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Assessment of Aquatic Food Web Recovery from Fish Introductions and Trophic Structure in Lakes of the Sierra Nevada, California Using Stable Isotopes

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Environmental Sciences

by

Paul David Koster II

March 2012

Thesis Committee:

- Dr. James O. Sickman, Chairperson
- Dr. Michael Anderson
- Dr. William Walton
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The Thesis of Paul David Koster II is approved:

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Committee Chairperson

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ABSTRACT OF THE THESIS

Assessment of Aquatic Food Web Recovery from Fish Introductions and Trophic Structure in Lakes of the Sierra Nevada, California
Using Stable Isotopes

by

Paul David Koster II

Master of Science, Graduate Program in Environmental Sciences
University of California, Riverside, March 2012
Dr. James O. Sickman, Chairperson

Stocking of salmonid fish in naturally fishless Sierra Nevada lakes over the last century has had a profound impact on aquatic foodwebs. Current research has focused on removal of fish from several lakes to observe recovery of zooplankton, benthic invertebrates and, in particular, the endangered southern mountain yellow-legged frog (*Rana muscosa*). Biological samples from two currently fish containing lakes and two lakes where fish have been removed were collected over the last 15 years to determine recovery rates of species found in the lakes. Due to the remote location of the lakes, the samples were preserved with ethanol (EtOH) for later analysis. However, long-term preservation methods can have significant impacts on the $\delta^{13}C$ values of stable isotopes by resulting in enrichment in isotope values. I developed a rinse method to remove EtOH from the archived samples and to reduce the impact preservatives on stable isotope

analysis. After removing the preservative, samples were rinsed for. The rinsing method resulted in enrichment of ^{13}C suggesting a much shorter rinsing time (< 1 day) as well as species-specific corrections for $\delta^{13}C$ content were required to improve the accuracy of stable isotope analysis of preserved samples.

I examined the trophic structure of lakes containing and lacking fish using carbon and nitrogen stable isotopes. $\delta^{15}N$ values revealed distinct trophic fractionation values for each type of lake. Trophic fractionation for fish-containing and fish-removed lakes was 2.03‰ ± 0.517 (n=116) and1.76‰ ± 0.384 (n=186) respectively. Nitrogen values in fish-containing lakes species tended to be enriched relative to fish-removed lakes, suggesting increased P availability. Time-series of $\delta^{15}N$ for multiple species indicated small variations in nitrogen suggesting changes in diet from year to year. However significant depletion of 4.42‰ was noted for the copepod *Leptodiaptomus signicauda and* correlated with the reemergence of *Daphnia melanica* post fish removal. Computations of trophic position indicated that frogs and fish occupy the same trophic level and likely rely on the same food sources. This finding suggests that fish cause extirpation of frogs both through direct predation and competition for food resources.

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Introduction

Invasive Species and their unintended consequences on aquatic ecosystems

Whether by accident or on purpose, introduction of non-native species can have unintended and deleterious consequences on ecosystems. Coupled with pollution, habitat modification, and land-use change, non-native species introductions are one of the biggest threats to global biodiversity (Chapin et al. 1997, Sala et al. 2000, Hartel et al. 2007). While some introduced species are benign and may even be beneficial to their new environment, more often than not they can alter water quality, interactions between populations of native species, induce bottom up or top down trophic cascades, shift nutrient flows, and in many cases result in the extirpation of native flora and fauna and reduce species diversity (Northcote 1988, McNaught et al. 1999, Knapp and Matthews 2000, Vredenburg 2004, Parker et al. 2006, Eby 2006, Hartel et al. 2007, Finlay and Vrendenburg 2007, Schabetsberger 2009).

Many species are intentionally introduced to control unwanted pests, to enhance recreational activities such as fishing and hunting, or for economic gains such as aquaculture. Some of the most commonly introduced organism in aquatic ecosystems belong to the family Salmonidae that includes many species of trout (Bahls 1992). Trout have been stocked for hundreds of years in a wide variety of aquatic ecosystems including historically fishless kettle lakes, headwater lakes and streams in the eastern United States, naturally fishless

streams in New Zealand and Australia and fishless alpine lakes in Europe and North and South America (Bahls 1992, Pister 2001, Simon and Townsend 2003, Eby et al. 2006, Schilling et al. 2009a, Schilling et al. 2009b). The earliest known records of fish stocking date back to the 15th century in the Alps. Species of brown trout (*Salmo trutta* f. *fario*) and Alpine charr (*Salvelinus umbla*) were carried up to naturally fishless lakes to provide live fish for hunting trips by local feudal landowners. Non-native fish stocking in North and South America alpine ecosystems occurred in the late 19th to early 20th centuries to enhance recreational use of National Parks and Forest Service lands (Bahls 1992, McNaught et al. 1999, Schabetsberger et al. 2009).

Fish Stocking in the Sierra Nevada

Nearly all mountain lakes in the Sierra Nevada and in western North America were formed during the late Pleistocene glaciation (Pister 2001). As the glaciers retreated, many of these newly formed lakes were left isolated from any downstream influences due to physical barriers and as such were fishless (Pister 2001). Prior to the mid-nineteenth century, most Sierra Nevada lakes above an elevation of 1800 m were devoid of fish (Knapp 1996, Pister 2001).

Sierra Nevada lakes were stocked with non-native trout from hatcheries or inter-basin transfers to enhance recreational fishing (Knapp 1996, Vredenburg 2004). Generally, lakes < 1 hectare were not stocked as they were too shallow and froze to the bottom each winter preventing fish reproduction. Initial stocking

was largely conducted by sporting clubs such as the Bishop Fish Planting Club, Sierra Club, and Visalia Sportsmen Club (Knapp 1996). Eventually the California Fish and Game Commission, the precursor of the California Department of Fish and Game (DFG), took over stocking operations in the Sierra Nevada. The DFG has a current mandate to maintain existing trout populations outside of National Parks (Knapp 1996).

Little concern was initially given to the consequences of introducing non-native species on Sierran lake ecosystems but later research showed that fish introductions initiated a top down alteration of lake food webs (Northcote 1988, Bahls 1992, Eby et al. 2006, Parker and Schindler 2006). Major impacts included a shift towards dominance by smaller zooplankton and benthic macroinvertebrate species and extirpation of several amphibian species including the endangered mountain yellow-legged frog (*Rana muscosa, Rana sierrae*) (Stoddard 1987, McNaught et al. 1999, Knapp and Matthews 2000, Donald et al. 2001, Knapp et al. 2001b, Vredenburg 2004, Eby et al. 2006, Finlay and Vredenburg 2007). However, there is also evidence that fish stocking impacts extend to terrestrial ecosystems. Loss of native lake fauna results in loss of food for birds, snakes, as well as several other terrestrial species (Epanchin et al. 2010).

Trout stocking in the Sierra Nevada was phased out starting in 1969 in Yosemite and in 1991 in Sequoia and Kings Canyon National Parks, yet stocking continues in the majority of the Sierra Nevada administered by the US Forest Service (Leopold 1963, Knapp and Matthews 2000). However, even without

restocking, nearly 63% of lakes above 1800 m in the Sierra Nevada currently have introduced fish while 52% are still stocked regularly to maintain sport fishing (Bahls 1992, Knapp 1996). In 1996, Dr. Roland Knapp of the Sierra Nevada Aquatic Research Laboratory (SNARL) along with Dr. Orlando Sarnelle, with permission of the DFG initiated a study of 5 lakes in Humphreys Basin with removal of non-native fish to observe native faunal recovery (Sarnelle and Knapp 2005). This study provided the motivation and the majority of samples for my thesis research.

Stable Isotopes in Ecology

With humans encroaching into more undisturbed areas and thus affecting natural systems, the need to understand and mitigate any negative impacts is essential. One tool to assess ecosystem change is the measurement of stable isotopes in foodwebs. Isotopes are variants of atoms of the same element where the numbers of neutrons present differ in the nucleus (e.g. ^{14}N and ^{15}N) (Peterson and Fry 1987, Fry 2006). The different number of neutrons results in slightly different masses between the isotopes. The slight difference in mass dictates how fast atoms and molecules will react in kinetic and equilibrium reactions. In kinetic reactions, compounds containing more of the lighter isotope will react more quickly relative to heavier isotopes (Peterson and Fry 1987, Fry 2006). In exchange reactions, bonds are more easily broken in elements with lighter isotopes (Peterson and Fry 1987, Fry 2006). In kinetic reactions

differences in reactions rate result in the products containing a higher percentage of lighter isotopes whereas the heavy isotopes concentrate in the residual reactants. This process is referred to as isotopic fractionation.

Just as isotopes are separated by fractionation, they also recombine by mixing. For example, as heterotrophic organisms consume food, they take on the heavy to light isotope ratios (e.g. $^{15}N/^{14}N$) of the organisms they consume, thus, allowing one to discern energy pathways and food sources in foodwebs.

Stable isotope ratios are reported using delta notation (δ) and are expressed as parts permil (‰)**.** This notation compares the ratio of the heavier isotope to the lighter isotope in a sample to that of a standard and can be summarized as:

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

The ratio of heavy to light isotopes in the sample is defined as R_{SAMPLE} and the ratio of heavy to light isotopes for the international standard reference material is denoted as $R_{STANDARD}$. For $\delta^{13}C$ and $\delta^{15}N$, data are expressed relative to Vienna PeeDee Belemnite (VPDB) and atmospheric nitrogen (N_2) standards, respectively. These international standards are given δ values of 0‰, so delta values that are positive are enriched in the heavy isotope relative to the standard and negative values indicate samples depleted in heavy isotopes relative to the standard.

Food Webs

Food webs represent the structure of an ecosystem based on feeding relationships and trophic position on a community level (Elton 1927, Vander Zanden and Rasmussen 1999). In constructing a food web, species or populations are assigned to one of several distinct trophic levels based on their niche. Most food web relationships are inferred by observation of feeding behavior or gut content analysis, but it is often difficult to observe all possible interactions in a given ecosystem (Hobson and Welch 1992, Grey 2006). As a result, food webs based solely on field observation and gut contents can be oversimplified.

The ratios of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) in organisms can be used to elucidate food web structure by inferring animal diets and energy pathways, respectively (Kling et al. 1992, Vander Zanden et al. 1999). Stable isotope analysis of food web organisms offers several advantages to the study of aquatic ecosystems. Since consumers take on the isotopic signature of their diets, stable isotopes allow for time-integrated analysis of a consumer's diet. Unlike stable isotopes, gut content analysis provides information on a consumer's diet over a short length of time. One complication to the use of stable isotopes is that as food is metabolized, light isotopes are preferentially excreted while heavy isotopes concentrate in the consumer; this process is known as trophic fractionation or diet discrimination. Thus, consumers will have higher $\delta^{15}N$ and higher $\delta^{13}C$ than their food. The mean trophic fractionation for nitrogen is

often assumed to be 3.4‰ but, this value has been shown to vary from +1.4‰ to 5.4‰ depending on the consumer (Minagawa and Wada 1984, Peterson and Fry 1987, Vander Zanden and Rasmussen 1999). Carbon is generally considered to exhibit lower trophic fractionation (0.0-1.0‰) during metabolic processes (Peterson and Fry 1987, Vander Zanden and Rasmussen 1999, Post 2002, Vander Zanden et al. 2003, Carabel et al. 2006, Smyntek et al. 2007, Schmidt et al. 2009). Quantitative estimates of consumer diets require that the trophic fractionation be known and corrected for in isotope mixing analyses. Isotopes and isotope modeling software are allowing scientists to begin untangling the complicated dynamics of food webs.

If there are only two or three possible food sources, determining the diet of a consumer is fairly straightforward. However, ecosystems and food webs are often highly complex with multiple food sources contributing to the isotopic composition of a consumer (Peterson and Fry 1987). To account for multiple food sources, computer mixing models are used to estimate likely food mixtures in a consumer's diet using $\delta^{15}N$ and $\delta^{13}C$ isotope values of food sources (Moore and Semmens 2007).

Objective

The main objectives of my thesis research were to compare the trophic structure of Sierra Nevada lakes where non-native fish are present versus lakes where they were removed, and to determine how the presence of introduced fish

has affected the lake food webs. I also assessed how the food webs of lakes respond isotopically over time after the fish were removed. My study is one of the first to compare the food webs of fish-containing and fish-removed lakes in the Sierra Nevada using stable isotopes of carbon and nitrogen.

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Chapter 1

Methods for Preparation of EtOH-Preserved Biological Samples for Stable Isotope Analyses

Abstract

Stable isotope analysis is key to understanding trophic interactions. Typically fresh samples are used for analysis; however, samples are sometimes collected in remote locations and need to be preserved for later analysis. Samples are usually preserved in ethanol (EtOH) or formalin. However, long-term preservation methods have significant impacts on the values of stable isotopes of $\delta^{15}N$ and $\delta^{13}C$. In an attempt to overcome preservation artifacts, we conducted a rinse and soak method to remove EtOH in our samples and reduce the impact of preservatives on isotope analysis. Twelve species of zooplankton, benthic macroinvertebrates, and frogs were analyzed for this report. Samples were collected for a prior food web analysis and had been preserved for up to 13 years. Additional samples were collected in 2010, split with one half frozen and unpreserved and one half preserved in 70% EtOH for 3 months. All samples were then rinsed for multiple days and then analyzed on the mass spectrophotometer. The rinsing method resulted in enriched values for $\delta^{13}C$ from 0.02‰ to 5.48‰ relative to control samples and results for $\delta^{15}N$ were depleted to slightly enriched: -1.31‰ to 1.04‰ relative to control samples. Preservation artifacts were not consistent based on the size of an organism or across the different species indicating that corrections for specific species may be needed.

The resulting enrichment of $\delta^{13}C$ indicates a multiple day rinse and soaking may not be necessary and only a quick rinse with deionized water should be done.

Introduction

Over the last several decades, stable isotope analysis (SIA) has become an integral tool in understanding aquatic ecosystem function and structure (Feuchtmayr & Grey 2003). Naturally occurring stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are frequently used by ecologists to elucidate energy sources, physiological changes, trophic relationships, migratory patterns, and anthropogenic impacts on ecosystems (Peterson & Fry 1987, Hobson et al. 1993, Cabana & Rasmussen 1996, Vander Zanden & Rasmussen 1999, Fry 1999, Post 2002, Bodin et al. 2007, Fanelli et al. 2010). With increasing impacts of introduced species, SIA also offers an excellent opportunity for museums and research institutions that hold preserved, archived samples to reconstruct historical food webs and to look at ecological impact of invasive species over time (Ventura and Jeppesen 2009, Syväranta et al. 2011).

Stable isotopes in biological material cannot be analyzed in the field; consequently, samples need to be preserved prior to analysis. Therefore, one potential limitation for the application of SIA to food web studies is the preservation methods of biological samples. The most commonly used forms of preservation are ethanol (EtOH), formalin, salt, freezing, or drying (Hobson et al. 1997, Arrington and Winemiller 2002, Jardine et al. 2003, Feuchtmayr and Grey 2003). However, since freezing is not always a viable option in the field, biological samples collected for long-term studies are usually preserved with a fixative such as EtOH or formalin (Carabel et al. 2009).

One constraint when using preserved samples for SIA is the impact of preservatives on carbon and nitrogen stable isotopes. Recent studies provide evidence that preservatives can lead to biased results for $\delta^{13}C$ and $\delta^{15}N$ values (Sweeting et al. 2004, Kelly et al. 2006, Barrow et al. 2008). Several studies showed enrichment in $\delta^{13}C$ and $\delta^{15}N$ values; others reported depletion of $\delta^{13}C$ and $\delta^{15}N$ values and some studies reported no significant preservation effects (Table 1.1). It is noteworthy that, in most of the studies that reported no effect of preservatives on isotope values, the samples were rinsed with deionized water (DIW) prior to SIA. Better understanding of how preservatives affect isotope measurements and methods for minimizing preservative artifacts is critically needed.

To date, many studies have reported on the problems of preservation methods on SIA, but few studies have addressed how to reduce these impacts. The magnitude and direction of preservation impacts in the scientific literature are highly variable. Analysis of preserved, unwashed *Drosophila melanogaster* samples demonstrated preservation effects of -2.0‰ ± 0.1‰ for $\delta^{13}C$ and 0.17‰ ± 0.08‰ for $\delta^{15}N$ (Ponsard and Amlou, 1998). Syvaranta et al. (2007) collected macroinvertebrates and zooplankton samples, placed samples in EtOH for 2 weeks to 12 months, and washed the samples for <1 minute. Preservation artifacts reported for zooplankton were +0.70 ± 0.15‰ for $\delta^{13}C$ and +0.12 ± 0.08‰ for $\delta^{15}N$. For benthic invertebrates, the differences from the control values were +0.31 ± 1.6‰ for $\delta^{13}C$ and -0.08 ± 1.0‰ for $\delta^{15}N$. Another study of samples

preserved for 12-15 years then rinsed for 3-5 days showed changes in $\delta^{13}C$ and $\delta^{15}N$ values of -0.8‰ and 0.4‰ respectively (Edwards et al. 2002). Overall, these studies demonstrate that preservation artifacts for nitrogen isotopes are small relative to anticipated trophic level fractionation of 3.4‰ (Minagawa and Wada 1984, Vander Zanden and Rasmussen 2001). However, preservative artifacts for carbon are more pronounced and in some cases exceed the generally accepted trophic fractionation of 1.0 to 2.0‰ (Rau et al. 1983, Fry and Sherr 1984, Edwards et al. 2002).

For my study, I report results from an experimental rinsing technique used on EtOH-preserved samples. I measured $\delta^{13}C$ and $\delta^{15}N$ in lakes samples collected from Lake Elsinore (2009, Riverside county) and from four Sierra Nevada lakes collected between 1997-2007. The Sierra Nevada samples were collected by Dr. Roland Knapp of the Sierra Nevada Aquatic Research Laboratory (SNARL) as part of a long-term experiment on trout removal and were preserved in 70% EtOH. The experimental lakes, Marmot, Mesa, Summit, and Square are located in Humphreys Basin of the Inyo National Forest. I also collected fresh and EtOH-preserved samples from the same Sierra Nevada lakes in the summer of 2010. I used samples collected in 1997, 1998, 2001, 2005, and 2007 for SIA analysis. A total of 16 species consisting of zooplankton, benthic macroinvertebrates, and frogs were analyzed (Table 1.3).

Because of the variability of preservative effects reported in previous research and differences in rinsing methods employed, I hypothesize that a

standardized, soak and rinse procedure will reduce preservation artifacts and improve SIA analyses of lake food webs. In order to analyze stable isotopes of preserved samples from the Sierra Nevada I sought to: 1) Develop a standardized rinsing method, 2) reduce impacts of preservation methods, and 3) observe the efficacy of rinsing with different types of organisms

Methods

Lake Sampling Protocol

Because of the limited amount of biological material available from Humphreys Basin study, I performed my initial rinsing tests on zooplankton samples collected from Lake Elsinore in Riverside County, California during 2009. In order to obtain enough material from Lake Elsinore, bulk zooplankton samples were collected with multiple tows (20-30) using a 64-μm (mesh opening) zooplankton net. Organisms collected and tested from Lake Elsinore included copepods, cladocerans, and rotifers and were morphologically similar to zooplankton species found in Humphreys Basin Lakes.

Because we were interested in general effects of the preservation medium, the copepods and cladocerans from Lake Elsinore were not separated from each other nor were they identified by genus or species but kept together to increase overall weight of sample material. Half of the samples collected were placed in 70% EtOH in the field and the remaining half were kept fresh in lake water and placed in a cooler with ice. Upon returning to the lab, the fresh samples were

placed under a dissecting microscope and all dead organisms and detritus were removed. The fresh samples were in a freezer -20°C. The preserved samples were kept in EtOH for approximately 30 days to simulate long-term preservation conditions. The frozen samples were lyophilized at -80ºC for 24 hours, thirty days after collection.

