Early Detection of a New Invasive Mesogastropod, *Assiminea parasitologica*, in Pacific Northwest Estuaries

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South Slough National Estuarine Research Reserve
with
The Confederated Tribes of the Coos, Lower Umpqua, and Siuslaw Indians

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Executive Summary

Assiminea parasitologica is a small intertidal estuarine mesogastropod snail native to Japan. This species was first documented in the Coos Bay Estuary, Oregon in June of 2007. The discovery of this new non-native species in the Coos Estuary catalyzed a summer 2008 expedition lead by Dr. James T. Carlton and colleagues to search for *A. parasitologica* in estuaries in Oregon and Washington. The aforementioned work generated the basis for this study; to conduct a systematic baseline survey of the distribution, ecology, and life history of *A. parasitologica* in Southern Oregon Estuaries.

The arrival of A*ssiminea parasitologica (AP)* may pose ecological problems as the snails develop interactions with the existing community of mesogastropods in the salt marshes and other intertidal habitats of Coos Bay. Several species of small brackish water mesogastropods currently inhabit the intertidal zone of the Coos Estuary, including the native *Angustassiminea californica (AC) and Littorina subrotundata (LS),* and non-native *Myosotella myosotis (MM).* The potential of this new invader to interact with or displace existing mesogastropods directed this study to collect baseline data on these four species of snails.

The primary goal of the study was to obtain a current (2009) snap shot of the status of *Assiminea parasitologica* in Pacific Northwest Estuaries. The project focused on characterizing: 1) the abundance, distribution and habitat used by *AP* in the Coos Bay Estuary 2) the reproductive biology of AP in the Coos Bay Estuary 3) the distribution of AP in Pacific Northwest Estuaries.

The surveys to determine the distribution, abundance and habitat use of AP in the Coos Estuary employed a two-tiered sampling strategy: (1) A superficial and extensive Rapid Assessment Method, with evenly spaced sampling to cover all extents of each salt marsh and (2) A thorough and spatially limited Detailed Assessment Method, using randomly generated points within marshes having known high AP abundance. Both methods included habitat classification and abundance measurements for the four snail species.

The primary goals of the Rapid Assessment were to classify the habitat and create distribution boundaries of the new invasive and existing mesogastropod populations that were present. The Detailed Assessment allowed us to quantify specific snail abundances and further examine potential mechanisms of distribution.

The environmental and/or habitat signature was unique to each area sampled in both methods. This uniqueness was not due to one environmental factor but a combination of variables. Environmental data from areas sampled were grouped and proved to be statistically different by spatial assignment. Although individual waterways had multiple salinity zones, each region of the estuary (consisting of several waterways) had a dominant zone. This dominant salinity zone may have been the main component to the unique signature of each region.

The tidal marsh habits of the Coos Estuary are highly influenced by local topography. In addition, marshes in the Coos Estuary have been highly altered by historic land uses including diking, filing, draining, and encroachment or alteration by transportation infrastructure. The result is that today waterways of the Coos Estuary support a mix of broad and compressed marshes. The combination of land use and topography along an individual waterway typically resulted in one marsh type dominating that waterway. All areas sampled had a range of marsh strata and dominant vegetation. The salt marshes of Coos Bay have distinct topographic features including tidal channels, large rises and low shallow pools. Marsh strata and vegetation vary within an individual marsh of the Coos Estuary. As the marsh topography changes so do the salt marsh plants; exhibiting classic salt marsh vegetation stratification.

We found that the distribution, relative abundance, and absolute abundance of the mesogastropods examined in this study reflected the variation in habitat found in the marshes of the Coos estuary. The species-specific snail distribution, relative abundance, and abundance patterns reflect this variation in habitat type. Our correlations of specific habitat types with specific species strongly suggest that all four snail species' distribution patterns are dictated by species-specific responses to environmental factors. Of all the

factors measured in this study, salinity appears to be the dominant factor influencing the distribution of these four mesogastropods species.

AP was found in all areas sampled within the Coos Bay Estuary. Both assessments revealed similar results and patterns about the distribution of AP. This new invader was found in highest concentration at the Isthmus Slough (approximately 750 snails per $0.5m^{2)}$ compared to any other area of Coos Bay and in polyhaline-dominated waters. However, AP also persisted in a wide range of salinity regimes. AP was found across marsh types, marsh strata, and vegetation.

According to the data generated from this study, AP is by an order of magnitude the most abundant mesogastropod examined in the Coos Bay system. AP shows preference for more brackish water but can tolerate a wide range of salinity regimes. AP and AC had similar patterns of abundance and distribution across the estuary and appear to be able to persist in a variety of habitats. LS and MM were found in much lower numbers than AP or AC across the estuary. The data suggests that LS is more persistent in a euhaline-type habitat, with low marsh topography and vegetation. MM, although low in total numbers, was widely distributed and occupied a variety of habitats.

This is the first study to collect baseline data on the abundance and species-specific habitat correlations of these Snail Guild species. Therefore, assessing or determining change in the existing populations of AC, LS and MM was not possible. Although there are biases in the design toward the collection of AP and high variation between plots, the pattern is still clear that AP is abundant in the Coos Bay estuary. Given the high densities and range of habitats that AP can inhabit there is striking potential for AP to move into new areas of the estuary and possibly displace already-existing mesogastropod species. The data from this survey strongly suggest that: AP could displace AC as it persists and is successful in similar habitats and AP could displace LS and MM as these species populations are low in comparison to AP.

Questions about *A. parasitologica's* reproductive biology arose quickly after this non-native species was found in Oregon. At the time *Assiminea parasitologica* was detected in Oregon, very little was known about the reproductive biology of *A. parasitologica* in Japan. Live *A. parasitologica* collected in the Coos Estuary were brought to the Oregon Institute of Marine Biology in Charleston for observation; AP was observed to lay egg capsules in the sediment from which planktonic larvae hatched out as veligers (James T. Carlton, pers. comm., 2008).

Until recently only two of the Japanese Assimineids' reproductive biology had been examined, *Assiminea hiradoensis* and *Angustassiminea castanea* both of which have planktonic larvae. Our primary goal was to gain any further information, either qualitative or quantitative about the reproductive strategy of AP. We examined the gametogenic activity of select populations of AP within Coos Bay to investigate the seasonality of this organism's reproductive cycle. We were curious to know if the gametogenic activity of AP was synchronous across the Coos Bay population. It was found that AP is broadly synchronous across Coos Bay with active egg production in June-July and ovary content steadily declining from August through November. Variability between sampling sites appears to be correlated with differences in sun exposure during the summer. Individual females were found to contain over 40 eggs. Abundant young of the year AP were documented in August.

In order to assess the distribution and possible invasion of AP in other Pacific Northwest Estuaries we conducted presence/absence surveys of shoreline habitats for AP and other mesogastropod snails in the Umpqua, Smith, Siuslaw and Willapa systems.

Carlton et al. (2008) detected AP in three Oregon estuaries in 2008. We detected AP in five Pacific NW estuaries in 2009. Two estuaries with newly detected populations, the Coquille and Smith, are rather small waterways that do not experience commercial boat-based shipping traffic. These waterways do however experience relatively high levels of recreational fishing and boating traffic. Although not experimentally examined, these

findings support the hypothesis that AP is being transported to other estuaries via human equipment.

This study successfully met the objective of collecting baseline data on a recently detected invasive species. The data generated here reveals that *A. parasitologica* has successfully invaded the Coos Bay estuary, inhabits a wide breadth of habitats, making it advantageous in its invasion, reproduces on a seasonal cycle, and is expanding its colonization into other estuaries in the Pacific Northwest. In addition, this study yielded distribution and habitat use data for the existing mesogastropod snails that occur in Pacific Northwest Estuaries. We hope the information, data, and observations generated by this project will be of value to future studies that examine mesogastropods and habitat in the salt marshes of Pacific Northwest estuaries. We believe this data will play a critical role as we track this model organism's invasion of estuaries throughout the west coast of North America.

1. Introduction

1.1 Project Components and Personnel

Project Title: Early Detection of a new invasive mesogastropod, *Assiminea parasitologica* in Pacific Northwest Estuaries

This project was funded by NOAA and administered through the Pacific States Marine Fisheries Commission. The study was conducted via a cooperative agreement between the South Slough National Estuarine Research Reserve and the Confederated Tribes of the Coos, Lower Umpqua and Siuslaw Indians, one of three federally recognized Indian tribes on the Oregon coast.

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Report compiled by: Alix Laferriere & Heidi Harris

Contributing Team Members and Roles:
Principal investigator Graybill and Office Manager Robin Elledge coordinated grant administration through the Pacific States Marine Fisheries Commission. The team meet monthly to review materials and protocols and to discuss future directions of the project. Principal Investigators Graybill and Crombie advised Project Leader Laferriere on project decisions and protocols. Laferriere coordinated South Slough NERR and Confederated Tribes activities including experimental design, site selection, data collection, analysis, interpretation and written and oral communication of project. Laferriere lead the work of two Biological Technicians, Harris and Schaefer. Harris led the Geographical Information System (GIS) analysis and construction of the Geodatabase. Harris assisted in all field work, data interpretation and report writing. Schafer lead the reproductive module and assisted in data collection in the Umpqua, Smith and Siuslaw estuaries.

In addition, members of the team greatly benefited from the assistance of Dr. James T. Carlton, director of Williams Mystic maritime Studies Program, who provided much guidance and expertise to the project in aspects of invasion biology, experimental design, data analysis and interpretation. Dr. Steven Rumrill, Research Coordinator at the South Slough National Estuarine Research Reserve, advised the reproductive module methodology, and assisted in data analysis and interpretation. Stacy Galleher, biologist at the Oregon Department of Fish and Wildlife provided invaluable GIS assistance.

Project Timeline: The principal investigators first met in the fall of 2007 to discuss collaboration and the potential to characterize the status of an invasive snail first described in the Coos estuary in the summer of 2007. Discussion with NOAA's aquatic invasive species program began in late 2008. The award start date for this project was November 1, 2008. Project Leader Laferriere started in March of 2009. Field work started in May and continued through October 2009. End date for the project was January 31, 2010.

1.2 Project Foundation: Invasion History and Project Stimulus

Oregon's shallow estuaries and protected embayments are highly susceptible to colonization by non-indigenous species. For example, commercial shellfish mariculture operations have resulted in numerous deliberate and inadvertent introductions of non-native species into several different types of estuarine habitats including rip-rap and docks, salt marshes, eelgrass beds, tideflats, and subtidal channels. Moreover, intensive human settlement and industrial shoreline development in the Oregon estuaries that support deep-draft maritime traffic (*i.e.*, Coos Bay, Yaquina Bay, Columbia River) have been coupled with global increases in shipping, reductions in transoceanic transit times, and chronic introductions of non-native species over the past century (Rumrill, 2006). The increased frequency of non-native species associated with ballast water transport has created many opportunities for non-indigenous species to invade new habitats that are characterized by frequent disturbance and perturbations by anthropogenic activities (Carlton and Geller, 1993; Ruiz *et al.*, 1997).

In particular, the Coos estuary (Coos Bay) is currently inhabited by about 80 species of non-indigenous marine and estuarine organisms, and by about 25 species that are cryptogenic in origin (Hewitt, 1993; Carlton, 2001). Only San Francisco Bay (CA) contains a substantially greater number of non-indigenous aquatic species. The extent of colonization by non-native species in Coos Bay is comparable to several other major urbanized estuaries located along the Pacific coast of North America including San Diego Bay (CA), Humboldt Bay (CA), and the Columbia River estuary (OR-WA; Cohen and Carlton, 1998; Ruiz *et al.*, 2000). Introduction of new species occurs within Coos Bay on a regular basis, and the estuary is considered to be highly susceptible to invasion by non-indigenous species in the future.

During a recent rapid-assessment survey of non-indigenous marine and estuarine organisms in Coos Bay (J.T. Carlton, *pers. comm.* July 2007), several populations of a small brackish-water snail were discovered in the upper mesohaline/riverine regions of

the estuary where they were estimated to occur at densities in excess of thousands of individuals per square meter. Specimens of the unknown snail were sent to Dr. H. Fukuda (Okayama University, Japan) for taxonomic identification, and the snails were identified as *Assiminea parasitologica* (no common name). Of special interest; this snail in its native country is host to the parasitic lung fluke, *Paragonimus ohirai.* This is the first documented occurrence of *A. parasitologica* in Oregon estuaries and in North America.

This finding catalyzed an expedition by Dr. Carlton and colleagues to seek out the presence of *A. parasitologica* in estuaries in Oregon and Washington in the summer of 2008. The expedition team found that *A. parasitologica* had invaded the Umpqua and Yaquina systems and was not yet present in the Smith, Siuslaw, Alsea, Siletz, Schooner Creek, Devils Lake, Nehalam, Tillamook, Columbia and Willapa Systems. No parasites were found in any of the specimens and the production of planktonic larvae was observed in the laboratory. The findings from this ten day expedition in 2008 generated the basis for this study, to conduct a systematic survey to collect baseline data on the distribution, ecology and life history strategy of *A. parasitologica.*

The new arrival of *A. parasitologica* may pose ecological problems as the snails develop interactions with the existing community of mesogastropods in the salt marshes and other intertidal habitats of Coos Bay. Several species of small brackish water mesogastropods currently inhabit the intertidal zone of the Coos estuary, including the native *Angustassiminea californica* and the native *Littorina subrotundata*, and the non-native *Myosotella myosotis*. All of these native and non-native gastropods graze detritus that accumulates within salt marshes, and empirical observations indicate that success of the non-native *M. myosotis* in the higher elevations has not resulted from competition with the native snails (Berman and Carlton, 1991). However, it is not clear whether the recent arrival of a second non-native species (*A. parasitologica*) will result in interactions that may include competitive displacement of the native species, competition with the non-native snail, or occupation of an un-occupied ecological niche (Byers, 2000). The potential of this new invader to displace existing mesogastropods dictated the study to

collect baseline data on all four species. The suite of native (*Angustassiminea californica, Littorina subrotundata*) and non-native (*Assiminea parasitologica, Myosotella myosotis*) will hereafter be referred to as the Snail Guild. (For detailed information on each species please refer to Appendix 9.1: Snail Guild Information).

1.3 Project Objectives

The primary goal of the study was to obtain a current (2009) snap shot of the status of *Assiminea parasitologica (AP)* in Pacific Northwest Estuaries. The objectives of the study to obtain the goal were: (1) Determine the spatial distribution & abundance of AP and the snail guild within the estuarine environment of Coos Bay, Oregon (2) Correlate the species-specific distributions and densities with environmental parameters. (3) Survey the Umpqua, Siuslaw, Smith and other Pacific Northwest estuaries for the presence of *AP*. (4) Investigate the life history strategy of *AP*.

This study was divided into three parts: 1) The Main Survey of *A .parasitologica* in the Coos Bay Estuarine system. 2) Seeking the presence of *A. parasitologica* in other Pacific Northwest estuaries. 3) Examination of the life history strategy of *A. parasitologica* in the Coos Estuary.

2. Distribution and habitat use by Assiminea parasitologica in the Coos Bay Estuarine system

2.1 Objectives

This survey was designed to assess the spatial extent of colonization of AP within the Coos Bay Estuary, collect baseline data on the distribution and density of existing mesogastropods and to observe habitat use by each member of the snail guild. The objectives of the survey were to: (1) Determine the spatial distribution and abundance of each species of the snail guild within the estuarine environment of Coos Bay, Oregon (2) Correlate the species-specific distributions and densities with environmental parameters. These objectives were the basis of our sampling and statistical design.

2.2 Sampling Strategy and Statistical Design

All of our sampling strategies are based on a general two-factor design of comparing across and among regions and marsh strata for each mesogastropod species. Our sampling design was two tiered: (1) A superficial and extensive Rapid Assessment Method (RAM), with evenly spaced sampling to cover all extents of each salt marsh and (2) A thorough and spatially limited Detailed Assessment Method (DAM), using randomly generated points within marshes having known high AP abundance.

Our goal was to survey all marshes within the Coos system. Salt marsh habitat is found in all upper reaches of the estuary, becoming limited or absent in the lower estuary (Figure 1). We defined sampling sites, as marshes that were adjacent to individual waterways. We selected sampling locations, based on the following criteria (1) waterways with clearly defined physical boundaries (i.e. South Slough) (2) waterway margins having appropriate mesogastropod habitat: composed of salt marsh vegetation or rocky rip rap (3) waterways located in the middle to upper reaches of the estuary (4) marshes with sufficient spatial extent to collect a statistically viable number of sample, which were independent of one another, (5) marshes we expected to support target Guild Snails at some population levels.

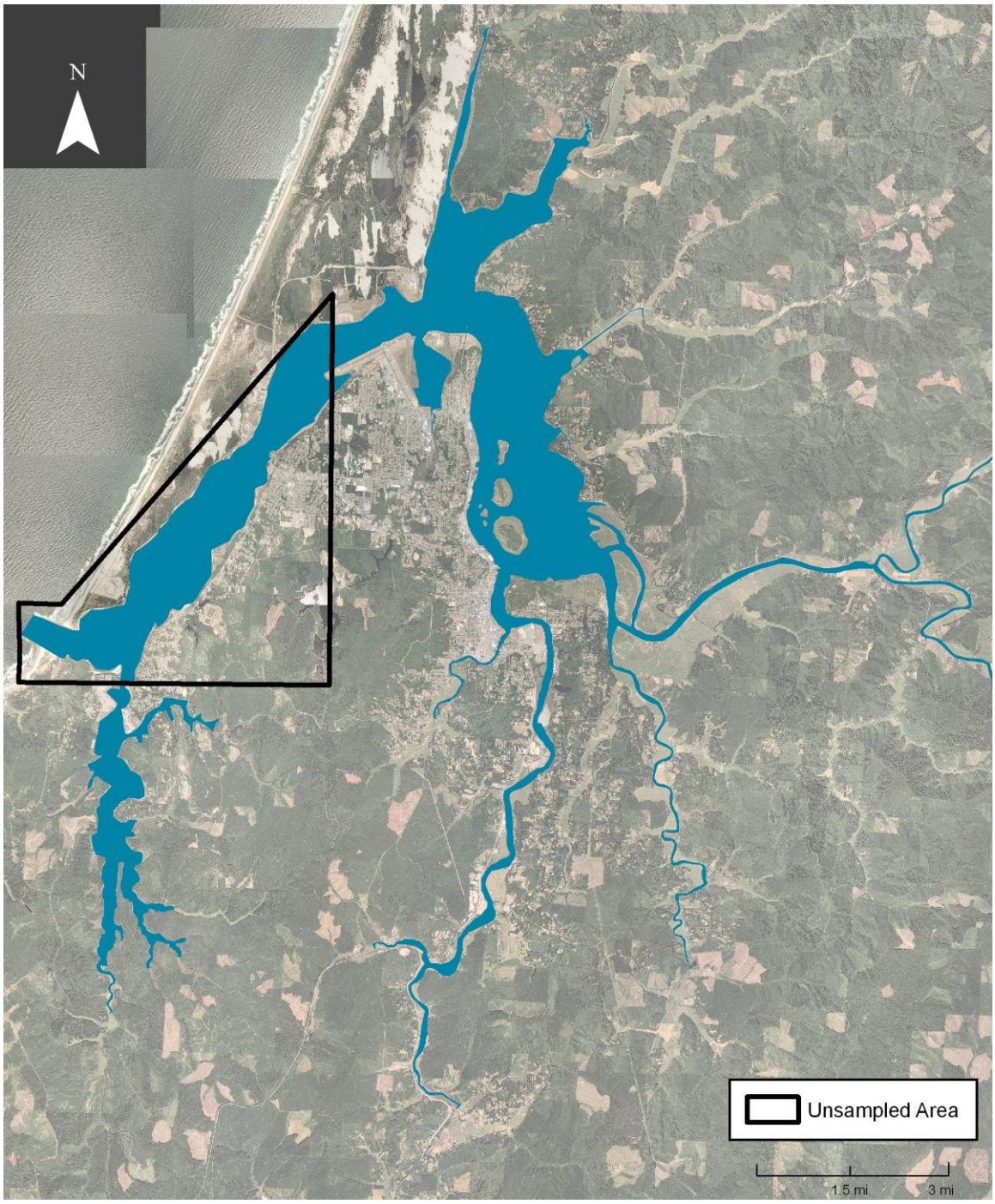

Figure 1: There is no appropriate Guild Snail habitat within the Unsampled Area

The Rapid Assessment Method utilized an evenly spaced grid sampling to cover the extent of each marsh. Individual grids were drawn over each site, which consisted of a waterway and adjacent marshes. The grid was a cell size was 800m alongshore (transect)

and 15m upshore along waterway axis (Figure 2). From these grids, points were selected for sampling, based on appropriate habitat. At each sampling point habitat classification, environmental variables and the relative abundance of mesogastropods was recorded in a 0.5m^2 quadrat. The collection of relative abundance data as a methodology was chosen over presence/absence data so that we could perform nonparametric statistics on the results. This design allowed us to systematically and rapidly (at the experimental unit level) sample waterways throughout the Coos Estuary. Sites were classified into five regions based on arbitrary spatial arrangement: North, South, East, West and Central and were unbalanced in quantity per site (See Appendix 9.2: Sampling Locations).

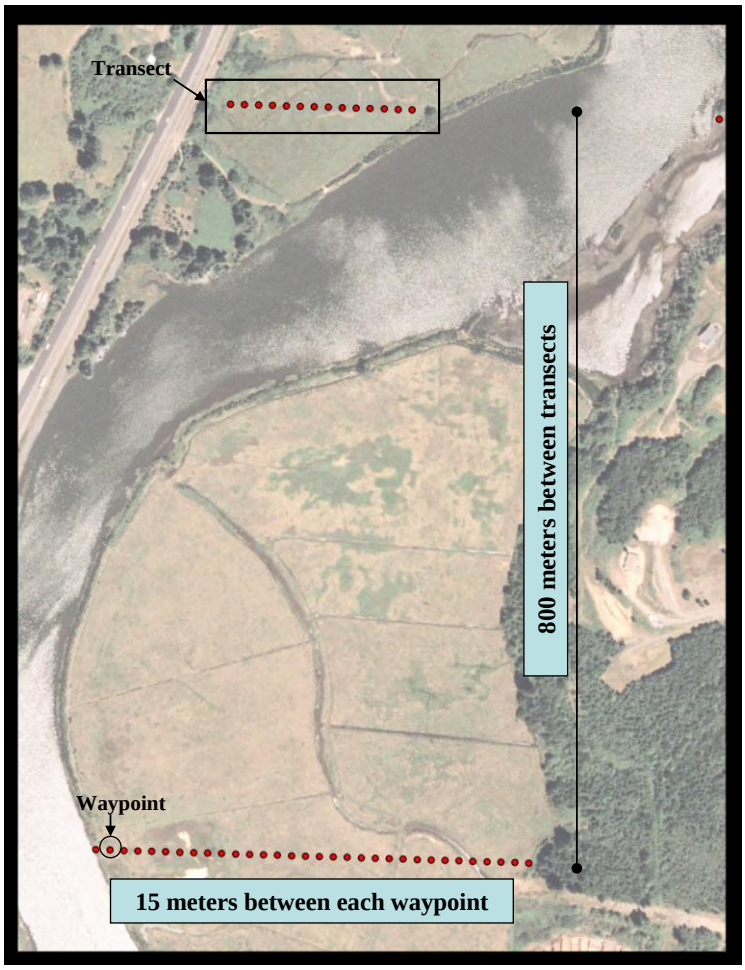

Figure 2: Rapid Assessment grid spacing

Data and results from the Rapid Assessment were analyzed to identify sites of known high AP density that became the focus of the Detailed Assessment. One site per region was chosen for the Detailed Assessment, high density AP areas were encircled with polygons utilizing GIS. Fifty random waypoints were generated within each marsh. Points were then randomly selected using a random number table and ground-truthed for suitability. Twenty random waypoints were sampled in each of the five sites selected: ten high marsh and ten low marsh per site. At each sampling point the Rapid Assessment Method was replicated and the Detailed Assessment Method was employed. The Detailed Assessment Method excavated snails from the plot allowing us to perform biometrics and generate abundance numbers for each species. This design, although slower in data collection, allowed us to have a more statistically rigorous balanced stratified random design.

We generated waypoints for both the Rapid and Detailed Assessments using ESRI ArcGIS v. 9.3 and National Park Service (NPS) AlaskaPak v. 2.2 software programs. For all points, latitude and longitude were generated by NPS AlaskaPak and transferred to Garmin GPSMap76 using the DNRGarmin GPS application software v. 5.4.1. See detailed protocol (Appendix 9.8.7)

2.3 Methods

Using ArcGIS-generated waypoints and handheld GPS units we navigated to the sampling locations. Depending on the waterway, points were assessed from shore from the nearest road or by water using a sea kayak or small motorized watercraft. At the conclusion of sampling each area, boots were scrubbed down with a toilet bowl brush to prevent transferral of the organisms from one location to another. Whole transects and/or individual waypoints were categorized as suitable for sampling or were excluded from sampling. A transect or waypoint was excluded if: (1) the waypoint was in non-suitable substrate (mud, trees, road), (2) the waypoint was unsafe or inaccessible due to soft mud or vertical banks, (3) the waypoint was on private property.

2.3.1 Habitat Classification and Environmental Variables

At each transect head we classified the habitat and measured salinity. At each sampling point we measured additional environmental variables to further classify the habitat. Habitat classification was based off of an amalgamation of the NERRS Habitat and Land Use Classification System (Appendix 9.4) and the state of Oregon's current Estuary Habitat Classification system (Appendix: 9.3). We utilized these two classification systems in developing our sampling design so that we would be able to classify each waypoint in accordance with repeatable standards. We chose additional environmental variables (modifiers) that were qualitatively or quantitatively recorded for each sampling event. At the sampling point variables were measured over a $0.5m^2$ quadrat for both our Rapid and Detailed Assessment Methods.

Salinity was determined with a handheld refractometer at each transect head for the Rapid Assessment and at each polygon for the Detailed Assessment (salinity measurements were often taken on an incoming or high tide). Marsh topography was classified into marsh type as broad or compressed and marsh slope was determined as flat, inclined or vertical. The presence of tidal channels and anthropogenic structures such as bridges, docks, dikes or pilings were noted.

At each sampling point the environmental variables sampled included temperature of the air and sediment at the surface, slope of the ground, dominant sediment classification (determined by feel whether it was composed of sand, mud, or a mixture of the two= Smud), marsh strata classification (high or low), and the presence of algal wrack and tidal channels. Over a $0.5m^2$ area, we documented percent cover of: salt marsh vegetation, separated by species, and the overlying substrate separated into the following categories: gravel, cobble, bark, flotsam and decayed plant material.

2.3.2 Rapid and Detailed Assessments

Our Rapid Assessment Method (RAM) was designed to allow broad spatial scale sampling across the Coos Bay Estuary salt marshes. The primary goals of the Rapid Assessment were to classify the habitat and create distribution boundaries of invasive and existing mesogastropod populations that were present. Target habitats were salt marsh vegetation and rocky riprap that would provide suitable habitat for Guild Snails. Using handheld GPS units, we navigated to a waypoint, sampled environmental variables for habitat classification (described above), and sampled biological variables that could be determined by visual observation and slight adjustment of the vegetation.

We quantified relative abundance of the snail guild over two spatial scales, on the substrate and on the vegetation. Three areas within each quadrat were randomly chosen for examination of target organisms, the quadrat was examined for a period of two-three minutes. The relative abundance was determined independently for AP, AC, LS, MM and NZMS (New Zealand Mud Snail). Relative abundance categories were as follows: Absent=0, Rare=1-10, Common=11-100, Abundant=101+.

The presence of other species was also noted on the data sheet.

Our Detailed Assessment Method (DAM) was based on a random stratified sampling design, which allowed for rigorous statistical analysis. One DAM sampling site was chosen per region: Haynes Inlet, Kentuck Inlet, Coos River North, Isthmus Slough and South Slough. Site-specific RAM data was used to generate polygons that encompassed known areas of known high AP abundance. Within polygons, random waypoints were generated, waypoints were randomly assessed for marsh strata and sampled until ten high marsh and ten low marsh points had been sampled, yielding twenty DAM sampling waypoints per site and 100 DAM sampling waypoints total for Coos Bay.

Our objectives at a DAM site were two-fold: (1) to replicate our RAM effort and (2) to extract snails for absolute identification and biometrics in the laboratory. A $0.5m^2$

quadrat was placed on to the substrate at the waypoint and the RAM was replicated including the relative abundance measurements. In a randomly chosen $0.025m^2$ sub-quadrat, all snails and materials were removed from the plot, packaged, and taken to the laboratory for analysis. In the laboratory, samples were sieved through a 600 micron mesh sieve and then frozen until analysis could be conducted. Snails were identified and enumerated under a dissecting microscope and the total count and weight was recorded for each species from each waypoint.

2.3.3 Data Analysis

RAM and DAM biological and environmental data were mapped using ESRI ArcMap. RAM data was used to generate relative abundance estimates for all species of the snail guild. To compare abundance data and apply statistics to the data; relative abundance categories (absent, rare, common and abundant) were assigned numbers respectively. To obtain an estimate of relative abundance, we calculated the average relative abundance per unit area for all sampling sites within region, marsh type, marsh strata, salinity zones and dominant vegetation. DAM biological data was used in generating abundance estimates for each species of the snail guild. To obtain an estimate of abundance, we extrapolated from the sub-quadrat area (0.0025m2) to the standard quadrat area (0.5m2), which generated a mean abundance for each species by 0.5m2 area by factor selected for analysis. Factors selected for analysis included site, marsh type, marsh strata, salinity zone and dominate vegetation.

Multivariate analyses were performed using PRIMER® (v. 6.1) to examine patterns of environment and snail abundance across and among regions and salinity zones. Multi-Dimensional Scaling plots with ANOSIM post-hoc analysis were developed from Euclidean distance resemblance matrices (normalized environmental variables) to determine if region or salinity of a site were defining the community structure. Non-parametric, Wilcoxan statistical tests were used for the RAM data and ANOVA statistical tests were used to examine the environmental correlates with individual species distribution patterns.

2.4 Results

2.4.1 Rapid Assessment

Habitat Classification

The Rapid Assessment Method covered five major spatial regions: North, South, East, West and Central (Figure 3). Grids were sampled at 16 sites across the regions (Appendix 9.2: Sampling Locations). The transects sampled and excluded are shown in Figure 4. Transects were excluded if the transect lay across private property, an inaccessible area (cliff or dangerously soft mud) or incorrect habitat (mudflat, sand dune, purely terrestrial environment, road) (Figure 5). Many portions of the South Slough and the Coos River upper reaches did not have the appropriate habitat for mesogastropods. Downtown Coos Bay and certain parts of Isthmus Slough could not be sampled due to private property and or incorrect habitat, typically farmland. All waterways had some portion that was inaccessible to sampling, often due to dangerously soft mud.

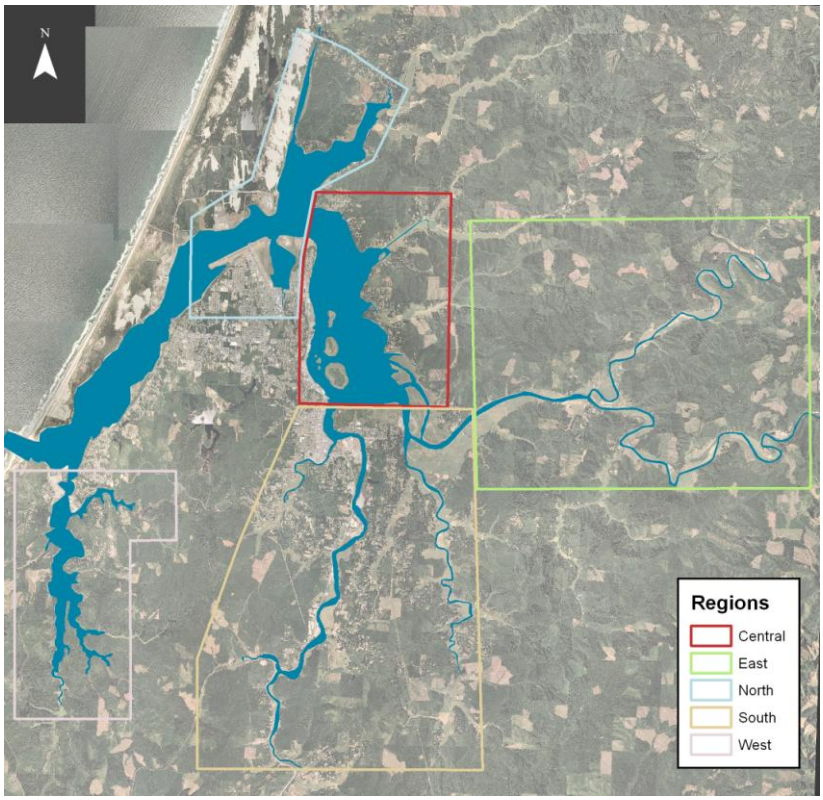

Figure 3: Rapid Assessment regions across the Coos estuary

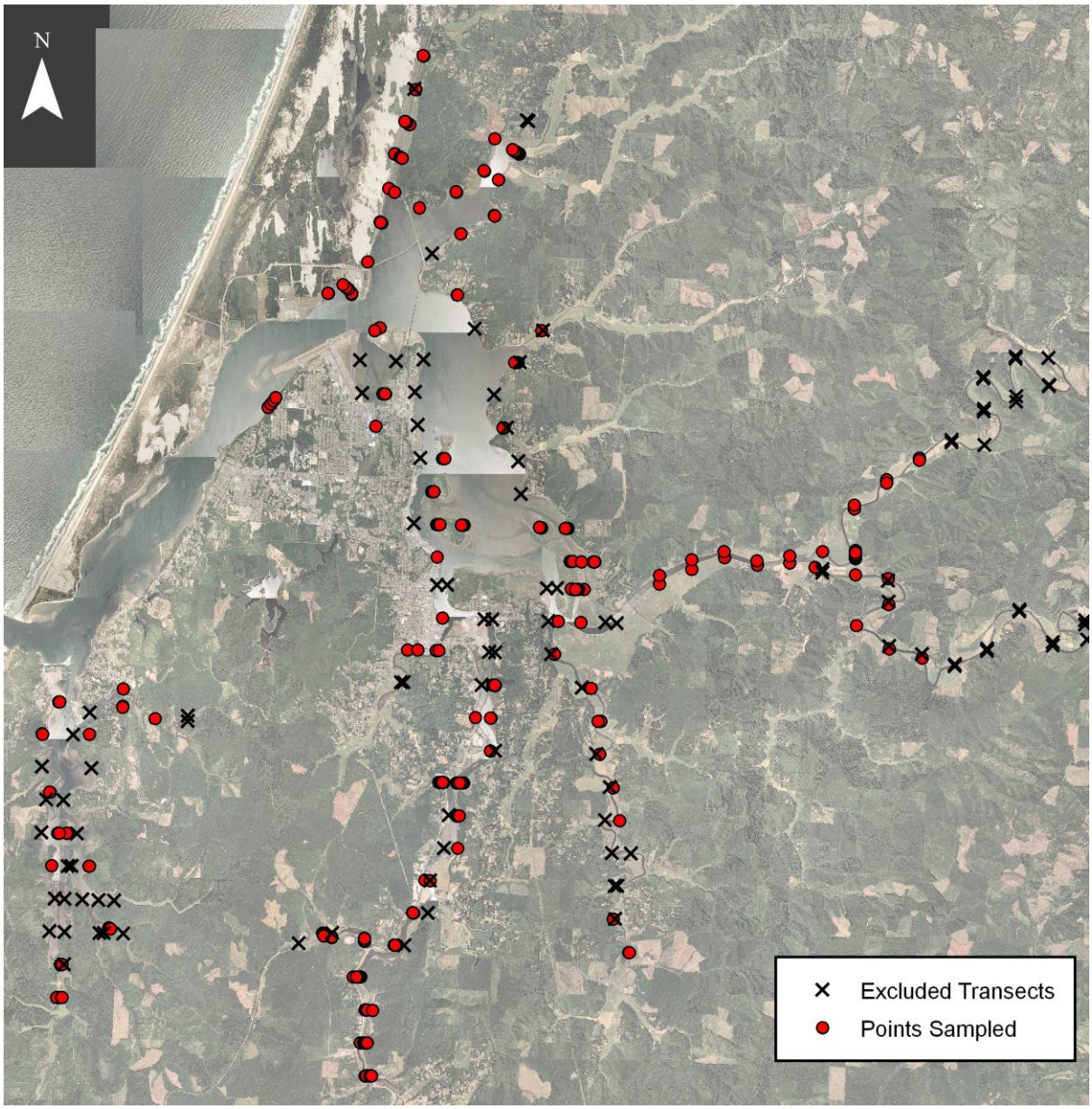

Figure 4: Excluded and sampled transects in the Rapid Assessment of the Coos estuary

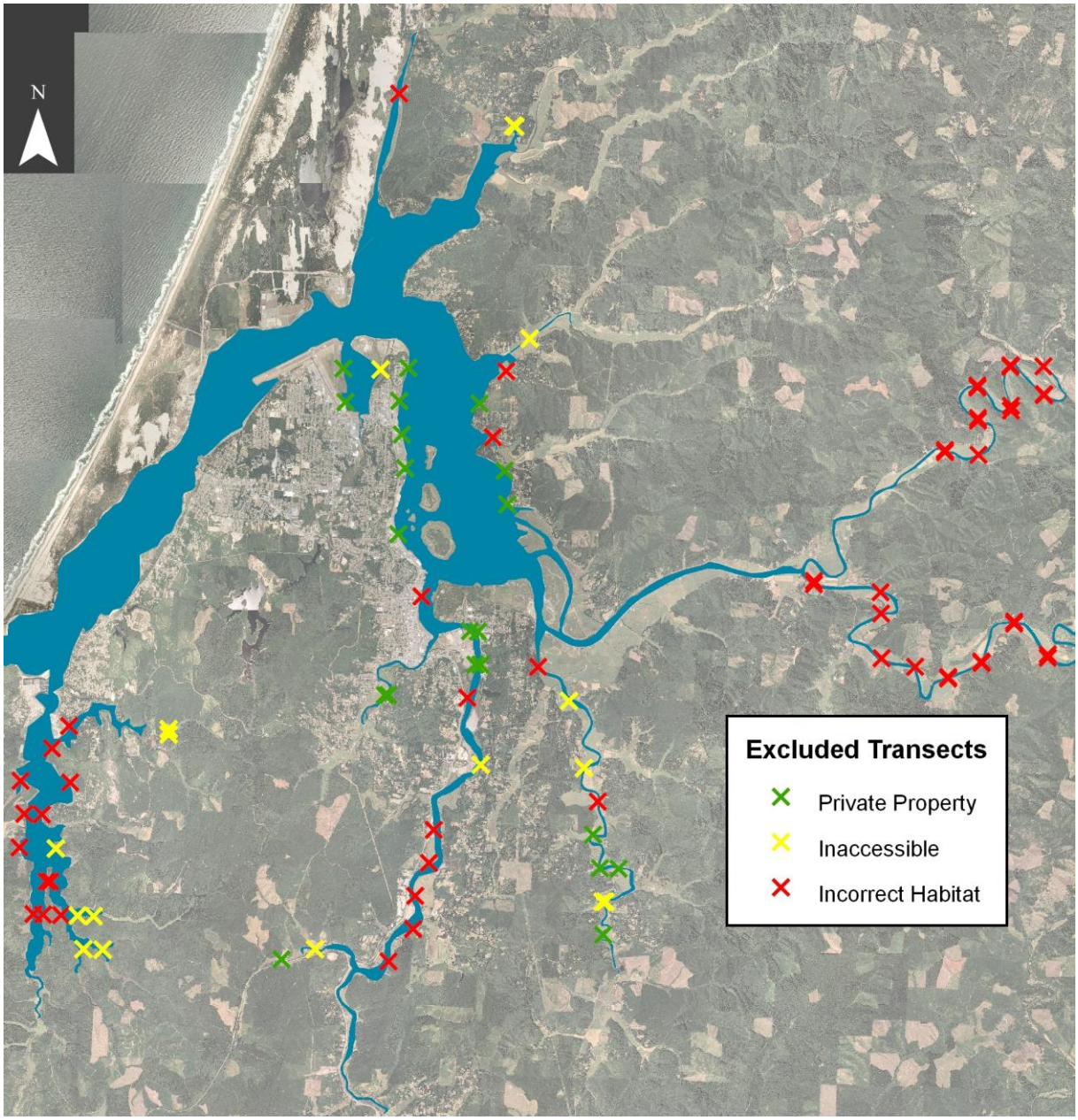

Figure 5: Reasons for transect exclusion during Rapid Assessment

Habitat maps of the five regions sampled by RAM show the similarities and differences across regions for each habitat characteristic (Figures 6-9). For salinity zone and marsh type maps, the symbol represents the qualitative or quantitative assessment of that variable for an entire transect. For marsh strata and dominant vegetation maps, the symbol represents the qualitative or quantitative assessment of that variable for a sampling point.

Salinity concentrations (measured in parts per thousand), used to define zones were: Euhaline (31-35), Polyhaline (19-30), Mesohaline (6-18) and Oligohaline (0.5-5). These zones were adopted from the NERR Habitat Classification (Appendix 9.4).

Salinity zones by region are shown in Figure 6. Examining salinity across regions shows that there is a wide salinity range, from euhaline-dominated regions to sites with oligohaline conditions. Examining within region reveals that each region is not homogenous and is often composed of one or more salinity zones. As expected, salinity zonation is often stratified along the estuarine gradient of a given waterway. The West and the North regions are predominantly composed of euhaline and polyhaline water. The Central is mainly composed of polyhaline water except in some of the fringing marshes on the east side. The South region is a mixture of polyhaline and mesohaline water. The East region is rather fresh with low numbers in the mesohaline zone and some transects in the oligohaline zone.

Marsh type was defined as broad or compressed. Marsh type is not currently part of either the NERR or Oregon Habitat Classification systems used but were added as modifiers to further classify marshes for an additional comparison variable. The following lengths were used to define marsh type: Compressed <30meters, Broad > 30 meters. Marsh type classification is mapped and shown in Figure 7. Marshes sampled within Coos Bay do not have a unifying marsh type. Both marsh types are present in the West, South and North regions. The Central region is dominated by broad marshes and the East region is dominated by compressed marshes.

Marsh stratum is an important component of the NERR Habitat Classification Scheme. We classified the strata as either high or low at every sampling point. We defined strata by the presence of algal wrack, tidal inundation and composition of vegetation. A high stratum quadrat was assigned when algal wrack was absent, plants were of the higher elevation composition (Rumrill, 1997), and there was little evidence of a strong tidal inundation. A low stratum quadrat was assigned when algal wrack was present, plants

were of a lower elevation composition and there was evidence of tidal inundation. Marsh strata across all sampling points are depicted in Figure 8. Marsh strata varies within region, within site and within a given transect. Based on the mapping of marsh strata it is apparent that marshes are rather heterogeneous in their composition, made up of high, dry points to fully submerged tidal channels.

Dominant vegetation was determined from the percent cover data at the quadrat level: when a particular plant covered 50 percent or more it was assigned as the dominant vegetation. Dominant vegetation across all regions is illustrated in Figure 9. *Carex* spp. is dominant in the upper reaches or the riverine-dominated portion of several regions. *Salicornia virginica* is dominant in the lower euhaline and polyhaline sections of particular regions. *Triglochin maritimum* appears to be rather patchy in its distribution throughout the bay.

Based on data collected and the habitat maps generated, each region is fairly heterogeneous in its composition of salinity, marsh type, marsh strata and dominant vegetation. Within region there is salinity stratification and patches of broad and or compressed marshes. Vegetation and marsh strata appear to vary within and across the marsh. The additional environmental variables were determined for each sampling point: dominant substrate, overlying substrate (% cover), air temperature (C), sediment temperature (C), slope (degrees). Further statistical analysis of environmental structure will follow in the *mechanisms of distribution* section.

