling.

As described above, we are collecting detailed information on tidal inundation patterns at each USACE release site. A complete set of those data, however, are not yet available for analysis. Therefore, we used tidal levels predicted by computer software (described on page 21 and Figure 7 in Garono et al. 2006), available LiDAR data, and our land cover classification to show how the release sites look at different tidal levels. Figures 18 a-aj show all sites at MLLW, MSL and MHHW (values for Knappa Slough). As tidal gauge data are processed, we plan to refine these views.

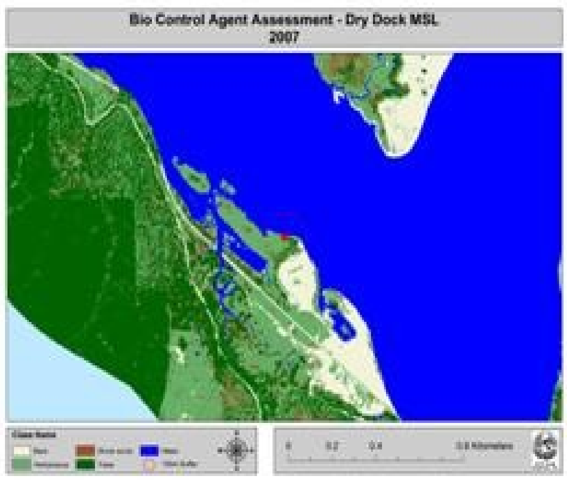
Water levels, as predicted by the Tides and Currents tidal software, exceeded MHHW ~ 3% of the time in 2006 (Garono et al. 2006). The timing of these high water events is critical and we plan to match these with the phenology of *Lythrum* and biocontrol agent populations. Figures 18f, l, o, u, and ad suggest that during high water levels, biocontrol agents at the Devils Elbow, Fitzpatrick Island, Karlson Island, and Miller Sands Downstream release stakes may be washed from host plants and prevented from moving into nearby suitable habitat. Svensen Island, although shown as flooding, is diked and probably will not flood at MHHW. In addition, the GIS model suggests that there may be areas near the release stake on Fitzpatrick Island (Fig 18 l) and other sites where biocontrol agents may be more successful in establishing their populations because it is slightly higher, wider and host plants are present.

We recommend that the GIS predictive model be developed from more detailed data describing land cover including *Lythrum* distribution, tidal inundation, and water velocity. Understanding how water moves onto and off of the release sites will help to determine how current release sites may act to inoculate adjacent sites (as described below in Section 3.3.3 – Tenasillahe example).

Figure 18. Computer generated images showing degree of flooding at each of the 15 USACE release sites. Shown are water levels predicted by Tides and Currents software for MHHW, MSL and MLLW for Dry Dock (A-C), Devils Elbow (D-F), Eureka Bar Upstream and Downstream (G-I), Fitzpatrick Island (J-L), Karlson Island (M-O), Marsh Island (P-R), Miller Sands Upstream and Down Stream (S-U), Mott Island (V-X), Pillar Island Upstream and Downstream (Y-AA), Svensen Island (AB-AD), Tenasillahe Island (AE-AG), and Wallace Island (AH-AJ). Values were derived from the Knappa Slough tide station.


