## Title

Spatial and Temporal Variability in Benthic Invertebrate Assemblages and Population Genetics in a Lake and Stream System



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# Spatial and Temporal Variability in Benthic Invertebrate Assemblages and Population Genetics in a Lake and Stream System

by

Natalie Janelle Stauffer-Olsen

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Professor Vincent H. Resh, Chair
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Abstract

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An understanding of the spatial and temporal diversity of benthic invertebrates is necessary to understand, manage, and protect freshwater habitats. Benthic invertebrates are important components of aquatic ecosystems and are frequently used in bioassessment and biomonitoring programs. Benthic invertebrates can also play a role in nutrient cycling in lentic environments through bioturbation activities. This dissertation uses a range of techniques and analyses to understand the arrangement of benthic invertebrate diversity in Upper Klamath Lake, Oregon, and several watersheds in northern southern California.

Upper Klamath Lake (UKL) is a large, shallow, naturally eutrophic lake that has experienced declines in water quality, which has led to annual cyanobacterial blooms of *Aphanizomenon flos-aquae*. Benthic invertebrates can increase autochthonous nutrient cycling through benthic bioturbation activities. In order to better understand the role that benthic invertebrates play in UKL, I studied the density, taxonomic richness, and species composition of benthic invertebrate assemblages in three geographic regions (north, central, and south) and three habitats (littoral, open-water and trench) across UKL. I also characterized sediment composition and water quality at each collection site and determined which environmental variables correlated with differences in benthic invertebrate composition. This research is located in Chapters 1 and 3 of this dissertation.

Like benthic invertebrates in UKL, the mayfly *Baetis tricaudatus* is an abundant and ecologically important organism of freshwater ecosystems. Despite its widespread distribution, *B. tricaudatus* cannot be consistently and accurately identified and belongs to a species group known to have cryptic species diversity. While previous studies have examined the spatial distribution of this diversity, none have studied the temporal distribution. To better understand the temporal arrangement of diversity at the cytochrome oxidase subunit 1 (CO1) mitochondrial gene region, I collected *B. tricaudatus* specimens from 3 sites over 4 years and used haplotype networks to visualize diversity. Because my results were different than those from other studies on the same taxon, for my final chapter I analyzed *Baetis rhodani* group COI sequences from northern and southern California using Bayesian phylogenetic analyses and haplotype networks. This research contributes to our understanding of genetic diversity, which is an important component to biodiversity.

DEDICATION

This dissertation is dedicated to Bo Stauffer-Olsen

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# INTRODUCTION

Freshwater habitats provide humans with arguably the single most important natural resource: water. Furthermore, although freshwater only covers 0.8% of the Earth's surface, this area supports nearly 6% of all known diversity- at least 100,000 species (Dudgeon *et al.*, 2006). Despite the importance of freshwater resources to society and the natural world, these fragile ecosystems and their associated species are being severely degraded by human activities (Dudgeon *et al.*, 2006; Moyle, Katz & Quiñones, 2011). Human demands on freshwater are so great that declines in biodiversity are much worse in freshwater ecosystems than in terrestrial ecosystems (Dudgeon *et al.*, 2006). In order to sustainably manage freshwater environments, our knowledge of their assemblage structures and the species living within them needs to be further developed.

Benthic macroinvertebrates are integral components of aquatic ecosystems. As important links between primary production and larger organisms, benthic invertebrates break down leaf litter, graze on periphyton, and provide food for a wide variety of predators such as fish (Cummins *et al.*, 1989; Power, Parker & Dietrich, 2008). Benthic invertebrates are also very diverse and respond to a variety of environmental changes (Resh, 2008). Because of their essential roles within the food web, their widespread distribution within a range of freshwater habitats, and their interactions with the environment (*e.g.* Kuwabara *et al.*, 2016), benthic invertebrates are often used as biological indicators in bioassessment and biomonitoring programs designed to estimate the health of aquatic ecosystems (Carter & Resh, 2013).

Collecting and identifying benthic macroinvertebrates for use in bioassesment and biomonitoring programs is done all over the world (Buss & Salles, 2007; Resh, 2008). In the United States, benthic macroinvertebrates are used in bioassessment and biomonitoring programs done by regulatory agencies including the EPA, the US Forest Service, the National Park Service, and state departments of Fish and Game (Carter & Resh, 2001). These widely used bioassesment programs are based on the assumptions that species respond to environmental conditions and that specimens in samples can be identified correctly and consistently.

