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Selenium Partitioning and Food-Chain Transfer at the Salton Sea

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by

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ABSTRACT OF THE THESIS

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by

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With the habitat at the Salton Sea, California, expected to decline to conditions unsuitable for wildlife as the Sea shrinks in coming years, efforts are being made to restore this valuable wildlife area. Current plans involve construction of a series of Species Conservation Habitat (SCH) ponds at the south end of the Sea to replace some of the habitat lost and evaluate options for further restoration. There is a concern for the accumulation of selenium (Se) in the SCH ponds to levels potentially toxic to the birds they are meant to protect.

In order to evaluate potential Se risk in SCH ponds, this study employed a novel selenium modeling approach in which Se concentrations in food web organisms were linked to stable isotope data. Two freshwater lakes in Imperial County, CA, Finney Lake and Ramer Lake, were chosen as surrogates for the proposed SCH ponds. Organisms from the aquatic food webs of the two lakes were collected and analyzed for both Se concentration and carbon and nitrogen stable isotope composition. Se concentration was plotted against $\delta^{15}N$ for each lake to develop an empirical relationship between position in the food web and Se concentration, then used to predict Se concentrations in piscivore and invertivore birds.

Results provide direct evidence for the bioaccumulation of Se in freshwater habitats at the Salton Sea, but indicate that birds should be at little or no risk from Se toxicity in freshwater systems such as those at Finney and Ramer Lakes. Even though multiple food web components had Se concentrations above established toxicity thresholds, predicted bird concentrations were either below or only slightly above a widely used threshold for reduced hatchability.

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Chapter 1
Introduction

The Salton Sea, California's largest lake, is located in the southeast corner of the state. Created at the beginning of the 20th century when the Colorado River was accidentally diverted into the Salton Sink for over a year in 1905 to 1907 (Woerner, 1989), this unique body of water has served as important habitat for migrating and resident birds, especially in a state that has lost most of its native wetlands (Shuford et al., 2002). Since it is a terminal basin in an arid region and is supplied almost entirely by agricultural wastewater from the Imperial, Coachella, and Mexicali valleys, the Salton Sea has become progressively saltier since its formation, with salinity currently estimated at about 52 ppt (Cohen et al., 1999; Schroeder et al., 2002; Woerner, 1989; Sickman et al., 2011). Aside from the high salinity, the Salton Sea faces other challenges brought about as a result of its source water, including the potential for selenium toxicity.

Ever since selenium toxicity in aquatic ecosystems was brought to light in the 1980s with the notorious poisoning at Kesterson National Wildlife Refuge (Ohlendorf, 2002; Young et al., 2010), selenium has been a concern to those managing for wildlife. Although an essential trace nutrient, the narrow margin between essentiality and toxicity results in selenium quickly becoming a poison at elevated levels (Janz et al., 2010). Toxicity is thought to be caused by two mechanisms: formation of faulty proteins as a result of Se serving as an analogue to sulfur and generation of oxidative stress via redox cycling (Spallholz and Hoffman, 2002). Making selenium even more dangerous is its tendency to bioaccumulate up the food web, with higher trophic levels often containing higher selenium concentrations than lower trophic levels (Luoma and Presser, 2009). Oviparous vertebrates, such as fish and birds, tend to be especially sensitive to Se toxicity, with reproductive effects manifesting first (Janz et al., 2010; Skorupa et al., 2004). Reduced hatchability is the most sensitive endpoint in birds, with the characteristic terata of eye,

bill, and limb deformations, known as the Kesterson Syndrome, occurring at higher embryo selenium levels (Ohlendorf et al., 1986; Ohlendorf and Heinz, 2011, Skorupa, 1998). In contrast, hatching rate of fish is generally not affected by Se. Teratogenesis, especially deformities of the spine, fins, and craniofacial sutures, edema, and larval mortality are the most sensitive endpoints of Se toxicity in fish (Janz et al., 2010; Lemly, 1997). Since the youngest stages are affected first, selenium toxicity can go unnoticed, with populations declining even though adults are perfectly normal and healthy (Lemly, 1997; Luoma and Presser, 2009a). It is therefore crucial that any site with elevated levels of selenium be carefully monitored for both Se levels and effects on wildlife.

For the Salton Sea, the source of selenium is the Colorado River water that is used to irrigate crops in the surrounding area, which contains about 2-3 µg Se/L, primarily in the form of selenate (Schroeder et al., 2002). Evaporative concentration of irrigation water on fields generates elevated levels of selenium in the agricultural wastewater (Frankenberger and Engberg, 1998), and this wastewater is then carried via drains and the Whitewater, Alamo, and New Rivers to supply the Sea and various other nearby ponds and wetlands. Selenium has become of increasing concern at the Salton Sea, as studies conducted throughout the past two decades have shown bird egg selenium levels above the 6 mg/kg dry weight (dw) threshold established for reduced hatchability in a common shorebird, the black-necked stilt (*Himantopus mexicanus*) (Skorupa, 1998), and that birds are experiencing reduced hatchability and possibly immune system depression due to Se (Anderson, 2008; Bennett, 1998; Bruehler and de Peyster, 1999; Henny et al., 2008; Roberts, 1997; Roberts and Berg, 2000). As a result, the Salton Sea has been classified as embryotoxic (Seiler et al., 2003), so it is imperative that the problem of Se is addressed and managed.

The future of this accidental water body is uncertain, though it is certain to change. Because of the future decrease in water supply due to the Quantification Settlement Agreement

(QSA) of 2003, which requires California to reduce its use of Colorado River water, selenium problems currently experienced by the Salton Sea are only expected to worsen (DOI, 2007; Cohen, 2009; DWR, 2010a). However, legislation also requires restoration of the Salton Sea to alleviate negative effects from declining conditions and loss of wildlife habitat (CRA, 2005). Current plans for accomplishing this mandate include construction of a series of shallow ponds, each 50 to 250 hectares in area, in the footprint of the Sea as it recedes in the near future (Sickman et al., 2011). These Species Conservation Habitat (SCH) ponds will be located on the south and/or north end of the Sea and supplied by river and Sea water (DWR, 2010b).

Many of the details for the management of the SCH ponds have yet to be worked out, including how to minimize selenium toxicity to the animals the ponds are intended to benefit. Managing for selenium risk is greatly complicated by the tremendous variation in selenium behavior between sites. As a consequence, selenium concentration in water alone is not enough to predict how selenium will accumulate up the food web and whether or not higher trophic levels are expected to suffer from toxic effects (Skorupa and Ohlendorf, 1991). Food web studies are critical for accurate modeling and understanding of selenium behavior in a system, as higher trophic level animals obtain Se nearly exclusively from their diet, not the water (Ohlendorf and Heinz, 2011; Stewart et al., 2010; Bowie et al., 1996; Besser et al. 1993).

Very little is known about movement of selenium through freshwater ecosystems at the Salton Sea. Since selenium behavior tends to be site-specific (Adams et al., 1998), only data collected from the area can give an accurate portrayal of how selenium can be expected to behave in the SCH ponds. Since no SCH ponds have been constructed, decision makers must turn to modeling approaches to predict what Se concentrations in higher trophic levels of the SCH ponds will be and whether or not these levels pose a significant hazard. A popular approach for predicting Se concentrations in a food web is the progressive model developed by Presser and

Luoma (2010). This modeling approach involves the progressive solution of a series of simple equations, each representing a step of Se accumulation in the food web. The first step involves the incorporation of dissolved selenium into particulate matter. This is described by a partitioning coefficient, K_d (or an enrichment function, EF (Stewart et al., 2010)), which is the ratio between the Se concentration in particulate matter at the base of the food web, such as algae, and that in the water column. All the steps after this involve the use of a species-specific trophic transfer factor/function, TTF, which is the ratio between Se concentration in a consumer versus that of its diet. A TTF greater than one means that the consumer accumulates Se to greater levels than that found in its diet. Water concentration is multiplied by the K_d value to get an estimate of Se concentration in the base of the food web and then this selenium concentration is multiplied by a TTF to get the concentration in primary consumers and so on up the food web.

Progressive models can pose some difficulties. TTF and K_d values both vary with Se concentration and food web composition (Stewart et al., 2010), so there is no universal value for any of these variables that will work for all sites or all times. Particularly for sites in which selenium concentrations in various components of the food web and food web composition are not well studied, the use of a progressive model can involve some guess work when choosing which values to use for modeling. Thus, there can be uncertainty in the results when a progressive model is applied to a system that is not well understood prior to modeling (Presser and Luoma, 2010). A progressive modeling approach was used to predict Se concentrations in SCH ponds (Sickman et al., 2011). However, there was not sufficient data from the Salton Sea region to derive site-specific K_d and TTF values to the level of accuracy needed for successful modeling.

In order to eliminate this shortcoming, this study employed a novel type of Se modeling in which Se accumulation was directly studied in two freshwater lakes in the Imperial Valley.

These lakes, Finney Lake and Ramer Lake, have existed for approximately 50 years, allowing study of well-established aquatic ecosystems that are supplied with the same kind of freshwater or agricultural wastewater that the SCH ponds are expected to receive. The aquatic food webs in each of the lakes were sampled as comprehensively as possible, up through fish, and analyzed for both Se concentration and carbon and nitrogen stable isotopes. An empirical relationship between Se concentration and $\delta^{15}N$ was developed that was used to model Se concentrations in invertivore and piscivore birds at proposed SCH ponds. The results can be used to aid in determining the level of risk from Se and in making decisions about how best to manage the SCH ponds.

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Chapter 2
Se Accumulation in Freshwater Ecosystems of the Salton Sea

Introduction

The Salton Sea, a large lake located in the southeast of California, provides habitat for thousands of migrating and resident birds (Shuford et al., 2002) as well as a variety of other animals, including the endangered desert pupfish (*Cyprinodon macularius*). However, this habitat will soon be lost if measures are not taken to replace it. As a result of water conservation and the Quantification Settlement Agreement of 2003, inputs to the Salton Sea have been declining and will continue to decline. Already saltier than the ocean, salinity of the Sea will continue to rise as the Sea shrinks until the waters can no longer support wildlife. Recent legislation has been enacted that requires the restoration of this irreplaceable body of water, with plans to build a series of Species Conservation Habitat (SCH) ponds in the footprint of the Sea that will be supplied with river and Sea water. (CRA, 2005; DWR, 2010)

While the basics of the SCH pond design have been worked out, the details of how they will be managed and operated have not currently been defined. Of special concern is how to manage the ponds to minimize risk to wildlife from Se, an essential trace nutrient that becomes toxic at elevated levels (Janz et al., 2010). Particularly worrisome are the bioaccumulative and biomagnifying tendencies of Se, meaning that higher trophic levels often have higher concentrations of Se than lower trophic levels (Luoma and Presser, 2009a). Selenium is brought to the region with the Colorado River water that is used for irrigation, evapoconcentrated on the fields, and released into drains and rivers at elevated levels (Schroeder et al., 2002). With recent studies showing negative effects in birds, one of the more Se sensitive groups of animals (Anderson, 2008; Bennett, 1998; Bruehler and de Peyster, 1999; Henny et al., 2008; Roberts,

1997; Roberts and Berg, 2000), any attempt at the restoration of Salton Sea habitat by building SCH ponds must include plans to protect wildlife from potential Se toxicity.

Management of selenium is greatly complicated by the immense variability in its behavior between sites. Water concentration alone is not enough to predict Se concentrations in organisms and determine level of risk (Skorupa and Ohlendorf, 1991; Stewart et al., 2010). Factors such as pH, redox potential, source and site water Se speciation, sediment composition, Se concentrations in the various compartments, residence time, and composition of the food web all influence the extent to which Se will accumulate in higher trophic levels and thus whether or not toxicity will develop. Selenium is found primarily in four forms in aquatic ecosystems: selenate (SeO_4^{2-} or Se[VI]), selenite (SeO_3^{2-} or Se[IV]), organo selenides (Se[-II]), and elemental Se (Se[0]) (Cai, 2003; Luoma and Presser, 2009a; Maher et al., 2010). Selenium speciation and partitioning between the aqueous and particulate phases are determined by pH, redox potential, sediment composition, and the microbial community, as microbes play a key role in the cycling of Se (Besser et al., 1989; Maher et al., 2010).

Speciation of Se is important in determining potential for toxicity because the different dissolved forms of Se vary in their bioavailabilities. Organic forms of Se are the most bioavailable, followed by selenite, then selenate (Besser et al., 1993; Ohlendorf and Heinz, 2011; Riedel et al., 1991). Thus, toxicity will develop at lower water Se concentrations when the speciation is dominated by organic forms and selenite than when it is dominated by selenate (Skorupa, 1998; Besser et al., 1993). Residence time further influences speciation. Selenium is introduced into the food web via uptake by primary producers, such as algae, which convert the selenium into organic forms (Young et al., 2010; Presser and Luoma, 2010). When the primary producers die, selenite and organo-selenides are released back into the water column. The longer the residence time, the more recycling occurs, resulting in potential accumulation of the more

bioavailable forms since oxidation back to selenate is very slow (Young et al., 2010; Luoma and Presser, 2009b). Longer residence times, therefore, can potentially result in higher Se concentrations and toxicity in the food web because Se is transformed into species that are more easily taken up and accumulated.

Structure of the food web can also have profound influence on how Se will accumulate. For example, different groups of algae have been shown to accumulate Se to different extents, so the composition of the phytoplankton community can determine how much selenium is accumulated at the base of the food web and is available to higher trophic levels (Baines and Fisher, 2001; Stewart et al., 2010). In addition, higher level consumers and predators vary in the extent to which they accumulate Se, meaning that the food items which are available at a site determines how much Se a predator takes in with its diet, and various physical parameters of the predator species determine how much Se is retained from the diet (Stewart et al., 2010; Janz et al., 2010; Presser and Luoma, 2010). With so many different parameters influencing Se behavior, predicting how selenium will accumulate in a particular food web is exceedingly difficult unless the selenium speciation and food web composition at the site of interest are well known.

Food web studies can greatly enhance understanding of Se movement and help build more robust models that can be used for risk assessment and management decisions. Animals acquire selenium nearly exclusively through the diet (Ohlendorf and Heinz, 2011; Stewart et al., 2010; Bowie et al., 1996; Besser et al., 1993). Consequently, knowing what an animal is feeding on is far more important than simply looking at water concentrations when determining potential selenium accumulation and risk in that animal (Janz et al., 2010; Maher et al., 2010). Since the exact composition of species present differs between sites, as well as through time at a single site, samples used for Se accumulation studies should be taken from the location in question and as close together in time as possible (Janz et al., 2010, Young et al., 2010). Previous studies have

linked stable isotope data with selenium concentrations in food web organisms to accurately describe trends in Se concentration with trophic level (Campbell et al., 2005; Jarman et al., 1996; Orr et al., 2006; Stewart et al., 2004). The heavier isotope of nitrogen, ^{15}N, tends to become enriched with movement up the food web (Post, 2002). Consequently, measurement of $\delta^{15}N$ in an organism's tissues can give an idea of what trophic level one organism is feeding at relative to others from the same site. When $\delta^{15}N$ is plotted against Se concentrations, the plot shows how Se concentration changes with movement up through the food web. Use of stable isotope data allows visualization of an actual, rather than assumed, food web (Orr et al., 2006) and when coupled to selenium data gives a true understanding of how selenium concentrations change with trophic level, which can then be used to predict Se concentrations in higher trophic level organisms, such as birds.

For this study, data on food chain selenium concentrations and stable isotope composition, as well as water column, sediment, and sediment pore water selenium concentrations, were taken from the Salton Sea area to use for modeling selenium concentrations in the proposed SCH ponds. Since no SCH ponds have yet been built, two freshwater lakes supplied by water with similar properties to that which will be used to supply the SCH ponds were chosen to gain an understanding of food webs and selenium accumulation in such systems. Stable isotope data was linked to Se data to develop a relationship between tropic position and Se concentration. This relationship was then extrapolated to predict Se concentrations in invertivore and piscivore birds and determine risk to birds from expected conditions in the SCH ponds. Results of the modeling were also taken into context of variation in basal food web composition, water chemistry, and management between the two lakes to help determine how the SCH ponds should be managed to minimize risk from Se toxicity.