Sampling protocols for Humphreys Basin samples for all years except 2010 can be found in Knapp et al. (2001). In 2010, I collected additional biological samples in order to compare isotopic values among preserved and duplicate fresh samples collected at Marmot, Square, Summit, and Mesa Lakes. The 2010 samples consisted of multiple zooplankton tows (30-40) and D-net sweeps (20-30). One half of species collected were placed in 70% EtOH (henceforth denoted as preserved) and the other half kept in lake water (denoted as fresh). In the field, the fresh samples were packed in snow to minimize the chance of the organisms dying and decomposing.

The fresh samples from Humphreys basin were returned to the laboratory within 12-24 hours, observed through a dissecting scope and all dead organisms and detritus were removed. The remaining live organisms were identified to species, placed into separate vials containing lake water and then placed into a freezer for future analysis. Dr. Roland Knapp also supplied two unpreserved and frozen frogs collected from Marmot Lake from an earlier experiment in 2009 and 2 EtOH-preserved frogs from an earlier collection in 2010. The unpreserved frogs were kept frozen. Previous research indicates that freezing has little impact on

$\delta^{13}C$ and $\delta^{15}N$ isotope vales when compared to fresh, unfrozen samples and is a viable way to store biological samples long term (Ponsard and Amlou 1998, Bosley and Wainright 1999, Kaehler and Pakhomov, 2001, Sweeting et al. 2004, Barrow et al. 2008). Thus, I have considered the frozen samples as controls in evaluating the effects of EtOH-preservations and DIW rinsing. All EtOH-preserved samples collected in 2010 were stored in a refrigerator.

Rinsing Protocol

The main objective of the initial rinsing/soaking tests on Lake Elsinore samples was to determine how much preservative or organic material was leached from samples over 5 consecutive days of soaking (Figure 1.1). These rinsing experiments included rinsing of preserved samples only (in contrast we also rinsed fresh samples from Humphreys basin in later tests described below). To test the efficacy of DIW rinsing of EtOH preserved Lake Elsinore samples, EtOH-preserved samples were removed from the refrigerator, placed on a 64-µm-mesh and rinsed for 30 seconds with DIW and then placed into 40-ml vials containing DIW, capped and placed in a refrigerator for 24 hours. Each day, the preserved specimens were removed from the 40-ml vials and placed into a new 40-ml vial of DIW. The previous day's water sample was acidified to pH 2 with concentrated HCl and stored for later analysis of dissolved organic carbon (DOC); we assumed that EtOH rinsed from the specimens would be detectable as DOC. The rinsing/soaking process continued for 5 consecutive days. After 5

days, the rinse water samples were analyzed on a Shimadzu Total Organic Carbon analyzer (TOC) analyzer to assess the removal of organic preservative from the samples. After rinsing for 5 days, the Lake Elsinore specimens were lyophilized at -80ºC for 24 hours. Between 0.1-0.8 mg of dry mass was weighed and loaded into tin capsules for isotopic analysis.

Based on the results from the Lake Elsinore samples, we altered the rinsing methods for the Humphreys Basin food samples in several respects (Figure 1.2). First, a portion of the 2010 samples were preserved for 3 months instead of 30 days to better duplicate longer-term storage conditions for preserved samples from 1997-2007. Second, while none of the fresh samples from Lake Elsinore were rinsed, we performed DIW rinses on frozen specimens collected from Humphreys Basin to determine how much native organic material was leached from the samples by DIW which allowed for better understanding of the effects of rinsing. Lastly, we separate out individual species in the rinsing experiments with Humphreys Basin samples.

The EtOH-preserved samples from 1997-2010 were rinsed with DIW. After rinsing, samples were lyophilized at -80°C for 24-48 hours. Zooplankters were too small to run singly; so multiple individuals were included in the sample to obtain sufficient weight for stable isotope analysis (SIA). Benthic organisms were crushed and homogenized. Leg muscle from frogs and ventral muscle from fish were dissected and used for SIA. After lyophilizing the samples, between 0.06-

2.0 mg of dry mass was weighed for each sample and loaded into tin capsules for isotopic analysis.

Isotope analysis protocol

After Lake Elsinore and Humphreys Basin samples were combusted in 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 stable carbon and nitrogen isotopes.

Stable isotope ratios are reported using delta notation (δ) and are expressed as parts permil (‰). This notation compares the ratio of the heavier isotope to the lighter isotope in a sample to that of a standard and can be summarized as:

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

$\delta^{13}C$ and $\delta^{15}N$ values are expressed relative to Vienna PeeDee Belemnite VPDB) and atmospheric nitrogen (N_2) standards, respectively. These international standards are given delta values of 0‰ so delta values that are positive are enriched in the heavy isotope relative to the standard and negative values indicate samples that are depleted in heavy isotopes relative to the standard. National Institute of Standards and Technology (NIST) peach leaves

SRM 1587) were used to correct for mass-dependency of delta values in small samples. The samples delta values were normalized to the international standard scale by using USGS40 and USGS41 standard L-glutamic acid.

Results

Lake Elsinore Rinse Results

From the Lake Elsinore zooplankton tows, three EtOH-preserved samples (LE-1 to LE-3) and two frozen samples (LE-6 to LE-7) were prepared and used for: 1) testing of the sequential rinsing method and 2) isotopic analysis to identify preservative artifacts. A plot of DOC concentration in DIW rinses versus time indicated that most of the EtOH was removed within the first day and that DOC generally decreased until day 3 (Figure 1.3). Isotope analyses for LE-1, LE-2, LE-6, and LE-7 were run in duplicate while a single duplicate was run for LE-3 owing to limited sample availability. We detected significant differences in $\delta^{13}C$ between the frozen and EtOH-preserved samples. The EtOH-preserved samples were enriched about 1‰ relative to the frozen samples: $\delta^{13}C \pm SE$ = -20.60 ± 0.06‰ vs. -21.57 ± 0.03‰; $p < 0.001$. Smaller, and less significant isotopic enrichment was observed for $\delta^{15}N$ between EtOH-preserved and frozen samples: $\delta^{15}N$ SE = 11.35 ± 0.15‰ vs. 10.93 ± 0.11‰, respectively; $p = 0.07$. Based on the DOC and isotope results from Lake Elsinore we determined that rinsing should be limited to 1-5 days.

Humphreys Basin Rinse Results

Based on the results from the Lake Elsinore rinse procedure, the rinse water from the first day of soaking for the Humphreys Basin preserved samples was discarded. The DOC concentration in the first rinse from the Lake Elsinore samples was many times that of the subsequent rinses and therefore we determined the first day's rinse from the Humphreys Basin samples was not needed for analysis.

Overall, there was a progressive decrease in DOC concentrations in rinses for all Humphreys Basin samples from all years (Figure 1.4). Except for *Strictotarsus striatellus, Pisidium casertanum,* and *Leptodiaptomus signicauda*, which showed the greatest variability from rinse-to-rinse, all samples generally had the same overall reduction in DOC concentrations.

Rinsing of the frozen samples collected in 2010 was conducted over 3 days. Since there was no EtOH to leach off, any leaching of DOC would indicate loss of organic matter from the specimens themselves which could potentially alter their isotopic composition. For all species, the DOC measurements indicated there was organic material leaching off the samples. The amount of DOC on the first rinse day ranged from 0.10 to 1.76 mg/L and then generally declined (Figure 1.5).

For the EtOH-preserved samples from 2010 we measured isotopes in a single replicate for most species and duplicates were measured for several species chosen at random. Frozen samples (control group) were compared to

the 2010 and 1997-2010 isotope data from the preserved samples to observe preservative artifacts. For the 2010 samples, nitrogen isotope values for the rinsed EtOH-preserved specimens were generally depleted relative to the frozen control specimens while carbon values tended to be more highly enriched relative to frozen specimens (range of differences: -1.31‰ to 0.44‰ for nitrogen and 0.02‰ to 6.83‰ for carbon) (Table 1.4a, Figure 1.6a). When 1997-2010 preserved samples were compared to frozen samples from 2010, differences for nitrogen isotopes were mixed while carbon was enriched (-1.04‰ to 0.50‰ and 0.02‰ to 5.48‰ respectively) (Table 1.4, Figure 1.6b).

Discussion

Rinsing Methods

The results from the Lake Elsinore rinsing were encouraging even though the number of samples analyzed was small. The analysis gave good insight into the time EtOH took to leach off the samples. Most EtOH appeared to rinse off in the first day with a significant concentrations loss of approximately 3400 mg/L from day 1 to day 2 and a much slower rate of loss in EtOH concentrations of 24 mg/L from day 2 to day 5. The Lake Elsinore species were not separated since we hypothesized DOC leaching would be minimal and leaching of EtOH would not differ significantly from species to species. However, coupled with the amount of time required removing preservatives, the leaching of preservatives and/or DOC may be species specific. However, two questions arose during our analysis

of the Lake Elsinore samples: 1) is the reduction in concentrations we observed preservatives or biological material? And 2) more importantly, when does the loss of preservatives cease and the losses of biological material begin?

Rinsing each species individually from Humphreys Basin allowed us to observe that removal rates for EtOH are species specific. Based on data in Figures 1.4 and 1.5, we also determined that not only was EtOH was being removed but biological (DOC) material as well.

DOC concentrations in rinses of preserved soft body organisms were fairly low < 1.37 mg/L for *Dicosmoecus atripes* to 0.21 mg/L for Oligochaeta on day 2 to 0.93 mg/L for *L. signicauda* to 0.13 mg/L for *Daphnia melanica* on day 5. With the exception of *L. signicauda* and *Calibaetis ferrugineus*, all soft body species exhibited a steady decline or consistently low DOC concentrations. The higher DOC concentrations for *C. ferrugineus* are most likely a result of slower leaching caused by the relatively large size of this macroinvertebrate species. Observed increases in DOC for *L. signicauda* from day 2 to day 3 are most likely a result of handling of the organisms and a break in the exoskeleton.

Except for *Pisidium casertanum*, which had a much higher DOC concentration of 6.4 mg/L relative to the other organisms, other hard-bodied organisms. *Strictotarsus striatellus*, *Sanfilippodytes terminalis, and Cenocorixa kuiterti* exhibited low rates of DOC leaching (< 0.4 mg/L); however, I observed an increase in DOC in rinses of *S. striatellus* from day 4 to day 5. This was most

likely due to handling and a break in the exoskeleton allowing biological material to leach out.

The rinsing results from Humphreys Basin, while similar to the Lake Elsinore results, would have been more definitive if the first day's rinse water would have been analyzed. It would have been easier to determine if the different types of organisms had significant EtOH concentrations on the first day or a simple rinsing rather than a long soaking would have sufficed.

Long-term preservation of biological specimens for research and museums not only involves EtOH but also formalin. Standard preservation methods used by museums include placing species in 10% buffered formalin solution for several weeks, and then placed in EtOH for long-term preservation (Sarakinos et al. 2002, Carabel et al 2009). Samples are placed in formalin to maintain cell turgidity, stop any physical and chemical changes that would affect tissue upon death, and to maintain overall physical form (Simmons 1995). Samples then are placed in EtOH for long-term to stop decay or decomposition (Stoddard 1989).

Although formalin preservation analysis was beyond the scope of our current research, we felt it important to mention impacts of formalin compared to EtOH on long-term preservation of biological samples. As noted in Table 1, many samples prepared for long-term preservation for use in museums are generally preserved in formalin prior to placement into EtOH.

When comparing the results from the different studies shown in Table 1, several patterns become apparent. Formalin preservation tends to significantly deplete $\delta^{13}C$ values while $\delta^{15}N$ values are either slightly depleted or enriched (-2.50‰ to -0.48‰ and -1.0‰ to 0.16 respectively) (Bosley and Wainright 1995, Kaehler and Pakhomov 2001, Sarakinos et al. 2002, Syvaeranta et al. 2011, Bicknell et al. 2011). Differences in preservation effects from different mediums present a significant problem when trying to determine how to correct for these impacts. In this study we found rinsing samples for more than one day probably results in loss of native organic matter and should be avoided.

Preservatives and Rinsing Artifacts

After the fresh samples from Humphreys Basin were rinsed and analyzed, I determined any future rinsing and soaking duration should be significantly reduced based on $\delta^{13}C$ results. Fresh samples were compared to all preserved samples from 1997-2010 and the effect for nitrogen and carbon isotopes was within the same magnitude as reported in the literature reviewed earlier (-1.31‰ to 1.04‰ and -0.39‰ to 1.41‰, respectively).

The effects of EtOH preservation on carbon isotopes were more significant than I expected. While I anticipated some native DOC to leach out, and even though the DOC concentrations in the DIW rinses from the frozen samples seemed insignificant, the resulting cumulative effect of leaching of DOC over 5 days resulted in highly enriched $\delta^{13}C$ isotope values relative to controls

and the enrichment seemed larger than those reported in the literature (0.02‰ to 6.83‰).

When EtOH-preserved samples are separated from 1997-2010 and 2010 only, we find depletion for $\delta^{15}N$ for most species from 2010 and 1997-2007 and enrichment for $\delta^{13}C$ for all species (Table 1.4a – 1.4b). Further analysis shows there was little change in $\delta^{15}N$ values for species preserved for 90 days in 2010 when compared to species preserved for up to 13 years. When we compared $\delta^{15}N$ for both 2010 and 1997-2010 EtOH-preserved data against the $\delta^{15}N$ values of the 2010 frozen control samples, we found there was no significant difference in the slopes from 1 for 2010 ($p = 0.47$) and for the 1997-2010 ($p = 0.33$) (Figure 1.6a). In addition, the slight variations in $\delta^{15}N$ caused by EtOH preservation are not significant when compared to the 3.4‰ trophic level fractionation that is used to determine feeding relationships (Minagawa and Wada 1987). So I determined there was no need to correct the $\delta^{15}N$ data of the EtOH preserved samples for preservation or rinsing effects.

When the same data sets are compared for $\delta^{13}C$, there is also no significant difference from a slope of 1 for the 2010 frozen control to the 1997-2010 preserved group ($p = 0.97$) (Figure 1.6b). When $\delta^{13}C$ values in the 2010 frozen samples are compared to the 2010 EtOH-preserved samples there is a slight difference ($p = 0.09$) from a slope of 1 and a significant difference from an intercept of zero ($p = 0.05$) indicating there is a statistically significant enrichment in $\delta^{13}C$ in the EtOH-preserved samples for 2010 (Figure 1.6b).

In an attempt to standardize a rinsing method to reduce preservation artifacts, the extended rinsing/soaking not only resulted in removal of preservatives, it also resulted in significant leaching of organic material. The resulting enrichment of $\delta^{13}C$ from EtOH preservation is most likely a loss of lipids due to tissue hydrolysis (Hobson et al. 1997, Bosley and Wainright 1999, Arrington and Winemiller 2002, Sarkinos et al. 2002). Lipids are generally depleted in $\delta^{13}C$ due to fractionation during biosynthesis of pyruvate to acetyl coenzyme A and are easily removed with a polar solvent such as EtOH (DeNiro and Epstein 1977, Murry et al. 2006, Ventura and Jeppesen 2009). Research where lipids were extracted from biological samples prior to SIA, showed that $\delta^{13}C$ values of the specimens tended to be enriched (DeNiro and Epstein 1977, Gloutney and Hobson 1998, Smyntek et al. 2007, Post et al. 2007, Logan et al. 2008). Biological material such as liver, gonad, or white muscle frequently varies in lipid content due to different uptake rates and is typically extracted to correct for $\delta^{13}C$ of consumers to better reflect diet $\delta^{13}C$ (Post et al. 2007, Ingram et al. 2007). Research also indicates lipid extraction can also cause significant increase in $\delta^{15}N$ values from non-extracted controls (Murray et al 2006, Smyntek et al. 2007). Because of the limited amount of material available for my isotope analyses, I determined that no lipid extraction on any of the organisms could be conducted prior to rinsing or soaking.

Conclusions

Our rinsing experiments were designed to determine how long it would take for preservatives to be removed from biological material and if the rinsing process produced artifacts in stable isotope values of biological specimens. The rinsing method we used provided good insight into how biological material is affected over time when trying to correct for preservation artifacts. It is clear our rinsing and soaking method can be used as an “upper limit” to how long biological material can be processed before isotopic analysis. Rinsing/soaking times should be greatly reduced if not eliminated all together to minimize leaching of any biological material. Samples analyzed by Syvaranta et al. (2008) showed no effect with rinsing times less than a minute whereas Edwards et al (2002) showed both enrichment and depletion in samples soaked and rinsed for 3-5 days. Yet, samples rinsed for 1-2 minutes ended up being enriched (Ventura and Jeppesen 2009). Based on these rinsing times and our results, we recommend that only a simple rinsing is sufficient and a prolonged rinse/soaking process is not needed. It is difficult if not impossible to discern when the removal of preservation ends and the removal of biological material begins. However, we compiled a table of Sierra Nevada lake species and recommended rinsing times for each species if EtOH-rinsing is needed (Table 1.5). Our results helped to support previous research that removal of preservatives is complicated and great care needs to be used while preparing samples for stable isotope analysis. Preparation of samples for SIA should be

addressed on a species-specific basis and corrections for each species may be needed as a simple rinsing removes most preservatives but a longer rinsing time will be dictated by type and size of sample.

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Table 1.1 Reported impacts of preservation treatment on stable isotope values of aquatic species.

Source	Species/Taxa	Time of preservation	Ethanol preserved		Rinsed in DIW
			$\delta^{15}N$	$\delta^{13}C$	
Sarkinos et al., 2002	*Catotomus occidentalis* (fish)	3 days - 6 months*	enriched	no effect	no
Sarkinos et al., 2002	*Corbicula fulminea* (mollusk)	3 days - 6 months*	depleted	enriched	no
Sarkinos et al., 2002	*Hydropsyche sp.* (aquatic insect)	3 days - 6 months*	no effect	no effect	no
Carabel et al., 2009	*Himanthalia elongata* (seaweed)	6-24 months**	depleted	depleted	no
Carabel et al., 2009	*Patella vulgata* (mollusk)	6-24 months**	enriched	no effect	no
Carabel et al., 2009	*Mytilus galloprovincialis* (mollusk)	6-24 months**	enriched	depleted	no
Carabel et al., 2009	*Anemonia sulcata* (anemone)	6-24 months**	enriched	depleted	no
Kaehler & Pakhomov, 2001	*Octuopus vulgaris* (mollusk)	1-12 weeks***	no effect	enriched	yes
Kaehler & Pakhomov, 2001	*Argyrosomus hololepidotus* (fish)	1-12 weeks***	enriched	enriched	yes
Kaehler & Pakhomov, 2001	*Ecklonia radiata* (kelp)	1-12 weeks***	no effect	enriched	yes
Bosley & Wainright, 1999	*Pleurinectes americanus* (fish)	2-4 months‡	enriched	depleted	no
Bosley & Wainright, 1999	*Cragon septemspinosa* (decapod)	2-4 months‡	no effect	no effect	no
Edwards et al., 2002	Percidae (fish)	up to 15 years‡‡	enriched	depleted	yes
Syväranta et al., 2008	Cladocera (zooplankton)	2 weeks - 12 months‡‡‡	no effect	no effect	yes
Syväranta et al., 2008	Cyclopoida (zooplankton)	2 weeks - 12 months‡‡‡	no effect	no effect	yes
Syväranta et al., 2008	Calanoida (zooplankton)	2 weeks - 12 months‡‡‡	no effect	no effect	yes
Syväranta et al., 2008	*Asellus quaticus* (crustacean)	2 weeks - 12 months‡‡‡	no effect	no effect	yes
Syväranta et al., 2008	Sialidae (aquatic insect)	2 weeks - 12 months‡‡‡	no effect	no effect	yes
Syväranta et al., 2008	*Chironomidae sp.* (aquatic insect)	2 weeks - 12 months‡‡‡	no effect	no effect	yes
Syväranta et al., 2008	Tricoptera (aquatic insect)	2 weeks - 12 months‡‡‡	no effect	no effect	yes
Ponsard & Amlou, 1998	Drosophila melanogaster (fly)	10 days - 6 weeks§	enriched	depleted	no
Ventura & Jeppesen, 2009		2 - 6 months§§	enriched	enriched	yes
Sweeting et al., 2004	*Gadus morhua* (fish)	1 day - 21 months§§	no effect	no effect	yes
Arrington & Winemiller, 2002	Mugil cephalus, Cynoscion nebulosus, Arius felis, Dorosoma cepedianum (fish)	6 weeks§§§	enriched	depleted	yes

*Samples in 10% formalin for 2 months then placed in 90% EtOH for additional 2 months.

