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OSMB AIS Funds Final Report: Task 1 Boat Ramp Monitoring for New Zealand Mud Snails

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Final Report prepared by Valance Brenneis, Sam Cimino, and Angela Strecker

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Abstract

The New Zealand mud snail (*Potamopyrgus antipodarum*; NZMS) is an invasive species found in a variety of ecosystems in Oregon, including brackish estuaries, heavily used recreational rivers, and highly trafficked coastal freshwater lakes. NZMS are an invasive species of concern because once established, they may out-compete native invertebrate grazers, such as native insect larvae that provide important food resources for fish, and NZMS themselves provide little nutritional value. Monitoring for the presence and population density of NZMS was performed at boat ramps located along several water bodies in 2006 – 2007. These water bodies were then re-sampled during the summer of 2012 and winter of 2013 to investigate changes in population densities, as well as the potential spread of NZMS. In addition, six brackish estuaries and six freshwater coastal lakes were sampled in the summer of 2013 once again for population densities but also for stable isotope analysis to better understand the influence NZMS may have on these different ecosystem food webs. This work addresses how NZMS densities differ across invaded sites, how densities vary over time, how community diversity in rivers and estuaries varies between sites and over time, whether there is a correlation between NZMS densities and invertebrate diversity, and whether NZMS density correlates with decreasing dietary specificity for competing benthic invertebrates. Samples were sorted, identified, and counted, and stable isotope analyses from the summer 2013 field season were conducted on macroinvertebrates, zooplankton, and primary producers at the UC Davis Stable Isotope Facility. NZMS densities were found to be dynamic, with population densities increasing and decreasing over time and space. Additionally, two waterbodies (the lower Siuslaw River and the Nestucca River Estuary) previously without reported NZMS presence were identified to have established NZMS populations. Across invaded sites during the 2006 and 2012 sampling periods, there was a strong negative correlation between NZMS density and the diversity of the benthic invertebrate community. Within sites, no significant correlation was detected. High NZMS population densities were found to have a significantly negative relationship with detritivore density populations in the freshwater lake sites sampled in the summer of 2013. There were no significant relationships between NZMS and the density of any particular macroinvertebrate feeding habit group in estuaries. Additionally, stable isotope analyses indicated that NZMS at high population densities in the freshwater lakes share a similar stable isotope signature with macroinvertebrate herbivores, but NZMS had a more specific or narrow dietary range suggesting macroinvertebrate herbivores had more generalized diets than NZMS when at high densities. Conversely, NZMS population densities in the brackish estuary ecosystems showed no discernable patterns from the stable isotope analyses to indicate that their presence, either at high or low density, had an effect on the estuarine food webs. We concluded that continued research on long term population dynamics of invasive species like NZMS and their effects on native food webs remain crucial to future management and mitigation.

Introduction

The New Zealand mud snail (*Potamopyrgus antipodarum*; NZMS) is an invasive species found in a variety of ecosystems in Oregon, including brackish bays of the Columbia River Estuary, heavily used recreational rivers including the Deschutes and Umpqua, and highly

trafficked coastal freshwater lakes such as Devils Lake in Lincoln City. These sites are popular with boat users and anglers, and several sites have signage advising boaters to be aware of New Zealand mud snails and wash their gear and boats before entering another water body. New Zealand mud snails are an invasive species of concern because once established, snails may out-compete native invertebrate grazers, such as native insect larvae that provide important food resources for fish, and the snails themselves provide little nutritional value (McCarter 1986, Hall et al. 2006). NZMS are very small (<5 mm in length) and are very tolerant of desiccation (Phillips and Lambert 1987), which allows the snails to be easily moved between water bodies by water users, such as fishers and boaters, who may unknowingly transport them on their gear (Loo et al. 2007b). NZMS are very tolerant of a wide range of abiotic conditions (Dybdahl and Kane 2005, Zaranko et al. 1997, Jacobsen and Forbes 1997), and are currently found in rivers, lakes, and brackish estuarine systems in the State of Oregon (Figure 1). The impact of these snails is likely to differ between these different types of systems and the associated biological communities. NZMS were first documented in the Western US in the mid-1980s in the Snake River drainage (Bowler 1991) and are now found in lakes, rivers and estuaries of many states (Zaranko et al. 1997) (Figure 2).

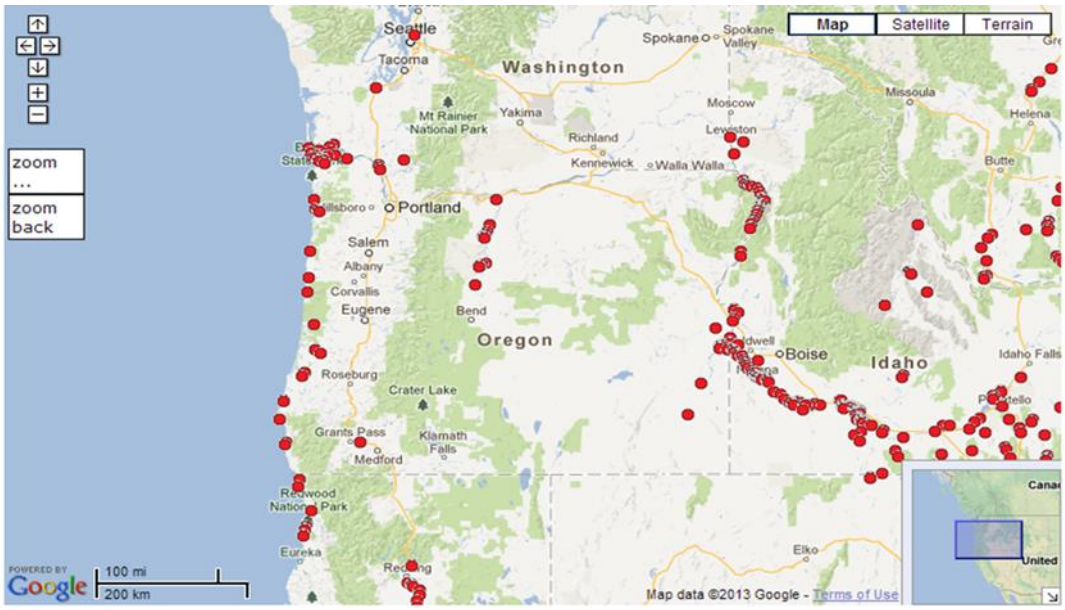


Figure 1: Pacific Northwest New Zealand mud snail distribution. Source: Benson, A. J. 2014. New Zealand mud snail sightings distribution. Retrieved 6/24/2014 from newzealandmudsnaildistribution.aspx

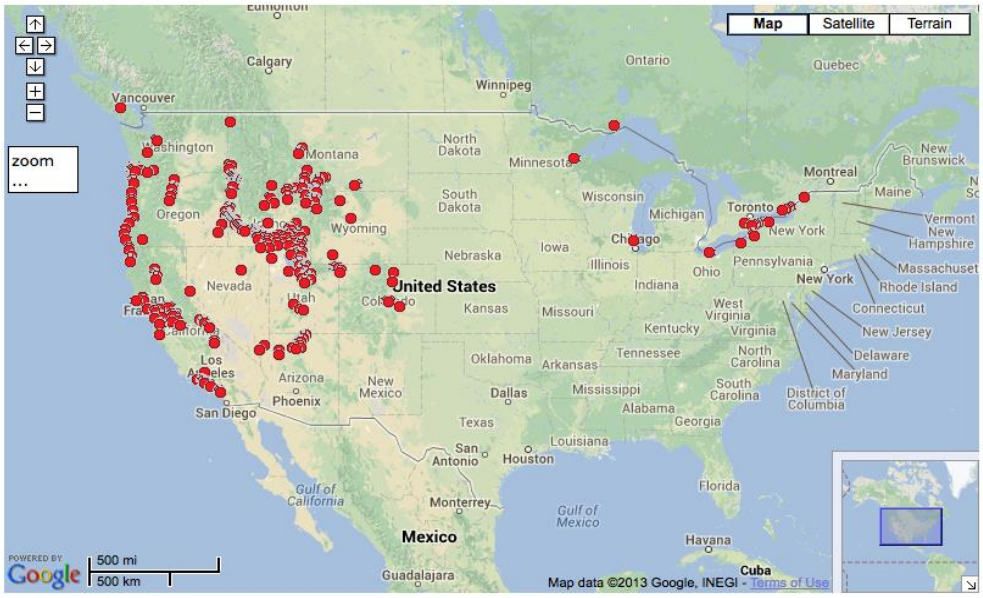


Figure 2: North American New Zealand mud snail distribution. Source: Benson, A. J. 2011. New Zealand mud snail sightings distribution. Retrieved 7/24/2013 from newzealandmudsnaildistribution.aspx.

Baseline monitoring for the presence and density of New Zealand mud snail (NZMS) was performed at boat ramps located along several water bodies in 2006 – 2007. As part of this grant, we re-sampled these sites during the summer of 2012 to look for changes in population densities, as well as the potential spread of this species (Figure 3). A few of these sites were re-sampled during the winter of 2013. In addition, five new brackish estuaries and six new freshwater coastal lakes were sampled as well as a re-sampling of the Columbia River Estuary at Youngs Bay in the summer of 2013 (Figure 4**).** The objectives of this survey were to quantify: 1) benthic invertebrate diversity and density across two pairs of rivers, six estuarine sites, and six lake sites 2) compare New Zealand mud snail (*Potamopyrgus antipodarum*; NZMS) population and benthic invertebrate community data between surveys performed during the summers of 2006 and 2012 and 3) compare the effect NZMS have on the benthic food webs of the estuarine and freshwater lake systems sampled in the summer of 2013.

The 2006 and 2012 sampling sites included two estuarine sites in Youngs Bay in the Columbia River Estuary and multiple sites located in upstream and downstream reaches of two pairs of rivers (Figure 3); these rivers were located in adjacent watersheds with similar abiotic conditions but different invasion status (Table 1). These pairs of rivers were the Umpqua (NZMS present) and Siuslaw (NZMS absent) and the Deschutes (NZMS present) and John Day (NZMS absent). Rivers with a history of NZMS invasion were paired with rivers in adjacent watersheds where there was no history of NZMS. Reaches with and without NZMS were selected in each river and described as upstream or downstream. Sites were chosen based on access, in the form of a fishing access, boat ramp, or public trail. In some rivers, public access was more limited than others (i.e. the John Day River) and for this reason, all 'sites' were located at one access point, whereas in other rivers (i.e., the Siuslaw River), there were more access points and each access point represents a different site.

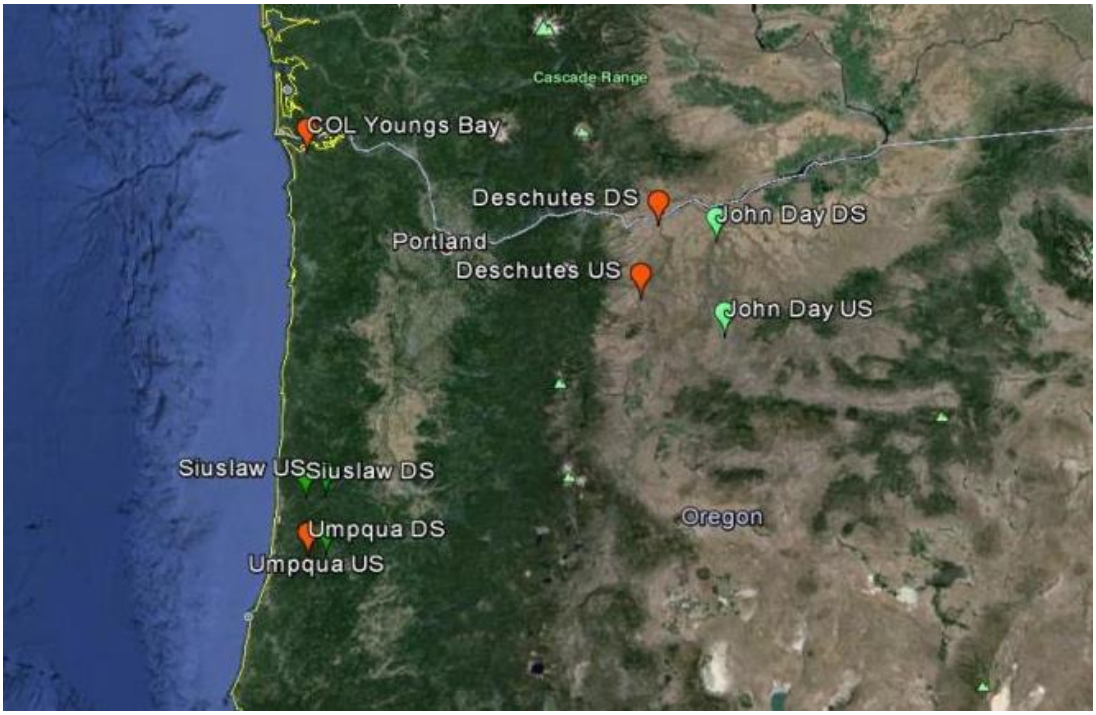


Figure 3: Sampling reaches in the 2006 and 2012 portion of this study.

Table 1. Sampling design for NZMS and benthic invertebrate surveys in the 2006 and 2012 sampling period. The number of sites sampled in each reach is indicated in parentheses.

Region	River	Reaches (fishing access/boat ramps)	NZMS in 2006?	NZMS in 2012?
Coastal rivers	Siuslaw River	Upstream sites (4): Rocky substrate	No	No
		Downstream sites (4): Tidally influenced, muddy substrate		Yes, at Tieman
	Umpqua River	Upstream (4): Rocky substrate	No	No
		Downstream (4): Tidally influenced, muddy substrate	Yes	Yes, but at lower densities.
Columbia Plateau rivers	John Day River	Upstream (4)	No	No
		Downstream (4)	No	No
	Deschutes River	Upstream (5)	Yes	Yes, lower densities at most sites.
		Downstream (4)	Yes, very low densities	Yes, low densities
Columbia River Estuary	Youngs Bay	East side: Yacht Club (1) West side (1)	Yes, high densities	Yes, high densities

Figure 4: Summer of 2013 sampling locations along the coast of Oregon, USA: The six yellow markers indicate estuaries sampled and the six blue/green markers indicate freshwater lakes sampled.