Materials and Methods

Study Sites

Two freshwater lakes located south of the Salton Sea near Brawley in Imperial County, CA were chosen as surrogates for the proposed Species Conservation Habitat ponds. Finney and Ramer Lakes are both popular recreation areas for fishing, hunting, camping, and birding. Ramer Lake is supplied by freshwater from the Narcissus 22 and 23 IID freshwater drains and has a typical inflow of 2 acre-feet per day and an outflow of 0.5 to 1 acre-feet per day. Finney Lake is supplied by agricultural water from the Melva and Mayflower drains, has an inflow of 4-6 acre-feet per day, and an outflow of 1.5-2 acre-feet per day (Ramon Redondo, Wister Office, personal communication). The lakes are within the size proposed for the SCH ponds but differ somewhat in surface area, from approximately 160 acres (Finney Lake) to 230 acres (Ramer Lake) (Table 1). Although detailed bathymetry measurements were not made, depth was recorded at a number of sites and suggests mean depths near 3.5 ft for Finney Lake and 5.5 ft for Ramer Lake, yielding lake volumes of roughly 560 and 1265 af, respectively (although it should be noted these are first order approximations and depending upon results, more detailed area-depth-volume measurements may be needed in future studies here). Notwithstanding, these values combined with outflow estimates provided by Ramon Redondo (pers. comm.) suggest residence times of potentially 1-4 yrs (Table 1). Ramer Lake was sampled on May 6, 2011, while Finney Lake was sampled on May 19, 2011.

Table 1: General information on the lakes used in the study

Lake	Latitude	Longitude	Area (acres)	Shoreline (mi)	Volume (af)	Residence Time (d)[a]
Finney	33.060597°	-115.503320°	160	2.8	560	320
Ramer	33.076430°	-115.511654°	230	2.7	1265	1690

[a]*based upon average outflow data provided by Ramon Redondo, Wister Office*

Figure 1: Overhead view of the lakes used in the study

Sampling

One or more locations around each lake were chosen for sampling of fish, plants, and larger invertebrates. A D-net with 243 µm mesh and small pet store fish nets were used to collect fish and invertebrates. Western mosquitofish (*Gambusia affinis*) and invertebrates were sorted in the field by placing net contents into plastic tubs partly filled with lake water then plucking organisms out with forceps and placing them into labeled Nalgene plastic bottles filled with deionized (DI) water. Periphyton was either scraped off of substrates at the site with metal spatulas and stored in plastic bottles or branches containing periphyton were collected and stored in Ziploc bags then scraped off in the lab. Terrestrial macrophytes growing along the shore were sampled by plucking leaves and twigs from multiple plants and placing them into labeled Ziploc bags. All samples were immediately placed on ice in a cooler after collection and then placed into a freezer upon return to the lab.

Portable inflatable boats were used to collect zooplankton and phytoplankton samples from the lakes. Zooplankton samples for Se and stable isotope analyses were taken by performing horizontal tows with an 80 μm mesh opening zooplankton net to collect as much biomass as possible. These samples were rinsed with a squirt bottle filled with lake water into large, acid-washed Nalgene plastic bottles. Vertical zooplankton tows for characterization and quantification of the zooplankton community were also taken at Ramer Lake, with organisms transferred to plastic Nalgene bottles with 70% ethanol. Finney Lake was too shallow to collect meaningful vertical samples however.

Phytoplankton and seston samples for Se and stable isotope analyses were collected via horizontal tows using a 20 μm mesh open opening net and the samples stored in large acid-washed plastic bottles. Lake water was separately collected in amber Nalgene bottles for characterization of phytoplankton communities. All samples were placed in a cooler on ice until return to the lab. Phytoplankton samples that were taken for community characterization were preserved with Lugol's solution before being placed in a refrigerator. All samples collected for Se and stable isotope analysis (benthic invertebrates, phytoplankton, periphyton, zooplankton, fish, etc.) were stored in a freezer at -20°C until further sorting and sample preparation in the laboratory.

Sediment and water samples were also collected from the two lakes. One sediment core from each lake was collected using an Aquatic Research universal corer from an inflatable boat for determination of sediment and pore water selenium concentrations. Upon return to the shore, the core was sub-divided into 2 cm sections, each of which was stored in a labeled plastic bag and placed in a cooler until returned to the lab. Lake water was collected into a large plastic container. 50 mL of water from each lake was filtered through a 0.45 μm mixed cellulose ester

filter, preserved with trace metal grade nitric acid to a concentration of 1% and stored in a refrigerator for determination of total selenium concentration.

Sorting and Preparation of Biota

Invertebrates and fish were removed from samples with forceps, rinsed with DI water, and sorted into numbered, acid-washed KIMAX glass beakers. Beakers were kept on ice during long periods of sorting to keep the samples cool. Once sorted, the beakers were covered with Parafilm and placed in a freezer. These samples were used for both stable isotope and Se analysis.

For zooplankton and phytoplankton, separate samples were prepared for stable isotope and Se analysis. Zooplankton samples for Se analysis were removed from the water they were collected in by carefully pouring them over 180 µm mesh netting held in place over a large glass jar with a rubber band. In a few cases, when only the larger copepods and cladocerans were desired, a 350 µm mesh was used. The zooplankton that remained on the mesh were carefully washed with DI water using a squirt bottle. Obvious debris was removed with forceps and the sample then carefully scraped from the mesh with forceps into a beaker and stored as above. Examination of random portions of the samples under a dissecting scope confirmed that they were nearly entirely zooplankton. For stable isotope analysis, different types of zooplankton, including ostracods, were removed by hand with forceps under a Nikon SMZ800 dissecting scope and placed into new small plastic vials to ensure sample purity. These samples were stored in a freezer.

Periphyton samples from the field were also purified with visible chironomids and debris removed with forceps. The periphyton was then rinsed with DI water and placed into a beaker, covered with Parafilm, and stored in a freezer for Se analysis. The periphyton samples for stable

isotope analysis were examined under a dissecting scope, cleaned of all invertebrates and debris, rinsed with DI water, and placed into new small plastic vials and stored in a freezer.

Zooplankton and debris were removed from phytoplankton samples by pouring the sample through 180 µm mesh netting placed over clean I-CHEM glass jars. Zooplankton stayed on the mesh while phytoplankton passed through into the jars. Lids were placed on the jars and the samples allowed to settle in a refrigerator overnight. The settled phytoplankton were pipetted into acid-washed glass beakers, covered with Parafilm, and stored in a freezer.

Following their clean-up and preparation, all samples were freeze-dried and homogenized. Clean stainless steel scissors, which were rinsed with DI water and dried between each sample, were used to homogenize the samples in the beakers until they reached the approximate consistency of coarse sawdust. Samples were not ground to homogenize because in most cases only a small mass of sample was able to be collected and any potential loss to the grinding apparatus could not be risked. Samples were then covered with fresh Parafilm and stored in a desiccator until analysis.

Stable Isotope Analysis of Biota

Samples of the food web were rinsed and lyophilized prior to isotope analysis. 0.5-1.0 mg of each sample was weighed out into Sn tins to be analyzed. Whenever sample size permitted, a duplicate was prepared for analysis as well. Multiple small pieces were used for each sample to try and get as isotopically homogenous a sample as possible. Ostracods, periphyton, and phytoplankton were not fumigated with hydrochloric acid to remove inorganic carbon because accurate $\delta^{15}N$ values were needed for comparing Se concentration to trophic position.

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios were measured using a Costech ECS 4010 elemental analyzer (EA) coupled to a Thermo Delta-V Advantage isotope ratio mass

spectrometer (IRMS). The use of a continuous flow IRMS allowed for simultaneous measurements of both carbon and nitrogen isotopes. Stable isotope ratios are reported using delta notation (δ) and are expressed permil (‰). This notation compares the ratio, R, of the heavier isotope of an element (X) to the lighter isotope in a sample to that of a standard and can be summarized as:

$$\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000 \quad (1)$$

Results for $\delta^{13}C$ are expressed relative to Vienna PeeDee Belemnite (VPDB) and $\delta^{15}N$ values are expressed versus atmospheric nitrogen. These international standards are given delta values of 0.0‰ so delta values that are positive are enriched in the heavy isotope relative to the standard and negative values are depleted in heavy isotopes relative to the standard. National Institute of Standards and Technology (NIST) 1587 peach leaves were used to correct for non-linearity in isotope ratios caused by small sample size. Samples of USGS40 standard L-glutamic acid ($\delta^{13}C$ = -26.39 ± 0.09‰ and $\delta^{15}N$ -4.52 ± 0.12‰) were included in all analytical runs and used as our primary isotope reference. Since nitrogen stable isotopes are important in our evaluation of selenium movement through the food web, in cases where the standard deviation between the first sample and duplicate was greater than 0.5‰ for $\delta^{15}N$, one or two more replicates were weighed out and analyzed and the average of all replicates was used to represent the sample.

Selenium Digestion and Analysis of Biota

Depending on the amount available, either 0.20, 0.10, or 0.02 g of each sample was weighed out into an acid-washed 50 or 25 mL glass KIMAX Erlenmeyer flask. When sample size permitted, a duplicate was prepared for each. Three duplicates of standard oyster tissue (NIST 1566b) were prepared for each sample weight used as well as three blanks for quality

control. Trace metal grade nitric acid was added (8 mL for 0.20 g of sample, 3 mL for 0.10 g of sample, and 2 mL for 0.02 g of sample) and then a small, acid-washed glass funnel was placed over the top of each sample and the samples allowed to sit in a fume hood overnight to digest.

The next morning, samples were placed on hot plates in a fume hood and heated at 60 to 90°C for 3 to 4 hours, during which time the solution and interior of the flasks turned orange from the NO_x compounds that formed during the destruction of the nitric acid. After these 3 to 4 hours, a small amount of Barnstead filtered water was squirted into each funnel to remove sample that had accumulated in the tip. The glass funnels were removed and the heat increased until the samples came to a gentle boil. Orange then white vapor was given off as the acid was destroyed. Samples were heated until they turned a very light yellow and had significantly reduced in volume (approximately 1-2 mL for the 0.20 g samples, less than 1 mL for the 0.10 g samples and approximately 0.25 mL or less for the 0.02 g samples). This was to minimize nitrate interference during analysis. Samples were rinsed into new or acid washed centrifuge tubes and diluted using a squirt bottle filled with Barnstead filtered water. 0.20 and 0.10 g samples were diluted to approximately 16 mL while 0.02 g samples were only diluted to 10 mL. 2 mL of trace metal grade hydrochloric acid was added to samples that contained sediment (periphyton) to free Se from iron and manganese oxides. The weight of solution and solution density were taken for each sample to determine exact sample volume for calculations. Samples were then either centrifuged at 10,000 rpm for 15 minutes or allowed to settle overnight to remove any sediment or precipitates from solution.

For analysis, 0.5 or 1 mL aliquots of sample solution were placed into 15 mL polypropylene centrifuge tubes with 0.2 mL of 0.2M $K_2S_2O_8$. The solution was diluted with DI water and concentrated HCl to obtain 6M HCl and then heated at 90 degrees Celsius in a water bath for 20 minutes. Following heating, the solution was quickly cooled in a room-temperature

water bath and then 0.3 mL of 5% sulfanilamide solution was added before Se measurement to remove possible nitrate interference. Se concentrations were determined using hydride generation atomic absorption spectrometry (HGAAS).

Data Analysis and Modeling

Selenium concentration versus $\delta^{15}N$ was plotted for both lakes for each food web component in which enough tissue was collected for determination of both. Terrestrial plants and leeches from Ramer Lake were left out of this plotting. Leeches have a style of feeding that was viewed as not representative of normal food-chain transfer and Se accumulation. Best fit lines and linear regression equations were generated and these regression equations were used to predict Se levels in piscivore and invertivore birds. Analytical data for these linear regressions, as generated by SigmaPlot, are given in Appendix B.

For linear regression modeling, a trophic fractionation value of 3.4‰ for $\delta^{15}N$ was used (Post, 2002). For piscivores, 3.4‰ was added to the $\delta^{15}N$ value of mosquitofish to give a value for piscivorous birds, such as double-crested cormorants (*Phalacrocorax auritus*). This $\delta^{15}N$ value was plugged into the regression equations to get an estimate of selenium concentration in these birds. For invertivores, 3.4‰ was added to the $\delta^{15}N$ value for corixids and this value plugged into the regression equations to get estimates of selenium concentration in birds such as black-necked stilts (*Himantopus mexicanus*).

A progressive model was also run for both piscivore and invertivore birds for comparison. The water concentrations for Ramer and Finney Lakes were used, as well as the K_d and TTF values for the general model in Sickman et al. (2011) (K_d = 588, $TTF_{algae\ to\ inverts.}$ = 2.75, $TTF_{fish\ to\ birds}$ = 1.31, $TTF_{food\ to\ birds}$ = 1.8).

Determination of Selenium in Sediments

For Finney Lake, sediment Se concentration and speciation were determined with HGAAS using the parallel extraction method developed by Zhang and Frankenberger (2003). Samples from Ramer Lake were analyzed with HGAAS from determination of total Se concentrations only.

Determination of Selenium in Lake Water and Pore Water

Water from Ramer Lake and sediment pore water from Finney Lake were speciated using a method developed by Zhang et al. (1999). Other site water and pore water samples were analyzed with HGAAS for determination of total dissolved Se only.

Characterization of Zooplankton and Phytoplankton Communities

For Ramer Lake, slide counts using 1 mL aliquots of sample pipetted onto a 1 mm^2 Gridded Sedgewick Rafter slide were conducted with a Nikon E600 compound microscope to determine zooplankton species density and proportions. For Finney Lake, since vertical tows could not be taken, a small amount of the horizontal tow sample was counted for determination of species proportions only. At least three 1 mL subsamples were counted for each lake. Preserved phytoplankton samples were sent to Dr. Danuta Bennett, freshwater ecologist at UC Santa Barbara, for determination of species present. Chlorophyll was measured with an *Aqua*Fluor™ Handheld Fluorometer and solid secondary standard.

Results

Water Chemistry

The two lakes demonstrated differing water chemistries (Table 2). Finney Lake had a higher total dissolved Se concentration (2.9 µg/L) in the water column than did Ramer Lake (1.8 µg/L). The dissolved Se in the water at Ramer Lake was 49.4% selenate, 22.0% selenite, and 28.7% organo-selenides. Finney Lake water had a chlorophyll concentration of 174 µg/L while Ramer Lake had a chlorophyll concentration of 96 µg/L.

Table 2: Information on general water chemistry for the two lakes

	Ramer Lake	Finney Lake
Chlorophyll Concentration (µg/L)	96.4	174.1
Dissolved Se Concentration (µg/L)	1.8	2.9
Water Column Se Speciation	49.4% Se(VI)	--
	22.0% Se(IV)	--
	28.7% Se(-II)	--

Community Composition

Phytoplankton composition differed between the lakes. A full list of species found is shown in Appendix A. The phytoplankton in Finney Lake was dominated by the diatoms *Cyclotella/Thalasiosira* and a species of *Fragilaria*. The diatoms *Cyclotella meneghiniana* and *Nitzschia* were subdominant, as was the green algae *Scenedesmus*. At Ramer Lake, the phytoplankton community was dominated by the cyanobacteria *Raphidiopsis curvata*, while an *Oscillotoria* species was subdominant. Unfortunately, no periphyton sample from Ramer Lake

was left after processing for examination, but a *Lyngbya* species (cyanobacteria) and a *Nitzschia* species (diatom) dominated the periphyton community at Finney Lake. Epiphyton at Finney Lake was dominated by a green algae species of the genus *Oedogonium* and a *Nitzschia* species, which was densely attached to the *Oedogonium* surface.