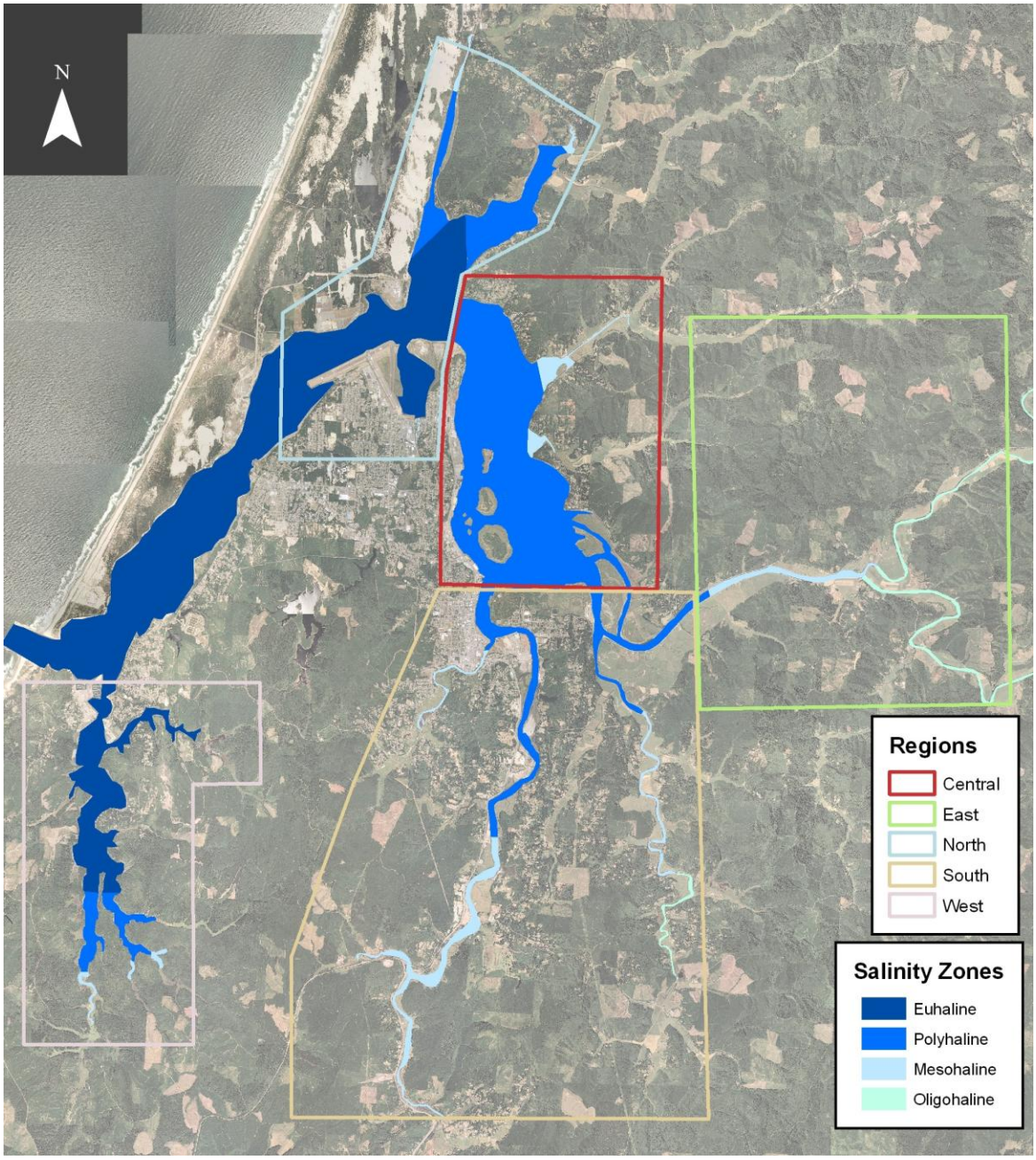

Figure 6: Salinity zones by region across the Coos estuary

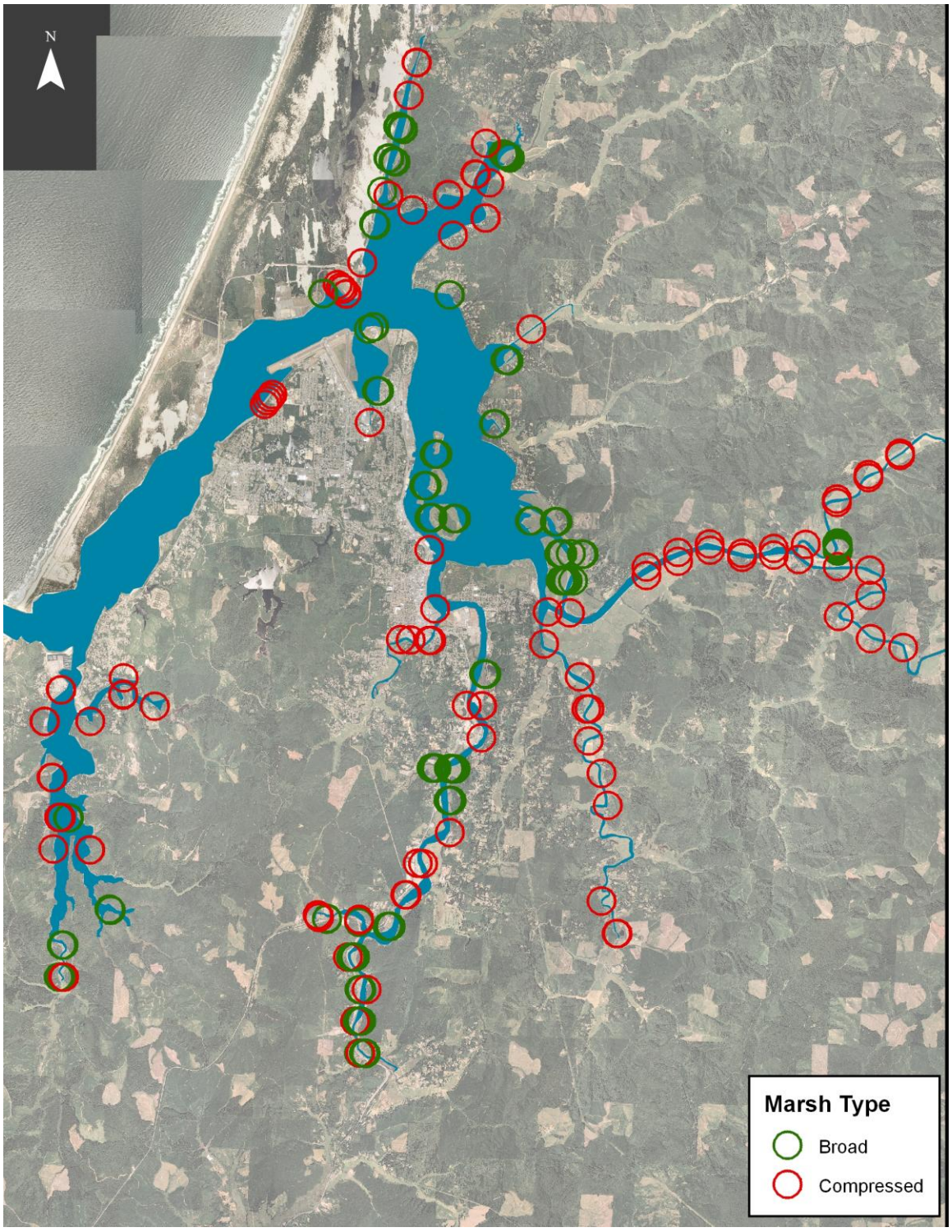

Figure 7: Marsh type of transects sampled across the Coos estuary

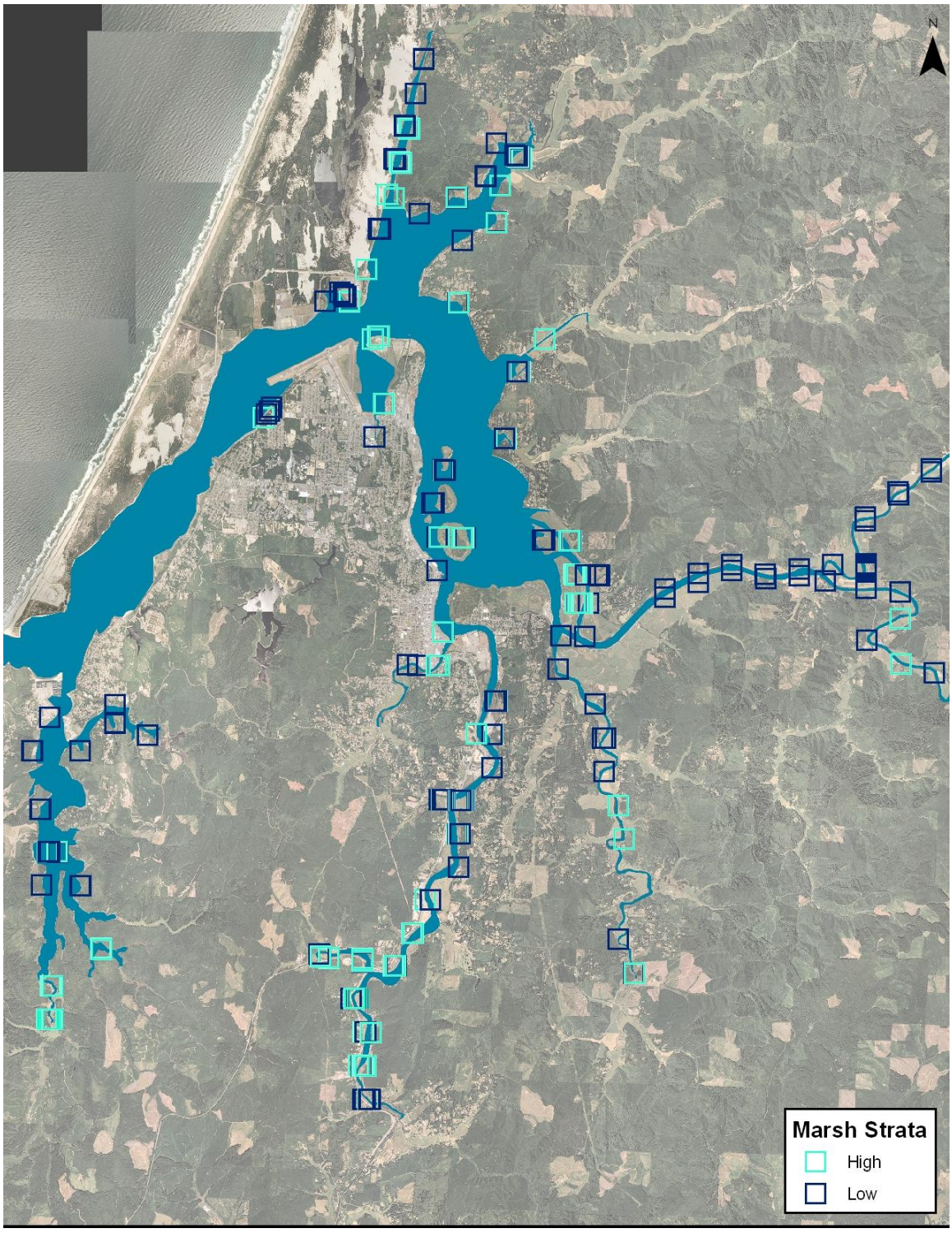

Figure 8: Marsh strata of quadrats sampled across the Coos estuary

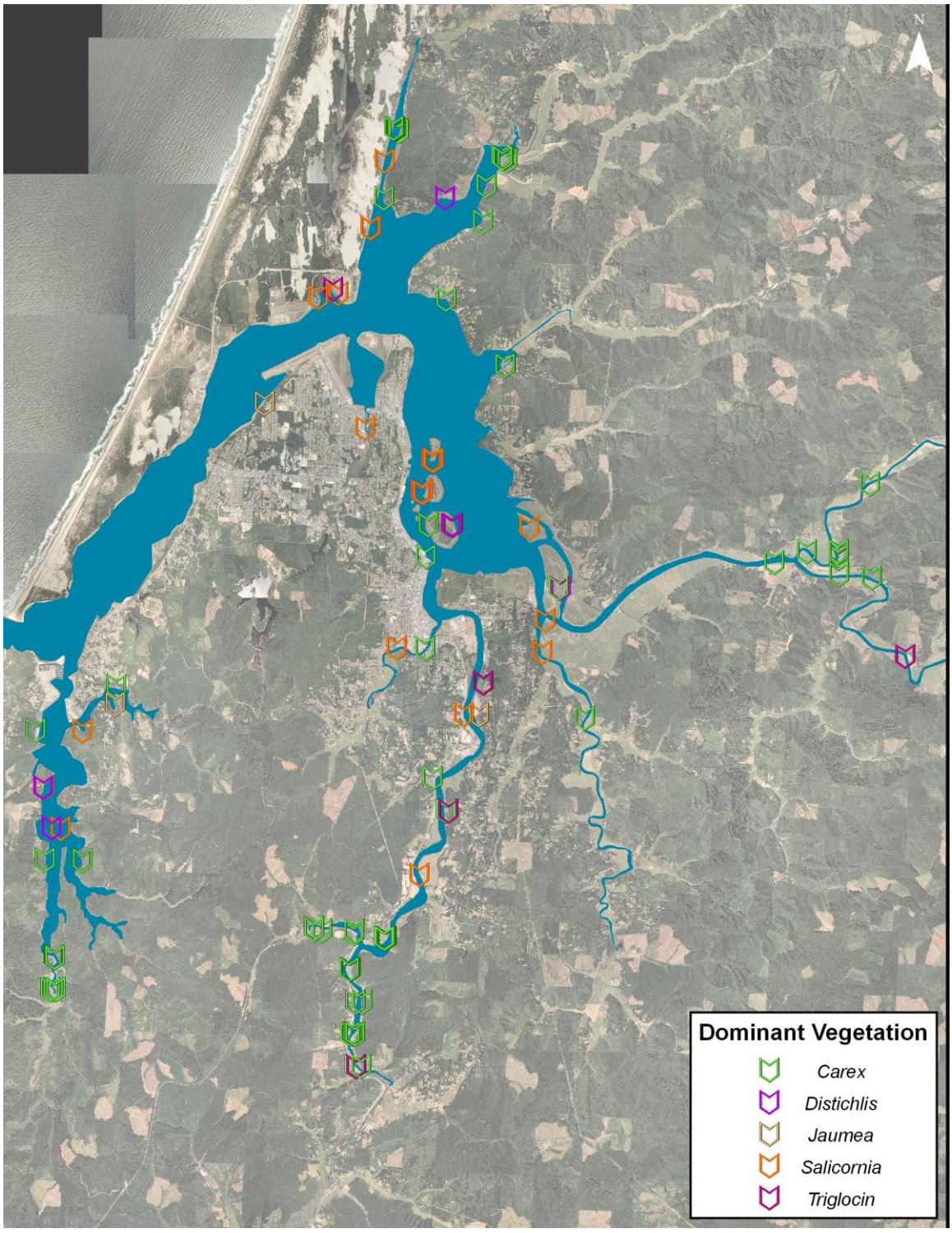

Figure 9: Dominant vegetation of quadrats sampled across the Coos estuary

Guild Snails

We mapped the relative abundance of Guild Snails at each sampling point by species composition (Figure 10-11). The map of species composition shows that the North, Central and estuarine dominated section of the South region had the most diverse assemblages of the snail guild species. These areas had euhaline and polyhaline dominated waters (Figure 11).

Relative abundance of each species is separately mapped and the respective distributions are overlaid over salinity zones (Figure 12-15). All species were present in all regions but not in all sites. AP were most abundant in the South region with highest abundance at the Isthmus Slough site, a mesohaline dominated site. AC were most abundant in the Central and South region in areas dominated by polyhaline waters. LS had a wide breadth of distribution across region and salinity zones. MM were most abundant in the central region and in the euhaline and polyhaline dominated areas of the bay.

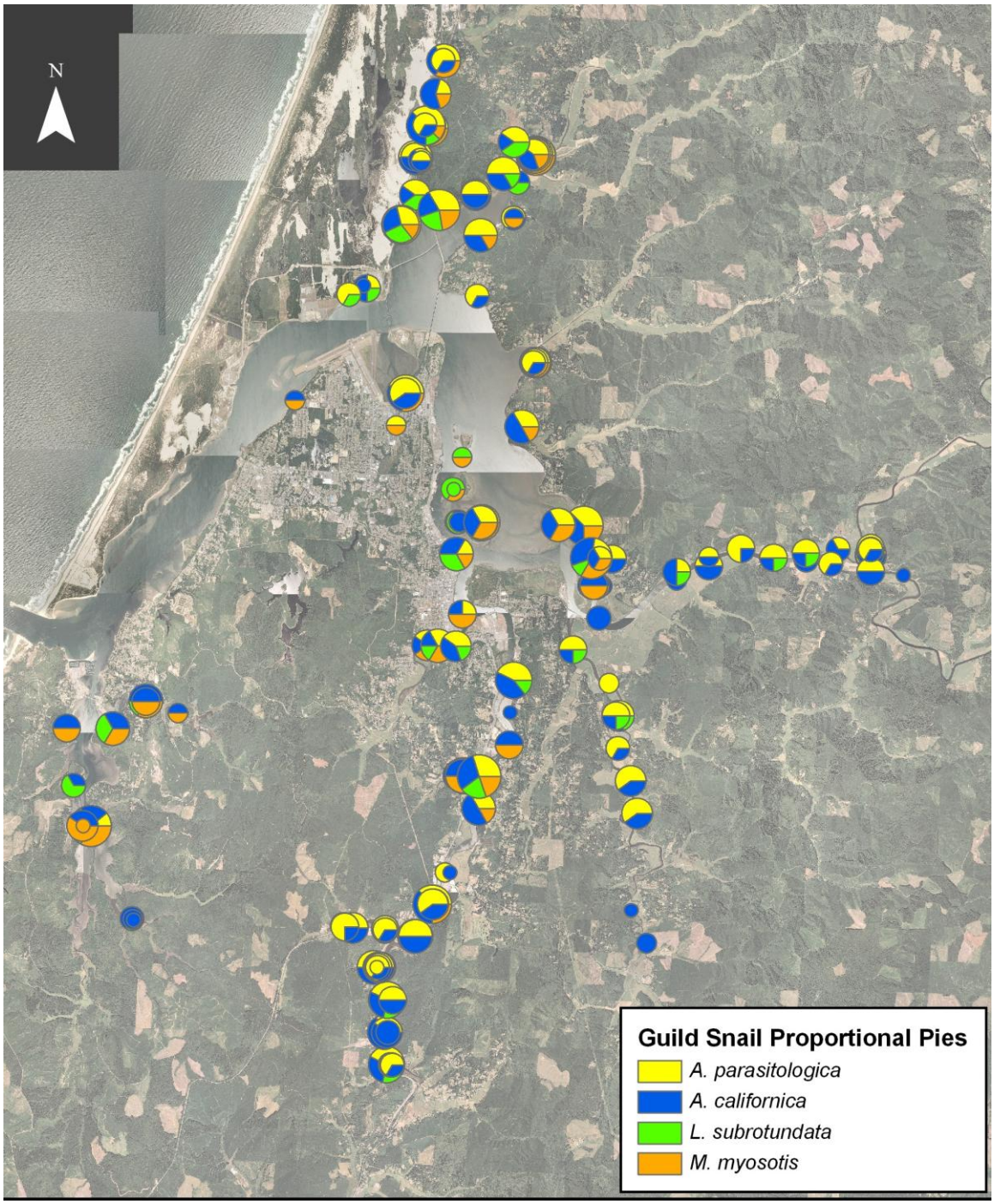

Figure 10: Proportional pie Charts of Guild Snail populations across the Coos estuary

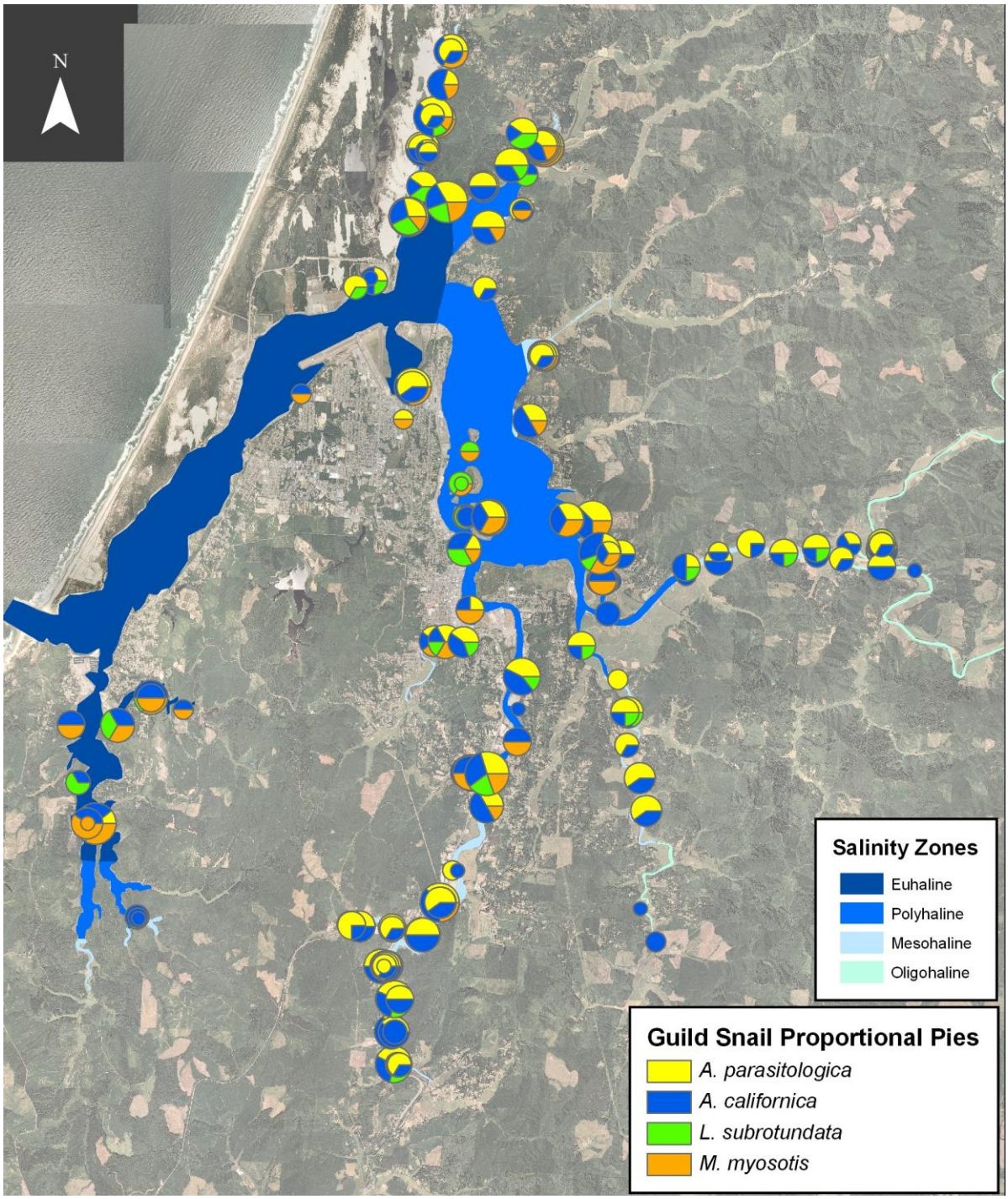

Figure 11: Proportional pie charts of Guild Snail populations and salinity zones across the Coos estuary

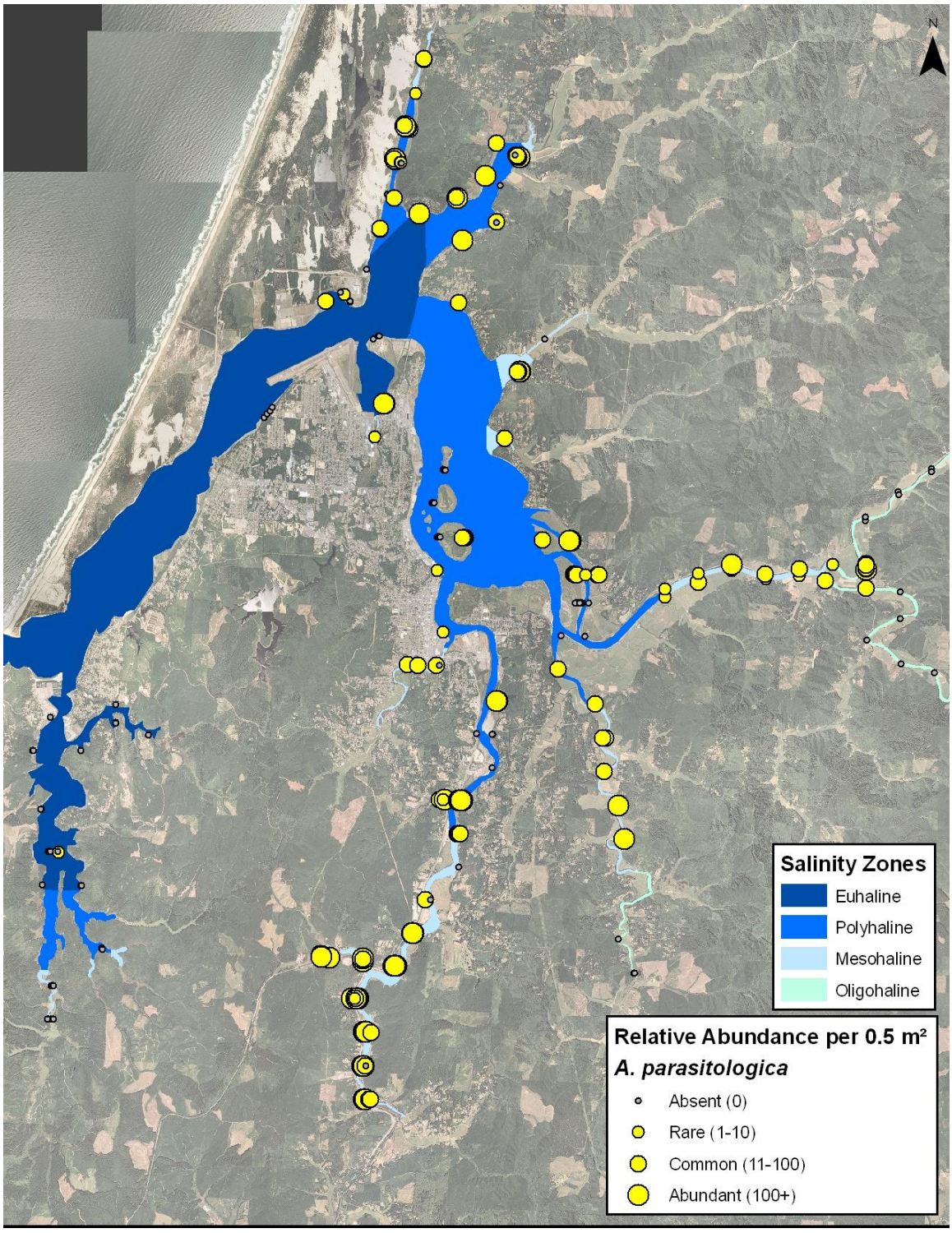

Figure 12: Relative abundance of *A. parasitologica* and salinity zones across the Coos estuary

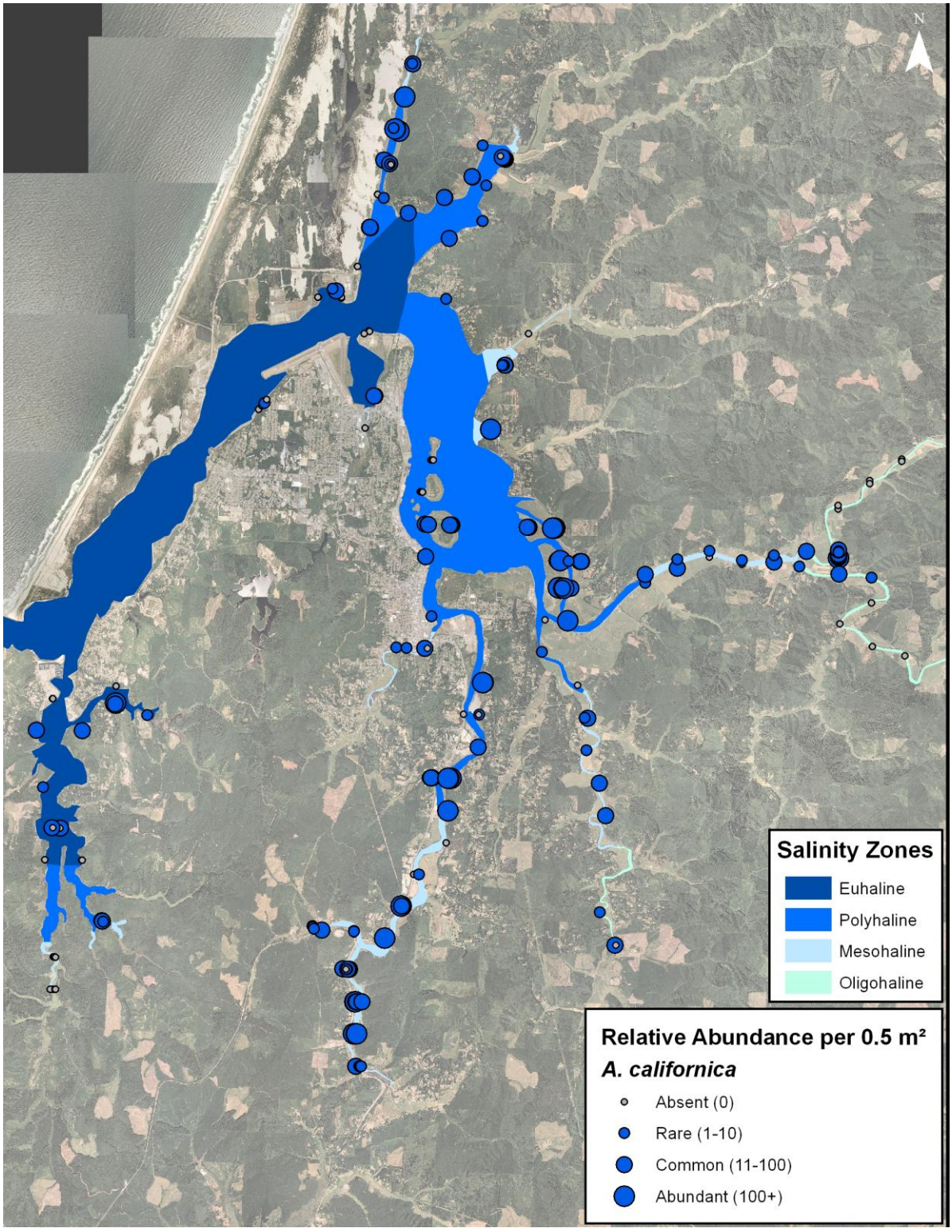

Figure 13: Relative abundance of *A. californica* and salinity zones across the Coos estuary

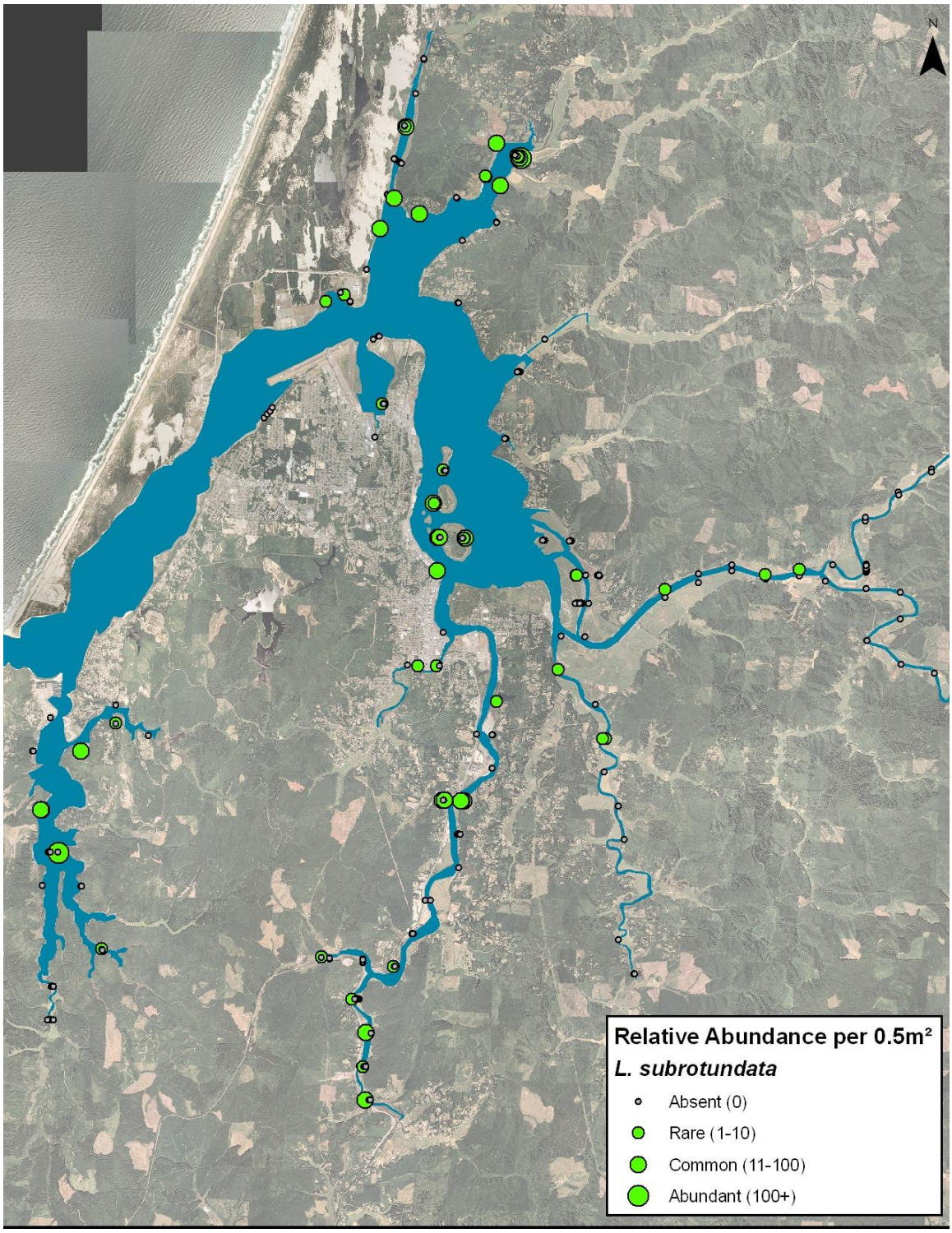

Figure 14: Relative abundance of *L. subrotundata* and salinity zones across the Coos estuary

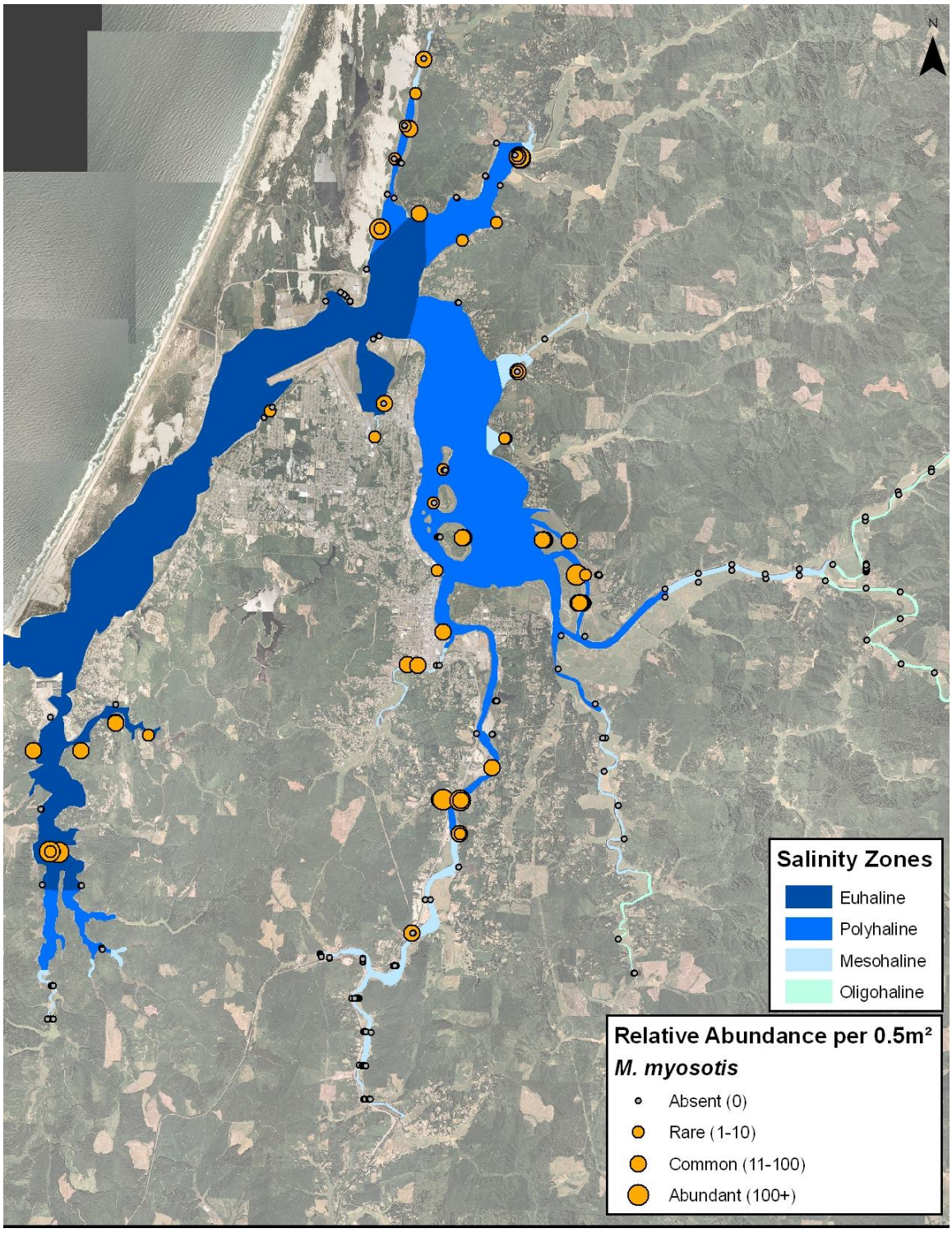

Figure 15: Relative abundance of *M. myosotis* and salinity zones across the Coos estuary

Mechanisms of Distribution

While the results explained above show the bay-wide habitat classification and patterns of relative abundance distribution of Guild Snail species, we also wanted to better understand the correlations between Guild Snail species and environmental variables to identify potential mechanisms of distribution. To address this question, we used two approaches: 1) Community analysis using the software PRIMER (v.6.1) and 2) Standard non-parametric statistical tests to test the species distribution values against certain environmental variables.

PRIMER applies a pair-wise comparison process to data sets that have multiple biological and environmental variables, in which the software compares each sample to every other sample within the data set to derive a resemblance number for each pair-wise comparison. The output is a matrix, which summarizes the resemblance statistics for all pair-wise comparisons. We used PRIMER, resemblance matrices for environmental variables using Euclidean distance. From these resemblance matrices, we made Multi-Dimensional Scaling (MDS) plots, which show how closely related samples are to each other by plotting all data points on a 2D plot using a clustering routine. Samples that are very similar to each other are clustered together; sample clusters that are different from other sample clusters are separated by space on the 2D plot. The "stress" of each plot is one indication of the statistical significance of how well the 2D plot represents the resemblance matrix data. The analysis of similarity (ANOSIM) routine, applies a confidence interval to how different the sample clusters are from each other.

To understand whether regions had distinctly different environmental signatures, we plotted the environmental variable resemblance matrix with the point color-coded by region. Figure 16 shows a weak correspondence between region and the environmental variables used in this analysis (Stress=0.21), although Analysis of Similarity (ANOSIM) test indicated that region was significant in describing our environmental signature (R=0.19, %=0.1). Salinity zone was examined across all regions to determine if there was a difference in environmental structure. The MDS plot shows weak correspondence

between salinity zone and the environmental variables (Stress=0.21), however ANOSIM confirmed that there was a significant difference in environment by Salinity zone (R=0.341, %=0.1) (Figure 17).

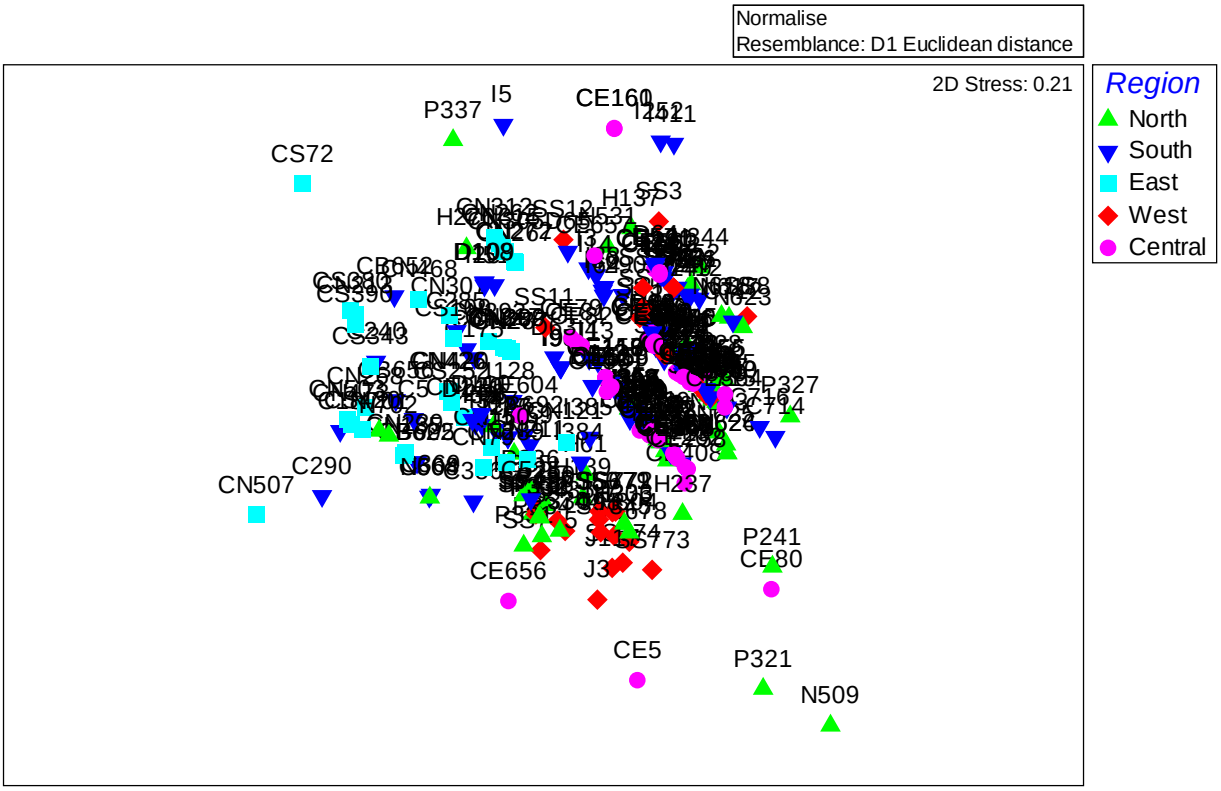

Figure 16: MDS plot showing the correspondence between environmental variables and sampling region

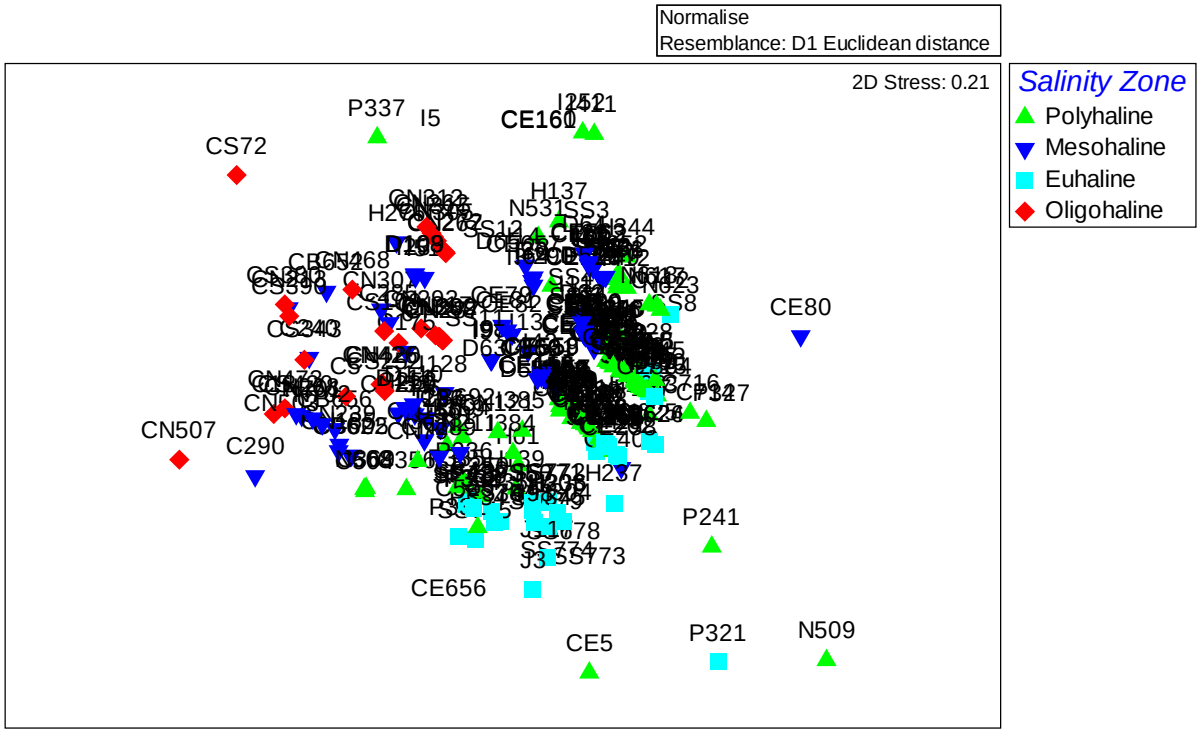

Figure 17: MDS plot showing the correlation between environmental variables and salinity zone

Once it was understood that there was a difference in the environmental structure by region and by salinity zone we examined the distribution of each individual species. Environmental variables were assessed for importance for each snail's relative abundance distribution. Environmental variables selected for analysis were: region, salinity zone, marsh type, marsh strata, dominant vegetation and sediment temperature (°C). A Wilcoxan non-parametric statistical test was employed for each snail species analyzing these selected environmental variables to determine what variables may be driving distribution. For a full list of environmental variable results, refer to Table 1.

AP distribution was significantly different by region (p= 0.0001), salinity zone (p=0.0001), marsh type (p=0.0001) and dominant vegetation (p=0.0063) (Figures 18-21). AC distribution was significantly different by region (p=0.0001), salinity zone (p=0.0003), marsh type (p=0.0001) and dominant vegetation (p=0.05) (Figures 22-25). LS distribution was significantly different by salinity zone (p=0.0011), marsh strata (p=0.02), and dominant vegetation (0.0183) (Figures 26-28). MM distribution was

significantly different by region (p=0.0001), salinity zone (p=0.0001), marsh type (p=0.0229) and dominant vegetation (p= 0.0009) Figures (29-32).

Table 1: Wilcoxan non-parametric statistical tests of factors with Snail Guild Species. P-values for selected environmental data correlated with the relative abundance of Guild Species.