New Zealand mud snails provide an interesting case study of an invasive species that is tolerant of both brackish and freshwater conditions, and they are likely to have different impacts in estuarine versus lake communities. In order to investigate the impacts in these different systems, the summer of 2013 sampling locations included six estuaries along the Oregon coast from Astoria (Youngs Bay in the Columbia River Estuary) to Gold Beach (Rogue River Estuary) and six freshwater coastal lakes (Figure 4). Sampling locations for this study were determined using the USGS New Zealand mudsnail distribution map (Figure 1).

All coastal freshwater lakes in Oregon with reported New Zealand mudsnail sightings were selected for this study: Coffenbury Lake, Lake Lytle, Devils Lake in Lincoln County, and Garrison Lake (Figure 4). For comparison, four brackish water estuaries in close proximity to each freshwater lake with varying densities of NZMS were selected: Columbia River Estuary at Youngs Bay, Memaloose Point in the Tillamook River Estuary, Yaquina Bay, and the Lower Rogue River Estuary (Figure 4). In addition to the sites with NZMS present, reference sites without previously reported NZMS sightings were also sampled. These included two sites for the estuarine system, Nestucca River Estuary and the Coquille River Estuary at Bandon, and two coastal freshwater lakes, Mercer Lake and Cullaby Lake (Figure 4). Upon sampling, New Zealand mud snails were found to be present at low densities in the Nestucca River Estuary. This detection was reported to the U.S. Geological Survey as well as the Oregon Department of Fish and Wildlife (ODFW).

Ecologists have long been interested in the relationship between community diversity and the ability of invasive species to become established (invasibility) (Elton 1958). Invasibility is

not equivalent to the vulnerability of systems to negative impacts from invaders once they do become established (Elton 1958). After initial surveys of aquatic communities in Oregon where NZMS have been introduced and successfully established, there appeared to be a negative correlation between native benthic invertebrate diversity and NZMS density. From the 2006 and 2012 sampling period, the sites with the highest recorded NZMS density were in highly disturbed estuarine sites (Youngs Bay, Columbia River Estuary) where native diversity is fairly low and the community is dominated by a few common species. NZMS have also become established in highly diverse benthic communities, such as those found in the Deschutes River, but have not maintained high-density populations at these sites.

This work addresses both applied and basic ecological questions, specifically:

1. How does the density of NZMS differ across invaded sites with varying environmental and biological characteristics?
2. How does NZMS density vary over time?
3. How do the benthic invertebrate communities differ between these sites? Over time? With NZMS density?
4. Is there a correlation between NZMS density and benthic invertebrate diversity within systems? Across all systems?
5. Does NZMS density correlate with decreasing dietary specificity for competing benthic invertebrates at invaded sites?

Methods

Benthic invertebrates were sampled quantitatively in rivers and lakes by disturbing a fixed area of substrate and collecting organic and inorganic materials in D-nets. Vertebrates, such as larval fish, were removed from the nets, and the contents were sieved and preserved in 70% ethanol (in 2012 only). We took special care to disinfect our gear between sites. These samples were then sorted, identified, and counted in the Aquatic Bioinvasions Research and Policy Institute (ABRPI) lab located at Portland State University (PSU). Abiotic conditions, including a rapid riparian assessment and measurements of salinity (‰), conductivity ($\mu S \cdot cm^{-1}$), dissolved oxygen ($mg \cdot L^{-1}$), pH, and temperature (°C), were recorded during sampling trips and will be used in conjunction with longer-term data sets available through the USGS National Water Information System. In addition to analysis of the change in New Zealand mud snail population densities over time, we calculated univariate metrics and looked for correlations between NZMS density and benthic invertebrate community diversity and abundance. In addition to this univariate approach, we used multivariate statistics and ordination approaches to look at changes in community structure and composition over time and space. To better understand benthic food web structure in the invaded systems, samples of invertebrates and their food sources were collected at the estuarine and coastal lake sites in 2013 and analyzed for their stable carbon and nitrogen isotope ratios.

The ratio of the common carbon isotope (carbon-12 or ^{12}C) and the rare and heavier stable carbon isotope (carbon-13 or ^{13}C) is useful in determining the primary production source or sources responsible for energy flow in the ecosystem, denoted as $\delta^{13}C$, (Vander Zanden and Rasmussen 1999, Fry 2006, Michener and Kaufman 2007). In simpler terms “you are what you

eat" (Fry 2006). A primary consumer feeding solely on one primary producer will have a very similar $\delta^{13}C$ signature as the primary producer (McCutchan et al.2003, Fry 2006). The stable nitrogen isotope ratio, denoted as $\delta^{15}N$, shows trophic level position as $\delta^{15}N$ (the ratio of light,common^{14}N and the rare and heavier stable isotope ^{15}N) enriches or has more of the heavier ^{15}N in species as they move up in trophic level (Vander Zanden and Rasmussen 1999,Fry 2006, Michener and Kaufman 2007). Different consumers will have different $\delta^{13}C$ signatures due to variances in diet and variance in digestion during assimilation and metabolic processes, but these consumers may have very similar $\delta^{15}N$ if they are at the same trophic level (Vander Zanden and Rasmussen 1999, McCutchan et al. 2003, Fry 2006). Using stable isotopes to map food webs can show potential impacts abiotic or biotic disturbance (like the establishment of an invasive species) have on the food webs (Fry 2006). For instance, benthic invertebrates in competition with New Zealand mud snails have shown depleted $\delta^{13}C$ signatures; this depletion is due to less availability to feed on enriched $\delta^{13}C$ producers like periphyton (Moore et al. 2012). Stable isotope ratio analysis can provide a snapshot of an ecosystem's trophic structure and therefore a better understanding of the impact an invasive species may have on the community.

Table 2. Sampling design for NZMS and benthic invertebrate surveys for the 2013 sampling period. Each location was sampled at six different sites with various substrates indicated by the numbers in parentheses.

Ecosystem	**Location**	**Sampling sites: Predominate Substrates**	**NZMS?**	**Relative densities (Individuals·m^2)**
Coastal Freshwater Lakes	Coffenbury Lake	Littoral (5): Muddy/organic substrate, Pelagic (1)	Yes	Low (1- 100)
	Cullaby Lake	Littoral (5): Sandy/silty substrate, Pelagic (1)	No	Absent (0)
	Lake Lytle	Littoral (5): Muddy/organic substrate, Pelagic (1)	Yes	Moderate (101-1,000)
	Devils Lake	Littoral (5): Sandy/rocky substrate, Pelagic (1)	Yes	High (1001+)
	Mercer Lake	Littoral (5): Organic/sandy , Pelagic (1)	No	Absent (0)
	Garrison Lake	Littoral (5): Organic/sandy substrate, Pelagic (1)	Yes	Moderate (101-1,000)
Brackish Estuaries	Columbia River Youngs Bay	Mudflats (3), Rocky Shore (2), Pelagic (1)	No	High (10,001+)
	Tillamook River Estuary	Mudflat (2), Rocky Shore (3), Pelagic (1)	Yes	Low (1-1,000)

Ecosystem	**Location**	**Sampling sites: Predominate Substrates**	**NZMS?**	**Relative densities (Individuals·m^2)**
	Nestucca River Estuary	Mudflat/Organic (3), Rocky Shore (2), Pelagic (1)	Yes	Low (1 – 1,000)
	Yaquina River Estuary	Mudflat/Organic (5), Pelagic (1)	Yes	Low (1 – 1,000)
	Coquille River Estuary	Mudflat/Rocky (3), Rocky Shore (2), Pelagic (1)	No	Absent (0)
	Rogue River Estuary	Mudflat/Rocky (2), Sandy Shore (3), Pelagic (1)	Yes	Moderate (1,001 - 10,000)

Sampling design July-August 2006 and 2012

For Youngs Bay, two sites were sampled, one on either side of the bay. For rivers, two rivers with adjacent watersheds were selected in two regions (central Oregon coast and Columbia Plateau). One of these rivers in each region had documented NZMS presence as of 2006. Sites were sampled initially during July-August of 2006 and re-sampled in July-August of 2012. Within each river, upstream and downstream reaches (relative to each other) were selected (Table 1). Within each reach, four sites were selected based on accessibility. When access sites (e.g., boat ramps or campgrounds) were abundant (e.g., upstream Deschutes reach), sampling sites were spread out (one site per access point). When access sites were sparse, multiple sites were located at one access point; efforts were made to locate the sampling sites as far apart as possible (e.g., John Day river sites). Sites therefore represent only accessible, wadeable portions of the river segment selected. We took the following general precautions to prevent spreading of invasive species between water bodies. We sampled sites without (known) NZMS prior to sites with known NZMS populations, we used different shoes and D-nets between different water bodies sampled on the same day, and we scrubbed gear between sites when possible and allowed to dry completely in hot sun between watersheds. At all sites we completed a rapid assessment and measured temperature, salinity and conductivity using aYSI-80 probe. In addition to this summer sampling effort, three sites were re-sampled in the February 2013 (SIU-DS1, UMP-DS1 and UMP-DS2) to follow up on interesting changes in NZMS density.

Sampling design Summer 2013

Sampling locations for this study were determined using the USGS New Zealand mudsnail distribution map (Figure 1). For estuaries, six different estuaries were sampled. Four of the estuaries had documented NZMS presence as of 2013, and two of the estuaries did not have reported NZMS presence. For lakes, six different coastal lakes were sampled. Four of these lakes had documented NZMS presence as of 2013, and two of the lakes did not have reported NZMS presence. Sites were sampled during August-September of 2013. Within each estuary six sites were sampled. Five of the estuarine sites were in exposed mudflats or rocky shoreline and one sampling site was pelagic Table 2. Exposed shoreline sites were selected adjacent to boat access ramps. Within each lake, six sites were also sampled. One coastal lake littoral sampling site was

selected near a public boat ramp and then the lake perimeter was divided into four more sections and one site was chosen in each section to characterize the lake (Table 2). When diverse habitats were present in estuaries and lakes (weed beds, bedrock, cobble, riffle, run) an effort was made to sample across all habitat types to incorporate the maximum amount of diversity present at the location. We took the following general precautions to prevent spreading of invasive species between water bodies. We scrubbed gear between locations with a bleach water solution, rinsed off all equipment, and allowed to dry completely in the sun before entering the next waterbody.

Protocol for riverine sites 2006 and 2012

The sampling method used for these sites was modified from the OR DEQ protocols for wadeable streams (OWEB 1999). Within each site, 8 subsamples were collected (each representing a 1ft^2 area of benthic substrate) and pooled into one sample for the site. For each site, benthic invertebrates were sampled from grids located in the littoral and wadeable (<1 m deep) zone of the river (Figure 5). When feasible, grid placement was located randomly along accessible portions of each site (~10 - 100 m distance depending on site). In 2012, the distance from the access point was determined by using a random number generator (iphone app). The first grid will be located farthest downstream, at a randomly generated number of paces (~0.8 m) downstream of the access point. Invertebrates were sampled by placing a D-frame kick net (250 μm) against the stream bed with the net opening facing upstream, and then disturbing a 30.5 cm by 30.5 cm (1 ft^2) area of substrate upstream of net opening using hands/feet to a depth of up to 5 cm (depending on substrate type). When large rocks were present in the sample grid, they were scraped to remove invertebrates. When aquatic vegetation was present, the D-net was swept over the grid area several times. Two squares chosen from pre-selected positions in a three-by-three grid of nine 1 ft^2 squares were sampled; a total of four grids were placed along each site for a total of eight sub-samples (total substrate area = 0.74 m^2), these were then pooled in a 5 gallon bucket. The spacing between grids ranged between 10 and 20 m. When diverse habitats were present (weed beds, bedrock, cobble, riffle, run) an effort was made to locate the grids across all habitat types to incorporate the maximum amount of diversity present at the site. This was done by adjusting the distance from the bank at the randomly specified location along the stream. Samples of aquatic vegetation were collected and placed in ziplock bags and frozen for subsequent stable isotope analysis.

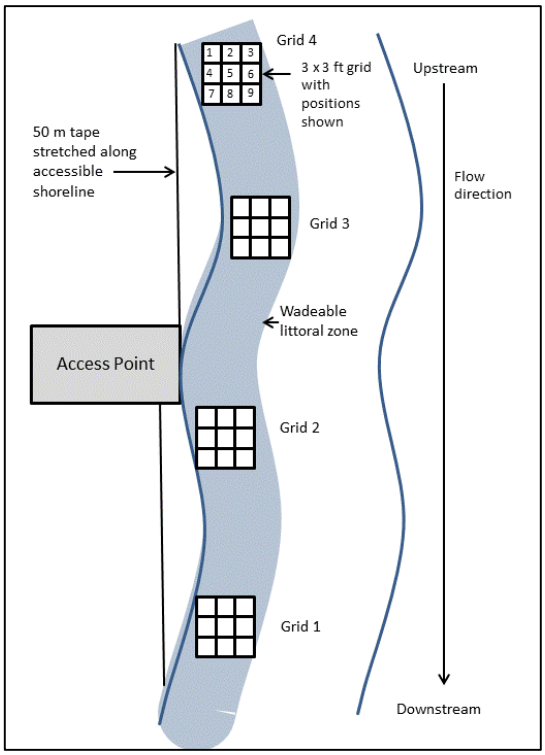


Figure 5: Placement of imaginary grids for collection of eight 1ft^2 samples substrate for benthic invertebrate collection.

Protocol for estuarine sites 2006 and 2012

In Youngs Bay, two sites were sampled; one on the west side of the Old HWY 101 bridge, and the other on the east side at the Astoria Yacht Club. Sampling of the exposed mudflats was performed using a circular PVC ring (0.073 m^2 in area, 30.5 cm in diameter, created by sawing off the top of a 5 gallon bucket). The sampling ring was placed at 3 locations within the accessible and exposed intertidal zone, incorporating both cobble and mud substrate at each site. Location of the first ring was determined by counting off the number of paces from the access point, determined by a random number generator (~1- 10). Direction was determined by using a random number generator (1 – 4) to indicate direction (1:N, 2:E, 3:S, 4:W). For each subsequent ring, location was determined similar, but based on the previous sampling location for a total of three rings (total area = 0.219 m^2). The top 2 cm of substrate was removed from the inside of the ring using a trowel and all material was placed in a 5-gallon bucket. Rocks were washed to remove any attached organisms. The contents of the bucket were then poured into a 500 μm sieve, and remaining material was placed in 500 mL bottle with ~90% ethanol. Samples were collected from 3 rings at each site; the contents of these samples were all combined (~2 bottles per site).