Morphological identification of freshwater invertebrates used in many bioassesment and biomonitoring programs is exceptionally difficult because many are small and/or have numerous instars, dynamic life histories and systematic uncertainties (McCafferty *et al.*, 2008). Furthermore, species-level identifications generally require adult specimens, not the aquatic larval forms (Baird & Sweeney, 2011; Webb et al. 2012; Stein et al. 2014). Genetic sequencing techniques, such as "barcoding" the cytochrome oxidase subunit 1 mitochondrial gene region, have been found to be effective at identifying a wide variety of organisms, including benthic macroinvertebrates (Hebert *et al.*, 2003; Hebert & Gregory, 2005; Sweeney *et al.*, 2011; Webb *et al.*, 2012). Integrating genetic and morphological methods can help assuage challenges with identifying benthic macroinvertebrates (Jackson *et al.*, 2014).

*Baetis,* a widely distributed mayfly genus that is common in bioassessment and biomonitoring programs, contains several taxa that are difficult or impossible to correctly and consistently identify (Brittain, 1982; Webb *et al.*, 2012; Jackson *et al.*, 2014; Spitzer, 2014). Baetids have been found to have differing responses to aquatic stressors (*e.g.* Chang et al. 2014, Macher et al. 2015) and play key roles in freshwater ecosystems as

consumers of periphyton and food sources for fish and predatory macroinvertebrates ((Peckarsky *et al*., 2005)). Despite its importance, many *Baetis* species, including *B. tricaudatus,* cannot be accurately and consistently identified using traditional morphological methods. The current suggestion is to identify suspected *B. tricaudatus* larvae to the *Baetis rhodani* group (Webb et al. 2014). In order to accurately identify this taxon and to understand the true biodiversity of freshwater environments, more research linking together morphology and genetic patterns is necessary.

Benthic macroinvertebrates have been found to be very abundant and ecologically important in a lake plagued by poor water quality and high water demands-Upper Klamath Lake (UKL) (Wood *et al*., 2013; Kuwabara *et al*., 2016). UKL, a naturally shallow, eutrophic lake in south central Oregon, has experienced severe declines in water quality and has become hypereutrophic during the last century (Bradbury, Colman & Dean, 2004; Colman, Bradbury & Rosenbaum, 2004). Studies have identified that internal (autochthonous) nutrient loading caused by flux from the benthos accounts for a significant portion of the nutrients in the lake and likely contributes to poor water quality (Kuwabara *et al*., 2009). Benthic invertebrates can increase diffusive nutrient fluxes several-fold as a result of bioturbation, the physical disturbance of the sediment by biota living on or beneath the sediment-water interface (Wood *et al*., 2013). Additionally, benthic invertebrates can recycle phosphorus back to the water column from the benthos through metabolic excretion (Wood *et al*., 2013). In order to understand the role that benthic macroinvertebrates have in autochthonous nutrient loading in UKL, more information that characterizes their assemblages and relationships to environmental conditions is necessary.

*Overview of Chapters*

In **Chapter 1,** I determined the spatial and temporal distribution of benthic invertebrate density and richness values collected from 21 sampling sites that spanned three habitat types (littoral, open water and trench), and regions (north, central and south) in Upper Klamath Lake, Oregon. Benthic invertebrate samples were collected in triplicate over 3 summer sampling trips in May, June and July 2013, for a total of 189 samples. For this study, over 50,000 benthic invertebrates were collected and identified. Environmental parameters, including sediment particle size, water quality, and organic matter content, were also measured and analyzed in this study. Results indicated that benthic invertebrate densities varied among habitats and regions, but not sampling periods. Taxonomic richness also appeared to differ among habitats and regions. By determining whether density and richness values differed throughout the lake, I was able to suggest whether models estimating total maximum daily load models of nutrients, such as phosphorous, need to be spatially explicit and contribute important information about food availability for two endangered fish species endemic to Upper Klamath Lake.