**Samples placed in 10% formalin for 2 days then placed in 70% EtOH long term.

***Samples placed in 70% EtOH.

‡Samples placed in 10% formalin for 2 weeks then placed in 90% EtOH for up to 2 months

‡‡Samples placed in 10% formalin for 10 days, then placed in 35% EtOH for 2 weeks then in transferred to 70% EtOH.

‡‡‡Samples placed in 80% EtOH.

§ Samples placed in 95% EtOH.

§§ Samples placed in 80 and 100% industrial EtOH (95% EtOH & 5% methanol)

§§§ Samples placed in 10% formalin for 2 weeks then placed in 70% EtOH long term.

Table 1.2 Results from isotope analysis of replicate Lake Elsinore samples.

Sample ID	Treatment	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰
LE-1	EtOH preserved plus 5-day rinse	11.43	-20.79
LE-1	EtOH preserved plus 5-day rinse	11.50	-20.48
LE-2	EtOH preserved plus 5-day rinse	11.53	-20.52
LE-2	EtOH preserved plus 5-day rinse	10.75	-20.67
LE-3	EtOH preserved plus 5-day rinse	11.52	-20.54
LE-6	EtOH preserved plus 5-day rinse	11.26	-21.49
LE-6	Frozen	10.80	-21.62
LE-7	Frozen	10.78	-21.63
LE-7	Frozen	10.88	-21.53

Table 1.3 List of species used for the rinsing experiment and isotopic analysis from Humphreys Basin.

Species
Rana muscosa (Sierra Nevada yellow-legged frog)
Agabus tristis (beetle)
Stictotartsus striatellus (beetle)
Sanfilippodytes terminalis (beetle)
Callibaetis ferrugineus (mayfly)
Cenocorixa kuiterti (water boatman)
Desmono mono (caddisfly)
Dicosmoescus atripes (caddisfly)
Polycentropus variegatus (caddisfly)
Pisidium casertanum (clam)
Oligochaeta (several species of earthworms)
Chironomidae (many species of midges)
Acari (several species of mites)
Leptodiaptomus signicauda (copepod)
Hesperodiaptomus shoshone (copepod)
Daphnia melanica (cladoceran)

Table 1.4 Isotope results for frozen and EtOH-preserved specimens from Humphreys Basin for 2010 and 1997-2010. EtOH-preserved samples were preserved for 90 days. Deviations are computed as: EtOH ‰ –minus Frozen ‰.

Species	2010 Frozen control		EtOH-Preserved 2010		Deviation		EtOH-Preserved 1997-2010		Deviation from control	
	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰
Rana muscosa (Sierra Nevada yellow-legged frog)	5.85	-18.10	5.20	-17.60	-0.65	0.50	5.20	-17.60	-0.65	0.50
Agabus tristis (beetle)	3.57	-18.95	3.13	-18.06	0.44	0.89	3.44	-18.25	0.13	0.70
Stictotartsus striatellus (beetle)	1.40	-17.10	1.69	-16.65	0.29	0.45	1.79	-16.24	0.39	0.86
Callibaetis ferrugineus (mayfly)	1.35	-19.00	1.01	-16.33	0.34	2.67	1.85	-18.27	0.50	0.73
Cenocorixa kuiterti (water boatman)	2.31	-21.80	1.00	-20.00	-1.31	1.80	2.31	-18.73	0.00	3.07
Dicosmoescus atripes (caddisfly)	-1.58	-22.56	-0.74	-17.10	-0.01	5.46	-0.48	-14.61	1.04	5.48
Pisidium casertanum (clam)	1.20	-17.47	1.08	-13.98	-0.12	3.49	1.36	-15.00	0.16	2.44
Chironomidae (many species of midges)	2.22	-19.36	1.90	-17.20	-0.32	2.16	2.67	-17.58	0.45	1.78
Acari (several species of mites)	3.91	-20.33	3.98	-16.76	-0.07	3.57	4.40	-18.30	0.39	2.03
Leptodiaptomus signicauda (copepod)	1.90	-28.11	-1.14	-26.46	-0.60	1.65	2.70	-26.16	0.46	1.69
Hesperodiaptomus shoshone (copepod)	1.15	-27.01	0.53	-26.99	-0.62	0.02	0.53	-26.99	0.62	0.02
Daphnia melanica (water flea)	-0.81	-32.88	-0.03	-32.91	0.78	-0.03	-0.66	-26.58	0.15	5.67

Table 1.5 Species list with recommended rinsing and soaking times as detailed in the experimental section.

Species	Suggested rinsing/soaking times
Rana muscosa (Sierra Nevada yellow-legged frog)	1-2 day*
Agabus tristis (beetle)	1 day*
Stictotartsus striatellus (beetle)	1 day
Sanfilippodytes terminalis (beetle)	1 day*
Callibaetis ferrugineus (mayfly)	1 days
Cenocorixa kuiterti (water boatman)	< 1 day
*Sialidae occidens (*alderfly)	1 day
Desmono mono (caddisfly)	1 day*
Dicosmoescus atripes (caddisfly)	2 days
Polycentropus variegatus (caddisfly)	< 1 day*
Pisidium casertanum (clam)	1 days*
Oligochaeta (several species of earthworms)	< 1 day*
Chironomidae (many species of midges)	< 1 day
Acari (several species of mites)	1 day*
Leptodiaptomus signicauda (copepod)	< 1 day
Hesperodiaptomus shoshone (copepod)	< 1 day*
Daphnia melanica (cladoceran)	< 1 day

* Samples were only rinsed as either preserved or fresh and recommended rinsing and soaking times are based on species of similar size.

Figure 1.1 Schematic representation showing collection process for samples and rinsing procedure for Lake Elsinore samples. Fresh samples were split with one-half placed in preservatives and the other half frozen.

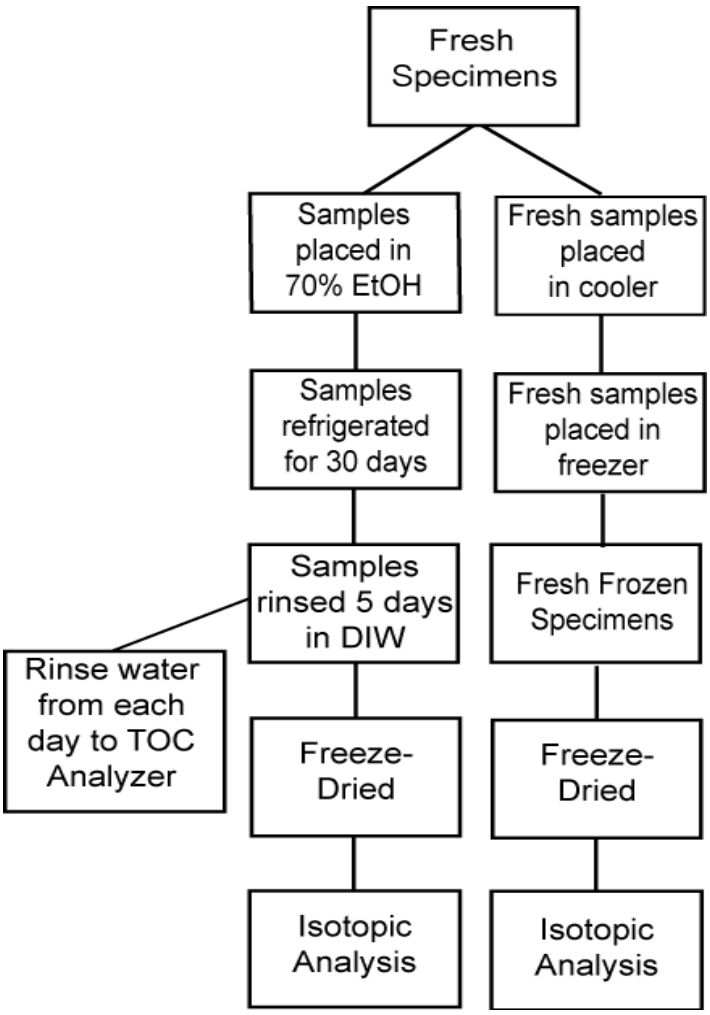


Figure 1.2 Schematic representation showing the rinse method for Humphrey's Basin samples.

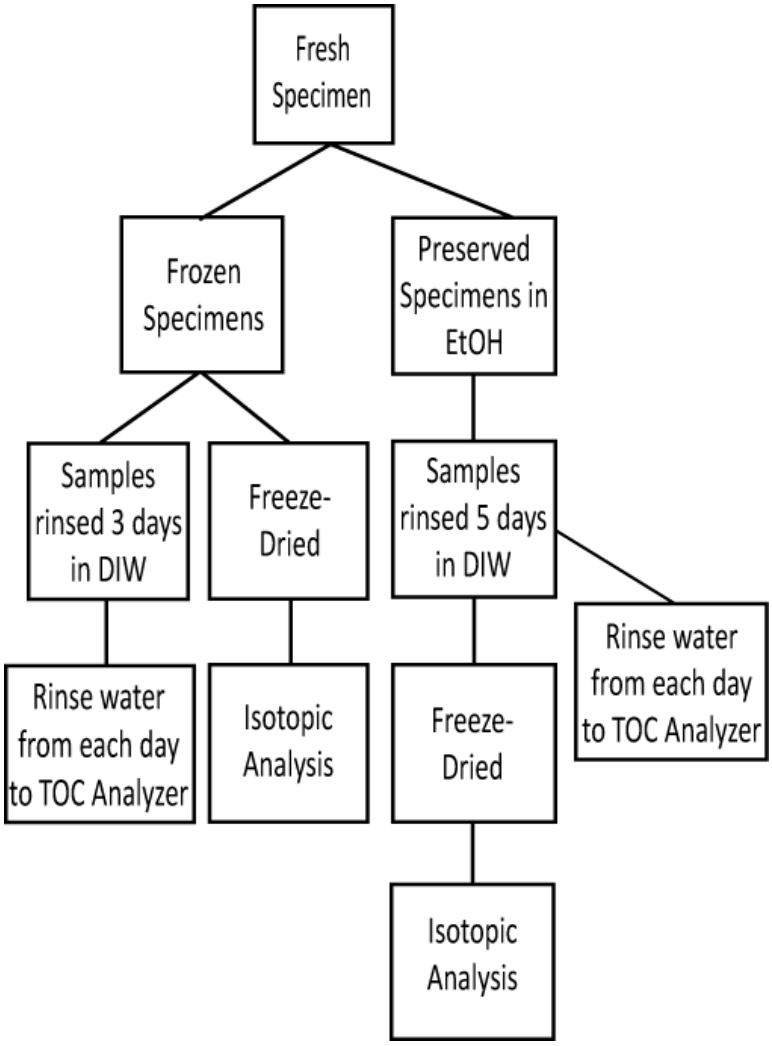


Figure 1.3 Semi-log plot of dissolved organic carbon (DOC) concentrations over time for zooplankton samples collected from Lake Elsinore for testing of rinsing method.

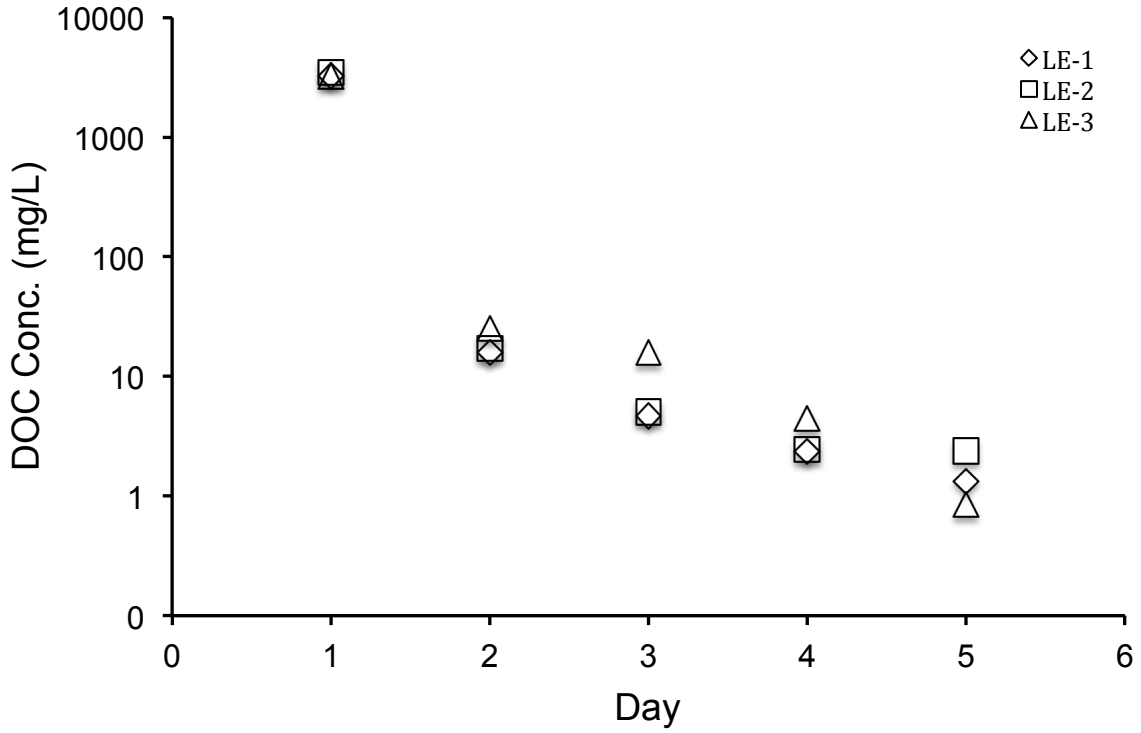


Figure 1.4a Measured concentration of DOC for Humphrey's Basin preserved samples from 1997-2007. Samples were rinsed using the multiple day rinse method. First day's rinse results not shown since the magnitude was many times higher then the following days. BD refers to below detection limit.

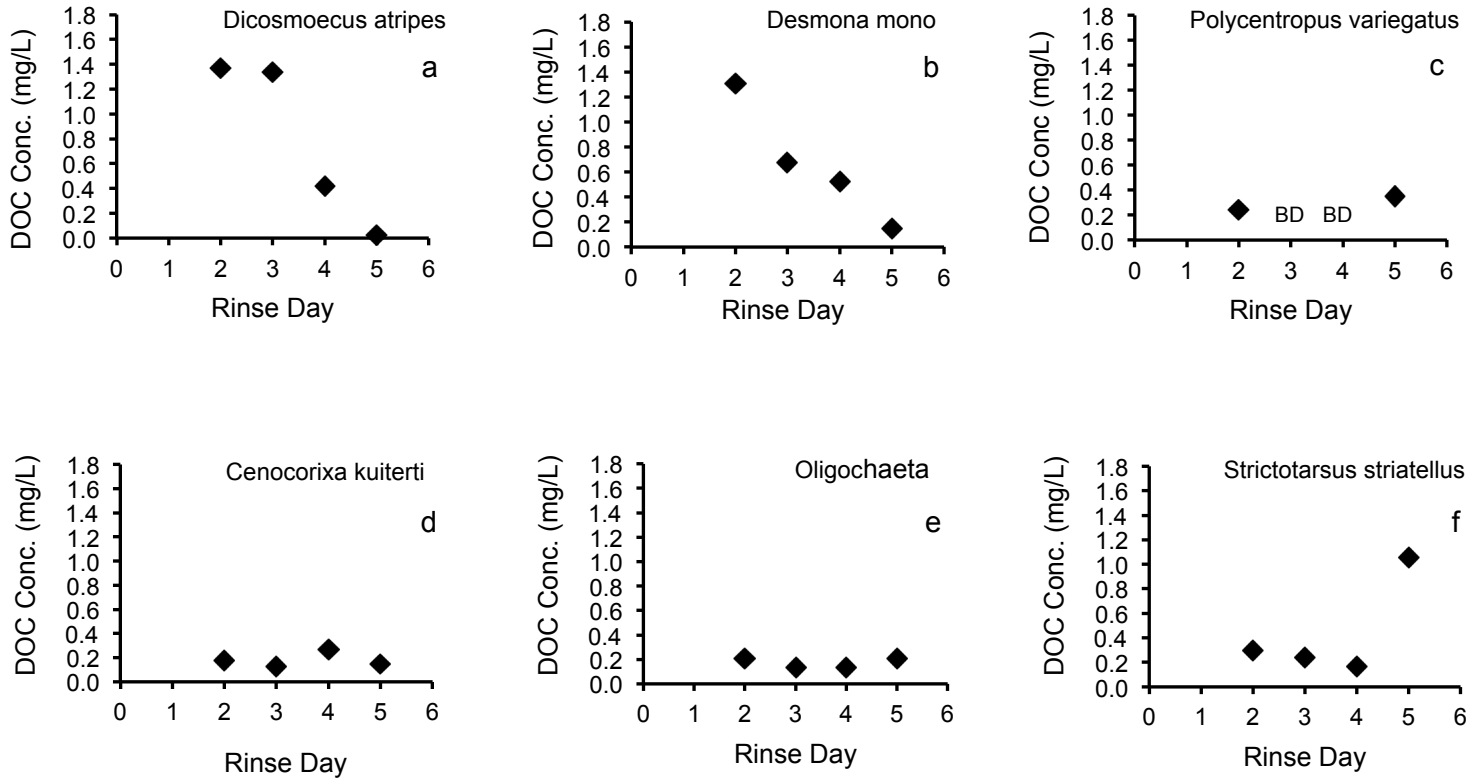


Figure 1.4b. Measured concentration of DOC for Humphrey's Basin preserved samples from 1997-2007. Samples were rinsed using the multiple day rinse method. First day's rinse results not shown since the magnitude was many times higher then the following days. BD refers to below detection limit.