Protocol for quantitative sampling at coastal lake sites 2013

The quantitative sampling method used for these sites was modified from the OR DEQ protocols for wadeable streams (OWEB 1999). Within each location (lake), five littoral sites were sampled for macroinvertebrate densities. Eight subsamples were collected from each of the five sites (each representing a 1ft^2 area of benthic substrate) and pooled into one sample. Two randomly selected 1ft^2 sections ona 9 ft^2 grid were chosen for benthic sampling with the D-net. This sampling method was repeated four times at each of the five sites. Benthic invertebrates were collected in the littoral and wadeable (<1 m deep) zone of the lake. The first sampling site was located near a public boat launch, and the remaining four sample sites were accessed by canoeing around the edge of the lake. Invertebrates were sampled by using a D-frame kick net (250 μm) and disturbing a 30.5 cm by 30.5 cm (1 ft^2) area of substrate to a depth of up to 5 cm (depending on substrate type). When large rocks were present in the sample grid, they were scraped to remove invertebrates. When aquatic vegetation was present, the D-net was swept over the grid area several times. A total of four grids were placed along each site for a total of eight sub-samples (total substrate area = 0.74 m^2), these were then pooled in a 5 gallon bucket, sifted through using a 250 μm stainless steel mesh, and then preserved in 500mL Nalgenes at a concentration of 70% ethanol . Macroinvertebrates collected for quantitative sampling at the coastal lakes were counted at Portland State University using a subsampling method. All subsampling was done using the Caton standardized subsampling apparatus which consists of a standardized gridded screen (370 μm opening) and a tray (Caton 1991).

Protocol for qualitative sampling at coastal lake sites 2013

Samples for qualitative stable isotope analyses were collected at each site the quantitative macroinvertebrate densities were collected from each coastal lake. In addition, a pelagic sample was collected for qualitative analysis at each coastal lake using an Ekman grab sampler. For qualitative analysis, macroinvertebrates were sampled using a D-frame kick net (250 μm) and

disturbing the areas previously sampled for quantitative analysis in an attempt to capture the same species. The contents of the D-net were then sifted through a 250 μm stainless steel sieve and preserved in a 500 mL Nalgene at a concentration of 70% ethanol. Samples of aquatic vegetation were collected from the five littoral sites and placed in ziplock bags and frozen for subsequent stable isotope analysis. Baited minnow traps were used at the five littoral sites to catch secondary invertebrate consumers like crayfish, which were then preserved in 500mL Nalgenes at a concentration of 70% ethanol. Periphyton samples were collected at each of the five littoral sites by brushing periphyton off rocks and other smooth surfaces and filtering the periphyton on to glass fiber filters. The filters where then wrapped in tinfoil, put in ziplock bags, and flash froze and preserved using dry ice. Phytoplankton were collected at the pelagic site using a Van Dorn and filtered using an amber hour glass filter on to a glass fiber filter and wrapped in tinfoil. The phytoplankton sample was then frozen and preserved using dry ice. Zooplankton, also sampled at the pelagic site, were collected with a vertically towed 250 μm plankton net. Zooplankton were preserved at a final concentration of 70% ethanol in 90mL containers.

Protocol for quantitative sampling at estuarine sites 2013

Sampling of the exposed mudflats and rocky shoreline was performed using a circular PVC ring (0.073 m^2 in area, 30.5 cm in diameter, created by sawing off the top of a 5 gallon bucket). The sampling ring was placed at 5 different sites within the accessible and exposed intertidal zone at low tide, incorporating cobble and mud substrate at each estuary. A spade was used to dig up 2cm depth within the 30.5cm core benthic ring. Any large rocks within the core ring were scraped and cleaned for invertebrates. All material collected was then sifted through using a 250 μm stainless steel sieve. Macroinvertebrates captured were preserved in 500mL Nalgenes at a final concentration of 70% ethanol. Macroinvertebrates collected for quantitative sampling at the estuaries were counted at Portland State University using a subsampling method. All subsampling was done using the Caton standardized subsampling apparatus which consists of a standardized gridded screen (370 μm opening) and a tray (Caton 1991).

Protocol for qualitative sampling at estuarine sites 2013

Separate macroinvertebrate samples from each of the different estuarine substrates were also collected for stable isotope analysis. These macroinvertebrates were preserved in the same fashion as the quantitative samples in 500mL Nalgene in a 70% ethanol concentration. At the different substrates, periphyton were scraped off rocks and filtered on to glass fiber filter paper to be wrapped in tinfoil and then flash froze in dry ice. Macrophytes were also collected during low tide at the differing sampling sites and preserved in ziplock bags in an ice-packed cooler. Zooplankton were collected with a vertically towed 250 μm mesh plankton net during high tide in the pelagic reaches of the estuary. Zooplankton were preserved at a final concentration of 70% ethanol in a 90mL container. Minnow traps (5) were used during higher tide and at depths that were still submerged during low tide for secondary consumer collection. Phytoplankton were sampled during high tide with a Van Dorn and filtered using an amber hour glass filter on to glass fiber filter paper and wrapped in tinfoil. The phytoplankton sample was then frozen and preserved using dry ice.

Sample Processing and Analysis

Complete benthic invertebrate samples were later sorted in the lab. Organisms were identified to lowest level of taxonomic classification possible (often family) and counted. All samples from sites with NZMS were sorted analyzed and we will retain voucher specimens for confirmation. Students at Portland State University were trained to perform this work and created a photographic key for common species at our sites. This photographic key is available upon request.

Benthic invertebrate abundances were converted to densities (number of individuals per square meter) and a site by species matrix was created using Excel. The presence and density of NZMS was determined for all sites, and then the NZMS densities were removed from the matrix for further analysis of benthic invertebrate community structure. Several univariate metrics were calculated (excluding NZMS) for the 2006 and 2012 field season data. These included the total number of individuals, taxonomic richness, community diversity (using the Shannon Diversity Index, Eq. 1), as well as native snail diversity and richness.

Equation 1: Shannon Index $$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

For invaded sites and sampling dates in the 2006 and 2012 field seasons, we analyzed the correlation between the density of NZMS and the diversity of the recipient benthic community. We also analyzed the correlation between the density of NZMS and the density of individuals in the recipient community. Finally, we use non-metric multidimensional scaling (NMDS) to compare multivariate community structure between sites and years using R.

The program R was also used to perform multivariate principal component analyses. Principal component analyses (PCA) were used to determine correlations between environmental variables in the freshwater coastal lakes and NZMS densities (number of individuals per square meter) as well as environmental variables in brackish estuaries and NZMS densities from the 2013 field season. Univariate analyses were also calculated for the 2013 field season data. NZMS densities in freshwater lakes and brackish estuaries and the densities of other benthic macroinvertebrates of specific feeding habits (predators, collector-gatherers, detritivores, etc.) from respective habitats were analyzed through the program R.

Stable isotopes analyses of nitrogen (N) and carbon (C) were performed on preserved invertebrate, macrophyte, algal, and terrestrial vegetation samples. Samples were dried at 60°C for 24 to 48 hours until a constant dry weight was achieved and homogenized with a mortar and pestle. For invertebrates, 1 mg of dry weight was needed for stable isotope analysis and 2-3 mg of dry weight was needed for primary producer samples. All samples were then analyzed for $\delta^{15}N$ and $\delta^{13}C$ at the University of California at Davis Stable Isotope Facility. The UC Davis Stable Isotope Facility expresses measuring error as the long term standard deviation of 0.2 ‰ $\delta^{13}C$ and 0.3 ‰ $\delta^{15}N$. Additionally, all benthic invertebrate samples were preserved in 70% ethanol and this preservation process can alter isotopic signatures. To correct for altering of isotopic signatures, a constant adjustment factor can be used (Ventura and Jeppesen 2009). Preservation in ethanol was adjusted by subtracting 0.39 ‰ from $\delta^{15}N$ and 1.18 ‰ from $\delta^{13}C$ as advised by Ventura and Jeppesen 2009. We expected an enrichment of ~3.4 ‰ for N and ~1‰ for C between trophic levels (Fry 2006).

Stable isotope results from the 2013 field season were organized and analyzed in Excel. Macroinvertebrate taxa were categorized into specific feeding habits (predators, collector-gatherers, detritivores, collector-filterers, herbivores, omnivores, and NZMS). Feeding habit classification was primarily determined using the classification table developed by Poff et al. (2006) and "A Guide to Common Freshwater Invertebrates of North America" by Voshell (2002). Some macroinvertebrate taxa that were numerous throughout a sampling location and with distinct isotopic ratio signatures became their own category. Primary producers were also categorized into groups (aquatic moss, floating macrophytes, shoreline macrophytes, submerged macrophytes, periphyton, phytoplankton, matted algae, and terrestrial leaf litter). Some primary producers numerous throughout a sampling location and with distinct isotopic ratio signatures became their own category. For each estuary and lake, food webs were mapped using the stable isotope signatures from the benthic invertebrates, zooplankton, macrophytes, and algae. This information can be used to improve understanding of food web structure of these benthic lake and estuary systems, including potential competitors and predators of NZMS.

Results

Initial qualitative field assessments at sampling sites revealed some dramatic changes between 2006 and 2012. Notably, we detected NZMS in the lower Siuslaw River for the first time at the Tiernan boat ramp. We contacted the local watershed council and reported this sighting to both the national and Oregon invasive species hotlines. Conversely, the densities of NZMS at many of the Deschutes River and Umpqua river sites appeared to be much lower. NZMS densities remained high in Youngs Bay. Please see Appendix A for a list of specific sampling sites with notes on invasive species signage, NZMS presence and site photographs. The results of the quantitative analysis of NZMS density are shown in the graphs below (Figure 6, Figure 7, and Figure 8; note differences in y-axis scales).

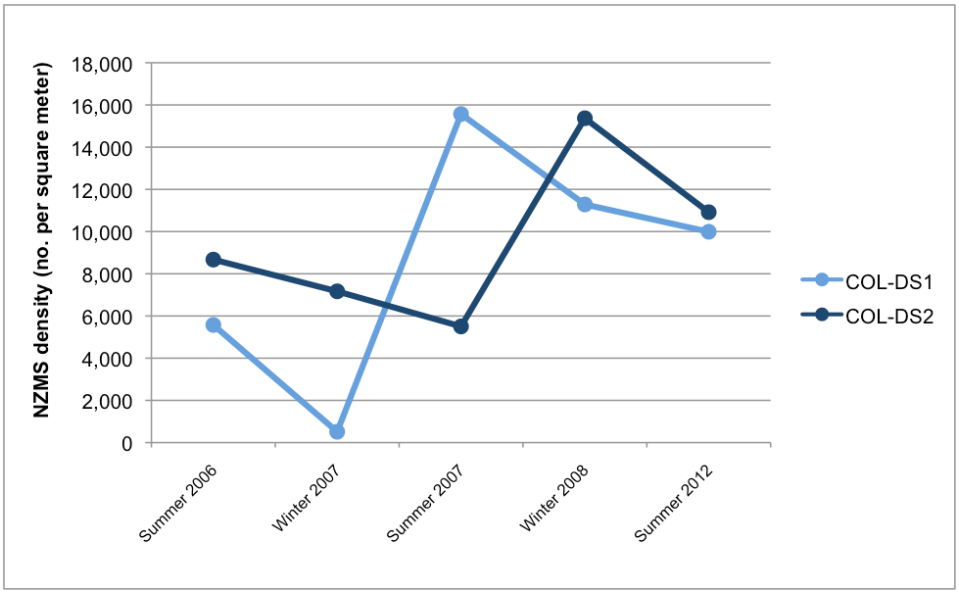


Figure 6: Youngs Bay New Zealand mud snail densities over time. COL-DS1 and COL-DS2 represent the two sties sampled in Youngs Bay between 2006 and 2012.

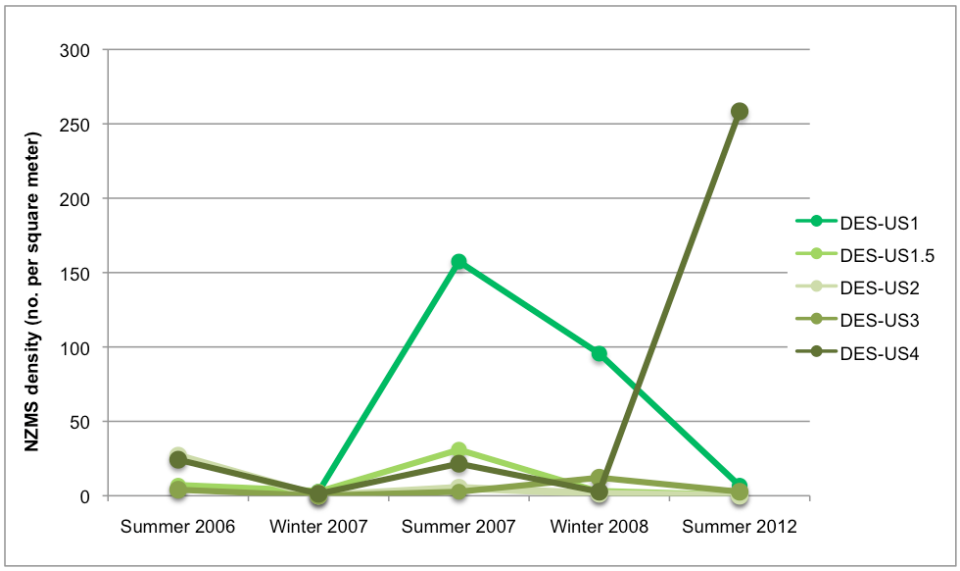


Figure 7: Deschutes River upstream sites New Zealand mud snail density over time.

As we can see from Figure 6, Figure 7, and Figure 8, NZMS density is dynamic, with population densities increasing and decreasing over time and space. While NZMS densities seem to increase or fluctuate around a mean value in Youngs Bay, the NZMS densities in the portions of the tidally influenced lower Umpqua River have plummeted in recent years while new populations have become established in the lower Siuslaw River. In the Deschutes River, NZMS populations maintain very low densities in the littoral habitats sampled.