In **Chapter 2,** I examined the cytochrome oxidase I (COI) mitochondrial gene region of *Baetis tricaudatus*, an abundant and widespread mayfly. Although *Baetis tricaudatus* is common in bioassessment and biomonitoring programs, it is impossible to accurately identify aquatic larvae. Furthermore, previous work done mostly on the east coast and in southern California, has found evidence of cryptic genetic diversity in this and related taxa. In order to determine whether there was cryptic diversity in *Baetis tricaudatus* populations in northern California and whether that diversity was structured

temporally, I generated cytochrome oxidase I (COI) mitochondrial gene sequences from specimens collected in northern California over 3 years. The three main sites of the study were located in the Russian River watershed and samples were collected monthly, for a total of 37 sampling trips. Diversity statistics and haplotype networks of collected gene sequences were to used examine the temporal arrangement of genetic diversity and search for cryptic species.

In **Chapter 3**, I characterized benthic invertebrate assemblage composition across Upper Klamath Lake. Like in Chapter 1 of this dissertation, sampling sites spanned three habitats, three regions, and three summer sampling dates. In addition to characterizing benthic fauna, assemblages were compared among habitats, regions and sampling events. Differences in assemblage composition were correlated with 21 measured environmental variables, including sediment particle sizes, water quality parameters, and benthic fluxes of macronutrients such as soluble reactive phosphorous and iron. By correlating the composition of benthic fauna with abiotic variables including benthic nutrient flux values, I was able to determine what variables were related to benthic invertebrate distribution and whether there was evidence for a relationship between benthic invertebrates and benthic nutrient fluxes, which can contribute to poor nutrient quality in Upper Klamath Lake.

In **Chapter 4**, I compared cytochrome oxidase I (COI) mitochondrial gene sequences from specimens collected from northern and southern California to search for patterns in genetic diversity. Methods used to catalogue and understand genetic information have changed rapidly over recent years and are often inconsistent among studies, which unsurprisingly often yield differing results. In this chapter, I used genetic statistics and haplotype networks, in addition to species identification based on tradition morphological methods, to compare genetic sequences from northern and southern California. Genetic sequences from northern and southern California were remarkably different and shared few haplotypes. I also found evidence for 7 putative species.

My dissertation contributes to our understanding of benthic invertebrate diversity, distribution, and ecology, as well as how they can best be utilized to answer important questions about freshwater ecosystems. Information included in this dissertation will be helpful in understanding, managing, and protecting freshwater ecosystems now and into the future.

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# CHAPTER 1

## Spatial and Temporal Variability in Benthic Invertebrate Assemblages in Upper Klamath Lake, Oregon

**Spatial and Temporal Variability in Benthic Invertebrate Assemblages in Upper Klamath Lake, Oregon**


## ABSTRACT

Upper Klamath Lake (UKL) in southern Oregon has experienced declines in water quality due to excessive nutrient loading. This has led to annual cyanobacterial blooms, primarily of *Aphanizomenon flos-aquae*. Benthic invertebrates are important food resources for benthic feeding fishes; however, they can increase autochthonous nutrient cycling in lakes and as a result might be contributing to poor water quality in UKL. This study determined the density and taxonomic richness of benthic invertebrate assemblages in three geographic regions (north, central, and south) and three habitats (littoral, open-water and trench) across UKL. Sediment composition and water quality were also characterized at each of the 21 benthic invertebrate collection sites. Three sampling trips were made from May - July 2013. Mean lake-wide invertebrate density was 12,617 ±7,506 individuals $m^{-2}$ (n=63, based on 189 Ekman grabs) with oligochaetes, chironomids, and leeches representing 97% of all individuals. Mean invertebrate richness per sample was 16 ±4 (n=63). Two and three-way repeated measures ANOVAs identified differences in invertebrate densities and richness among regions, habitats, and sampling periods. There were no differences in total density among sampling periods. Total density was higher in littoral compared to open-water habitats, and in the northern region, proximal to all riverine inputs to the lake, compared to the central or southern regions. Although variances were heterogeneous, the number of taxa appeared to differ between habitats and regions.



**Key words:** spatial distributions, temporal distributions, hypereutrophic lake


## INTRODUCTION

Upper Klamath Lake (UKL), located in south-central Oregon, USA, is one of the largest freshwater lakes west of the Rocky Mountains. UKL has experienced massive blooms of the cyanobacterium, *Aphanizomenon flos-aquae* (AFA), during the summer months since the mid-1900s (Bradbury et al. 2004a, 2004b; Colman et al. 2004). These blooms severely impair water-quality (Kuwabara et al. 2009, Simon et al. 2009), which in turn, negatively impacts resident fauna (Perkins et al. 2000), including two endangered fish species, the Lost River Sucker (*Deltistes luxatus*: Catostomidae) and the Shortnose Sucker (*Chasmistes brevirostris*: Catostomidae) (Janney et al. 2008). High mortality among juvenile suckers has reduced their recruitment into adult populations (Rasmussen 2011). One potential cause of the decline in sucker populations is the negative effects of poor water quality on juvenile suckers.