Results of zooplankton community composition as based on slide counts are shown in Figure 2. Finney and Ramer Lakes had similar zooplankton communities dominated by cyclopoid copepods, identified to genus *Acanthocyclops*, and *Brachionus* rotifers, primarily of the species *B. rotundiformis*. Abundances for Ramer Lake are as follows: *B. rotundiformis* = 341 ± 22 individuals/L, *B. angularis* = 11 ± 4 individuals/L, *Keratella cochlearis* = 1 ± 1 individuals/L, *Polyarthra* = 3 ± 1 individuals/L, *Acanthocyclops* = 172 ± 9 individuals/L, nauplii = 205 ± 10 individuals/L. Cladocerans of the species *Daphnia similis* were found in horizontal tow and invertebrate samples from Ramer Lake, while cladocerans of the genus *Moina* were found in horizontal tow and invertebrate samples from Finney Lake. However, cladocerans did not appear to be a prominent part of the zooplankton populations of either of these lakes. Although they did not appear prominently in slide counts, chydorid cladocerans, ostracods, and calanoid copepods were found in samples from both lakes. A portion of the copepods from both lakes exhibited cyst-like growths. Cysts were most often observed on the sides in between segments of the prosome. Counts from Finney Lake gave an average of 4% of copepods with cysts, though some with cysts may have been missed due to the angle of the copepod on the slide.

Finney and Ramer Lakes exhibited similar macroinvertebrate and vertebrate food web composition as well. Western mosquitofish (*Gambusia affinis*) were caught at both Finney and Ramer Lakes, as well as corixids, damselfly nymphs (all from the genus *Ischnura*), snails (genus *Physella*), chironomids, shrimp, and notonectids. Corixids at Finney Lake were a mixture of five instars of two predatory genera, *Trichocorixa* and *Corisella*. Amphipods, crayfish, leeches, and

one dragonfly nymph (genus *Sympetrum*) were collected only at Ramer Lake while oligochaetes were found only at Finney Lake. It is important to note that just because a certain type of organism was not found during sampling, this does not mean it is not present at the site.

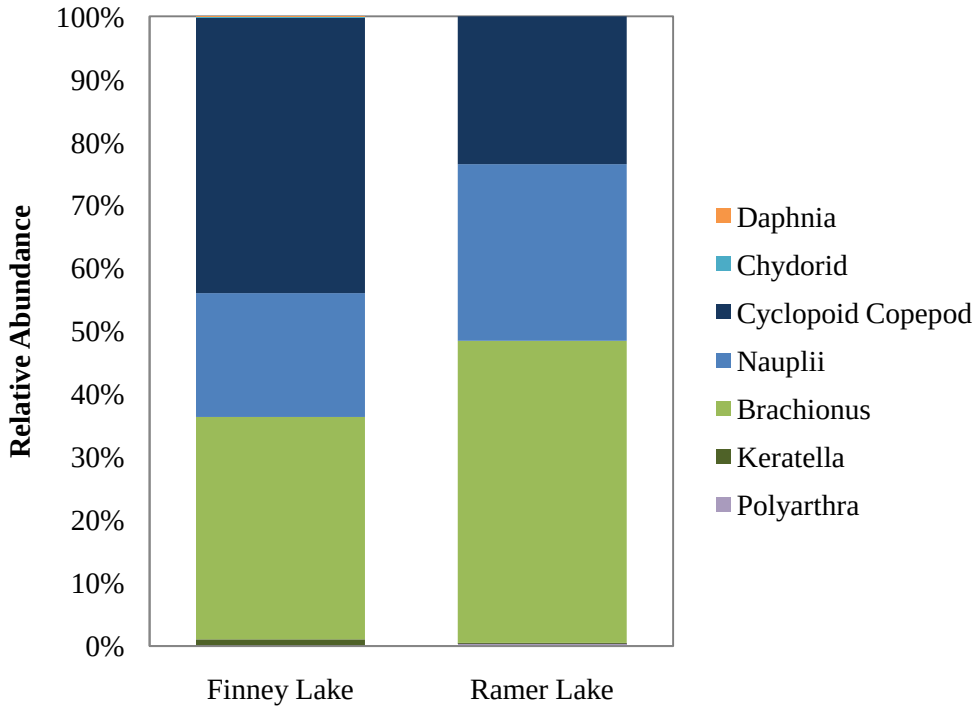

Figure 2: Results of slide counts for determination of zooplankton community composition. Since density was not determined for Finney Lake, only relative abundances are shown.

Stable Isotope Analysis

Stable isotope compositions differed between the lakes. Finney Lake showed higher enrichment in ^{15}N than Ramer Lake (Figure 3). Table 3 shows average $\delta^{15}N$ and $\delta^{13}C$ values for each organism analyzed. For Ramer Lake, $\delta^{15}N$ values range from 2.82 ± 0.04‰ for tamarisk to 10.58 ± 0.36‰ for mosquitofish greater than 2 cm in length, while $\delta^{13}C$ ranged from -13.47 ± 0.08‰ for periphyton to -26.65 ± 0.35‰ for arrundo. Finney shows ranges of 8.11 ± 0.32‰ (phytoplankton) to 13.01 ± 0.46‰ (shrimp) for $\delta^{15}N$ and -14.21 ± 0.26‰ (periphyton) to -29.90 ±

0.56‰ (Tamarisk) for $\delta^{13}C$. Results of t-tests using $\alpha = 0.05$ revealed that $\delta^{15}N$ was significantly higher in Finney Lake than in Ramer Lake for all organisms except copepods ($t = -1.21$, c.v. = 4.30). Ramer Lake was significantly more enriched in ^{13}C than Finney Lake for all organisms except corixids, periphyton, small shrimp, and snails.

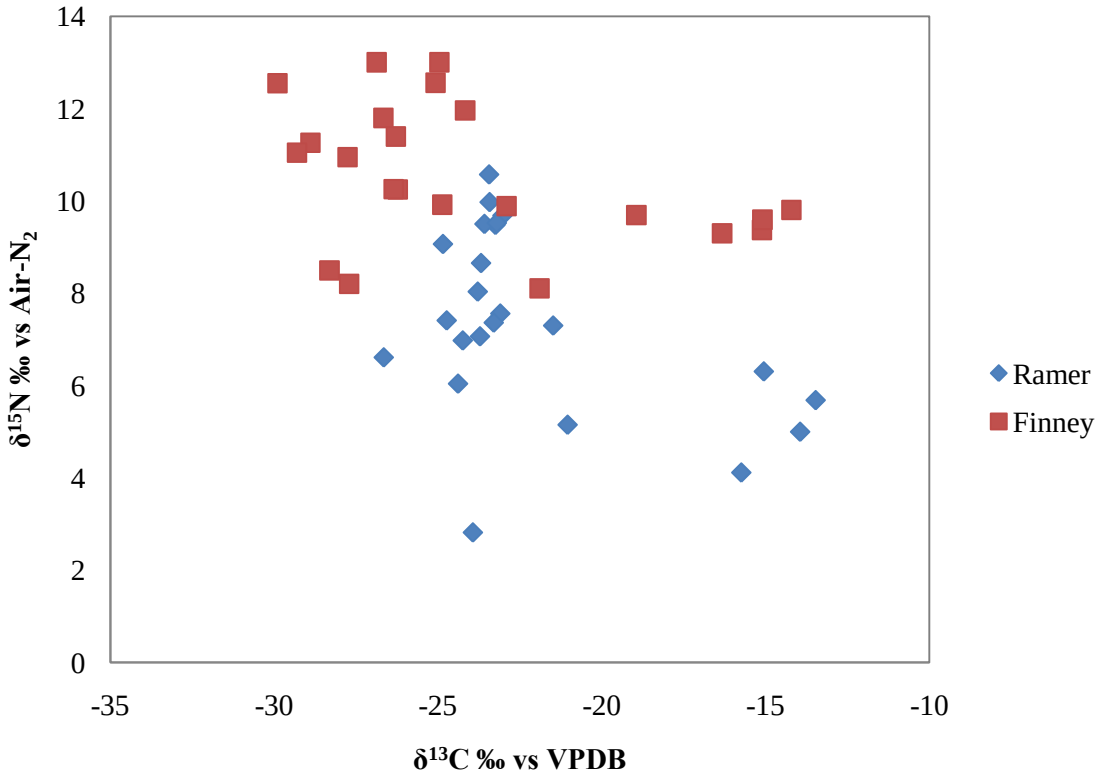

Figure 3: Summary of stable isotope results for Ramer and Finney Lakes

Table 3: Average $\delta^{15}N$ and $\delta^{13}C$ values (± standard deviation) for every organism collected

	Average $\delta^{13}C$ ‰ vs VPDB		Average $\delta^{15}N$ ‰ vs Air-N_2	
Organism	**Ramer Lake**	**Finney Lake**	**Ramer Lake**	**Finney Lake**
Amphipod	-21.04 ± 0.03	none collected	5.15 ± 0.03	none collected
Arrowweed	none collected	-27.71 ± 0.53	none collected	8.21 ± 0.06
Arrundo	-26.65 ± 0.35	none collected	6.61 ± 0.02	none collected
Brachionus	-24.73	-27.76	7.41	10.95
Cattail	none collected	-28.89 ± 0.47	none collected	11.26 ± 0.22
Chironomid Larva	-24.39 ± 0.51	-29.31 ± 0.10	6.04 ± 0.07	11.05 ± 0.00
Cladoceran (Mix)	-24.24	none collected	6.98	none collected
Copepod	-23.42 ± 0.04	-26.35 ± 0.40	9.98 ± 0.30	10.26 ± 0.13
Corixid	-23.30 ± 0.22	-24.87 ± 2.03	7.37 ± 0.39	9.92 ± 0.24
Crayfish	-23.71 ± 0.26	none collected	7.07 ± 0.08	none collected
Damselfly Nymph	-23.78 ± 0.29	-26.67 ± 0.22	8.04 ± 0.14	11.80 ± 0.00
Dragonfly Nymph	-23.10 ± 0.35	none collected	7.56 ± 0.05	none collected
Leech	-23.20 ± 0.20	none collected	9.53 ± 0.41	none collected
Mosquitofish (<2cm)	-23.24 ± 0.27	-25.07 ± 0.27	9.48 ± 0.47	12.56 ± 0.18
Mosquitofish (>2cm)	-23.44 ± 0.46	none collected	10.58 ± 0.36	none collected
Notonectid	-23.68 ± 1.66	-26.28 ± 0.70	8.66 ± 0.41	11.40 ± 0.05
Oligochaete	none collected	-24.17	none collected	11.96
Ostracod	-15.06 ± 0.23	-18.94 ± 0.12	6.31 ± 0.21	9.70 ± 0.14
Periphyton (Diatoms)	-13.47 ± 0.08	-15.11 ± 0.17	5.68 ± 0.39	9.37 ± 0.18
Periphyton (Diatoms)	none collected	-15.10 ± 1.34	none collected	9.59 ± 0.11
Periphyton (Green)	-13.95 ± 2.46	-14.21 ± 0.26	5.00 ± 0.01	9.81 ± 0.29
Periphyton (Mix)	-15.74 ± 0.43	-16.33 ± 2.71	4.11 ± 0.32	9.30 ± 0.33
Phytoplankton	none collected	-28.31 ± 0.47	none collected	8.50 ± 0.32
Phytoplankton (Green)	none collected	-21.90 ± 0.03	none collected	8.11 ± 0.32
Shrimp (Big)	-23.03 ± 0.30	-24.96 ± 0.20	9.69 ± 0.30	13.01 ± 0.46
Shrimp (Small)	-23.58 ± 0.03	-26.87 ± 1.43	9.50 ± 0.10	13.01 ± 0.25
Snail	-21.48 ± 0.14	-22.91 ± 0.95	7.30 ± 0.26	9.89 ± 0.35
Tamarisk	-23.93 ± 0.49	-29.90 ± 0.56	2.82 ± 0.04	12.55 ± 0.15
Zooplankton (Mix)	-24.85 ± 0.23	-26.22 ± 0.03	9.07 ± 0.14	10.25 ± 0.10

Selenium Analysis

Values for every organism that was analyzed for Se concentration are shown in Table 4. Insufficient biomass for Se extraction and analysis limited the number of samples available for Se determination. Se concentrations ranged from 0.24 ± 0.05 mg/kg dw for tamarisk to 12.44 ± 0.13 mg/kg dw for leeches collected from Ramer Lake (Table 4). Results of t-tests at the $\alpha = 0.05$ significance level showed no significant differences in selenium concentrations between lakes for any organism except tamarisk, where Finney Lake had significantly higher Se concentration in tamarisk than Ramer Lake ($t = -10.47$, c.v. = ±4.30). Cattail Se was in the 1 to 2 mg/kg dw range. Periphyton, phytoplankton, arrowweed, and corixids were in the 2-3 mg/kg dw range, while amphipods, chironomids, damselflies, mosquitofish, and shrimp were in the 3-5 mg/kg dw range. Copepods and the zooplankton mixture from Ramer Lake had Se concentrations between 6 and 7 mg/kg dw. With only a few exceptions (periphyton mix and zooplankton mix from Ramer Lake), standard deviations for Se concentrations were less than 0.5 mg/kg dw (Table 4).

Results of Se analysis using NIST oyster tissue standard reference material are shown in Figure 4. Digestion of 0.02 g of tissue averaged 93% recovery of the 2.06 mg/kg dw certified value while 0.10 g averaged 90% recovery and 0.20 g averaged 96% recovery. Analysis of three blanks each yielded readings within 0.1 of zero, indicating no Se contamination of equipment.

Table 4: Average Se concentrations (± standard deviation) for organisms collected from Ramer and Finney Lakes. A dash indicates that the organism was not collected at that lake or that there was not enough material for Se analysis. * Tamarisk had the only statistically significant difference in Se concentrations between lakes at $p \leq 0.05$

Organism	Se Concentration (mg/kg dw)	
	Ramer Lake	**Finney Lake**
Amphipod	3.67 ± 0.17	--
Arrowweed	--	2.37 ± 0.08
Arrundo	0.37 ± 0.09	--
Cattail	--	1.19 ± 0.01
Chironomid Larva	4.34 ± 0.40	3.29 ± 0.01
Copepod	6.80 ± 0.34	--
Corixid	3.02 ± 0.03	3.01 ± 0.01
Crayfish	3.96	--
Damselfly Nymph	3.50 ± 0.33	--
Leech	12.44 ± 0.13	--
Mosquitofish (<2cm)	5.11 ± 0.19	4.10 ± 0.53
Mosquitofish (>2cm)	4.35 ± 0.01	--
Notonectid	5.42	--
Ostracod	--	1.68 ± 0.06
Periphyton (Mix)	2.74 ± 0.56	2.73 ± 0.12
Phytoplankton	--	2.75 ± 0.34
Shrimp (Big)	4.39 ± 0.22	4.60 ± 0.18
Shrimp (Small)	--	4.12 ± 0.06
Tamarisk *	0.24 ± 0.05	1.73 ± 0.20
Zooplankton Mix	6.98 ± 1.39	4.44 ± 0.09

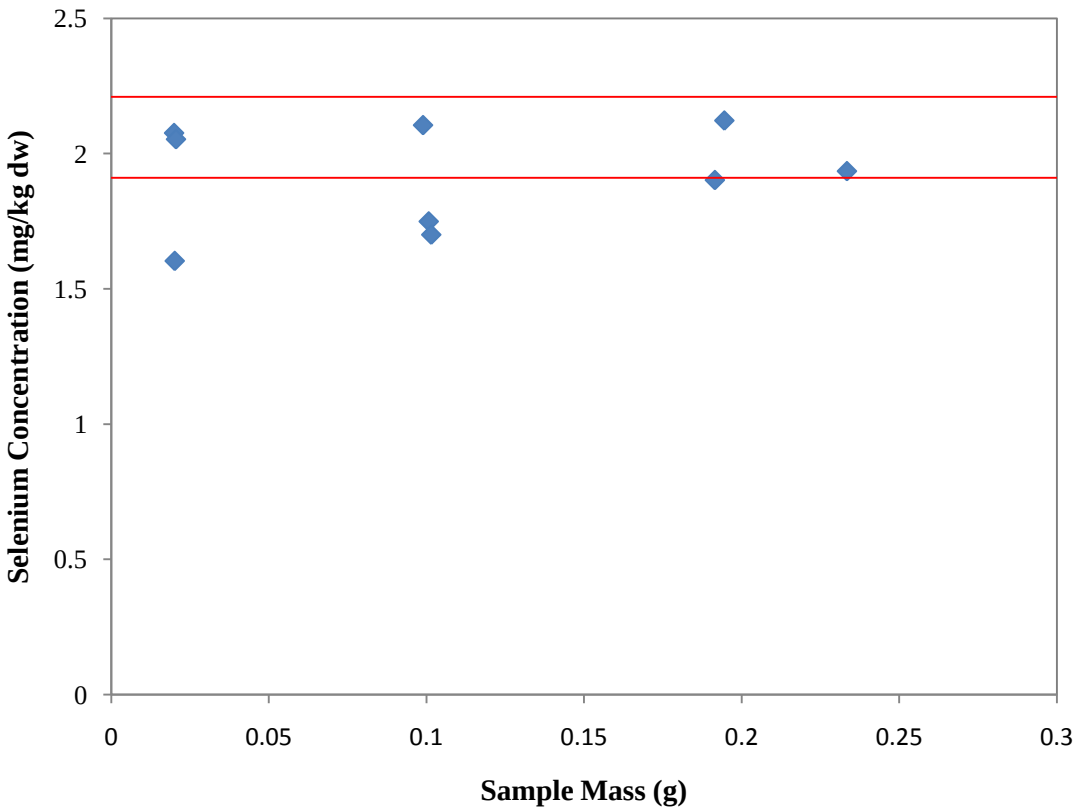

Figure 4: Results of Se analysis of standard oyster tissue (NIST 1566b), certified at 2.06 ± 0.015 mg/kg dw. Red lines indicate the boundaries of the certified range.