Factor	AP	AC	LS	MM
Region	0.0001	0.0001	NS	0.0001
Salinity zone	0.0001	0.0003	0.0011	0.0001
Marsh Type	0.0001	0.0001	NS	0.0229
Marsh Strata	NS	NS	0.0216	NS
Dominant Vegetation	0.0063	0.0542	0.0183	0.009
Sediment Temperature	NS	NS	NS	NS
Slope	NS	NS	NS	NS

Wilcoxan Non-parametric tests are significant when p > 0.05, NS = not significant

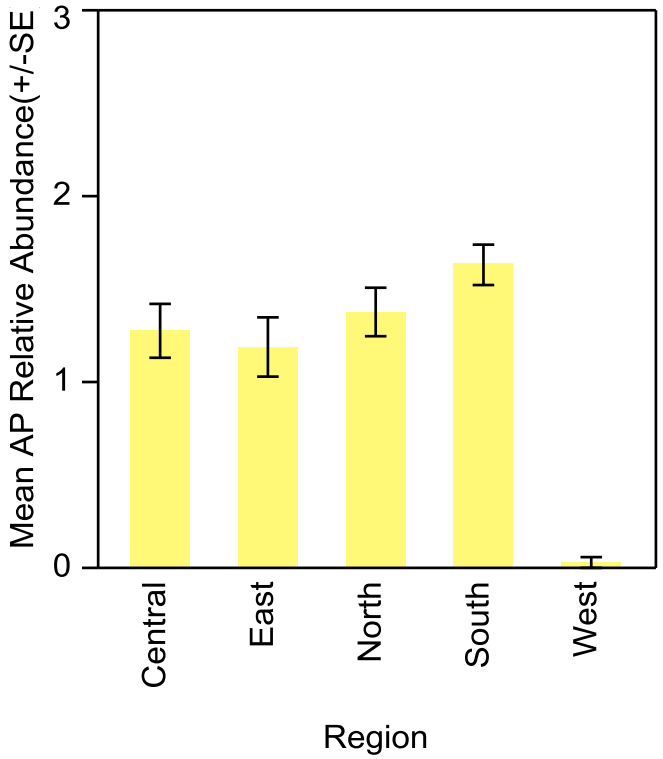

Figure 18: Distribution of *A. parasitologica* by region per 0.5 m^2 in the Rapid Assessment

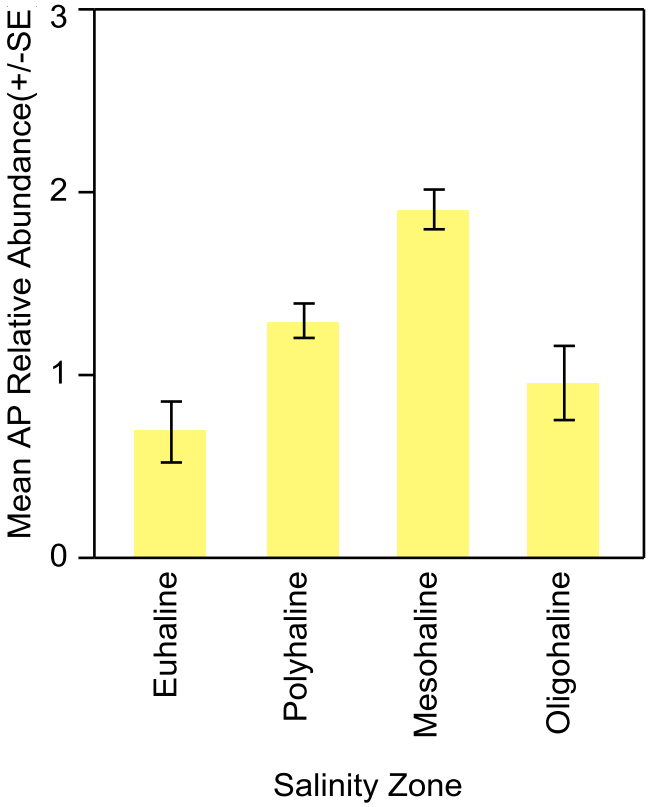

Figure 19: Distribution of *A. parasitologica* by salinity zone per 0.5 m^2 during Rapid Assessment

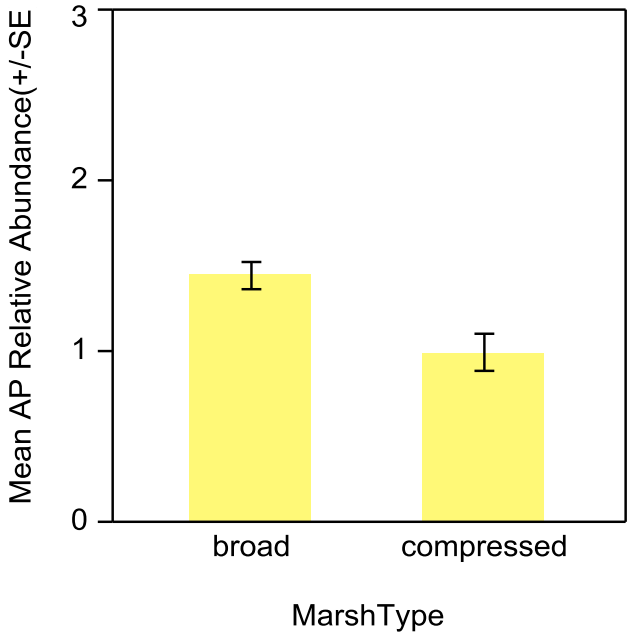

Figure 20: Distribution of *A. parasitologica* by marsh type per 0.5 m^2 during Rapid Assessment

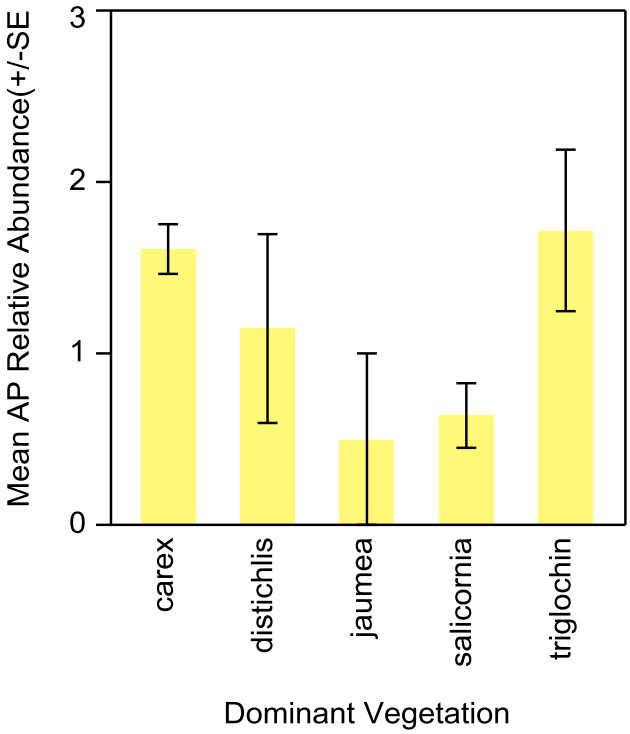

Figure 21: Distribution of *A. parasitologica* by dominant vegetation per 0.5 m^2 during Rapid Assessment

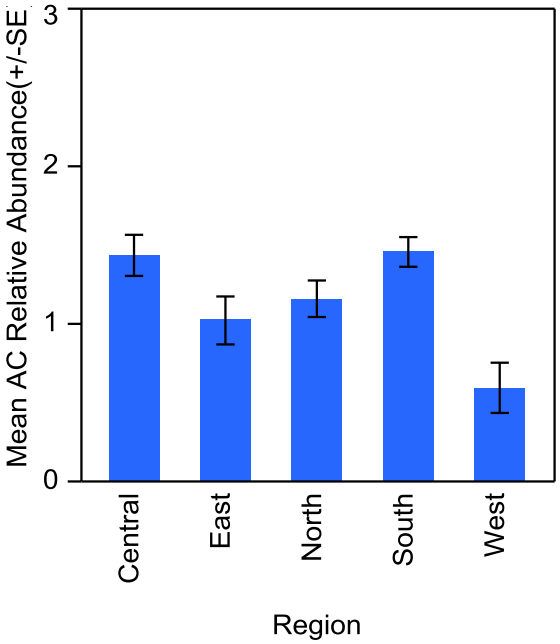

Figure 22: Distribution of *A. californica* by region per 0.5 m^2 during Rapid Assessment

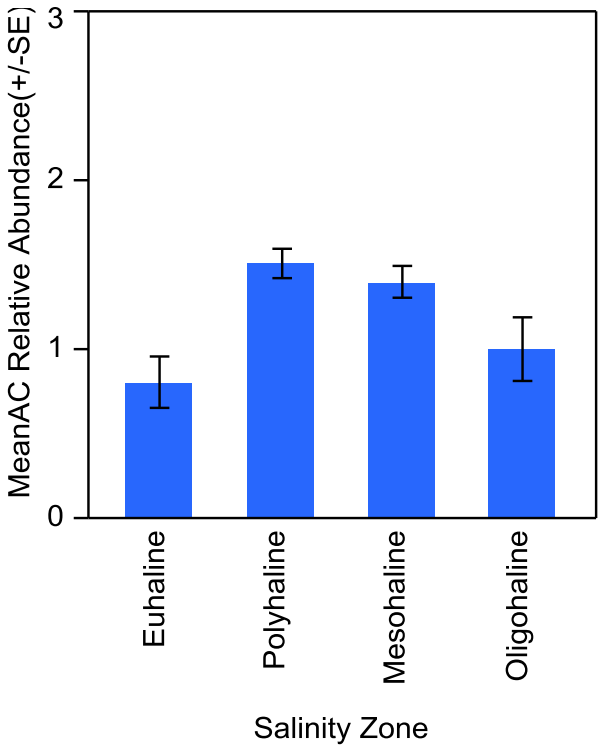

Figure 23: Distribution of *A. californica* by salinity zone per 0.5 m^2

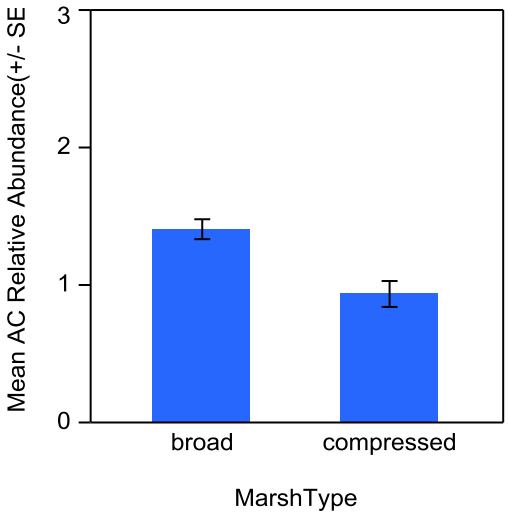

Figure 24: Distribution of *A. californica* by marsh type per 0.5 m^2

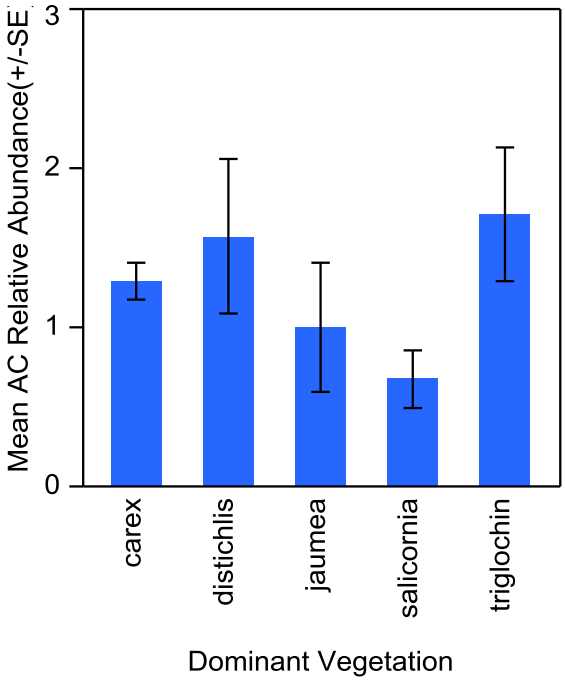

Figure 25: Distribution of *A. californica* by dominant vegetation per 0.5 m^2

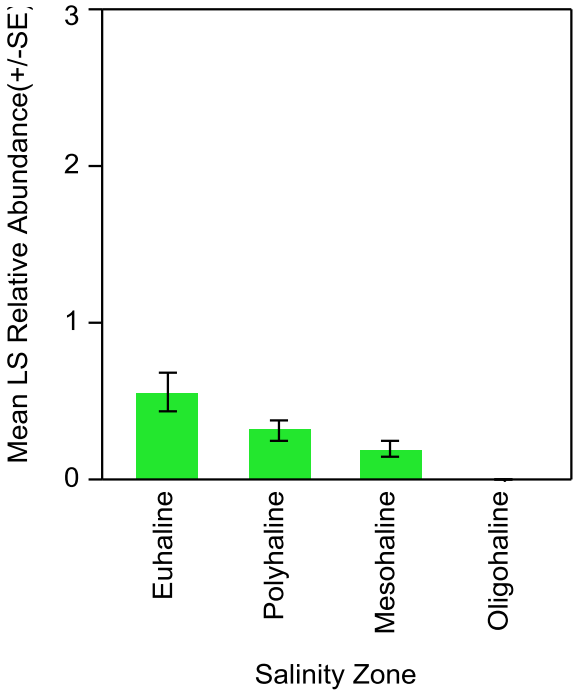

Figure 26: Distribution of *L. subrotundata* by salinity zone per 0.5 m^2

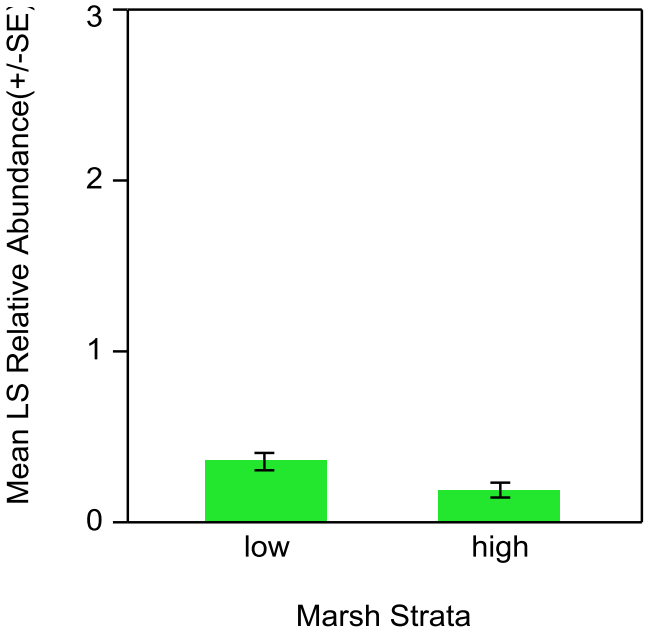

Figure 27: Distribution of *L. subrotundata* by marsh strata per 0.5 m^2

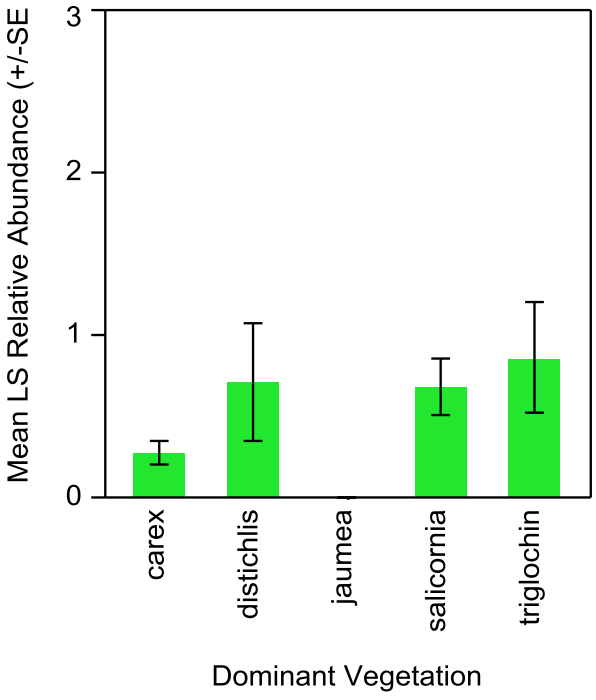

Figure 28: Distribution of *L. subrotundata* by dominant vegetation per 0.5 m^2

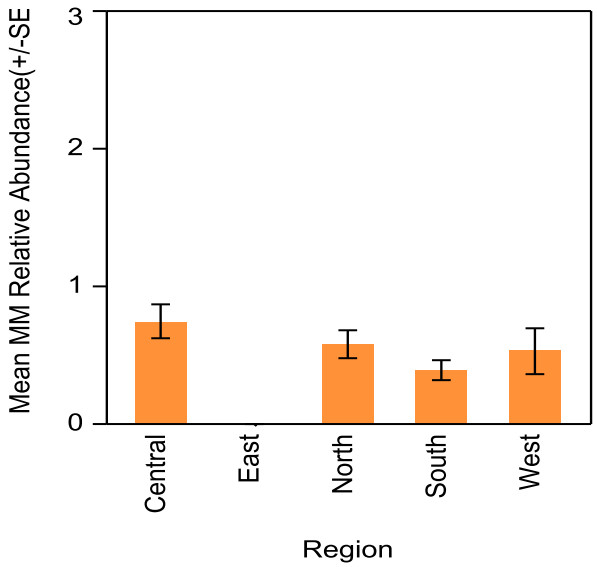

Figure 29: Distribution of *M. myosotis* by region per 0.5m2

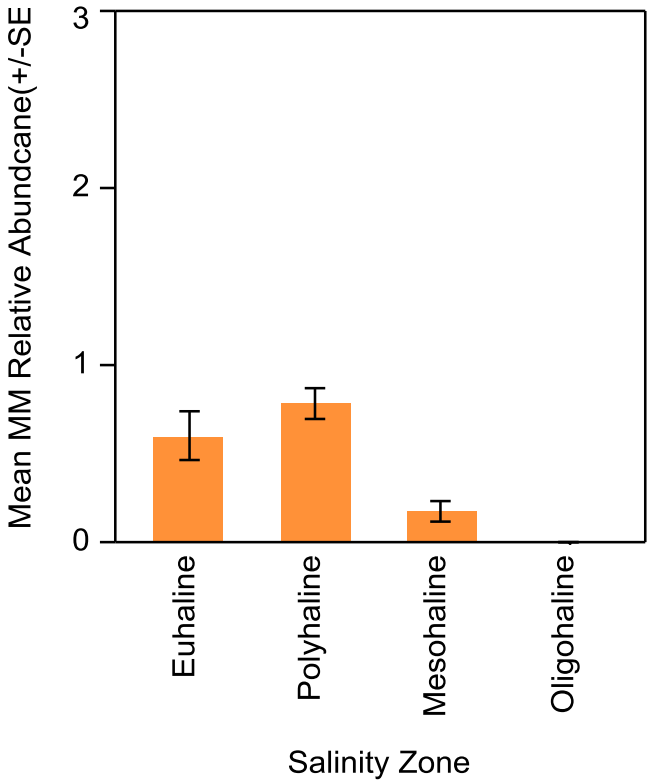

Figure 30: Distribution of *M. myosotis* by salinity zone per 0.5m^2

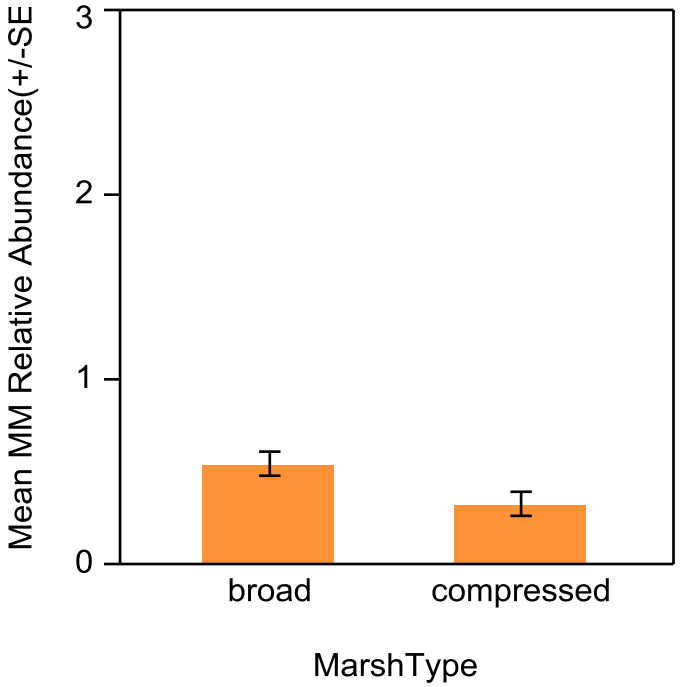

Figure 31: Distribution of *M. myosotis* by marsh type per 0.5m^2

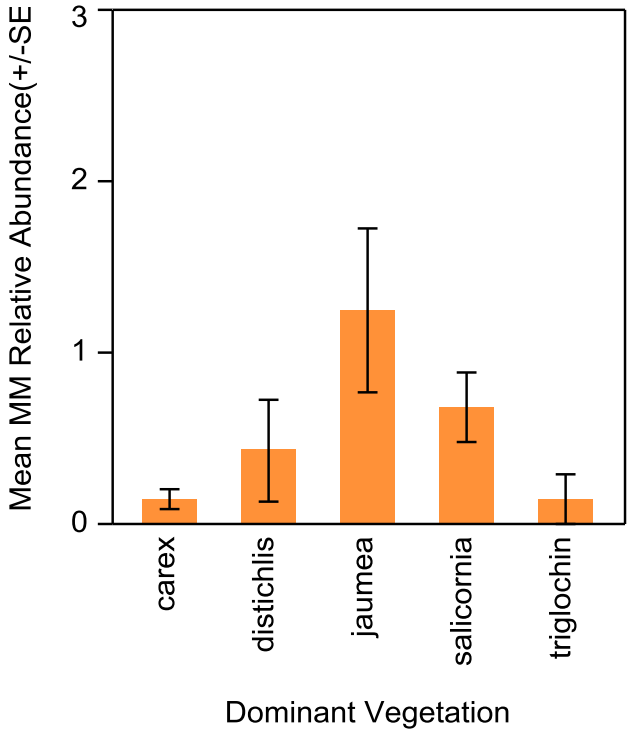

Figure 32: Distribution of *M. myosotis* by dominant vegetation per 0.5m^2

2.4.2 Detailed Assessment

One site per region was chosen for the detailed assessment: Isthmus Slough, Coos River North, Kentuck Inlet, Haynes Inlet and South Slough. The high AP concentration polygons delineated for the Detailed Assessment are shown in Figure 33. Three of the sites had one large continuous polygon: Isthmus Slough, Coos River North and Kentuck Inlet. Haynes Inlet and the South Slough sites had two polygons drawn per site. The Haynes Inlet sub-sites are called Haynes East and Haynes West. The South Slough sub-sites are called the Hinch Bridge and Valino Island. Each point was classified as high or low strata. When able, ten of each stratum were examined per site.

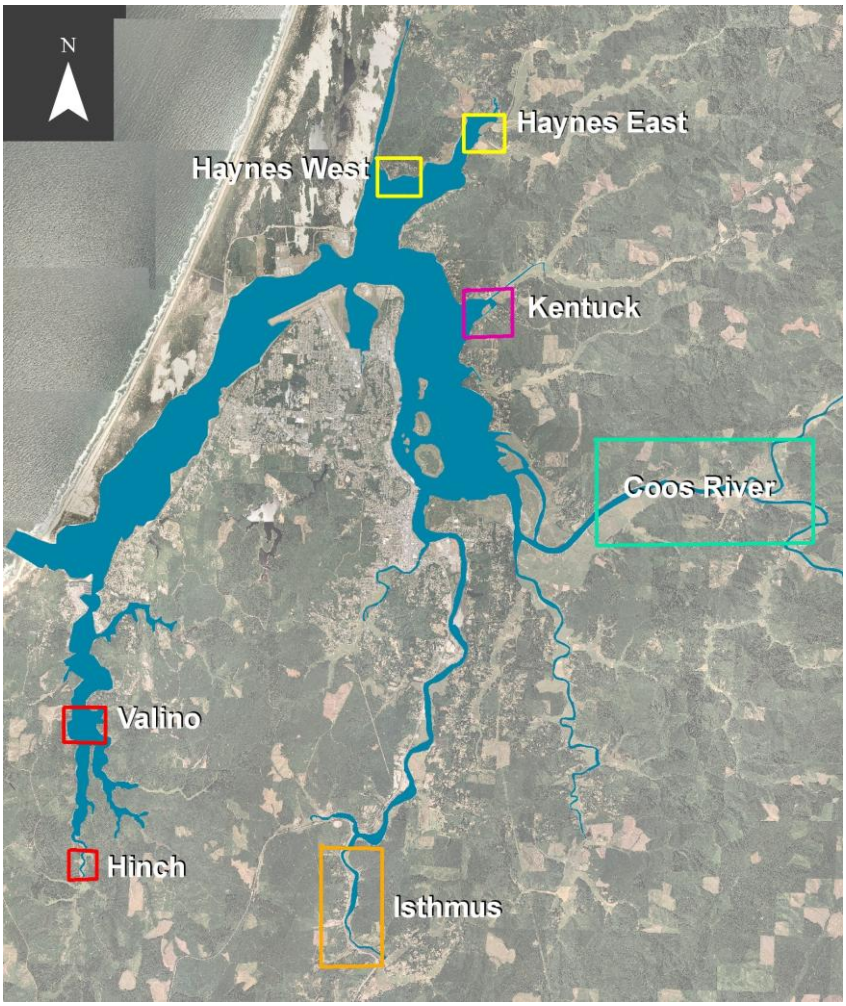

Figure 33: Detailed Assessment polygons by regional site across the Coos estuary

The Isthmus Slough polygon encompassed an area of approximately 125,000 square meters. The distribution of high and low sampling points is shown in Figure 34. The site was classified as having broad expansive marshes and was dominated by polyhaline water. Eight samples had dominant vegetation, which was *Carex spp;* these quadrats were of both high and low strata (Figure 35). Proportional pies of Guild Snails along the Isthmus Slough are shown in Figure 36. Individual species abundance distributions are shown in Figure 37. AP was more abundant than any other snail, with a mean abundance of 750 snails per $0.5m^2$; AC was also fairly abundant at this site, with a mean density of about 400 snails per $0.5m^2$ (Figure 38).

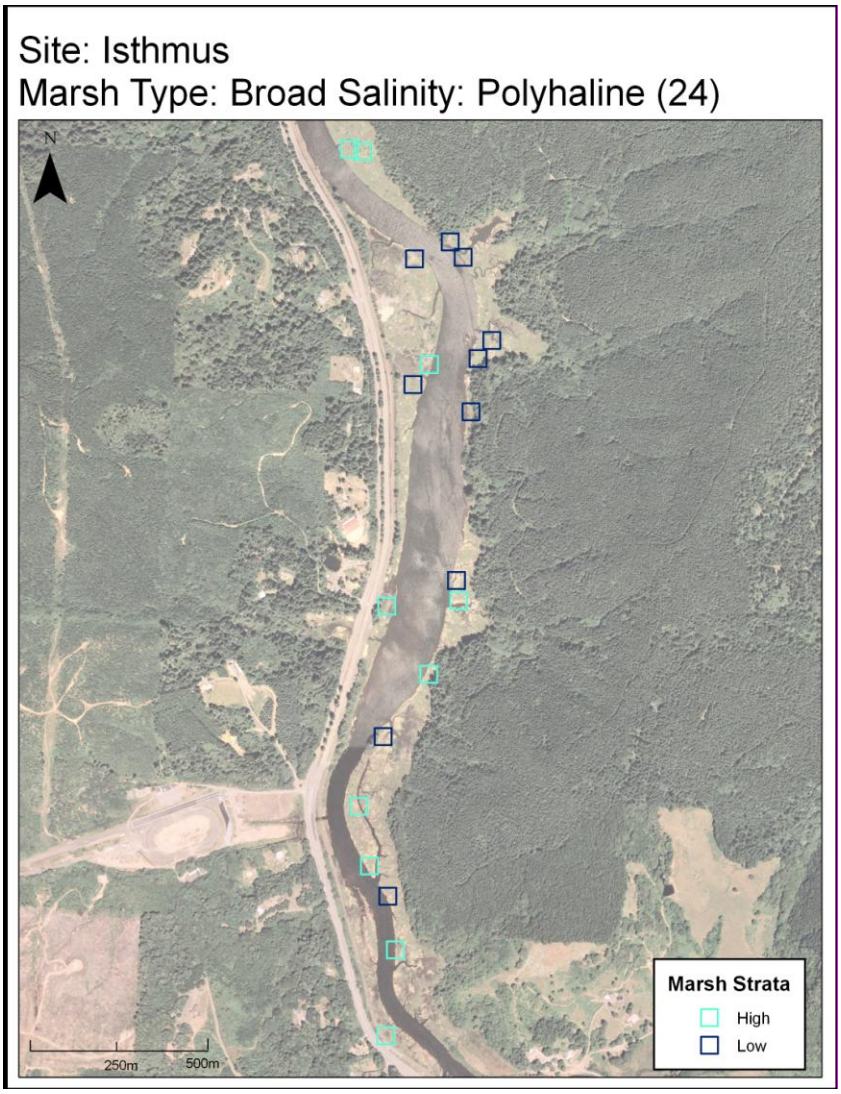

Figure 34: Marsh strata at each Detailed Assessment sampling point in the Isthmus site

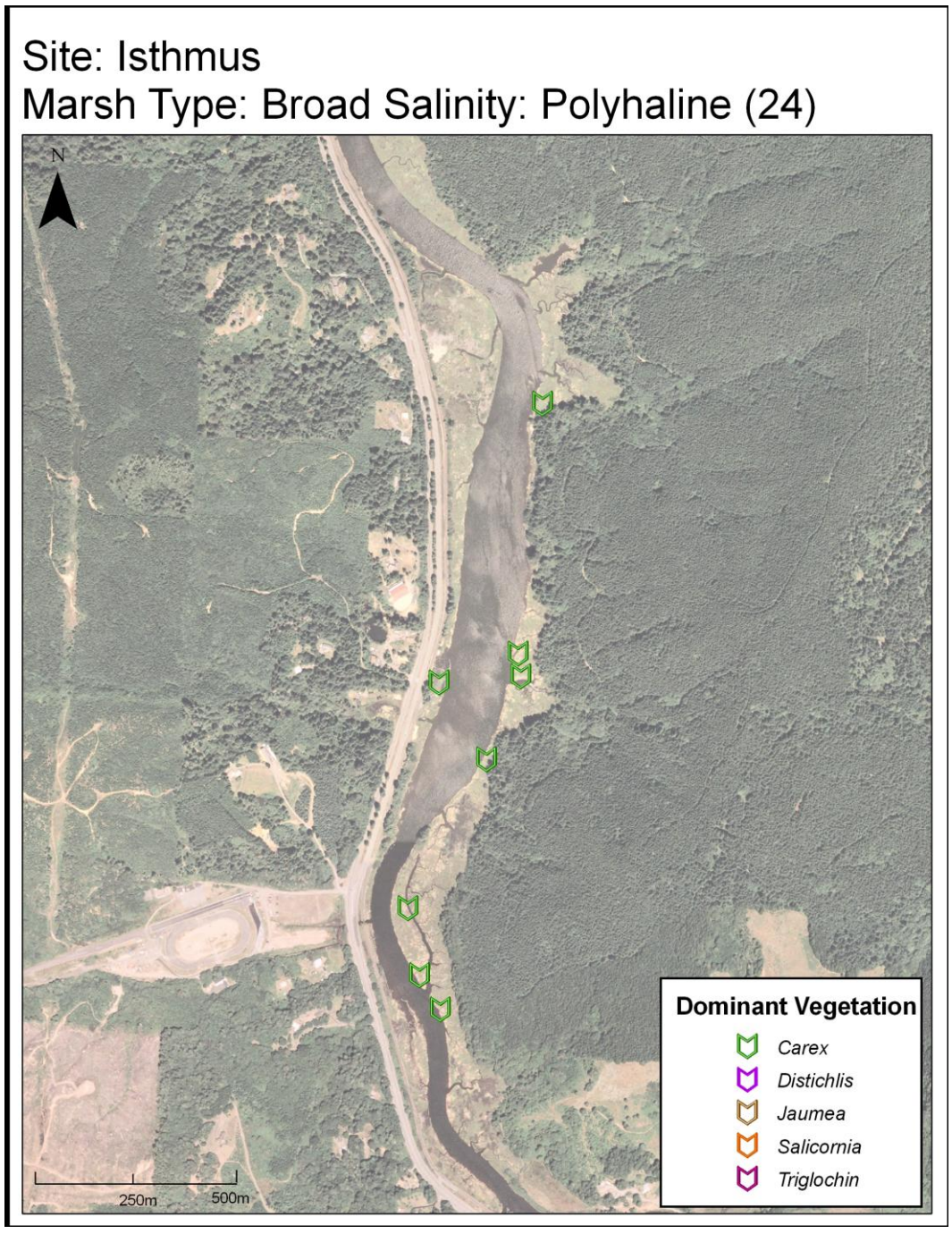

Figure 35: Dominant vegetation at the Detailed Assessment sampling points in the Isthmus site

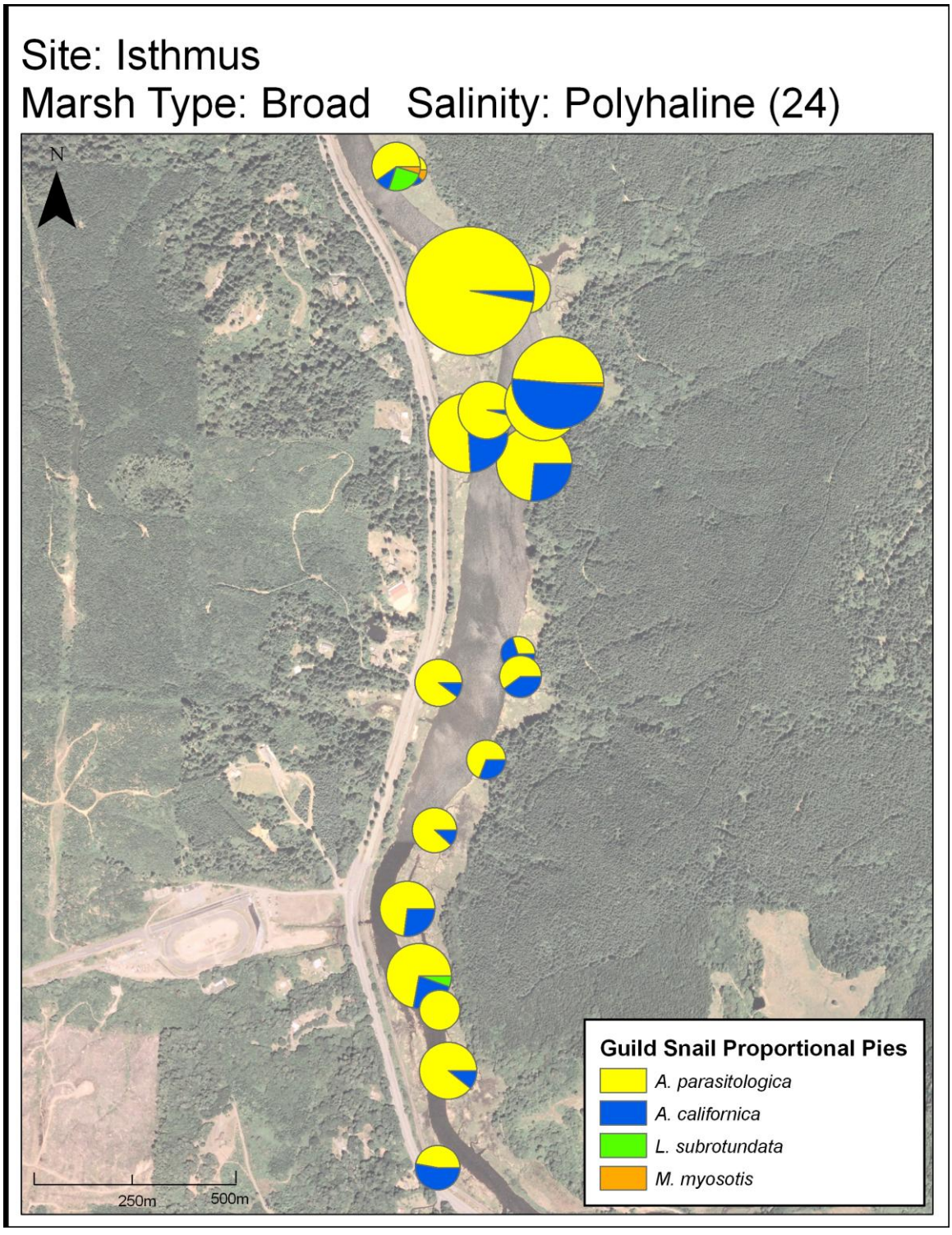

Figure 36: Proportional abundance of Guild Snails at each Detailed Assessment sampling point in the Isthmus site

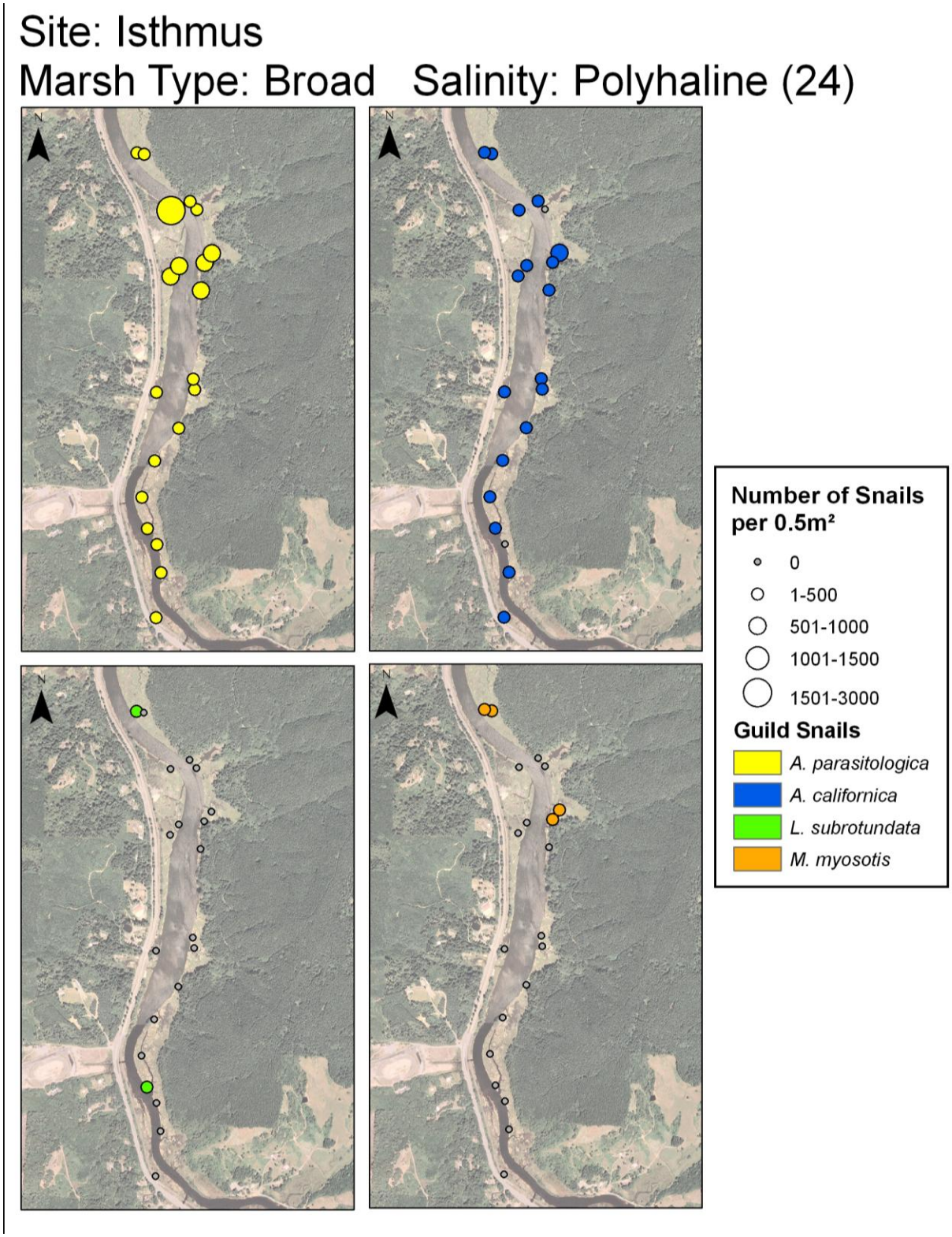

Figure 37: Individual species abundance distribution at each Detailed Assessment sampling point in the Isthmus site

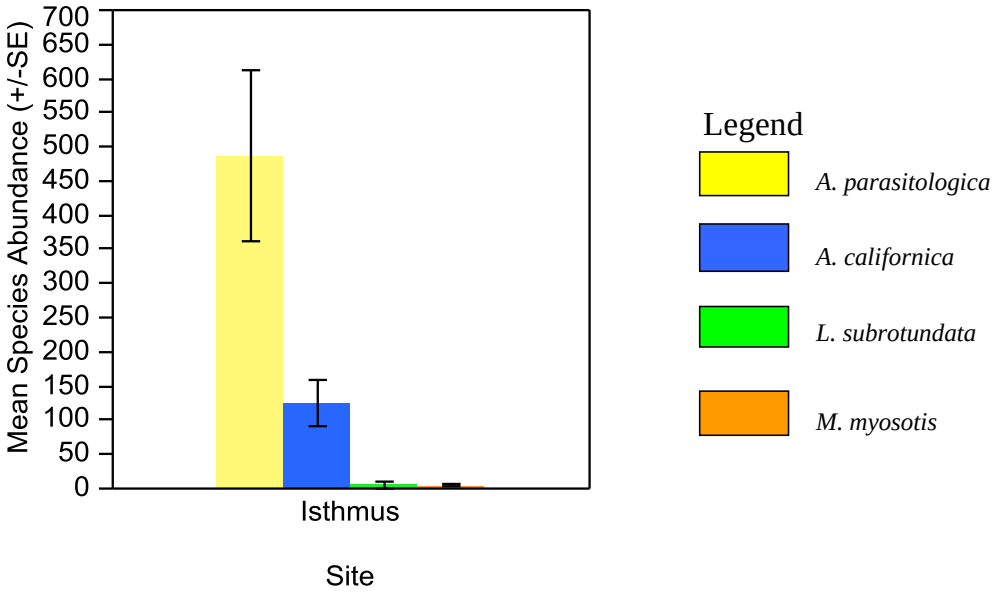

Figure 38: Mean abundance of Guild Snails per 0.5m^2 at the Isthmus site during the Detailed Assessment

The Coos River polygon encompassed an area of approximately 67,500 square meters. The Coos River site had compressed marshes on both sides of the waterway; the banks were frequently steep and often composed of rocky riprap. The distribution of marsh strata was unequal: we sampled five low strata and 15 high strata (Figure 39). The site was dominated by mesohaline water with an average salinity of 11. Six samples had dominant vegetation, five with *Carex spp.* and one with *Salicornia virginica* (Figure 40*)*. Proportional abundance of Guild Snails along the river axis is shown in Figure 41 and individual species' abundance distributions are shown in Figure 42. LS and MM were absent at this site. Grouping the 20 samples AP was more abundant than AC overall, but at specific quadrats AC was the more abundant species (Figure 43).

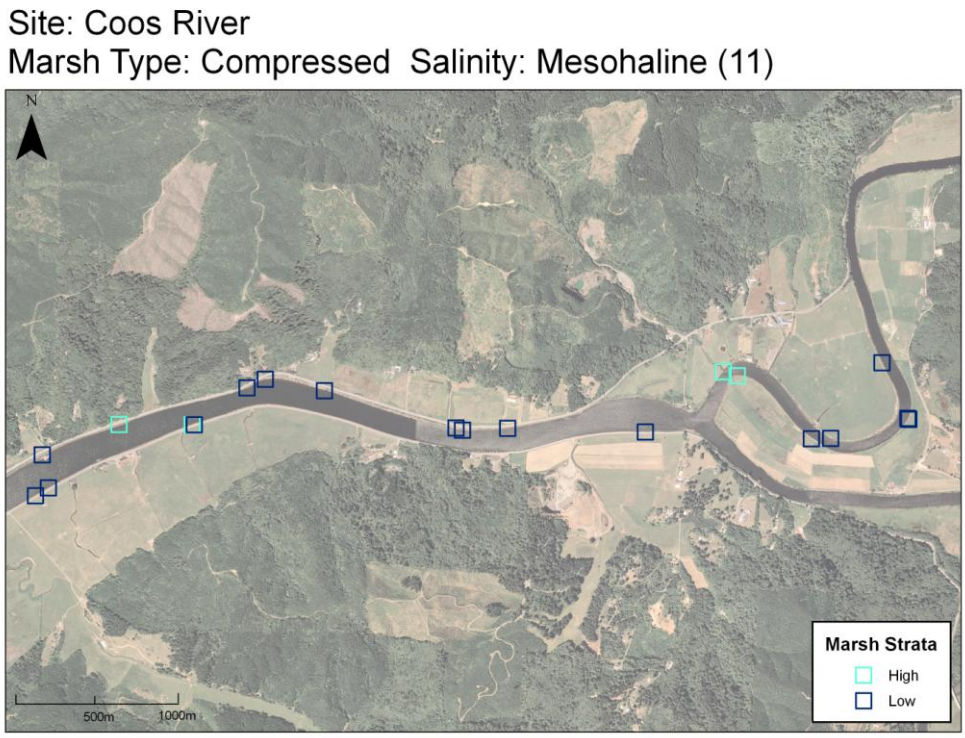

Figure 39: Marsh strata at each Detailed Assessment sampling point

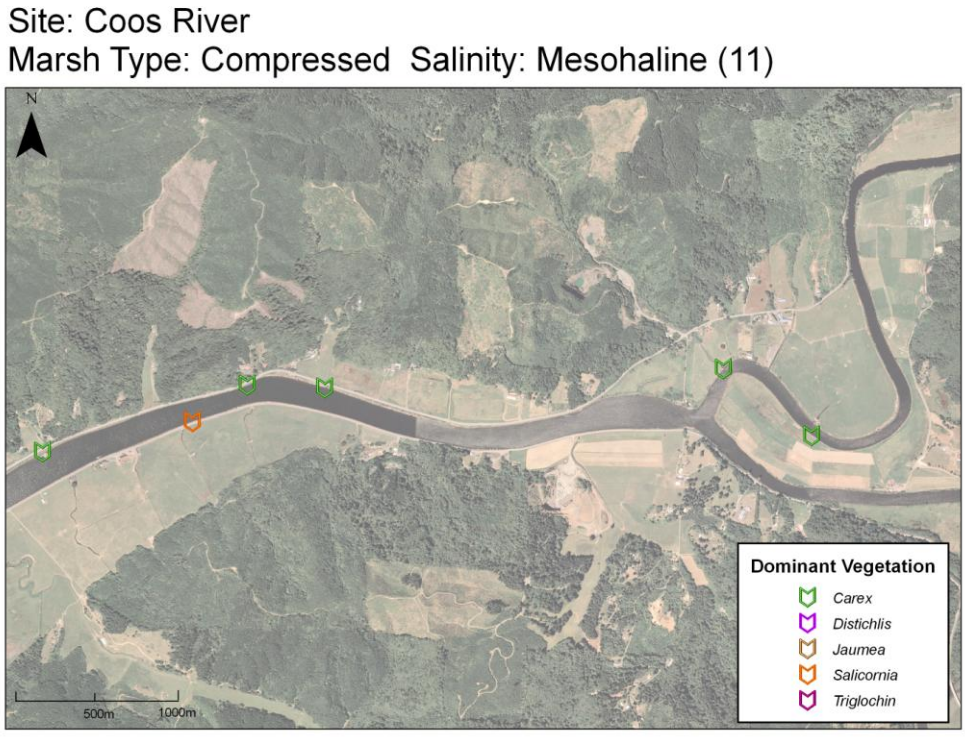

Figure 40: Dominant Vegetation of the Detailed Assessment of the Coos River site

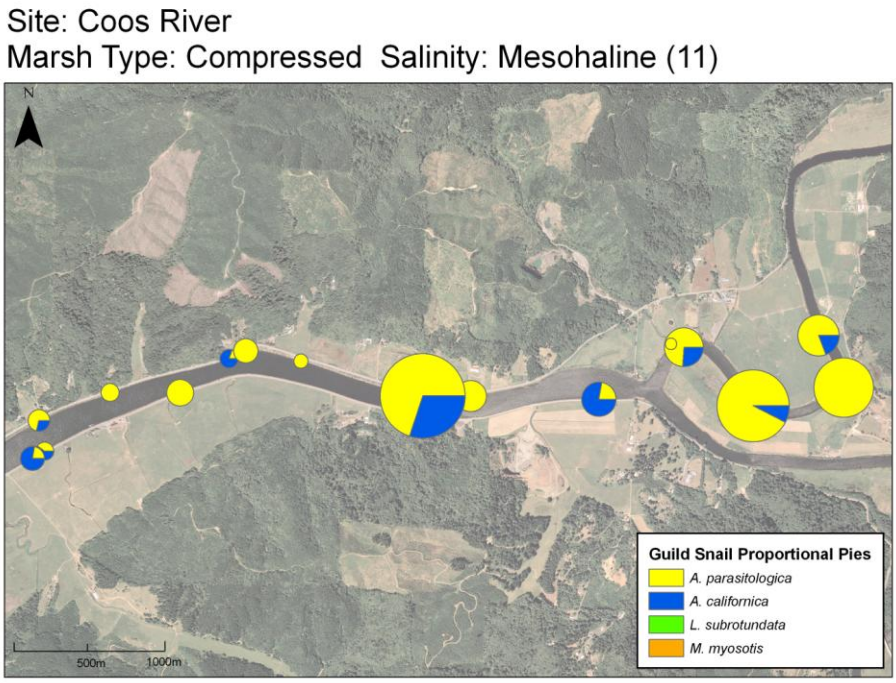

Figure 41: Proportional abundance of Guild Snails at each Detailed Assessment sampling point in the Coos River site

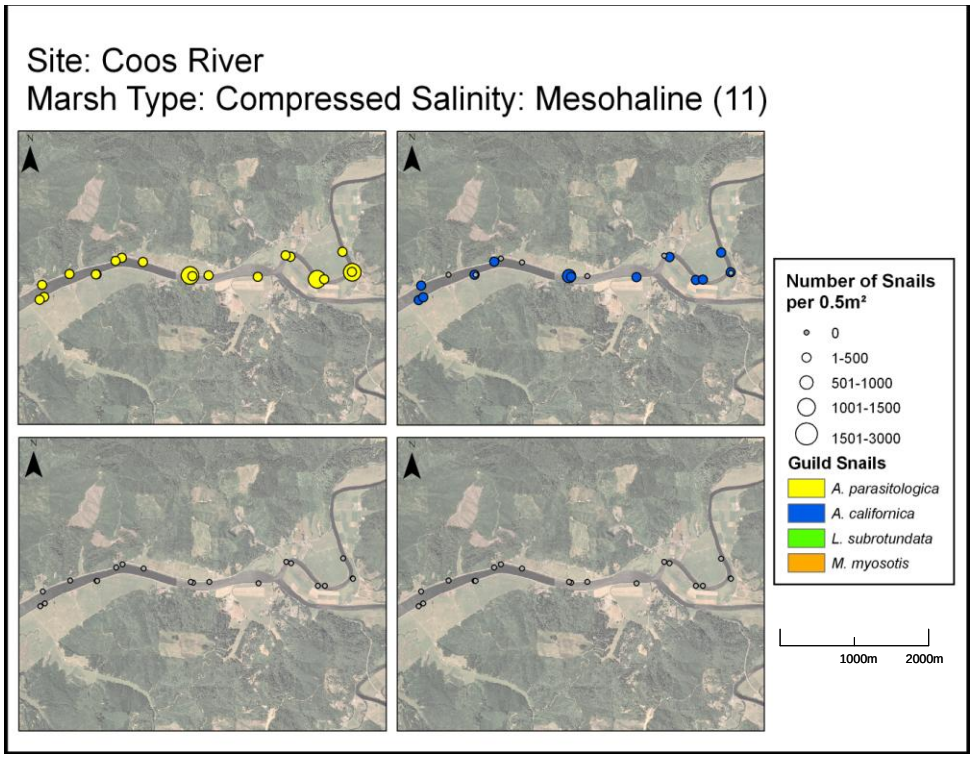

Figure 42: Individual species abundance distribution at each Detailed Assessment sampling point in the Coos River site

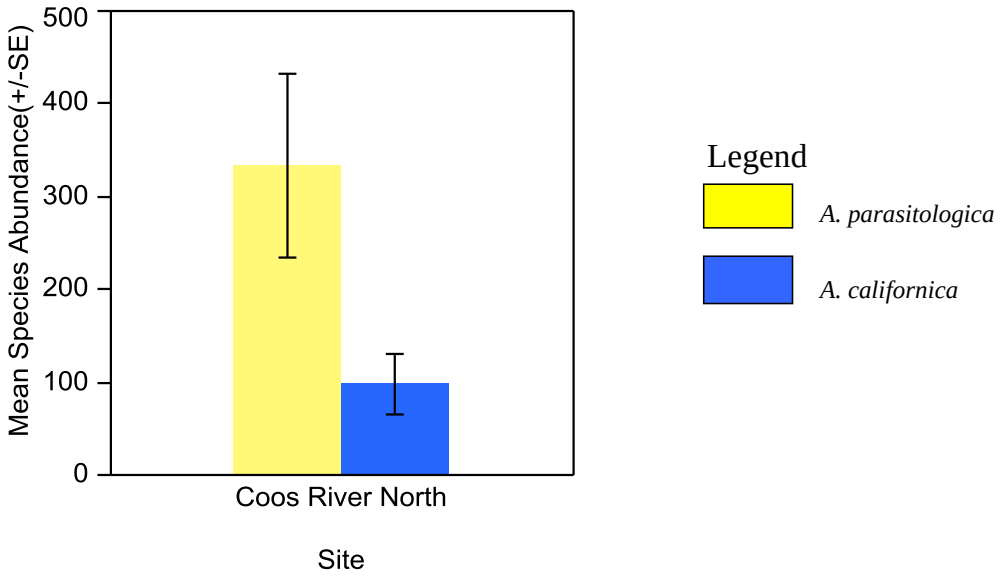

Figure 43: Abundance of *A. parasitologica* and *A. californica* per 0.5m^2 at the Coos River site during the Detailed Assessment

The Kentuck Inlet polygon encompassed an area of approximately 57,400 square meters. Kentuck Inlet was a broad peninsula shaped marsh with a dozen or more tidal channels. It was a euhaline site with an average salinity of 34. We collected 11 high marsh samples and nine low marsh samples (Figure 44). *Carex spp*. was the dominant vegetation on the marsh in six quadrats, then *Salicornia virginica* dominating three quadrats and *Distichlis spp*. dominant in one quadrat (Figure 45). Proportional abundance of Guild Snails across the marsh are shown in Figure 46 and individual species' abundance distributions are shown in Figure 47. AP was the most dominant at an average abundance of 125 snails, then AC at 75 snails and then MM at 25 (all species per 0.5m^2) (Figure 48). LS was absent in this site's samples.