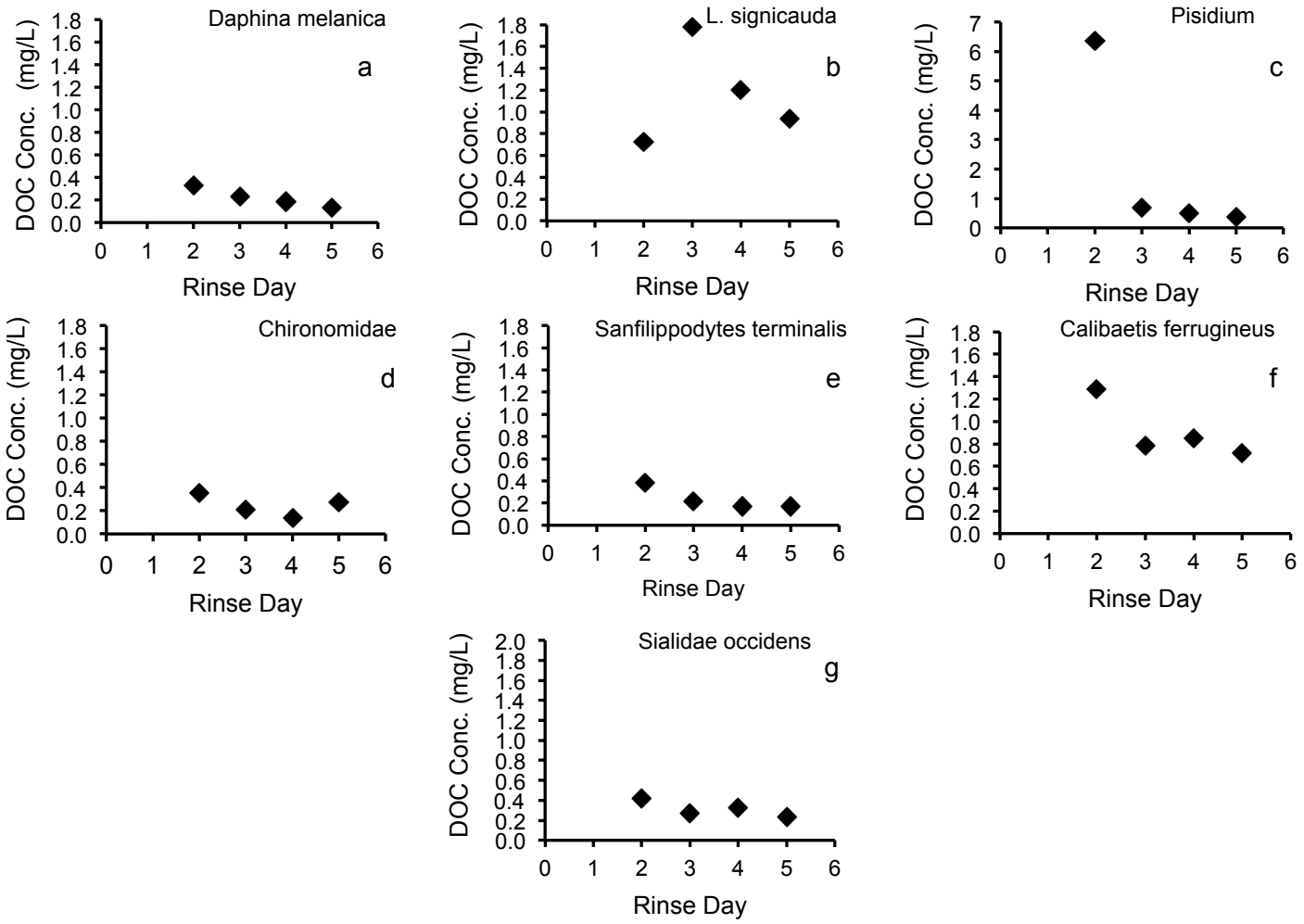
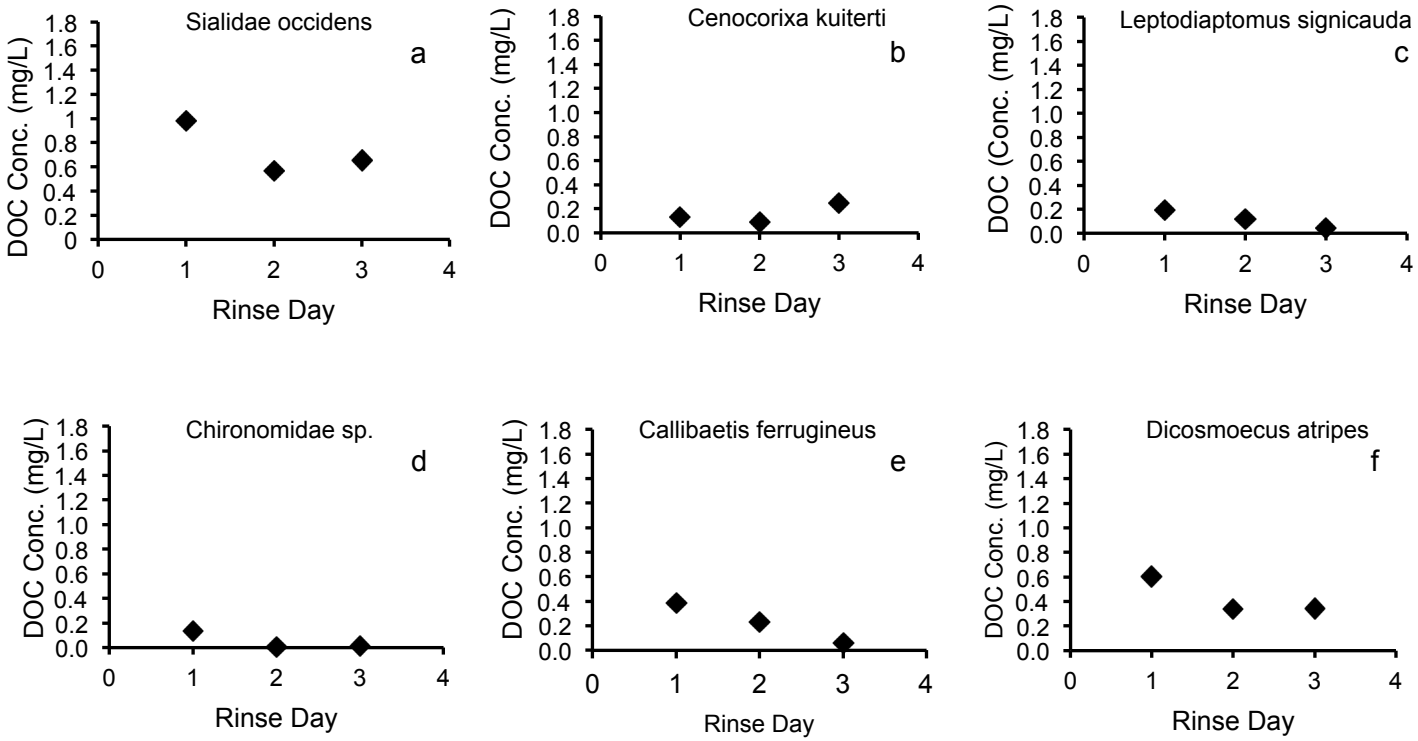
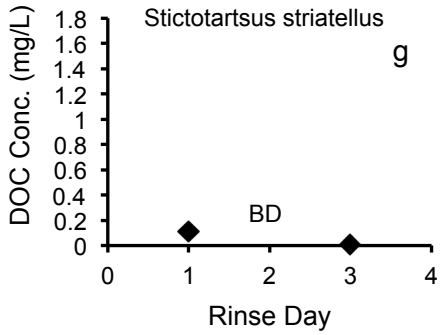
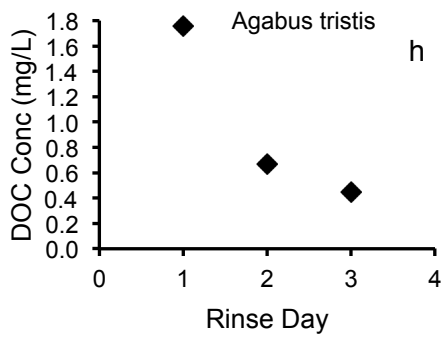
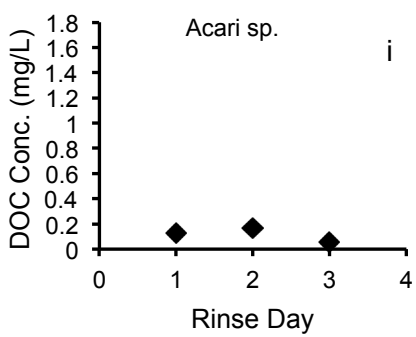


Figure 1.5 Measured concentrations of DOC for Humphrey's Basin fresh, frozen samples collected in 2010. Samples were rinsed using the multiple day rinse method. Rinsing was reduced to 3 days. BD refers to below detection limit.




Stictotartsus striatellus
g
BD
DOC Conc. (mg/L)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
Rinse Day


Agabus tristis
h
DOC Conc (mg/L)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
Rinse Day


Acari sp.
i
DOC Conc. (mg/L)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
Rinse Day

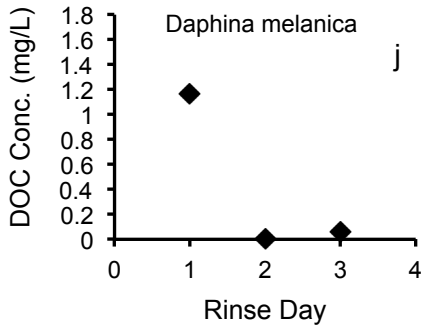
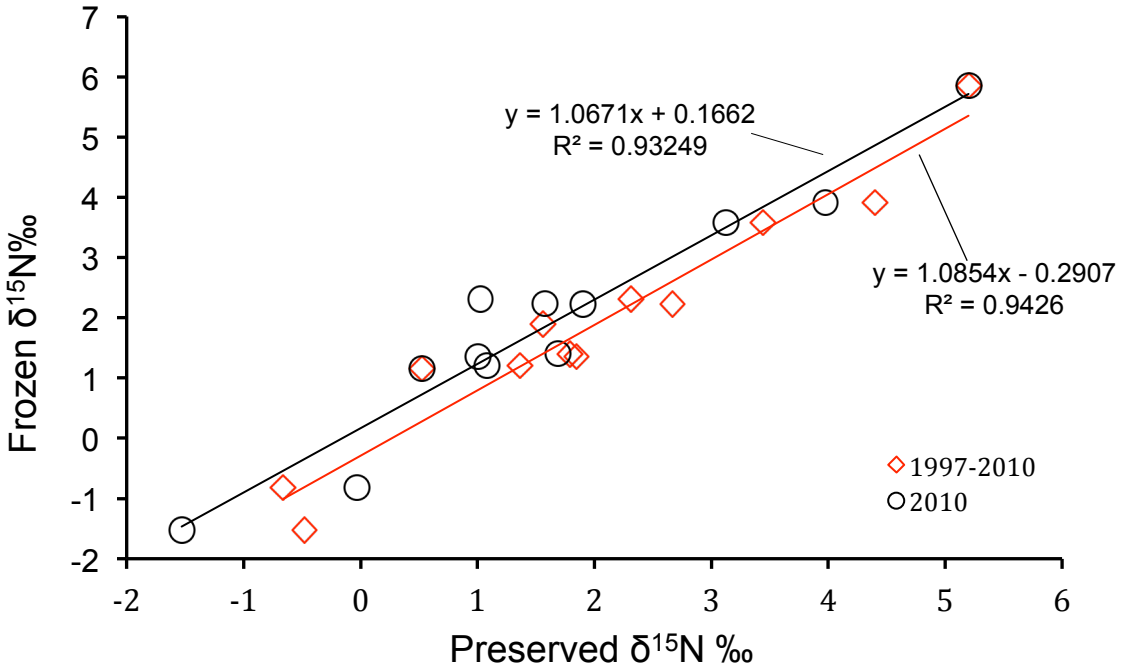
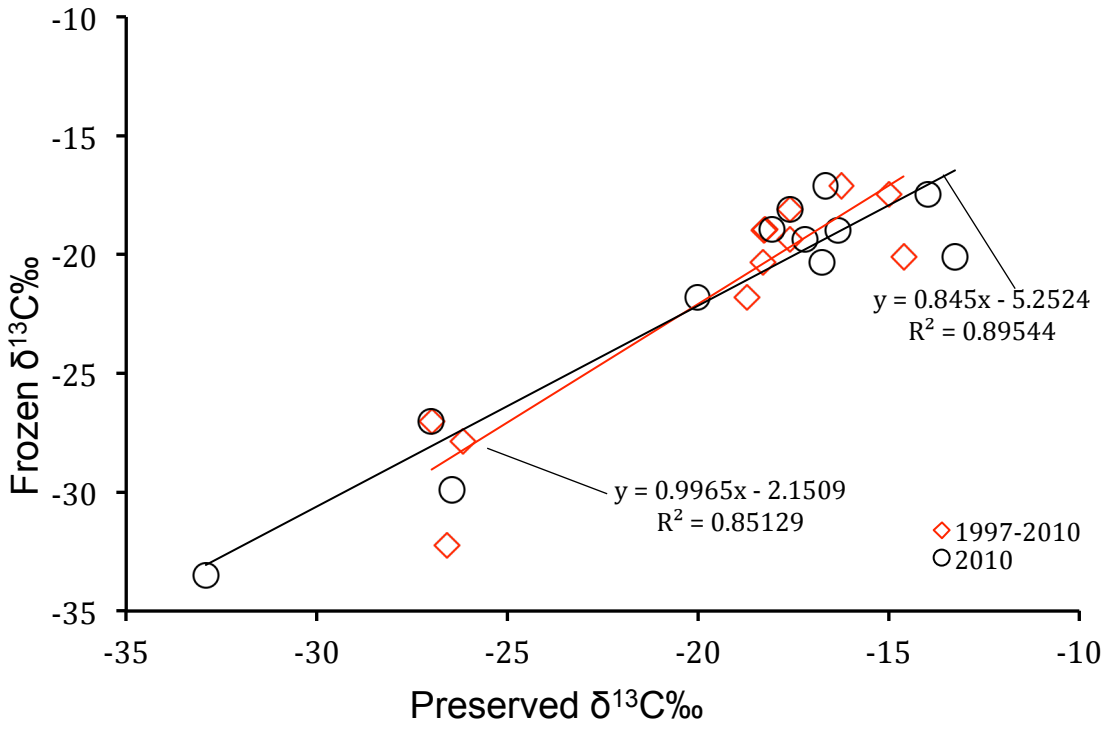

Daphina melanica
j
DOC Conc. (mg/L)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
Rinse Day

Figure 1.6a & 1.6b. Graphical representation of Table 1.4a & 1.4b showing the shift of isotope values from frozen specimens for Humphreys Basin for both nitrogen and carbon respectively. Shown are for 90-day preservation in 2010 and long-term preservation from 1997-2007. Each point represents a single species for which unpreserved and preserved samples were available.





Chapter 2

Food Webs in Sierra Nevada, Lakes and Their Response to Fish Removal

Abstract

Stable nitrogen ($\delta^{15}N$) and carbon isotopes ($\delta^{13}C$) were used to quantify trophic position and energy pathways in food webs in two lakes containing introduced trout and two lakes where introduced trout had been removed in the late 1990s. The stable isotope composition of eighteen species including zooplankton, benthic macroinvertebrates, fish, and frogs was measured in archived samples collected during 1997-2007 and new samples collected during 2010. Presence of fish resulted in a pronounced enrichment of $\delta^{15}N$ values for all levels of the foodweb, suggesting that fish introductions affect whole lake ecosystems. I hypothesize that this isotopic enrichment results from extirpation of large zooplankton by trout predation, with consequent effects on phytoplankton biomass and increased P availability. Increased P availability increases N limitation in phytoplankton and thereby reduces discrimination against ^{15}N during algal assimilation resulting in higher $\delta^{15}N$ values in phytoplankton that cascades up through the foodweb. Removal of fish and return of previously extirpated cladocerans resulted in significant depletion of 4.42‰ in $\delta^{15}N$ for a copepod, *Leptodiaptomus signicauda*. This isotopic shift suggests that *Daphnia melanica* may out-compete *L. signicauda* for food and forces the copepod to utilize different food sources.

Introduction

Whether by accident or on purpose, introduction of non-native species can have unintended and deleterious consequences ecosystems. Coupled with pollution, habitat modification, land use, and biotic exchange, non-native species introductions are among the biggest threats to global biodiversity (Chapin et al. 1996, Sala et al. 2000, Hartel et al. 2007). While some introduced species are benign and may even be beneficial to their new environment, more often than not introduced species can alter water quality, change interactions between populations of native species, induce bottom up or top down trophic cascades, shift nutrient flows, and in many cases result in the extirpation of native flora and fauna reducing species diversity (Northcote 1987, McNaught et al. 1999, Knapp and Matthews 2000, Vredenburg 2004, Eby 2006, Parker and Schindler 2006, Hartel et al. 2007, Finlay and Vrendenburg 2007, Schabetsberger 2009).

Some of the most commonly introduced organisms globally are members of the family Salmonidae that includes 14 species of trout. Stocking of fish has occurred for hundreds of years in all types of ecosystems, non-native fish stocking in North and South America alpine ecosystems occurred in the late 19th to early 20th centuries (McNaught et al. 1999, Schabetsberger et al. 2009, Bahls 1992). Many alpine lakes in the Sierra Nevada and in western North America result from late Pleistocene glaciation (Pister 2001). As the glaciers retreated, many of these newly formed lakes were left isolated from downstream influences due to physical barriers which prevented colonization by fish (Pister 2001). Prior

to the mid-nineteenth century, most Sierra Nevada lakes above 1800 m were devoid of fish (Pister 2001, Knapp 1996). Stocking of nearly all lakes in the Sierra Nevada with non-native species of trout as well as interbasin transfers of trout was attempted to enhance recreational fishing (Knapp 1996, Vredenburg 2004). Early stocking was conducted by sporting clubs such as the Bishop Fish Planting Club, Sierra Club, and Visalia Sportsmen Club (Knapp 1996). Eventually the California Fish and Game commission, the precursor of the California Department Fish and Game (DFG), took over stocking activities in the Sierra Nevada with the mandate to maintain existing trout populations (Knapp 1996).

Little concern was initially given to the consequences of introducing trout into Sierra Nevada lakes (Bahls 1992). Later research showed fish introductions initiated a top down alteration of lake food webs. Major impacts included a shift towards dominance of smaller zooplankton and smaller benthic macroinvertebrate species and extirpation of several amphibian species including the endangered mountain yellow-legged frog (*Rana muscosa*) (Stoddard 1987, McNaught et al 1999, Knapp and Matthews 2000, Donald et al. 2001, Knapp et al. 2001b, Vredenburg 2004, Eby et al. 2006, Finlay & Vredenburg 2007).

Trout stocking in the Sierra Nevada was phased out starting in 1969 in Yosemite and in 1991 in Sequoia and Kings Canyon National Parks, but stocking continues in lands managed by the US Forest Service (Leopold 1963, Knapp and Matthews 2000). However, even without restocking, 63% the lakes currently have self-sustaining fish populations while 52% are still stocked regularly (Bahls

1992). In 1996, Dr. Roland Knapp of the Sierra Nevada Aquatic Research Laboratory (SNARL) along with Dr. Orlando Sarnelle, and the DFG began an experimental removal of trout from lakes within the Humphreys Basin (John Muir Wilderness, Inyo National Forest) in order to observe rates of native faunal recovery (Sarnelle and Knapp 2005). This study provided the motivation and the majority of samples for my thesis research.

For the last 15 years the National Park Service (NPS) and the California Fish and Game have attempted to remove fish from Sierra Nevada lakes to increase populations of *R. muscosa* and restore the aquatic foodwebs to pre-introduction conditions. Knapp et al (2001b) compared the food webs in lakes: i) that were stocked and contained salmonids, ii) where stocked salmonids were removed by gill-netting, and iii) lakes that were never stocked, to examine the resistance and resilience of the native flora and fauna to fish introductions. The study documented the loss and subsequent recovery of two large zooplankton species, *Hesperodiaptomus shoshone* and *Daphnia melanica*, as well as several large benthic invertebrates following the removal of the fish (Sarnelle and Knapp, 2004, Knapp and Sarnelle, 2008). Although most native species recover following fish removal, some like *H. shoshone*, and *R. muscosa/sierrae* do not consistently recover and attempts to manually reintroduce *R. muscosa/sierrae* to now fishless lakes have not been completely successful. One question remains: *How do the foodwebs of lakes where fish were removed compare isotopically to lakes that maintain fish populations?* Answering this question may aid ongoing

efforts to restore lake ecosystems to their original fishless conditions and would contribute to general knowledge regarding the long-term trophic impacts of introduced fish on alpine lake ecosystems.

Given the known impacts fish have on lake food webs*, I hypothesize that the food web of lakes where non-native fish are removed will diverge isotopically from the food web of fish-containing lakes. Through time these differences will increase as native species return.* I anticipate removing the non-native fish as the top predator in the lakes will result in return of many species extirpated by the fish and will allow for a more diverse foodweb. This diversity in turn will result in less competition for limited food sources, thereby altering the isotopic composition of all species within the foodweb.

One way to assess changes in food webs through time is to measure how stable isotopes shift in different species over time within an ecosystem. Stable isotope ratios of nitrogen ($^{15}N/^{14}N$) can be used to elucidate food web structure, animal diets, and to indicate trophic position of individual species. (Peterson and Fry 1987, Kling et al. 1992, Vander Zanden et al. 1999, Vander Zanden and Rasmussen 1999). Carbon isotope ratios ($^{13}C/^{12}C$) can be used to trace energy pathways in a system and indicate food sources since the isotope ratios of a consumer are similar to its food sources (Kling et al. 1992, Vander Zanden et al. 1999, Vander Zanden and Rasmussen 1999, Post 2002, Carabel et al. 2006, Smyntek et al. 2007, Schmidt et al. 2009). In this chapter I used stable nitrogen ($\delta^{15}N$) and carbon isotopes ($\delta^{13}C$) to quantify trophic position and energy

pathways in food webs in two lakes containing introduced trout and two lakes where introduced trout had been removed in the late 1990s. The stable isotope composition of eighteen species including zooplankton, benthic macroinvertebrates, fish, and frogs was measured in archived samples collected during 1997-2007 and new samples collected during from 2010.