Modeling

Selenium concentration tended to increase with increasing $\delta^{15}N$ (Figure 5). Equation 2 gives the best-fit line for Ramer Lake while Equation 3 gives the best-fit line for Finney Lake:

$$Se\ (mg/kg\ dw) = 0.42\delta^{15}N + 1.19 \qquad (R^2 = 0.40) \qquad (2)$$

$$Se\ (mg/kg\ dw) = 0.42\delta^{15}N - 1.16 \qquad (R^2 = 0.54) \qquad (3)$$

Analysis with SigmaPlot revealed that the slopes of these lines are significant ($p = 0.024$ and 0.027 for Finney and Ramer Lakes, respectively) while the intercepts are not ($p = 0.497$ and 0.391 for Finney and Ramer Lakes, respectively) (Appendix B).

Use of the regression equations for selenium modeling provided slightly different results depending on which lake was used (Table 5). Adding a trophic fractionation factor of 3.4‰ (Post, 2002) to the $\delta^{15}N$ value of mosquitofish to get an estimate of $\delta^{15}N$ in piscivores and then substituting the piscivore $\delta^{15}N$ into the regression equations (Eqs. 2 and 3) yielded predicted Se concentrations of 7.06 mg/kg dw and 5.59 mg/kg dw for piscivore birds in Ramer and Finney Lakes, respectively (Table 5). For invertivore birds, 3.4‰ was added to the $\delta^{15}N$ of corixids to get an estimate of $\delta^{15}N$ in invertivores and then this $\delta^{15}N$ value was plugged into the regression equations, yielding estimates for Se concentrations of 5.71 mg/kg dw for Ramer Lake and 4.47 mg/kg dw for Finney Lake (Table 5). Predicted values have a wide 95% confidence interval, however (Table 5).

The progressive model predicted slightly lower Se concentrations than the linear regression model in both invertivore and piscivore birds for Ramer Lake, but nearly double the Se concentration predicted by the regression model for both types of birds for Finney Lake (Table 5). For Ramer Lake, the progressive model predicted Se concentrations of 5.24 mg/kg dw and 6.86 mg/kg dw for invertivore and piscivore birds, respectively. Predicted concentrations for Finney Lake were 8.44 mg/kg dw for invertivores (189% of the predicted concentration obtained using the regression model) and 11.06 mg/kg dw for the piscivores (198% of the predicted value obtained using the regression model).

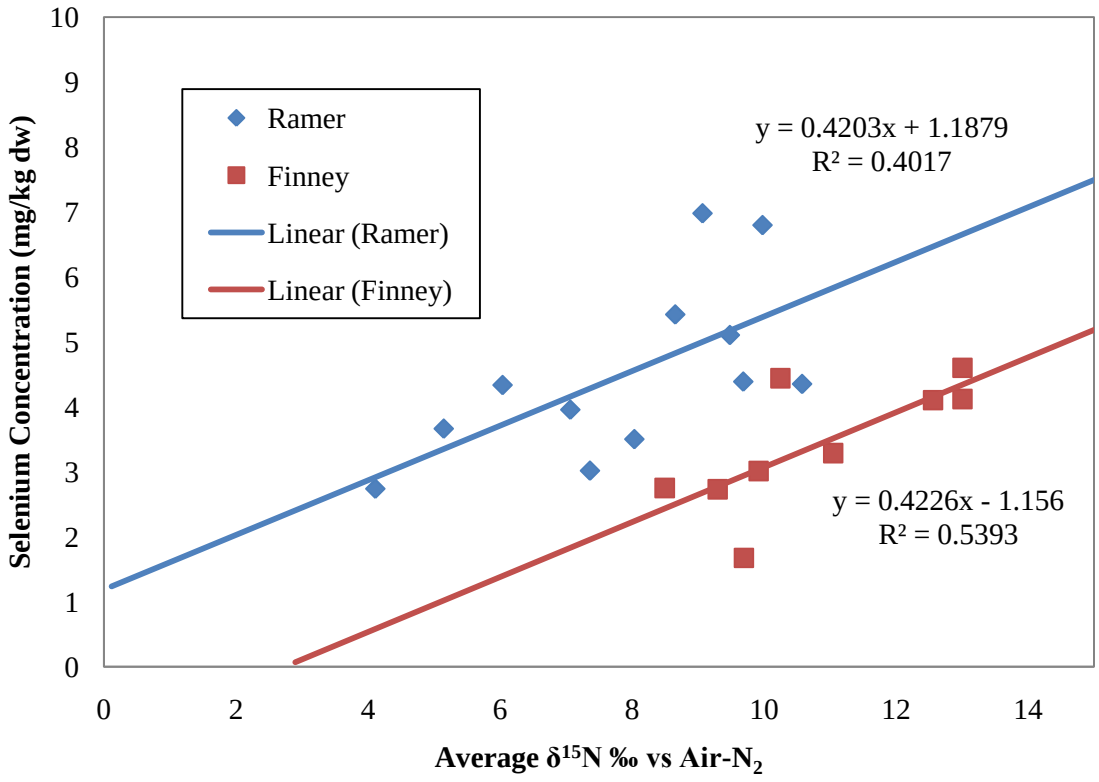

Figure 5: Se concentration plotted against $\delta^{15}N$ values for the lakes. Best fit lines, as well as R^2 values, are shown.

Table 5: Results of linear regression modeling from this study to predict Se concentrations in birds at the proposed SCH ponds. Predicted Se values are shown with the upper and lower limits of the 95% confidence interval in parenthesis. Also shown, for comparison, are the predicted Se concentrations from running a progressive model with the water concentrations from Ramer and Finney Lakes and the same K_d and TTF values as used in Sickman et al. (2011) for the general model.

	Invertivore Birds		**Piscivore Birds**	
	Progressive (mg Se/kg dw)	**Regression (mg Se/kg dw)**	**Progressive (mg Se/kg dw)**	**Regression (mg Se/kg dw)**
Ramer	5.24	5.71 (2.96-8.35)	6.86	7.06 (3.77-10.01)
Finney	8.44	4.47 (2.55-6.39)	11.06	5.59 (3.36-7.92)

Discussion

Community Composition

Ramer and Finney Lakes showed quite similar food webs. Both had zooplankton communities dominated by cyclopoid copepods and *Brachionus* rotifers. Cysts such as those found on the copepods at both lakes have been attributed to puncture wounds from an ellobiopsid parasite (Bridgeman et al., 2000). Though likely not related to Se levels, it has also been suggested that such growths are the result of a carcinogen in the water (Omair et al., 1999). While it is not known exactly how much of a detriment these cysts are to a copepod population, such growths likely exert a stress on these important food-web organisms.

Macroinvertebrate and vertebrate communities were quite similar as well. This is not surprising, despite the fact that the lakes receive different source water, as the lakes are adjacent to each other (Figure 1). The algal communities at both lakes were dominated by cyanobacteria and diatoms, though the species composition differed between lakes and the phytoplankton community at Ramer Lake was dominated by cyanobacteria while that at Finney Lake was dominated by diatoms. Finney Lake had higher chlorophyll levels than Ramer Lake (Table 2), indicating higher concentrations of phytoplankton. As selenium uptake has been shown to be proportional to both selenium concentration and phytoplankton biomass (Baines and Fisher, 2001; Riedel et al., 1996), this finding suggests that selenium could potentially be more effectively taken into the food web in Finney Lake, though no significant differences in Se concentration were observed for aquatic organisms (Table 4).

The phytoplankton and periphyton community at a site is important because its composition is a critical factor in determining how much Se is taken into the base of the food web for transfer to higher levels. Different groups of phytoplankton accumulate Se to different extents. Chlorophytes, green algae, tend to show the lowest Se enrichment, while

prymnesiophytes, prasinophytes, and dinoflagellates exhibit some of the highest Se enrichment (Baines and Fisher, 2001). Algal composition tends to vary throughout the year, with diatoms dominating in the colder winter months, green algae dominating in the warmer spring months, and cyanobacteria dominating in the hot summer months (Wetzel and Likens, 1991). This seasonal succession of algal community can result in seasonal variation in Se concentrations in herbivores that is then passed up the rest of the food web (Baines and Fisher, 2001; Stewart et al., 2010). Because of the warm conditions at Salton Sea, it is not surprising that the phytoplankton community at Ramer Lake was dominated by cyanobacteria, even in May. It is somewhat more surprising that diatoms were dominant in the phytoplankton community at Finney Lake. This may be the result of there being sufficient quantities of silica and nutrients, carried into the lake with the agricultural water, to sustain the diatom community later in the season than they would typically be found. Since chlorophytes were not dominant in the communities at the time of sampling, it can be assumed that enrichment of Se at the base of the food web was, perhaps, about as high as it gets in these systems and that Se concentrations throughout the rest of the food web may be near maximum as well. However, because communities change with season, risk from Se toxicity could vary throughout the year (Baines and Fisher, 2001). The results presented here only apply to the specific conditions and communities that were present at the time of sampling. Further study is needed to assess the threat at other times, although the typically warm conditions in the region suggest that late spring – early summer conditions would likely be applicable to much of the year.

Selenium and Stable Isotope Analysis

Selenium concentrations in organisms, sediment, and water collected from these freshwater lakes are not alarmingly high, though many fall above established thresholds for the

protection of higher trophic levels from bioaccumulation of Se. Chironomids, mosquitofish (>2cm), and shrimp from Ramer Lake, as well as mosquitofish (<2cm), shrimp, and zooplankton from Finney Lake, all fall in the 4-5 mg/kg dw moderate hazard range (Table 4). Ramer Lake zooplankton, leeches, mosquitofish (<2cm), and notonectids fall above the high hazard threshold (> 5 mg/kg dw) established for dietary organisms (Lemly, 1996). Though not a focus of this study, core data were taken to evaluate sediment and pore water selenium concentrations and how they vary with depth (Appendix C). For both lakes, sediment selenium concentration in the surface layer was about 6 mg/kg dw. This places them into the high hazard category for selenium accumulation from the sediments into the benthic community (Lemly, 1996). Water Se concentrations in Finney Lake, but not Ramer Lake, are above the 2 µg/L toxicity threshold (Lemly, 1996). Measured Se concentrations in these food webs suggest that birds feeding in them are at risk from Se toxicity.

The two lakes showed very little difference in Se accumulation. The significantly higher Se concentration found in tamarisk from Finney Lake could be due to a difference in age. Tamarisk collected at Finney Lake was much smaller and younger than that collected at Ramer Lake and thus may have had different nutrient dynamics or distributions than the older plants. Excluding this exception, Se concentrations in sediments and aquatic food web organism were the same. Pore water concentrations at the surface of the cores were approximately 10 µg/L for both lakes, with Finney Lake just a little higher than Ramer Lake (Appendix C). It is notable that Finney and Ramer Lakes showed such similar sediment and pore-water Se concentrations in the upper core layer and that Se concentrations in aquatic organisms that were able to be compared between lakes showed no statistically significant differences. Se concentrations in the water column, however, were different between the lakes, with Finney Lake having a higher concentration than Ramer Lake (Table 2). It appears the difference of only 1 µg/L Se

concentration in the water column is not enough to affect food chain accumulation in these systems.

Finney and Ramer Lakes showed more differences in stable isotope composition than in selenium concentrations. The most notable difference between the two lakes was in $\delta^{15}N$ values. While the source water may not be different enough in Se concentration or speciation to affect Se accumulation in the two food webs, it is different enough in stable isotope composition to result in organisms from Finney Lake being more enriched in ^{15}N than those from Ramer Lake (Figure 3). This is most likely due to Finney being supplied by agricultural wastewater, while Ramer is supplied only by freshwater (Ramon Redondo, Wister Office, personal communication). Manure, which may be applied to agricultural fields in the area, has $\delta^{15}N$ values ranging from +5‰ to +25‰ (Xue et al, 2009). $\delta^{15}N$ values for Finney Lake fall within this range (Table 3). Other processes, such as denitrification, can enrich ^{15}N in agricultural drain water between the field and receiving water bodies (Nestler et al., 2011). Both these possible explanations can easily explain why Finney Lake is more enriched in ^{15}N than Ramer Lake.

With a few exceptions, Se concentration tended to increase with increasing $\delta^{15}N$. Results of statistical analysis indicate that this positive correlation of Se increasing with trophic level is significant (Appendix B). The relatively high Se concentration found for leeches at Ramer Lake was unexpected. However, in mammals Se is transported in the blood (Shamberger, 1983), so it may be that shrimp haemolymph is a rich source of Se. Because the leeches were viewed as special cases that varied from typical food web accumulation, they were left out of the regression analysis. For both Ramer and Finney Lakes, zooplankton plotted above the best-fit line, indicating a higher Se concentration than would be expected for their $\delta^{15}N$ value. This could be due to direct uptake of Se from the water column, as has been demonstrated for cladocerans (Besser et al., 1993; Tsui and Wang, 2007; Yu and Wang, 2002), or it may be a result of there

being two different basal food webs in these lakes, one pelagic and one littoral. The pelagic food web would consist of the phytoplankton, cladocerans, rotifers, and copepods, while the littoral food web would consist of the periphyton, chironomids (most of which were collected from the periphyton and were observed feeding on it), amphipods, and other such organisms. These two basal food webs may have different selenium accumulation dynamics, as has been shown in a marine system in San Francisco Bay, where a bivalve-based food web accumulated higher Se concentrations than a crustacean-based food web (Stewart et al., 2004). Higher consumers, such as mosquitofish, likely feed off both food webs, and bring them together when modeling up through piscivorous birds. Ostracods from Finney Lake fell below the regression line, having lower Se concentration than would be expected for their $\delta^{15}N$ value. This may be because the bulk of the weight of an ostracod is composed of inorganic carbon, which would not be expected to contain much, if any, Se. The regression line for Finney Lake (Eq. 3) showed a better fit than that from Ramer (Eq. 2), as all organisms except the just mentioned zooplankton fell closely to the regression line. While there is some deviation from a straight line, results show that biomagnification is occurring in these systems, with higher trophic levels containing higher concentrations of Se.

Comparison to Other Recent Studies at the Salton Sea

Concentrations of Se in mosquitofish, crayfish, corixids, chironomids, and algae are similar to those found in recent studies of ponds, wetlands, and drains at the Salton Sea and surrounding area (Johnson et al., 2009; Miles et al., 2009; Saiki et al., 2010). A summary of the findings of these studies is presented in Table 6. In a study of the Imperial wetlands, Johnson et al. (2009) found that an inflow Se concentration range of 2.7 to 5.4 µg/L resulted in median Se concentrations of 0.3 mg/kg dw in sediments, 4.3 mg/kg dw in mosquitofish, 5.2 mg/kg dw in

glass shrimp, and 4.1 mg/kg dw in corixids. For the Brawley wetlands, which had a Se concentration range of 2.2 to 3.9 µg/L in the inflow, median Se concentrations of 0.4 mg/kg dw in sediments, 3.8 mg/kg dw in mosquitofish, 3.8 mg/kg dw in glass shrimp 2.6 mg/kg dw in crayfish, and 3.7 mg/kg dw in corixids were found. The Imperial wetlands are supplied with agricultural drain water while the Brawley wetlands are supplied with New River water (Johnson et al., 2009).