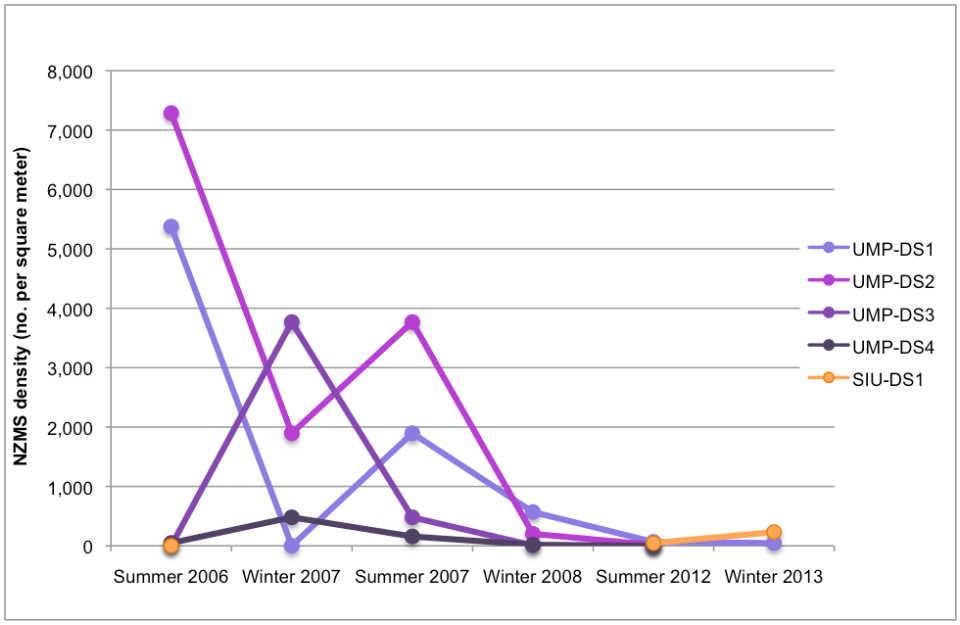


Figure 8: Umpqua River and Siuslaw River downstream sites: New Zealand mud snail density over time.

The relationship between NZMS density and the diversity (H') of recipient benthic invertebrate communities of invaded sites can be shown in several ways. Figure 9 shows the average NZMS density and diversity for each sampling reach (all sites averaged) for samples collected during the summers of 2006 and 2012. Figure 10 shows the relationship between these two variables across all invaded sites during all sampling events (2006 – 2013) when NZMS were detected. The Pearson correlation coefficient was calculated to show the relationship between NZMS density and benthic invertebrate diversity at all invaded sites (Table 3). Across all invaded sites and sampling periods, there is a strong negative correlation between NZMS density and the diversity of the benthic invertebrate community ($r=-0.44$, $N=57$, $p<0.001$). Within sites, no significant correlation was detected. We also examined the correlation between NZMS density and the total density of benthic invertebrates at invaded sites (Figure 11). Here, there was a strong positive correlation ($r=0.47$, $N=57$, $p<0.001$) for all sites and times (Table 4).

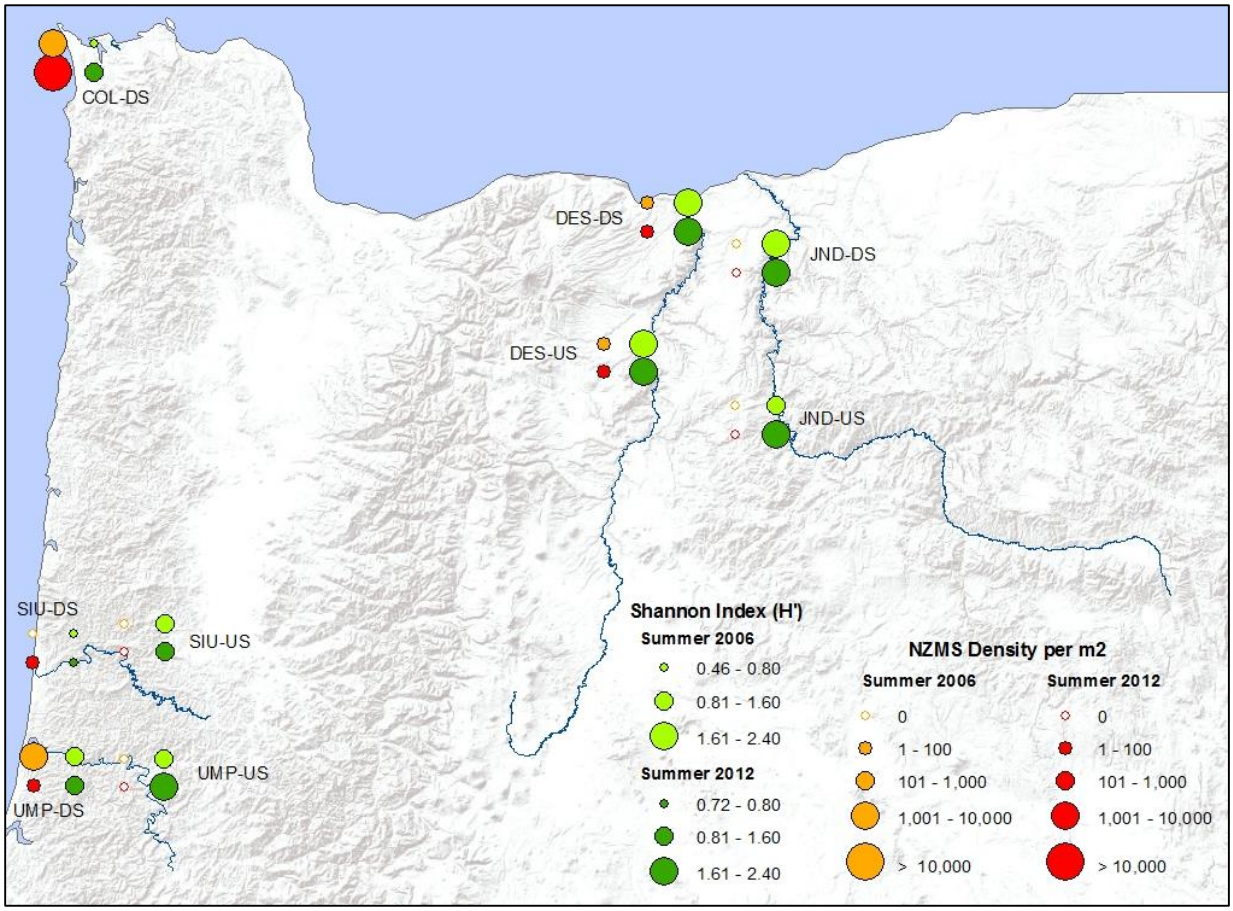


Figure 9: Average NZMS density and diversity (H') for all reaches in 2006 and 2012. Image created by Cindy Moomaw-Nerf.

Table 3. Pearson correlation coefficient for NZMS density and diversity (H') across all invaded sites and times (2006 – 2013).

	r	***N***	***p***
All sites/dates	-0.44	57	<0.001
Youngs Bay	0.38	10	0.3
Deschutes R.	-0.14	24	0.5
Umpqua R.	-0.35	20	0.1

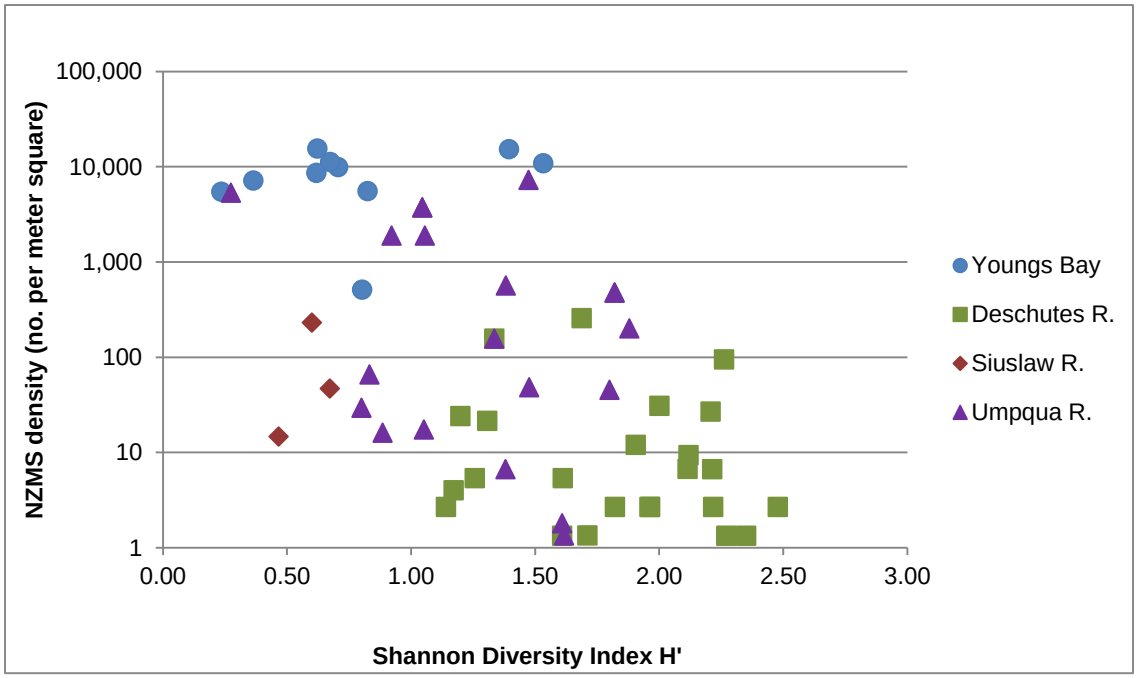


Figure 10. Scatter plot showing the relationship between NZMS density and diversity (H') for all invaded sites and times (2006-2013). See Table 3 for correlation coefficients.

Table 4. Correlation between NZMS density and the total density of benthic invertebrates across all invaded sites and times (2006 – Winter 2013).

	r	***N***	***p***
All sites/dates	0.47	57	<0.001
Youngs Bay	0.21	10	0.56
Deschutes R.	0.00	24	1
Umpqua R.	-0.04	20	0.87

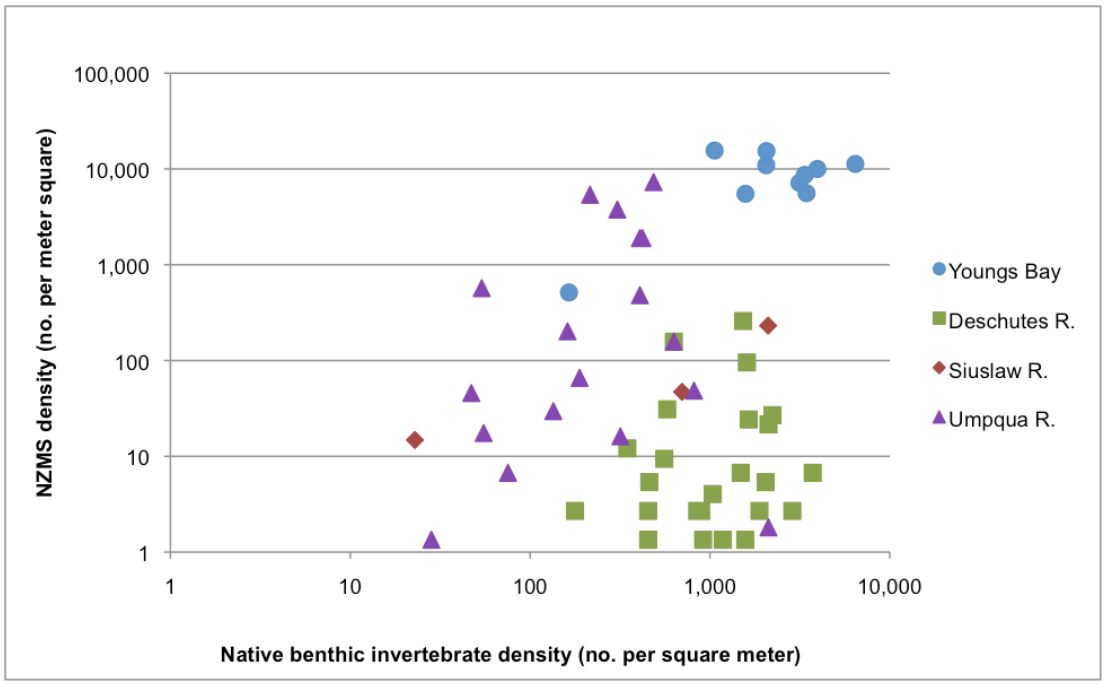
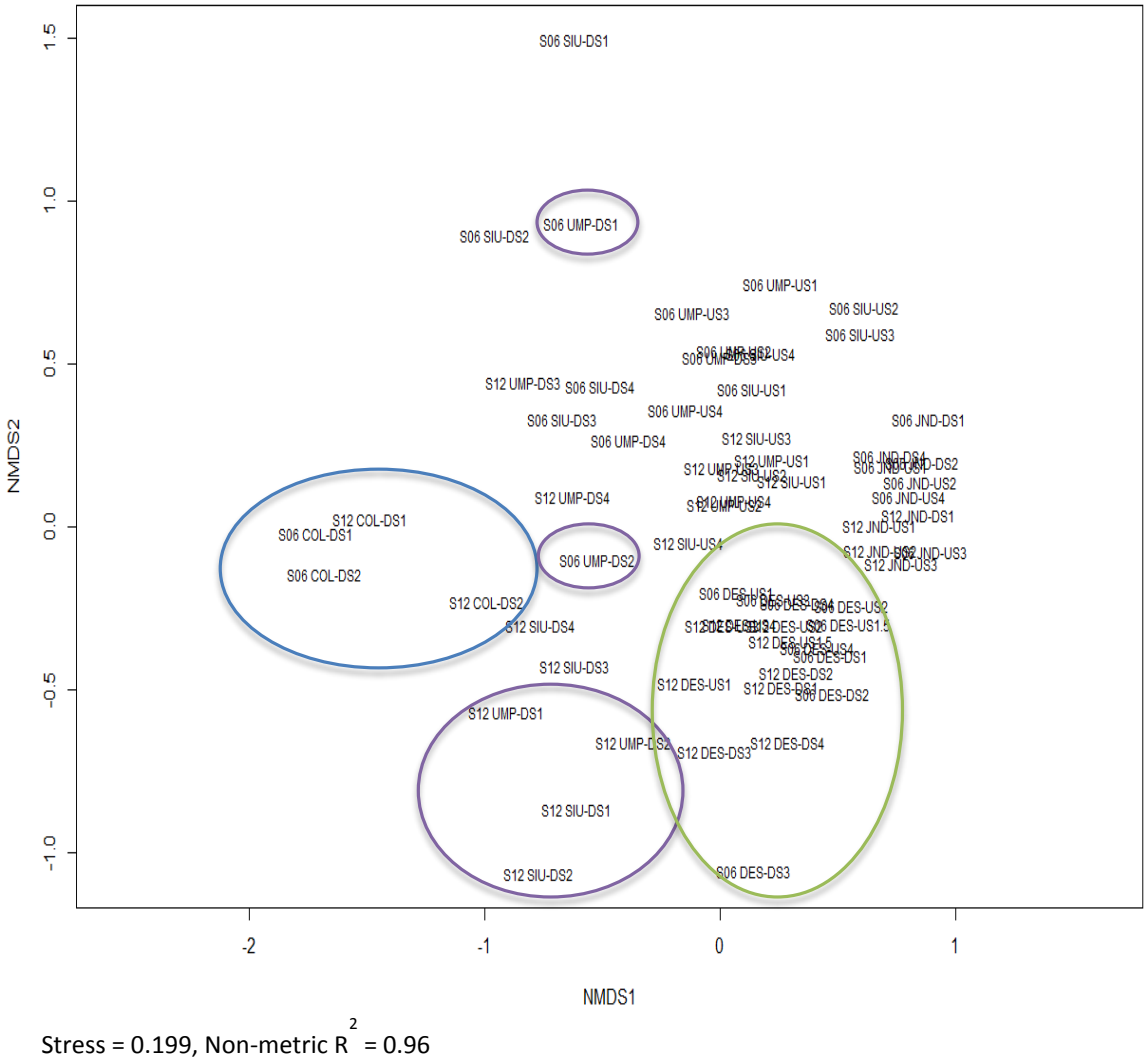