Methods

Study Site

Dr. Roland Knapp and his research group have conducted a fish removal experiment in Humphreys Basin located in the John Muir Wilderness between since 1996. Four lakes were selected for my study: two lakes from which fish were removed (Marmot and Square, henceforth denoted “fish-removed” lakes) and two lakes containing introduced trout that were stocked much earlier in the 20th century (Summit and Mesa, henceforth denoted “fish-containing” lakes). All four lakes were selected due to similar species composition when fish were present (Table 2.1) and physiography (Table 2.2). Archived samples from 1997, 1998, 2001, 2005, 2007 were analyzed and I collected additional samples from the same lakes in 2010 (Figure 2.1). Fish were removed from Marmot and Square lakes using gill netting during the summers of 1997-1998; by 1999 no fish remained in these lakes (Sarnelle and Knapp 2004).

Research was initially conducted to determine species recovery, particularly *R. sierrae*, following fish removal from Marmot and Square Lakes.

Coupled with the fish removal, zooplankton and zoobenthos samples were also collected to determine recovery rates and densities of species present with fish and to determine what native species would return once fish were removed. Special attention was paid to two of the three main extirpated zooplankton species: *Hesperodiaptomus shoshone* and *Daphnia melanica*. Historical presence of these two species in Marmot and Square lakes prior to fish introductions was confirmed through sediment core sampling.

Historical Lake Sampling Protocol

Roland Knapp and his research team collected all 1997-2007 species used in my foodweb analysis. Zooplankton samples were collected using a 29.5-cm diameter; 64-μm mesh net with one to five tows made to ensure sufficient numbers of zooplankton were present in the sample for enumeration (Knapp et al. 2001, Knapp and Sarnelle 2008). Benthic samples were collected with 15 standard sweeps using a D-net with a 0.5 mm mesh size bag. Each sweep consisted of a 1-meter sweep in one direction and a 1-meter sweep in the opposite direction (Knapp et al. 2001, Knapp and Sarnelle 2008). The zooplankton and zoobenthos samples were preserved in 70% ethanol while in the field. Samples were identified to genus and species by Roland Knapp and were stored at his laboratory at SNARL at room temperature.

In addition, Dr. Orlando Sarnelle from Michigan State University supplied to me seston filter samples from 1998 and 2002 from a previous research project

at the same lakes. The seston samples were comprised primarily of phytoplankton and organic detritus and were collected onto pre-ashed glass fiber filters from water collected from the center of the lakes (Sarnelle and Knapp 2005). Isotope values of the 1998 and 2002 seston samples were compared to seston samples I collected in 2010 to determine if there were any significant changes in overall isotopic values through time.

Current Lake Sampling Protocol

To understand the effects of preservatives on the isotopic composition of the samples, ethanol-preserved and duplicate frozen and unpreserved biological samples were collected during the summer of 2010 using the same sampling protocols as described above. In an attempt to collect all species previously identified, the 2010 samples consisted of multiple zooplankton tows (30-40) and D-net sweeps (20-30). Duplicate samples were collected with one half of the samples placed in 70% EtOH (henceforth denoted as EtOH-preserved) and the other half kept in lake water (henceforth denoted as unpreserved). In the field, the fresh samples were packed in snow to minimize the chance of death and decomposition. Two fish were also collected: one from Summit and one from Mesa Lakes and one tadpole from Marmot Lake. These samples were also packed in snow.

The fresh samples were brought back to the laboratory within 36 hours, observed through a dissecting scope and all dead organisms were removed. The

remaining live organisms were identified to genus and species, put into separate vials, then placed into a freezer for future analysis. Dr. Knapp also supplied two frozen and unpreserved frogs from 2009 and two EtOH-preserved frogs from 2010 that were collected from Marmot Lake. The unpreserved frogs had been kept frozen at -20°C since their collection. Previous research indicates that freezing has little impact on $\delta^{13}C$ and $\delta^{15}N$ isotope vales and is a viable way means for long-term preservation (Ponsard and Amlou 1998, Bosley and Wainright 1999, Kaehler and Pakhomov 2001, Sweeting et al. 2004, Barrow et al. 2008).

Sediment, phytoplankton and seston samples were also obtained from each of the four lakes during 2010. Sediment samples were collected near the shore in water of approximately 0.5 m depth. I collected the upper 0-1 cm of the water/sediment interface. Sediment samples were dried at 60°C for 24 hours, powdered with mortar and pestle, and lyophilized at -80°C for 24 hours. Powdered samples were then placed in tin capsules for isotopic analysis.

Phytoplankton samples were obtained using a 60 ml bottle by grab sampling 0.5 meters in depth with 5% formalin added to the water to preserve the specimen for identification. Samples were then placed in a refrigerator until analysis. The preserved phytoplankton samples were sent to Dr. Danuta Bennett at University of California, Santa Barbara to determine species composition and abundance. Seston samples were collected by vacuum-filtering lake water through a 47 mm Whatmann GF/F filter. Filters were taken back to the lab,

observed through a microscope to remove any benthic or zooplankton species present, dried and then placed into tins for isotopic analysis.

Food Web Isotope Analysis

All EtOH-preserved Humphreys Basin samples were rinsed for 5 consecutive days as described in Chapter 1. Rinsed EtOH-preserved samples and unpreserved samples stored in the freezer were lyophilized at -80°C for 24-48 hours. After lyophilizing, the specimens were weighed and placed in tin capsules for isotope analysis. To obtain adequate sample weight, each zooplankton sample consisted of 150-200 individuals. One or two whole zoobenthos specimens were loaded into each tin capsule. Larger zoobenthos specimens were crushed and powdered prior to being placed into tin capsules. Leg muscle from the frogs and ventral muscle from the fish were also lyophilized at -80°C for 24-48 hours. After drying, each sample was powdered and placed into tins for isotope analysis. Dry weights for all samples ranged from 0.1 mg to 4.0 mg. Samples were combusted in 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 stable nitrogen and carbon isotopes. Stable isotope analysis was conducted by the protocol described in Chapter 1 methods.

Food Web Trophic Position

Trophic fractionation (*λ),* for $\delta^{13}C$ and for $\delta^{15}N$, are generally accepted to be 0.5 - 1.0‰ and 3.4‰ per trophic level, respectively (Minagawa and Wada 1984, Peterson and Fry 1987, Jacob et al. 2005). The trophic positions for each species in the Humphrey Basin lakes were calculated relative to the baseline $\delta^{15}N$ value using the following equation:

$$TP_{consumer} = ((\delta^{15}N_{consumer} - \delta^{15}N_{baseline}) / \lambda) + TL \quad \text{Equation 2.1}$$

where $TP_{consumer}$ is consumer trophic position; $\delta^{15}N_{consumer}$ is $\delta^{15}N$ of consumer; $\delta^{15}N_{baseline}$ is $\delta^{15}N$ of baseline nutrient sources; λ is the trophic fractionation factor per trophic level, and TL is trophic level (Vander Zanden and Rasmussen 1999, Post 2002, McCutchan et al. 2003, Gorokhova et al. 2005).

In Chapter 1, I summarized the carbon isotope artifacts caused by EtOH preservation and rinsing of samples with DIW. EtOH preservation and DIW rinsing produced enrichment of $\delta^{13}C$ and the magnitude of the enrichment was large relative to the trophic fractionation expected for C isotopes in foodwebs. Therefore, I applied correction factors to the $\delta^{13}C$ isotope values of preserved samples from 1997-2003 and 2010. Corrections for carbon were done by subtracting the offset between EtOH-preserved and frozen unpreserved samples collected in 2010, on a species by species basis. For species found in the 1997-2003 samples but not collected during 2010, I used the correction factors for

similar species. The nitrogen isotope artifacts produced by EtOH preservation and DIW rinsing were smaller than the carbon isotope artifacts both in absolute size and in relation to expected trophic fractionation. Therefore, no corrections were made to the $\delta^{15}N$ values of EtOH-preserved samples.

Results

Isotopic Composition of Foodwebs

Isotope results for each species in both the fish-removed and fish-containing lakes were compiled with the mean and standard error calculated (Table 2.3a and 2.3b, respectively). In the table I lumped the data for the two fish-removed lakes and the two fish-containing lakes. The carbon isotope values represented in Table 2.3 have been corrected for preservation effects as described in the Methods. Because $\delta^{15}N$ isotope values for several species (*D. mono*, *D. melanica*, *P. casertanum,* and *D. atripes*) were depleted relative to seston and sediment isotope values, another resource must have been responsible for these lower values. A literature review was conducted and the most likely source for these lower isotope values was input of terrestrial materials due snow and rain runoff. To account for the depleted values, carbon and nitrogen isotope values for terrestrial organic matter are also presented in the tables; $\delta^{13}C$ values are the mean for C3 plants as reported in Fry (2008) and $\delta^{15}N$ are from terrestrial biological materials measured in Sierra Nevada watersheds by Sickman et al. (2003).

Mean nitrogen isotope values of samples for fish-containing lakes ranged from -1.62‰ ± 1.26‰ to 7.06‰ ± 0.09‰ and for fish-removed lakes ranged from -1.62‰ ± 1.26‰ to 5.53‰ ± 0.15‰. Carbon isotope values for fish-containing lakes ranged from -17.98‰ ± 1.07‰ to -28.0‰ ± 0.0‰ and for fish-removed lakes ranged from -15.9‰ ± 0.01‰ to -28.00‰ ± 0.0‰. Mean $\delta^{13}C$ and $\delta^{15}N$ ± SE from Table 2.3a and 2.3b for each species were plotted to show energy pathways and trophic relationships for fish-removed and fish-containing lakes (Figures 2.2a and 2.2b). Species were then combined into specific groups based on feeding relationships (i.e., benthic grazers, zooplankton, seston, invertebrate predators, and sediments) and plotted in Figure 2.3. In both figures removal of fish was associated with a general depletion in $\delta^{15}N$ and enrichment in $\delta^{13}C$ for all species and groups that were found in both types of lakes. The sole exception was zooplankton where $\delta^{15}N$ decreased, while $\delta^{13}C$ increased. Similar species in both fish-containing and fish-removed lakes were compared to each other to determine how significant the depletion of $\delta^{15}N$ was.

Species Trophic Position

Trophic fractionation for both fish-containing and fish-removed lakes was determined by dividing the total variation in $\delta^{15}N$ seen in Figures 2.2a and 2.3a for each type of lake by the number of trophic levels in each lake. For our lakes, I determined there were four trophic levels in both fish-containing and fish-removed lakes, based on current ecological understanding of Sierra Nevada lake

ecosystems. Also included in these calculations was nitrogen isotope data for tadpole, frog, and fish from Finlay and Vredenburg (2007). Based on the Humphreys Basin lakes, trophic fractionation of nitrogen for fish-containing and fish-removed lakes were 2.03‰ ± 0.52 (n=116) and 1.76‰ ± 0.38 (n=186) respectively, which are lower than the generally accepted value of 3.4 proposed by Minagawa and Wada (1984). The $\delta^{15}N$ baseline used in both lakes included seston and terrestrial inputs. The baseline $\delta^{15}N$ was 0.15 ‰ for fish-containing lakes and -0.34‰ for fish-removed lakes.

The mean trophic fractionation factors for both fish-containing and fish-removed lakes were then used in equation 2.1 to determine each species' trophic position (Figure 2.4a&b). A species trophic position is based primarily on feeding habits. For example, primary producers are assigned a trophic level (TL) value of 1; primary consumers (herbivores) a TL value of 2; secondary consumers (predators) a TL value of 3; and tertiary consumers (predators like trout and fish) a TL value of 4. For each type of lake, the average TP shift from species to species was minimal for both fish containing and fish-removed lake. However, a significant increase occurs from *Agabus tristis* with a TP of 5.2 to a TP of 7.1 for *R. muscosa* in fish-removed lakes and from a TP of 5.0 for *S. occidens* to a TP of 7.1 for *O. mykiss* (Figure 2.4a&b).

Fish and Phytoplankton Analysis

Gut analysis of fish caught from lakes in July 2010 showed a diet of predominantly chironomid larvae and pupae while a fish from Mesa Lake appeared to have remains of a dytiscid in the stomach. Examinations of the stomachs of the two frogs were inconclusive while the stomach of the tadpole contained detritus and plant material.

Analysis of algal/phytoplankton community from Square Lake indicated low densities of algae with few diatoms - primarily genus *Pinnularia* and *Aulacoseria*. Marmot Lake also had extremely low phytoplankton densities and was dominated by an armored dinoflagellate *Peridinium* and the cyanobacterium *Microcystis*. The phytoplankton population analysis in Mesa Lake was dominated by 3 species of *Aulacoseria*. *Dinobryon* (gold algae) and a filamentous alga *Zygnema* (green) were also identified. Summit Lake had low to medium algal densities but a high diversity of diatoms (*Epithemia sorex*, *Synedra ulna*, and 3 species of *Navicula*) and the cyanobacterium *Anabaena.*

Species Isotopic Shifts over Time

For each species for which I had a minimum of three samples between 1997 and 2010, the $\delta^{13}C$ and $\delta^{15}N$ values in each lake were plotted against year to determine if any significant shifts in $\delta^{13}C$ or $\delta^{15}N$ could be discerned over time either as a result of fish removal or other causes (Figures 2.5a - 2.5d). In all lakes, patterns in $\delta^{13}C$ of nearly all the species mirrored patterns in $\delta^{15}N$ values and

show that food source isotope values can fluctuate with energy influxes into the lakes. For both of the fish-containing lakes, monotonic shifts in isotope values, if present, were small and could be the result of the incompleteness of the record. Some larger, short-term shifts in $\delta^{13}C$ and $\delta^{15}N$ were observed for Chironomidae and Pisidium in fish-containing lakes, but the underlying causes are hard to identify.

However, I did observe noteworthy trends in $\delta^{13}C$ and $\delta^{15}N$ in the fish-removed lakes that appear to be related to fish removal and recovery of extirpated species. Natural return of *D. melanica* and artificial reintroduction of *H. shoshone* resulted in significant depletion in $\delta^{15}N$ for *L. signicauda* in Square lake and to a lesser degree in Marmot Lake. In Square Lake, from 2001, when *D. melanica* returned, to 2004, $\delta^{15}N$ values for *L. signicauda* decreased by 4.42‰. Co-occurring depletion in $\delta^{13}C$ in *L. signicauda* was also evident, but the changes were relatively small. Even with an increase in $\delta^{15}N$ of 1.31‰ for *L. signicauda* from 2004-2005 in Square Lake, $\delta^{15}N$ values remained significantly lower than prior to recovery of the larger zooplankton (Figure 2.6).

In Square Lake, the $\delta^{15}N$ values of *D. melanica*, *Dicosmoecus atripes* and *Polycentropus variegatus* decreased markedly starting in 2001 which coincided with the patterns observed for *L. signicauda* . In Marmot Lake, a depletion of 1.42‰ in $\delta^{15}N$ occurred for *L. signicauda* after fish removal has completed in 1998 (Figure 2.6b). However, unlike Square Lake, after return of *D. melanica* and reintroduction of *H. shoshone*, $\delta^{15}N$ values for *L. signicauda* in Marmot Lake

showed no significant long-term trend. Except for decline in $\delta^{15}N$ observed for *D. atripes*, isotope data for other species in Marmot Lakes showed little variability over time.

Discussion

Foodweb Structure in Sierra Nevada Lakes

The concept of food webs is based on observed interactions between organisms within a given system. Food chains, food webs, and the concept of a "pyramid of numbers" are ways to represent the structure of an ecosystem based on feeding relationships and trophic position on a community level (Elton 1927, Vander Zanden and Rasmussen, 1999). Species or populations are assigned to one of several distinct trophic levels (1, 2, 3... etc.). One problem with these concepts however is they oversimplify variability in an ecosystem.

Ecosystems are very complex and dynamic and placing organisms or species into 3 or more distinct levels, while adequate, is not complete. Most feeding relationships are inferred by observations, but it is often difficult to observe all possible interactions in a given system. Simple food webs do not take into consideration biogeochemical cycling, changes in interannual species diet, or introduction of non-native species. Applying these concepts with the previously mentioned nitrogen fractionation average of 3.4‰ per trophic level determined by Minagawa and Wada (1984) and equation 2.1 allows for a more quantitative analysis of feeding relationships in Sierra Nevada lakes. For example both

zooplankton species *L. signicauda* and *H. shoshone* can be considered primary consumers and be placed at trophic level 2. However, *H. shoshone* is known to be more predaceous so they would have a higher overall trophic position than L. signicauda, such as a TP of 3.1 for *H. shoshone* and a TP of 2.9 for *L. signicauda* as shown in Figure 2.4. This methodology can be the first step to observing who eats whom.

While a $\delta^{15}N$ trophic fractionation of 3.4‰ is a good starting point to use for food web studies, recent research indicates that actual trophic fractionation may be unique to a given system (Vander Zanden and Rasmussen 1999, Post 2002, McCutchan et al. 2003, Vandeklift and Ponsard 2003). Trophic fractionation for $\delta^{15}N$ can also be smaller or larger than 3.4‰ depending how the samples are processed (McCutchan et al. 2003, Finlay and Vrendenburg 2007). Along with acidification of samples to remove inorganic carbonates, analysis of a whole organism compared to just analyzing white muscle or organs of the same animal all tend to produce variability in $\delta^{15}N$ isotopic values (Pinnegar and Polunin 1999, Vander Zanden and Rasmussen 2001).

The original analysis conducted by Minagawa and Wada (1984) to obtain the 3.4‰ was limited in scope (n=27) and the only aquatic organisms included were phytoplankton, zooplankton and fish. A meta-analysis from 32 published articles consisting of 134 samples of mammals, birds, insects, fishes, crustacean, and molluscs resulted in an overall $\delta^{15}N$ of 2.54 ± 0.11‰ (Vanderklift and Ponsard (2003). Additionally, McCutchan et al. 2003 reviewed published studies

and compiled 83 samples of consumers fed controlled diets. The overall $\delta^{15}N$ fractionation determined from their analysis was 2.30‰ ± 0.18. Both of these studies were much broader-based than the Minagawa and Wada (1984) and they provide insight into how $\delta^{15}N$ isotopic variability arise from variation in forms of N excretion, differences in diets, different taxa, sample preparation, environment, and the use of different tissues and organs when sampling (McCutchan et al. 2003, Vanderklift and Ponsard 2003). It was clear that I needed to determine the trophic fractionation for each type of our lakes. If I were to have applied the $\delta^{15}N$ value of 3.4‰ for our study, most species defined as primary consumers or primary predators would then be placed at the same level and based on their known feeding habits, this would not be realistic or accurate.

Prior to determining the trophic fractionation in my lakes, I wanted to verify that our N isotope data for tadpoles, frogs and fish were similar to other research since I had very few data points for these species. To confirm my isotope results, I compared my data to research conducted by Finlay and Vrendenburg (2007) in Sixty Lake Basin approximately 30 miles south of Humphreys Basin. Due to concerns over low populations of *R. muscosa*, Finlay and Vredenburg obtained non-lethal toe clippings from *R. muscosa* as well muscle tissue from a related and abundant species of bullfrogs (*Rana catesbeina*)(n=51), They also obtained dorsal muscle tissue from two trout species: *O. mykiss* and *O. aquabonita* (n=22) and *R. muscosa* tadpoles (n=32). T-tests were run to determine if my frog, tadpole, and fish nitrogen isotope data were similar to that reported by Finlay and

Vrendenburg (2007) and they showed there was no significant difference for either tadpoles (p=0.27) or frogs (p=0.06), however there was a significant difference between $\delta^{15}N$ in the two data sets for fish ($p < 0.001$). However, since different parts of an organism metabolizes at different rates, I can assume the isotopic differences for the fish samples are a result of different metabolic fractionation rates and not from sampling error.