Table 6: Summary of findings from recent studies of freshwater habitats located near the Salton Sea

			Selenium Concentration				
Location	**Study**	**T_r (days)**	**Water (µg/L)**	**Sediment (mg/kg dw)**	**Corixids (mg/kg dw)**	**Chironomids (mg/kg dw)**	**Mosquitofish (mg/kg dw)**
Drains	Saiki et al., 2010	<1	0-5	0-3	1.0-4.0	2.0-10.0	2.0-8.0
Brawley wetlands	Johnson et al., 2009	9	2.2-3.9	0.4	3.7		3.8
Imperial wetlands		18	2.7-5.4	0.3	4.1		4.3
SHP Pond 1	Miles et al., 2009	9.9	2.0-3.0	1-2.3	3.5-5.5		
SHP Pond 2		36.3	1.5-2	0.9-1.6	3.0-5.0		
SHP Pond 3		70.2	1.5-2.5	1.7-3	3.5-5.25		
Finney Lake	This study	320	2.9	5.9	3.01	3.29	4.1
Ramer Lake		1690	1.8	6.1	3.02	4.34	4.35-5.11

Saiki et al. (2010) performed an extensive study of drains at the south end of the Salton Sea. In this study, concentrations of dissolved Se from 0 to 5 µg/L resulted in approximate ranges of mean Se concentrations from about 0 to 3 mg/kg dw in sediments, 2 to 7 mg/kg dw in particulate organic detritus, 1 to 5.5 mg/kg dw in filamentous algae, 2 to 10 mg/kg dw in chironomid larvae, 1 to 4 mg/kg dw in net plankton (which consisted of anything in the water column large enough to be retained in the net, including zooplankton, insect larvae, corixids, and

plant debris), and 2 to 8 mg/kg dw in mosquitofish. Salinities varied over time in these drains, but means were typically between 0 and 5 ppt, indicating generally freshwater or brackish water systems.

In a third recent study, Miles et al. (2009) examined Se concentrations in water, sediment, and biota in a series of four ponds (known as shallow water saline habitat ponds, or SHP) built to examine the feasibility of restoration of Salton Sea habitat via shallow ponds supplied by a mix of Alamo River and Sea water. Pond 1 had a salinity of approximately 5 to 25 ml/L and Se concentrations of approximately 2 to 3 µg/L in water, 1 to 2.3 mg/kg dw in sediments, and 3.5 to 5.5 mg/kg dw in corixids. Pond 2 (salinity of 10 to 30 ml/L) had Se concentrations of about 1.5 to 2 µg/L in water, 0.9 to 1.6 mg/kg dw in sediments, and about 3 to 5 mg/kg dw in corixids while Pond 3 (salinity of 30 to 70 ml/L) had Se concentrations of about 1.5 to 2.5 µg/L in water, 1.7 to 3 mg/kg dw in sediments, and approximately 3.5 to 5 mg/kg dw in corixids. Pond 4 was representative of hypersaline conditions.

There are some differences between the Se concentrations found during the study at Finney and Ramer Lakes and those found in other recent studies around the Salton Sea. In all the previous studies, sediment concentrations were lower than those found in this study. This is likely due to a difference in age or residence times. With the possible exception of the drains, Finney and Ramer Lakes are the oldest systems examined in these recent studies. The Brawley and Imperial wetlands were constructed in 2000 (Johnson et al., 2009) while the SHP ponds were constructed in 2006 (Miles et al., 2009). It was found that sediment Se concentrations increased over time in Ponds 1 and 2 of the SHP ponds, indicating that Se removal pathways develop over time (Miles et al., 2009). At the Brawley and Imperial wetlands, sediments were found to retain more Se than any other compartment (Johnson et al., 2009). It is possible that sediments accumulate Se over time. Residence time influences the amount of time sediments have to

interact with dissolved Se, and thus may influence how much Se is accumulated in sediments. Drains would be expected to have a very short residence time, as water is constantly flowing through them. The Brawley wetlands had a residence time of only 9 days, and the residence time of the Imperial wetlands was only slightly longer than this at 18 days (Johnson et al., 2009). Mean residence times for the SHP ponds were as follows: Pond 1 = 9.9 days, Pond 2 = 36.3 days, Pond 3 = 70.2 days (Sickman et al., 2011). It was estimated from available information that Finney Lake has a residence time near 320 days, while Ramer Lake has the longest residence time of all at about 1690 days (Table 1). Upon examination, it can be seen that the systems with the shortest residence time had the lowest sediment Se concentrations and those with the highest had the highest sediment Se concentrations (Table 6). The Brawley and Imperial wetlands had the lowest sediment Se, while the SHP ponds had intermediate sediment Se concentrations, and Finney and Ramer Lakes had the highest. A possible exception to this general trend is the drains, which had sediment concentrations ranging across those of the wetlands and SHP ponds at similar dissolved Se concentrations. Agricultural drains can show drastic changes in dissolved Se concentration across time (Saiki et al. 2010), so it may be that the higher sediment concentrations are residual from a time of higher dissolved Se concentration. The trend between residence time and sediment Se concentration is intriguing, but without a thorough examination of all differences between the systems it cannot be stated with any certainty that the higher sediment Se concentrations are the result of longer residence time and not some other parameter.

Selenium concentrations in samples of biota, though generally quite similar between Ramer and Finney Lakes and the other sites studied within the past decade, also showed some differences. Concentrations in corixids from the wetlands and SHP ponds were all slightly higher than those found in this study. Shrimp from the Brawley wetlands had slightly lower Se concentrations and those from the Imperial wetlands had slightly higher Se concentrations than

those in Ramer and Finney Lakes, while crayfish from the Brawley wetlands had slightly lower Se concentrations than those in Ramer Lake. Mosquitofish from the Imperial and Brawley wetlands had Se concentrations quite similar to those found in this study. The variation in the Se concentrations found at drains does not allow direct comparison of numbers, but in all cases the Se concentrations found in Finney and Ramer Lakes were within the range of those found in the same organisms for the study by Saiki et al. (2010) when water Se concentrations were between 0 and 5 µg/L. Despite subtle differences, selenium concentrations in biota are not different enough to make any conclusions, without examination of variation in all parameters, other than Se concentrations in the food web do vary to some extent between systems. However, with the exception of the less stable drain systems, dissolved Se concentrations of 0 to 5 µg/L in the water generally result in concentrations of 3 to 5 mg/kg dw in corixids and about 4 to 5 mg/kg dw in mosquitofish and shrimp.

In planning the management of the SCH ponds, there is debate over whether or not longer or shorter residence times are safer from a selenium perspective. Longer residence times can potentially lead to increased recycling of selenium with an accumulation of more bioavailable forms (Luoma and Presser, 2009b; Young et al., 2010), while short residence times could potentially elevate risk due to increased Se loading. Unfortunately, not every study gave information on Se speciation to compare residence time to proportion of selenate, selenite, and organo-selenides. However, Saiki et al. (2010) found that speciation in drain water was generally 82% selenate, 9% selenite, 8% organic Se, and 1% particulate Se across all drains sampled. In this study, it was found that the dissolved Se in Ramer Lake was 49.4% selenate, 22% selenite, and 28.7% organic Se (Table 2). Therefore, Ramer Lake, which has a much longer residence time than agricultural drains, does have about 13% more selenite and 20% more organic Se than typical agricultural drain water, demonstrating that increased residence time does result in

accumulation of more bioavailable forms in aquatic ecosystems around the Salton Sea. However, based on the concentrations measured in biota, this does not appear to have a detrimental effect on wildlife. A potential explanation is that selenite is more easily adsorbed to sediment particles than selenate (Maher et al, 2010). Thus, selenite, although more bioavailable, may be more easily removed from the water column into the sediments, as evidenced by the higher sediment Se concentrations in Ramer and Finney Lakes. The shorter residence time, more variable drains exhibited wider ranges in average Se concentrations in biota than any of the wetlands, lakes, or ponds. This indicates that systems with longer residence times are more able to buffer Se concentrations due to removal pathways while removal pathways are not developed in systems with very short residence times and concentrations in biota can change quickly depending on changes in inflowing Se concentration. Without more study, no definite conclusions can be made about whether or not shorter residence time increases Se loading. That said, the similar Se concentrations found in biota of all wetland, pond, and lake systems indicate that Se loading is not a major problem in systems with shorter residence times. Based on available evidence, as long as residence times in the SCH ponds are at least as long as those in the Imperial and Brawley wetlands and inflow Se concentrations do not exceed 5 µg/L, Se concentrations in wildlife at the SCH ponds should not exceed those currently found in similar habitats in the Salton Sea area.

Regression Modeling

Results of food web modeling using regression equations indicate that piscivorous birds in freshwater SCH ponds may be at risk from decreased reproduction due to Se toxicity, but that invertivore birds should not be in danger. With the exception of the zooplankton, data from Finney Lake plotted very linearly (Figure 5). Because of its better fit, the model for Finney Lake more accurately reproduced Se concentrations in organisms that were collected than the model

for Ramer Lake. Using the Finney regression equation (Eq. 3) yielded predicted Se concentrations in mosquitofish and corixids to within 0.05 mg/kg dw of the actual measured concentrations (1.2% and 1.0%, respectively), and gave a predicted value of 5.59 mg/kg dw for piscivorous birds and 4.47 mg/kg dw for birds consuming invertebrates such as corixids (Table 5). Data from Ramer Lake did not plot as tightly as that from Finney Lake, but followed the same trend and yielded the same slope of 0.42 (Figure 5). When the Ramer regression equation was used to make predictions, it overestimated the Se concentrations in mosquitofish and corixids by about 1.3 mg/kg dw (29.4% and 42.1%, respectively) and also yielded higher predicted concentrations for birds (7.06 mg/kg dw for piscivores and 5.71 mg/kg dw for invertivores)(Table 5).

For both lakes, estimates for invertivores fall below the reduced hatchability threshold of 6 mg/kg dw generated for eggs of the invertebrate consuming shorebird black-necked stilt (Skorupa, 1998). Therefore, modeling results suggest that Species Conservation Habitat ponds designed similarly to these lakes should not pose a risk to invertivore birds. Estimated Se concentrations for piscivore birds using the Ramer equation fall slightly above the 6 mg/kg dw threshold, indicating that piscivores may be at risk from low levels of reproductive impairment in these systems. However, the exact sensitivity of birds such as pelicans and double-crested cormorants to Se is not known. They may be more or less sensitive than stilts, though a study of American white pelicans at Pyramid Lake, Nevada found no evidence of impaired reproduction at egg Se concentrations below 6 mg/kg dw (Wiemeyer et al., 2007). Also, studies have indicated that fish-eating birds are not as sensitive to Se as various species of ducks and shorebirds (Skorupa et al., 2004). This suggests that the 6 mg/kg dw threshold should be protective of piscivores, and that they may not develop symptoms of toxicity at the slightly higher levels

predicted. Based on modeling from the food webs collected, Se toxicity should not be a major problem for birds in similar freshwater systems.

There are a few additional considerations when interpreting the modeling results. Only a few species of birds were modeled here and only two similar food webs were modeled. If the design of the SCH ponds includes cattails and other aquatic vegetation, such as these lakes, more sensitive species, such as ducks (Skorupa, 1998), may utilize the ponds. Although this model could be used to predict Se concentrations in ducks, such predictions were not made. At the same time, if no aquatic vegetation is included (i.e., strictly pelagial), the food webs may be different than those modeled here and may exhibit different Se accumulation dynamics.

Another factor to keep in mind is that this modeling does not specify a specific tissue in birds. Egg concentrations are the most reliable tissue for predicting risk from Se toxicity (Skorupa, 1998), so eggs would be the best tissue to model. For this modeling, it is assumed that egg Se accumulation follows that of the adult tissue, with the same increase with trophic level as established for the rest of the food web by the regression equations; that is, Se is not preferentially accumulated in bird eggs to levels above those that would be expected based on their stable isotope composition. Since it has been found that most of the selenium in avian eggs comes from the diet of the mother rather than from mobilization of Se from maternal tissue and that egg production is not a significant pathway of Se excretion (Hobson et al., 1997; Janz et al., 2010), this assumption seems to be valid. However, if Se is preferentially accumulated in eggs, this model will likely underestimate Se concentrations in bird eggs. Also, since selenium is an essential nutrient in eggs, it is likely that there is regulation between the amount of selenium in the mother's diet and the amount of selenium that reaches the egg (Ramirez et al. 2011). Knowledge of the relationship between egg selenium concentrations and stable isotope

compositions in relation to those of the mother's diet would allow improved modeling of egg Se concentrations based on dietary data.

The only way to validate or disprove our assumption that egg Se can be modeled using a regression equation for $\delta^{15}N$ versus selenium concentration would be to add into our study the sampling and analysis of bird eggs. Jarman et al. (1996) conducted such a study on the Farallon Islands off the coast of California. They found that selenium concentrations increased up to the level of fish and then decreased in higher trophic levels, such as bird eggs. Therefore, it could be that actual egg selenium concentrations will be lower than those predicted in this study. It should be noted, though, that the birds sampled in the Jarman et al. (1996) study were marine species. Marine birds have been found to store proportionately more selenium in their livers and less in their eggs when compared to freshwater species (Janz et al., 2010), so comparison to our freshwater food webs may not be valid. Without collection of eggs to assess the predictive value of this model, the results generated by its use must be interpreted with caution and viewed as only possible rather than actual.

It is also important is to stress that this model only represents a snapshot in time of the conditions present on the days of sampling. Se concentrations, food web structure, and various other parameters vary through time. This model only applies to the conditions present on the days of sampling and may not be valid for making predictions throughout the rest of the year. Fortunately, the dates of sampling were ideal for a study of Se, an element which primarily effects reproduction in birds, as many egrets and cormorants were currently nesting on Ramer Lake. There is still much we do not know about the behavior of Se in freshwater habitats in the Salton Sea area, however. Future studies conducted at multiple times throughout the year and for a wider variety of habitats are needed to fully assess the applicability of this model and gain a

better understanding for how Se accumulation changes with varying parameters to develop more dynamic models.

It is interesting to note that the best-fit lines generated for Ramer and Finney have nearly identical slopes. The line for Ramer has a slope of 0.420 while that for Finney has a slope of 0.423 (Figure 5). Therefore, there seems to be agreement for an increase of 0.42 mg/kg dw of Se for every 1‰ increase in $\delta^{15}N$ for the food webs modeled. However, this slope is not universal. In a study of San-Francisco Bay, Stewart et al. (2004) found an increase in Se concentration of 2.5 mg/kg dw for every 1‰ increase in $\delta^{15}N$ for a clam-based food web and an increase of 0.73 mg/kg dw in a crustacean-based food web. When studying freshwater systems exposed to Se from coal mining operations, Orr et al. (2005) found an exponential increase in Se concentration with increasing $\delta^{15}N$. It's hard to say whether or not the striking similarity in the slopes of the best-fit lines for Finney and Ramer Lakes is simply coincidence or due to a close resemblance between the food webs and other parameters, but it seems clear from the results of similar studies that the way in which Se accumulates can vary a great deal between food webs and systems. Since the food web in Finney Lake was more enriched in ^{15}N than that in Ramer Lake (Figure 3), its best-fit line is shifted right and down of that from Ramer Lake. In order to make direct comparisons between the lakes, $\delta^{15}N$ values would have to be converted to trophic positions.