Figure 11: Scatter plot showing the relationship between NZMS density and benthic invertebrate density for all invaded sites and times (2006-2013). See Table 4 for correlation coefficients

While the diversity of these benthic invertebrate communities can be describe by calculating an index (e.g., the Shannon Index (H')), this tells us very little about the actual species composition of benthic invertebrate communities at these various sites. The composition and structure of the benthic invertebrate community (excluding NZMS) can be shown in two-dimensional space by using Non-Metric Multidimensional Scaling (NMDS) to reduce the dimensionality of the data and map sites out in relationship to the species that compose the communities present at these sites (Figure 12). This type of analysis can provide information about the changes in taxonomic patterns that drive differences in community composition.

The most notable patterns to emerge from the NMDS analysis are the similarity between the community composition between Youngs Bay samples, and their difference from the other communities sampled. At these brackish, intertidal sites, the community is dominated by the native amphipod, *Americorophium salmonis* and the native isopod, *Gnorimosphaeroma insulare*, as well as the invasive NZMS (Figure 12). Similarly, the Deschutes River sites (upstream and downstream) cluster together (Figure 12). These sites include those with low densities of NZMS as well as sites where NZMS were not detected. The uninvaded upstream reaches of the Umpqua River and the Siuslaw River, which have similar substrate (primarily bedrock), also have similar benthic invertebrate community composition (Figure 12). Only in the downstream reaches of the Umpqua and Siuslaw Rivers do we see marked differences in community composition between the summer of 2006 and 2012 (Figure 12). NZMS were not included in the creation of these plots. Therefore, NZMS density does not inform the community structure.



Stress = 0.199, Non-metric R^2 = 0.96

Figure 12: Non-metric multidimensional scaling plot showing all sites sampled in Summer 2006 and Summer 2012. Stress of 0.199 indicates that the data is represented fairly well in the reduced dimensions. Sites with NZMS present are enclosed in colored ovals; Youngs Bay sites are enclosed in blue, the Deschutes River upstream and downstream sites are in green, and Siuslaw and Umpqua sites are in purple.

Here, it is the changes in the density of the native snail *Juga*, as well as the detection of a new invasive snail species, most likely *Assiminea parasitologica*, drive these differences in community structure (Figure 12). In 2006, *Juga* was much more abundant in the downstream Umpqua River sites (as were NZMS); densities were reduced in 2012. *Assiminea parasitologica* was not detected in 2006, but appeared in the summer 2012 and winter 2013 samples from the lower Siuslaw River. These changes appear to be driving the change along the NMDS2 axis for these same sites between 2006 and 2012.

Sampling in the summer of 2013 provided interesting results in understanding the food web structure of coastal lakes and estuaries with a gradient of NZMS densities. As expected, the

brackish water estuarine ecosystems and the coastal freshwater lake ecosystems had greatly different abiotic components (Figure 13). A principal component analysis conducted on the abiotic variables at all the sample locations in the summer 2013 field season produced two very distinct sampling location groups separated by specific conductance and salinity levels (Figure 13). The brackish water estuaries had much higher specific conductance and salinity levels than the freshwater lakes as can be depicted along the x-axis in PC I (Loadings PC I: specific conductance = 0.917, salinity = 0.396; Loadings PC II: pH = -0.985) (Figure 13) (Table 5). Abiotic variability within the lake sites was minimal (Table 5). Estuarine sites had large variability in specific conductance and salinity (Table 5). High and low densities of New Zealand mud snails were observed at both brackish estuarine and freshwater lake sampling sites. Thus, it was important to analyze these systems separately.

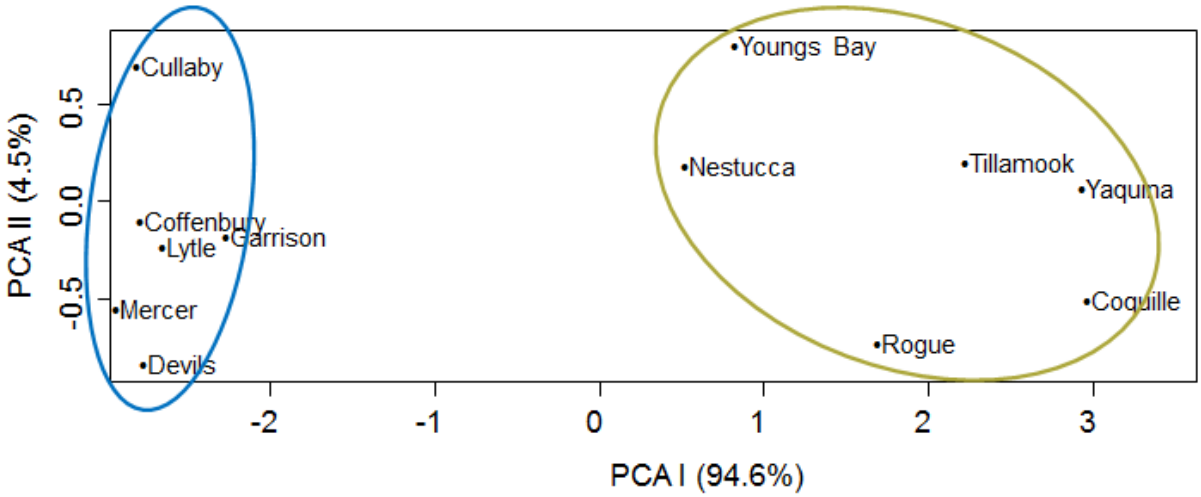


Figure 13: A principal component analysis (PCA) plot of the six freshwater lake sampling locations (circled in blue) and the six brackish estuary sampling locations (circled in tan) sampled in the summer of 2013. PC I describes 94.6% of the variation between the sampling locations, and PC I is represented primarily by specific conductance and salinity. PC II only describes 4.5 % of the variation between sites and is represented primarily by pH.

Table 5. The averages and standard deviations (SD) of abiotic variables in the freshwater coastal lakes and brackish estuaries sampled in the summer of 2013 showing the range of variability within each system.

	Temperature (°C)		Dissolved Oxygen ($mg \cdot L^{-1}$)		Specific Conductance ($\mu S \cdot cm^{-1}$)		Salinity (‰)		pH	
System	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Lakes	21.3	1.15	8.10	0.45	130.5	37.04	0.08	0.04	7.55	0.53
Estuaries	20.0	1.47	7.78	0.75	10485.9	6836.0	6.00	4.18	7.56	0.52

Separate PCA biplots depicting NZMS densities and abiotic components for coastal freshwater lakes (Figure 14) and brackish estuaries (Figure 15) indicate which environmental factors correlate most with high NZMS densities in each of these distinct ecosystems. Average NZMS densities varied greatly within each system (Table 6). Relative densities for coastal freshwater lakes and the brackish estuaries were on different scales due to much higher densities of NZMS in the brackish estuaries than the freshwater lakes (Table 6). In the freshwater lake sampling locations, lakes with relatively higher pH typically correlated with higher densities of NZMS (Figure 14). Higher specific conductance also correlates with higher NZMS densities in some of the freshwater lakes (Figure 14). However, both the trends of higher pH and higher specific conductance correlating with higher NZMS densities in the coastal freshwater lakes do not hold true throughout all sampling locations (Figure 14). In the brackish estuary sampling locations, NZMS densities were highest at relatively low specific conductance and salinity as well as low pH (Figure 15). Again, these trends of relatively low specific conductance and low salinity as well as low pH correlating with high NZMS densities do not hold true throughout all sampling locations (Figure 15).

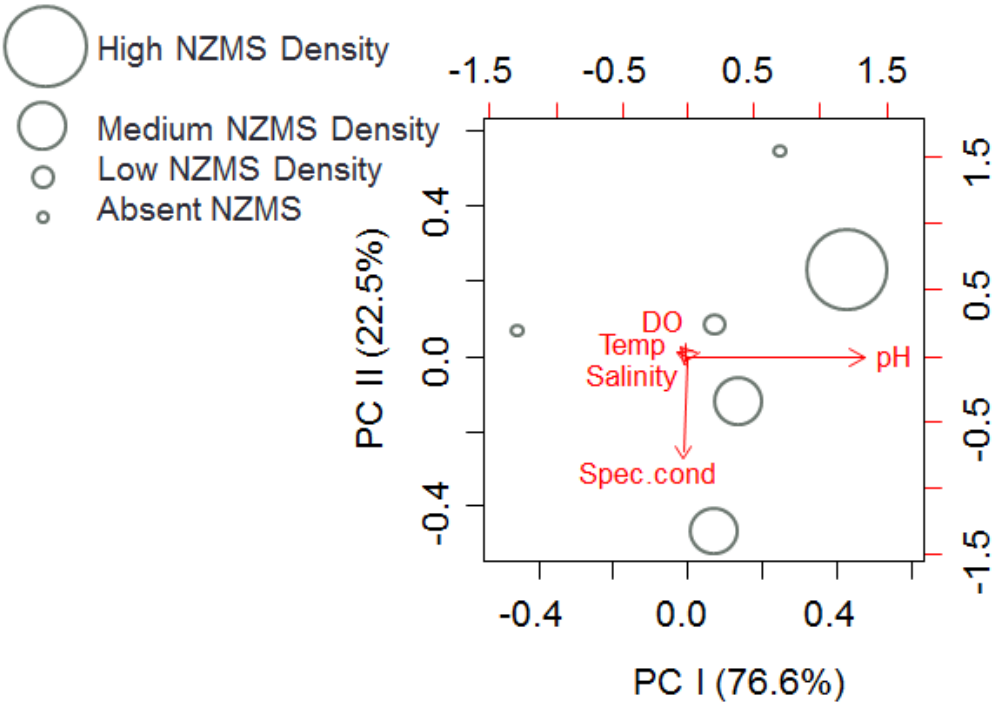


Figure 14: PCA biplot of the coastal freshwater lake sampling locations depicting environmental factors recorded at each lake and relative NZMS densities. PC I, driven largely by pH, describes 76.6% of the variation between the lakes. PC II driven primarily by specific conductance describes 22.5% of the variation between the sample lakes. Larger circles represent higher relative NZMS densities.

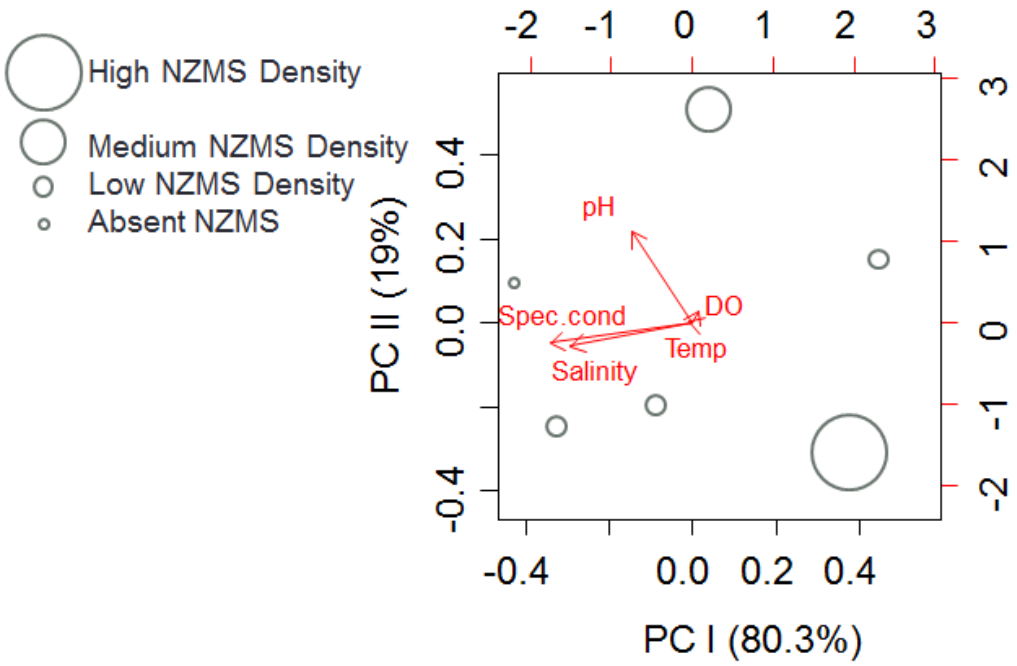


Figure 15: PCA biplot of the brackish estuary sampling locations depicting environmental factors recorded at each estuary and relative NZMS densities. PC I, driven primarily by specific conductance and salinity, describes 80.3% of the variation between the estuaries. PC II, which is represented mostly by pH, describes 19% of the variation between the sample estuaries. Larger circles represent higher relative NZMS densities.