Integrating the two data sets, I obtained trophic fractionation of 2.03‰ ± 0.52 (n=132) for fish-containing and 1.78‰ ± 0.38 (n=264) for fish-removed lakes. These values are time-integrated means of each species' $\delta^{15}N$ over 13 years and includes mean $\delta^{15}N$ data for tadpoles, frogs, and fish from Finlay and Vredenburg (2007). Although these values are slightly lower than those previously reported in Vanderklift and Ponsard (2003) and McCutchan et al. (2003) and significantly lower than the often used 3.4‰ proposed by Minagawa and Wada (1984), I am confident that our data is representative of the overall nitrogen trophic fractionation in foodwebs of Sierra Nevada lakes.

One complication with my trophic fractionation estimates is the manner in which I prepared samples of fish and frog tissues for isotope analysis. While a smaller organism can be used whole, larger organisms cannot. Since I only sampled the muscle tissue of fish and frogs, their isotopes values might be slightly biased. Turnover rates of dietary nitrogen occur over different time periods in different tissues such as muscle or organs (Sweeting et al. 2004). As such, using one and not the other can induce a bias when trying to calculate

mean trophic fractionation. Using multiple tissue samples from the same animal would allow for a more accurate $\delta^{15}N$ value for the animal. However I feel that due to the number of overall samples I obtained, the impact in overall mean fractionation for each type of lake would be insignificant.

Combining the traditional food web concepts and my trophic fractionation estimates and isotope data, I propose there are four trophic levels for each type of lake. In both types of lakes, fish and frogs represent the tertiary consumer or TL 4. When equation 2.1 is applied to the $\delta^{15}N$ values, each species unique trophic "niche" or position can be calculated. Although nitrogen isotope values are enriched for fish relative to frogs, the larger trophic fractionation in fish-containing lakes results in a lower trophic position of 7.1 for the fish vs. 7.3 for the frogs in fish-removed lakes. The slightly larger TP for frogs results from the lower fractionation factor for fish-removed lakes when calculating using equation 2.1. The abrupt increase in TP observed for frog and fish relative to *A. tristis* and *S. occidens* in Figure 2.4 suggests that both frogs and fish may obtain part of their diets from terrestrial sources or I have missed an important aquatic food source.

Another complication in my food web analysis is that the Chironomidae were only identified to family level and Oligochaeta were only identified to subclass level. Because of this, $\delta^{15}N$ and $\delta^{13}C$ values for both Chironomidae and Oligochaeta were somewhat higher than anticipated. Both types of consumers were on the same level as predaceous beetles for both types of lakes. Because

Oligochaeta live at the water/soil interface, their isotope values may be a result of normal feeding on enriched substrate material. Isotope results for Chironomidae larvae for both lakes indicate they most likely were opportunistic omnivores, feeding on small plants and animals as well as detritus (Merritt et al. 2008). This makes sense, as the feeding habits of chironomid species are quite diverse.

General Effects of Non-Native Fish on Lake Foodwebs

Stocking of non-native fish is not unique to Sierra Nevada alpine aquatic ecosystem nor are the consequences. Fish stocking and subsequent species loss have occurred throughout North America and Western Europe (Persson et al. 1993, Mittelbach et al. 1995, Elser et al. 1995, Winder et al. 2003, Hartel et al. 2007, Schabetsberger et al. 2009). Alpine lakes are: 1) highly oligotrophic, 2) species poor, 3) cold (<20°C), and 4) have short growing seasons (3-4 months) and therefore have low productivity (Stoddard 1987, Knapp at al. 2001b, Epanchin et al. 2010, Parker and Schindler 2006). The loss of species and alteration of food webs not only impacts the nutrient cycling and food webs within lakes, but can also reduce biological links to terrestrial ecosystems (Epanchin et al. 2010, Eby et al. 2006). Research in Humphreys Basin by Epanchin et al. (2010) shows that loss of mayflies (*Callibaetis ferrugineus* and *Ameletus edmundsi*) may be a result of non–native fish stocking. Epanchin et al. also suggest that the Rosy-Finch (*Leucosticte tephrocotis dawsoni*), is dependent on the mayflies as a primary source of food and they found that finch populations

were 6-times larger at lakes without fish due to the importance of adult mayfly as a food source for finches. Matthews et al. (2002) also postulate that declines in garter snakes numbers(*Thamnophis elegans*) are due to predation by salmonids on *Rana muscosa*.

Analysis of stable isotope distributions of foodwebs of fish-containing versus fish-removed lakes can be used to elucidate the impacts that fish stocking has had on Sierra Nevada lake ecosystems. I determined there were 3 types of foodweb impacts as a result of fish stocking; 1) there are distinct differences in $\delta^{15}N$ isotopic composition values between fish-containing and fish-removed lakes, 2) removal of fish results in an overall depletion in $\delta^{15}N$ values for most species and slight enrichment in $\delta^{13}C$ in other species, 3) fish and frogs occupy the same trophic position, tertiary predator, in their respective lakes.

In the Humphreys Basin, removal of fish from lakes resulted in generally depleted $\delta^{15}N$ and slightly enriched $\delta^{13}C$ isotope values for most species common to both lakes. Changes in isotopic values were greater for nitrogen as compared to carbon as removal of fish allowed for recovery of 7 species that were lost as a result of fish stocking. The change in carbon energy pathways appears less impacted from fish stocking and fish removal produces less significant changes in $\delta^{13}C$ in food webs. Similar isotopic shifts have been observed in other aquatic ecosystems when species are added or removed. Good examples include introductions of primary consumers such as *Dreissena polymorpha* (zebra mussel) and *Cercopagis pengoi* (cladoceran) (Schimdt et al.

2009, Gorokhova et al. 2005) to lakes. Removal of non-native trout from alpine lakes in the Canadian Rockies resulted in the recovery of several zooplankton species (Knapp et al. 2001, Donald et al. 2001, Vredenburg 2004, Finlay and Vredenburg 2007). Isotopic analysis also indicates removal of invasive fish resulted in lower $\delta^{15}N$ values for copepods and cladocerans (Mitchell et al. 1996, McNaught 1999, Gorokhova et al. 2005, Schimdt et al. 2009).

Removal of non-native fish from naturally fishless lakes in Sixty-Lake Basin (approximately 35 miles south of Humphreys Basin) resulted in a strong recovery of *R. muscosa* in a majority of lakes. Early research by Vredenburg (2004) indicated larval stages of amphibians are vulnerable to predation by fish and therefore this was assumed to be the main factor as to why fish and frogs could not co-occur in small alpine lakes. However, using stable isotope analysis and a mixing model, Finlay and Vredenburg (2007) were able to show the diets of *R. muscosa* and trout coincide strongly even though they occupy different parts of the lakes. Fish feed mainly in the pelagic zone while frogs feed on the periphery of a lake. With limited resources due to low lake productivity and abundant frog offspring, the fish not only eat tadpoles but they can out-compete frogs for food. The trophic positions estimated for fish and frogs in my study also closely align and support the results of Finlay and Vredenburg.

Recovery of Extirpated Species and Their Affects on Lake Foodwebs

Introduced trout have strong effects on zooplankton communities in Sierra Nevada lakes. However, three of the six commonly occurring zooplankton taxa species are generally <1.0 mm and therefore are not a primary food source for trout and they can coexist with introduced trout (Knapp et al. 2001). It is interesting then, that removal of trout from Square had significant effects on isotopic values of several benthic species as well as the smaller zooplankton species *L. signicauda*. Prior to fish removal, *L. signicauda* was the dominant zooplankton species in all four lakes (Knapp et al. 2001). The shift in $\delta^{15}N$ for *L. signicauda* in Square Lake correlates well with the removal of fish from the lake and natural return of *D. melanica.* The initial $\delta^{15}N$ depletion in 1999-2001 after fish were removed and slight subsequent enrichment in 2002 could be the result of season to season variability. The first observations of strong decline of $\delta^{15}N$ for *L. signicauda* coincided with the return of *D. melanica* in 1999 when their numbers increased from approximately 5,000 individuals/m^3 with significant to 20-25,000 individuals /m^3 in 2000-2001 (Sarnelle and Knapp 2004)(Figure 2.6).

Depletion in $\delta^{15}N$ values was not limited to L. signicauda in Square Lake. Lower $\delta^{15}N$ values were also observed for *Dicosmoecus atripes*, *Polycentropus variegatus,* and *D. melanica* (Figure 2.5a panels a, b, and h). Although both *D. atripes* and *P. variegatus* are from different families (Limnephilidae and Polycentropodidae respectively), they have similar diets. Each species molt through 4-5 instar stages before pupation into adult forms. Food sources for early

instars 1-2 are primarily comprised of detritus (25-35%) and diatoms (60-75%) with very minimal uptake of algae (<10%) and animal material (<5%). With later instar stages 3-5, uptake of algae (<1%) and diatoms (5-20%) significantly decrease while uptake of animal and detritus increases (Gotceitas and Clifford 1982, Merritt et al. 2008). Even in late instar stages, particularly for *D. atripes*, 50-80% of dietary needs are primarily from detritus with the rest coming from animal material (Gotceitas and Clifford 1982, Merritt et al. 2008). I speculate that my samples contained younger instars that relied heavily on diatoms as a food source.

With the reemergence of *D. melanica* in Square Lake (Figure 2.6), competition for food among all zooplankton likely increased. I hypothesize that *L. signicauda* and *D. melanica* responded by altering their diets. Because *L. signicauda* made up nearly the entire zooplankton biomass while fish were present, they did not have strong competition for food resources and as such were able to be less selective in their diet and to eat higher on the foodweb. Once *D. melanica* reappeared, *L. signicauda* now had to compete for specific food sources due to competition with *D. melanica*. Most freshwater Cladocera are generally 0.2 and 3.0 mm in size with *Daphnia* being on the larger side: 1.0 to 3.0 mm long, while copepods range in size from 250 µm to 4 mm long with *L. signicauda* generally <1.0mm (Smith 2001, Thorp and Covich 2010). The preferred diet of cladocerans consists of small algae or diatoms generally 1.0-25 µm, but they will also consume bacteria less than 1.0 µm, ciliate algae, small

rotifers, and copepod nauplii up to 100 μm (Smith 2001, Thorp and Covich 2010). Copepod diet is similar to cladoceran diet with algae, bacteria, rotifers, and detritus, and nauplii comprising most of their food sources but they will also feed on dipteran larvae such as Culicidae and Chironomidae (Thorp and Covich, 2010). Particle food size for *L. signicauda* usually ranges from a few microns for small algae and bacteria to 1.0 mm for macrozooplankton, although due to *L. signicauda's* size, ingested particles would most likely be limited to 100-150 μm (Thorp and Covich 2010)

Removal of trout from Marmot Lake also resulted in a slight depletion of $\delta^{15}N$ in *L. signicauda* in 1999. Reemergence of *D. melanica* also occurred in Marmot Lake in 1999 but their numbers remained below 5,000 individuals/m^3 throughout the sampling period (Sarnelle and Knapp 2004). I hypothesize the higher densities of *D. melanica* in Square Lake compared to Marmot Lake were the driving force behind the pronounced $\delta^{15}N$ depletion in *L. signicauda* in Square Lake.

While some argument could be made that *H. shoshone* aided in the depletion of $\delta^{15}N$ values for *L. signicauda*, the fact is, depletion in $\delta^{15}N$ values began in 2001 before *H. shoshone* was reintroduced manually into Marmot and Square Lakes during 2003 (Knapp and Sarnelle 2008). After reintroduction, *H. Shoshone* densities ranged from 0.01 individuals/m^3 to 10 individual/m^3 and were likely too low to have impacted the behavior of *D. melanica* or *L. signicauda*. Except for a slight enrichment of approximately 1.0‰ in $\delta^{15}N$ values for *L.*

signicauda in 2004, nitrogen isotope values in Marmot Lake remained similar to those measured for 2003 prior to *H. shoshone* introduction.

I investigate several other possible factors to explain the $\delta^{15}N$ trends observed for *D. melanica* and *L. signicauda* in Square Lake. These factors included changes in phytoplankton communities, variations in the seasonal snowpack and changes in the timing of lake sampling. One scenario I considered was that the removal of trout and reemergence of *D. melanica* could have altered the phytoplankton community structure in Square Lake and favored an increase in N-fixing blue-green algae. However, personal communications with Roland Knapp and Orlando Sarnelle indicate the types of blue-green algae that may cause changes in C-N isotopic signatures are not found in Sierran lakes and if they were, they are not a preferred diet for *D. melanica*.

A Pearson Product Moment Correlation analysis was also used to see if the variability in $\delta^{13}C$ or $\delta^{15}N$ for foodweb organisms in Square and Marmot lakes might be a result of changes in seasonal snow depth. For Square Lake there was a significant relationship between $\delta^{15}N$ and snow depth for *S. striatellus* ($n = 12$, $r = -0.561$, $p < 0.057$). For Marmot Lake there was a significant relationship for *L. signicauda* for both $\delta^{13}C$ ($n=12$, $r = 0.600$, $p < 0.04$) and $\delta^{15}N$ ($n=12$, $r = 0.652$, $p < 0.02$) and snow depth. However, there were not enough data points for any other species to draw any definitive conclusions indicating snow pack as a primary driver for the shifts in isotopic values observed in the fish-removed lakes.

To determine if sampling in early season compared to late season resulted in the in $\delta^{15}N$ isotope trends observed from both fish-removed Marmot and Square lakes, additional samples for *L. signicauda* and *S. striatellus* in years where different sampling dates were available were also analyzed. For both lakes $\delta^{15}N$ values for *L. signicauda* in Marmot Lake varied from 0.07‰ to 0.40‰ and 0.29‰ to 0.84‰ in Square Lake. Minimal variability in Marmot Lake occurred in 1997 with sampling occurring 22 days apart and the largest variability occurring in 2003 with 28 days between samples. The smallest variability for Square Lake also occurred in 1997 with sampling occurring 33 days apart and the large variability of 0.84‰ in Square Lake occurred in 2003 with sample times 22 days apart. There was no pattern to the shifts in isotope values. Some early season samples were depleted relative to late season samples and other early season samples were enriched compared to late season samples. I speculate the largest change in isotope values in 2003 for Square Lake may be the recovery of sufficient densities of *D. melanica* from their reemergence in 2001. *S. striatellus* also exhibited slight variability in $\delta^{15}N$ values. For 1997, variability was 0.43‰ with sampling occurring 22 days apart. In 2000, the variability was 0.17‰ over a sampling interval of 17 days. Overall, these data suggest that the variations in the timing of collection of foodweb organisms could not produce the strong temporal changes in $\delta^{15}N$ seen at Square Lake.

The reemergence of *Daphnia* into Square Lake in such abundant numbers coupled with their ability to filter large volumes of water per day really gives them

a competitive advantage over *L. signicauda* as well as several benthic species when it comes to selecting preferred dietary needs. Depending on *Daphnia* size, each individual can process up to 4ml/hr and a healthy population can filter a significant volume of algae from a lake every day (Porter et al. 1982, Porter 1977). The result is *L. signicauda, D. atripes,* and *P. varigaetus* having to be more selective in their feeding habits and thus having to feed "lower" in the food web based on the depletion of $\delta^{15}N$ values.

Impacts of Fish on Lake Nutrient Cycling

In recent years, a great deal of emphasis has been placed on fish introductions and their impacts on nutrient cycling (Brabrand et al. 1989, Schindler et al. 2001). In oligotrophic alpine lakes, nitrogen (N) and phosphorous (P) tend to be key limiting nutrients (Schindler 1977, Stoddard 1987, Vitousek et al. 2010, Carey and Rydin 2011). Stocking of fish into fishless lakes can alter nutrient cycling, and increase overall lake productivity by introducing a previously unavailable source of P (Schindler et al. 2001, Carpenter et al. 1992). The increase in diversity of algae and phytoplankton species in fish-containing lakes compared to fish-removed lakes for our research supports this position. Fish not only provide P for algae and phytoplankton through excretion but to benthic and pelagic organisms when they die and decay on the lake floor (Carpenter et al. 1992). Fish predation on benthic and terrestrial organisms also makes available nutrients previously unavailable to algae and phytoplankton (Schindler et al.

2001). Bioturbation by Chironomidae larvae and fish on the sediment-water interface can also increase available P into the overlying water column (Wetzel 2001).

As a result of increased P availability after fish introduction, nitrogen now becomes the more limiting nutrient for primary producers in Sierra Nevada lakes. I hypothesize that increased P availability increases N limitation in phytoplankton and thereby reduces discrimination against ^{15}N during algal assimilation of inorganic N resulting in higher $\delta^{15}N$ values in phytoplankton that cascades up through the foodweb. When species found in both types of lakes are compared, $\delta^{15}N$ values for nearly all species are higher in fish-containing lakes relative to fish-removed lakes. My time-series analysis also showed that benthic invertebrates, sediments, phytoplankton, and the zooplankton species *L. signicauda* showed significant increases in $\delta^{15}N$ values in fish-removed lakes with changes ranging from 0.92‰ for *P. casertanum* to 1.58‰ for *D. atripes*. The only decrease in $\delta^{15}N$ values was observed in Chironomidae at 0.18‰ and no basically no change was observed in *S. striatellus* at -0.01‰. The changes in Chironomidae falls in the range of what I see in general variability in a species from year to year. The depletion in Chironomidae may also be a result that Chironomidae was only identified to family and not to genus and species.

Conclusion

Stocking of fish in naturally fishless lakes has a significant impact on all the organisms not only those lost through predation but by those that remain as well. Through isotopic analysis, I was able to show that once fish were removed, the entire lake foodweb was altered as evidenced by enrichment of $\delta^{15}N$ values in nearly all species. While removal of fish was the ultimate cause of the isotopic shifts, the proximal cause of the substantial changes in $\delta^{15}N$ in Square Lake was the reemergence of *Daphnia melanica* following fish removal. My research also showed that trout and R. muscosa rely on the same food to survive and that extirpation of these frogs occurs via fish predation and competition with fish for food. The need to remove fish from lakes is paramount. Not only is *R. muscosa'*s habitat dwindling but also the emergence of chytridiomycete fungus (*Batrachochytrium dendrobatidis) has resulted in a large decline in overall amphibians numbers in remaining frog habitats (Wake and Vrendenburg 2008, Vrendenburg et al. 2010).*

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Table 2.1. Species list for the two fish-containing lakes: Summit and Mesa and the two fish-removed lakes: Marmot and Square. Species absent from the fish lakes are a result of fish stocking.

Taxon	Fish-removed Lakes	Fish-containing Lakes
Rana muscosa	X	
Oncorhynchus mykiss aguabonita		X
Callibaetis ferrugineus	X	
Cenocorixa kuiterti	X	
Agabus tristis	X	
Stictotartsus striatellus	X	X
Sanfilippodytes terminalis	X	
Desmona mono	X	X
Dicosmoescus atripes	X	X
Polycentropus variegatus	X	X
Sialis occidens	X	X
Chironomidae *sp.*	X	X
Pisidium casertanum	X	X
Acari *sp*.	X	X
Oligochaeta *sp*.	X	X
Hesperodiaptomus shoshone	X	
Leptodiaptomus signicauda	X	X
Daphnia melanica	X	

Table 2.2 Physical data for all four lakes used for this study.

Lake Name	Lake Area m^2	Lake Area (ha)	Lake Perimeter (m)	Shoreline development	Elevation (m)	Max Depth (m)	Mean depth (m)	Avg temp (°C)
MARMOT LAKE	30659	3.06	833	1.34	3583	7.8	3.6	13.96
MESA LAKE	118915	11.89	1484	1.21	3444	5.8	3.0	15.20
SQUARE LAKE	13765	1.38	488	1.17	3444	3.5	1.9	15.68
SUMMIT LAKE	31599	3.16	776	1.23	3420	3.0	1.5	14.76

Temperature data is averaged from 1997, 2000, 2002, 2004, and 2005 between 7/9 and 8/23 each year.

Table 2.3a. Nitrogen and carbon isotope results for the Humphrey's Basin samples for the years 1997-2007 and 2010. Fish-containing lakes are Mesa and Summit and fish-removed lakes are Marmot and Square. Results are stated as the mean with standard error with n as the number of samples.