Bio Control Agent Assessment - Dry Dock
2007

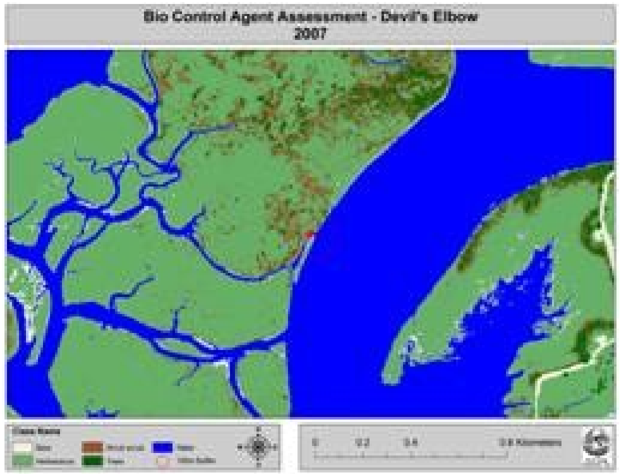
A


Bio Control Agent Assessment - Dry Dock MSL
2007

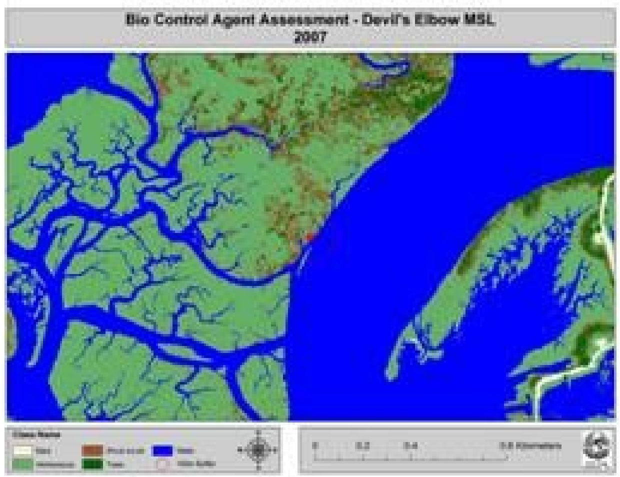
B


Bio Control Agent Assessment - Dry Dock MHHW
2007

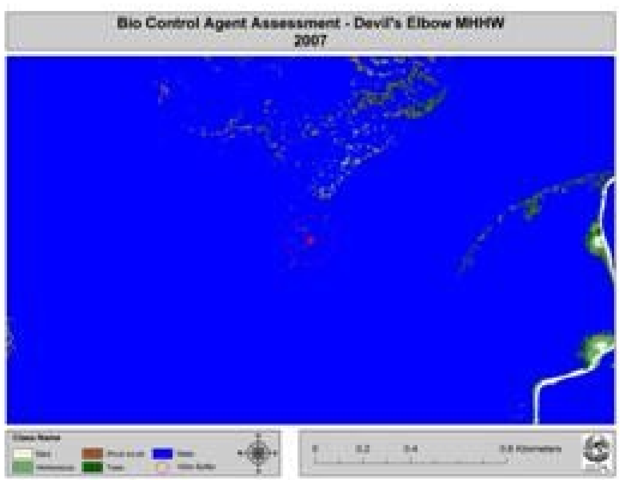
C


Bio Control Agent Assessment - Devil's Elbow
2007

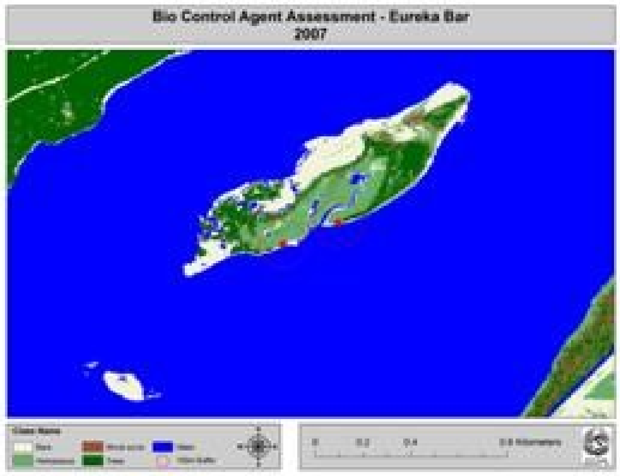
D


Bio Control Agent Assessment - Devil's Elbow MSL
2007

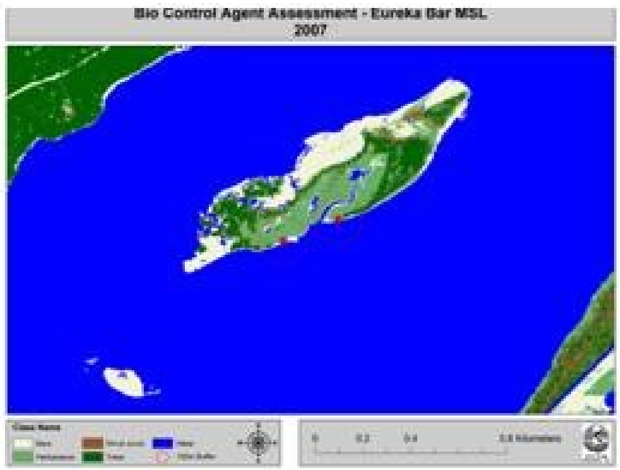
E


Bio Control Agent Assessment - Devil's Elbow MHHW
2007

F


Bio Control Agent Assessment - Eureka Bar