Table 6. The average densities and standard deviation (SD) of New Zealand mud snails per square meter at each sampling location during the summer 2013 field season. Each location was sampled at five different sites. Relative densities were determined within each ecosystem.

Ecosystem	**Location**	**Average Density (Individuals·m^{-2})**	**SD**	**Relative Densities (Individuals·m^{-2})**
Coastal Freshwater Lakes	Coffenbury	16.2	22.8	Low (1- 100)
	Cullaby	0	0	Absent (0)
	Lytle	776.2	497.1	Moderate (101-1,000)
	Devils	2469.9	204.8	High (1001+)
	Mercer	0	0	Absent (0)
	Garrison	135.9	192.2	Moderate (101-1,000)
Brackish Estuaries	Youngs Bay	14,814.4	7,270.7	High (10,001+)
	Tillamook	200.8	176.6	Low (1-1,000)
	Nestucca	976.6	1,208.9	Low (1 – 1,000)
	Yaquina	365.0	362.7	Low (1 – 1,000)
	Coquille	0	0	Absent (0)
	Rogue	4,274.9	53.2	Moderate (1,001 - 10,000)

The average New Zealand mud snail densities at the coastal freshwater lake sampling locations showed no relationship to the densities of the other benthic sampled macroinvertebrates ($R^2 = 0.02$, p =0.79) (Figure 16). Similarly, NZMS densities at the brackish estuary sampling locations showed no relationship to the densities of the benthic macroinvertebrates sampled at those estuarine sites ($R^2 < 0.001$, p = 0.90) (Figure 16). Furthermore, NZMS densities when compared to macroinvertebrates of specific feeding habits only had a significant relationship (negative) to freshwater lake detritivore densities ($R^2 = 0.79$, p = 0.03) (Figure 17). Estuarine detritivore densities were also compared to NZMS densities but did not result in a significant relationship ($R^2 = 0.03$, p = 0.79) (Figure 17). Herbivore densities were compared to NZMS densities in the freshwater lake and estuary sampling locations as well, and these densities also had no significant relationship with NZMS densities (lakes: $R^2 = 0.11$, p = 0.53; estuaries: $R^2 = 0.01$, p = 0.85) (Figure 17). The densities of the NZMS and other sampled macroinvertebrates were log transformed for scaling purposes (Figure 16 & Figure 17).

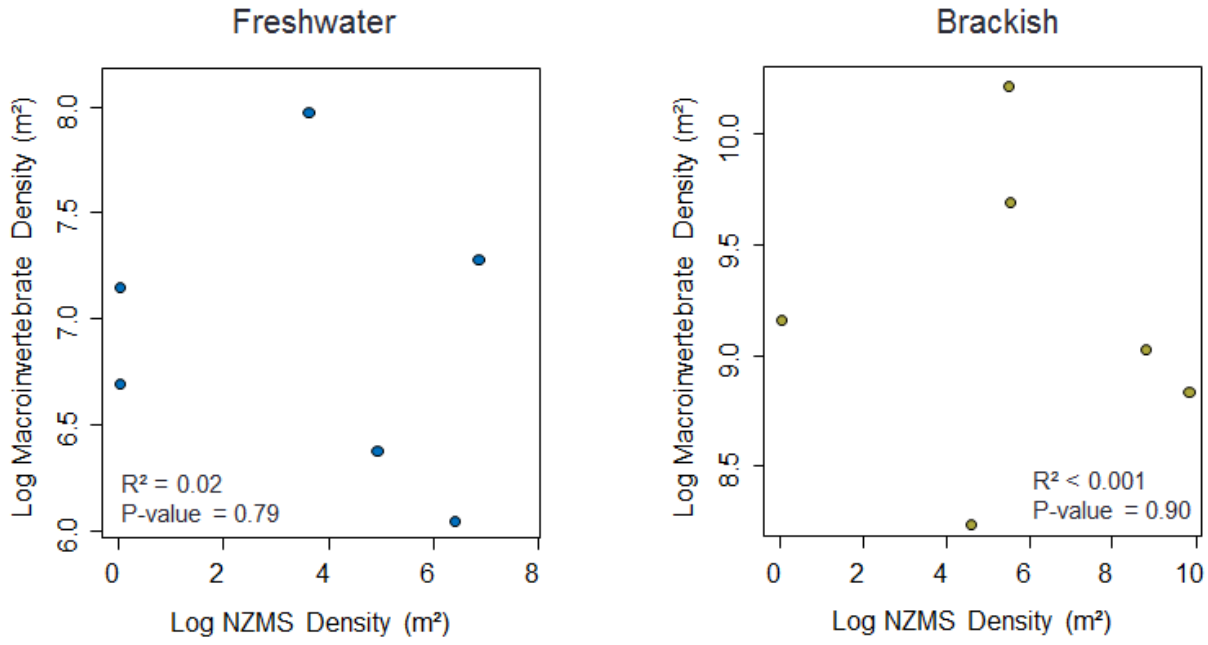


Figure 16: The average log transformed NZMS densities at each location compared to the average log transformed densities of all the benthic macroinvertebrates at each freshwater lake and brackish estuary location. The R^2 value represents the relationship between NZMS density and macroinvertebrate density, and the P-value represents whether or not the relationship is significant. A P-value ≤ 0.05 is significant.

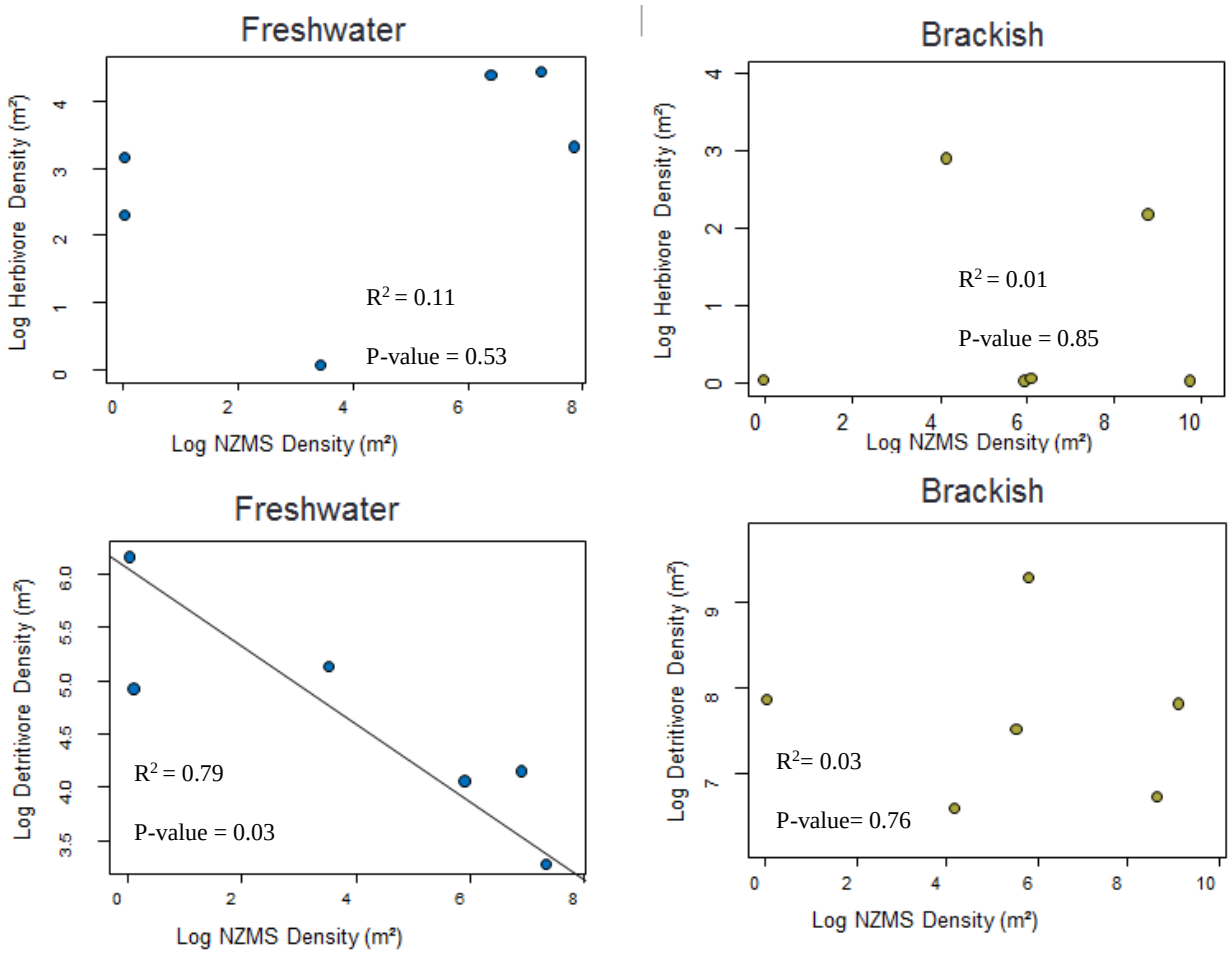


Figure 17: The average log transformed NZMS densities at each location compared to the average log transformed densities of macroinvertebrate herbivores and detritivores at each freshwater lake and brackish estuary location. The R^2 value represents the relationship between NZMS density and macroinvertebrate density, and the P-value represents whether or not the relationship is significant. A P-value ≤ 0.05 is significant.

Results of stable isotope analyses conducted at the UC Davis stable isotope facility were plotted on x-y graphs with average stable isotope signatures and standard error bars for $\delta^{13}C$ and $\delta^{15}N$ (Figure 18 & Figure 19). The freshwater lake sampling locations contained a greater number of species than the estuaries (Figure 18 & Figure 19). New Zealand mud snails at their highest density (Devils Lake) share a similar signature to herbivores though with a more specific or narrower range (less standard error for $\delta^{13}C$) (Figure 18). NZMS also appear to be feeding primarily on periphyton at Devils Lake (Figure 18), although without using a mixing model this apparent diet can only be assumed. At moderate densities (Lake Lytle and Garrison Lake), NZMS have a wide range of $\delta^{13}C$ values and these $\delta^{13}C$ signatures encompass multiple potential food sources such as periphyton, phytoplankton, floating macrophytes, and even the submerged macrophyte *Elodea canadensis* (Figure 18). The food source for NZMS at low density (Coffenbury Lake) was not captured in the stable isotope analysis (Figure 18). In these

freshwater lake stable isotope biplots, there was a wider range in values along the y-axis, which represents the depletion and enrichment of $\delta^{15}N$, in lakes with moderate to high NZMS densities (Garrison Lake, Lake Lytle, and Devils Lake) than the two NZMS absent lakes (Cullaby Lake and Mercer Lake) and the low density Coffenbury Lake (Figure 18). The y-axis in these graphs depicts variation in trophic level as well as the sources of nitrogen in the food web. However, stable isotope values can also be the result of kinetic and equilibrium reactions, and therefore the chemistry of the nitrogen and carbon cycle can play a role in the variations in these axes.

The brackish estuary sampling locations were characterized by low diversity (Figure 19). New Zealand mud snails, at their highest densities (Youngs Bay), have a narrow of range of $\delta^{13}C$ and $\delta^{15}N$ signatures. At Youngs Bay there is not a single clear food source or $\delta^{15}N$ signature directly below NZMS (Figure 19). However, there does appear to be predation on the NZMS at Youngs Bay by omnivores and macroinvertebrate predator species (Figure 19). At the Rogue River Estuary where NZMS are at relatively moderate densities there is not a clear predator of the NZMS or food source assuming that the NZMS is not consuming detritivores (Figure 19). At the sampling locations with low NZMS densities (Nestucca, Tillamook, and Yaquina) there were no obvious similarities or trends in stable isotope signatures other than predatory macroinvertebrates tend to be at a higher trophic level (enriched $\delta^{15}N$) and detritivores are typically the most $\delta^{15}N$ depleted (Figure 19). Similarly, the Coquille River Estuary stable isotope signatures displayed no clear pattern (Figure 19). Lastly, it is interesting to note that at most estuaries sampled macroinvertebrates (squares) had more depleted $\delta^{13}C$ signatures than the primary producers (circles) sampled (Figure 19) may indicate more benthic sources of carbon such as periphyton.