Fish-removed Lakes

Family	Genus	n	$\delta^{15}N$	$\delta^{13}C$
Ranidae	*Rana muscosa*	8	5.53 ± 0.05	-18.13 ± 0.11
Ranidae	*Rana muscosa (tadpole)*	2	2.89 ± 0.60	-15.90 ± 0.00
Limnephilidae	*Dicosmoecus atripes*	15	-0.47 ± 0.39	-21.80 ± 1.05
Limnephilidae	*Desmona mono*	3	-0.96 ± 0.28	-22.56 ± 3.24
Limnephilidae	*Polycentropus variegatus*	6	2.45 ± 0.89	-24.37 ± 1.11
Sialidae	*Sialis occidens*	5	3.06 ± 0.89	-18.94 ± 0.43
Baetidae	*Callibaetis ferrugineus*	7	1.85 ± 0.19	-19.00 ± 0.77
Corixidae	*Cenocorixa kuiterti*	6	2.62 ± 0.53	-18.79 ± 0.68
Pisidiidae	*Pisidium casertanum*	5	0.41 ± 0.63	-15.34 ± 0.36
Dytiscidae	*Agabus tristis*	11	3.54 ± 0.15	-18.81 ± 0.33
Dytiscidae	*Stictotartsus striatellus*	24	2.92 ± 0.18	-18.67 ± 0.27
Dytiscidae	*Sanfilippodytes terminalis*	4	2.53 ± 0.26	-17.73 ± 0.36
Chironomidae	Many	11	3.13 ± 0.46	-19.08 ± 0.61
Acari	Many	2	3.76 ± 0.68	-21.73 ± 1.75
Oligochaeta	Many	7	1.95 ± 0.22	-18.83 ± 0.68
Diaptomidae	*Hesperodiaptomus shoshone*	5	1.68 ± 0.42	-27.69 ± 0.71
Diaptomidae	*Leptodiaptomus signicauda*	37	1.20 ± 0.23	-28.12 ± 0.22
Daphniidae	*Daphniidae melanica*	9	-0.70 ±0.69	-32.88 ± 1.11
Seston	N/A	2	0.95 ±0.52	-24.83 ± 0.46
Sediments	N/A	2	1.43 ±0.62	-20.55 ± 1.22
Terrestrial	N/A		-1.62 ± 1.29	-28.00

Table 2.3b. Nitrogen and carbon isotope results for the Humphrey's Basin samples for the years 1997-2007 and 2010. Fish-containing lakes, Mesa and Summit and fish-removed lakes, Marmot and Square. Results are stated as the mean with standard error with n as the number of samples.

Fish-containing Lakes

Family	Genus	n	$\delta^{15}N$ ‰	$\delta^{13}C$ ‰
Salmonidae	*Oncorhynchus mykiss aguabonita*	7	7.06 ± 0.09	-18.18 ± 0.30
Limnephilidae	*Dicosmoecus atripes*	6	1.11 ± 0.78	-24.44 ± 0.19
Limnephilidae	*Polycentropus variegatus*	4	3.88 ± 0.44	-21.51 ± 0.70
Sialidae	*Sialis occidens*	10	4.13 ± 0.39	-18.72 ± 0.36
Pisidiidae	*Pisidium casertanum*	10	1.33 ± 0.12	-17.98 ± 1.07
Dytiscidae	*Sanfilippodytes terminalis*	1	3.84 ± 0.00	-25.28 ± 0.00
Dytiscidae	*Stictotartsus striatellus*	17	2.93 ± 0.16	-18.95 ± 0.22
Chironomidae	Many	18	2.95 ± 0.31	-20.29 ± 0.33
Oligochaeta	Many	12	2.36 ± 0.20	-20.45 ±0.47
Diaptomidae	*Leptodiaptomus signicauda*	17	2.51 ± 0.20	-28.10 ± 0.47
Acari	Many	6	3.73 ± 0.41	-20.97 ± 0.54
Seston	N/A	2	1.92 ± 0.68	-25.25 ± 0.55
Sediments	N/A	2	2.47 ± 0.18	-22.03 ± 0.17
Terrestrial	N/A		-1.62 ± 1.29	-28.00

Figure 2.1 Location map for Humphrey's Basin and selected research lakes located in the Eastern Sierra Nevada range of California.

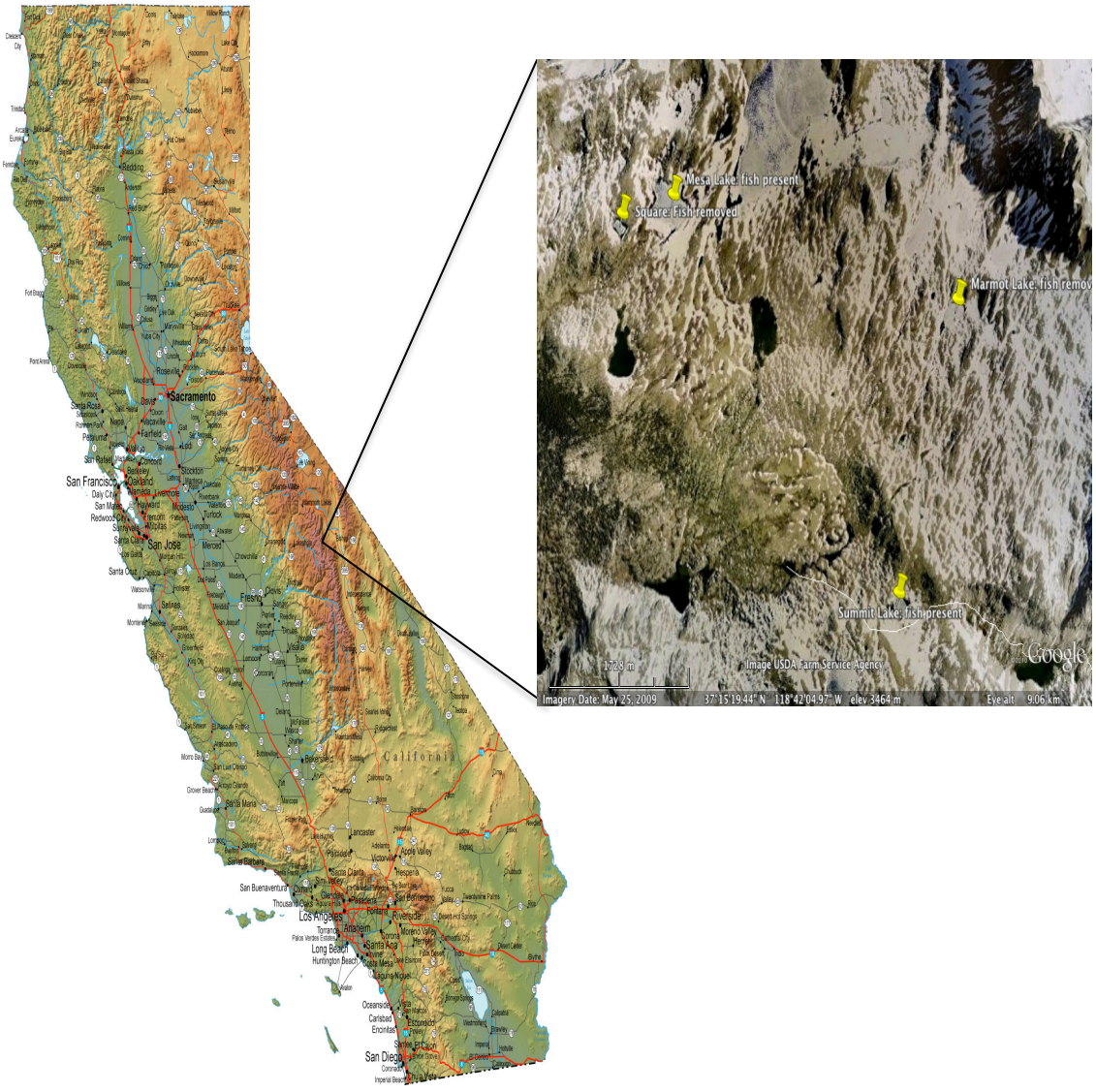
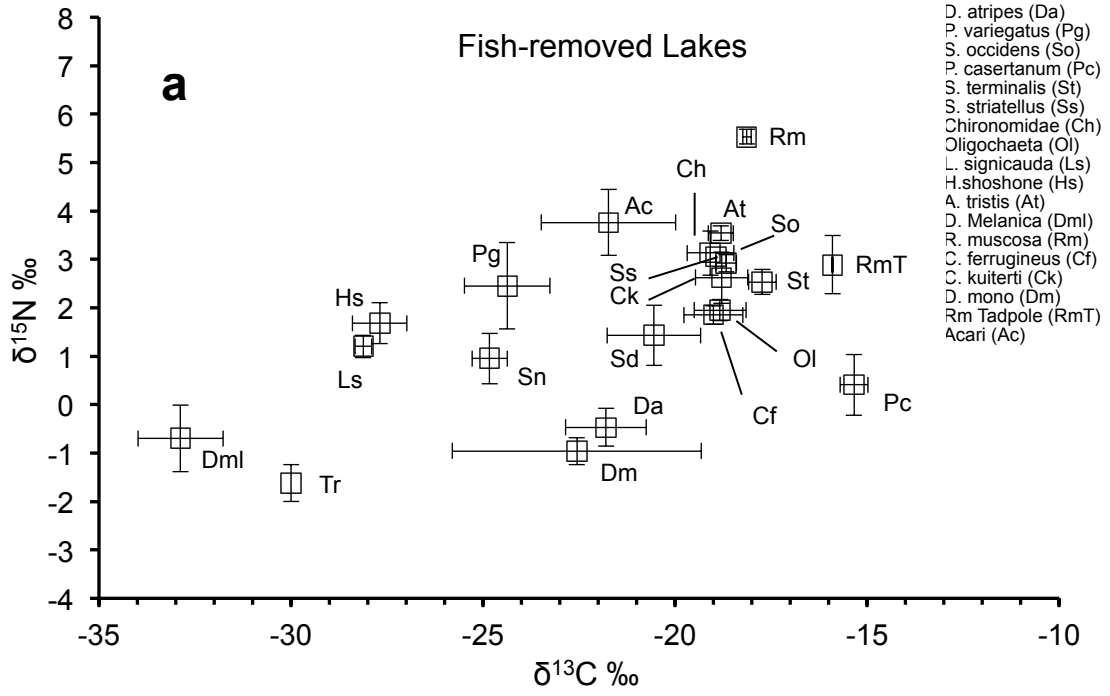


Figure 2.2a & 2.2b Dual isotope bi-plots of mean $\delta^{15}N$ and $\delta^{13}C$ values for individual species in both fish-removed (Square & Marmot) and fish-containing (Summit & Mesa) lakes in Humphreys Basin.



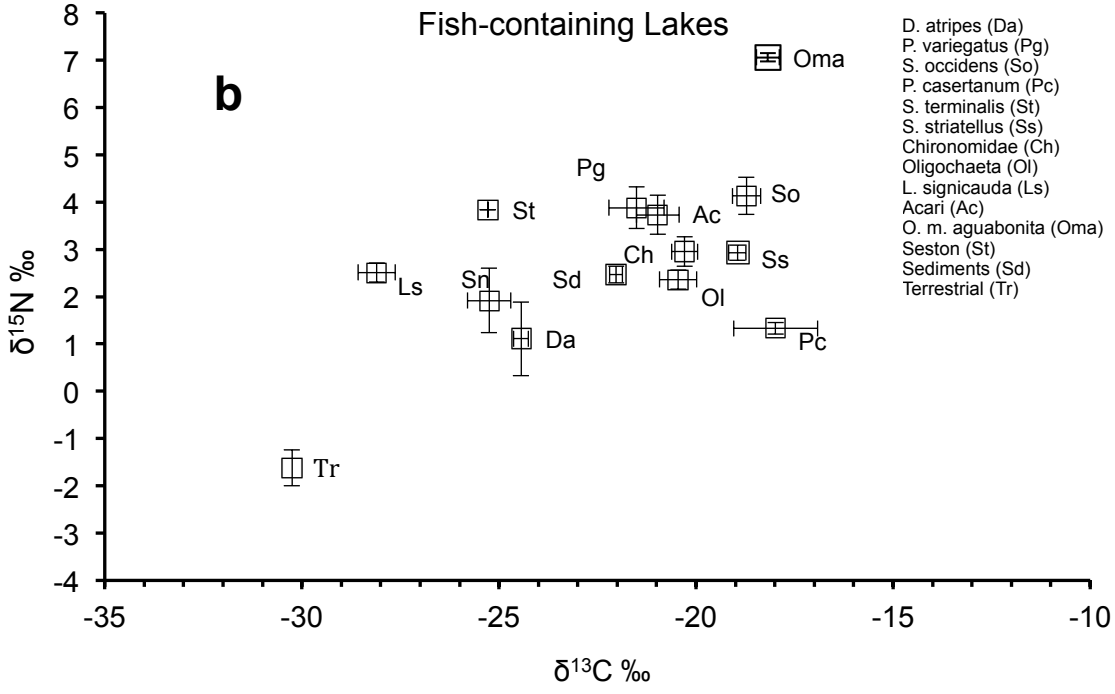


Figure 2.3. Bi-plot illustrating feeding relationship and energy pathways for species combined into similar groups based on feeding habits for fish-containing (Summit & Mesa) and fish-removed (Square & Marmot) lakes. Data includes sample mean isotope values from 1997-2010 represented in Table 2.3a.

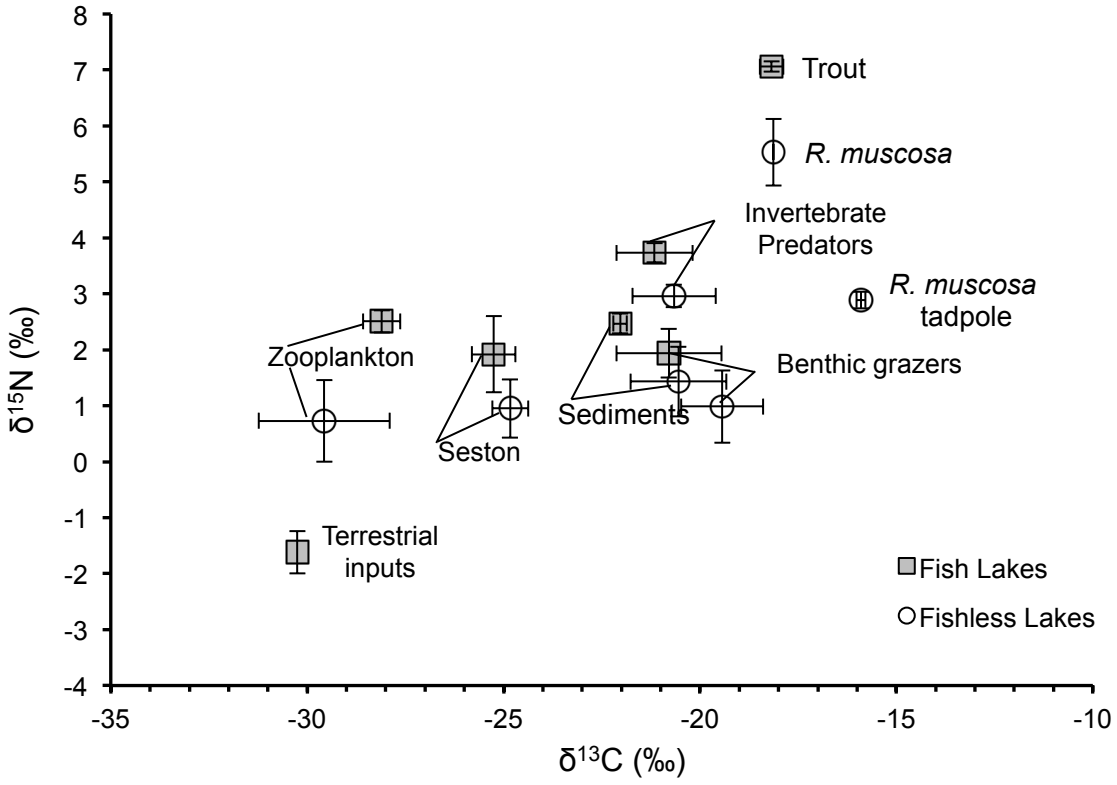


Figure 2.4a & 2.4b. Calculated trophic position for each species in both fish-removed (2.4a) and fish-containing (2.4b) lakes using the trophic fractionation for each type of lake.