Accounts by settlers from the mid-1800s indicate that UKL has long been eutrophic (Colman et al. 2004). The natural eutrophic condition is likely a result of high natural phosphorus concentrations in groundwater and springs in the basin (Kuwabara et al. 2009). Over the last century, nutrient availability has increased because of human development and increased agriculture in the basin (Bradbury et al. 2004a, 2004b).

Excess nutrients have further degraded water quality, increasing summer bloom densities and changing the lake to a hypereutrophic condition (Snyder and Morace 1997).

Over the last decade, research has identified several sources of nutrients to the lake, some of which have been quantified (Kuwabara et al. 2007). These sources include external tributary inputs (Kann and Walker 1999, Wood et al. 2013) and internal benthic sources (Kuwabara et al. 2007, Kuwabara et al. 2009, Wood et al. 2013). Internal benthic sources include both diffusive and advective flux (Kuwabara et al. 2009), as well as nutrients released by benthic invertebrates (Wood et al. 2013).

Benthic invertebrates (*e.g.*, worms, insects, mollusks) are an integral component of lake ecosystems. They live on and in lake sediments, often occurring at the sediment-water interface. They represent multiple heterotrophic levels and most functional feeding groups including filterers, grazers, deposit feeders, piercers and engulfers. Benthic invertebrates represent an important food resource for fish, birds and some mammals, and are a major component of the diet of juvenile suckers in UKL (Markle and Clausen 2006).

There have been very few recent studies addressing the lake-wide distribution of benthic invertebrates in UKL (but see Hazel (1969). Therefore, the purpose of this research included: 1) providing an unbiased estimate of the spatial distributions of benthic invertebrates in UKL, 2) determining whether habitat differences influence those distributions, and 3) determining whether those distributions change over time.

## METHODS

*Study location*

Upper Klamath Lake (UKL) is located in south-central Oregon east of the Cascade Mountains (Figure 1.1). It is the remnant of a large pluvial lake that receded approximately 10,000 years ago. UKL is one of the largest water bodies in the western United States, with a surface area of approximately 260 $km^2$ and a mean depth of approximately 2.8 m. Although generally shallow, there is a narrow trench with a depth of approximately 15 m along the western edge of the central region of UKL. The Williamson and Wood Rivers discharge into the northern region of the lake (Figure 1.1). Most water from UKL discharges to the south into the Link River through Link Dam and eventually drains into the Klamath River.

Historically, UKL was surrounded by widespread marshes and wetlands (Bradbury et al. 2004b, Colman et al. 2004). Many of the surrounding wetlands have been filled and are now cattle ranches or large-scale farms. Watershed development has likely contributed to it becoming hypereutrophic (Bradbury et al. 2004b). Hypereutrophic lakes are characterized by low secchi disk transparency with annual means ≤1.5 m, high total phosphorus with means ≥100 mg $m^{-3}$, and high chlorophyll levels with annual means ≥25 mg $m^{-3}$ (Meybeck and Beim 1996, Carlson 1977). High phosphorus loading has created a favorable environment for the development of dense blooms of AFA to occur during the summer.

Sediment in the lake varies according to habitat (Hazel 1969). Open-water habitat is composed of mostly fine siliceous sediment while littoral habitat can range from pebble, to mostly sand, to highly organic sediment. Trench habitat ranges from soft silt to

fine gravel. Water currents in the lake are mostly wind-induced and hence mix the lake water column from the surface to the bottom (Wood et al. 2013).

*Sampling design*

To determine the distribution of benthic invertebrates, Upper Klamath Lake was first partitioned into three approximately equal-area geographic regions (north, central, and south) and three major habitat-types (littoral, open-lake, and trench) (Figure 1.1). Littoral sites were defined as sites that were located near shore at a depth of approximately 2 m. Limiting depth to 2 m in the spring ensured that sites remained inundated and accessible by boat even as water levels decreased during the summer. In 2013, lake water elevation decreased by approximately 1 m from its highest level in June to its lowest toward the end of summer. Open-water sites were defined as locations that were >2 m but <8 m in depth. Trench sites were >8 m in depth, associated with this western bathymetric feature.