Calculating trophic positions rather than simply using $\delta^{15}N$ values has some advantages. Trophic positions standardize values relative to the baseline $\delta^{15}N$ of each site, allowing direct comparison between locations (Jardine et al., 2006; Post, 2002). Calculating trophic positions also gives a more accurate idea of feeding relationships than $\delta^{15}N$ values, as trophic fractionation values can vary by species and diet (Jardine et al., 2006; McCutchan et al., 2003). It is, therefore, not safe to assume that an increase of 3.4‰ in $\delta^{15}N$ represents an increase in one trophic level, or that organisms with the same $\delta^{15}N$ value are at the same trophic level, even if the organisms were

taken from the same site on the same day. This modeling would benefit by converting $\delta^{15}N$ values to trophic positions using the two-habitat equation presented in Post (2002), and then plotting trophic positions against Se concentrations to give a more clear portrayal of Se accumulation up the food web. The slope of a best fit line generated for such a plot would represent the change in selenium concentration from one trophic level to the next, thus allowing easier and more accurate predictions by correcting for variations in stable isotope composition in the food web (Jardine et al., 2006). Unfortunately, there were not enough data on the food webs present in the lakes to accurately determine trophic positions.

Conversion to trophic positions was hampered by a few difficulties. Stable isotope signatures for organisms at the base of the food web show considerable spatial and temporal variation (Post, 2002). Only sampling on a single day for each site, as was done in this study, could thus give misleading information. One method to account for this variation and get better model results is to standardize values to the stable isotope composition of long-lived primary consumers, which integrate temporal variation in primary producer isotope signature, when performing calculations of trophic position (Post, 2002). It is suggested that snails be used to represent the littoral food web and mussels be used to represent the pelagic food web. Although snails were found at both lakes, there are no appropriate primary consumers to represent the pelagic food web. Continuing to sample over a year or two to achieve an understanding of how stable isotope signatures and selenium concentrations vary across time would be beneficial. Although less important in the context of this study, one notes that accurate $\delta^{13}C$ values were not obtained for algae and ostracods. The decision not to remove the inorganic carbon, which resulted in the relatively enriched $\delta^{13}C$ values in these organisms (Table 1), was to ensure that accurate $\delta^{15}N$ values were obtained to use in modeling. Gut content analysis would also help to build a food web and achieve better modeling results by helping to accurately determine trophic

position. Though this study is a step forward in understanding Se accumulation in freshwater food webs at Salton Sea, more data is needed to refine models and expand their use to the varying conditions found through time.

Comparison with Progressive Modeling Results

Modeling results from this study predicted lower Se concentrations for birds in freshwater SCH ponds than the progressive model used in Sickman et al. (2011). Sickman et al. predicted Se concentrations in piscivores of 12.9 mg/kg dw using New River water and 21.4 mg/kg dw using Alamo River water, and Se concentrations in invertivores of 9.8 mg/kg dw using New River water and 16.3 mg/kg dw using Alamo River water. The difference in concentrations predicted by the empirical Se-stable isotope model and the progressive model can be explained in a number of ways.

The progressive model used in Sickman et al. (2011) was developed by Luoma and Presser (2009b). It uses a partitioning coefficient (K_d) to describe the ratio between Se concentrations in the aqueous and particulate phases, and trophic transfer factors (TTF) to describe the ratio of Se concentrations between a consumer and its diet. Expected water Se concentrations were multiplied by a K_d, as determined from the combined results of multiple recent studies at Salton Sea, to predict the Se concentration in particulates, such as algae, at the base of the food web. Then the Se concentration of this first trophic level, and each level after, was multiplied by a TTF to predict the Se concentration in the next trophic level. The trophic transfer factor used to predict bird egg concentrations, 1.8, was developed for the mallard (*Anas platyrhynchos*) (Presser and Luoma, 2010). However, trophic transfer factors are species-specific (Luoma and Presser, 2009a), so a TTF developed for mallards, an herbivore duck (DeGraaf et al., 1985), may not be applicable to invertivores and piscivores. Also, the type of fish piscivores will

be most likely to eat at the SCH ponds, tilapia, is an omnivore, feeding on detritus, algae, phytoplankton, and invertebrates (CRA and USACE, 2011). Tilapia would, therefore, be at a trophic position somewhere in between 2 and level 3. In the progressive modeling, fish were treated as strictly invertivores, which may result in predicted Se concentrations being higher for both them and the birds that feed on them. This demonstrates some of the difficulties inherent in using a progressive approach to model a system under development, for which food webs and selenium accumulation dynamics are not known.

When the progressive model using the same parameters as Sickman et al. (2011) (K_d = 588, $TTF_{algae\ to\ inverts.}$ = 2.75, $TTF_{fish\ to\ birds}$ = 1.31, $TTF_{food\ to\ birds}$ = 1.8) was applied to water concentrations found in this study, predicted bird concentrations were about twice as high as those predicted by the linear regression model for Finney Lake and slightly lower than those predicted by the linear regression model for Ramer Lake (Table 5). Thus, it seems that the predictive ability of progressive models using constant K_d and TTF values depends strongly on the water concentration and constant values used. This is not surprising, since K_d (or enrichment function, EF) and TTF values vary depending on water and diet Se concentration, respectively (Stewart et al., 2010). When K_d and TTF values were calculated with data from this study, Ramer had a K_d of 1522 L/kg while Finney had a K_d of 945 L/kg. TTF values were 1.1 for both lakes for algae to corixids and 1.69 (Ramer) and 1.36 (Finney) for corixids to fish. Both these values are different from those used in Sickman et al. (2011). Current mean Se concentrations in the Alamo and New Rivers that were used in the modeling of Sickman et al. (2011) are about 5 µg/L and 2 µg/L, respectively (CRA and USACE, 2011). Based on comparison with a study of the Imperial Wetlands (Johnson et al., 2009), which had inflow Se concentrations up to about 5.4 µg/L, slightly higher water concentrations from those found at Ramer and Finney Lakes did not result in noticeably different Se concentrations in the food web (Table 6), though statistical

analysis was not able to be conducted and concentrations of Se in the inflow to Finney and Ramer Lakes were not measured. It therefore appears that use of a fixed K_d value will accentuate slight differences in water concentration and carry them through predictions of Se concentrations for the rest of the food web, potentially resulting in much higher or lower predicted Se concentrations than may be found in nature.

These differences emphasize once again the variability between systems and how one set of constants cannot be used to model all situations. Rather than using mean values, the progressive model of Sickman et al. (2011) may be improved by determining how K_d and TTFs varied with concentration in the studies used to develop model parameters and using different values for the different water concentrations modeled. The regression model used here did not rely on constants to predict levels in the base of the food web, as concentrations were directly measured, so the uncertainty in going from water to food web concentrations was eliminated. However, both models used to predict Se concentrations in birds at the SCH ponds have uncertainty in their applicability to varying conditions and in the conversion of diet to bird concentrations, since birds were not included in the food web sampling and analysis.

Conclusion

The food webs from two freshwater lakes near the Salton Sea and adjacent to the Alamo River were sampled and organisms analyzed for total Se concentrations and stable isotope compositions. Statistically significant linear relationships were found between Se concentration and $\delta^{15}N$. These findings, therefore, provide direct evidence for Se bioaccumulation and biomagnification in these lakes and also provide an empirical model for which to make predictions of Se concentrations in higher trophic level organisms, such as birds. Although sediment and food web selenium concentrations suggest moderate to high risk from selenium

toxicity to birds in the systems studied, based on threshold values, results from modeling using linear regression equations developed from plots of $\delta^{15}N$ versus Se concentration indicate that invertivore and piscivore birds will be at little or no risk in freshwater habitats similar to those studied. Additional sampling would allow development of a more dynamic model that can be used to assess Se risk across time and habitats. Comparison with the results of a progressive model using fixed values for K_d and TTFs revealed that this type of modeling will accentuate differences in dissolved Se concentration, potentially yielding predictions of Se concentrations in birds that are much higher or lower than those actually found in nature at these water concentrations.

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Chapter 3
Conclusions

Two freshwater lakes in Imperial Valley were studied to gain knowledge of how Se may accumulate in freshwater Species Conservation Habitat (SCH) ponds, which are planned to replace some of the wildlife habitat lost as the Salton Sea shrinks due to decreasing water inputs. These lakes, Ramer Lake and Finney Lake, are located south of the Salton Sea near Brawley, California. Finney Lake is supplied by agricultural drain water, while Ramer Lake is supplied by freshwater, thus allowing study of systems supplied by the types of water expected to supply the SCH ponds. Water, sediment, and biota were collected and data on Se concentrations and carbon and nitrogen stable isotope composition obtained. Using these data, an empirical relationship between Se concentration and $\delta^{15}N$ was derived, which was then used to predict Se concentrations in birds, a type of animal that is especially vulnerable to Se toxicity. Direct evidence for the bioaccumulation and biomagnification of Se was obtained for these freshwater ecosystems, as linear regressions for Se concentration plotted against $\delta^{15}N$ had significant positive slopes.

Results indicate that while sediment and some food web components have Se concentrations above widely accepted thresholds for the protection of higher trophic level organisms from the bioaccumulation of Se, birds feeding in systems similar to those studied should not be greatly harmed by selenium toxicity. Predicted Se concentrations for piscivore and invertivore birds were mostly below the 6 mg/kg dw egg concentration established for reduced hatchability in the black-necked stilt, a common shorebird at the Salton Sea which is expected to nest at SCH ponds should they be built. The only predicted Se concentration that was above this threshold was for piscivore birds using the linear regression equation derived from data collected

at Ramer Lake, and this was only about 1 mg/kg dw over the threshold. Since piscivores are not thought to be as sensitive to Se as many shorebirds (Skorupa et al., 2004), it is likely these levels will not impact piscivores at the SCH ponds.

Until more is known about Se concentration in relation to $\delta^{15}N$ in eggs of birds nesting at the Salton Sea, it is uncertain exactly how well the results of this empirical modeling predict Se concentrations in bird eggs. Since whole organisms were analyzed to acquire the data used for modeling, results obtained from the linear regression equations theoretically predict Se concentrations in whole adult birds. For risk assessment in this study, it was assumed that stable isotopes and Se in eggs would be derived only from the diet of the mother and that stable isotopes and Se in eggs would follow the same pattern as the rest of the food web without modification going from adult to egg. Validation of the model to bird eggs would require completion of studies like this that include that sampling of bird eggs.

Both progressive modeling and the regression modeling used in this study have advantages and disadvantages. Progressive modeling has been shown to give results with surprising accuracy and has the benefit of allowing backward modeling to determine safe Se concentrations in the water on a site-specific basis. However, it requires a measured partitioning coefficient and prior knowledge of the food web being modeled to reduce uncertainties (Presser and Luoma, 2010). The regression model presented here avoids the uncertainty in going from water Se concentrations to Se concentrations in the base of the food web. Since all needed data are directly measured, any uncertainty presented in choosing K_d and TTF values is eliminated. If K_d and TTF values used are incorrect, errors will be propagated at each step in a progressive model. This can be seen in a comparison of the results between lakes based on using a progressive model with fixed values. The slightly higher water Se concentration in Finney Lake resulted in progressive model predictions of Se concentrations in birds nearly twice those

predicted for Ramer Lake. Based upon the results of recent studies in the Salton Sea region, the small difference in dissolved Se concentrations should not result in a two-fold higher Se concentration in the food web at Finney Lake than that at Ramer Lake. The only source of uncertainty in the regression model, as used here, is where exactly bird eggs fit in relation to the rest of the food web. Once the model is adjusted to account for any potential processing between adult birds and eggs, this regression modeling can be a valuable tool for making predictions of bird egg Se concentrations to use in risk assessment in cases where eggs cannot be collected and directly measured.

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APPENDIX A: ALGAE SPECIES FOUND AT THE LAKES

Sample	**Taxon**	**Notes**
Finney Lake Phytoplankton	***Cyclotella* or *Thalasiosira***	Dominant, small centric
	***Fragilaria* sp.**	Dominant, most likely *F. capucina* or *F. vaucheria*
	Cyclotella meneghiniana	Subdominant
	***Scenedesmus* group**	Subdominant, *S. acustus*, *S. quadricauda*, & others
	***Nitzschia* sp.**	Subdominant
	Pediastrum boryanum	Abundant
	Rhapidiopsis curvata	Abundant
	Merismopedia punctata	
	Oscillatoria aghardii	
	Pediastrum tetras	
	Leptolyngbya sp.	
	Synedra sp.	
	Navicula sp.	
	Scenedsesmus acuminatus	
	Scenedesmsu sp. 1	
	Cosmarium sp.	
	Cryptomonas sp.	
	Chloromonas sp.	
	Stephanodiscus sp.	
	Epithemia sorex	
	Oscillatoria sp. 1	
	Synedra ulna	
	Gleocapsa sp.	

Sample	Taxon	Notes
Finney Lake Periphyton	***Lyngbya* sp.**	The major filamentous component in this sample
	***Nitzschia* sp.**	Dominant, epiphyte
	Nitzschia amphibia	Subdominant
	***Navicula* sp. 1**	Subdominant
	Gomphonema gracilis	Abundant, epiphyte
	Encyonema gracilis	Abundant, needs futher verification
	Synedra ulna	
	***Oedogonium* sp.**	The 2nd most common - major filamentous alage
	Navicula cf. *capitata*	Needs futher verification
	Navicula sp. 2	
	Anabaena sp.	
	Cymbella sp.	
	Amphora sp.	Most likely A. ovalis
	Navicula cf. *tripunctat*a	Needs futher verification
	Encyonopsis sp.	
	Navicula sp. 3	
	Diploneis sp.	
	Oscillatoria aghardii	
	Cyclotella meneghiniana	
	Diatoma vulgaris	
	Cocconeis pediculus	
	Gomphonema parvulum	
	Rhapidiopsis curvata	
	Fragilaria sp.	Most likely *F. capucina* or *F. vaucheria*
	Achnanthes sp.	
	Cyclotella	Most likley *meneghiniana*
	Cocconeis pediculus	
	Scenedesmus quadricauda	
	Scenedesmus sp.	

Sample	Taxon	Notes
Finney Lake Epiphyton	***Oedogonium* sp.**	The major filamentous component in this sample
	***Nitzschia* sp.**	Dominant, densly attached to Oedogoinum
	Gomphonema gracilis	Subdominant
	Leptolyngbya sp.	Abundant
	Synedra ulna	
	Scenedesmus acuminatus	
	Cocconeis pediculus	
	Rhoicosphenia curvata	
	Melosira varians	
	Oscillatoria sp. 1	
	Epithemia sorex	
	Oscillatoria aghardii	
	Navicula cf capitatoradiata	
	Rhopalodia gibba	
Ramer Lake Phytoplankton	***Raphidiopsis curvata***	Dominant
	***Oscillatoria* sp. 1**	Subdominant
	Oscillatoria aghardii	
	*Chaetocero*s sp.	Prefers brackish and saline water
	Cyclotella sp.	
	Spirulina sp.	
	Amphora ovalis	
	Hantzschia amphioxis	
	Synedra ulna	
	Nitzschia sp.	
	Leptolyngbya sp.	
	Gleocapsa sp.	