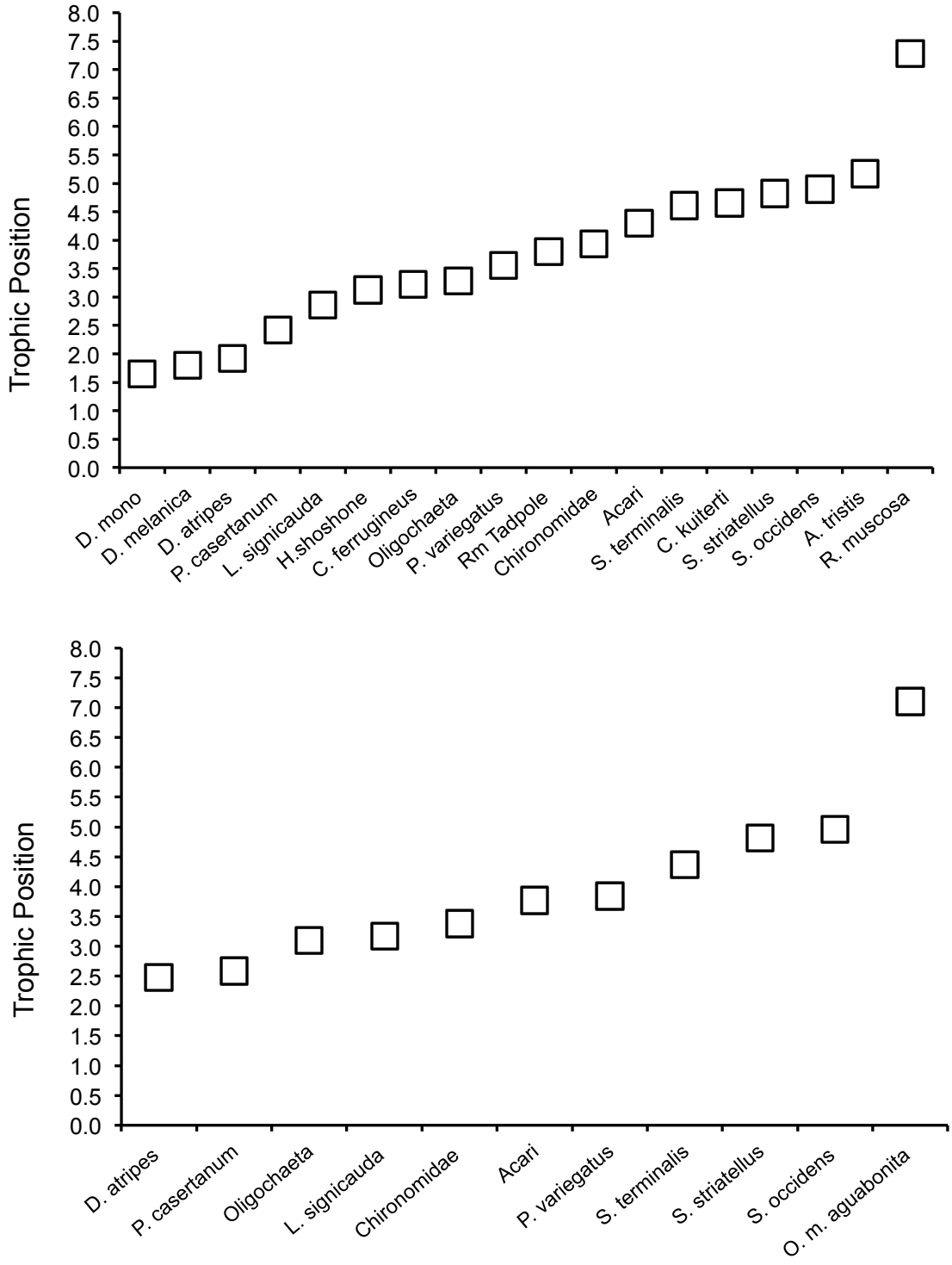
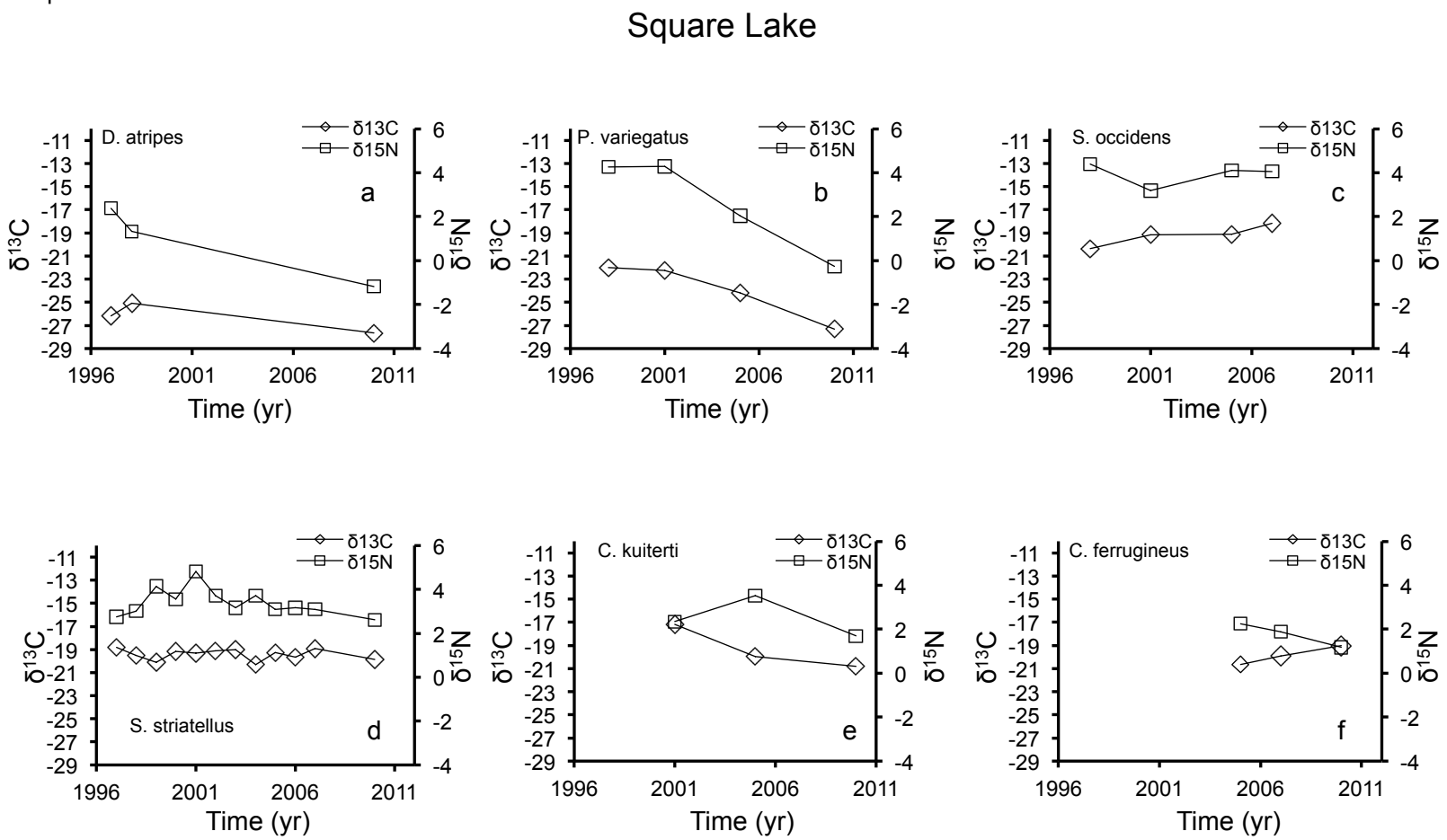


Figure 2.5a Bi-plots of mean $\delta^{15}N$ and $\delta^{13}C$ values versus time for each species that had 3 samples collected from 1997-2007 and 2010 for Square Lake.



Square Lake (con't)

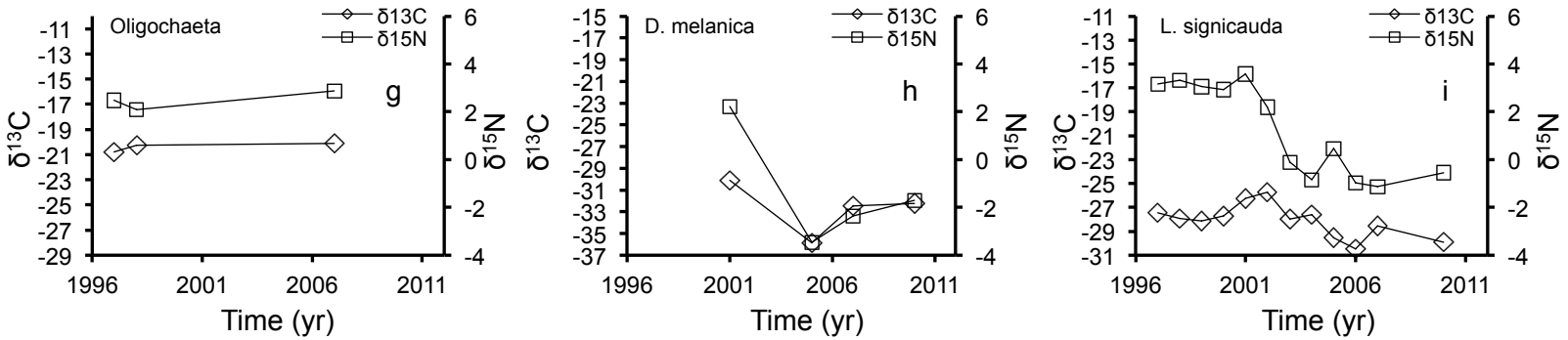
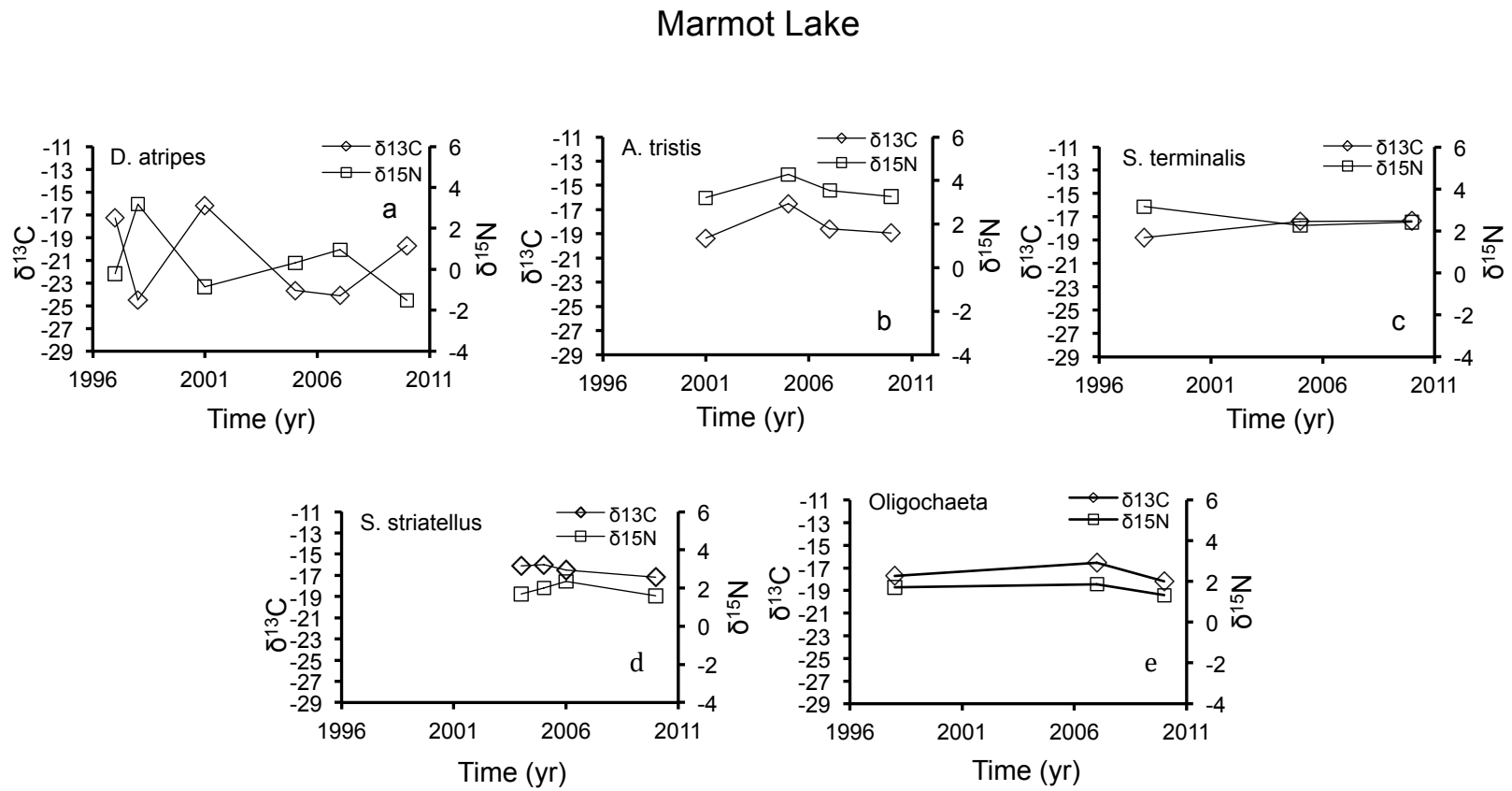


Figure 2.5b Bi-plots of mean $\delta^{15}N$ and $\delta^{13}C$ values versus time for each species that had 3 samples collected from 1997-2007 and 2010 for Marmot Lake.



Marmot Lake (con't)

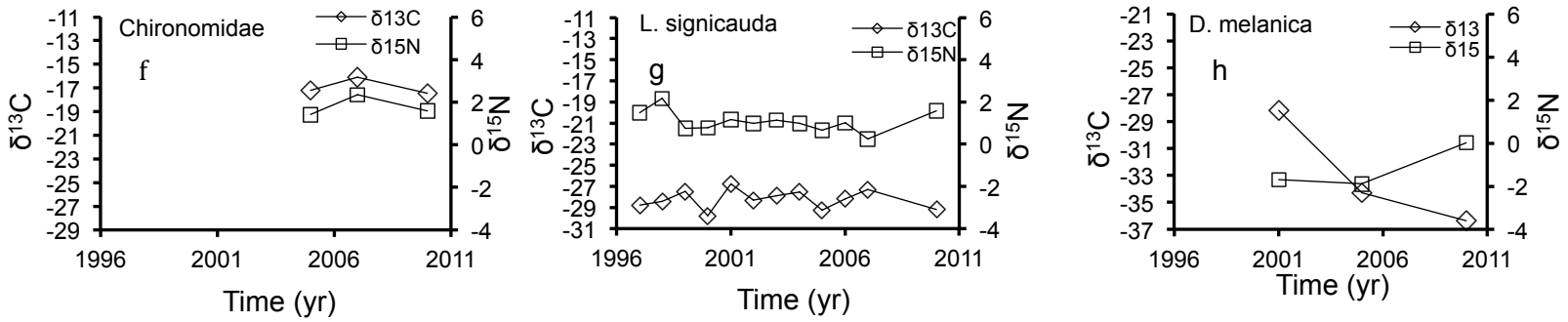


Figure 2.5c. Bi-plots of mean $\delta^{15}N$ and $\delta^{13}C$ values versus time for each species that had 3 samples collected from 1997-2007 and 2010 for Mesa Lake.

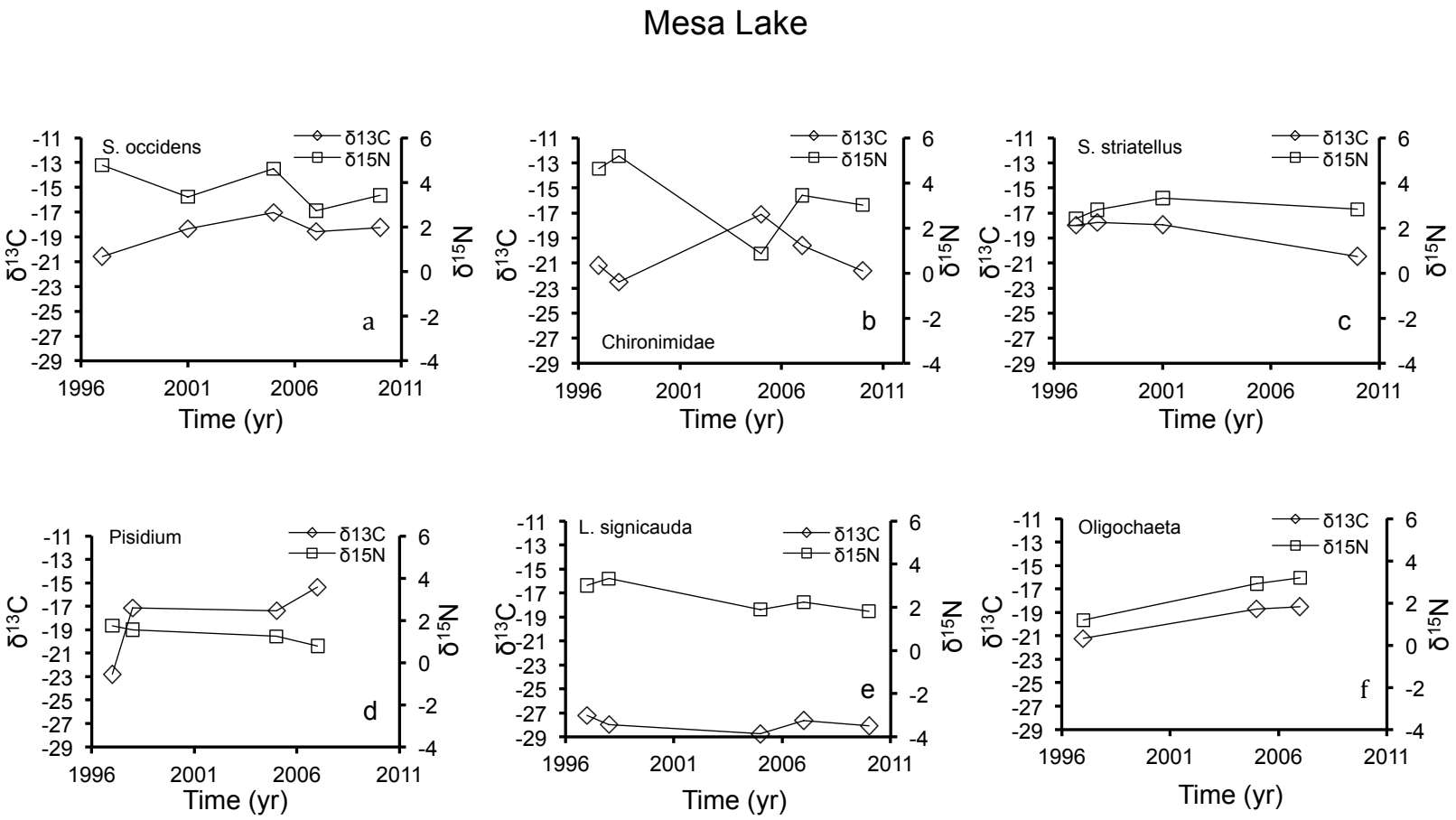


Figure 2.5d. Bi-plots of mean $\delta^{15}N$ and $\delta^{13}C$ values versus time for each species that had 3 samples collected from 1997-2007 and 2010 for Summit Lake.

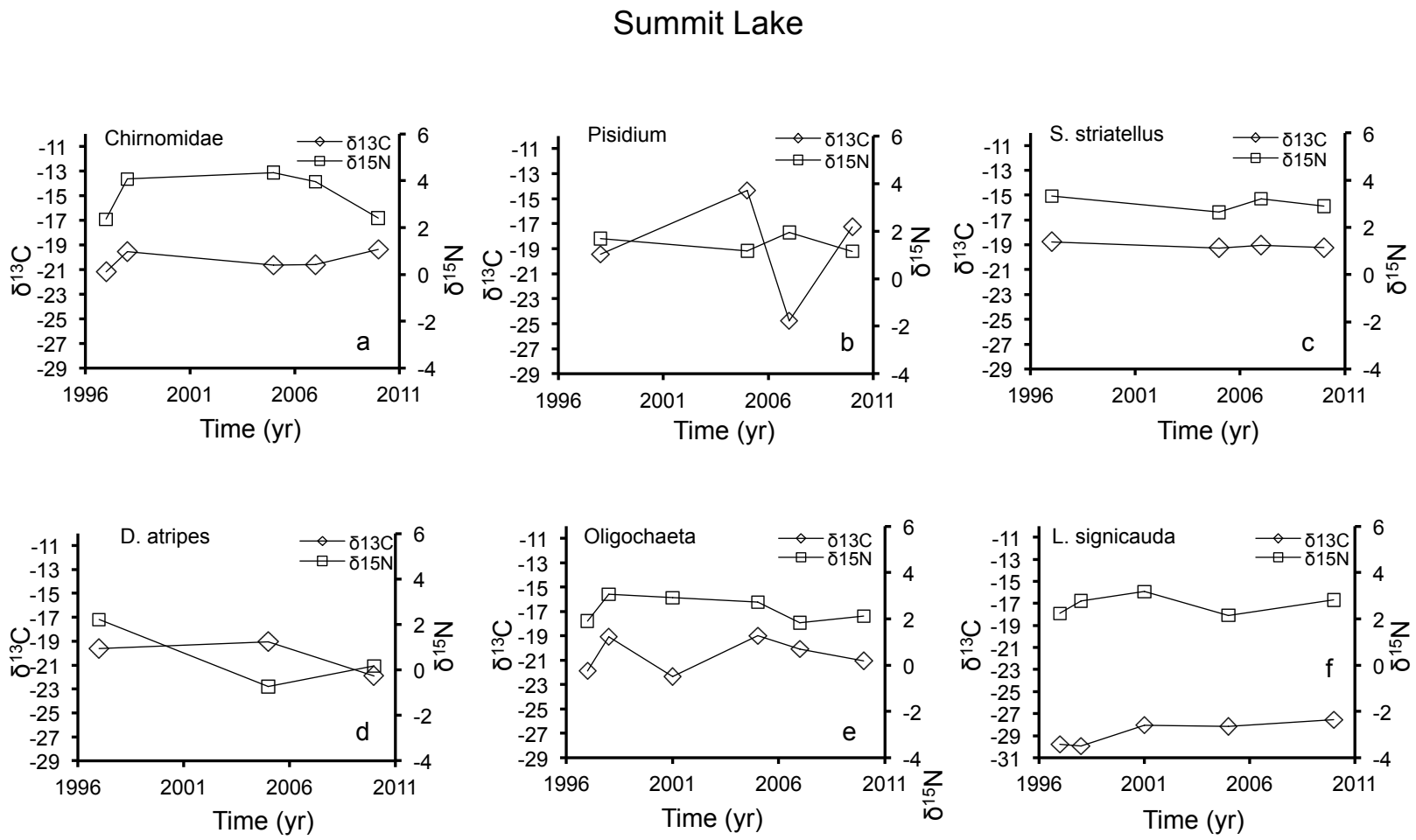


Figure 2.6. Bi-plot of $\delta^{15}N$ and $\delta^{13}C$ values versus time for *L. signicauda* for both Marmot and Square Lakes. Fish were removed from 1996-1998, *D. melanica* returned in 2001 and *H. shoshone* were reintroduced in 2004. Graphs on right are from Sarnelle and Knapp (2004) and are presented to show relationship and timing of D. melanica emergence and decrease in $\delta^{15}N$ values for L. signicauda. Arrows represent first detection of *Daphina* in each lake and open symbols shows fish density decline.