2007

G

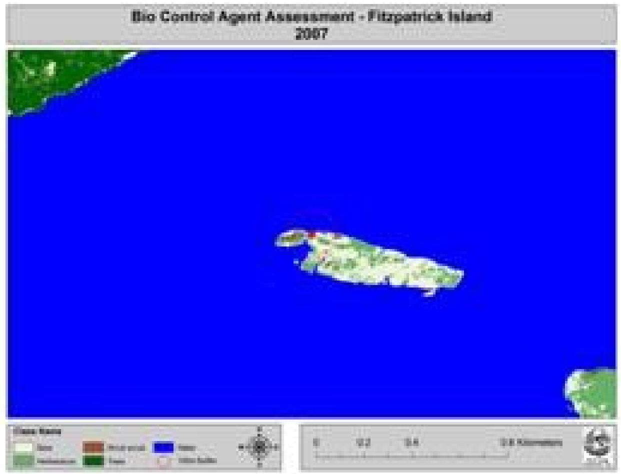

Bio Control Agent Assessment - Eureka Bar MSL
2007

H

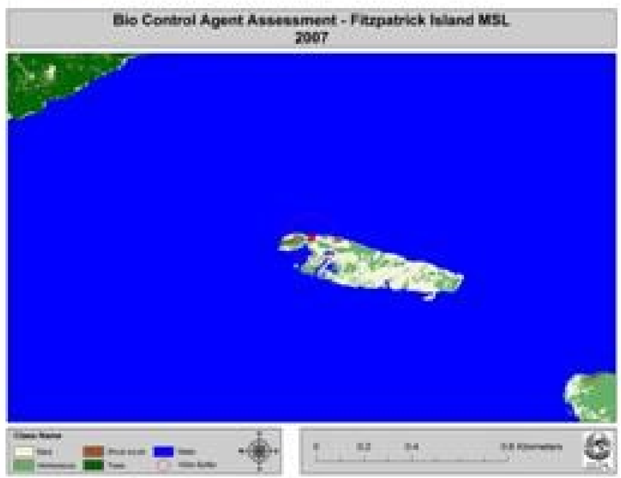

Bio Control Agent Assessment - Eureka Bar MHHW
2007

I

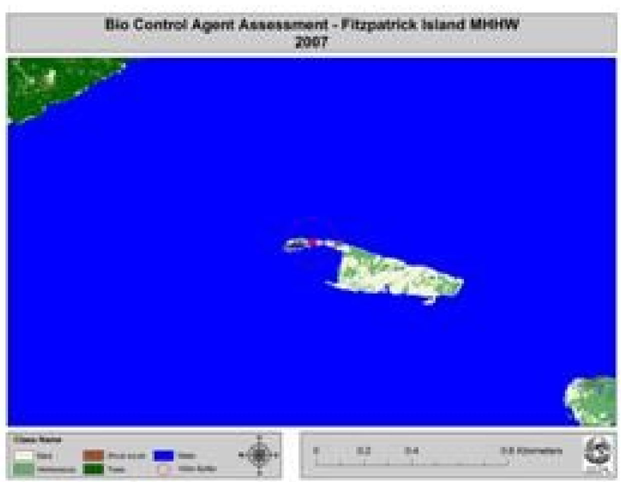
J


Bio Control Agent Assessment - Fitzpatrick Island
2007

K


Bio Control Agent Assessment - Fitzpatrick Island MSL
2007

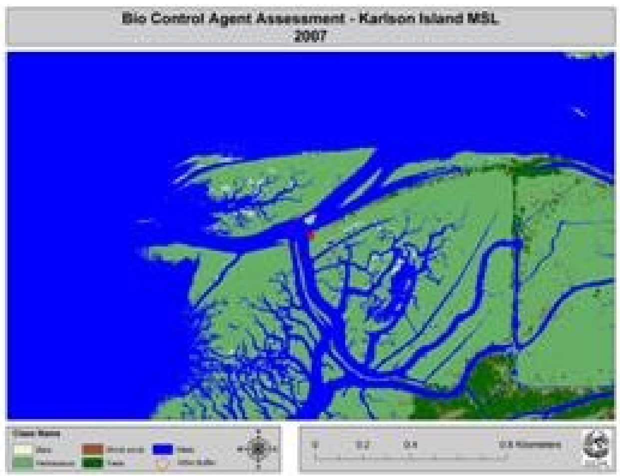
L


Bio Control Agent Assessment - Fitzpatrick Island MHHW
2007

M

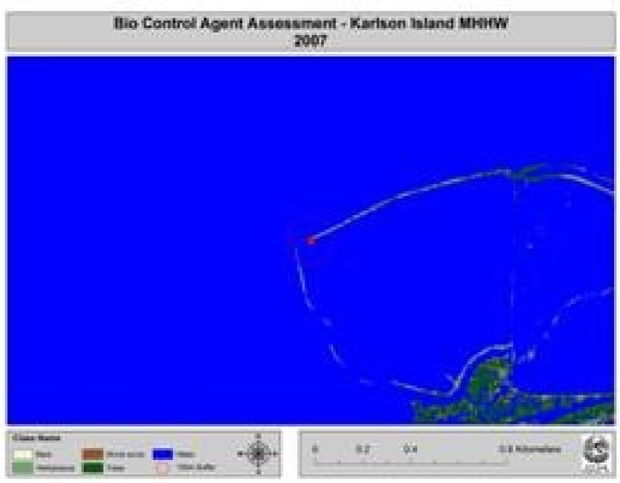
Bio Control Agent Assessment - Karlson Island
2007
0.8 Kilometers