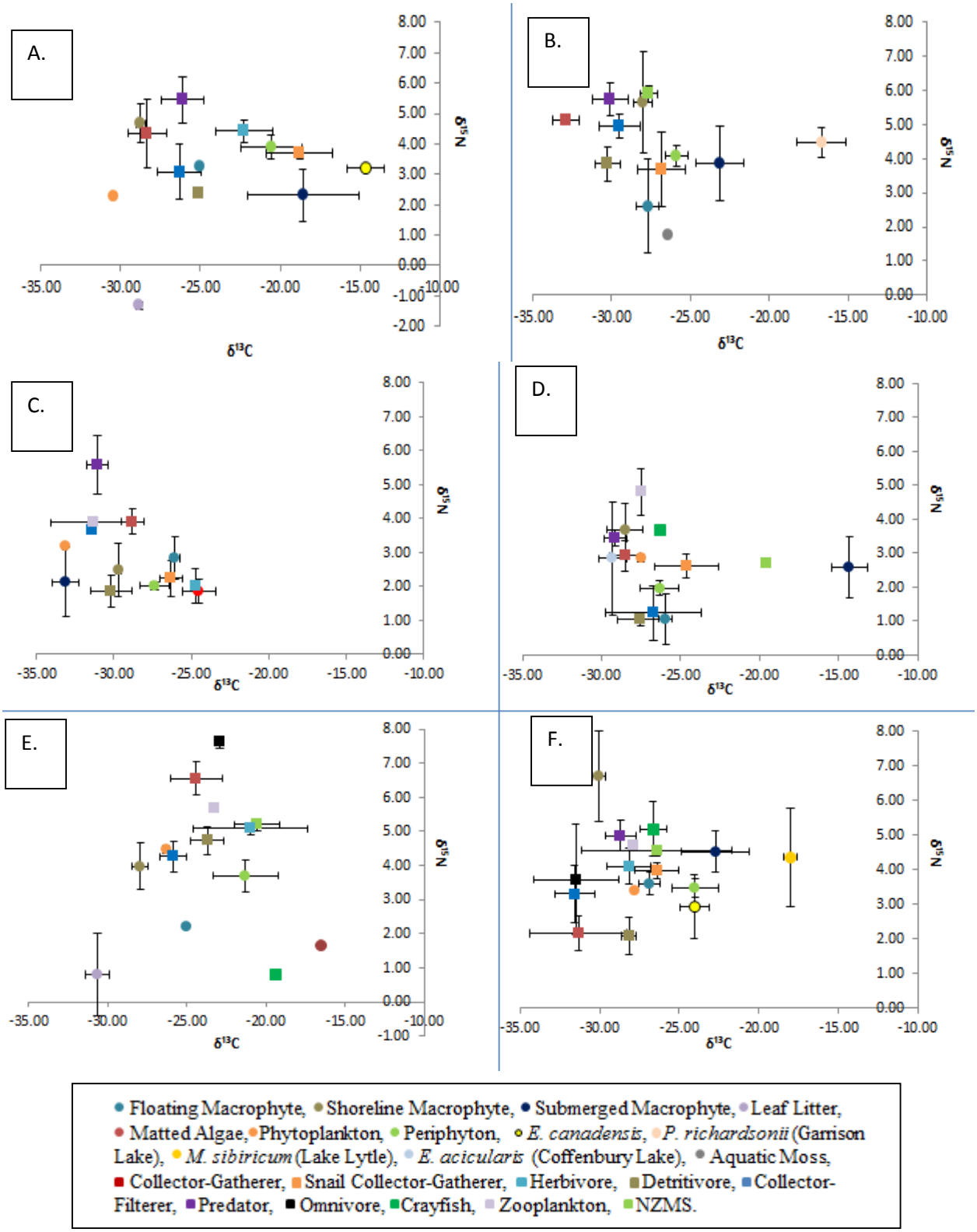


Figure 18: Clockwise from top left: A. Cullaby Lake, B. Coffenbury Lake, C. Devils Lake (Lincoln City) , D. Lake Lytle, E. Mercer Lake, and F. Garrison Lake. Average stable isotope signatures of the benthic food webs with standard error bars for $\delta^{13}C$ and $\delta^{15}N$.

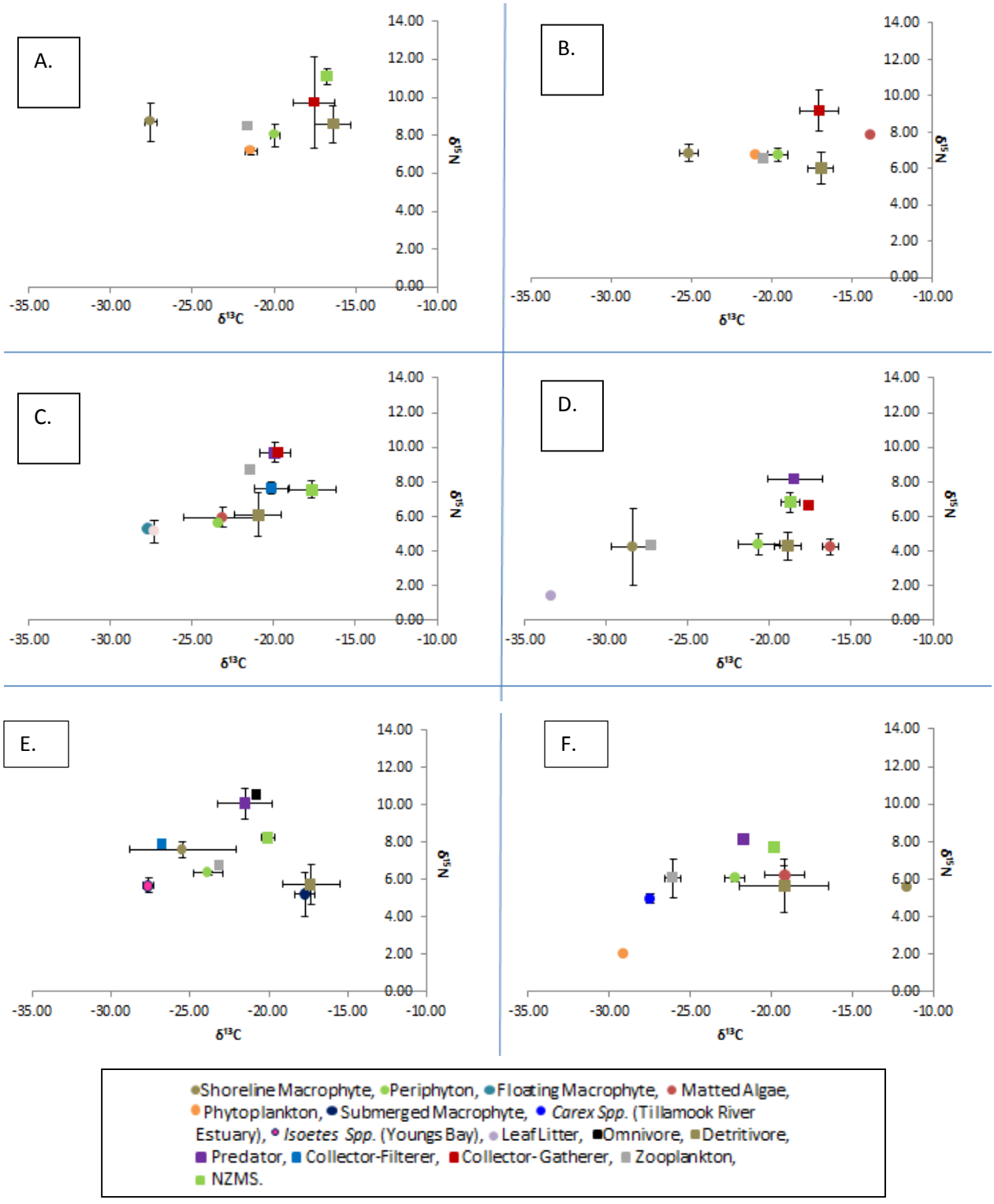


Figure 19: Clockwise from top left: A. Rogue River Estuary, B. Coquille River Estuary, C. Yaquina River Estuary, D. Nestucca River Estuary, E. Columbia River Estuary at Youngs Bay, and F. Tillamook River Estuary at Memaloose Point. Average stable isotope signatures of the benthic food webs with standard error bars for $\delta^{13}C$ and $\delta^{15}N$.

Discussion:

In regards to our original research questions, we can draw the following conclusions. Not surprisingly, NZMS densities vary over time, and while populations continue to increase and spread in some systems (the Siuslaw River), we found decreases in other systems (the Umpqua River). NZMS densities do not always increase; they have decreased notably at several sites and increased slightly at others, however densities are patchy in distribution meaning that sampling efforts may not accurately represent overall densities in these systems. Among sites sampled, NZMS are at highest densities in estuarine sites and tidally influenced riverine sites. These estuarine sites are characterized by low diversity and high levels of disturbance. NZMS have maintained low densities at many riverine sites sampled (primarily the Deschutes River). These riverine sites are characterized by high diversity. Similarly, NZMS had low densities at the freshwater lake sites.

New Zealand mud snails can survive and thrive in a variety of different ecosystems, and the sampling results from the summer 2013 field season indicate the wide range of this invasive macroinvertebrate. To better understand the New Zealand mud snail's role and influence on these vastly different systems, as noted in Figure 13, it was essential to analyze the systems separately. As expected, the variation of the freshwater lakes and the brackish estuaries was described by specific conductance and salinity levels. Within each system there was also variation between locations despite the effort to choose sites with similar abiotic and biotic components. The four coastal freshwater lakes with reported NZMS presence chosen for the summer 2013 sampling were the only coastal Oregon lakes with a previous report of New Zealand mud snails; therefore there was not much opportunity to pick and choose lakes with the greatest similarities. Although there were more estuaries with previously reported NZMS presence, it was similarly difficult to minimize variations in abiotic and biotic conditions where the NZMS were actually found along the estuary. Additionally, one estuary (Nestucca River Estuary) that had previously not been reported with NZMS presence was discovered to have NZMS through our sampling efforts. This additional presence of NZMS did not affect this study as the densities at Nestucca were relatively low and the studies objective was to sample a gradient of NZMS density locations.

Higher densities of New Zealand mud snails in freshwater lakes were correlated with higher pH and also showed a significantly negative relationship with detritivore densities. The New Zealand mud snail is a generalist feeder (both grazing herbivore and detritivore) that utilizes the same food resources as many other macroinvertebrates (Haynes and Taylor 1984, Kearns et al. 2005). At high densities NZMS have been observed to compete with other herbivore grazers for periphyton in an invaded system (Kerans et al. 2005), conversely this negative relationship between NZMS densities and detritivore densities is more analogous to the New Zealand mud snail's native range where their diet is more dependent on a higher proportion of detritus to algae (Talbot and Ward 1987). However, it is still unclear without further investigation whether this relationship of NZMS densities and detritivore densities was due to the abundance of NZMS or just a function of the abiotic conditions.

In the estuarine systems sampled, high densities of New Zealand mud snails were correlated with relatively low specific conductance and salinity as well as relatively low pH. This correlation is consistent with other studies results indicating the tolerance for salinity but only to a certain extant (Dybdahl and Kane 2005, Zaranko et al. 1997, Schreiber et al. 2003). There were no significant relationships between New Zealand mud snails and the density of any particular

macroinvertebrate feeding habit group in estuaries. The sampled estuaries from the 2013 field season are characterized by high disturbance, and a lack of relationship between NZMS and other feeding groups may just indicate that those species that can withstand the conditions are present and densities are not controlled by competition. Additionally, very few herbivores were captured in the estuarine sites making any sort of comparison between herbivore density and NZMS density difficult.

Stable isotope analysis helped to better understand the food web structure of littoral and benthic freshwater coastal lakes and brackish estuaries. Stable isotope ratios of organisms from the freshwater lakes indicated that when NZMS were at high densities they appeared to overlap in diet with other macroinvertebrate herbivores for periphyton. Furthermore, the herbivores had a greater range in $\delta^{13}C$ suggesting their diets were more generalized than the NZMS's diet. This observation is similar to other studies in which high densities of NZMS were shown to force competing species to change their dietary habits (Hall et al. 2006, Moore et al. 2012). In contrast to the Moore et al. (2012) paper, freshwater sampling locations with high NZMS densities showed more enrichment in $\delta^{15}N$ signatures in macroinvertebrates including the NZMS. In the freshwater lake ecosystems, established New Zealand mud snail populations may have an observable effect on the food web and especially on competing herbivores and detritivores. Conversely, high or low NZMS population densities in the brackish estuary ecosystems showed no discernable patterns from the stable isotope analyses to indicate that their presence has an effect on these food webs. In both the lakes and estuaries sampled, not all food sources were captured for stable isotope analysis, and therefore may not represent the most accurate portrayal of each system.

The New Zealand mud snail can survive and thrive in a variety of different habitats. Continued research on long term population dynamics of invasive species like the New Zealand mud snail and their effects on native food webs remain crucial to future management and mitigation. Given that invasive species are one of the greatest agents of global change with annual costs to the United States of almost $120 billion (Pimentel et al. 2005), it is essential to understand the potential long-term processes of New Zealand mud snail invasions.

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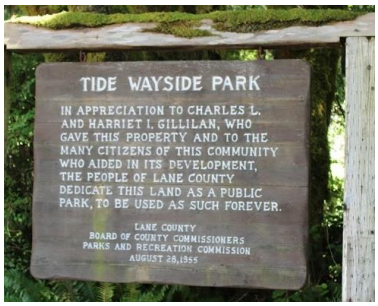
Appendix A. Sampling sites, notes and photos.

Siuslaw River: Thursday, July 12th, 2012

SIU-US1: Farnham landing. Clear invasive species signage posted.

SIU-US2: Tide boat ramp. No invasive species signage.



SIU-US4: Austa landing boat ramp. Lacking clear signage.

SIU-DS1: Tiernan Boat ramp (upstream) *TIDAL* - New detection of NZMS at low density.

SIU-DS2: Tiernan Boat ramp (downstream) – New detection of NZMS

Clear signage regarding invasive species. *SITE REVISITED ON FEB. 1, 2013.

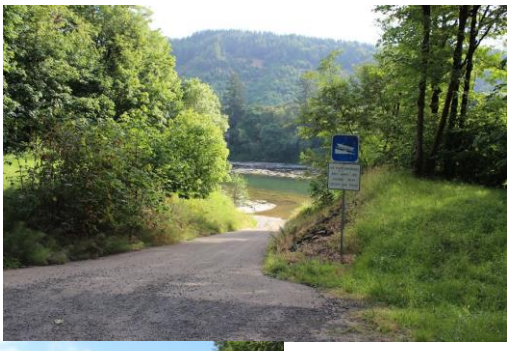
SIU-DS3: Mapleton Boat ramp (upstream) *TIDAL – sampled at ~1 pm low tide

SIU-DS4: Mapleton Boat ramp (downstream)

Umpqua River: Friday, July 13th, 2012

UMP-US1: Scott's Park (upstream) and UMP-US2: Scott's Park (downstream). No clear signage.

UMP-US3: Sawyer Rapids boat ramp (upstream) and UMP-US4: Sawyer Rapids boat ramp. No signage.




UMP-DS1: Umpqua Wayside (upstream) *Tidal, sample at 2:00 pm. Lower densities of NZMS this year.

UMP-DS2: Umpqua Wayside (downstream) NZMS present in woody debris, much lower densities. *SITES REVISITED ON FEB. 1st, 2013.