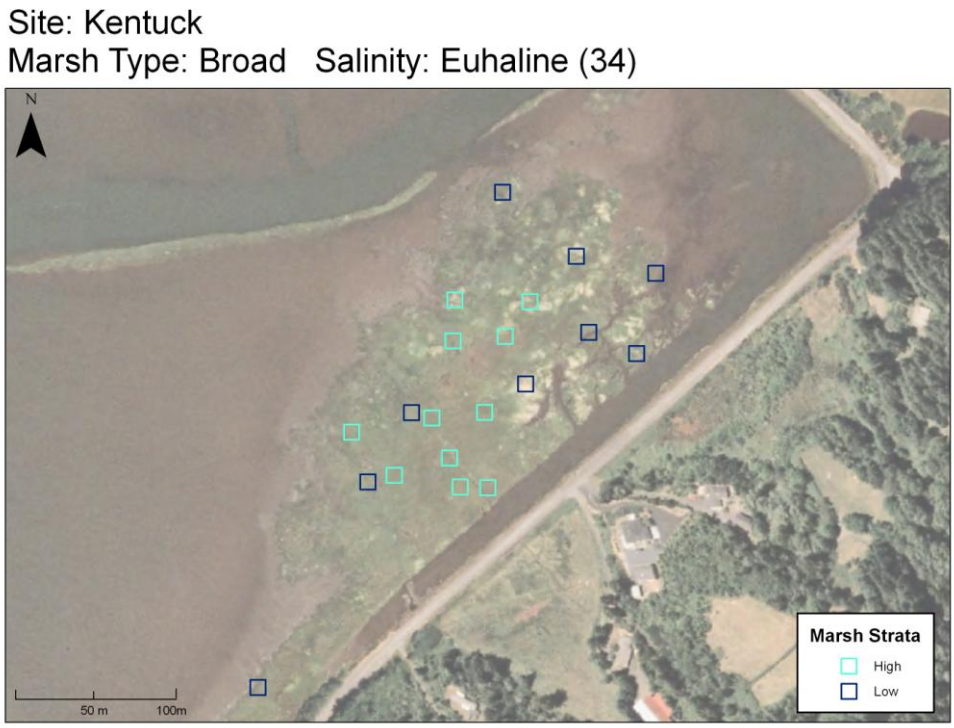

Figure 44: Marsh strata at each Detailed Assessment sampling point in the Kentuck site

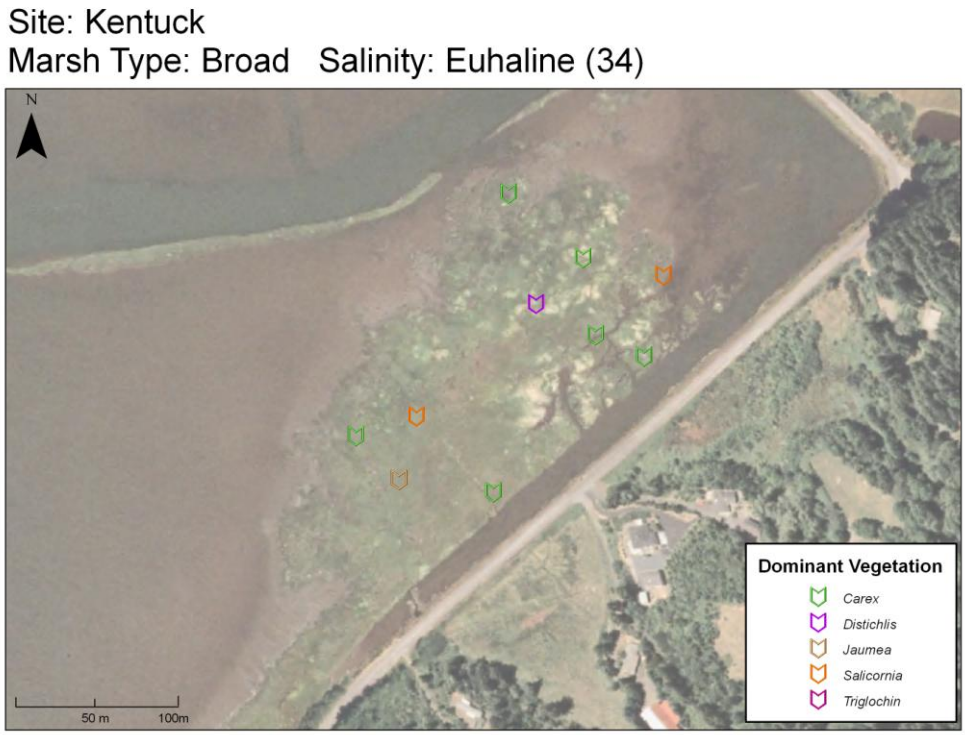

Figure 45: Dominant vegetation in the Kentuck Site

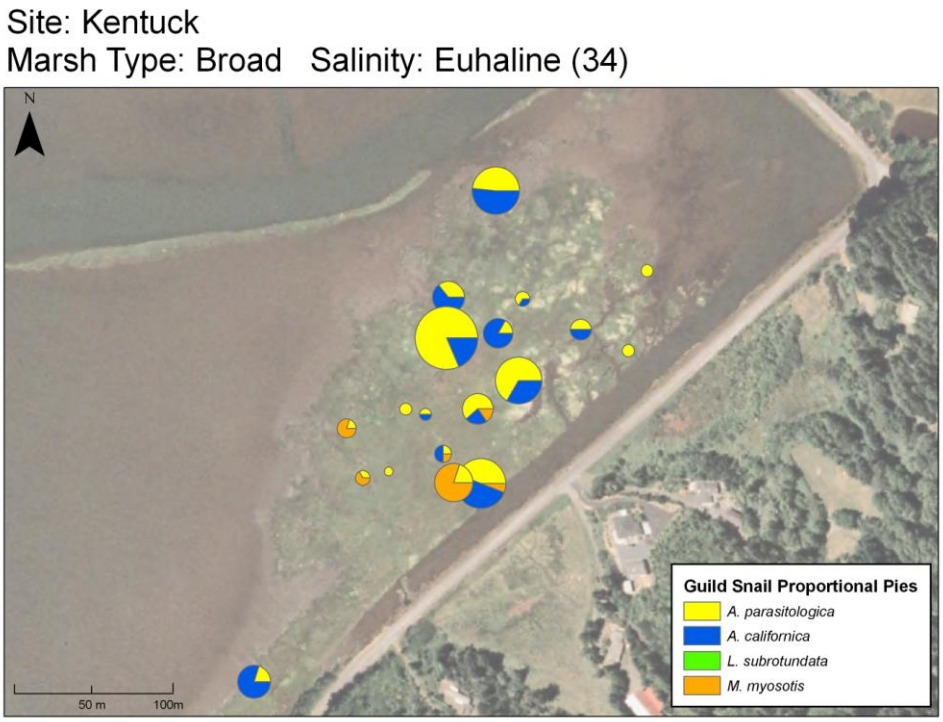

Figure 46: Proportional abundance of Guild Snails at each Detailed Assessment point in the Kentuck Site

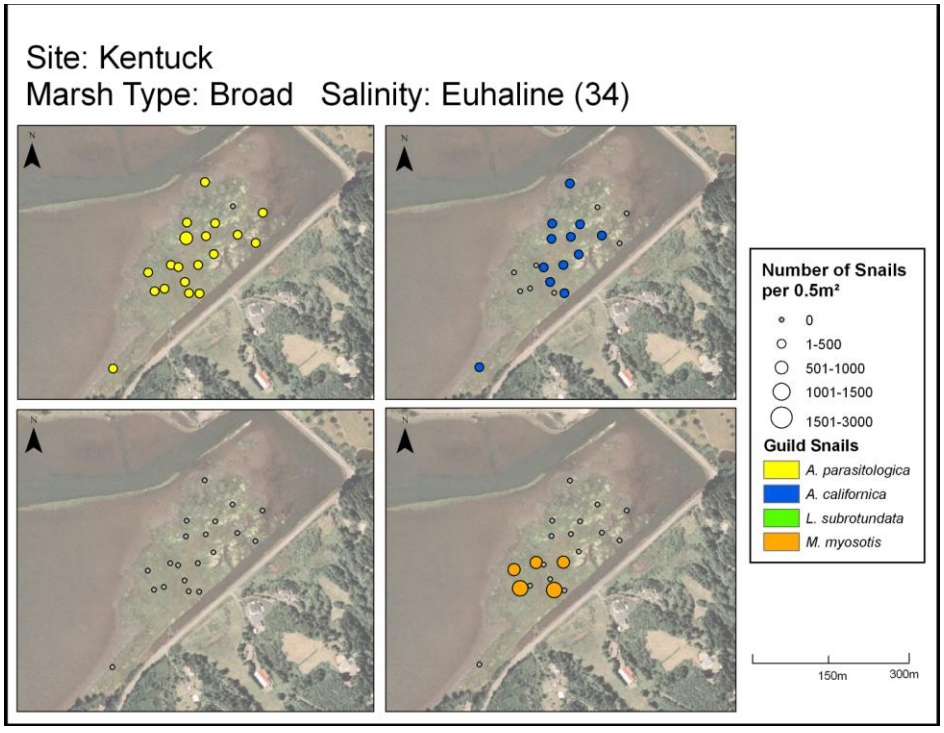

Figure 47: Individual species abundance distribution at each Detailed Assessment point in the Kentuck site

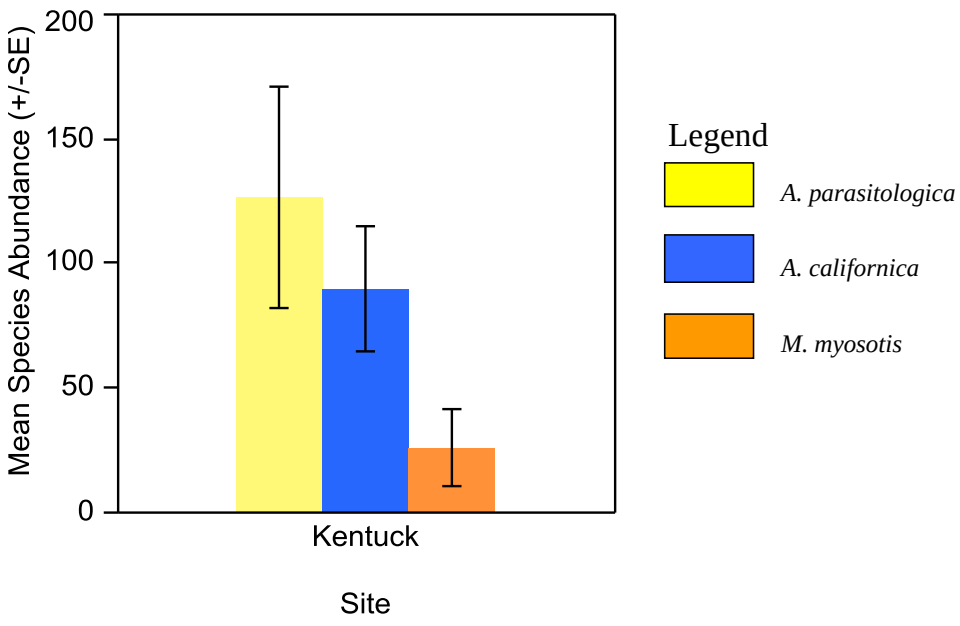

Figure 48: Abundance of *A. parasitologica, A. californica, and M. myosotis* per 0.5m^2 in the Kentuck site

Haynes Inlet was sampled at two sub-sites (ten samples per site): Haynes East and Haynes West. The Haynes polygons were approximately 58,500 square meters in area. This sub-site was a broad marsh in a polyhaline dominated section of the inlet with an average salinity of 22. We collected five high and five low strata plots from this site and there was no dominant vegetation (Figure 49). Proportional abundance of Guild Snails across the marsh is shown in Figure 50 and individual species' abundance distributions are shown in Figure 51. AP and AC were the dominant snails at 275-300 snails per 0.5m^2, MM were in lower abundance, 150 per quadrat, LS was absent in this site's samples (Figure 52). Haynes West was a compressed marsh with a rather small area of 374 square meters. This site was in a euhaline zone with an average salinity of 35. We collected samples from three high and seven low strata (Figure 53). Dominant vegetation was varied between quadrats: two *Carex spp*., two *Salicornia virginica* and one *Distichlis spp*. dominated plots (Figure 54). All four species were present: AP had noteworthy higher abundance than the other three snails present (Figure 55-57).

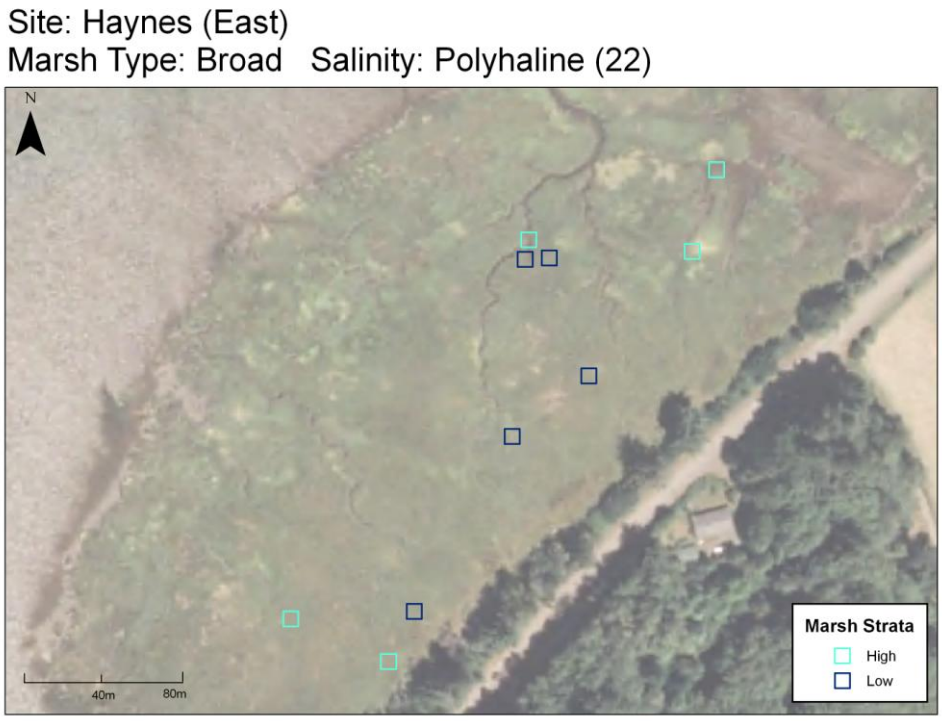

Figure 49: Marsh strata at each Detailed Assessment point in Haynes East

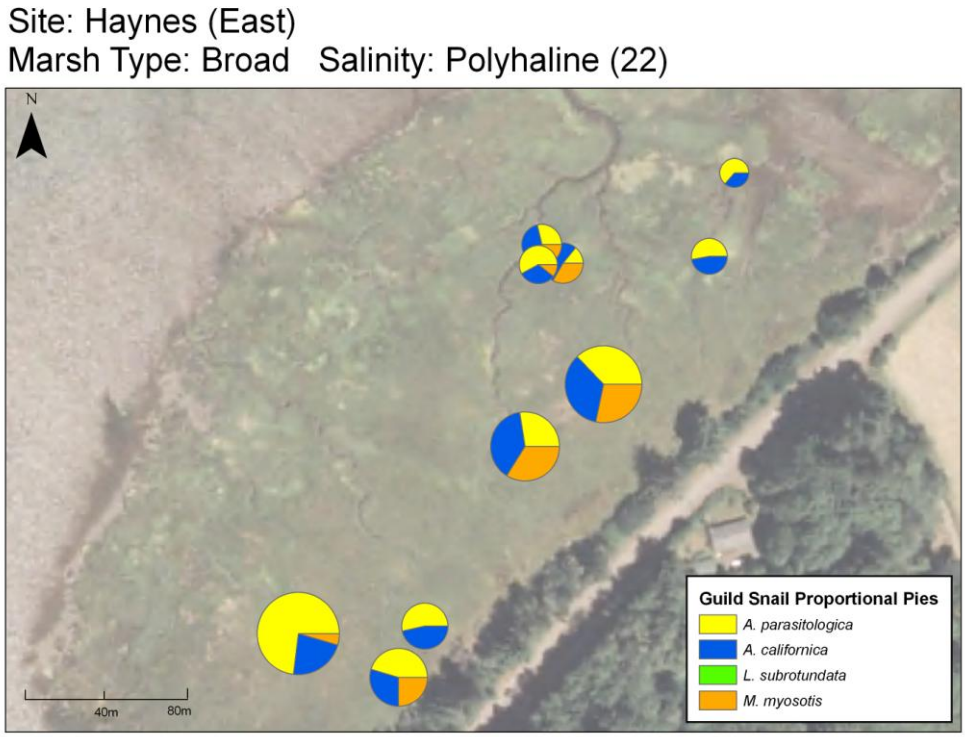

Figure 50: Proportional abundance of Guild Snails at each Detailed Assessment point in Haynes East

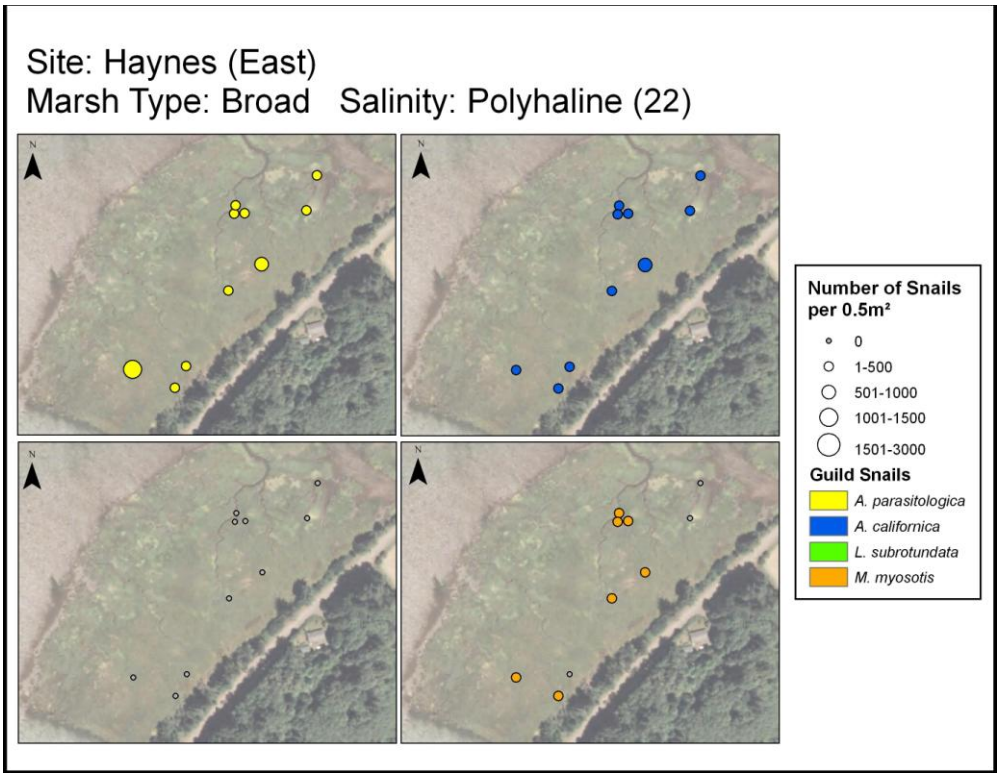

Figure 51: Individual species abundance distributions at each Detailed Assessment point in Haynes East

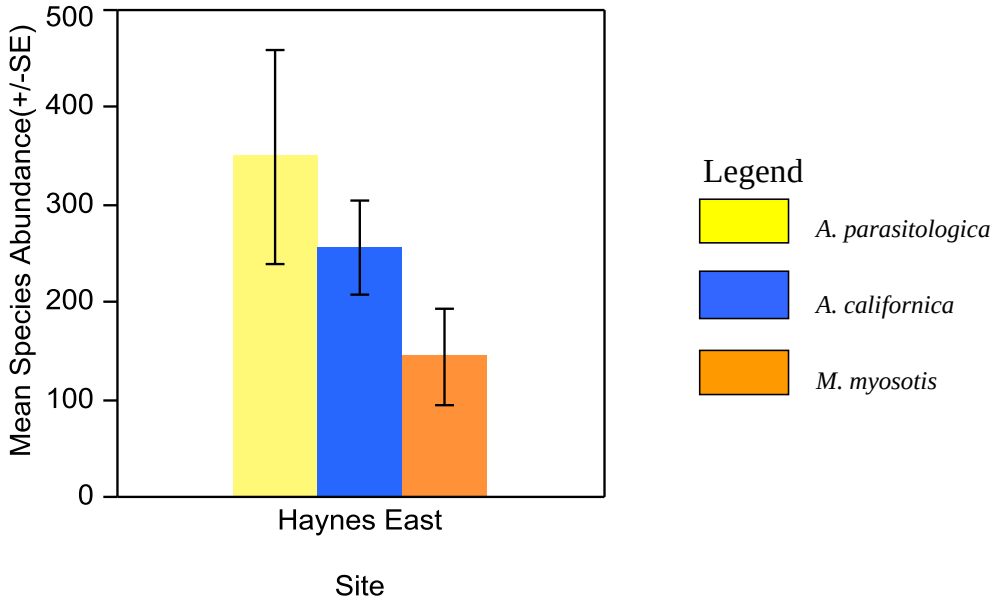

Figure 52: Abundance of *A. parasitologica*, *A. californica* and *M. myosotis* per $0.5m^2$ in Haynes East

Figure 53: Marsh strata at each Detailed Assessment point in Haynes West

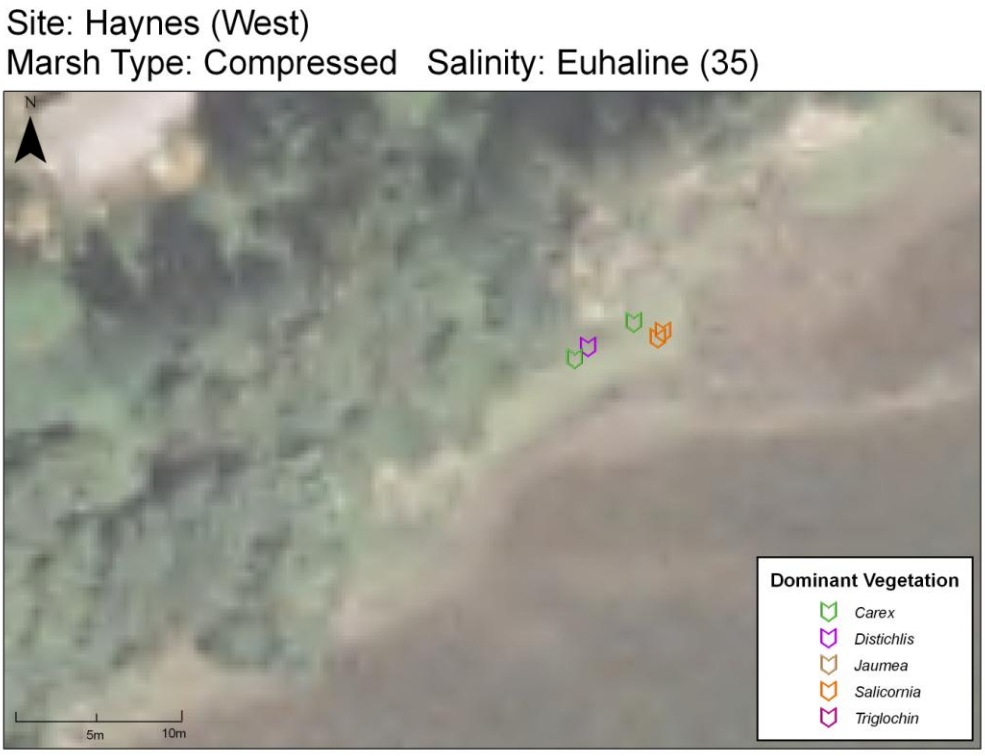

Figure 54: Dominant Vegetation in Haynes West

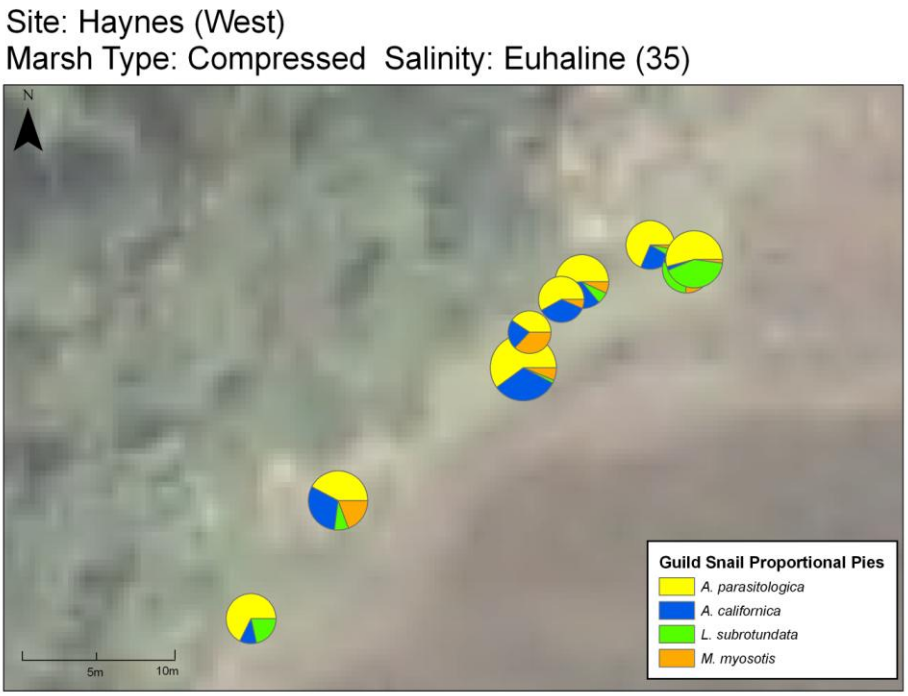

Figure 55: Proportional abundance of Guild Snails at each Detailed Assessment point in Haynes West

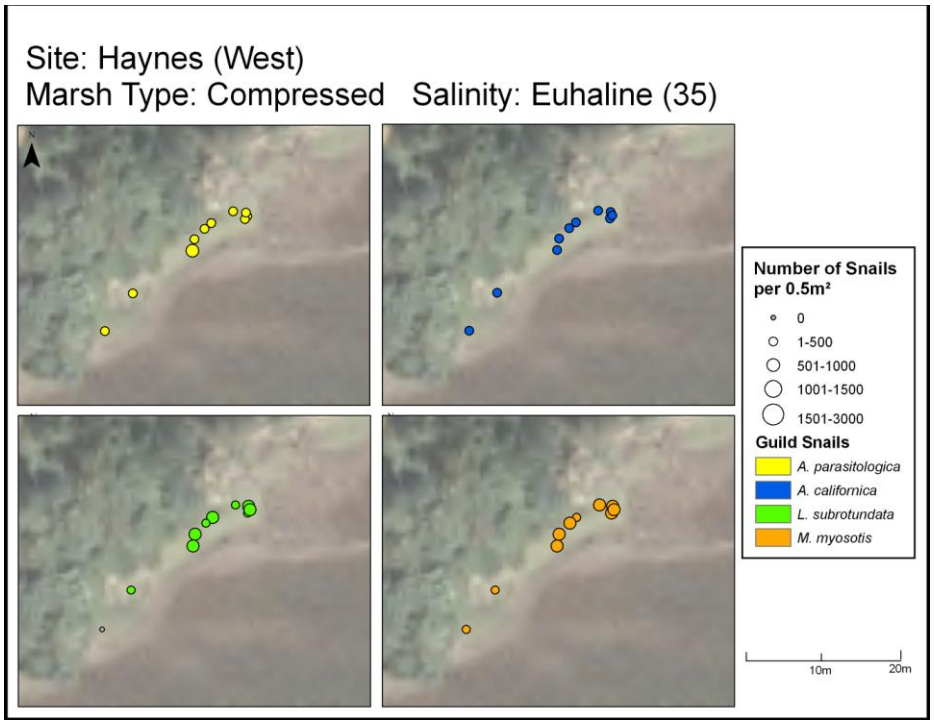

Figure 56: Individual species abundance distribution at each Detailed Assessment point in Haynes West

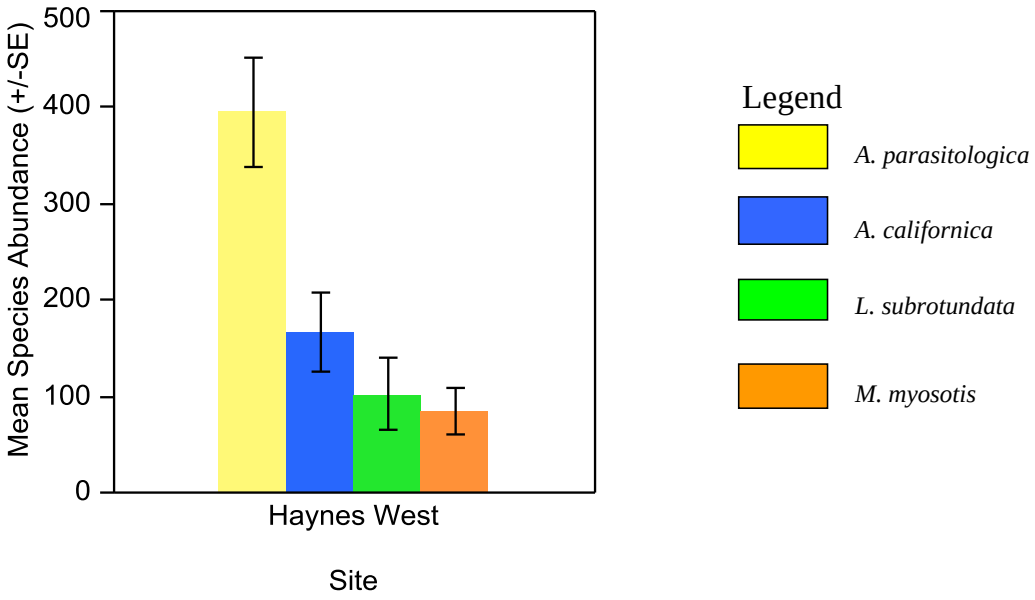

Figure 57: Abundance of *A. parasitologica*, *A. californica*, *L. subrotundata,* and *M. myosotis* per 0.5m^2 at Haynes West

South Slough was sampled at two sub-sites (ten samples per site): Hinch Bridge and Valino Island. The Hinch Bridge site was at the upper reach of the South Slough, in a mesohaline dominated area with an average salinity of 15. This site was approximately 19,800 square meters and was predominantly high marsh (Figure 58). The dominant vegetation was *Carex spp*. at five quadrats (Figure 59). Mesogastropods were only found in one quadrat; both AP and AC were present in low numbers (Figure 60). The Valino Island site is a compressed fringing marsh and the polygon was about 4,532 meters squared. We sampled nine low and one high marsh strata in this euhaline dominated site (Figure 61). Four of these plots were dominated by *Triglochin maritimum* and four by *Salicornia virginica* (Figure 62*)*. All four species were present at this site; AC was the dominant species followed by MM, AP and LS (figure 63-65).

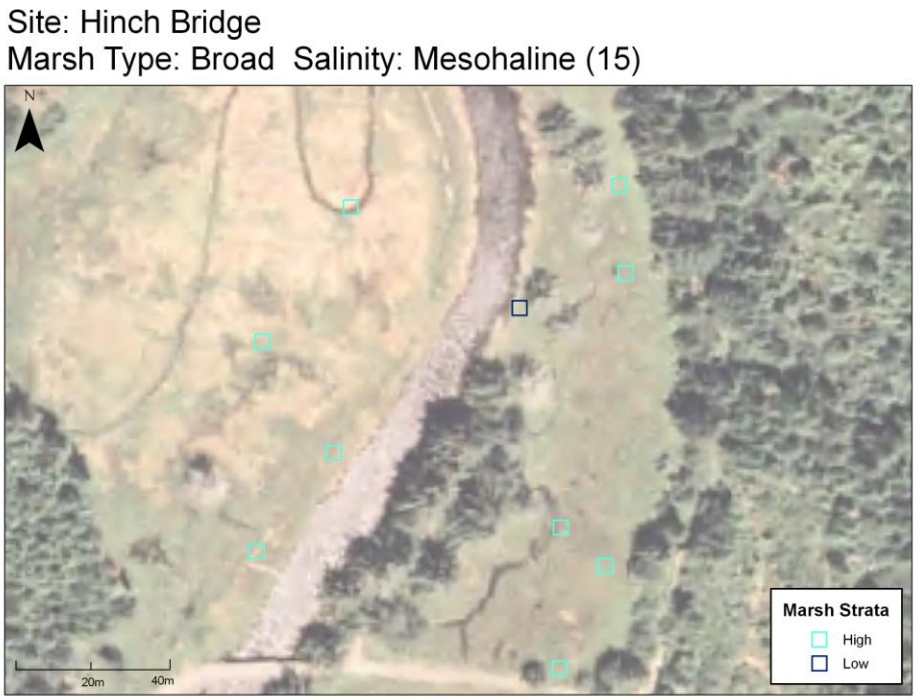

Figure 58: Marsh strata at each Detailed Assessment sampling point at Hinch Bridge

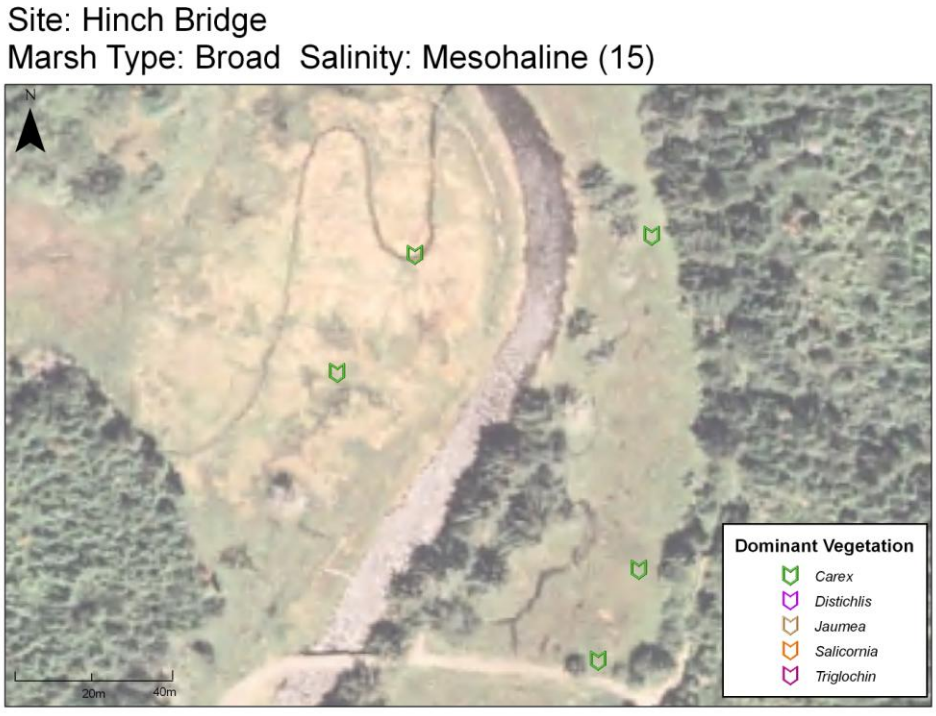

Figure 59: Dominant vegetation during Detailed Assessment at Hinch Bridge

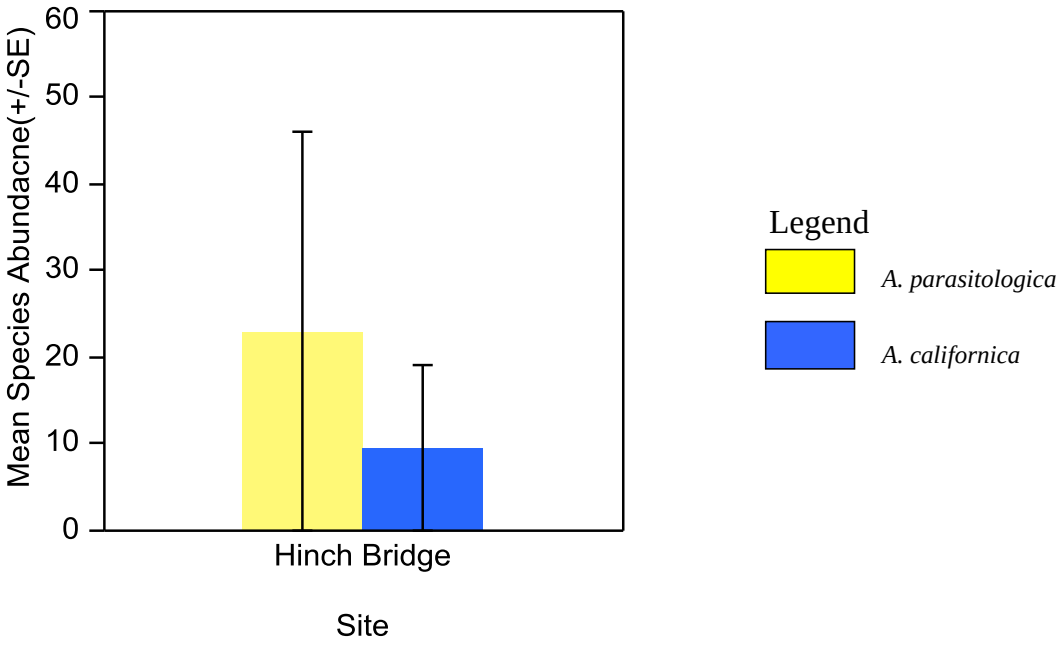

Figure 60: Abundance of *A. parasitologica* and *A. californica* per 0.5m^2 during Detailed Assessment at Hinch Bridge

Figure 61: Marsh strata at each Detailed Assessment sampling point at Valino Island

Figure 62: Dominant vegetation at Valino Island

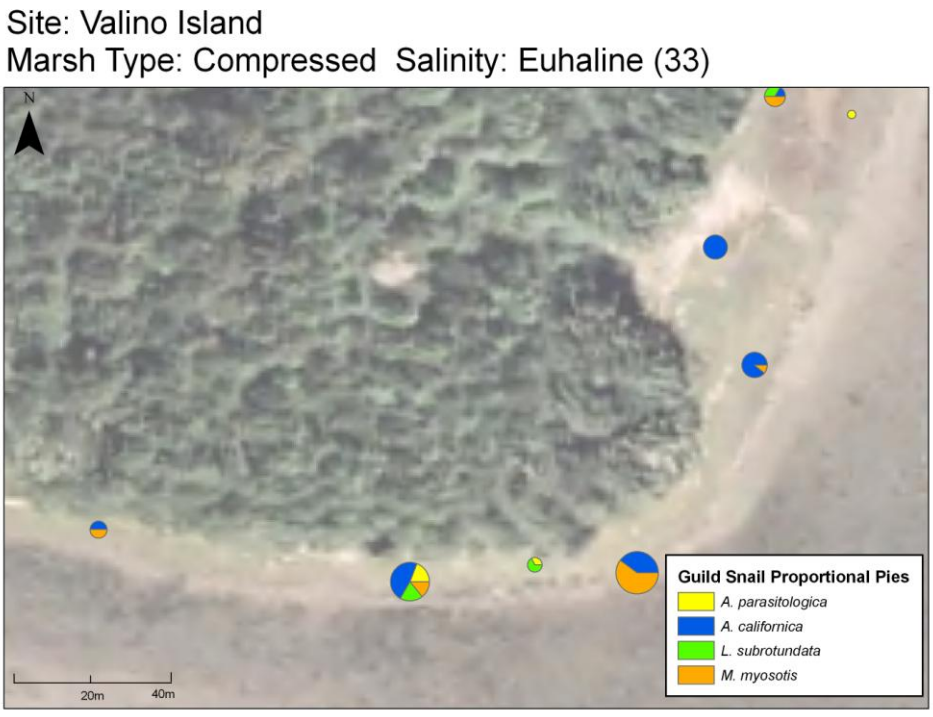

Figure 63: Proportional abundance of Guild Snails at each Detailed Assessment point at Valino Island

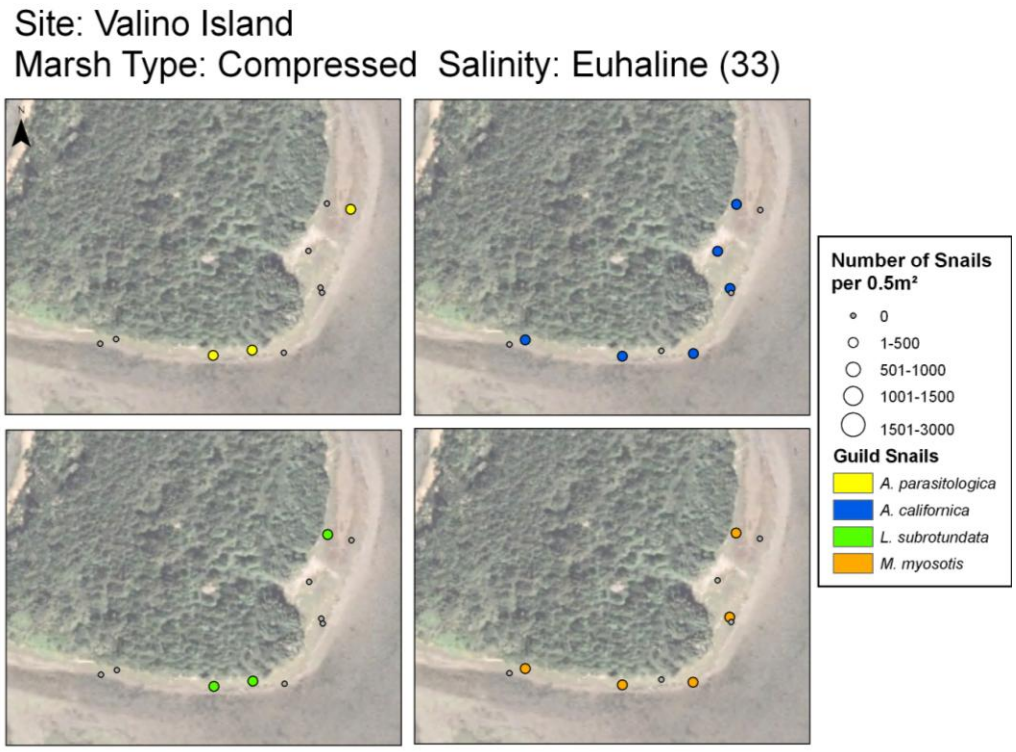

Figure 64: Individual species abundance distribution at each Detailed Assessment point at Valino Island

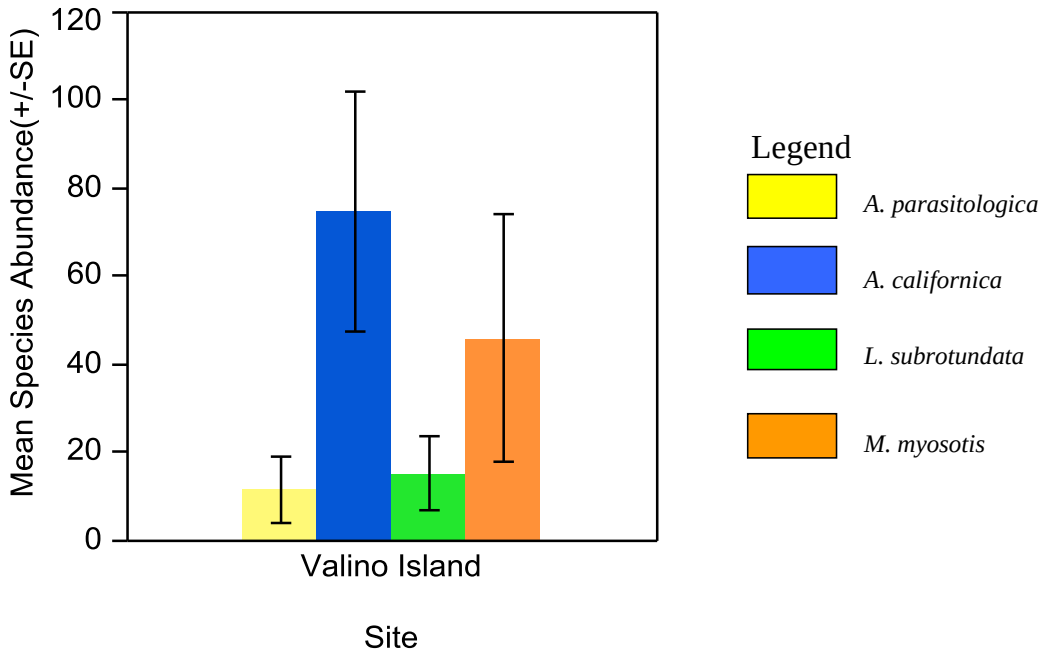

Figure 65: Abundance of *A. parasitologica*, *A. californica*, *L. subrotundata*, *M. myosotis* per 0.5m^2 during Detailed Assessment for Valino Island

The abundance of each species by site is shown in Figure 66. Across all regions AP was most abundant at all sites except for Valino Island, where AC was dominant. AC was the second most abundant at all remaining sites. Overall there were more MM collected than LS, but this result was not consistent across all sites. Haynes West had the highest species diversity (Figure 66). ANOVA statistical tests with post-hoc Tukey-Kramer analysis were employed to test the difference in abundance of each species by site. The abundance of AP was significantly different by region (p= 0.0019, Figure 67). Post-hoc analysis showed that the regions driving the significant difference in abundance were the large population at Isthmus Slough versus the smaller populations at Kentuck Inlet, Valino Island, and the Hinch Bridge. AC abundance was significantly different by site (p= 0.0023, Figure 68). The difference lay in the high abundance at Haynes West versus the lower at Coos River, Kentuck Inlet, Valino Island and the Hinch Bridge. LS was significantly more abundant at the Haynes site versus all other sampling locations (p=0.0001, Figure 69). MM abundance was significantly different by site (p= 0.0001) (Figure 70). MM was much more abundant at Haynes East than as the Isthmus or Coos River. All post-hoc, Tukey-Kramer results can be viewed in Appendix: 9.6.