N



Bio Control Agent Assessment - Karlson Island MSL
2007

O



Bio Control Agent Assessment - Karlson Island MHHW
2007

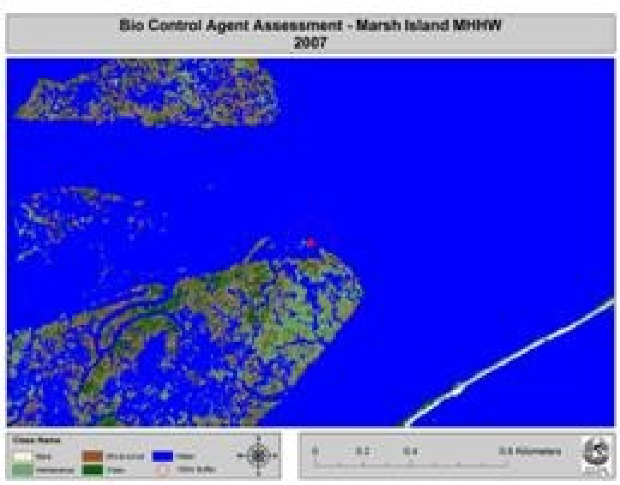
P


Bio Control Agent Assessment - Marsh Island
2007

Q


Bio Control Agent Assessment - Marsh Island MSL
2007

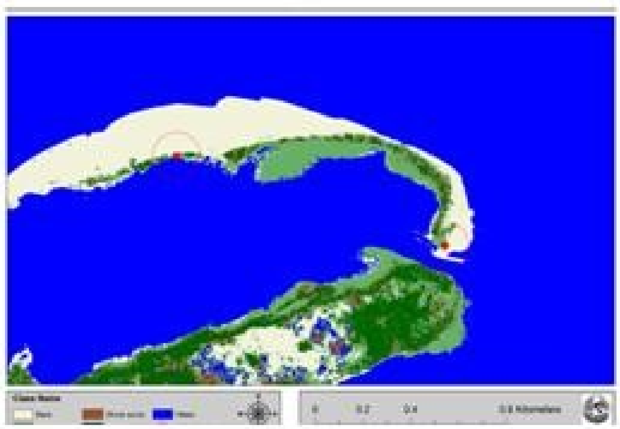
R


Bio Control Agent Assessment - Marsh Island MHHW
2007

S


Bio Control Agent Assessment - Miller Sands
2007

T



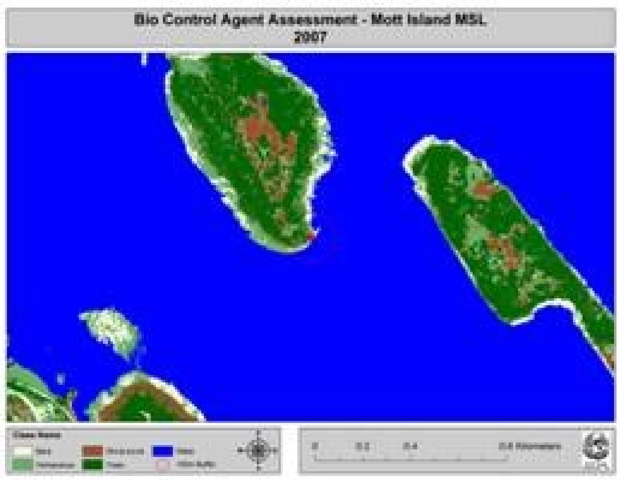
U


Bio Control Agent Assessment - Miller Sands MHHW
2007

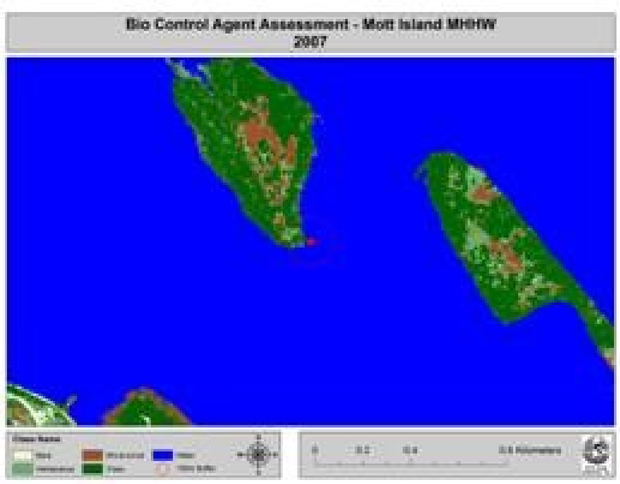
V


Bio Control Agent Assessment - Mott Island
2007
0 0.2 0.4 0.8 Kilometers

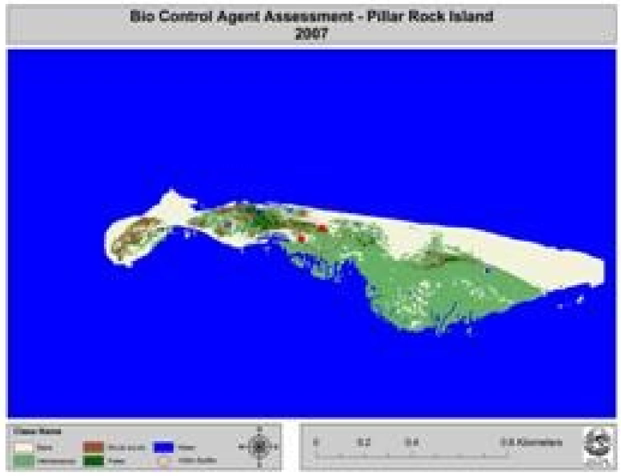
W


Bio Control Agent Assessment - Mott Island MSL
2007
0 0.2 0.4 0.8 Kilometers

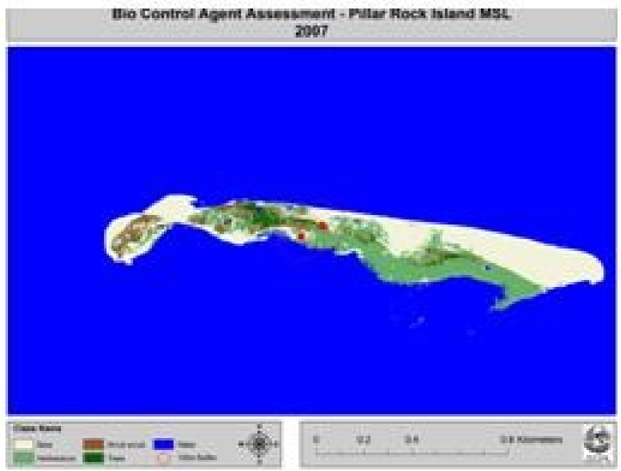
X


Bio Control Agent Assessment - Mott Island MHHW
2007
0 0.2 0.4 0.8 Kilometers

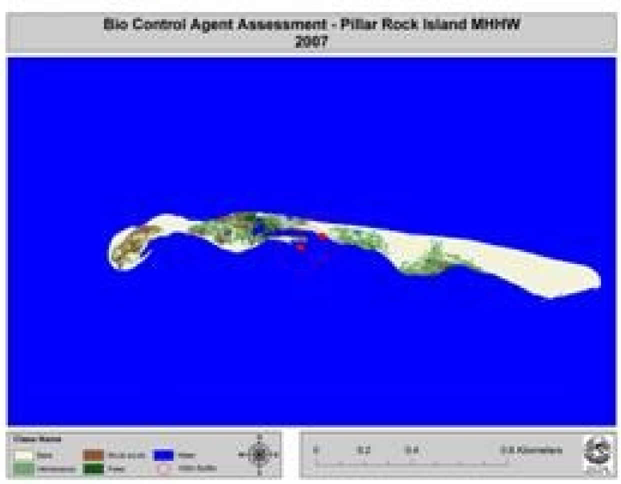
Y


Bio Control Agent Assessment - Pillar Rock Island
2007

Z


Bio Control Agent Assessment - Pillar Rock Island MSL
2007

AA


Bio Control Agent Assessment - Pillar Rock Island MHHW
2007

AB


Bio Control Agent Assessment - Svensen Island
2007

AC


Bio Control Agent Assessment - Svensen Island MSL
2007

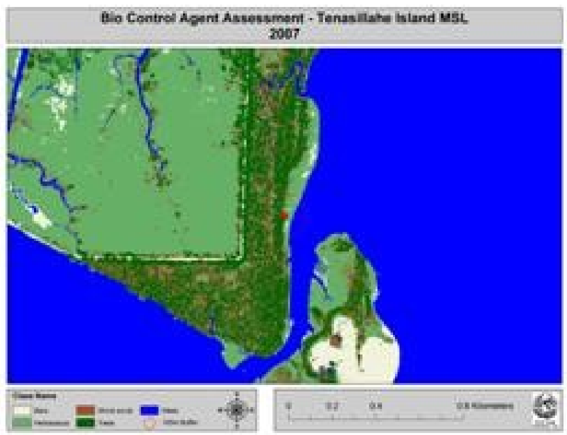
AD


Bio Control Agent Assessment - Svensen Island MHHW
2007

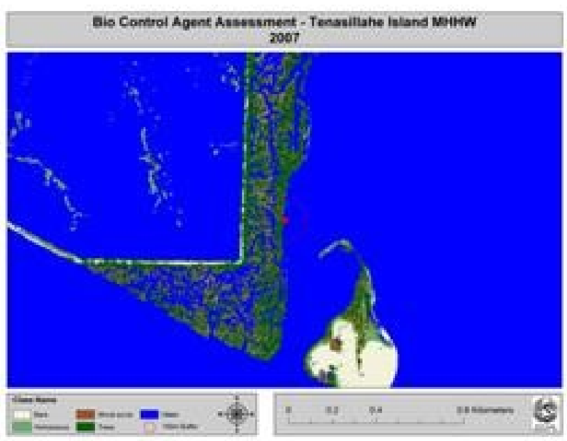
AE


Bio Control Agent Assessment - Tenisillahe Island
2007

AF

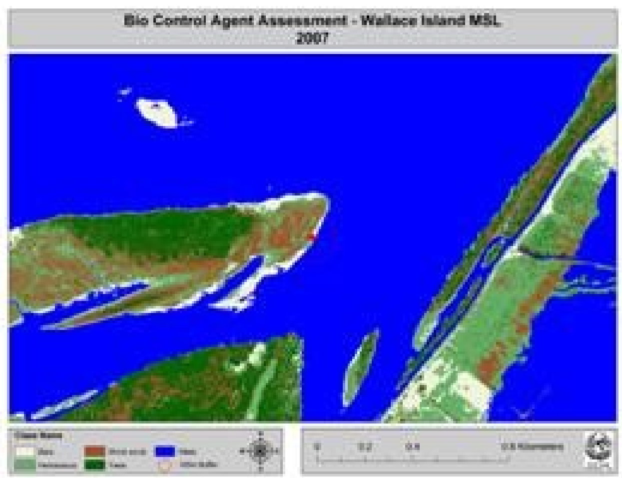

Bio Control Agent Assessment - Tenasillahe Island MSL
2007

AG


Bio Control Agent Assessment - Tenasillahe Island MHHW
2007


Bio Control Agent Assessment - Wallace Island
2007
0
0.2
0.4

AH


Bio Control Agent Assessment - Wallace Island MSL
2007
0
0.2
0.4

AI


Bio Control Agent Assessment - Wallace Island MHHW
2007
0
0.2
0.4

AJ

3.3 Site Summaries

3.3.1 Devils Elbow