APPENDIX B: SIGMAPLOT ANALYSIS OF LINEAR REGRESSIONS

Finney Linear Model

Linear Regression Thursday, August 18, 2011, 5:53:13 PM
Data source: Finney Se:15N Model in Notebook1

Finney _Se = -1.156 + (0.423 * Finney_15N)

N = 9
R = 0.734 Rsqr = 0.539 AdjRsqr = 0.474

Standard Error of Estimate = 0.705

	Coefficient	Std. Error	t	P
Constant	-1.156	1.613	-0.717	0.497
Finney_15N	0.423	0.148	2.863	0.024

Analysis of Variance:

	DF	SS	MS	F	P
Regression	1	4.068	4.068	8.195	0.024
Residual	7	3.475	0.496		
Total	8	7.543	0.943		

Normality Test (Shapiro-Wilk) Passed (P = 0.223)

Constant Variance Test: Passed (P = 0.709)

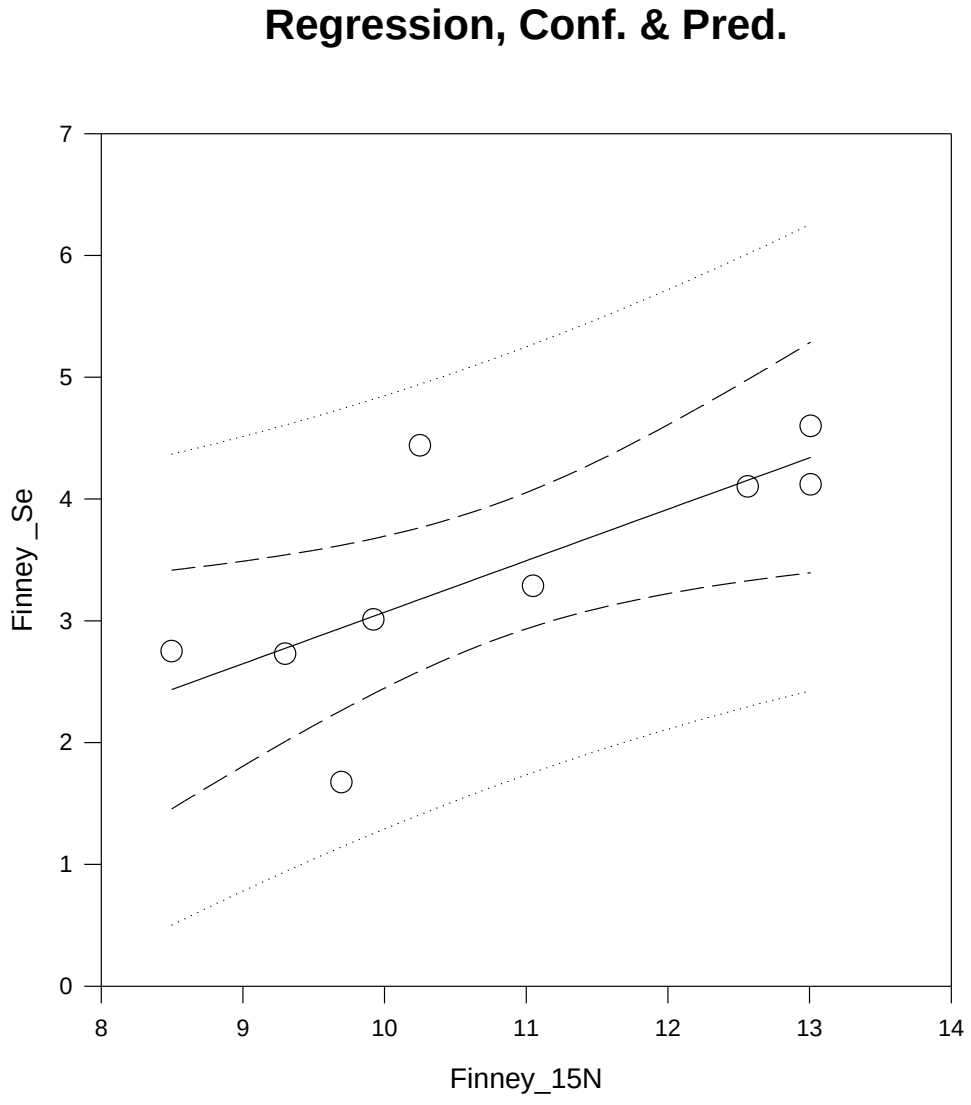
Power of performed test with alpha = 0.050: 0.632

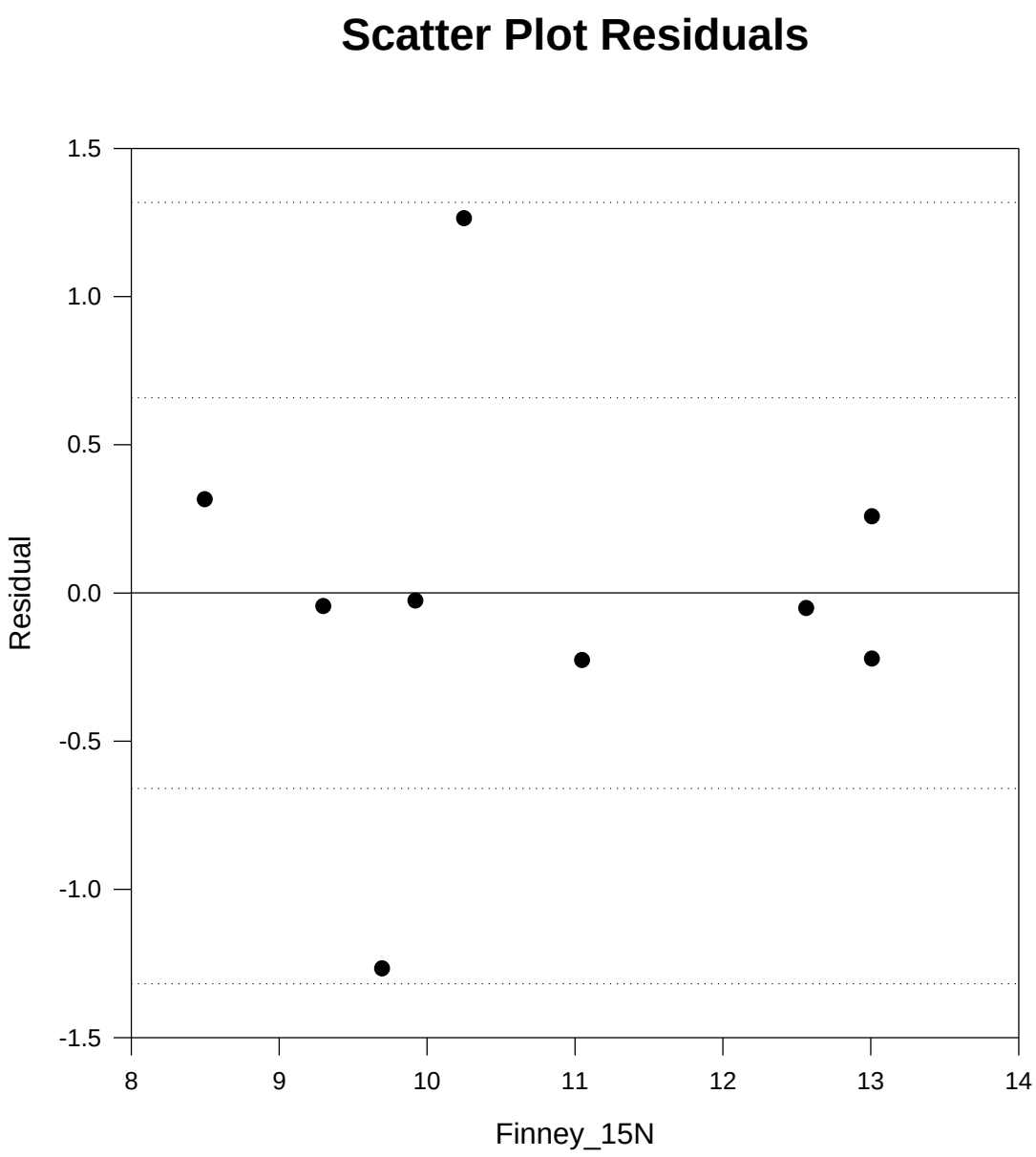
==========
Influence Diagnostics:
==========

Row	Leverage
1	0.125
2	0.114
3	0.346
4	0.146
5	0.323
6	0.323
7	0.166
8	0.246
9	0.211

==========
% Confidence Intervals:
==========

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
1	3.176	2.587	3.765	1.409	4.943
2	3.514	2.952	4.075	1.756	5.272
3	2.435	1.455	3.415	0.502	4.368
4	3.037	2.401	3.673	1.254	4.821
5	4.342	3.395	5.289	2.425	6.258
6	4.342	3.395	5.289	2.426	6.258
7	2.942	2.264	3.620	1.143	4.741
8	4.154	3.328	4.981	2.295	6.014
9	2.774	2.009	3.540	0.941	4.608




Scatter Plot Residuals
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
Residual
8
9
10
11
12
13
14
Finney_15N

Ramer Linear Model
Linear Regression Thursday, August 18, 2011, 6:16:48 PM

Data source: Data 1 in Notebook1

Ramer_Se = 1.188 + (0.420 * Ramer_15N)

N = 12

R = 0.634 Rsqr = 0.402 AdjRsqr = 0.342

Standard Error of Estimate = 1.093

	Coefficient	Std. Error	t	P
Constant	1.188	1.325	0.896	0.391
Ramer_15N	0.420	0.162	2.591	0.027

Analysis of Variance:

	DF	SS	MS	F	P
Regression	1	8.028	8.028	6.715	0.027
Residual	10	11.957	1.196		
Total	11	19.985	1.817		

Normality Test (Shapiro-Wilk) Passed (P = 0.501)

Constant Variance Test: Passed (P = 0.066)

Power of performed test with alpha = 0.050: 0.612

==========
Influence Diagnostics:
==========

Row	Leverage
1	0.254
2	0.237
3	0.136
4	0.151
5	0.175
6	0.0948
7	0.405
8	0.0836
9	0.0905
10	0.162
11	0.0999
12	0.112

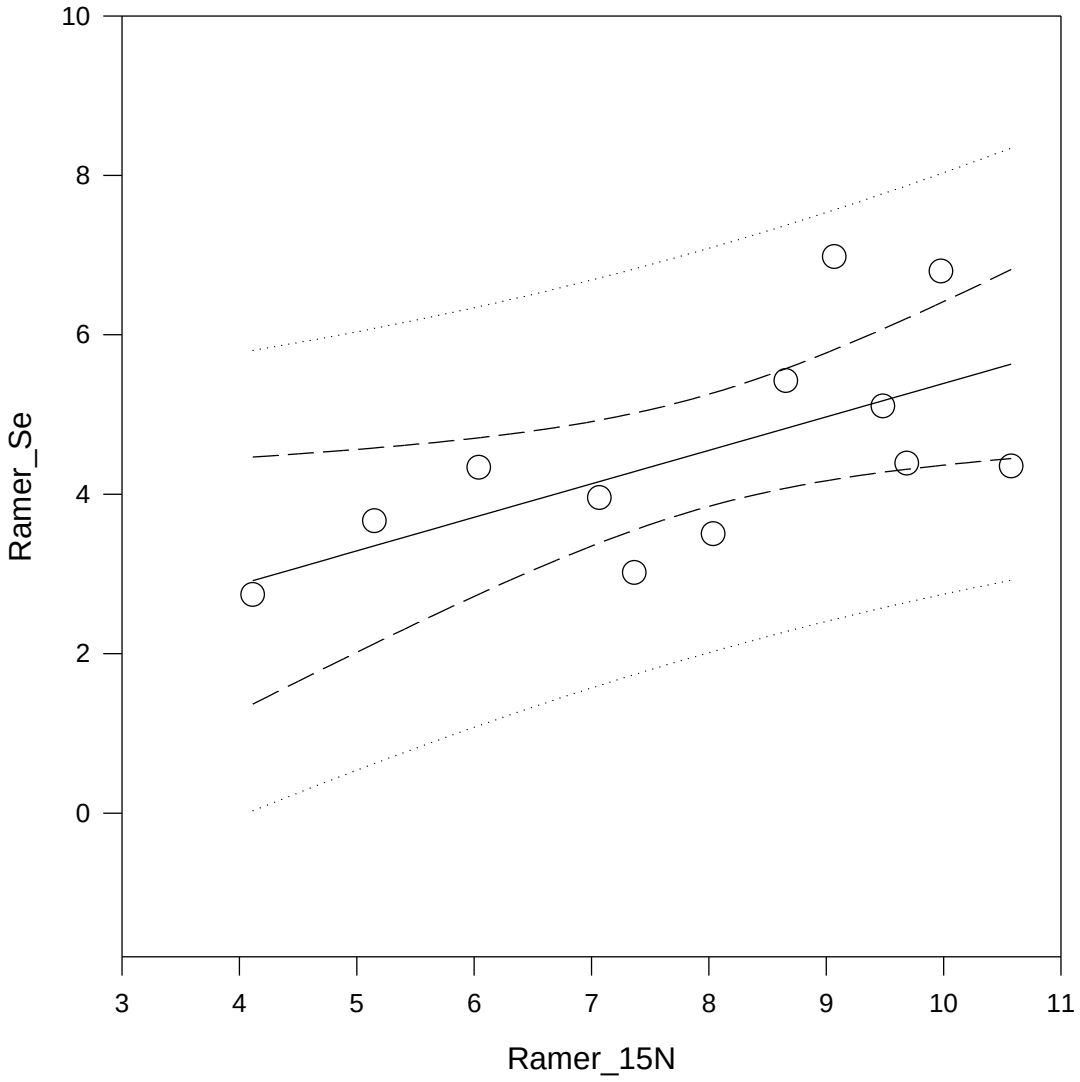
==========

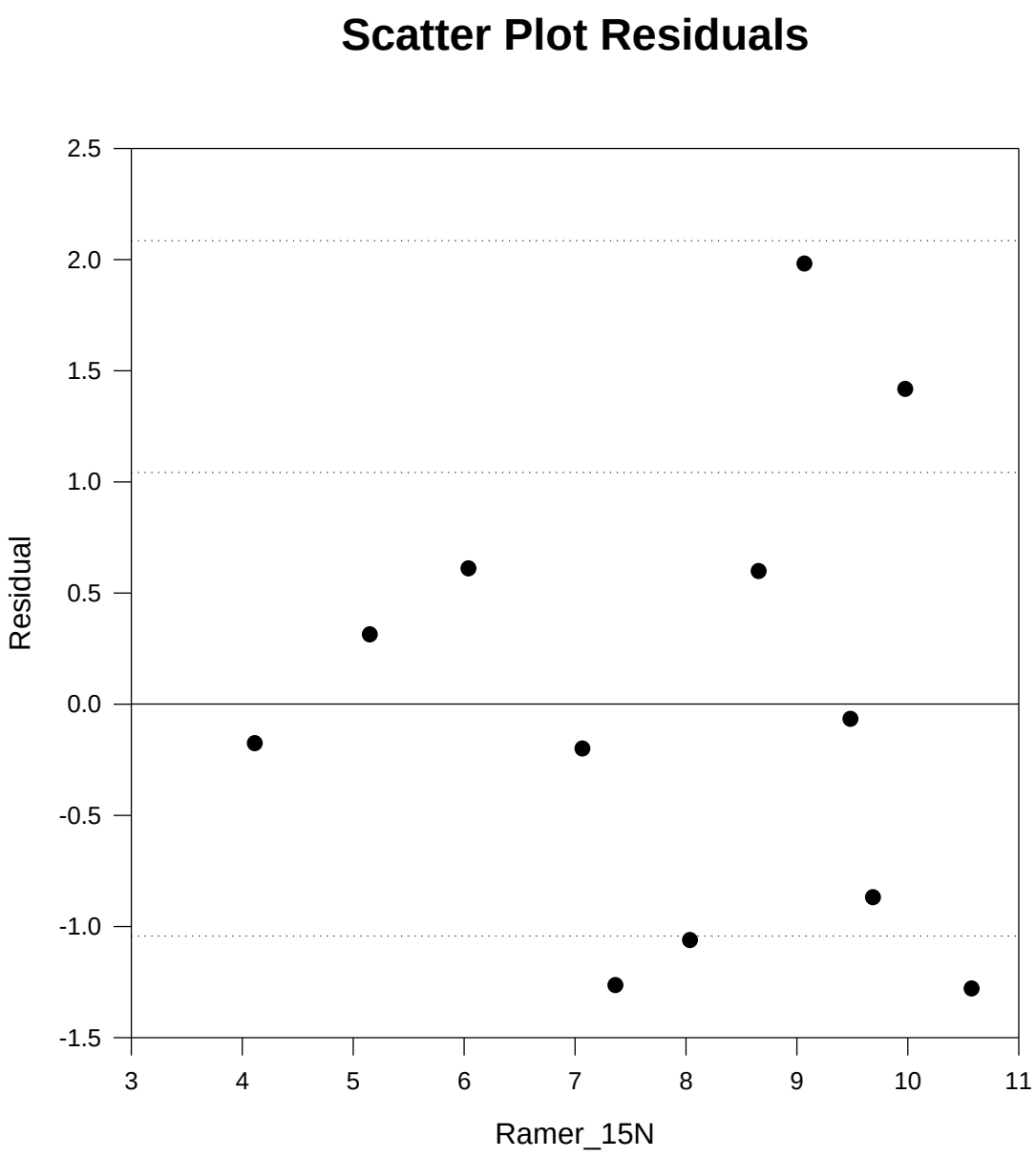
% Confidence Intervals:

==========

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
1	3.353	2.125	4.581	0.625	6.081
2	5.634	4.448	6.819	2.924	8.343
3	5.174	4.275	6.073	2.577	7.771
4	5.259	4.313	6.205	2.646	7.873
5	5.382	4.362	6.401	2.741	8.023
6	4.826	4.076	5.576	2.277	7.376
7	2.917	1.368	4.467	0.0299	5.805
8	4.566	3.862	5.270	2.030	7.102
9	4.284	3.551	5.016	1.739	6.828
10	3.727	2.746	4.709	1.101	6.354
11	4.158	3.388	4.929	1.603	6.714
12	5.000	4.186	5.814	2.431	7.569

Regression, Conf. & Pred.