Twenty-one sampling sites were chosen using a stratified random design by randomly sampling a 100 m x 100 m digital grid of the lake surface area. Three littoral and three open-water sites were randomly selected from within each geographic region (Figure 1.1). In addition, three sites were randomly selected from the trench, the deepest portion of the lake that is located in the central region. The sampling design was fully balanced for littoral and open-water habitats; however, trench sites were not present in the northern or southern regions of the lake. Once sites were chosen, site markers were placed in the lake to ensure constancy of site location for three sampling events. Sampling occurred from boats on May 23, June 13, and July 2, 2013. Dates were chosen to span the period of the initial summer AFA bloom (Morace 2007, Kuwabara et al. 2016).

*Sampling methods*

Benthic invertebrate samples were collected at each of the 21 sites using a standard, tall Ekman grab modified to limit collection depth in the unconsolidated lake substrate to approximately 10 cm. The sample volume per grab was 15.2 x 15.2 x 10 cm. Three replicate grabs were collected at each site-time combination for a total of 189 grabs. All grabs were sieved in the field using a 500 µm mesh sieve to remove sediment and were preserved with 10% buffered formalin. Results from the three replicate grabs were averaged to provide a single site-time measurement of benthic invertebrate density (n=63).

All grabs were individually sorted, counted, and identified to species or the lowest possible taxonomic level in the laboratory. In some cases, operational taxonomic units were created for consistency of identification. Andersen et al. (2013), Kathman and Brinkhurst (1998), and Davies and Govedich (2001) were used to identify chironomids, oligochaetes, and leeches respectively. The magnification used for sorting invertebrates from the sample debris ranged from 7x to 10x and sorting was not time-limited. Twelve grabs of the 189 collected (all from littoral sites in the northern region containing large volumes of coarse organic matter) were subsampled in the laboratory using standard procedures (Moulton et al. 2000).

*Sediment sampling and analysis*

Sediment cores were collected for physical and chemical analyses. A modified Ekman grab was used to retrieve sediment and a core (5 cm diameter × 10 cm long) was removed from the center of the grab. Core depths ranged from 2 cm to 10 cm. Sediment samples were collected June 13 and July 2 from each site, for a total of 42 samples. Sediment samples were put in plastic jars and stored in a cooler for transportation to the laboratory.

For particle size analysis, sediment subsamples (2 per site) were dried at 105°C for 48 hours, finely ground, and sieved with a 2 mm sieve to remove gravel, roots and coarse organic debris. Dried samples were then rewetted with deionized water, mixed with 30% hydrogen peroxide ($H_2O_2$) to remove organic matter and left for approximately 6 hours. Next, samples were centrifuged and the supernatant was decanted. Samples were re-suspended with 20 mL water and analyzed for particle size using a LISST-PORTABLE|XR (Version 1.00). Results for sediment grain size were reported as percentage clay (<2 μm), silt (2 – <50 μm), and sand (50 – 2,000 μm). The standard deviation of the percent of sand, silt and clay measured per site was used to represent sediment heterogeneity on a per-site basis. This estimate of sediment heterogeneity will henceforth be termed a standard deviation unit (s.d.u.).

Organic content of the sediment samples was measured as loss-on-ignition (Boyle 2004). Ten to 12 grams of wet sediment samples were weighed, dried at 105°C for 12 hours, ignited in an oven at 550°C for 2 hours, and then reweighed.

*Statistical analysis*

The 18 non-trench sampling sites were equally partitioned into three geographic regions (north, central and south) and two habitat types (littoral and open-water). Three-way repeated measures ANOVA with habitat, region and time as independent variables was used to determine if significant differences could be detected between sample partitions in total mean invertebrate densities and richness and mean densities and richness of each of the major taxa (oligochaetes, chironomids and leeches). All density data were $\log_{10}(x+1)$ transformed to approximate normality. Levene's tests were used to determine if variances were homogeneous. Newman-Keuls tests were used to identify specific differences when significant differences among regions were identified. All data are reported as means. Standard deviations and the number of samples (n) are reported in the text if they are not reported in the appropriate table.