In 2007, we observed most of the beetles within 25m of the release stake. Although we observed adult beetles during every survey trip, most of the adult biocontrol agent beetles were observed in April and August (Figure 8). Adult beetles were present within 10m of the release stake only during the April survey trip. Beetle damage patterns follow the observed presence of adult beetles and also occurred within 25m of the release stake (Figure 9). *Lythrum* densities observed in sampled quadrats were relatively sparse (Figure 4). Quadrats with relatively high densities of *Lythrum* are also where adult beetles were observed.

3.3.2 Dry Dock

We observed adult beetles during all three survey trips and there were adult beetles present within 10m of the release stake in April, July and August (Figure 8). Most of the adult beetles were detected on the western side of this site and during the spring and early summer surveys. Interestingly, during surveys of this site, we found insects and plant damage along the tree line on the eastern side of the site. We anticipate surveying this site in a more systematic survey in 2008. Although we observed more plant damage in August, when the beetles were not readily detected, the feeding damage matched patterns in the distribution of adult beetles (Figure 9). Distribution of *Lythrum* was somewhat patchy but matched the adult beetle distribution well within the western portion of the site (Figure 4).

3.3.3 Eureka Bar Downstream

We observed adult beetles during two of the three survey trips at this site (Figure 8). Eureka Bar Downstream is interesting because most of the adult beetles were located between 25m to 50m away from the release site, not at the stake itself, although there were plenty of host plants near the release stake (Figure 4). This suggests that some factor may be responsible for moving beetles back into the site away from the beach. Also interesting is that the feeding damage did not match the adult beetle distribution especially in August (Figure 9). We did observe most feeding damage in August.

3.3.4 Eureka Bar Upstream