UMP-DS3: Scottsburg Park boat ramp (upstream) * Tidal, sampled at 1:00 pm low tide.

UMP-DS4: Scottsburg Park boat ramp (downstream)

Signage present, not photographed. Only 1 NZMS found, previously present at higher densities.

John Day River

Upstream: Sampled on morning of Saturday, July 23rd, 2012. Clear invasive species signage.

JND-US1: Clarno boat ramp, access not as good as Cottonwood. Slower water. DS of bridge.

JND-US2: Riffle on R. side of channel, sedge hummocks.

JND-US3: Clarno, riffle in channel, sampled on bar side across from eroding bank.

JND-US4: Rush/sedge tussocks in shallow water.

Downstream: Sampled on morning of Monday, July 21st, 2012. Invasive species signage focused on plants (sign at right). Nothing explicit about aquatic invasive species.

JND-DS1: Cottonwood Canyon, eastside. Coarse gravel bar, littoral run (most upstream of DS sites)

JND-DS2: Cottonwood, eastside. Riffle 500 ft downstream of DS1.

JND-DS3: Cottonwood, flowing eddy below riffle, 400 ft downstream of DS2

JND-DS4: Cottonwood, run w/sedges in large cobble.

Deschutes River

Upstream: Wednesday, August 1st, 2012. Invasive species signage was posted only on the BACKSIDE of information kiosks, only visible to those who made a special effort to look for them.

DES-US1: Harpham flats

DES-US1.5: Wapinitia

DES-US2: Oasis group campsite

DES-US3: Blue Hole (Hard to sample, omit perhaps).

DES-US4: Oaksprings campsite

Downstream: Friday, July 27th, 2012. Very clear invasive species signage at fishing access site.

DES-DS1: Eastbank, accessed via Deschutes River Recreation Area. Just DS of gaging station.

DES-DS2: Eastbank, below huge old alder just US of power lines across from small island with alder.

DES-DS3: Westbank, upstream of heritage landing rocky area. Very slow water over silty bedrock. Best effort at 8 ft^2 composite sample.

DES-DS4: Westbank, upstream of DS3, underneath powerlines. Lots of little pools were sampled.

Youngs Bay: Friday, August 3rd. Low tide at 9:14 am (-1.1 ft).

COL-DS1: West side of old HWY 101 bridge. Not an official access point. No signage.


COL-DS2: East side, Astoria Yacht Club. Invasive species signage present.


Summer 2013 Sampling Locations

Cullaby Lake: August 18, 2013. NZMS absent.

CUL1: LAT: 46.085846, LONG: -123.907159, north of boat ramp near picnic area with floating macrophytes. Substrate sandy.

CUL2: LAT: 46.088866, LONG: -123.906772, residential area with woody debris, tall grasses, algal bloom. Substrate organic and sandy.

CUL3: LAT: 46.087429, LONG: -123.904397, eastern shore tall grasses. Silty, organic substrate.

CUL4: LAT: 46.078684, LONG: -123.899733, deciduous and coniferous overhanging trees southern edge of lake. Organic substrate.

CUL5: LAT: 46.082374, LONG: -123.906430, deciduous trees and ferns on shore, rotted old boat dock. Muddy organic substrate.

Coffenbury Lake: August 19, 2013. NZMS present at low densities.

COF1: LAT: 46.1795659, LONG: -123.9395343, north end of lake near boat ramp. Large deciduous tree shoreline. Substrate sandy with woody debris.

COF2: LAT: 46.1794343, LONG: -123.9472521, large floating macrophytes. Organic and sandy substrate.

COF3: LAT: 46.17188, LONG: -123.96074, southeast near swimming hole, emergent macrophytes. Sandy, silty, and organic substrate.

COF4: LAT: 46.16722, LONG: -123.95975, Southwest corner of lake. Large deciduous overhanging trees. Muddy, silty, and organic substrate.

COF5: LAT: 46.17358, LONG -123: 96380, coniferous trees fallen into water with floating macrophytes. Sandy and organic substrate.

Sampling equipment and canoe on Coffenbury Lake with emergent macrophytes.

Youngs Bay: August 20, 2013. NZMS present at high densities. Latitude: 46.17000, Longitude: -123.83385.

YOU1: End of east side of boat dock, 7 paces north toward shore, thick muddy substrate.

YOU2: East of boat dock, 6 paces north of site 1 toward shore, Muddy with vegetation and trash.

YOU3: East of boat dock, 9 paces from site 3 north toward shore. Mudflat with tall shoreling macrophytes.

YOU4: West of boat dock on rocky shore, 5 paces west of boat ramp. Some small grasses submerged at high tide.

YOU5: West of boat ramp, 4 paces west of site 4. Large rocks rocky substrate.

High Tide at 2:00pm (Noon)

Low Tide at 9:00 am

High tide, a fishing boat launching at the Astoria Yacht Club in Youngs Bay (L).

Large barge equipment, abandoned pilings, and visible garbage present at Youngs Bay (R).

Lake Lytle: August 26, 2013. NZMS present at moderate densities.

LYT1: LAT: 45.62612, LONG: -123.93956, Steep shoreling with bushes just south of boat ramp in NE corner of lake. Sandy, muddy substrate with a lot of submerged macrophytes.

LYT2: LAT: 45.62254, LONG: -123.93770, coniferous shoreline, tall grasses. Organic, muddy substrate.

LYT3: LAT: 45.62068, LONG: -123.94270, floating and emergent macrophytes, power lines overhead. Silty, muddy substrate.

LYT4: LAT: 45.62329, LONG: -123.94222, steep shore line just east of HWY 101, tall emergent macrophytes. Organic substrate.

LYT5: LAT: 45.62546, LONG: -123.94230, Just south of boat dock off of HWY 101. Large emergent macrophytes, rocky and sandy substrate.

Benthic macroinvertebrate D-Net sampling by Sam Cimino (L).

Emergent and floating macrophytes at Lake Lytle (R).

Clear NZMS caution signage at public boat ramp (L).

D-Net sampling littoral zone Sam Cimino (R).

Tillamook River Estuary at Memaloose Point: August 27, 2013. NZMS densities low. Latitude: 45.4743, Longitude: -123.89091.

TRE1: South of boat ramp, four paces south of bluff. Thick muddy flats.

TRE2: South of boat ramp, five paces south of site 1. Thick muddy flats.

TRE3: North of boat ramp, 4 paces from where the tall grass meets the boat ramp along the shore. Rocky substrate.

TRE4: North of boat ramp, 7 paces NE of site 3. Rocky substrate.

TRE5: North of boat ramp, 3 paces NE of site 4, Rocky substrate some large boulders.

High Tide at 4:30 pm

Low Tide at 11:30 am

High tide observations with minnow trap buoy to catch crayfish in the background (L).

Tillamook River Estuary with multiple culverts flushing into the system down stream of sampling (R).

Nestucca River Estuary at Nestucca Adventures Boat Launch: August 28, 2013. NZMS first identified at this location during sampling. Reported to USGS and ODFW. Latitude: 45.20649, Longitude: -123.96045

NRE1: South 4 paces from the North corner of the fishing dock. Tall grasses, mudflat, and woody debris.

NRE2: South of fishing dock, 9 paces south of site 1. Tall grasses and mudflats a lot of woody debris.

NRE3: South of fishing dock, 3 paces south of site 2. Directly below residential apartments/duplex, muddy-sandy substrate (NZMS first ID'd here).

NRE4: Just north of boat launch. Rocky substrate near private property boat dock.

NRE5: North of boat ramp, 8 paces north of site 4. Underneath and to the north of private boat dock. Large boulders.

High Tide at 5:45 pm

Low Tide at 12:35 pm

Minnow trap buoys for crayfish sampling (L).

Nestucca River boat ramp with canoe (R).

Sam Cimino preserving samples out of the back of the truck with Nestucca River Estuary in the background.

Devils Lake in Lincoln City: August 29, 2013. NZMS at high densities.

DEV1: LAT: 44.96768, LONG: -123.99847, Just south of east lake boat launch, Large coniferous trees. Woody debris and sandy substrate.

DEV2: LAT: 44.97354, LONG: -123.99805, North of park swimming area, residential manicured lawns. Substrate Clay and rocks with some sand.

DEV3: LAT: 44.97886, LONG: -123.99397, Large deciduous trees and tall grass shoreline. Sandy, rocky substrate.

DEV4: LAT: 44.97876, LONG: -123.98611, East arm of lakelarge boulders on shore. Sandy, woody substrate algal bloom.

DEV5: LAT: 44.97257, LONG: -123.99107, Tall shoreline macrophytes steep drop off from shore. Organic substrate.

Yaquina River Estuary at the Port of Toledo: August 30, 2013. NZMS at low densities. Latitude: 44.59137, Longitude: -123.94254.

YRE1: South side of dock, 4 paces from SE launch corner. Very thick mud a little grassy vegetation.

YRE2: South of dock, 7 paces east of site 1. Very thick mud some tall shoreline macrophytes.

YRE3: West of cliff bank, 3 paces west of cliff bank near creek. Thick, organic mud.

YRE4: West of cliff bank just beyond creek, 3 paces west of site 3. Deep, thick, and organic mud.

YRE5: West of cliff bank, 5 paces west of site 4. Thick organic mud with some macrophytes submerged at high tide.

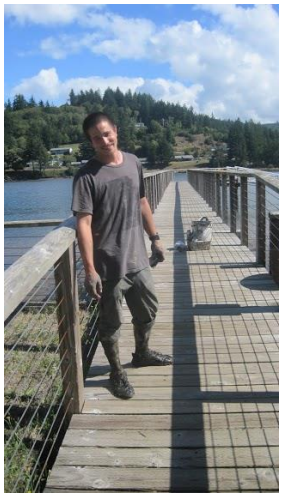
High Tide at 9:30 am

Low Tide at 2:30 pm

Yaquina at low tide (L).

Benthic core sampling ring at low tide (R).

Field assistant Jared Anderson after sampling.



Mercer Lake: August 31, 2013. NZMS absent.

MER1: LAT: 44.05277, LONG: -124.05349, Near public boat dock SE arm of lake, large native lily macrophytes. Organic, muddy substrate.

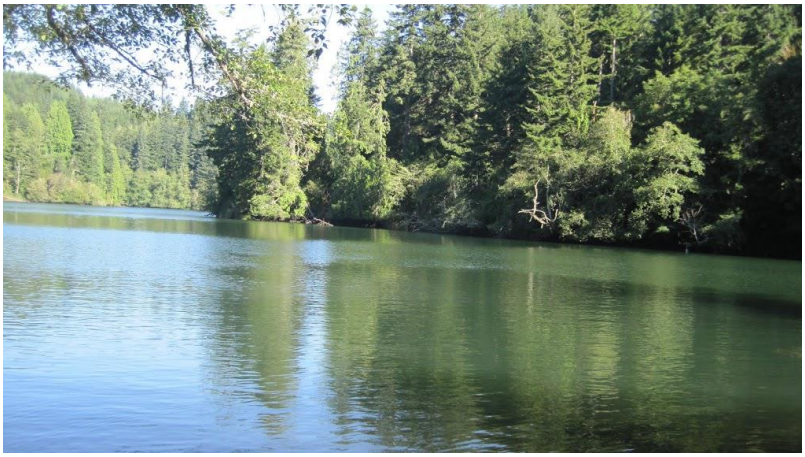
MER2: LAT: 44.05058, LONG: -124.05908, Steep edge cliff of shoreline, bushes and deciduous trees. Sandy, rocky substrate.

MER3: LAT: 44.05115, LONG: -124.06529, Over hanging deciduous and coniferous trees, cattails on shoreline. Substrate organic, woody debris.

MER4: LAT: 44.05709, LONG: -124.06677, Steep shoreline deciduous trees. Rocky, cobbled substrate.

MER5: LAT: 44.05186, LONG: -124:07473, Over hanging large deciduous trees. Sandy, cobbled substrate.

Mercer Lake from SE public boat ramp.



Coquille River Estuary at Bullard State Park: September 1, 2013. NZMS absent. Latitude: 43.14727, Longitude: -124.39928

CRE1: East five paces of eastern boat ramp. Slippery shallow mud with some vegetation.

CRE2: East of boat ramp, 4 paces east of site 1. Thick grassy area with mud and boulders.

CRE3: East of boat ramp, 9 paces east of site 2. Near fallen tree on edge of muddy-rocky hill.

CRE4: West four paces of western boat ramp at Ballard State Park, Substrate rocky with some gravel.

CRE5: West of western boat ramp, 8 paces west of site 4. Substrate rocky with thick, stinky mud.

High Tide at 10:45am

Low Tide at 3:30 pm

Garrison Lake: September 2, 2013. NZMS at moderate densities.

GAR1: LAT: 42.7844, LONG: -124.50861, Marsh area just north of boat ramp. Organic/Clay substrate.

GAR2: LAT: 42.75023, LONG: -124.50941, Dead stand of coniferous trees. Sandy substrate.

GAR3: LAT: 42.75497, LONG: -124.50941, Grassy emergent macrophytes. Substrate organic woody debris and sandy.

GAR4: LAT: 42.75774, LONG: -124.50452, Abundant floating and emergent macrophytes. Substrate organic.

GAR5: LAT: 42.74934, LONG: -124.51205, Beach area along dunes SW Lake. Sandy Substrate.

Rogue River Estuary at Port of Gold Beach: September 3, 2013. NZMS at Moderate densities. Latitude: 42.41993, Longitude:- 124.42245

NZMS Caution sign at Rogue River Estuary.

RRE 1: West of Boat launch, 6 paces North of where boulder meets dock/launch. Dark Muddy-Rocky substrate very little vegetation some gravel.

RRE2: West of Boat Launch, 3 paces north of Site 1. Dark, muddy gravel with a little vegetation.

RRE3: Sandy "grassy" substrate south of Coast Guard barge and bridge. Four paces from beginning of grassy flat in mudflat heading Northwest at bottom of steep, rocky hill.

RRE4: Sandy substrate 5 paces NW of site 3 in Sandy-grassy beach.

RRE5: Sandy substrate 6 paces NW of site 4. Soft spongy macrophytes present.

High Tide at 11:20 am

Low Tide at 5:00 pm