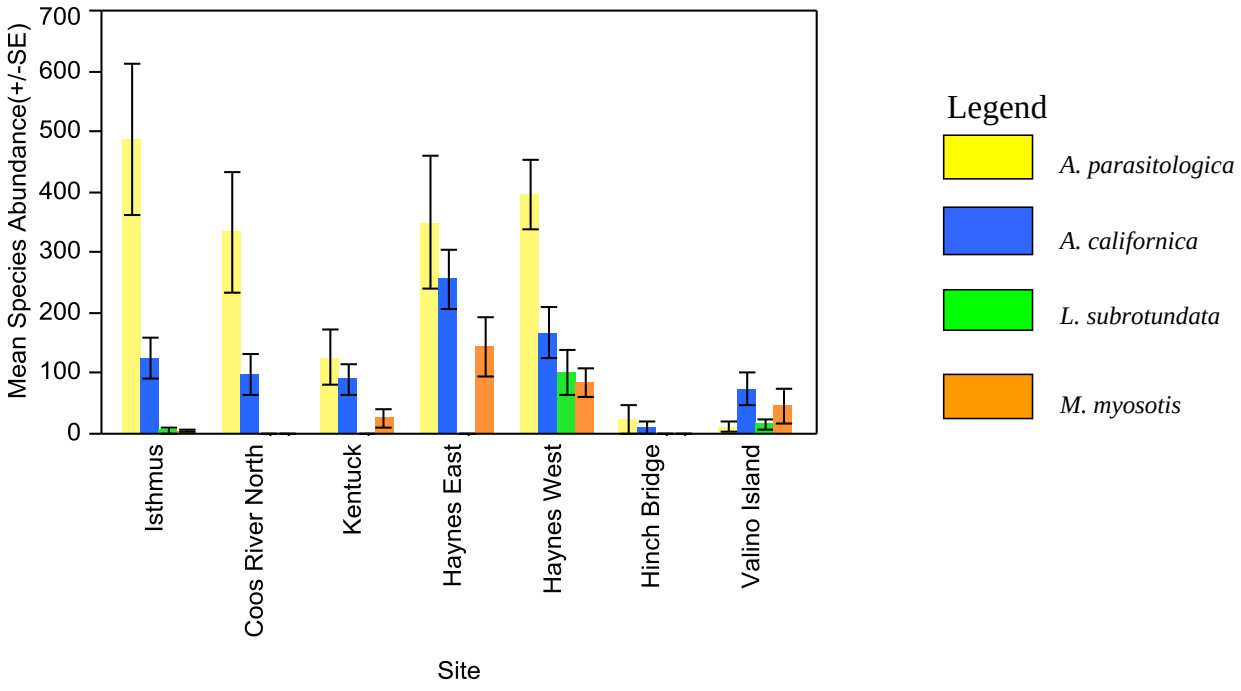

Figure 66: Guild Snail mean abundances by site per 0.5m^2 for Detailed Assessment

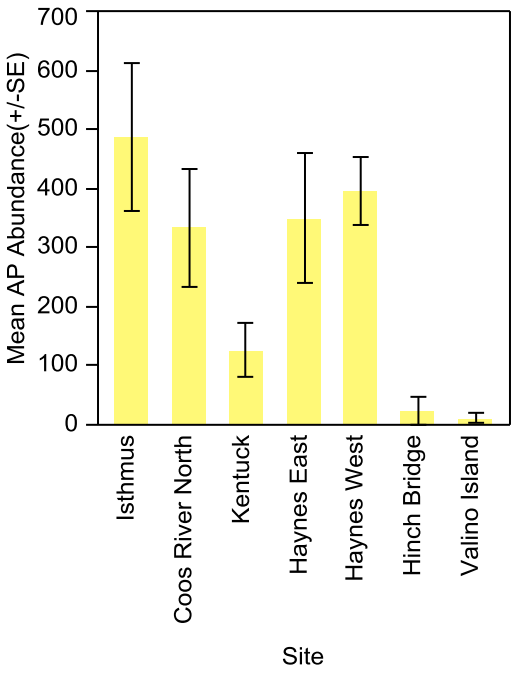

Figure 67: Mean AP abundance by site per 0.5m^2 during Detailed Assessment

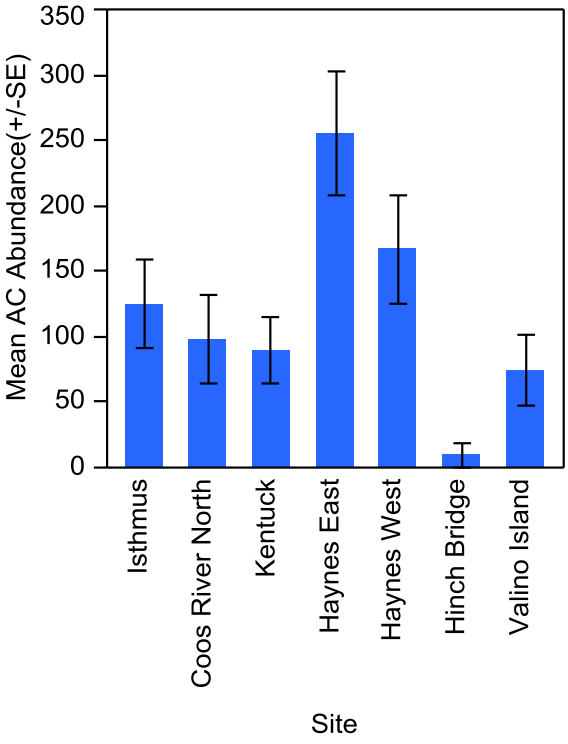

Figure 68: Mean AC abundance by site per 0.5m^2 during Detailed Assessment

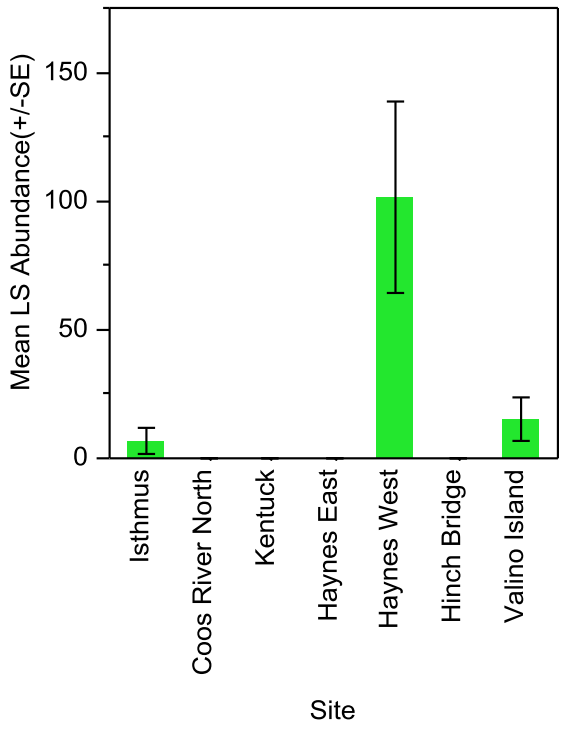

Figure 69: Mean LS abundance by site per 0.5m^2 during Detailed Assessment

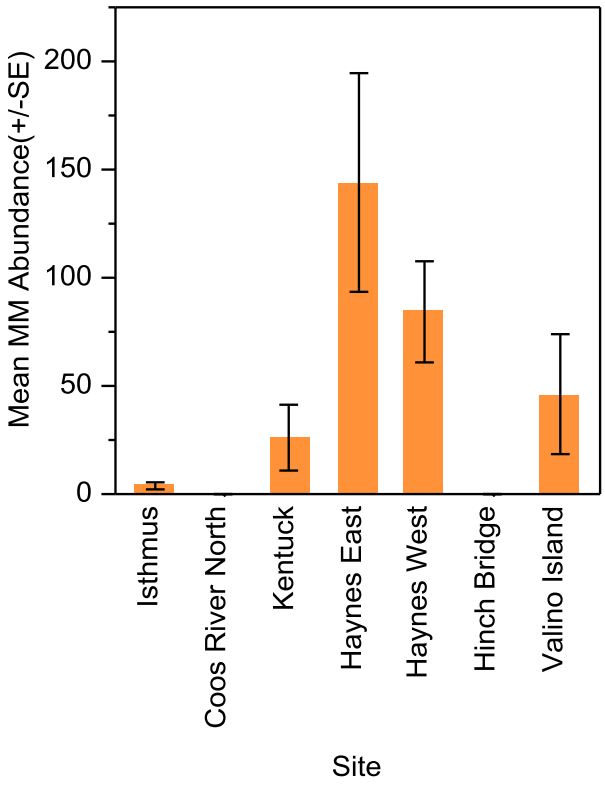

Figure 70: Mean MM abundance by site per 0.5m^2 during Detailed Assessment

The results explained above show the species-specific abundances by site, prompting us to examine the environmental signature of each site. We utilized Primer to compare the differences in environmental structure of each site. Figure 71 shows a strong correspondence between region and the environmental variables used in this analysis (Stress=0.15), ANOSIM test further confirmed that site was significant in describing the environmental structure (R=0.697, %=0.1). Salinity zone was examined across all sites to determine if there was a difference in environmental structure. The MDS plot shows a strong correspondence between salinity zone and the environmental variables (Stress=0.15), ANOSIM confirmed that there was a significant difference in environment by salinity zone (R=0.255, %=0.1) (Figure 72). Knowing that the environmental structure of the sites was different, and that this difference may have been due to salinity zone; we examined the abundance levels of each species by specific environmental variables. Environmental variables included: salinity zone, marsh strata, marsh type, marsh slope, dominant vegetation and sediment temperature. For a full list of environmental variable results refer to Table 2.

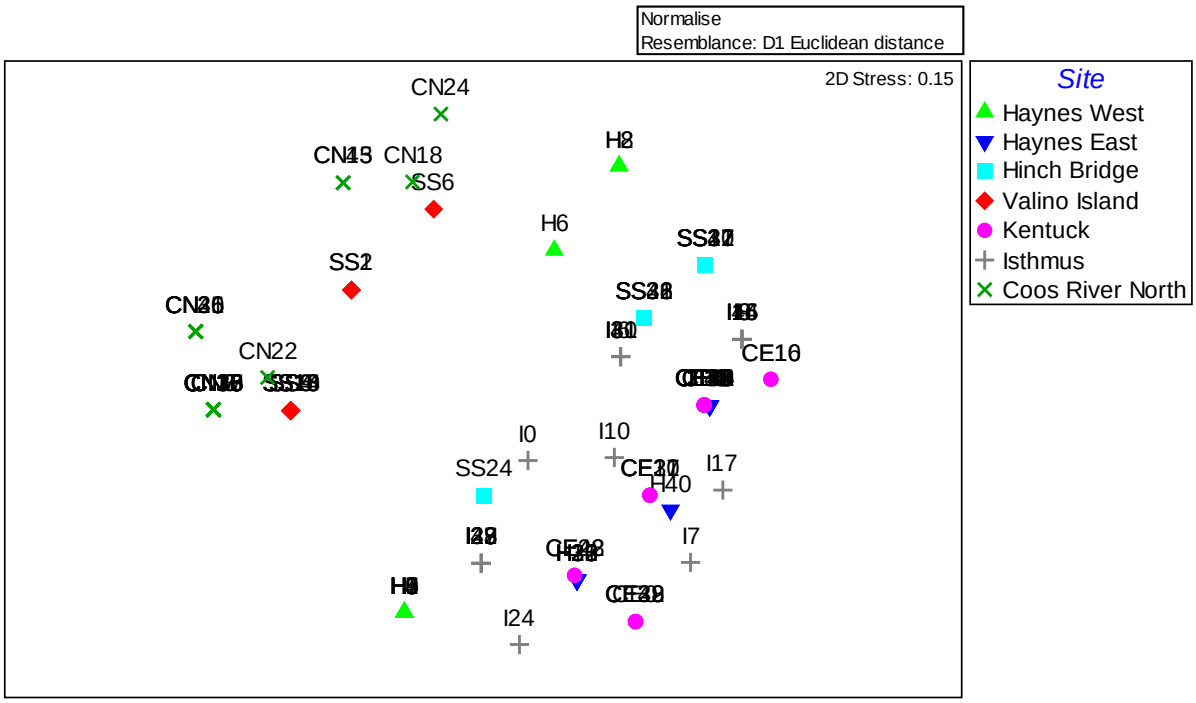

Figure 71: MDS plot showing the correspondence between environmental variables and site in the Detailed Assessment

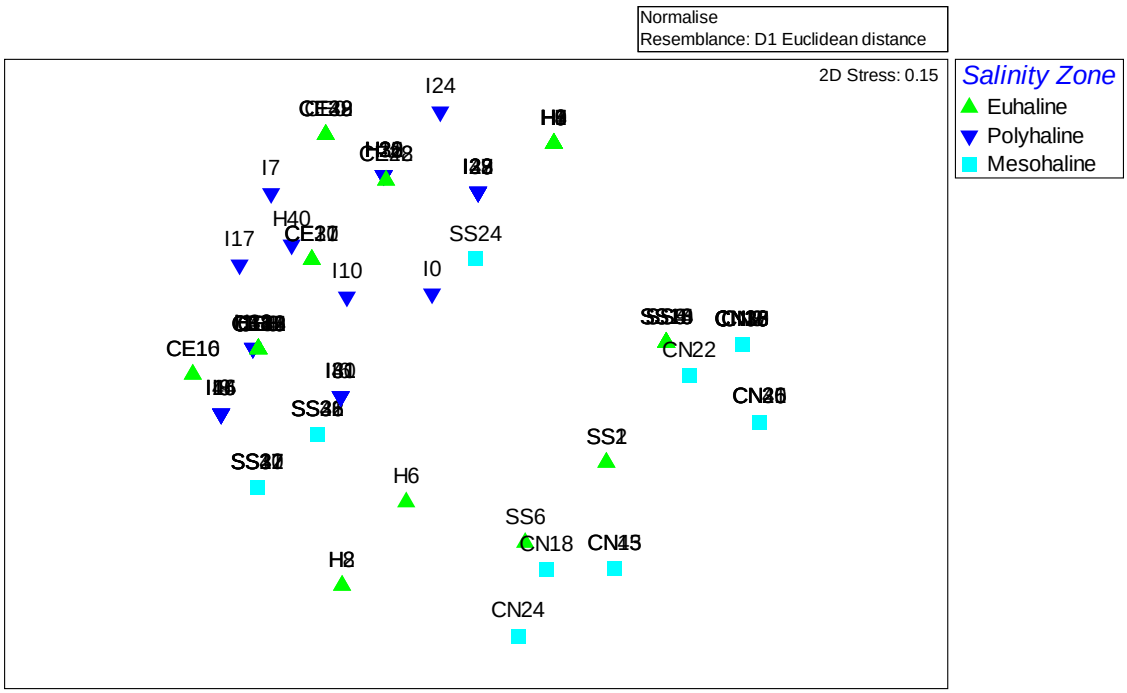

Figure 72: MDS plot showing correspondence between environmental variables and salinity zone in the Detailed Assessment

Table 2:
ANOVA statistical test results of factors with Snail Guild Species. P-values for selected environmental data correlated with species abundance

Factor	AP	AC	LS	MM
Salinity zone	0.0123	0.0477	NS	0.0010
Marsh type	NS	0.000	0.0055	NS
Marsh strata	NS	0.0513	NS	NS
Marsh slope	NS	NS	NS	NS
Dominant Vegetation	NS	NS	0.0216	NS
Sediment Temperature	NS	NS	NS	NS

1-WAY ANOVA tests are significant when $p < 0.05$, NS = not significant

AP abundance was significantly different by salinity zone (p=0.0123) (Figure 73). AC abundance was significantly different by salinity zone (p=0.0477) and marsh strata (p=0.0513) (Figures 74-75). LS distribution was significantly different by marsh type

(p=0.0055) and dominant vegetation (p=0.0216) (Figures 76-77) MM abundance was significantly different by salinity zone (p=0.0010) (Figure 78).

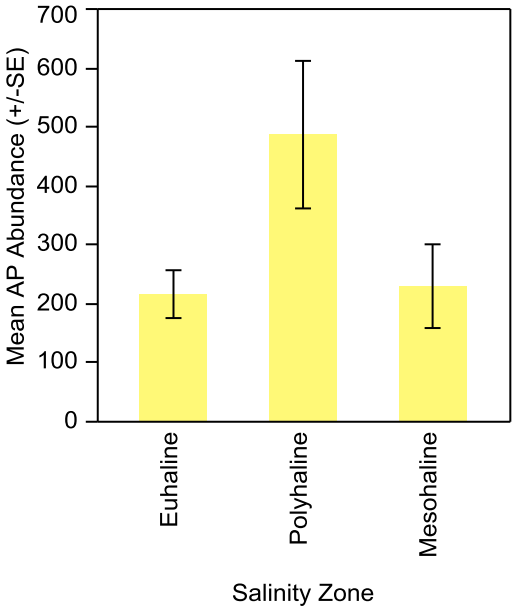

Figure 73: AP mean abundance by salinity zone per 0.5m^2 in the Detailed Assessment

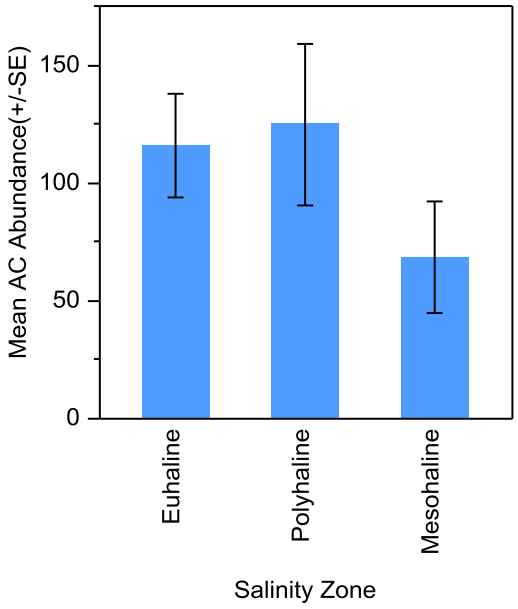

Figure 74: AC mean abundance by salinity zone per 0.5m^2 in the Detailed Assessment

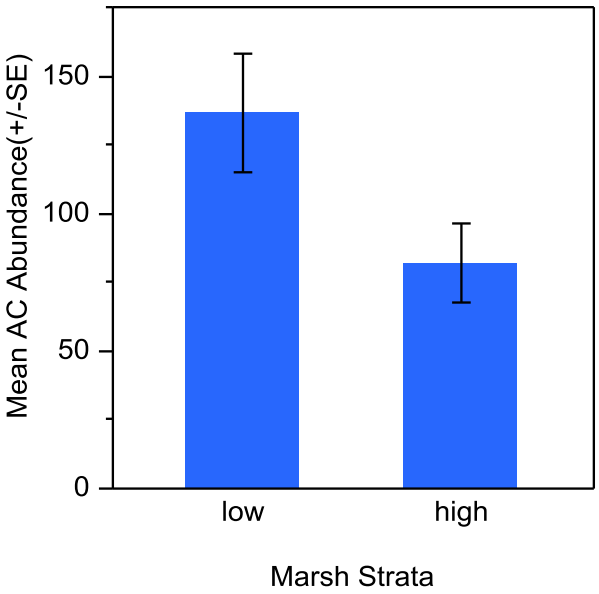

Figure 75: AC mean abundance by marsh strata per 0.5m^2 in the Detailed Assessment

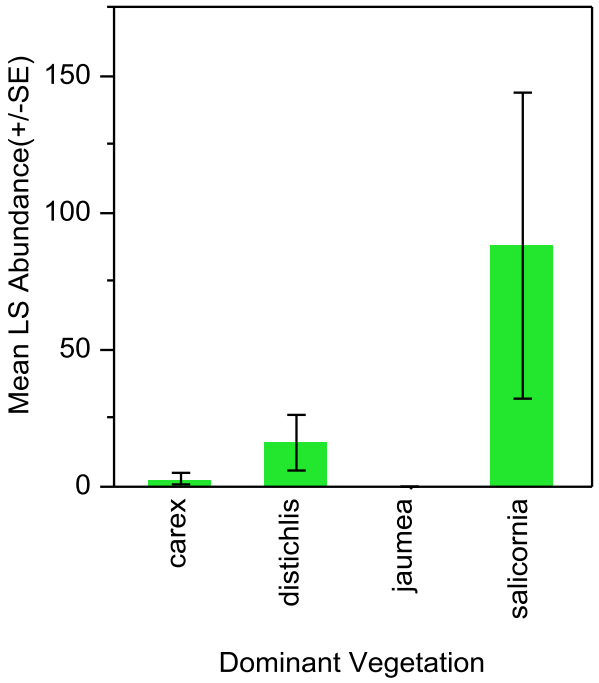

Figure 76: LS mean abundance by dominant vegetation per 0.5m^2 in the Detailed Assessment

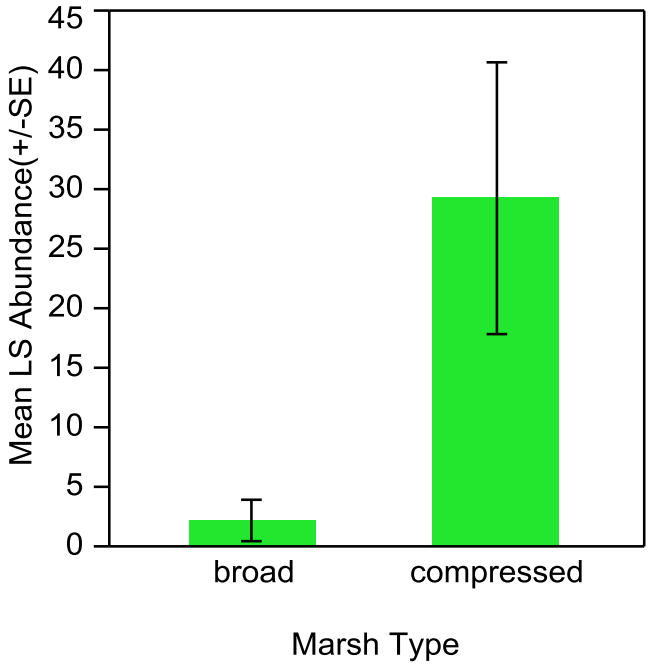

Figure 77: LS mean abundance by salinity zone per 0.5m^2 in the Detailed Assessment

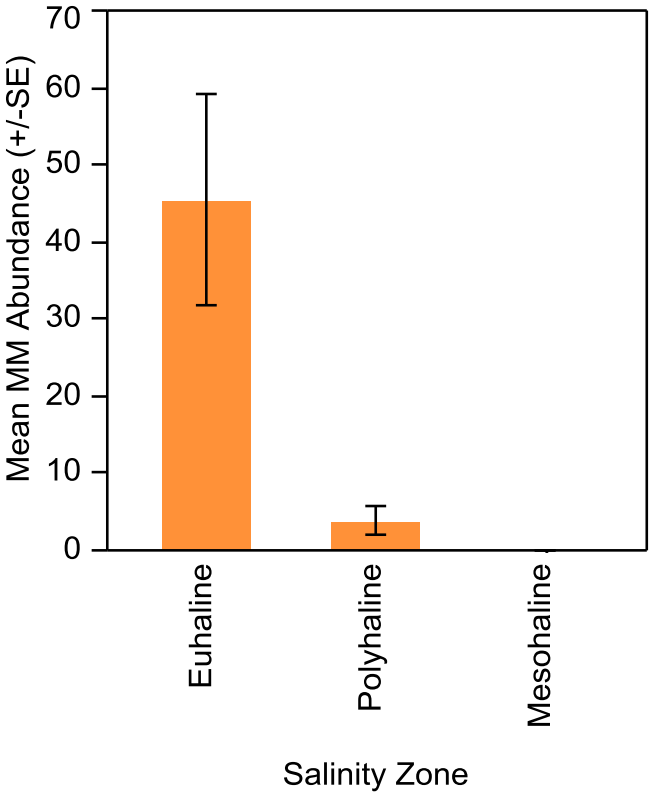

Figure 78: MM mean abundance by salinity zone per 0.5m^2 in the Detailed Assessment

2.5 Discussion

2.5.1 Habitat Characterizations

The survey designed to assess the extent of colonization of AP within the Coos Bay estuary was based on an analysis of 311 Rapid Assessment quadrats and 100 Detailed Assessment quadrats. In the Rapid Assessment, the full extent of the bay was covered by foot or by paddling along various waterways. In this initial assessment it was apparent that all regions assigned were very different in their environmental and habitat structure. The Rapid Assessment Method (RAM) enabled us to collect qualitative data about the habitat and biota, and visually map using ArcGIS to show distributional patterns. The Detailed Assessment Method (DAM) allowed us to quantify abundance and further examine mechanisms of distribution.

The environmental and or habitat signature was unique to each region in the Rapid Assessment. This uniqueness was not due to one environmental factor but a combination of variables. Due to the large spatial assignment of region, each region consisted of several salinity zones. The spatial layout of salinity zones followed the classic salinity stratification along an estuarine gradient. Although individual waterways and regions had multiple salinity zones, each region had a dominant zone, which may have been the main component to the unique signature of each region. In the Detailed Assessment, sites were chosen based on AP abundance and therefore each site had one averaged salinity measurement across the polygon. There were sites that fell into the same salinity zone: euhaline (Haynes West, Kentuck Inlet, and Valino Island), polyhaline (Haynes East, Isthmus Slough, and Hinch Bridge), and mesohaline (Coos River North).

The West, South, and North estuary regions of the RAM included both broad and compressed marshes. Often marsh types were specific to the waterway within a region. The Central region was dominated by large broad marshes with many tidal channels whereas the East region was dominated by compressed marshes along the shores of linear waterways. The compressed marshes within Coos Bay are often due anthropogenic

impacts; the construction of roads, dikes, levees and tidal gates. In the Detailed Assessment, each sites' marsh type was determined. Four sites had compressed marshes: Haynes West, Hinch Bridge, Valino Island and Coos River North. Three sites had broad marshes: Haynes East, Kentuck Inlet and Isthmus Slough.

All regions (RAM) and sites (DAM) had a range of marsh strata and dominant vegetation. Both marsh strata and vegetation vary within an individual marsh. The salt marshes of Coos Bay have distinct topographic features including tidal channels, large rises and low shallow pools. As the marsh topography changes so do the salt marsh plants. The plant composition of salt marshes of the west coast of North America is more heterogeneous than the marshes of the east coast. The Detailed Assessment included a much finer spatial analysis at the site level and revealed the same pattern of heterogeneous strata and vegetation composition within a marsh. This within-marsh variation makes across-region or across-site comparisons difficult due to the inherent variation.

Regions were randomly spatially assigned to the Coos Estuary as a tool for biological assessment. According to the data there is a statistical difference in the environmental signature of each region, this difference is not driven by one environmental factor but a matrix of factors. The combination of these factors generates a grouping of the sites into regions that allows us to determine they are significantly different from one another. Given that, when one examines the sites within a region we see a difference between sites as well. We would suggest that each site or waterway and even each marsh is unique in its environmental makeup. Although grouping is statistically viable, habitat does vary across a marsh, site and region.

The species-specific snail distribution, relative abundance and abundance patterns reflect this variation in habitat type. Our correlations of specific habitat types with specific species strongly suggest that AP, AC, LS and MM distribution patterns are dictated by species-specific responses to environmental factors. Of all the factors measured in this

study, salinity appears to be the dominant factor influencing the distribution of species in the Coos Estuary Snail Guild.

2.5.2 Rapid & Detailed Assessments

This survey employed a two-tiered sampling strategy, utilizing one method to design the second method. Relative abundance data is not an ideal method for analyzing abundances of organisms; organism numeration is the chosen methodology. However, measuring the relative abundance of organisms enabled us to cover a large area and map distribution of the Snail Guild species. These initial data were utilized to make design choices for the Detailed Assessment. During the Detailed assessment, the Rapid assessment method of determining relative abundance was also conducted. Doing both methods on a quadrat allowed us to examine our accuracy in determining relative abundance. Figure 79 highlights the difference in relative abundance classification versus enumeration of *A. parasitologica*. The figure illustrates that we often underestimated the abundance of AP. Due to this inaccuracy and the absolute confidence one can place on the enumeration method we placed more importance on the detailed data in the species-specific distributions. The distribution, abundance and potential mechanisms of distributions will be discussed on a species level and then as guild.

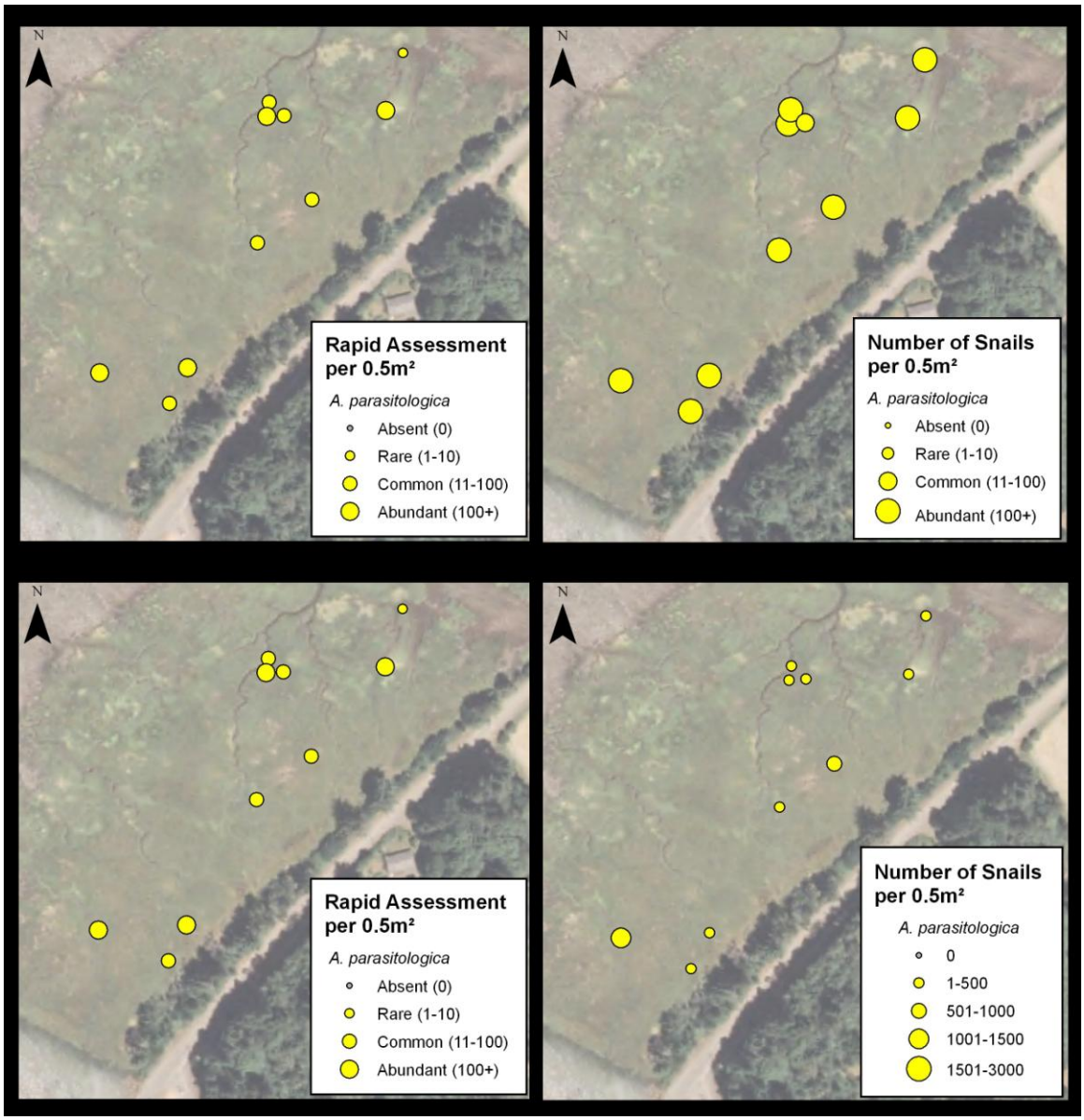

Figure 79: Comparison of the RAM and DAM results at the Detailed Assessment points of Haynes East. The top panel pair shows the Rapid Assessment and Snail Counts with an identical classification scale. The bottom panels show Rapid Assessment and Snail Counts with differing classifications to show a more specific representation of actual population.

AP was present in all sampled regions of the Coos Bay Estuary and in all sites except for Joney Slough. The relative abundance of AP was significantly different by region, salinity zone, marsh type and dominant vegetation. AP was most abundant in the South region with highest relative abundance at Isthmus Slough, a mesohaline dominated site.

Marsh topography was important on the larger scale with AP more abundant in broad marshes but did not appear to affect the distribution on the marsh strata level. AP was found in several types of vegetation, both of high and low marsh classification.

In the Detailed Assessment, AP was the most abundant Guild Snail in Coos Bay. AP was most abundant at the Isthmus Slough site with a mean density of 500 snails per $0.5m^2$. AP was significantly more abundant at the polyhaline sites, which were Haynes East and Isthmus Slough. Although AP did differ significantly by marsh strata there were more present in low marsh plots, approximately 300 snails in low plots versus 200 in high plots. AP was found in high abundance in all types of vegetation, AP can inhabit both high marsh plants such as *Carex spp.* and low marsh plants such as *Salicornia virginica*.

AP was found in all regions and all dedicated detailed sites. Both assessments revealed similar results and patterns about the distribution of AP. This new invader was found in highest concentration at the Isthmus Slough than any other area of Coos Bay and in polyhaline dominated waters but also persisted in a wide range of salinity regimes. AP was found across marsh types, marsh strata and vegetation.

Although AC was present in all regions and sites sampled within Coos Bay, the distribution of AC was significantly different by region, salinity zone, marsh type and dominant vegetation. AC was most abundant in the Central and South region in areas dominated by polyhaline waters. AC showed similar environmental correlated distribution patterns to AP, found in higher abundance in broad marshes and in several types of vegetation.

In the Detailed Assessment, AC was the second most abundant snail at all sites (except for Valino Island, where it was dominant). Maximum mean density of AC was at Haynes West with approximately 250 snails per $0.5m^2$. AC was the most abundant species at the Valino Island site, with a density of approximately 75 snails per $0.5m^2$. AC was found in higher abundance in the polyhaline zone, which included the Isthmus Slough and Haynes East sites. AC was more abundant in the low marsh plots than high marsh plots, which is

in accord with the distribution of AP by marsh strata. AC showed an even distribution with marsh type and vegetation.

AC was found in all regions and all dedicated detailed sites. The Rapid and Detailed assessments showed different patterns of AC distribution by site within the bay. However AC's habitat association results revealed similar patterns for both assessments. AC is more concentrated in polyhaline zones, but can persist in other salinity regimes. AC was found across marsh types, marsh strata and vegetation regimes. The distributions of AC both spatially and by habitat are similar to that of AP.

LS was present in all regions sampled in the rapid assessment. LS was widely distributed across region, marsh type and vegetation. LS was significantly more abundant in the euhaline dominated areas and in low marsh quadrats. These findings suggest that LS prefers more saline environments than other members of the Coos Estuary Snail Guild.

In the Detailed Assessment, LS was found at Isthmus Slough, Haynes West and Valino Island. Maximum mean abundance was found at the Haynes West site, with approximately 100 snails per $0.5m^2$. LS were evenly distributed in abundance across salinity zones and marsh level. However, LS density was higher in *Salicornia virginica* dominated plots, which is considered to be a low elevation euhaline plant. LS were found in higher concentration in compressed marshes than broad marshes.

Results from the rapid and detailed assessments for LS were not consistent. LS showed an even and yet patchy distribution across regions and habitats. Results from both methods suggest that LS is more abundant in euhaline dominated areas, in lower marsh plots dominated by low marsh elevation plants. These findings on LS suggest that this mesogastropod inhabits a different niche than AP and AC.

MM was found in all regions sampled in the rapid assessment. MM was most relatively abundant in the central region and in the euhaline and polyhaline dominated areas of the

bay. MM was more abundant in the broad marshes and was found in several different types of vegetation.

In the Detailed Assessment, MM was found in all sites, except for Coos River North and Hinch Bridge in the detailed assessment. MM was most abundant in the Haynes East site, with a mean maximum density of approximately 150 snails per $0.5m^2$. MM was significantly more abundant in the euhaline zone than the polyhaline zone and was not present in the mesohaline zone. MM was found evenly distributed across marsh types, stratum and within various vegetation.

MM was found in all regions sampled within Coos Bay, but was not found in all sites during the detailed assessment. Both methods reveled that MM is more abundant in the euhaline and polyhaline zones. MM had an even distribution across all other habitat characteristics.

According to the data generated from this study, AP is by an order of magnitude the most abundant species of the Snail Guild in the Coos Bay system. AP shows preference for more brackish water but can tolerate a wide range of salinity regimes. AP and AC have similar abundances and distribution patterns across the bay and appear to be able to persist in a variety of habitats. LS and MM were found in much lower numbers than AP or AC across the bay. The data suggests that LS is more persistent in a euhaline type habitat, with low marsh topography and vegetation. MM although low in total numbers, has a wide breadth of distribution spatially and by habitat.

Although AP is found to be the most abundant Guild Snail in the Coos Bay estuary it is important to consider the objectives and experimental design of the study. Sites for the Detailed Assessment were chosen from areas where AP was abundant. We did collect and analyze data on each member of the Snail Guild. However, these snails were collected from sites that were bias towards high levels of AP. If this studies' Detailed Assessment sites were chosen based on high-density areas of LS we could have observed

LS as the dominant mesogastropod of the guild. In saying that, we did observe species-specific distribution patterns and habitat.

Upon examination of the data by any factor, there is a great deal of variation in abundance between plots for all species. We would suggest that this variation between plots is likely due to the microhabitat from which snails are collected. Within a $0.5m^2$ plot there are great changes in the microhabitat: depressions, hills, pockets of shade and or moisture, changes in vegetation. We randomly allotted the sub-quadrat from which the snails were collected from and then extrapolated up to the 0.5m2 quadrat to generate abundance levels. We did not take microhabitat data from which these snails were collected; however we did qualitatively note the habitat. We observed snails clustered in large aggregations in depressions, or at the base of vegetation. These aggregations were variable and appeared to coincide with current temperature; aggregated in shade or pockets of moisture when the temperature was high and not when the temperature was cool. Although, this qualitative data (impressions on the minds of the field biologists) is not rigorous it poses a strong argument for examining microhabitat when comparing abundance levels and habitat distribution of these mesogastropods. A change in microhabitat for a 4mm snail might be of great importance and we would suggest examination in future studies.

2.5.3 Conclusion

This is the first study to collect baseline data on the abundance and species-specific habitat correlations of these Snail Guild species. Therefore assessing or determining change in the existing populations of AC, LS and MM was not possible. Although there are biases in the design toward the collection of AP and high variation between plots the pattern is still clear that AP is abundant in the Coos Bay estuary. Given the high densities and range of habitats that AP can inhabit there is striking potential for AP to move into new areas of the estuary and possibly displace these existing mesogastropod species. The data from this survey strongly suggest that: AP could displace AC as it persists and is successful in similar habitats and AP could displace LS and MM as these species populations are low in comparison to AP.

3. Seasonal Reproduction of *Assiminea parasitologica* in Coos Bay, Oregon

3.1 Introduction

Assiminea parasitologica (AP) is native to Japan and was first documented in North America in the Coos Bay estuary in June 2007. At the onset of the invasion very little was known about the reproductive biology of *A. parasitologica* in its native country. Upon finding AP in Oregon, specimens were brought into the laboratory for observations; AP was observed to lay egg capsules in the sediment from which planktonic larvae hatched out as veligers (James T. Carlton, pers. comm., 2008). Until recently only two of the Japanese Assimineids reproductive biology had been examined, *Assiminea hiradoensis* and *Angustassiminea castane*a both of which have planktonic larvae. The well studied European Assimineid. *Assiminea grayana* also has planktonic development. In contrast, the native mesogastropods *Angustassiminea californica* and *Littorina subrotundata* have direct development.

Questions about *A. parasitologica's* reproductive biology arose quickly after the new invasive was found. At the onset of the project we knew the following about AP's reproductive biology: the organism is dioecious with internal fertilization with copulation, they have sexual dimorphism (females larger than males), mating pairs seemed to be synchronous in the field, the gonad is in the spire of the organism, they lay egg capsules in the surface of the sediment and planktonic larvae hatch out at the veliger stage. Given that we had a basic understanding of the organism and were limited on time, our objectives for this module were rather simplistic.

Our primary goal was to gain any further information, either qualitative or quantitative about the reproductive strategy of AP. We examined the gametogenic activity of select populations of AP within Coos Bay to investigate the seasonality of this organism's reproductive cycle. We were curious to know if the gametogenic activity of AP was synchronous across the Coos Bay population. When gametogenic activity is synchronous, the population experiences three stages: "a resting stage, a period of active

gametogenesis, and a period when the gonad contains fertilizable or apparently mature gametes" (Webber, 1977). For the purpose of experimental design we applied these stages to all samples to ascertain if the population was synchronous. We also noted microhabitat and environmental variables to assess variation among populations. Between June and November of 2009 we monitored reproductive characteristics to investigate the reproductive cycle of AP in Coos Bay.

3.2 Methods

As this was a new endeavor, the experimental design went through several evolutions and iterations before settling into a sampling regime. We include our iterations to better educate the reader as to why we made the following changes. Preliminary work was started in late March 2009 with several discussions of sampling and dissections methods. Available literature was consulted as well as notes from Dr. James T. Carlton's 2008 expedition. The native snail *Angustassiminea californica* was found to be a very good model for the internal anatomy of *Assiminea parasitologica* (Fowler, 1980).

Reproductive sampling commenced on June 1 and continued through November 2009. Snails were collected during periods of low water every seven days throughout the study. Initially, we collected 30 snails each from one randomly chosen location at Haynes Inlet, Isthmus Slough, Cooston Marsh and South Slough (Figure 80. At each of these sites' sampling locations we characterized the habitat; noting the shade level, dominate vegetation, substrate and the presence of algal wrack. Upon examination of the data in late June, we decided to remove our Cooston Marsh and South Slough sites and focus on Isthmus Slough and Haynes Inlet. Due to time constraints, we needed to decrease our number of sites in order to increase our replication at the focused sites, therefore enabling us to examine the reproductive variability of sampled areas within a site.

Figure 80: Location of the original four sampling points for the Reproductive Module

At Isthmus Slough and Haynes Inlet we assigned three random locations from which to sample every seven days. These sampling locations are shown in Figure 81. At each sampling location the first 30 snails encountered were collected, placed into labeled Whirl-Pak bags and stored on ice until examination. Any notable changes in snail behavior such as male-female pairing or presence of young of the year were recorded. Air and sediment (~1 cm depth) temperature were measured and changes in habitat such as drying sediment and flotsam accumulation were observed and recorded.

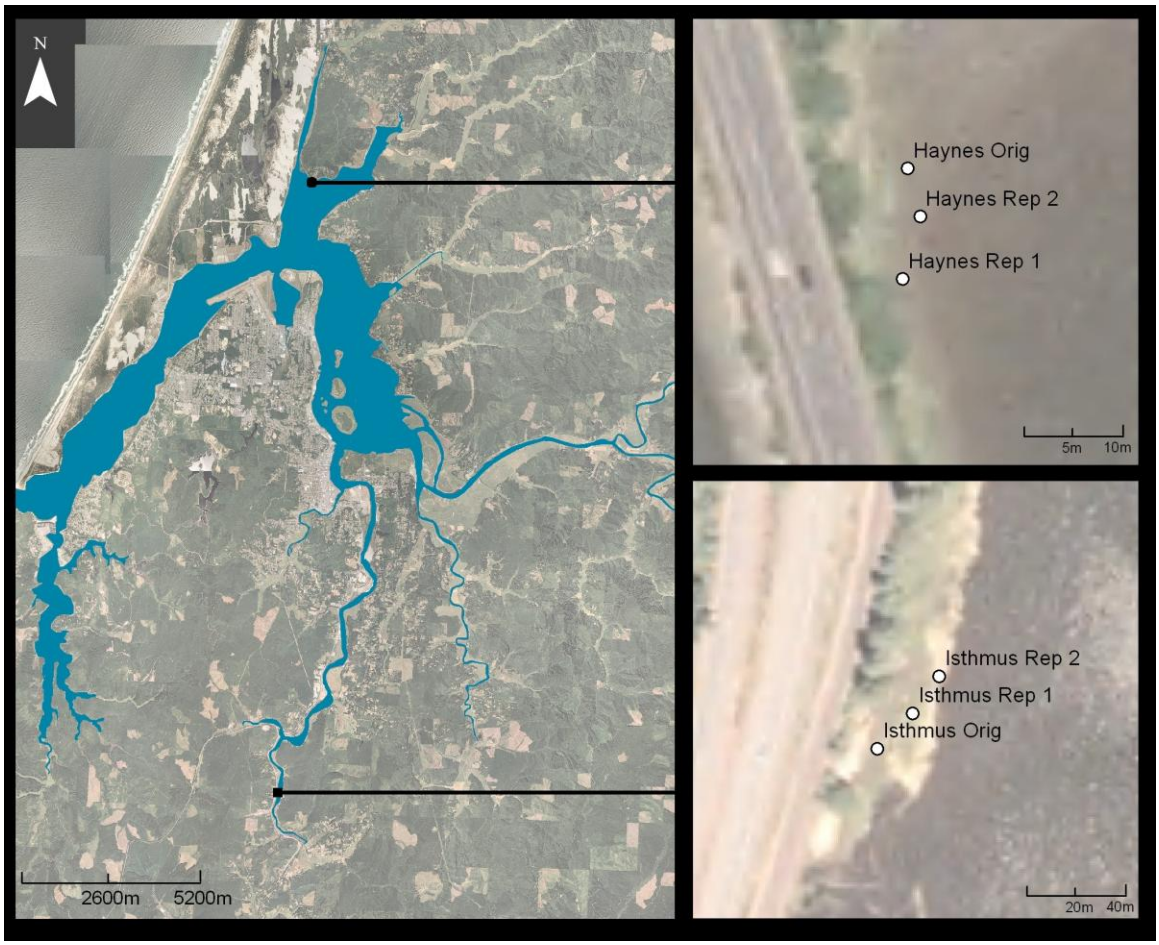

Figure 81: Location of the Haynes and Isthmus Reproduction sampling sites

The collected snails were brought back to the lab for immediate staging or frozen for later staging. Snails for staging were cleaned of mud, counted, positively identified as AP, and the total from each sampling area was weighed to the nearest 0.001 grams. Snail height was measured to the nearest 0.1 mm with digital calipers and/or sorted into 1 to 5 mm bins. Very small young of the year snails were generally not included in the samples as observations showed them to be consistently immature.

For dissection, each individual snail was placed between two glass slides with a drop of sea-water and firmly pressed with fingers until the shell just cracked. The top slide was then removed and the snail's body was extracted from the broken shell with fine forceps and a needle probe under 20x stereoscope magnification. The organism was sexed and staged.

Females were staged with the guidelines of the percentage of the ovary volume estimated for females and un-encapsulated, spherical, nucleate eggs counted when present. Irregular nucleate white "soft" eggs found when the ovary is torn were difficult to count as they quickly swelled then ruptured when exposed to the mounting water. This was especially true with distilled water wet mounts but swelling was slowed significantly with sea water mounts. A few encapsulated eggs were occasionally observed and assumed to be fertilized and ready for release.

Female reproductive stages were limited to 3 broad stages, "ripe", "active" and "spent" to facilitate rapid processing of large numbers of snails. Through the process of dissection and much hard work we defined and described the categories of "ripe", "active" and "spent" as follows:

Ripe: Spherical "hard" nucleate, unencapsulated eggs present (40 µm diameter), often abundant (=>10 count) spilling out of body when the shell is cracked (Figure 82). Full to nearly empty ovary (10-90+ %) with the coloration of ovary contents less white and less milky than the Active stage. Abundant "soft" nucleate eggs are often present if the ovary is torn (Figure 82). Snails with extremely reduced ovaries were occasionally observed producing enough spherical eggs and were considered Ripe (Figure 83).