As with the other Eureka Bar site, we also observed adult beetles during the same two (April and July) of the three survey trips at this site. All of the adult beetles were observed within 25m of the release site in 2007 and only within 10 m of the release stake in July (Figure 8). This is in contrast to the adjacent Eureka Bar Downstream site where beetles were located at some distance from the release site. Beetles were also observed in quadrats located along the beach. Feeding damage appears to be more widespread than what the presence of adult beetles would suggest (Figure 9). The heaviest damage was in August. Figure 4 shows that *Lythrum* was available over most of the site except for beach areas.

3.3.5 Fitzpatrick Island

We did not observe adult beetles at all on Fitzpatrick Island until the August field trip (Figure 8). This underscores the importance of multiple observations made at different times to detect beetles at low densities. All of the beetles were observed within 25m but not any closer than 10m to the release stake. We did not observe any feeding damage in April or July (Figure 9). There was feeding damage in August in the same area where adult beetles were observed. *Lythrum* distribution is patchy at this site; there are areas, however, with fairly dense stands (Figure 4). This will be an interesting site to see if Lythrum spreads out onto the southern sand flats. It is also a site that may benefit from another release on the eastern side of the Island (see discussion in Section 3.2.1.4).

3.3.6 Karlson Island

We only observed adult beetles in August in one quadrat that was about 12m from the release stake but none of the beetles were any closer than 10m (Figure 8). We did, however, observe beetle feeding damage within about 25m of the release stake during all three field surveys (Figure 9). The distribution of *Lythrum* at this site is sparse near the release site and is somewhat patchy within 100m of the release stake, and may, therefore, affect the spread of the biocontrol agents (Figure 4). In addition, this site may flood frequently thereby reducing beetle populations (see discussion in Section 3.2.1.4). It would be interesting to determine if the height of the *Lythrum* plants is high enough to elevate beetle eggs above flood water.