Scatter Plot Residuals
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0.5
0.0
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3
4
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7
8
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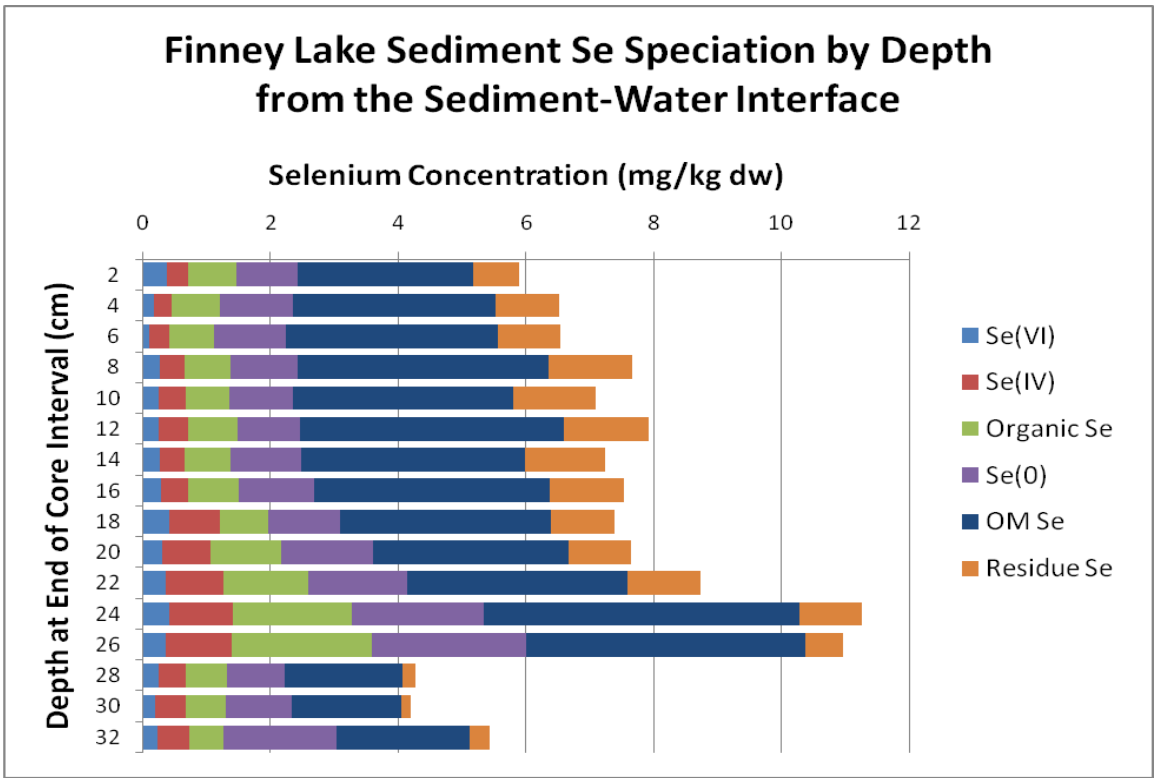
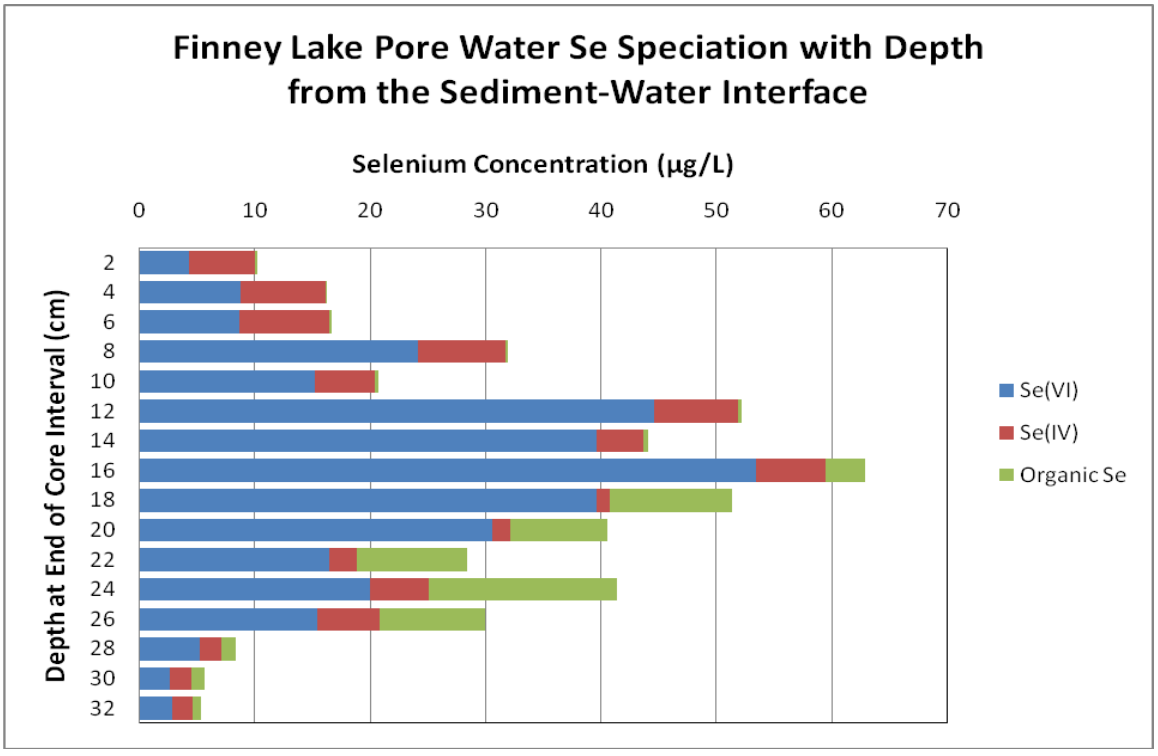
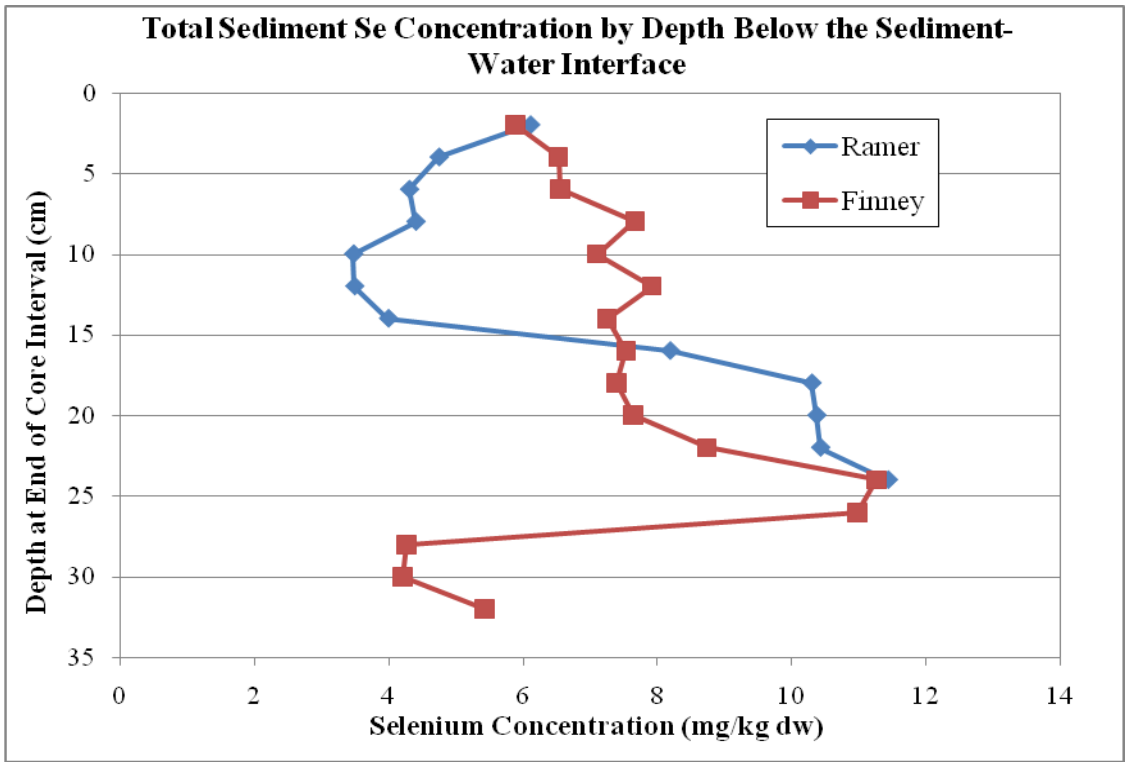
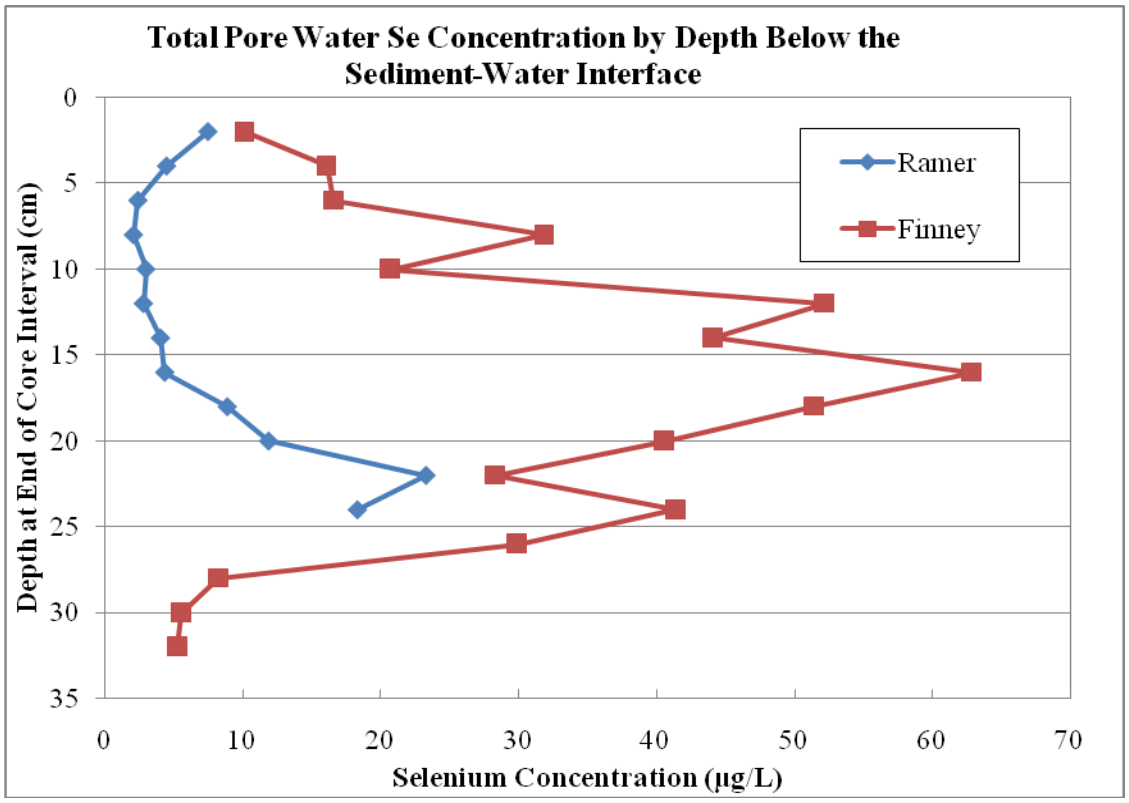
10
11
Ramer_15N

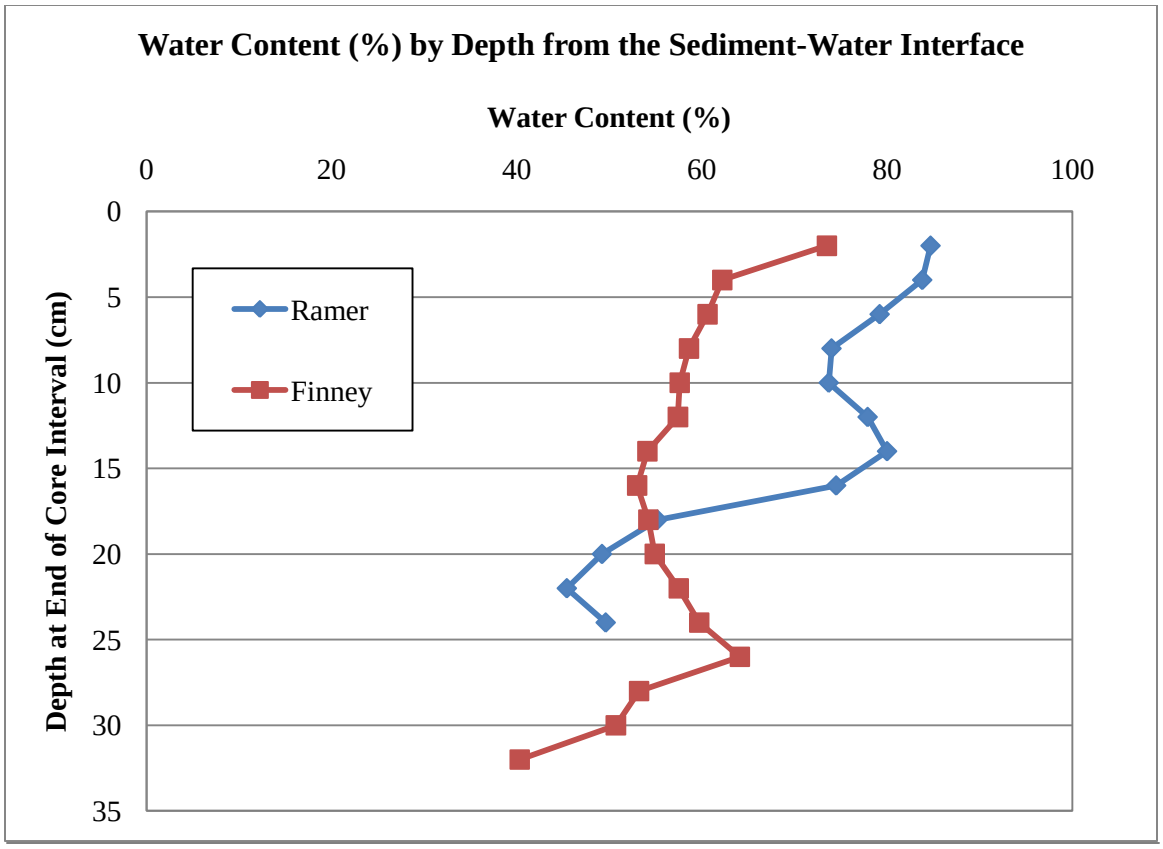
APPENDIX C: CORE DATA






Total Sediment Se Concentration by Depth Below the Sediment-Water Interface
Depth at End of Core Interval (cm)
0
5
10
15
20
25
30
35
0
2
4
6
8
10
12
14
Selenium Concentration (mg/kg dw)
Ramer
Finney


Total Pore Water Se Concentration by Depth Below the Sediment-Water Interface
Depth at End of Core Interval (cm)
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60
70
Selenium Concentration (µg/L)
Ramer
Finney


Water Content (%) by Depth from the Sediment-Water Interface
Water Content (%)
0
20
40
60
80
100
Depth at End of Core Interval (cm)
0
5
10
15
20
25
30
35
Ramer
Finney