Active: Transitional stage between Spent and Ripe. Ovary (10%-100%) with white areas often in distinctly lobed follicles when less than 60% full, usually milky when pressed or torn. Usually no spherical eggs, often a few soft eggs if ovary torn (Figure 84).

Spent: Ovary clearly depleted (<5-10%) often orange to red follicles in large individuals and no white gamete material. Spherical eggs can be present but few (<10 count) and no soft eggs present (Figure 85).

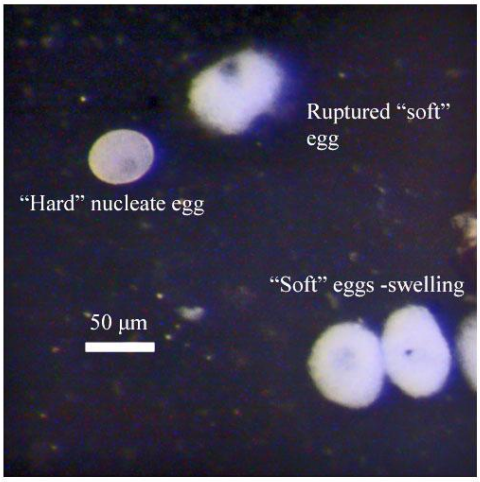

Figure 82: Two egg types observed during AP dissection

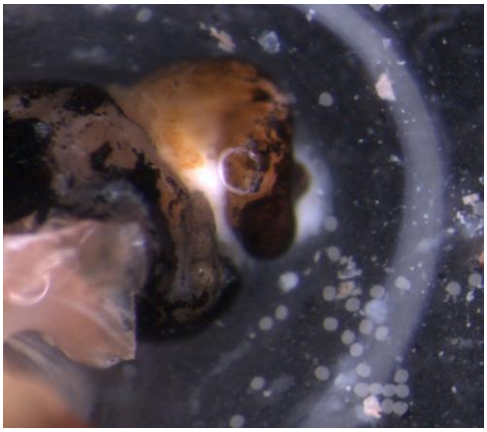

Figure 83: Ripe stage - Female: Abundant spherical eggs and some milky material leaking from torn ovary

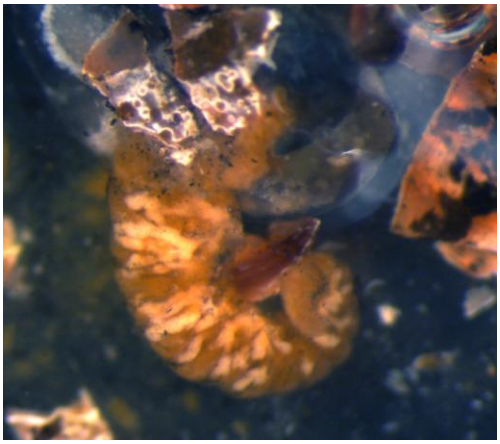

Figure 84: Active stage - Female: Partially filled white follicles in ovary and no eggs

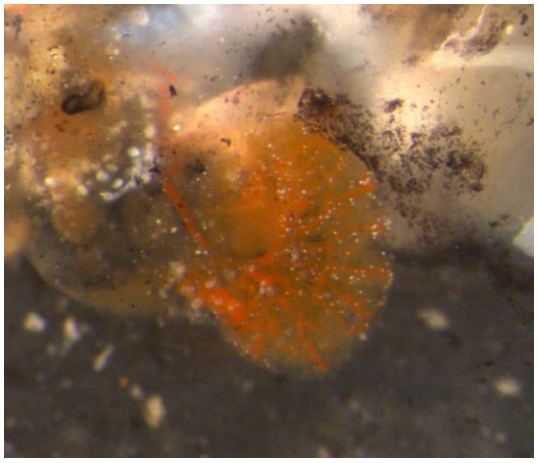

Figure 85: Spent stage - Female: No white material, often with red or orange follicles in ovary.

Male staging was less clear than the female stages and may not be important for this study. Males can be assumed to be active when females are receptive. The potential for long-term sperm storage in the females may also be a complicating factor. In the males the testis is rather variable in coloration but generally ranges from pale yellow to a brownish orange to a bright fluorescent orange with amber refringent material. The folded seminal vesicle portion of the vas deferens near the testis is filled with a bright white sperm filled fluid when ripe and tends to shrink and darken to a brownish-orange coloration when emptied (Figure 86). Individual sperm observed at high magnification are very similar in appearance to those of *Angustassiminea californica* (Fowler 1980) and are easily detected by the rapid motion of their tails.

Figure 86: Dissection of AP male with exposed testis.

Reproductive staging data were hand-written on printed data sheets and then entered into an Excel database. Periodic analysis of accumulated data was used to gauge the effectiveness of sample frequency, size, and replicates. These analyses lead to the elimination of two sample sites (South Slough and Cooston Marsh) and an increased focus on the Haynes and Isthmus slough sites with the addition of 2 replicates per site (Figure 81).

3.3 Results & Discussion

Habitat & Field Observations:

At each sites' sampling areas latitude and longitude were recorded and the salinity zone was defined. The habitat was characterized at the initial sampling period and changes in environmental conditions were noted on a weekly basis. Qualitative snail abundance and behavior was recorded at each site as well. Below the information is summarized by sampling area within site. A comparison of the two focused sites will follow the summary.

Haynes Inlet: Polyhaline

Location 1: (43 27' 0.248" N x 124 13' 28.224" W) Heavy vegetation, dominant species included *Triglochin maritmum* and *Salicornia virginica*. Abundant driftwood and algal wrack. Unshaded. Substrate, dark gritty mud. *Littorina subrotundata* and *Myosotella myosotis* very abundant.

Location 2: (43 25' 59.58" N x 124 13' 28.42" W) Approximately 16 meters south of Original site, parallel to Highway 101. Similar to the original site but almost completely shaded by a large willow tree. *Littorina subrotundata* and *Myosotella myosotis* very abundant.

Location 3: (43 26' 58.94" N x 124 13' 28.08"W) Approximately 16 meters south of Replicate 1, parallel to Highway 101. Very thin sediment over large rip-rap boulders. Unshaded. *Littorina subrotundata* and *Myosotella myosotis* very abundant but AP less abundant with AP becoming very scarce at the very end of the sampling season in November. Driftwood and algal wrack tended to accumulate and noticeably shift around between sampling weeks.

Isthmus Slough: Polyhaline

Location 1: (43 16' 15.710" N x 124 13' 38.603"W) Sparse vegetation, little tufts of short grasses *(Distichlis* spp.) but mostly a light colored, very sticky, sandy mud. Completely exposed, no shade, high intertidal. Mud completely dries and hardens during periods of low tidal exchange and warm, dry, summer weather. AP seems to endure considerable stress at this site and the snails are smaller and appear more pitted. During short dry periods AP clusters in shallow damp depressions and around the bases of the tufts of the short grasses. During extended dry periods, AP abandons the site and moves lower into heavier vegetation. Dead bleached shells were observed at this site but don't persist and are apparently washed away with the tide. This site is a well used access point for pigeon hunters and fishers. No other snail species noted.

Location 2: (43 16' 16.03" N x 124 13' 38.01" W) approximately 16 meters down-stream and 7 meters closer to the slough than the Location l site. Dense *Carex* spp. patches with some exposed dark mud patches. Abundant goose feces noted in July, which decayed slowly over many weeks and seemed to repel AP. Snails are larger and less pitted here than at Location 1. A few AC noted. No shade.

Location 3: (43 16' 16.31" N x 124 13' 37.64" W) Approximately 10 meters north from Location 2 on the very edge of the slough bank where the vegetation ends. Heavy claylike mud with isopod pits, *Carex spp* nearby. Occasional New Zealand Mud Snail (NZMS) *Potamopyrgus antipodarum* and AC observed. AP are less abundant than Location 2 and somewhat larger.

Site	Mean AP weight (g)	Mean AP shell height (mm)	Maximum AP shell height (mm)
Haynes (Location 1)	0.014	3.7	5.1
Haynes (Location 2)	0.012	Bin 3	Bin 4
Haynes (Location 3)	0.012	Bin 3	Bin 4
Isthmus (Location 1)	0.010	3.2	4.7
Isthmus (Location 2)	0.013	Bin 3	Bin 5
Isthmus (Location 3)	0.019	Bin 3	Bin 5
South Slough	0.018	3.9	5.5
Cooston Marsh	0.012	3.2	5.0

Table 3: Mean AP weight and shell height at all Coos Bay sample sites.

Additional Sites

Cooston: (43 23' 04.910" N x 124 10' 27.587" W**)** Grass dominated broad marsh with some driftwood and deep channels with abundant AP. Sampled only once (6/1/09)

South Slough: Mesohaline. Hinch Bridge area. Cooler average temperatures during sampling period when compared to all other sites during the same period. Large snails. Sampled 6/2/09-7/20/09**.**

Isthmus Slough and Haynes Inlet are both polyhaline sites with a range of habitat amongst its sampling areas. Haynes Inlet sampling locations were approximately spaced 16 meters from one another roughly parallel to the waterway. All three replicates had similar vegetation and LS and MM were noted as abundant. The location one site and location two had similar gritty mud substrate, whereas location three was an area of large boulder rip-rap. Location two was completely shaded compared to the un-shaded location one and three areas.

Isthmus Slough's sampling areas were more diverse than that of Haynes Inlet. The Isthmus Slough sites were closer in proximity to one another and were placed on a perpendicular orientation to the water-way. The location one site was the most distant from the water and exhibited classic high intertidal habitat: dry sandy substrate, tufts of vegetation and no algal wrack. The snails at this site appeared to be stressed, dry with extensive amounts of pitting. Location two and three were similar to one another; *Carex* spp. was the dominant vegetation, the substrate was muddy and the snails appeared to be healthier; less pitting and slightly larger.

AP Gametogenic Information

An analysis of all *Assiminea parasitologica* reproductive data in Coos Bay shows active egg production and male-female pairing in June-July with abundant (~0.5 mm) young of

the year (YOY) appearing in early August. Individual females were observed containing over 40 spherical nucleate eggs with an estimated 60% of the ovary contents still remaining for additional egg production. Based on this observation, females of 3.1-5.0 mm shell height could be expected to conservatively produce over 100 eggs during the summer spawning period. Occasionally, packets of densely packed nonmoving sperm were occasionally observed in females and are assumed to have been recently deposited by males. By September no male-female pairs were observed and ovary content was steadily declining through November. The average female in October seems to be absorbing the ovary contents instead of producing eggs but an occasional female will still produce up to 10 eggs. The ovaries of these late producing females are much reduced in size (~10-20% full) and result in few eggs, but hint at a continued low level of reproductive output throughout the year. The final samples on 11/23/09 showed some ovary recovery at most of the Haynes and Isthmus sites (Figure 87) but without continued sampling it is unclear if this was the beginning of a trend towards complete ovary recovery in the spring.

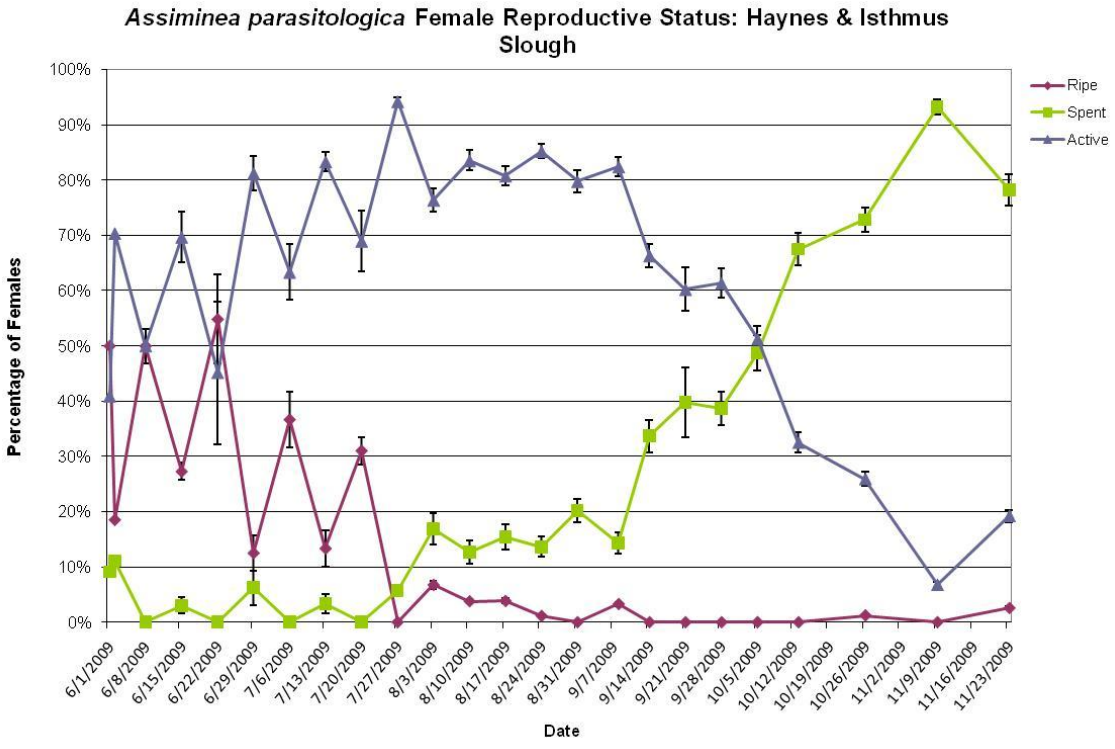

Figure 87: Haynes and Isthmus Slough overall reproductive status of *A. parasitologica* with standard error

At the un-shaded "Haynes Inlet Location 1" sampling site, female AP were spent approximately one month ahead of Location 2 and 3 starting in early September. This temporal variability nearly disappeared in November as AP became more synchronized between all Haynes Inlet reproductive sampling locations. A similar occurrence was observed at the "Isthmus Slough Location 1" sampling site. At the exposed Isthmus Slough Location 1 sample site, female AP were spent earlier than the more sheltered locations 2 and 3 (figure 89). AP is also smaller with extensive shell erosion and pitting at location 1. Despite the apparently negative effects of the high intertidal on the individual snails, the density of AP at Isthmus Slough location 1 appears to be remarkably high.

The Haynes Inlet and Isthmus Slough reproductive sampling sites are both considered polyhaline, but the presence of *Littorina subrotundata* at the Haynes Inlet sampling locations and *Potamopyrgus antipodarum* (NZMS) at the Isthmus Slough locations indicates that Haynes Inlet is a more marine influenced environment compared to the Isthmus Slough locations. Air and sediment temperatures were often much higher at the Isthmus Slough locations during the same sampling day.

Male-female ratios varied significantly between samples from 0-50% males to females. High numbers of males in the samples are correlated with high levels of male-female pairing and low numbers of males are correlated with an absence of pairing. Mature males are on average smaller (~2-3mm shell height) than mature females (~2.5-5mm shell height) and the low numbers of males in some samples are likely related to sampling bias towards the larger, easier to see females. With high levels of male-female pairing, each female collected will have a male attached, giving an average 1:1 male to female ratio. When all AP samples are combined and sorted into size classes, the male: female ratio approaches 1.0 for snails around 3.0 mm and smaller. The overall male: female ratio for all size classes is 0.20.

Shell Height	Male (N)	Female (N)	Male/Female
Bin 1 (under 2.0 mm)	13	8	1.63
Bin 2 (2.0-3.0 mm)	198	195	1.02
Bin 3 (3.1-4.0 mm)	144	917	0.16
Bin 4 (4.1-5.0 mm)	1	589	0.00
Bin 5 (greater than 5.0 mm)	0	29	0.00
All Bins	356	1738	0.20

Table 4: Male to female ratio by size class (bins) of all AP staged in Coos Bay

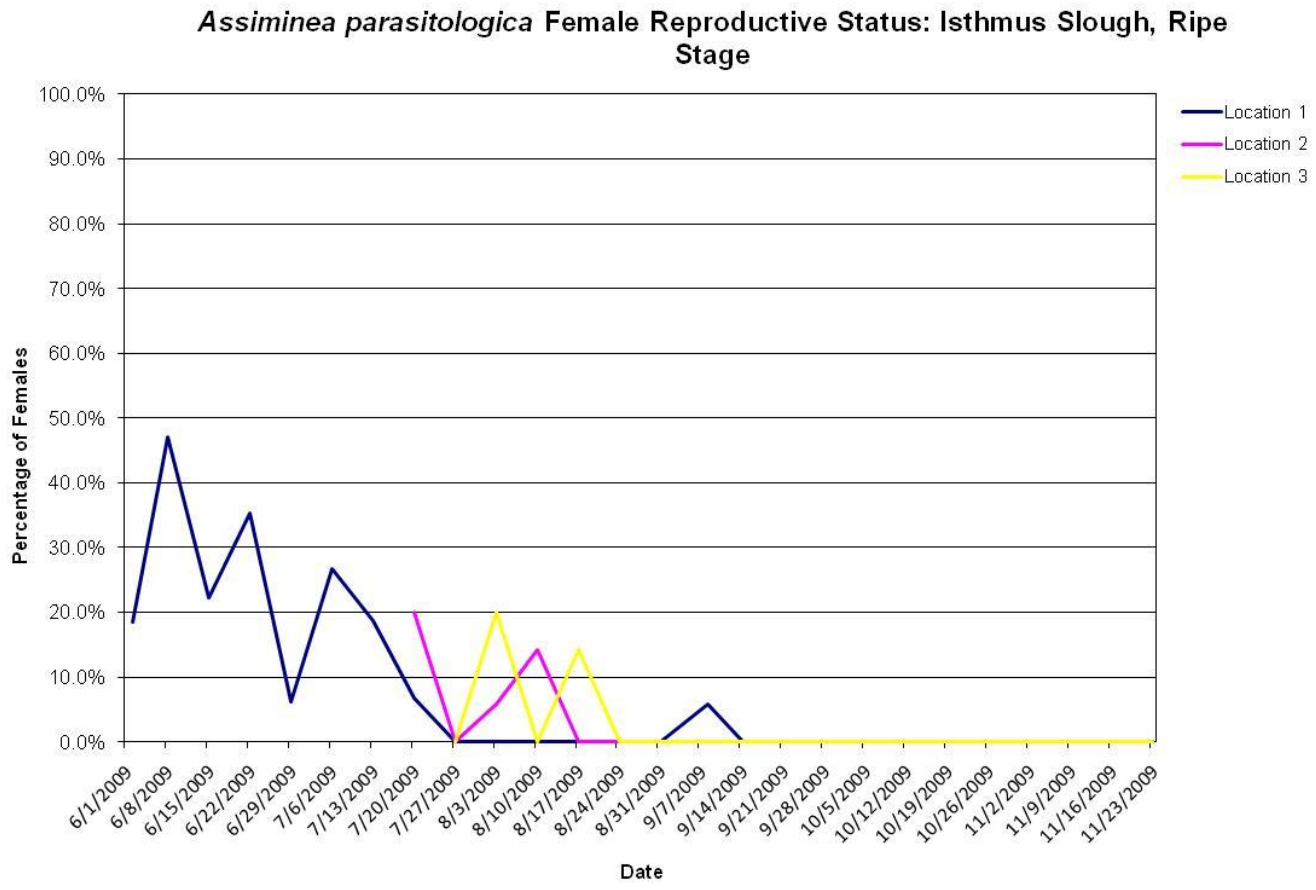

Figure 88: Comparison of Coos Bay, Isthmus Slough ripe stage between replicates

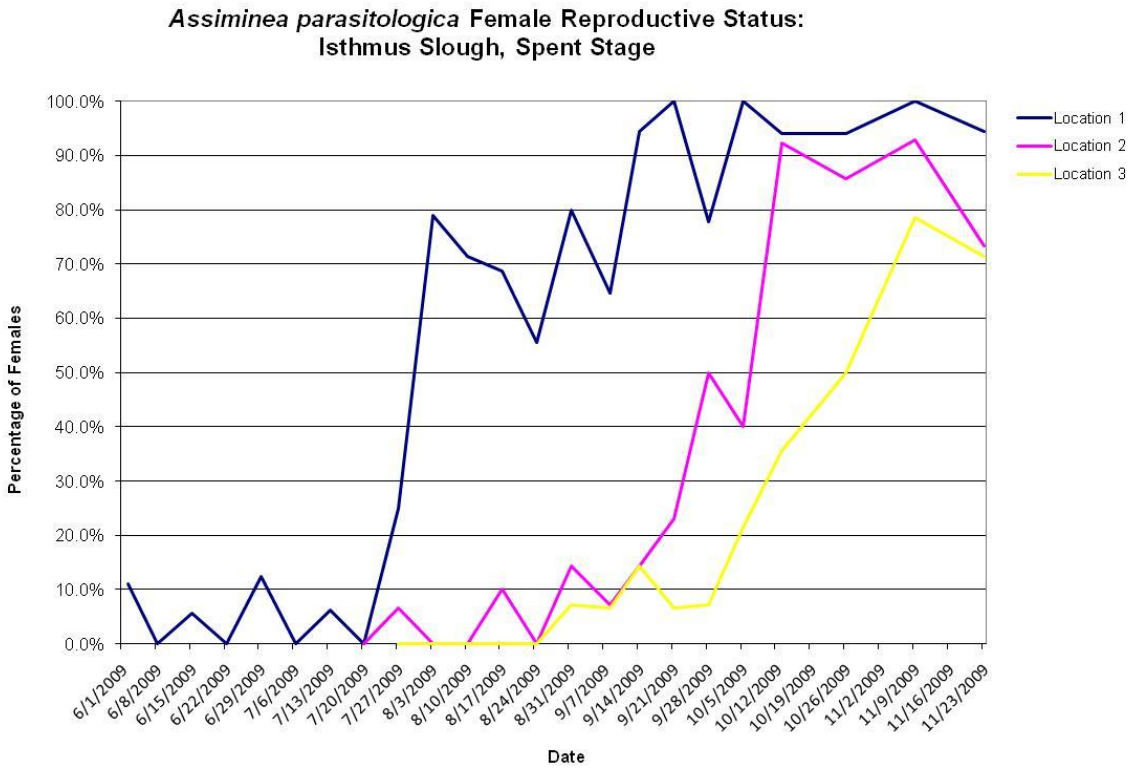

Figure 89: Comparison of Coos Bay, Isthmus Slough, spent stage between replicates

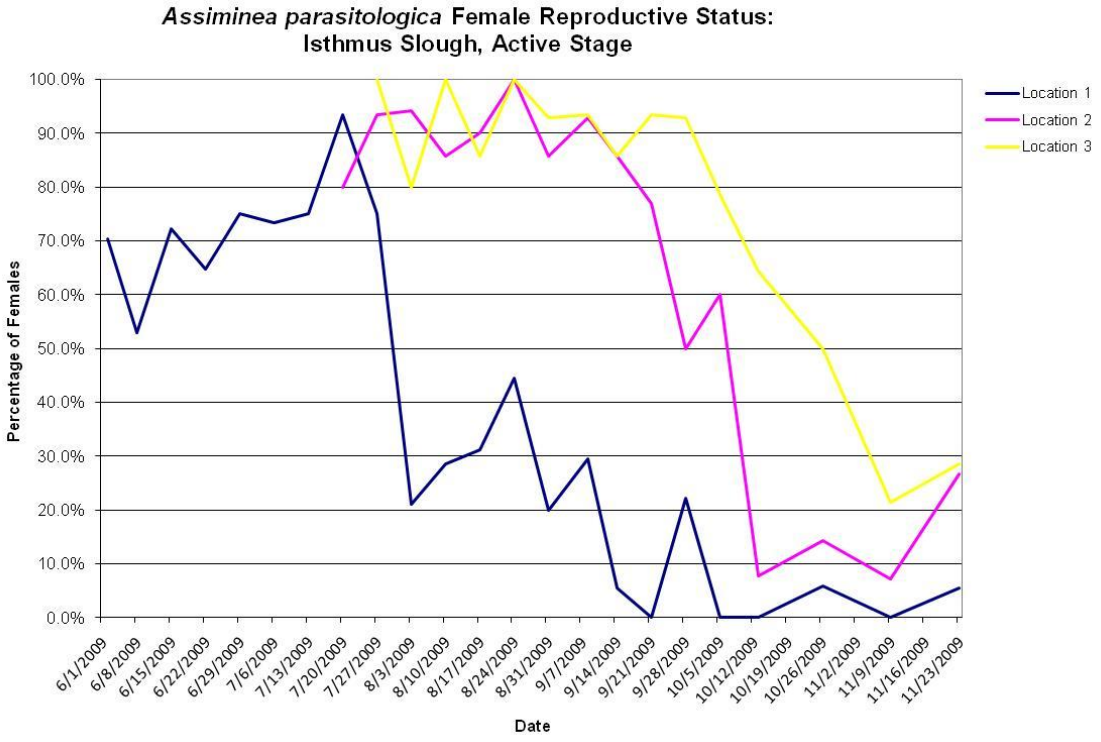

Figure 90: Comparison of Coos Bay, Isthmus Slough active stage between replicates

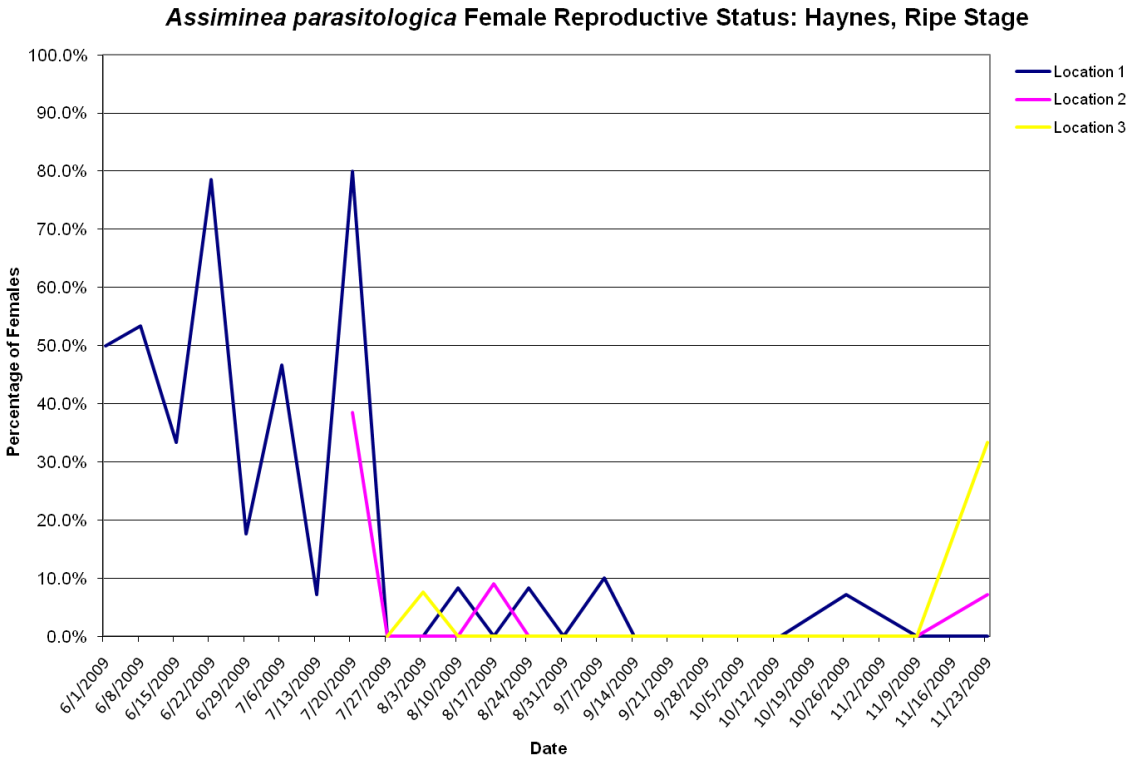

Figure 91: Comparison of Coos Bay, Haynes Inlet ripe stage between replicates

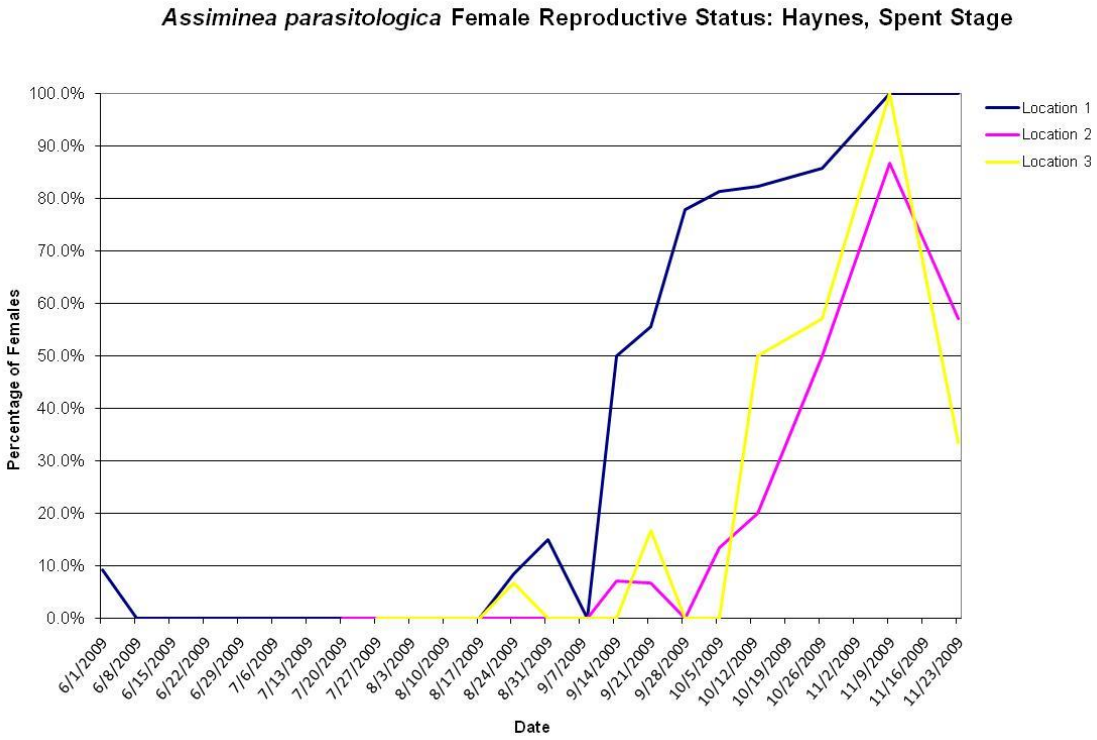

Figure 92: Comparison of Coos Bay, Haynes Inlet spent stage between replicates

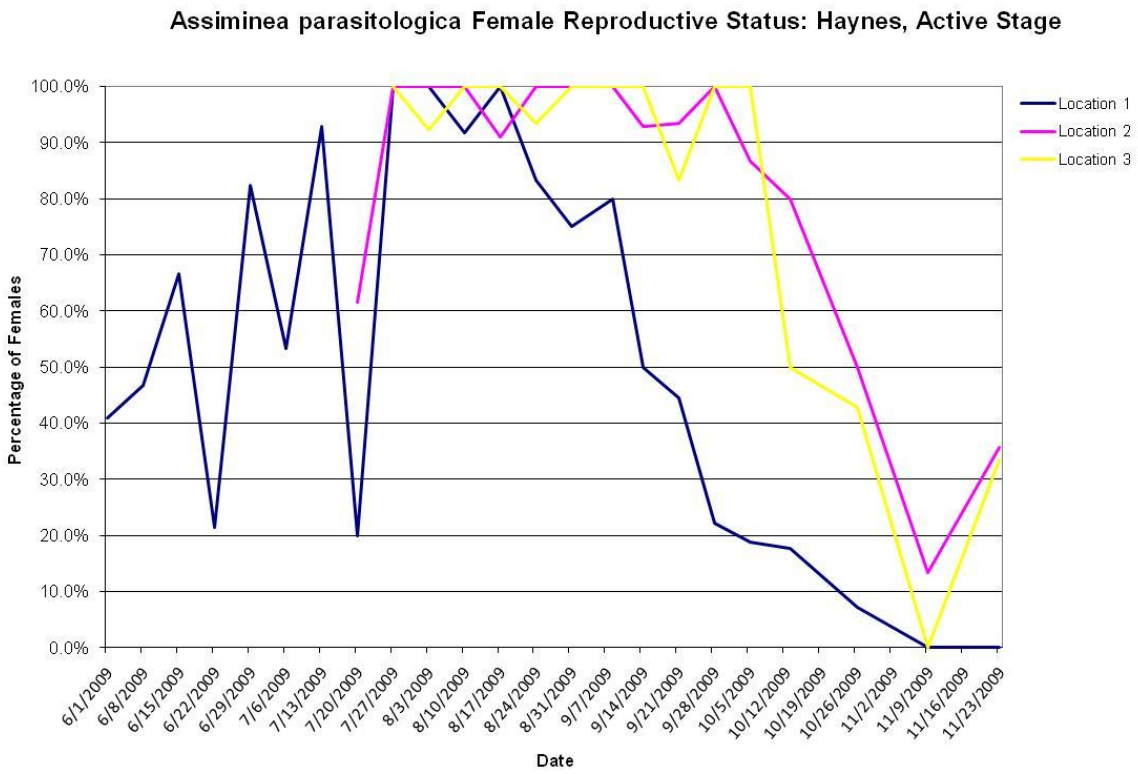

Figure 93: Comparison of Coos Bay, Haynes Inlet active stage between replicates

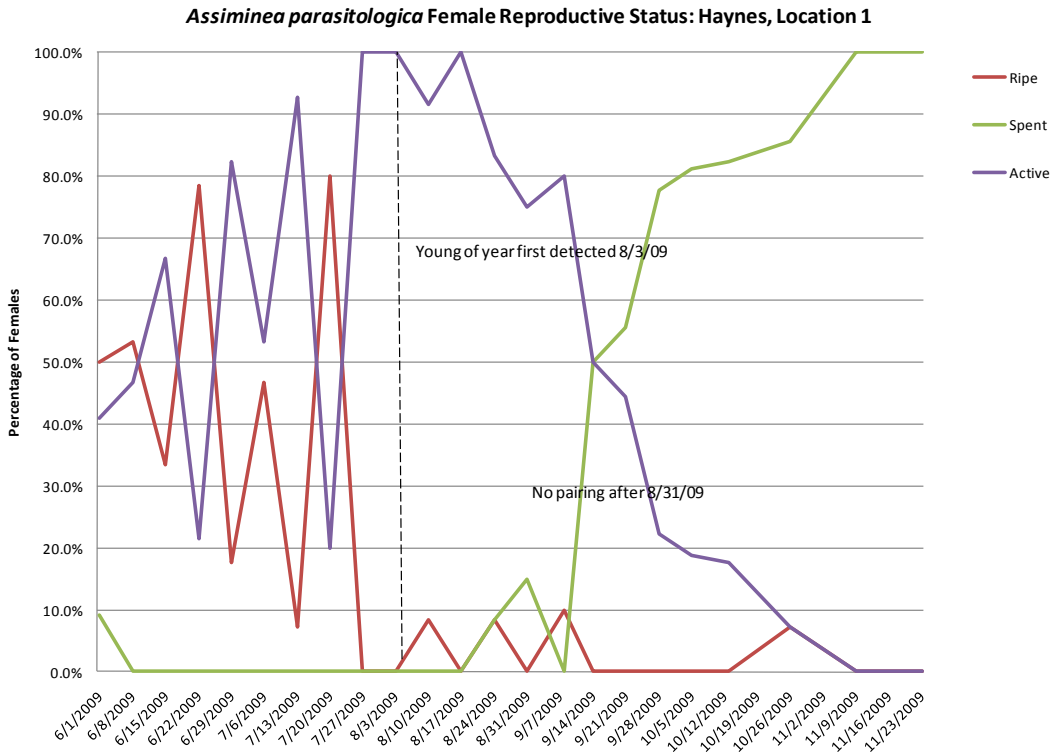

Figure 94: Reproductive status of AP, Coos Bay, Haynes Inlet location 1

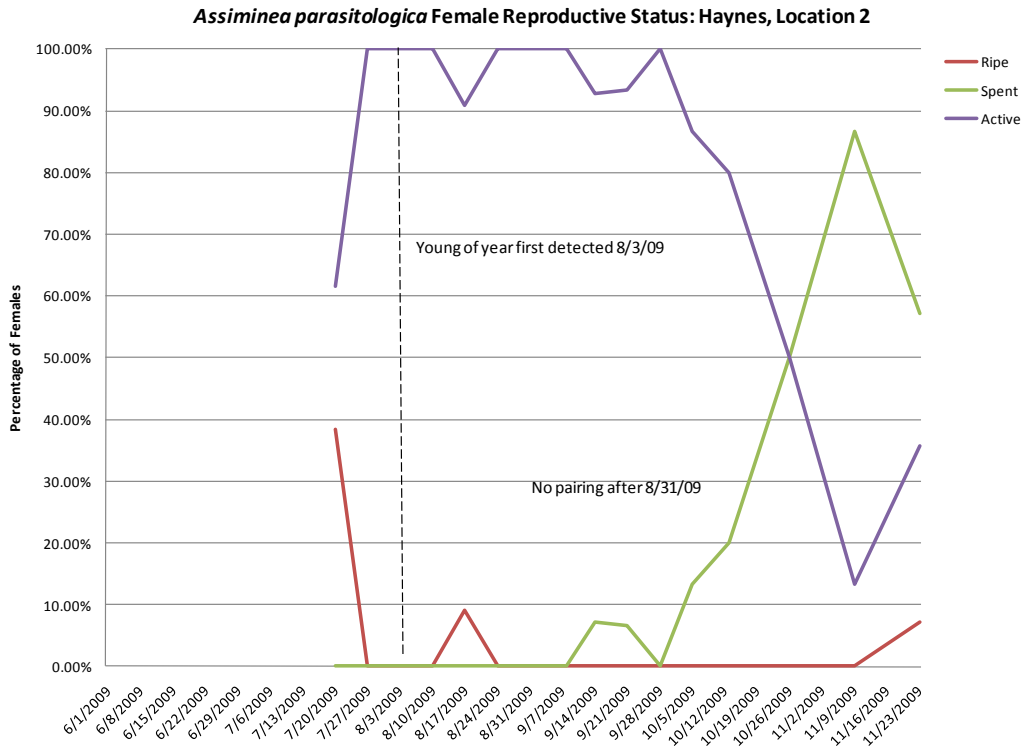

Figure 95: Reproductive status of AP, Coos Bay, Haynes Inlet location 2

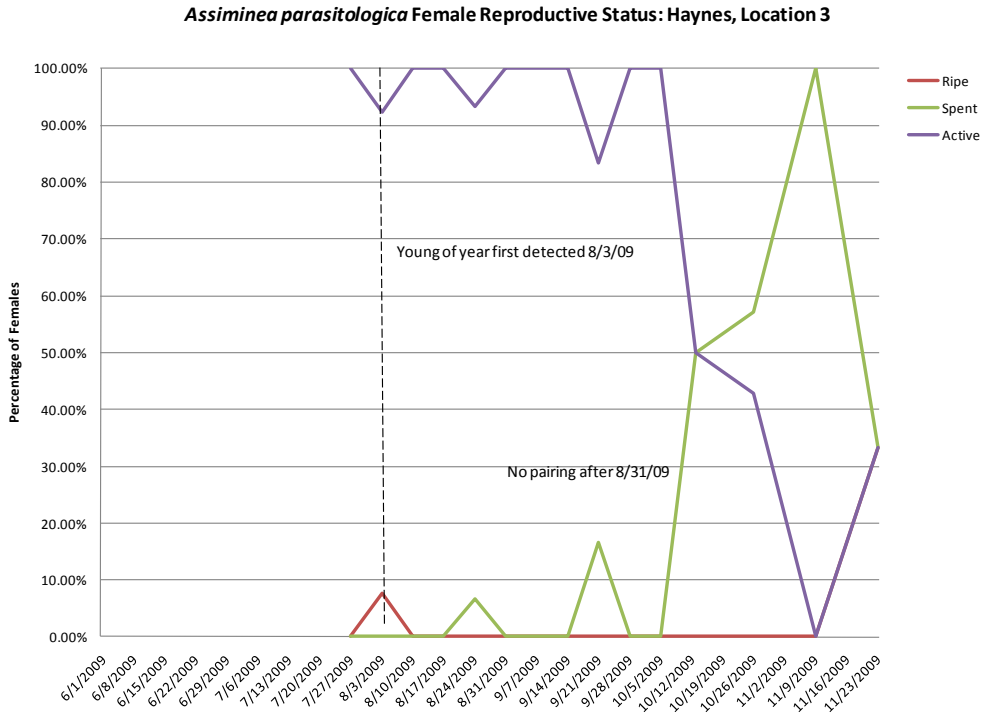

Figure 96: Reproductive status of AP, Coos Bay, Haynes Inlet location 3

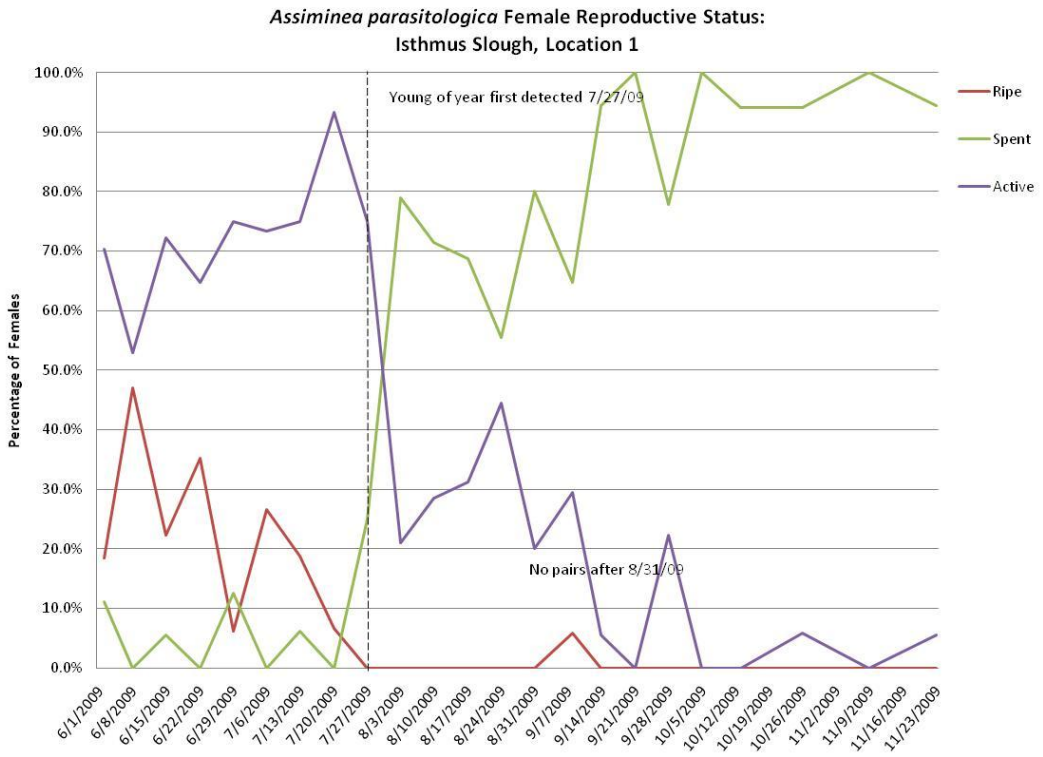

Figure 97: Reproductive status of AP, Coos Bay, Isthmus Slough location 1

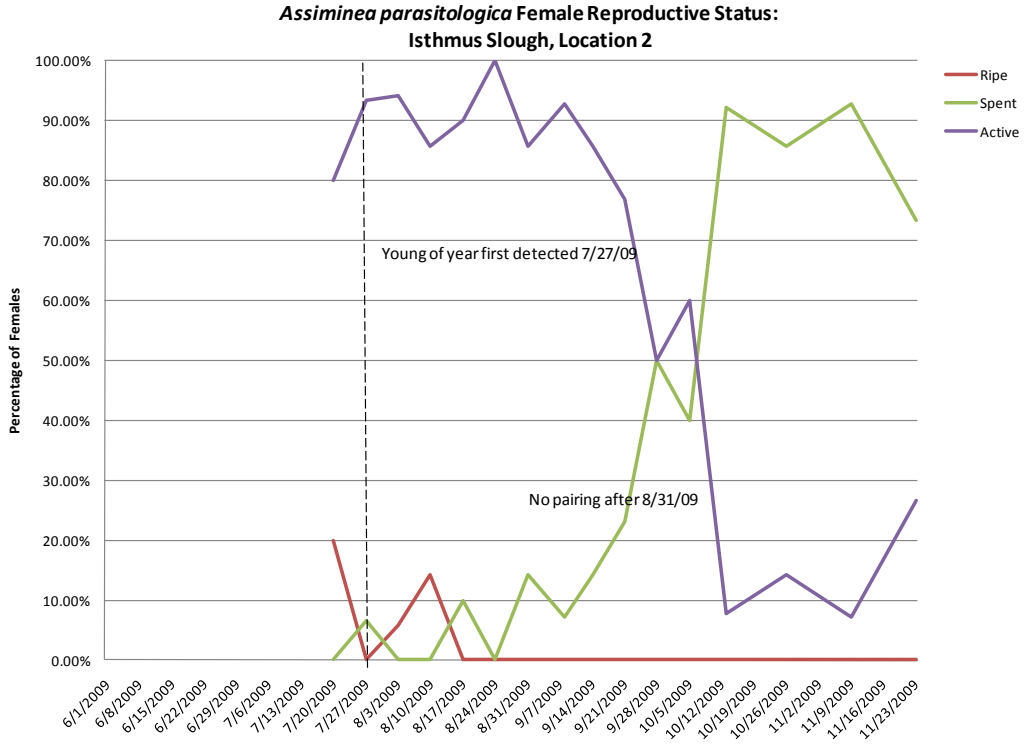

Figure 98: Reproductive status of AP, Coos Bay, Isthmus Slough location 2

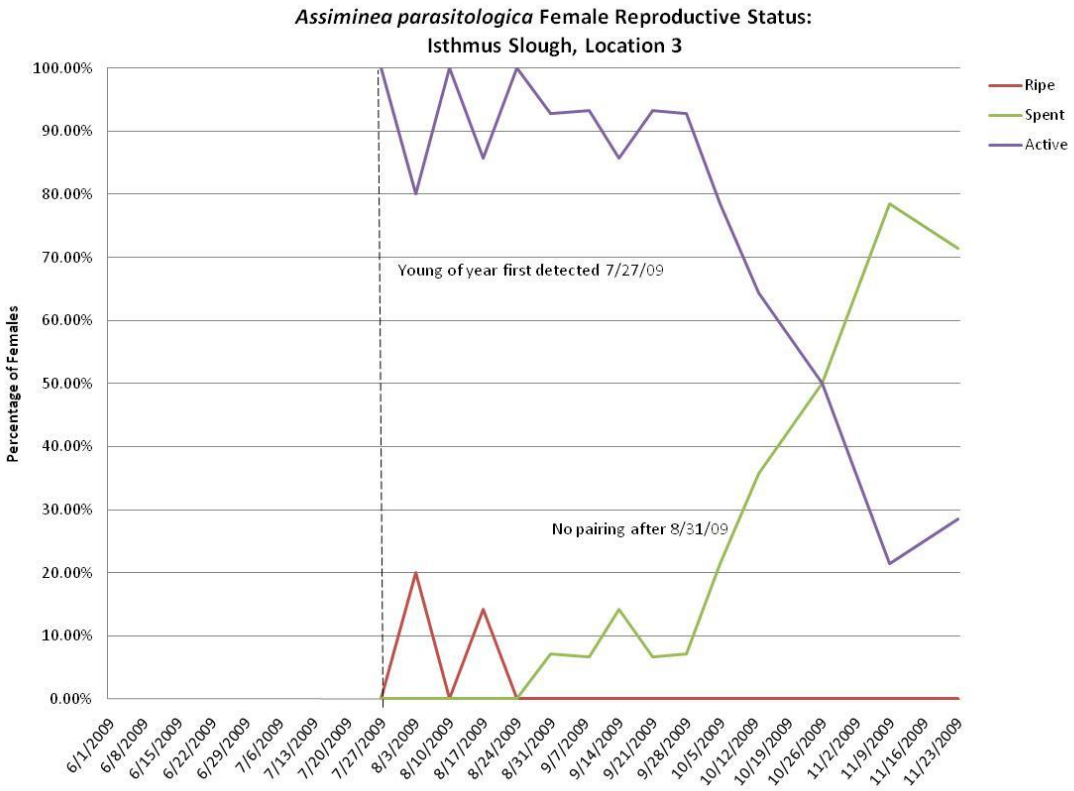

Figure 99: Reproductive status of AP, Coos Bay, Isthmus Slough location 3

Date	Cooston Marsh	South Slough	Haynes Loc 1	Haynes Loc 2	Haynes Loc3	Isthmus Loc 1	Isthmus Loc 2	Isthmus Loc 3	Subtotal
6/1/2009	20		22			27			69
6/2/2009		33							33
6/8/2009		14	15			17			46
6/15/2009		15	15			18			48
6/22/2009		14	14			17			45
6/29/2009		10	17			16			43
7/6/2009		17	15			15			47
7/13/2009		15	14			16			45
7/20/2009		24	10	13		15	20		82
7/27/2009			12	14	15	16	15	15	87
8/3/2009			10	10	13	19	17	20	89
8/10/2009			12	13	12	14	14	14	79
8/17/2009			14	11	13	16	14	14	82
8/24/2009			12	14	15	18	15	14	88
8/31/2009			20	13	15	15	14	14	91
9/8/2009			20	13	14	17	14	15	93
9/14/2009			18	14	14	18	14	14	92
9/21/2009			18	15	12	20	13	15	93
9/28/2009			9	15	6	18	14	14	76
10/5/2009			16	15	6	16	15	14	82
10/13/2009			17	10	10	17	13	14	81
10/26/2009			14	8	14	17	14	14	81
11/9/2009			16	15	11	18	14	14	88
11/23/2009			14	14	3	18	15	14	78
								Total Female AP staged	**1738**

Table 5: Numbers of female AP staged by date in all Coos Bay locations

3.4 Conclusion

AP reproductive activity is broadly synchronous with modest variability that appears to correlate with microhabitat differences. Active egg production was in June-July with ovary content steadily declining from August through November. Sites with full sun exposure experienced higher maximum temperatures and periodic drying during the summer. In the most extreme example, (Isthmus Slough high intertidal location one) this exposure period appears to have resulted in earlier spent animals and a smaller overall shell height with more shell pitting than all other sites. Shaded areas generally had larger, less pitted snails but the density of snails per meter appeared to be lower. The shaded and vegetation protected areas do not experience the drying or extremes in temperature from full summer sun exposure but AP may be food limited in these areas from the lower primary production. In November, when the weather cooled with more precipitation and cloud cover, the variability in reproductive status between sampling locations began to disappear. This is likely due to less stress from high temperature extremes and drying associated with summer weather.