3.3.7 Marsh Island

We did not observe any adults in 2007 at the Marsh Island site (Figure 8). We did, however, observe eggs. We also found possible feeding damage to almost 50m from the release stake in July and August mainly along the shore (Figure 9). Although we did observe isolated *Lythrum* plants south of the release site, we did not sample *Lythrum* in our quadrats in areas other than those to the northwest of the release stake (Figure 4). The patchy distribution of *Lythrum* may affect how the beetles move within this site. We did observe more *Lythrum* about 200m south of the release stake. This area may be a candidate for future releases.

3.3.8 Miller Sands Downstream

This was one of the few sites that had plenty of *Lythrum* distributed throughout the site in which we did not find any adults, larvae or eggs of the biocontrol agent, nor did we find any feeding damage (Figure 4, 8, 9). We did, however, find adult *Nanophyes* at this site. We speculate that this site has much of its vegetated area submerged during higher tides (see discussion in Section 3.2.1.4) and unlike other sites, the entire site is at the base of steep, sandy hill. There is very little *Lythrum* on the island proper. A GIS layer depicting the extent of *Lythrum* in the vicinity of this sight may be useful in re-establishing biocontrol populations on Miller Sands.

3.3.9 Miller Sands Upstream

We observed adult beetles at this site only within 25m but no closer than 10m to the release stake during the April surveys (Figure 8). Feeding damage was also observed in the spring and not during later surveys (Figure 9). Although there are numerous, widely distributed host plants extending more than 50m from the release stake, much of the site is either bare sandy beach or dense willow thicket (Figure 4). This seems like it is a rather high energy site evidenced by its position on the exposed point of Miller Sands and the sparsely vegetated dune just south of the release stake. This site might be a good candidate to watch over time to see if *Lythrum* stabilizes some of the sandy beach to the west of the release stake.

3.3.10 Mott Island

We did not observe adult *Galerucella* in sampled quadrats at this site in 2007 (Figure 8). However, we did observe both eggs and larvae. Feeding damage was confined to areas primarily along the shoreline as far as 50m from the release stake (Figure 9). *Lythrum* distribution was patchy for the most part and was most commonly encountered in our quadrats during the August field survey (Figure 4). This site has a low vegetated tidal marsh to the southeast of the release stake. We surveyed in the tidal marsh area in 2006 and 2007 hoping to establish whether the *Lythrum* population moves toward the water's edge.

3.3.11 Pillar Island Downstream

In contrast to the adjacent site (Pillar Upstream), we did not observe any adult beetles at this site in 2007 (Figure 8). Nor did we observe eggs or larvae. *Galerucella* also was not observed at this site in 2006. Lack of direct evidence of *Galerucella* and lack of the characteristic feeding damage (Figure 9) strongly suggest that the beetles are no longer present at this site although there are fairly dense stands of *Lythrum* (Figure 4). We recommend continuing monitoring of this site to see if biocontrol agents from the adjacent site, only ~100 m away, colonize this site.

3.3.12 Pillar Island Upstream

We observed adult beetles from 0m to 25m of the release stake during all three site visits in 2007 (Figure 8). Feeding damage patterns followed beetle occurrence (Figure 9). Feeding damage was the most widespread in August extending out to 50m. We observed dense *Lythrum* throughout the site (Figure 4).

3.3.13 Svensen Island

We observed adult biocontrol agents during all three field trips in 2007 at this site. Beetles were observed from 0m to 50m from the release stake (Figure 8). Feeding damage patterns generally follow the beetle distribution (Figure 9). We did note feeding damage on the west side of the dike suggesting that the beetles were able to move across 5m -10m of area that had no *Lythrum*. The host plants tended to occupy the eastern side of the dike down to the water edge. We extended our surveys out onto the mud flats, in order to determine if the *Lythrum* will colonize those low areas in the immediate future (Figure 4).

3.3.14 Tenasillahe Island

We observed adults during all field surveys in 2007. Adults were present out to a distance of 50m from the release stake in April (Figure 8). We observed beetles within 10m of the release stake in April and August. Feeding damage generally followed the occurrence of the adult beetles, especially along the tree line north and south of the release stake (Figure 9). We found that the most dense areas of *Lythrum* also occurred along the tree line and that *Lythrum* was relatively sparse in the low marsh to the east of the release stake (Figure 4). We extended a few of our transects into the low marsh to see if the *Lythrum* becomes more predominant in these areas during the next few years. We also observed an area of extensive biocontrol agent damage near, but not in this site (see Section 3.2.3). <u>We recommend that a more thorough survey be completed on this site because we often observed biocontrol agents and native *Galerucella* in areas adjacent to sampled quadrats.</u>

3.3.15 Wallace Island

We observed adult beetles during all three sampling events at Wallace Island (Figure 8). Beetles were present at distances from 0m to 50m from the release site. As with other sites, patterns of feeding damage tended follow the distribution of the beetles (Figure 9). Host plants were dense in the vegetated strip that runs parallel to the shoreline (Figure 4).

4 Conclusions and Recommendations

4.1 *Is the biological control of* Lythrum *progressing favorably? Key Questions Pertaining to Populations of Biocontrol Agents and Lythrum*

Is *Lythrum* continuing to spread and increase in abundance (density/biomass/cover) in the absence of the biological control agents?

We currently do not have the data needed to quantitatively examine the spread of *Lythrum* in the estuary. However, observation over the past five years suggests that it is continuing to spread. Stem density at some of the USACE sites did change from 2006 to 2007 but the change was not consistent with increases at some sites and decreases at others. It would be useful to have a number of randomly selected reference ("control" or "untreated") sites, where biological control agents are absent to compare with "treated" sites and to assess spread and abundance of *Lythrum* in the absence of the agents. <u>**We recommend that an estuary-wide survey of *Lythrum* be initiated, that control areas be monitored, or that the 35 transects surveyed by helicopter in 2006 be re-visited.**</u>

Are biocontrol agents persisting and creating self-perpetuating populations at the 15 sites?

Surveys in 2007 indicate that populations have persisted at 13 of the sites (Table 2a). However, results were variable throughout the year and may not indicate that the populations are self-perpetuating. Generally, at least three years of observations are necessary before agent establishment can be determined. Moreover, we are aware that other groups have been releasing biocontrol agents, including *Galerucella*, in the estuary. For this reason, we have completed development of a biocontrol agent spatial database (Moore et al., in review). This information will help us to interpret results from this USACE study. **We recommend that the surveys be continued in 2008.**

Are biocontrol agent populations increasing?

We believe that it is too early to conclude whether populations are increasing. At some sites we did observe higher evidence of biocontrol agents. In 2007, however, with only two years worth of observations we recommend that the surveys be continued in 2008 since at least three years of observations (Coombs persistence definition) are necessary to make claims about establishment and changes in population sizes.

Are biocontrol agents spreading from the point of release and how quickly are they moving?

Yes, biocontrol agents are spreading from the point of release (Figures 8 a-o; Section 3.2.3). We recommend continuing surveys in 2008 and conducting more detailed surveys at several of the release sites.

Is biocontrol agent damage to *Lythrum* increasing?

We have not been able to answer this question. We recommend continuing surveys in 2008.

Are biocontrol agents affecting *Lythrum* populations?

It is too early to tell. There is evidence of areas where relatively large areas of *Lythrum* have been killed by biocontrol agents; however, these are not USACE release areas. We recommend continuing surveys in 2008 and visiting areas between release sites. We also recommend an estuary-wide *Lythrum* survey. Other measures of impact of biocontrol agents, (e.g., cover, plant height, and seed production) and a more repeatable/ quantitative measure of leaf damage, may be worth evaluating.

To fully answer this question we need to develop a management goal. The impact of an invasive plant is a product of its spatial extent, density, and *per capita* impact (Parker et al. 1999). A prior study of the impact of *Lythrum* on plant diversity indicates that reducing *Lythrum* to below 30% cover will result in plant diversity at levels similar to that of native vegetation (Schooler et al. 2006). Thus, if the biocontrol agents can maintain levels of *Lythrum* below 30% cover across the estuary, it is one measure of success. Partial success may be considered if biocontrol agents reduce *Lythrum* at some sites but not others.

4.2 What might be limiting the progress of biological control? Key Questions Pertaining to Environmental Factors Affecting Populations of Biocontrol Agents

What factors are related to persistence and damage of the biological control agents?

We believe that hydrologic disturbance and vegetation may be related to biocontrol agent population establishment and effective control. We have started to examine the relationship between biocontrol agent abundance and distribution and these environmental variables. We are currently developing the datasets necessary to answer these questions. Inundation studies and observations indicate that each of these variables affects the effectiveness of the biocontrol agents in the estuary. Additionally, more study is needed to examine where and when these factors will limit the effectiveness of the *Galerucella* beetles. In addition, the other agents may have different responses to these variables and may be important in controlling *Lythrum* in some habitats. We expect to generate and test hypotheses related to these observations. We recommend that both sites and selected areas between sites continue to be characterized.

Are the release locations at the sites optimal for beetle establishment (model of above matched to GIS)?

This question will be addressed with the datasets and analysis described in this report. Other issues: We also recommend evaluating other invasive plant species at release sites. We are concerned that selectively controlling one species (e.g., *Lythrum*) will lead to increasing abundance of others. Other invasive plants are present throughout the estuary. Prior observations of biological control of *Lythrum* indicate that *Phalaris* does limit the increase of plant community diversity as *Lythrum* density decreases (Schooler 1998). However, dense

Phalaris populations cannot withstand long periods of inundation and are limited to higher elevations than *Lythrum*. Our field teams have measured the presence of other invasive species at our field sites. We recommend that future work focus on the impact of these other invasive species (spread and density) on the desired state of the estuary (plant diversity/salmonid production).

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