4. Distribution of *Assiminea parasitologica* in Pacific Northwest Estuaries

4.1 Sampling Locations & Methods

AP was first documented in the Coos estuary in 2008 and it was thought to have invaded via ballast water. Dr. James Carlton and colleagues conducted a search for AP in the estuaries of Oregon and Washington in the summer of 2008. AP was found in three other systems in the Northwest. The presences of AP in these smaller estuaries, which do not experience shipping and or the exchange of ballast water, suggest that AP is being transported via another vector. We postulate that AP once in North America is being transported within an estuary and between estuaries by human footwear, recreational fishing and scientific sampling gear.

In order to assess the extent of AP in Pacific Northwest estuaries we conducted presence/absence surveys for AP and other Guild Snails. Focused surveys were conducted in the Umpqua, Smith, Siuslaw and Willapa systems. 8-16 areas along the estuarine gradient were examined dependent on waterway and accessibility. Areas were chosen based on appropriate habitat and the presence of human access points. Areas sampled were boat ramps, marshes adjacent to clam beds or wildlife refuges. These sites were chosen in keeping with the concept that AP could be transported from the Coos Estuary via boots or nestled in recreational fishing gear. At each area the following variables were determined: salinity, the presence of tidal channels, presence of man-made structures and the marsh topography was classified as described in 2.3.1. The presence of algal wrack and the relative abundance of Guild Snails were measured in a $0.5m^2$ quadrat area. Relative abundance categories used were the same as in the Coos Bay survey.

Additional spot checking for the presence of AP was conducted in the Coquille, Yaquina, Tillamook and Nehalam systems. These sites were at public boat ramps and access points. Dr. Jim Carlton and colleagues evaluated some of these sites in the summer of 2009. Carleton's sampling points are not located in the projects database.

4.2 Results

4.2.1 Siuslaw Estuary

Eleven areas were sampled along the estuarine gradient of the Siuslaw Estuary on August 5, 2009. When possible three or more 0.5m^2 quadrats were randomly placed and the presence of Guild Snails were observed and recorded. We did not find *A. parasitologica* in the Siuslaw estuary (Figure 80).

A. californica was observed in two sites that were in the euhaline and polyhaline sections of the estuary, AC was observed to be common and abundant at these sites (Figure 81).

L. subrotundata was common in two sites, both of which were on the ocean dominated end of the estuary in a Fucoid zone (Figure 82). *M. myosotis* was absent in the Siuslaw estuary.

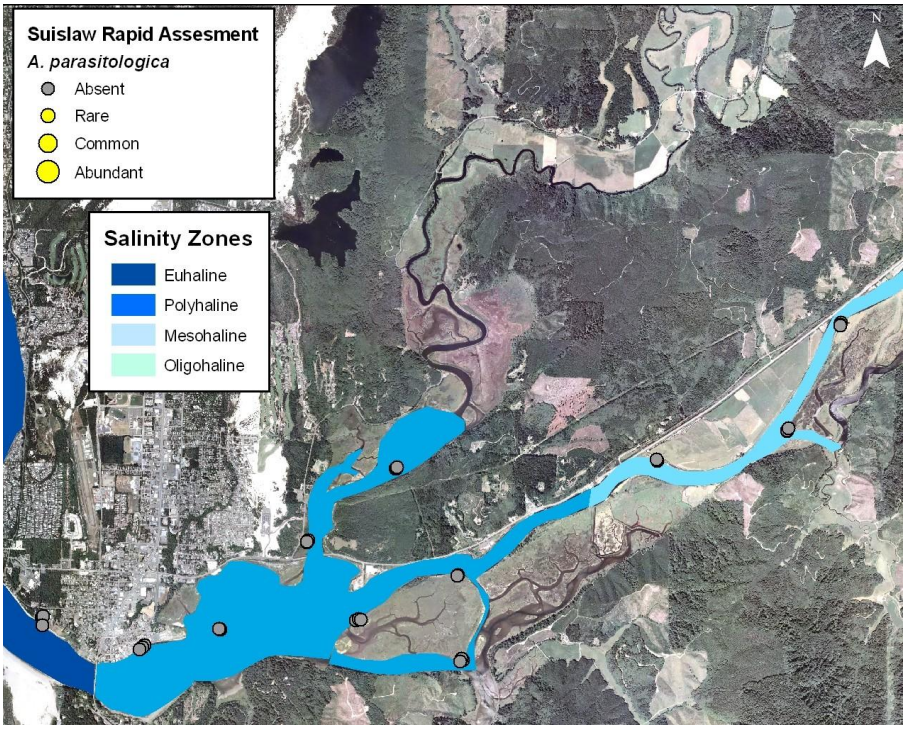

Figure 100: Relative abundance of *A. parasitologica* per 0.5m^2 in the Siuslaw estuary

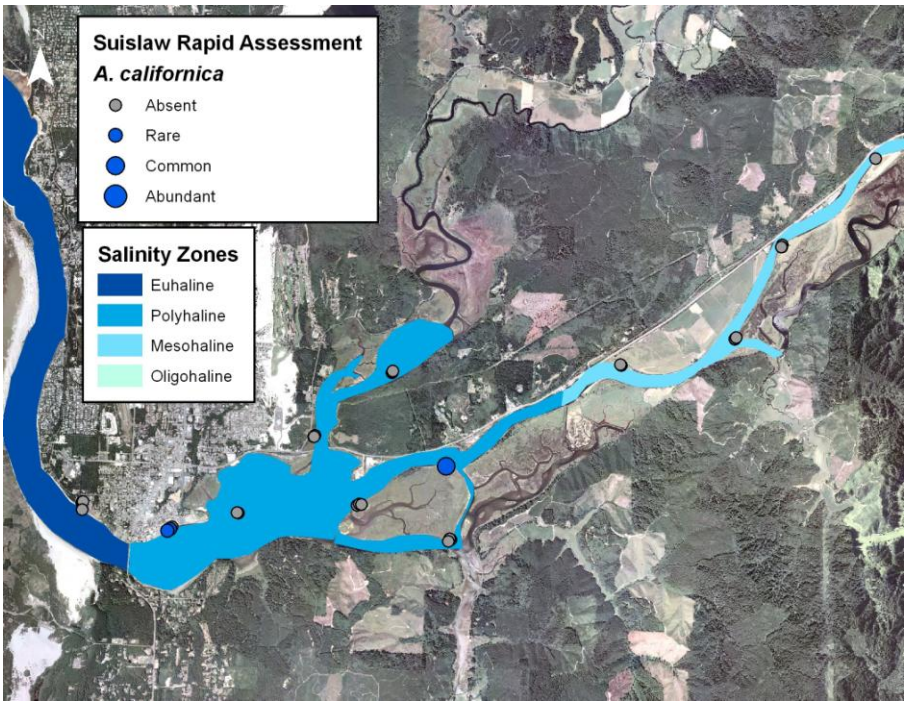

Figure 101: Relative abundance of *A. californica* per 0.5m^2 in the Siuslaw estuary

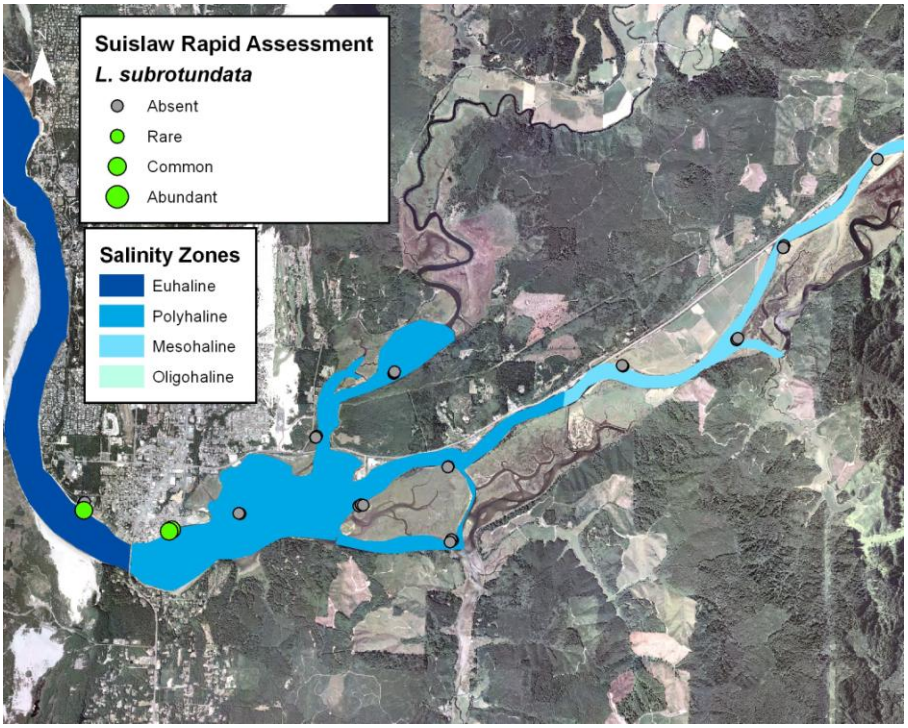

Figure 102: Relative abundance of *L. subrotundata* per 0.5m^2 in the Siuslaw estuary

4.2.2 Umpqua/ Smith Estuary

On August 6, 2009 16 areas were sampled in the Umpqua/Smith Estuary; 13 were in the Umpqua section and three were in the Smith section. Both systems were sampled along the estuarine gradient. Whenever possible three or more 0.5m^2 quadrats were randomly placed and the presence of Guild Snails were observed and recorded. *A. parasitologica* was observed in four areas, one of which was in the Smith portion, although rare in relative abundance (Figure 83). AP was common in sites, which were in the polyhaline zone. *A. californica* was observed to be common in two areas in the Umpqua system that was in polyhaline sections of the estuary (Figure 84). *L. subrotundata* and *M. myosotis* were absent in both the Umpqua and Smith systems. Although only qualitative data was collected; the New Zealand Mud Snail was found in high abundance, estimated at 1,000s per meter squared in the upper Estuary. In addition an unknown species (referred to as Species A in the projects database) was found in abundance in the mid to lower estuary.

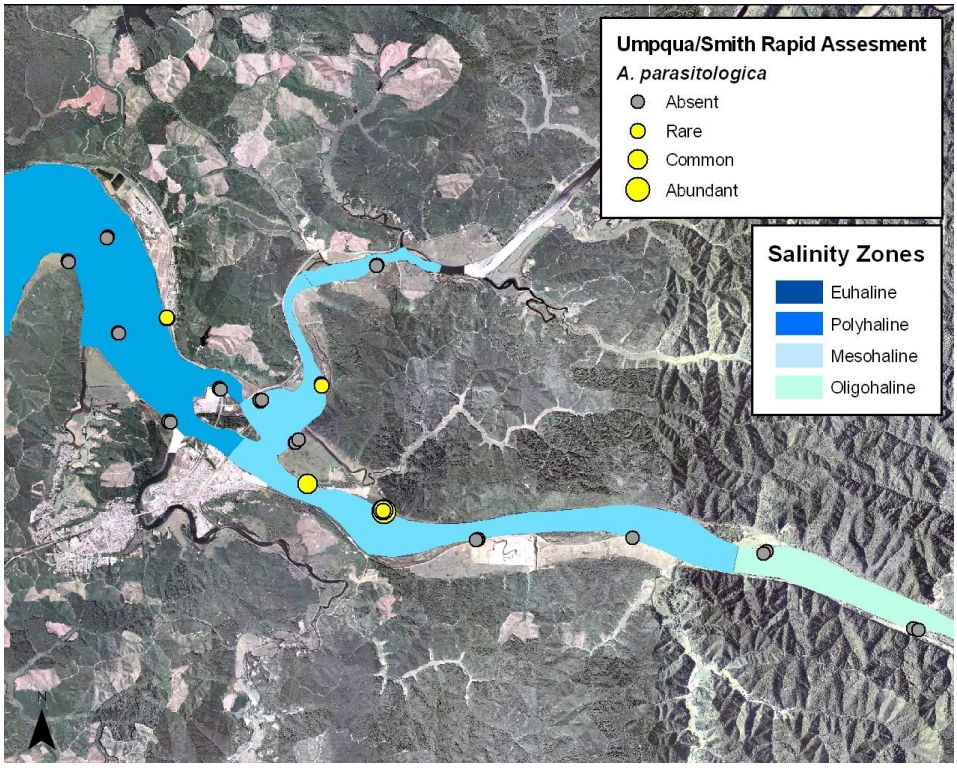

Figure 103: Relative abundance of *A. parasitologica* per 0.5m^2 in the Umpqua/Smith estuary

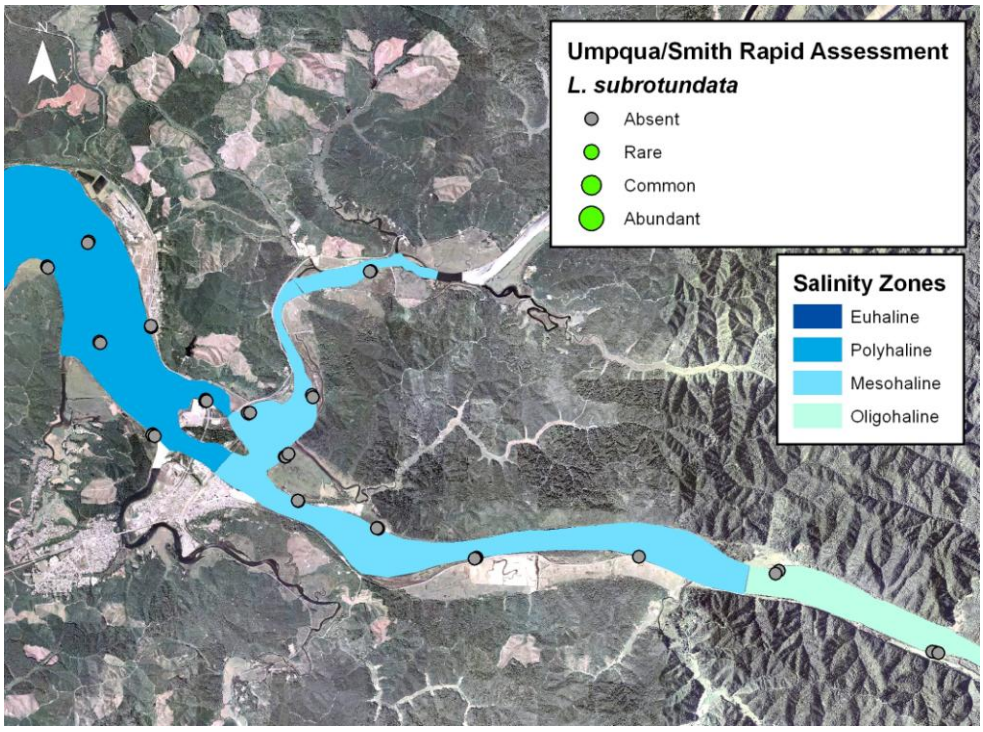

Figure 104: Relative abundance of *A. californica* per 0.5m^2 in the Umpqua/Smith estuary

4.2.3 Willapa Bay Estuary

Areas chosen for sampling in the Willapa system were based on Carlton's 2008 expedition and additional recommendations from Jen Ruesink, (Estuarine Ecologist, University of Washington). On October 14 and 15, 2009 eight areas were sampled in the Willapa System. When possible three or more 0.5m^2 quadrats were randomly placed and the presence of Guild Snails were observed and recorded. *A. parasitologica* was absent in the survey (Figure 85). *A. californica* was observed at six sites; abundant in three, common in two and rare in one (Figure 86). The AC observed in the Willapa system appeared to be larger and more robust than the Coos Bay population. LS were observed in all eight sites and MM was abundant in three sites (Figure 87).

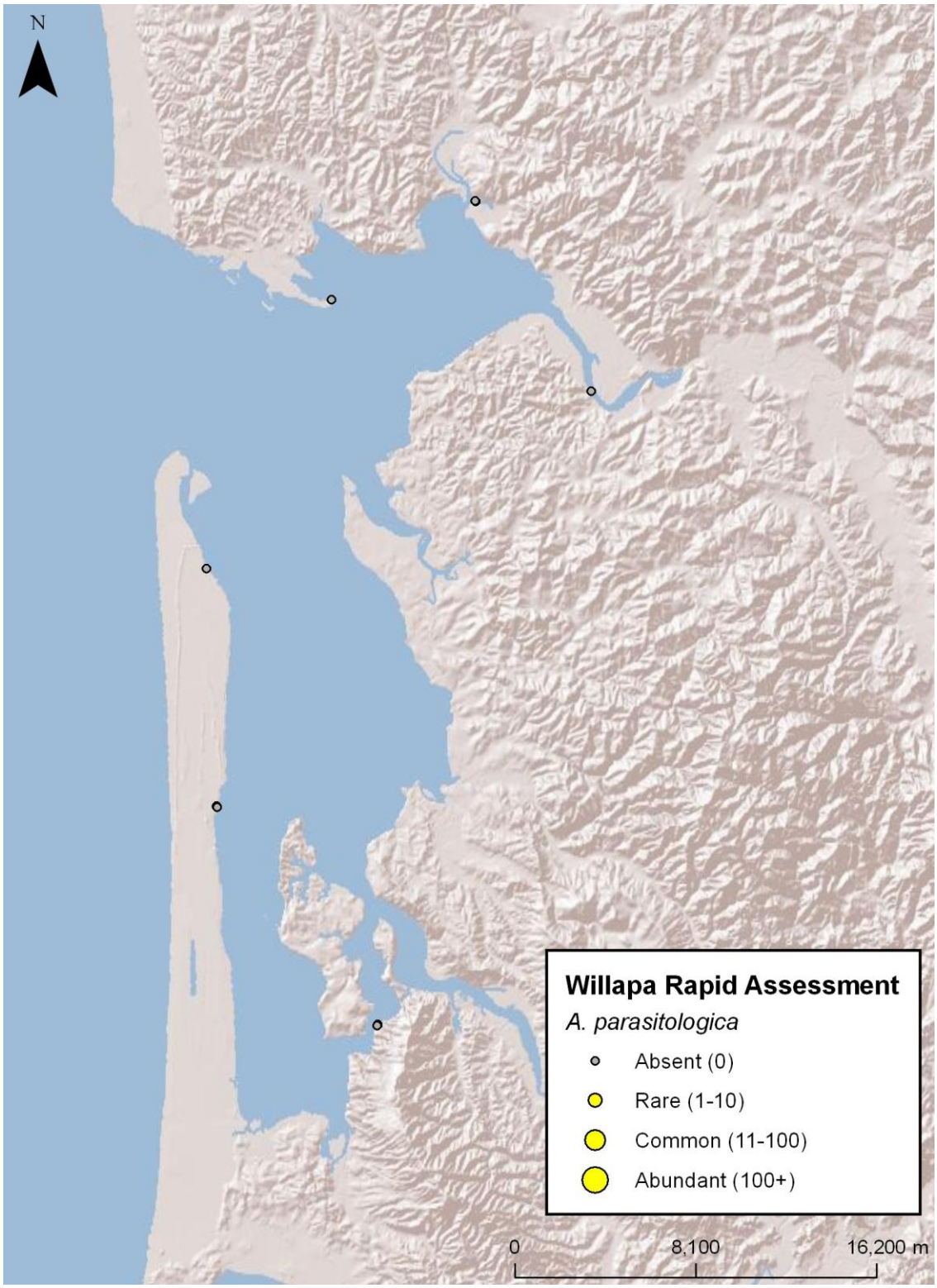

Figure 105: Relative abundance of *A. parasitologica* per 0.5m^2 in Willapa Bay

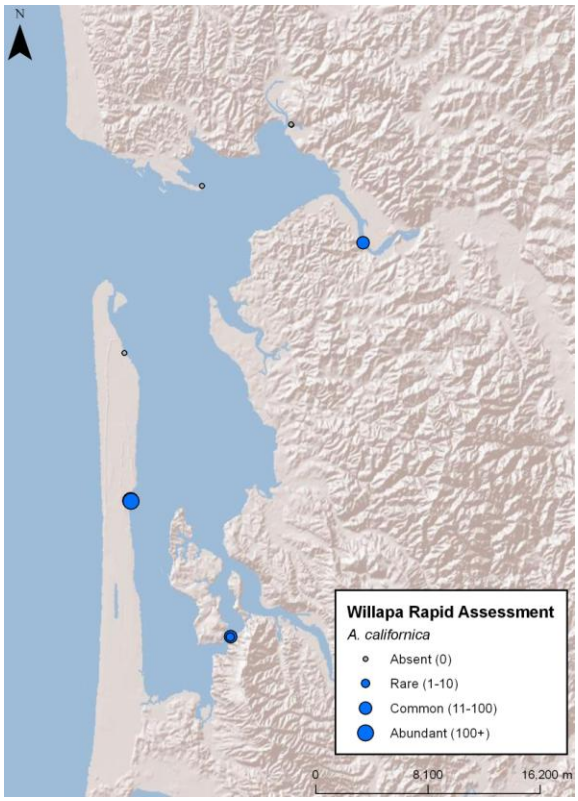

Figure 106: Relative abundance of *A. californica* per 0.5m^2 in Willapa Bay

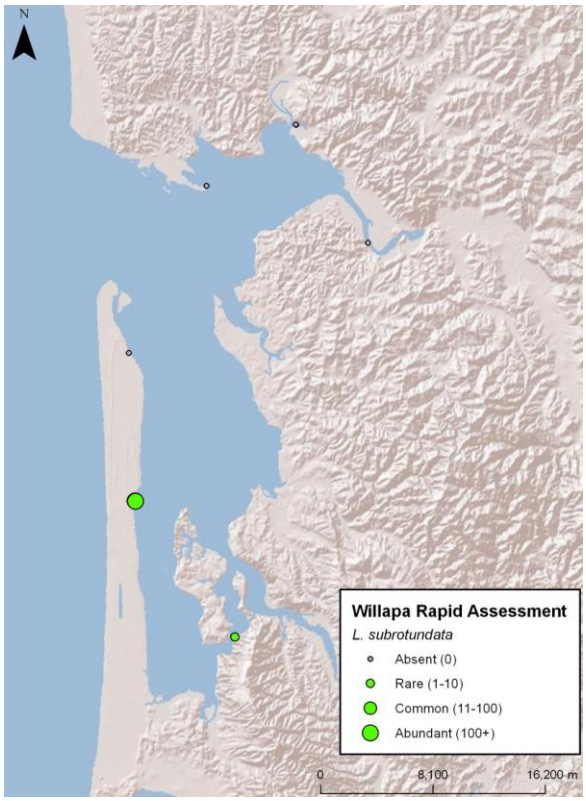

Figure 107: Relative abundance of *L. subrotundata* per 0.5m^2 in Willapa Bay

Figure 108: Relative abundance of *M. myosotis* per 0.5m^2 in Willapa Bay

4.2.4 Other Pacific Northwest Estuaries

The Coquille, Nehalam and Tillamook estuaries were all spot checked for the presence of AP. AP was present in the Coquille in high abundance and although qualitatively, individuals were noted as being large and robust in appearance (shiny shell with no pitting). AP was not found in the Tillamook or Nehalem systems.

Figure 109: The Presence of *A. parasitologica* in sampled Northwest estuaries as of October 2009

4.2.5 Conclusion

AP was present in three estuaries in 2008 and in five in 2009. The newly invaded estuaries, the Coquille, Smith and Alsea are rather small waterways that do not experience large shipping traffic. These waterways do however experience large amounts of recreational fisherman and boaters. Although not experimentally examined, these findings support the hypothesis that AP is being transported to other estuaries via human equipment.

5. Recommendations

The duration of this study was one year. Focused work with dedicated full time staff was from March through December of 2009. Although a vast amount of information was collected we would make several recommendations to better the study that was conducted, propose topics for future research, and advise on the prevention of the spread of *A. parasitologica.*

For the Coos Bay estuary survey, the Rapid Assessment method was useful to characterize the spatial extent of colonization of AP and the other Guild Snails. In the event of more time we would strongly suggest making the distance between transect lines less so as tease out fine scale variation along shore. In addition, relative abundance data is not dependable and, when able, actual counts are the preferred methodology. Additional Detailed Assessment surveys would generate more accurate abundance measurements. We would include all the environmental variables measured and add the following variables: re-dox potential of the sediment, canopy height of the dominant vegetation within the plot, food availability on the substrate. It would be prudent to develop a measurement of microhabitat, in regards to depressions, pockets of moisture and shade to which snails are aggregating. Current flow and tidal inundation time must play a role in the distribution of these mesogastropods; both field measurements and laboratory flume studies would be helpful in understanding the spatial distribution of these organisms. Furthermore, we would advise sampling with the detailed assessment method for randomly allotted sites, rather than driven by AP abundant areas. Having comparison sites will assist in teasing out the details of the snail guild distribution in Coos Bay, Oregon.

For the reproductive module, we would suggest collecting snails from several areas within a site. In addition we would suggest that transects run both parallel and perpendicular to shore. A full year sampling regime is recommended to capture a complete reproductive cycle. Continuous temperature data recorders placed at the sampling sites would give a better idea of how temperature affects reproduction and

behavior. Photographing a representative sample plot each week is recommended to capture behavior and recruitment between sampling periods. Reproductive stages could be more narrowly defined with the Active stage split into several sub-stages based on additional observations of ovary contents.

In regards to the northern estuaries sampled, we would add sites examined and limit spacing between sites sampled. In addition to measuring relative abundance of Guild Snails, we would collect snails for positive identification and numeration to generate abundance measurements.

In the future there is great opportunity to develop additional field and laboratory studies focused on *A. parasitologica*. We recommend looking into the following life history topics: gametogenesis, reproductive phenology, capsule formation, deposition and output, time of hatching, larval duration, dispersal and recruitment processes.

In addition, the distribution of adult AP populations by habitat should be examined to determine what factors may be preventing adults from living on the mudflat. As AP moves into new areas, there will be opportunities to examine species interactions and competition with other guild members. These studies of early colonization could contribute to the understanding of the potential impacts of this new invader. We would strongly suggest sampling estuaries examined in this study and adding additional estuaries, such as Humboldt Bay and San Francisco Bay.

A. parasitologica is present in high abundance in Pacific Northwest estuaries, but appears to thrive more in certain areas of an estuary. To prevent the spread of AP within and between estuaries we strongly recommend that researchers scrub their boots between field sites.

6. Overall Project Conclusion

This project was a rapid response study to collect baseline data on the new invasive, *Assiminea parasitologica*. The study was designed to examine the distribution and habitat characterization of AP, but did include the same foci on the members of the Snail Guild. In addition, the reproductive life history of AP was investigated to gain knowledge on this new invasive and to better understand the adult population. AP was first documented in Coos Bay, Oregon in 2007, however upon attempting to determine earliest possible arrival, it was found in 2006 salt marsh samples from the Coos Bay estuary (Tim Davidson pers. comm., 2008). With any invasive species there is often a lag between the invasion and human awareness and then response. Given that, the data collected here on AP is relatively soon after the organism was first detected in North America.

This is the first study to collect baseline data on the abundance and species-specific habitat correlations of these Snail Guild species in Pacific Northwest estuaries. Therefore assessing or determining change in the existing populations of AC, LS and MM was not possible. Although there are biases in the design toward the collection of AP, it is clear that AP is the most abundant mesogastropod examined in the Coos Bay Estuary. Given the high densities and range of habitats that AP can inhabit there is striking potential for AP to move into new areas of the estuary and possibly displace these existing mesogastropod species. The data from this survey strongly suggest that: AP could displace AC as it persists and is successful in similar habitats and AP could displace LS and MM as these species populations are low in comparison to AP.

The reproductive module generated a great deal of qualitative data and a general understanding of the organism. We now understand the general reproductive anatomy, output potential and seasonality. The data strongly suggests that AP is reproducing in a seasonal cycle and the population appears to be synchronous in its reproduction. The modest variation between areas sampled suggests that the reproductive timing may be correlated with temperature and/or microhabitat.

Since the response in documenting the invasion of *A. parasitologica* within Pacific Northwest estuaries, it has extended its colonization to the north and south of the Coos Bay Estuary. AP, to date, has been documented in five estuaries in Oregon. The information, data and thoughts generated from this project will be of most importance as we track this model organism's invasion throughout the west coast of North America.

7. Collaborations & Acknowledgements

- All kinds of help:
    - SSNERR STAFF Steve Rumrill, Ali Helms, Adam Demarzo, Craig Cornu, Hans Klausner, Mike Graybill, Robin Elledge, Pam Wilson and Kathy Andreason
    - Confederated Tribes of the Coos, Lower Umpqua and Siuslaw Indians: Howard Crombie, John Schaefer, Jeff Stump, The Tribal Council and The General council
    - ODFW Staff: Caren Braby, Stacy Galleher, Jean McCrae and Scott Groth
    - OIMB Staff: Alan Shanks and Barb Butler
    - Jim T. Carlton: an ecosystem based approach
- GIS
    - Stacy Gallaher, Oregon Department of Fish and Wildlife
    - Jeff Stump, Confederated Tribes of Coos, lower Umpqua and Siuslaw Indians (CTCLUSI)
    - Tanya Haddad (OCMP, ODLC)

- General consultation/experimental design and methods:
    - Brian Bingham, Western Washington University, (WWU)
    - Jim Carlton, Williams-Mystic of Williams College, (WC)
    - Jennifer Ruesink (UW)
    - Alan Shanks, (OIMB)
    - Caren Braby (ODFW)
    - Holly Keamerer (OIMB)
    - John Jameson (JJS)
- Field work volunteers
    - Evan Bloom
    - Paul Dunn (OIMB)

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9. Appendices

9.1 Snail Guild Information

Information	*Littorina subrotundata*	*Myosotella myosotis*	*Angustassiminea californica (Assiminea californica)*	*Assiminea parasitologica*
Taxonomy	Mesogastropod	Pulmonate	Mesogastropod	Mesogastropod
Adult size	6mm	8mm	4 mm	4mm (5.8mm in CB)
General Description	Small light brown and rounded	• shell color varies from light brown-violet-opaque white • interior is porcelainous • Olive shaped with pointed spire	• glossy chestnut shell color: nearly black spire/ body whorl almost transparent in color	Looks like californica But "distinctive yellow stripe below suture
Detailed description	• 4 whorls • chink between columella and inner lip/line is a diagnostic feature	• 3 "teeth" in columella	• Shell=globuse, stoutly conical, thin and smooth • operculum thin/colorless	• positive i.d.: from radular teeth and operculum
Latitudinal range(Pacific)	Neah Bay, WA-Humboldt Bay, CA	Boundary Bay, BC- Baja California	Vancouver, B.C.- Cabo San Lucas	• Found in Coos Bay, Smith-Umpqua river, Yaquina Bay
Guild intertidal range	low	•High • Found under rotting boards & debris	• low • found both in water & on moist substrate	• Sublittoral of mixed habitats • under plants and woody debris
Plant associations	Salicornia and Distichlis	Salicornia and Distichlis	Salicornia and Distichlis	?
Diet	Herbivorous	Herbivorous	Herbivorous	Herbivorous
Native or Non	Native	Introduced from Europe • 1st noticed in Coos Bay, 1965	Native	•Introduced from Japan • 1st noticed in Coos Bay in July 2008
Reproduction	Dioecious	Hermaphroditic	Dioecious	Dioecious
When	June-July	• mating occurs in spring & summer • eggs laid in summer	• Copulating pairs all year •	• know July, but when else
How many capsules	15-40 per egg mass	15-80 eggs per mass	? 1 egg per capsule	? • 1 egg per capsule
Egg description			Single, spherical, 0.5mm in diameter	• tiny white silt covered in mud

Laying style	Laid in moist locations, submerged at high tide	Eggs pushed into sediment or attached to emergent veg	•Females push capsules into the mud • capsules covered with a sticky film= mud etc adhere to them	• push into mud
Output	Crawl away within about 2 months	Crawl away	Crawl away	Hatch as free swimming planktotrophic veliger larvae

Native

Littorina subrotundata

Angustassiminea californica

Introduced

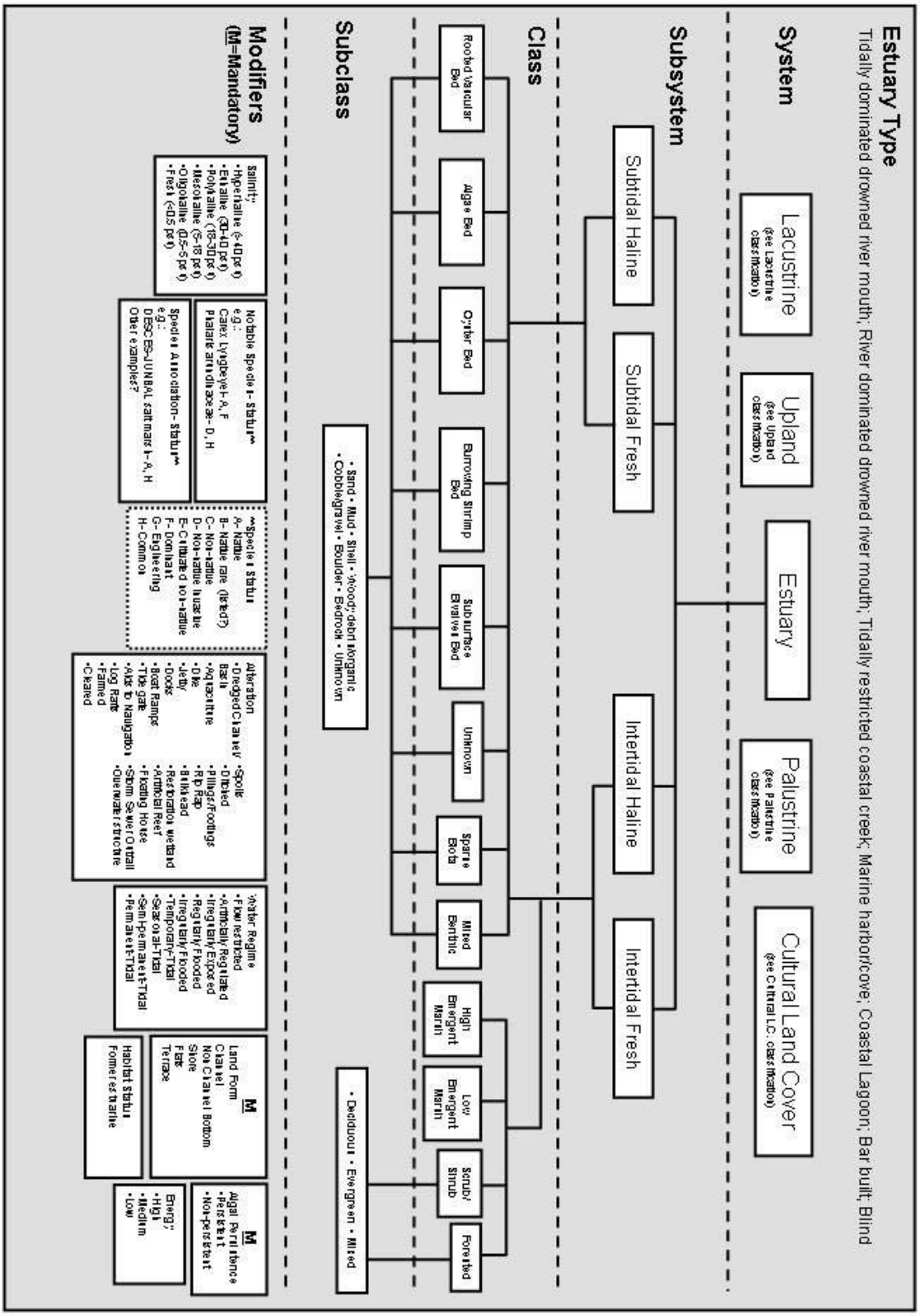
Myosotella myosotis (1967)

Assiminea parasitologica (2007)

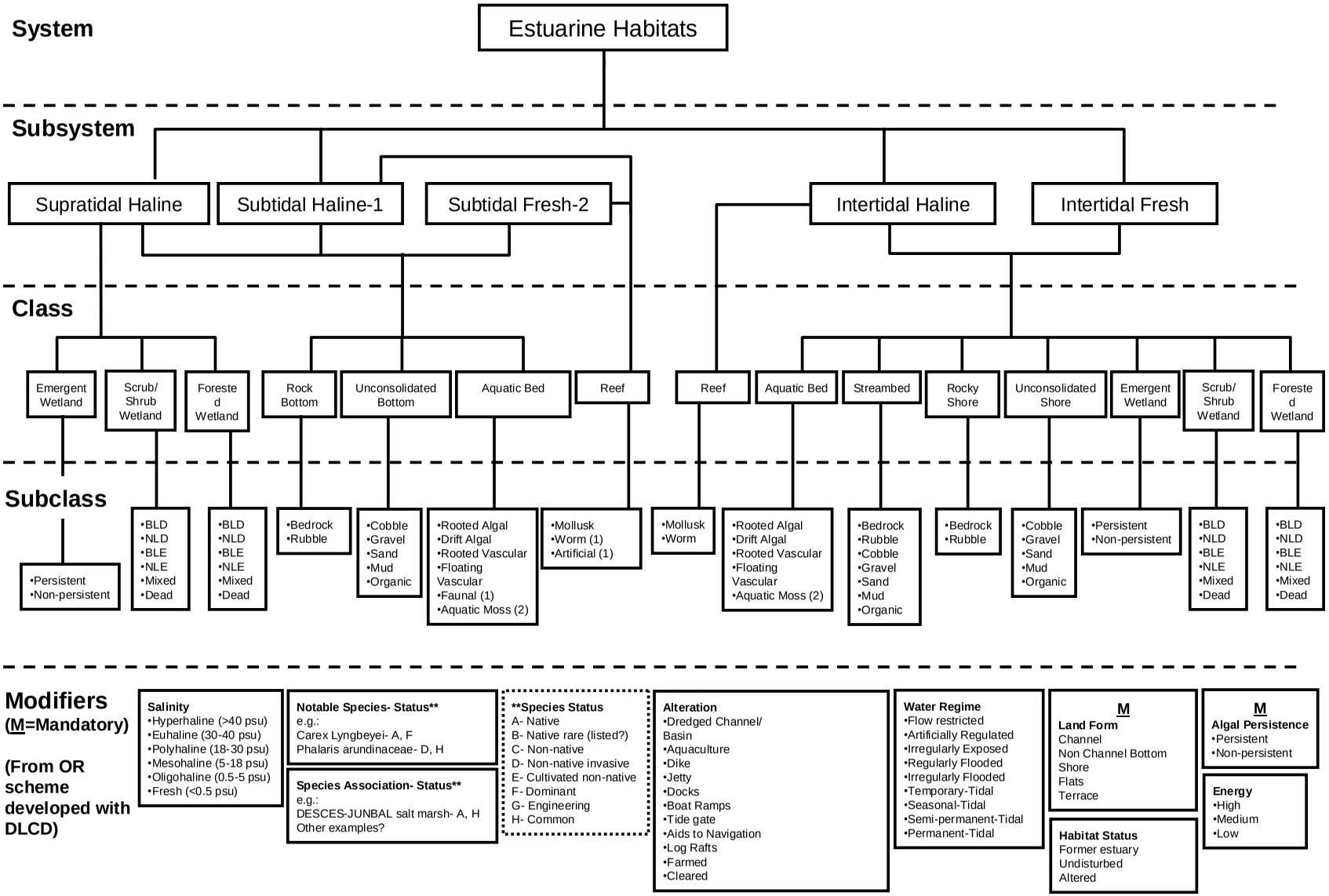
9.2 Sampling Locations

Region	Sites Sampled	Location Description
North	Haynes	Haynes Inlet
	North Slough	North Slough
	Pony	Pony Slough
	Airport	Marshes near Airport
	Jordan	Jordan Cove
South	Isthmus	Isthmus Slough
	Coal	Coalbank Slough
	Catching	Catching Slough
	Davis	Davis Slough
East	Coos River North	Coos and Millicoma River
	Coos River South	Coos River east of the Millicoma Fork
West	SS	South Slough
	JoNey	JoNey Slough
Central	Central	Central Coos Bay
	Islands	Spoil Islands
	Kentuck	Kentuck Inlet

9.3 Oregon Estuary Habitat Classification, Draft 4

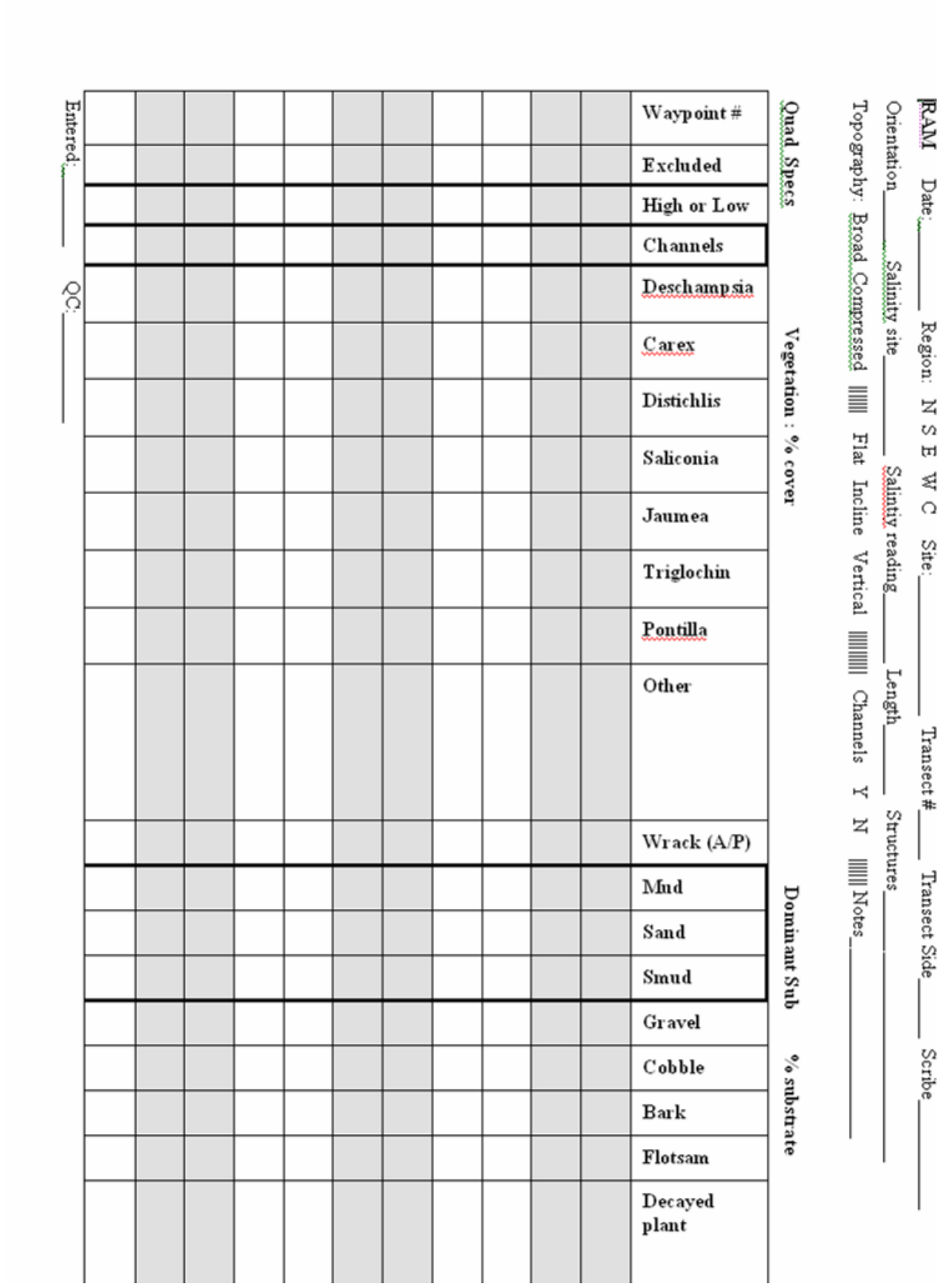


NERRS Habitat and Land Use Classification System (Kutcher, et al)



9.4 NERR Habitat Classification

9.5 Datasheets



Date_____ Site_____ transect #_____Transect side_____

Snails A=Absent(0), R=Rare(1-10), C=common (10-100), AB=Abundant (100+)

Waypoint	Air Temp	Sed Temp	Slope	AP	AC	LS	MM	NZM	Other	Notes

Entered:_____ QC:_____

DAM Date:_____ Region: N S E W C Site:_____ Scribe_____

Salinity site_____ Salinity reading_____ Length_____ Structures_____

Topography: Broad Compressed ||||||| Flat Incline Vertical |||||||||| Channels Y N ||||||| Notes_____

Quad Specs				Vegetation : % cover									Dominant Sub			% substrate				
Waypoint #	Exclnded	High or Low	Channels	Deschampsia	Carex	Distichlis	Saliconia	Jaumea	Triglochin	Pontilla	Other	Wrack (A/P)	Mud	Sand	Smud	Gravel	Cobble	Bark	Flotsam	Decayed plant

Entered:_____ QC:_____

DAM Date_____ Site_____

Snails A=Absent(0), R=Rare(1-10), C=common (10-100), AB=Abundant (100+)

Waypoint	Air Temp	Sed Temp	Slope	AP	AC	LS	MM	NZM	Other	Snails Collected

Entered:_____ QC:_____

Northern Estuaries Survey Date:_____ Estuary:_____ Scribe_____

Salinity site_____ Salinity reading_____ Structures_____

Topography: Broad Compressed |||||| Flat Incline Vertical |||||||| Channels Y N |||||| Notes_____

Quad Specs **Snails: Absent, Rare, Common, Abundant**

Waypoint #	High or Low	Wrack (A/P)	AP	AC	LS	MM	NZMS	Other

Northern Estuaries Survey Date:_____ Estuary:_____ Scribe_____

Salinity site_____ Salinity reading_____ Structures_____

Topography: Broad Compressed |||||| Flat Incline Vertical |||||||| Channels Y N |||||| Notes_____

Quad Specs **Snails: Absent, Rare, Common, Abundant**

Waypoint #	High or Low	Wrack (A/P)	AP	AC	LS	MM	NZMS	Other

Scope Sheet								
AP	AC	LS	MM		AP Weight	AC Weight	LS weight	MM Weight

9.6 Species by Site Results

Tukey-Kramer post hoc analysis for species specific distribution by site from detailed assessment. Levels not connected by same letter are significantly different.

AP Abundance

Level				Mean
Isthmus	A			486.72000
Haynes West	A	B		395.52000
Haynes East	A	B		349.44000
Coos River North	A	B		333.12000
Kentuck		B		126.72000
Hinch Bridge		B		23.04000
Valino Island		B		11.52000

AC Abundance

Level				Mean
Haynes East	A			255.36000
Haynes West	A	B		167.04000
Isthmus	A	B		124.80000
Coos River North		B		97.92000
Kentuck		B		90.24000
Valino Island		B		74.88000
Hinch Bridge		B		9.60000

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LS Abundance

Level				Mean
Haynes West	A			101.76000
Valino Island		B		15.36000
Isthmus		B		6.72000
Coos River North		B		0.00000
Haynes East		B		0.00000
Kentuck		B		0.00000
Hinch Bridge		B		0.00000

MM Abundance

Level				Mean
Haynes East	A			144.00000
Haynes West	A	B		84.48000
Valino Island		B	C	46.08000
Kentuck		B	C	25.92000
Isthmus			C	3.84000
Coos River North			C	0.00000
Hinch Bridge		B	C	0.00000

9.7 GIS Metadata

9.7.1 GIS report summary

The South Slough National Estuarine Research Reserve (SSNERR), along with support from the Confederated Tribes and under a grant from NOAA (number NA080AR4170927), documented the distribution and density of four mesogastropod snails in the Coos Bay estuary, particularly focusing on the new invasive species, *Assiminea parasitologica*. In addition, presence/absence surveys of the invasive were conducted in estuaries along the Oregon and southern Washington coasts. The targeted snail species are *Assiminea parasitologica*, *Angustassiminea californica*, *Littorina subrotundata*, and *Myosotella myosotis*.

All of our sampling strategies are based on a general two-factor design of comparing across and among regions, as well as including a reproductive experimentation module. Our sampling design was two tiered: (1) a superficial and extensive Rapid Assessment Method (RAM), with evenly spaced sampling to cover all possible marsh habitat in the Coos Estuary (2) a thorough and limited Detailed Assessment Method (DAM), with randomly generated points within known high populations of *A. parasitologica*. Both types of sampling designs were generated in ArcGIS v 9.3 using NPS AlaskaPak v 2.2 software. The RAM sampling points were generated with a series of grids drawn over all of Coos Bay with a cell size of 800 m along shore by 15 m between points. Each grid generally covered a particular waterway, allowing individual rotation of each to make transects lie perpendicular to the shore. Center points within each cell were used as waypoints. Detailed assessment sampling points were created by drawing polygons over selected RAM sampling area in each region and then generating random points using NPS AlaskaPak within each boundary. Approximately 50 random points were generated for each potential site. Points were then randomly selected using a random number table and ground truthed for suitability. For all points, latitude and longitude was generated by NPS AlaskaPak and transferred to Garmin GPSMap76 using the DNRGarmin GPS application software v 5.4.1.

For RAM, five regions were assigned across the entire estuary and each sampling waypoint was categorized as suitable for sampling or excluded. Waypoints were excluded if: (1) the waypoint was located on private and/or fenced or gated property, (2) the waypoint proved to be inaccessible, or (3) the waypoint was in a terrestrial, dune, or freshwater habitat. Habitat classification and environmental and biological variables that could be determined by visual observation were recorded for a 0.5 m^2 quadrat.

For DAM sampling five sites were chosen from within the five sampled RAM regions, based on high density populations of *A. parasitologica* found during RAM sampling. Our objectives at a DAM site were twofold; RAM assessment in a 0.5m² quadrat and core extraction of all snail species from within a randomly placed 0.025m² sub-quadrat. All target species were extracted, counted, and biometrics were measured in the lab.

The rapid assessment method (RAM) enabled us to collect data about the habitat and snail populations and visually map variables using GIS to show distributional patterns. The combined RAM and DAM survey allowed us to map the distribution and density of all four target species and other estuarine environmental factors. From our assessment we found a fundamental difference in the relative abundance and abundance calculations generated based on rapid visual assessment (RAM) compared to the actual number of snails counted (DAM).

9.7.2 Metadata for data clearinghouse

Abstract: The GIS dataset that is contained in this record is split up into the geodatabase file (AP Final Geodatabase.mdb) and the associated aerial photographs (.sid and .jpg) along with several JPEG and an interactive PDF map of RAM data for review and public presentation.

Use Constraints (same in every feature class): This product is for informational purposes and was not prepared for legal, engineering or surveying purposes. Users of this information should review or consult the primary data and information sources to ascertain the usability of the information. The Oregon Department of State Lands, South

Slough National Estuarine Research Reserve, Confederated Tribes, or any of the data providers cannot accept any responsibility for errors, omissions, or positional accuracy in the digital data or underlying records. There are no warranties, expressed or implied, including the warranty of merchantability or fitness for a particular purpose, accompanying any of these products.

This map was prepared by SSNERR staff under award number NA080AR4170927 from NOAA. The statements, findings, conclusions, and recommendations are those of the author(s) and do not necessarily reflect the views of NOAA or the US Government.

9.7.3 APGeoMaster.mxd map document Metadata abstract

Abstract: A map document containing symbolized key attributes as separate layers important for the Invasive Snail project, including all RAM, DAM, Coastal Estuaries, and Reproduction Module sampling points, SSNERR Boundary, Salinity Polygon, and Aerial photography data.

The aerial photographs used came from SSNERR (coos-shalf.sid) and Oregon Imagery Explorer (Siuslaw and Umpqua estuaries), projected in Oregon Lambert (NAD83) Int. Feet, using the GCS_North_American_1983 geographic coordinate system.

9.7.4 All other layer metadata abstracts

CoastalEstuaries_PresenceAbsence:
Abstract: Data determining the presence or absence of new invader, A. parasitologica for a series of estuaries along the Oregon/Washington coast ranging from the Coquille River, OR to Willapa Bay, WA.

CoastalEstuaries_RapidAssessment:
Abstract: A feature class created from latitude and longitude data linked to all environmental and biometric data taken during RAM (Rapid Assessment) sampling (covering the entire Coos Bay Estuary) including transect data, waypoint data (environmental classifications), and estimated snail counts within a 0.5m² quadrat.

CoastalEstuaries_Salinity:
Abstract: Salinity Zone polygons for the Siuslaw and Umpqua/Smith Estuaries

CoosBay_Salinity:
Abstract: Roughly drawn polygons covering the Coos Bay waterway with specified salinity zones from field data taken in the summer of 2009.

Detailed Sites:
Abstract: Rough polygons over the locations of the Detailed Assessment sites, across the Coos Estuary

DetailedAssessment_Data:
Abstract: A feature class created from latitude and longitude data linked to all environmental and biometric data taken during DAM (Detailed Assessment) sampling including transect data, RAM (Rapid Assessment) data replication at each DAM site within a 0.5 m^2 quadrat, and individual snail counts and biomass measurements within a 0.025m^2 core sample.

Excluded_Transects:
Abstract: All transects in the RAM (Rapid Assessment) survey excluded because of incorrect habitat, inaccessibility, or private property.

RapidAssessment_Data:
Abstract: A feature class created from latitude and longitude data linked to all environmental and biometric data taken during RAM (Rapid Assessment) sampling (covering the entire Coos Bay Estuary) including transect data, waypoint data (environmental classifications), and estimated snail counts within a 0.5m^2 quadrat.

Rapid Assessment_SamplingPts:

Abstract: Sampling points generated from 800m by 15m grids overlaid over 5 regions in Coos Bay. Transects selected upshore as far as the tree line or obvious obstruction. Sampling points run 15m apart upshore, each transect 800m apart along shore.

Reproduction_SamplingPts:
Abstract: Locations of reproduction module sampling sites, including their specific "rep" sampling points

Sampling_Regions:
Abstract: Sampling points generated from 800m by 15m grids overlaid over 5 regions in Coos Bay. Transects selected upshore as far as the tree line or obvious obstruction. Sampling points run 15m apart upshore, each transect 800m apart along shore.

SSNERR_Boundary:
Abstract: Administrative boundary of the South Slough National Estuarine Research Reserve.

Unsampled_Area:
Abstract: A rough polygon covering the lower Coos Bay, the area of the estuary which was not sampled for the Invasive Snail Project

9.8 GIS Protocols

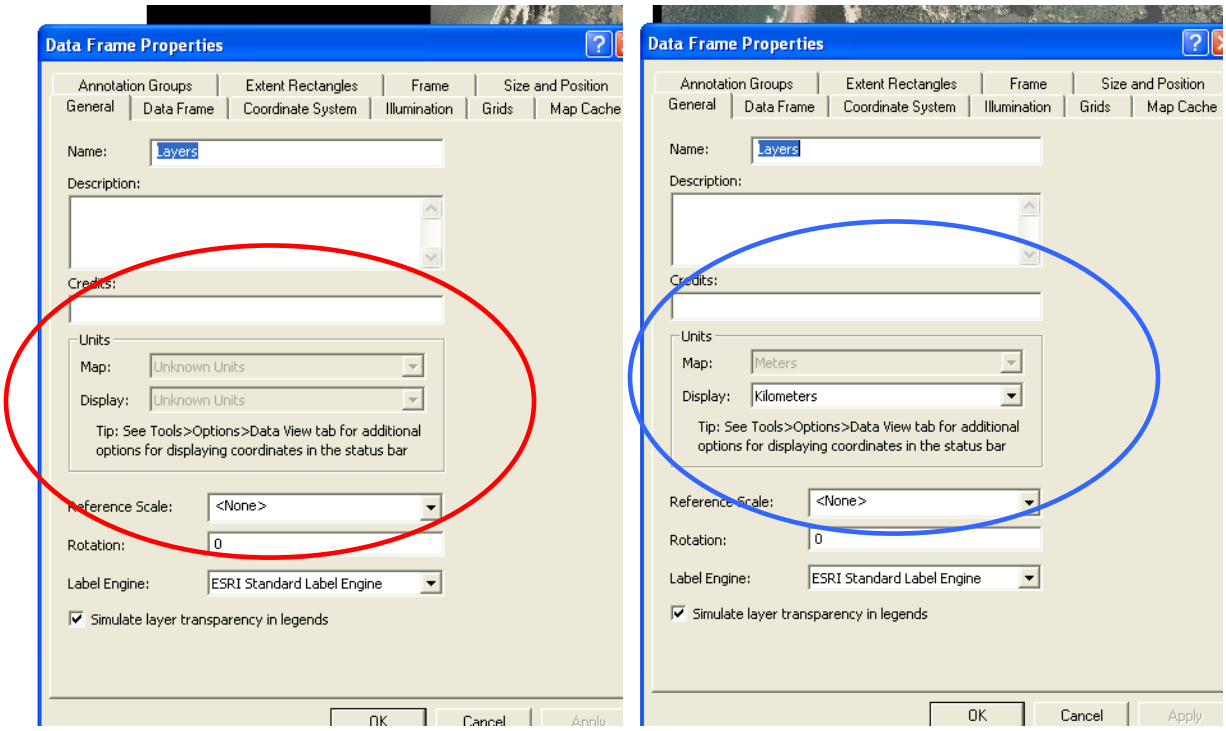
9.8.1 Important Information About Base Layers

Check to see if your ArcMap Document has its units properly set:

View→Data Frame Properties.

Under the "General" tab, the "Map Units" should be set to "Feet/Meters/something unit-like" (it will be grayed out because these units are *automatically* set by the image you first load.

As long as your Map Units are set, you can then select the "Display Units" you'd prefer—ArcMap will calc that out automatically for you. So, if your Map Units are automatically set in "Feet" but you want any measurements or your scale bar to read in meters, you simply select "Meters" in the "Display:" drop down menu.



9.8.2 Important Information about Map Projections

The projection set for the first layer you add to a map document will automatically become the projection of the entire data frame (all the subsequently created layers and the entire.mxd file).

9.8.2.1 Adding a layer with a different projection than that set in your data frame.

- Transform, or "project" it as a new layer file, and then add the new file into your map document. You CANNOT just "set it to a different projection" in properties (while in ArcCatalog). That is how you transport your data magically to Taiwan. It's not a nice place for data to go.
- First, open ArcCatalog.
- Open the "ArcToolbox"
- Data Management Tools → Projections and Transformations → Feature → Project
- Your "Input dataset" is the layer you want to re-project to match your data frame/map document.
- After browsing for that layer, the program should automatically fill in the "Input coordinate system"
- In "Output dataset or feature class" browse to the proper folder and specify the name you wish for your new layer. It is a good idea to include a shorted version of the projection you are transforming into within the name. Examples: CoosRiver_Lamb or RockyMtn_NAD83
- Output Coordinate system → Browse/Import the new projection/the projection of your data frame.
- If you need a "Geographic Transformation", a green dot will appear next to the field and a drop-down list will be available.
    - This list shows a number of transformation options (NAD83 to UTM, for example). Depending on your input and output files, select the pairing that fits.
    - Example: You wish to change a layer set in WGS84 to North American Datum 1983 (NAD83). You will have to select the "NAD83 to WGS84" transformation. Even though you are transforming your layer "backwards" so to speak (from WGS to NAD, rather than NAD to WGS as the transformation says), it is the pairing of geographic coordinate systems you have to select, not the direct of transformation.
- OK

9.8.2.2 Adding data from a table (excel spreadsheet, etc.) to your Map Document

There are two ways to make a shapefile or feature class from tables.

Method 1

In ArcMap → Tools → Add XY Data

Browse for your table → Specify which columns correspond to X and Y coordinates

Important: You must set the "Coordinate System of Input Coordinates" to the Projection system those coordinates were made with, not necessarily the projection of your data frame.

Set projections must always correspond to the data's original projection, not the one which you want it to conform to. If you try and force data into a projection that it wasn't made in, ArcMap will automatically place it in a noticeably incorrect location (ie South America) until you set it correctly.

Example: If you are entering XY data taken from points in the field from a GPS set to WGS84 (most GPS are defaulted here), then your "Coordinate System of Input Coordinates" should be set to WGS84.

Your points should then display "on the fly" in the correct places, labeled as an "event" in your Table of Contents.

After clicking "OK" and making sure your data appears in the correct place, you still need to save this as a shapefile. Right click on the "Event" in the Table of Contents → Data → Export Data… And follow the instructions being sure to now specify to use the "Same Coordinates as the DATA FRAME" to create a layer that can be used in your Map Document.

Method 2

Browse for your table in ArcCatalog.

Right click on the table → Create Feature Class → From XY Table.

Specify which columns correspond to X and Y coordinates (Long is X and Lat is Y. If you get confused, just imagine a Cartesian Coordinate system from your Algebra class. Y is the "up and down" and X is the "left and right").

Important: You must set the "Coordinate System of Input Coordinates" to the Projection system those coordinates were made with, not necessarily the projection of your data frame.

Set projections must always correspond to the data's original projection, not the one which you want it to conform to. If you try and force data into a projection that it wasn't made in, ArcMap will automatically place it in a noticeably incorrect location (ie South America) until you set it correctly.

Example: If you are entering XY data taken from points in the field from a GPS set to WGS84 (most GPS are defaulted here), then your "Coordinate System of Input Coordinates" should be set to WGS84.

Specify where you want your feature class saved and what to call it.

If your data is in a different projection that that in your Map Document, you will have to use the "Project" tool to transform it into the correct one. See "Adding a layer with a different projection than that set in your data frame" above.

Upload (or drag from ArcCatalog into ArcMap) your new feature class into your Map Document.

9.8.2.3 When creating a new shapefile

Specify the coordinate system to match the system you have chosen to use in your Map Document and Data Frame.

If you choose to set the coordinate system differently at its creation, you must use the "Project" Tool in the ArcToolbox to create a new layer with matching coordinates. Then add the newly projected layer and delete the old to keep your Map Document "happy."

9.8.2.4 Rasters and Projections

It is possible to re-project a raster layer (example: aerial photograph) using the "Project Raster" tool in the ArcToolbox.

However, this has only worked for me with very small aerials—it took over eight hours each to process and altered some of the coloration.

By far the best idea is to **find the base raster you need to use and conform all other shapefile layers to its already determined projection**, since feature projection transformations (with the "Project" tool) are easier to execute and do not produce any minor or major data loss, as experienced with raster transformations.

In sum, the goal is to **keep all your usable layers in the same, matching projection** system. If your layers do not match, some may look like they are placing themselves correctly "on the fly," but any analysis on these layers will hit major problems in the future. Other mismatched layers may not project "on the fly" at all, and leave your data stranded in Hong Kong.

Knowing what projection you wish to use from the beginning and *knowing how to transform already set feature classes and rasters* into newly projected layer files will keep you sane.

9.8.3 Making a Grid

First, be sure you have AlaskaPak downloaded to ArcMap (it's free): http://science.nature.nps.gov/nrgis/applications/gisapps/gis_tools/8x/alaskapak.aspx

-Open your ArcMap document and make sure you can see the AlaskaPak toolbar (Go to View→Toolbars and then select NPS AlaskaPak if it isn't open yet)

- Click the "Generate a Grid" icon in the toolbar.

-Select the area of the map over which you want to draw a grid by pulling a square over the area with your mouse.

-Specify which dimensions you want your grid to take in the window that pops up automatically.

-Note that you can randomize the origin of the grid lines and rotate the area you've selected if you'd like your grid to go at an angle (you can also rotate your grid later in Editor).

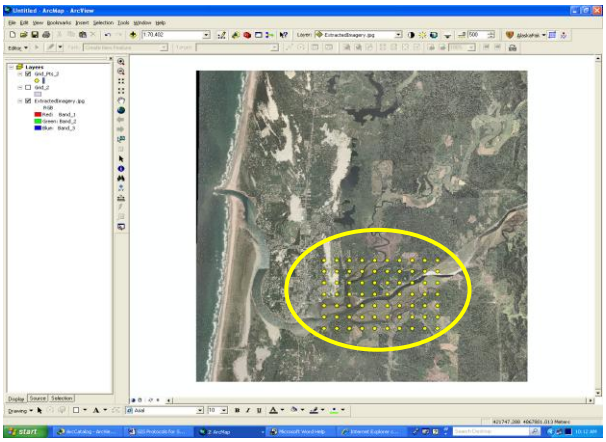
- Be sure to navigate to the correct save folder and call the Grid something that you will be able to recognize.

-After you click "Ok" ArcMap will ask you if you want to generate center points for the grid. Click yes. If you hit an error message, don't worry (this seems to happen a lot). Just x-out of the Error Log.

-Your map should have both a filled-in grid polygon (and another layer of center points if the first try worked).

-To generate centerpoints, go to the AlaskaPak menu.

- Select "Generate Grid Points." Again, specify which folder you want to save this new shapefile in and name it appropriately (like, no cuss words). OK



9.8.4 Drawing Polygons

- Open ArcCatalog

- Navigate to the folder in which you want to save your new polygon layer.
- Right click: New → Shapefile
- Name the new polygon and make sure the Feature Class is set to "Polygon"
- Edit: Set your Spatial Reference to match your other layers/data frame
- OK
- In ArcMap
  - Add your newly created polygon layer into your Map document
    - Nothing will appear on the map as you haven't drawn it yet, but it should be in your sidebar (also called the "Table of Contents").
  - Editor → Start Editing 

  - Find the folder in which you created your polygon layer. → OK
  - Make sure "Target" field matches the layer you wish to edit (red circle).

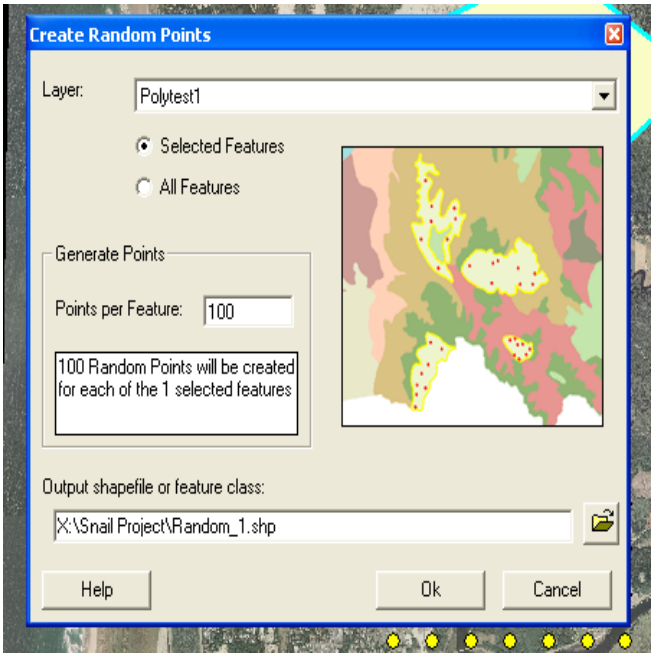
- Switch from the Pointer to the Pencil (blue circle) in the Editing toolbar.

- Click once to establish your beginning point and draw your polygon.
- Double-click again on your first point to close your polygon.
- Editor → Save Edits →Stop Editing

9.8.5 Generating Random Points

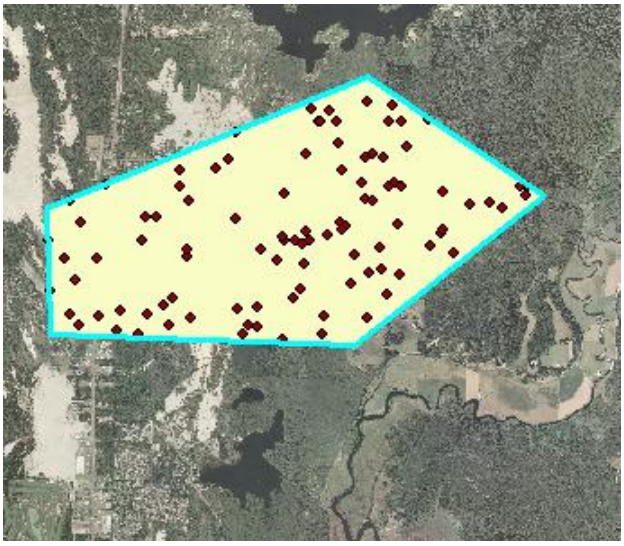
- Draw polygon(s) over the area in which you wish to create points.

- In the AlaskaPak menu select "Random Points."
- Identify the polygon layer within which you are generating points.
  - If you have multiple polygon shapes within a single polygon layer, consider whether you want to generate points in "selected features" or "all features". If your polygon layer is one shape, then it will automatically select "all features"



- Enter the number of random points you would like generated in each shape of your polygon layer.
- Specify where you would like your new random points layer saved, and any specific name you would prefer.
- OK

- "Add new shapefile as a layer?" → Yes. This will display your random points as a new point layer in your Map document automatically. Otherwise, you will have to browse in ArcCatalog to find your newly saved random points layer.



9.8.6 Adding Lat/Long to the Attribute Table of a Point Shapefile in ArcMap

At times it may be necessary to add Latitude and Longitude values into your layer, particularly in layers that you create from scratch. Since we generated all our grids and sampling points from AlaskaPak, our new layers did not have Lat/Long and we needed that information linked to each waypoint so we could later link the Waypoint and Lat/Long to all the field data.

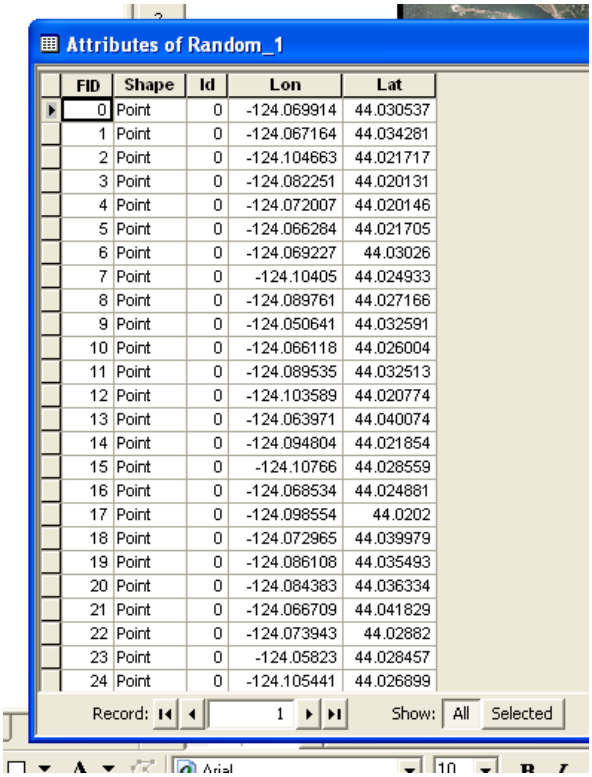
- Open AlaskaPak Menu

- "Add XY Coordinates"

- Select the layer to which you want to add Lat/Long data → Next

- Select your preferred coordinate format (decimal degrees, etc.) and make sure your fields match the data types (x with longitude, y with latitude)
- OK

- Check to see your new Lat/Long values by right clicking on the layer in the Table of Contents to the left → "Open Attribute Table"



Successful Lat/Long Addition

9.8.7 Uploading Points from ArcMap to Garmin GPS

If you generate your sampling points from ArcMap, you will need to upload them into a GPS system to accurately identify the site in the field.

Turn on GPS unit and plug into USB port connector.

Open ArcMap document and highlight in Table of Contents (one left click) the layer you wish to upload into unit.

Open DNR Garmin program.

File → Set projection to the projection of your data frame and/or particular layer to upload.

File → Load From → ArcMap → Layer

Waypoint → Upload

9.8.8 Downloading Points from Garmin GPS to ArcMap

Sometimes we went "off grid/off transect" to get sampling points. If our transect fell on bedrock, but a marsh was 200 meters north, we would go to the marsh to

sample instead. This, of course, meant that our data was going to land in a different spot on our Map, not on the pre-determined transects.

To solve the problem, we would save these points on the GPS while in the field and label our data according to the waypoint number we set on the GPS. After returning to the lab, we could then download these saved points to ArcMap and eventually merge them into the appropriate layers and with the correct field data while maintaining spatial integrity.

Here you can see where we obviously went "off grid/off transect" to get a good sampling of data in Jordan Cove. These points were downloaded from our Garmin GPS units.

To download waypoints:

- Turn on GPS unit and plug into USB port connector.
- Open DNR Garmin program.
- Waypoint → Download
- File → Save to… → ArcMap → And Choose Shapefile layer or Geodatabase Feature class

9.8.9 Calculating Attribute Table Fields in ArcMap

We used this function to create fields like “Quad-ID” which allowed us to give each point a unique identifier and thus link all our data accurately to the map itself. Essentially, in this case, we calc’ed a field to say “SS450” for South Slough, waypoint #450. Before calc’ing that field, we only had “450” in the South Slough attribute table, which didn’t allow us to properly link data since there were “450”s in Isthmus, Haynes, and Coos River. SS450, however, was completely unique.

The Field Calculator also allowed us to create "fid" values that would fit into our GPS systems. If, for example, we wanted to load two regions into our GPS's that day, we would have had to deal with the fact that each of those regions had "fid" values beginning at 1. We would not have been able to determine which "1" belonged to which region easily. Using field calculator, we simply created a new field containing values to which we had added "300" or "500". This then allowed us to upload waypoints 1-250 from Isthmus and waypoints 301-405 from Haynes. No overlap. Problem solved.

- Open your map document and find the layer you wish to work within the Table of Contents.

- Right click → Open Attribute Table

- Click "Options" → "Add Field"

- Name your new field (Do not use any spaces or more than ten characters)

    - To Decide which "Type" to use if your data is numeric:

Data type	**Storable range**	**Size (bytes)**	**Applications**
Short integer	-32,768 to 32,767	2	Numeric values without fractional values within specific range; coded values
Long integer	-2,147,483,648 to 2,147,483,647	4	Numeric values without fractional values within specific range
Single-precision floating-point number (float)	Approximately -3.4E38 to 1.2E38	4	Numeric values with fractional values within specific range
Double-precision floating-point number (double)	Approximately -2.2E308 to 1.8E308	8	Numeric values with fractional values within specific range

- You can also select "Text" or "Date" as field "Data Types."

- Some "Types" will ask you to specify the maximum length of any data set within your new Attribute field (this is measured in # of characters). If you know your field will contain numbers only up through the 9999, then specify a length of "4".

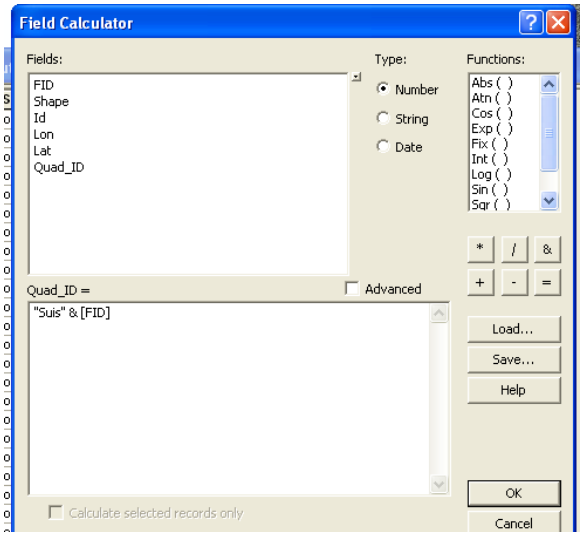
- Right Click on the heading of your new Field → Field Calculator.

Now, let's pretend that we want to create a new Attribute field that Attaches "Suis" to the set "Fid" numbers, giving us a unique "Quad_ID" that we can use for data sampling in a larger project.

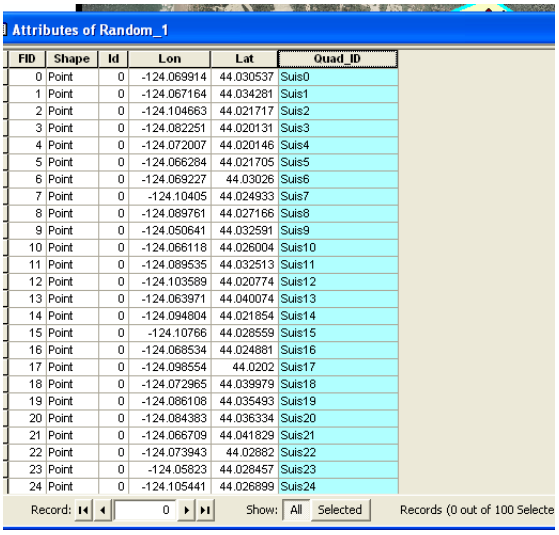
- Type "Suis" (in parenthesis) & (adds text or numbers onto other numbers, does not connote a mathematical function) and then double clicked "FID" in the "Fields" window above.

- OK

Of course, you can use any of the mathematical functions available to the right of the "Field Calculator" window. For example, you could easily create a new field called "Temperature Difference" and calculate:
[Temp_Monday] – [Temp_Tuesday]



An example calculation. Note the many functions available on the right side of the window.



The Results!

9.8.10 Getting GIS Lat/Long Into Your Access Database

To begin, you must have two things:

A UNIQUE ID for every waypoint that matches in the GIS attribute table and the Access database.

(Because we didn't think ahead on this, we had to calc out Unique IDs in ArcMap, which everyone will have to do regardless, *and* we had to do it in Access as well (it was a pain). If we had been entering our data with Unique IDs from the beginning, it would have saved some time.

All Lat/Long data in your ArcMap attribute tables, using the same Coordinate Format. See "Adding Lat/Long to the Attribute Table of a Point Shapefile in ArcMap."

Now, we must **A)** Export our GIS attribute tables to an easily edit-able program [Excel]. **B)** Edit our tables together (if you had multiple sampling regions that were created as different layers) so that they just display our UniqueID, Lat, and Long. **C)** Import this new excel table into our Access database where all field data is stored. **D)** Create a query that will draw the proper relationships between the Access and GIS UniqueID numbers and include all our field data as well as our Lat/Long in one nice table.

Exporting GIS Attribute Table

Open your attribute table → Options → Export

Specify where you want to save the exported .dbf file. → OK

DO NOT add it to your map document (I mean, it won't kill you, but it's sorta pointless).

Minimize ArcMap and open Microsoft Excel. File→Open (make sure you select the ability to see "All Files," .dbf files do not show up as excel files)

Locate your saved .dbf file and double click to open.

Editing tables

If you had to export multiple attribute tables that you want to combine, you'll have to open each one in a separate Excel document and then copy/paste them all into one.

Once all your tables are together in one big Excel file, Re-SAVE AS AN EXCEL FILE, not as a .dbf. You should always keep your UniqueID (whatever you call it), Lat, and Long fields. Any other fields can be deleted (FID/ID/Shape/etc.) The point is to just have a table with your Unique IDs and their spatial information.

Importing your GIS data/Excel table into Access.

Open your Access database.

File→Get External Data→Import

Be sure you are viewing Excel tables and navigate to where you saved your final edited Excel spreadsheet.

Import

Follow the Access Import Wizard to your specifications. Note: Be sure to save this as a New Table, do not try and add it to another table.

Queries

Now, be sure to draw the relationship between the UniqueID field of your new Lat/Long table and the table with all your field data. Then run a query to combine all the desired data from both tables into one MASTER table.

This master table will now have all your field data linked to Lat/Long data. And now you're just a right click away from creating a Feature Class from this new Access Table and adding it back into your map document. It will show all your points and your Attribute Table will contain all the data from Access.

See "Adding data from a table (Excel spreadsheet, Access table, etc.) to your Map Document".

9.8.11 Creating a Geodatabase

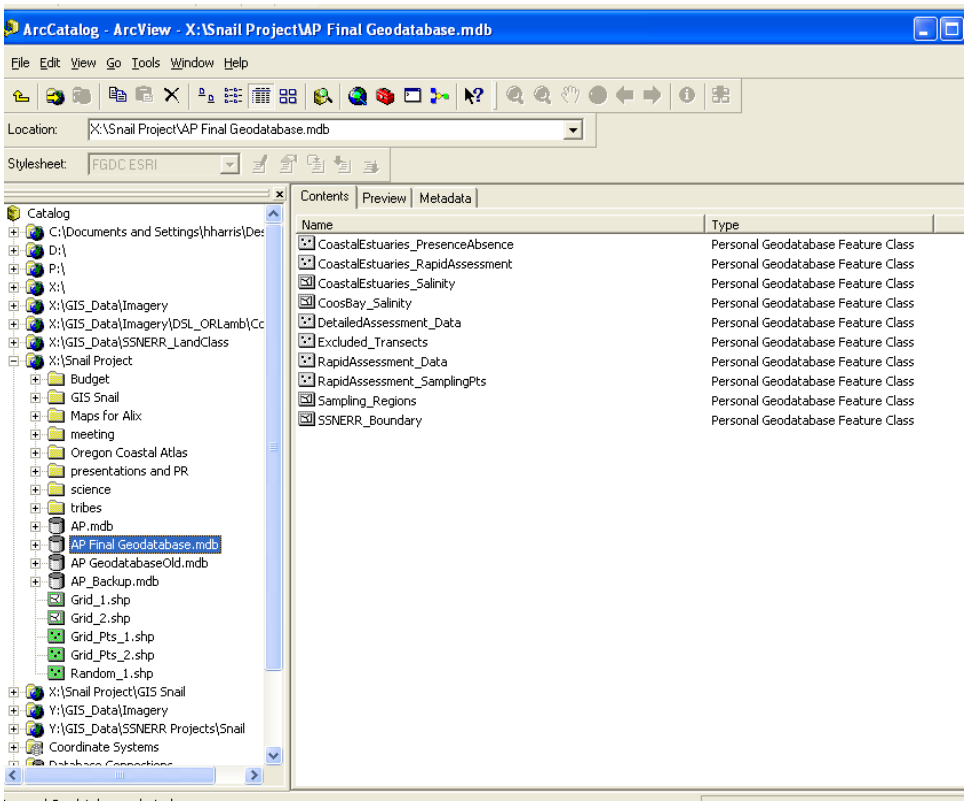
A geodatabase provides you with a single, organized location to put your spatial data (whether it is in table or shapefile format). An added bonus? After you load your data into a geodatabase, it can be generally accessed but the data itself cannot be edited, altered, or deleted by those who later view it.

There are two types of geodatabases that you may create:

A **file geodatabase** stores datasets in a folder of files on your computer. Each dataset is held as a file and can be up to 1 TB in size (and you can optionally configure a file geodatabase to store much larger datasets). File geodatabases can be used across platforms and can be compressed and encrypted for read-only, secure use.

NOTE: If you do not know what kind of geodatabase you want to create, this [File geodatabase] is a good default choice.

A **personal geodatabase** stores its datasets in a Microsoft Access .mdb file on disk. The storage sizes of personal geodatabases are effectively limited to between 250 and 500 MB for the entire geodatabase and are only supported on Windows. Users often need larger storage for their datasets, so they choose file or ArcSDE geodatabases.



Note how all the feature classes in this Geodatabase appear in a silver tone rather than the green of the shapefiles (you can see some shapefiles in the Table of Contents to the left). This shows the Geodatabase's slightly more "permanent" status; features are easily edited by their creator, but not able to be manipulated by others who may later wish to view the data.

To Create a Geodatabase:

- Open ArcCatalog and navigate to the location you would like to save your geodatabase.
- Right click → New → And select either Personal or File Geodatabase.
- Re-name your database something that will best identify your project.
- To populate your geodatabase:
    - You can create new tables or feature classes from within the geodatabase itself (Right click on the geodatabase → New → Feature Class or Table
    - OR, and probably more useful, you can put already created shapefiles/tables/rasters into the created geodatabase by importing or exporting.

To Import Files (Shapefiles/Tables/Feature Classes):

- Find your geodatabase in ArcCatalog,
- Right Click → Import → Select the type of file form you wish to import [Usually this will be a table or feature class(es)].
    - Input features: Browse for the shapefile you wish to import into your Geodatabase
    - Output feature class: Specify what you would like your new geodatabase-contained feature class to be named.
    - OK

To Export Files (Shapefiles/Tables/Feature Classes):

- In ArcCatalog, navigate to the file you wish to export into your geodatabase
- Right click → Export → To Geodatabase (single)
    - Output location: Browse to your geodatabase
    - Output feature class: Specify what you would like your new geodatabase-contained feature class to be named
    - OK

9.8.12 Retrieving an Aerial Photograph for a Small Area in Oregon

- Access http://imagery.oregonexplorer.info/DHTML.htm
- Select the area you wish to download.
- "Extract and Download" → Specify the projection
- Follow instructions and be sure to save to the folder you wish your aerial to download to.
- Note: There is a limit to the file size allowed for download. The area you choose *must* be pretty small. *Smaller than the total area of SSNERR.*

9.7 Reproductive staging

Female Reproductive stage descriptions:

Ripe: Spherical "hard" nucleate, unencapsulated eggs present (40 μm diameter), often abundant (=>10 count) spilling out of body when the shell is cracked. Full to nearly empty ovary (10-90+%) with the coloration of ovary contents less white and less milky than the Active stage. Abundant "soft" nucleate eggs are often present if the ovary is torn. Snails with extremely reduced ovaries were occasionally observed producing enough spherical eggs to be considered Ripe.

Spent: Ovary clearly depleted (<5-10%) often orange to red follicles in large individuals and no white gamete material. Spherical eggs can be present but few (<10 count) and no soft eggs present.

Active: Transitional stage between Spent and Ripe. Ovary (10%-100%) with white areas often in distinctly lobed follicles when less than 60% full, usually milky when pressed or torn. Usually no spherical eggs, often a few soft eggs if ovary torn. This stage includes apparent reabsorption of ovary contents without egg production starting in August and continuing through November.

Reproductive stages for males

Male staging is less clear than the female stages and may not be important for this study. Males can be assumed to be active when females are receptive. The potential for long-term sperm storage in the females may also be a complicating factor. In the males the testis is very variable in coloration but generally ranges from pale yellow to a brownish orange to a bright fluorescent orange with amber refringent material. The folded seminal vesicle portion of the vas deferens near the testis is filled with a bright white sperm filled fluid when ripe and tends to shrink and darken to a brownish-orange coloration when emptied. Individual sperm observed at high magnification are very similar in appearance to those of *Angustassiminea californica* (Fowler 1980) and are easily detected by the rapid motion of their tails. Packets of densely packed nonmoving sperm were occasionally observed in females and are assumed to have been recently deposited by males. On one occasion, individual sperm movements were detected within the digestive cecum of a female.

9.8 Photographs

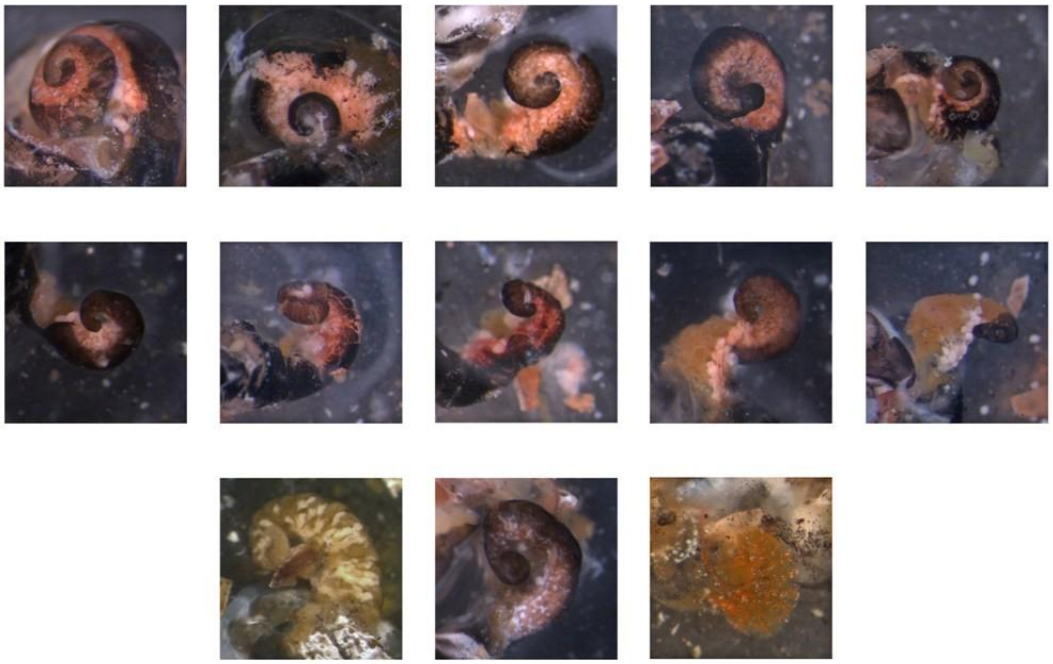
Typical Broad Marsh in the Coos Bay Estuary

Typical Compressed Marsh in the Coos Bay Estuary

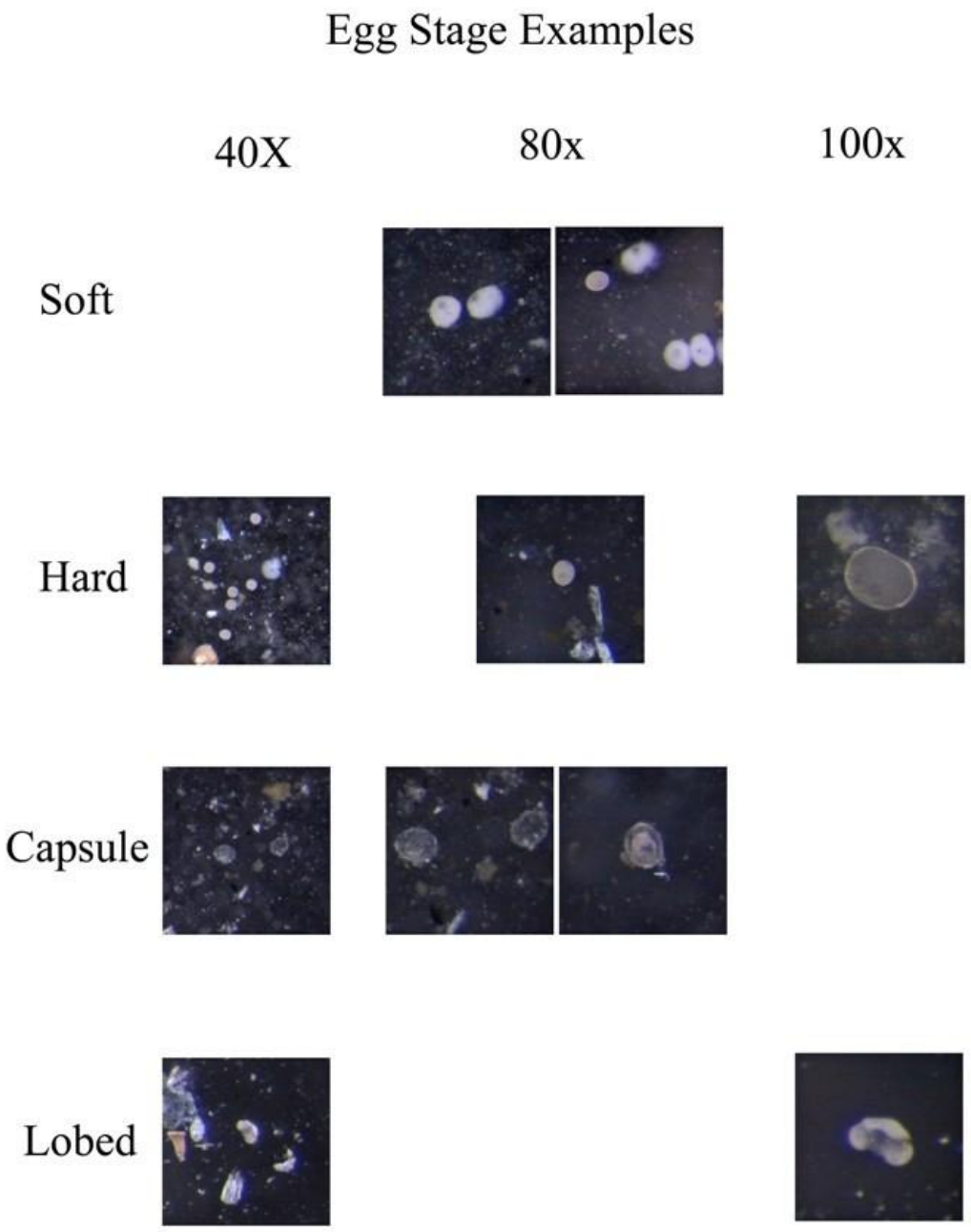
Range of Sizes for *A. parasitologica* in comparison to a Penny

A. Parasitologica, A. californica, and *M. myosotis* in comparison to a Penny

A. parasitologica Male and Female Pair Mating



A. Parasitologica Ovary Series



A. Parasitologica Egg Stages

9.9 Presentations, Outreach and Disseminations:

Presentations:

· Smith Watershed Project, Reedsport, Oregon 3-12/2009 Howard Crombie

· International Biological Invasions Conference, Portland, Oregon
August, 2009 A. Laferriere

· Pacific States Marine Fisheries Commission: 100th meridian Initiative meeting
Portland, Oregon, October, 2009 A. Laferriere

· Coastal and Estuarine Research Federation Meeting, Portland, Oregon
November, 2009 A. Laferriere

· South Slough National Estuarine Research Reserve Commission Meeting
November 2009 A. Laferriere

Outreach & Dissemination:

South Slough website http://www.oregon.gov/DSL/SSNERR/

Oregon Coastal Atlas *www.coastalatlas.net*

Education Materials with Oregon Sea Grant (in process)