Trench sites, confined to the western, central bathymetric feature, were only compared to littoral and open-water sites within the central region. Two-way repeated measures ANOVAs with habitat (littoral, open-water, and trench) and time as independent variables were used to determine if significant differences could be detected in total mean invertebrate densities and richness and mean densities and richness of each of the major taxa (oligochaetes, chironomids and leeches).

## RESULTS

*Density*

A total of 53,653 benthic invertebrates were identified and used in the analyses. Taxa excluded from this study included those that were abundant but too small to be reliably collected using a standard 500 μm sieve (*e.g.*, nematodes, *Manayunkia*) or were

contaminants from the sieving process (*e.g.*, planktonic microcrustaceans). Mean invertebrate density among all samples, with each sample based on the mean of three Ekman grabs per site-time combination, was 12,617 individuals $m^{-2}$ and ranged from 2,252 individuals $m^{-2}$ to 38,459 individuals $m^{-2}$ (Table 1.1). The most abundant higher taxonomic groups were oligochaetes, followed by chironomids, and then leeches (Table 1.1). Together, these three taxa represented 97% of the benthic invertebrates sampled. The remaining 3% was comprised of a variety of taxa including mayflies, caddisflies, amphipods, and mollusks.

Total invertebrate density differed between habitats and regions (Table 1.2). Mean invertebrate density in littoral habitat (Table 1.1) was higher than in open-water habitat (Table 1.2). Although the interaction between habitat and month was non-significant, density in littoral sites was only higher in June. Post hoc testing identified that northern sites, proximal to all surface water inflows to the lake, had higher mean density than central and southern sites (Table 1.2). The interaction between region and month was non-significant, but density in the northern region was only higher in June and not in May or July. There was no effect of month on total invertebrate density. There were no interactions between habitat × month, region × month, or habitat × region × month for any measures of density, so they are not reported in Table 1.2. Within the central region of the lake, total density differed among the three habitats (Table 1.3). Post hoc testing identified that trench and littoral habitats had higher total densities than open-water habitat. There was no interaction between habitat × month nor was there a month effect on density within the central region.

Densities of the three major taxonomic groups were influenced by one or more of the main factors (Table 1.2). Oligochaete density was higher in littoral than in open-water sites (Table 1.1), but there was no region effect, nor habitat × region interaction. There was, however, a significant month effect (Table 1.2). Post hoc testing indicated that oligochaete density was higher in May than in June and July (Table 1.1). In contrast, there was no habitat effect on chironomid density, but there was a region effect and a habitat × region interaction. Chironomid densities in central littoral sites were lower than at other habitat-region combinations. There was no month effect on chironomid density. Month was the only factor that had an effect on leech density. Leech density was higher in June and July than in May (Table 1.1). There were no interactions between factors for leech density. Total densities of oligochaetes and chironomids differed among the three habitats within the central region (Table 1.3). Oligochaete density was higher in littoral and trench sites than in open-water sites (Table 1.4). In contrast, chironomid density was higher in open-water and trench sites than in littoral sites. There was no effect of month on oligochaete or chironomid densities. There were no effects of habitat or month, or any interactions between these factors on leech density within the central region of the lake (Table 1.3). Lake-wide densities of the dominant taxa fluctuated over the span of the study. Oligochaetes generally decreased in density from May to July, while leeches generally increased in density during that time period. Chironomid density did not follow any lake-wide temporal pattern (Table 1.1).

*Taxonomic Richness*

A total of 73 benthic invertebrate taxa were collected from UKL over the study period of which 27% were identified to species (or equivalent OTU), 49% to genus and

19% to greater than genus (*e.g.*, tribe, subfamily, family) (Kuwabara et al. 2016). Seventy taxa were collected from littoral habitat, 45 taxa were collected from open-water habitat and 39 were collected from trench habitat. We identified 24 oligochaete taxa, 14 chironomid taxa, and 9 leech taxa (*i.e.*, 67% of the taxa, but 97% of the identified individuals). The remaining taxa included flatworms, mayflies, stoneflies, caddisflies, beetles, mites, and mollusks. Mean total richness per sample was 16 and ranged from 9 – 25. Mean richness per sample for oligochaetes, chironomids and leeches was 7, 4, and 3, respectively (Table 1.1).

Mean total richness appeared to differ between habitats and regions (Table 1.2). However, heterogeneity in the variances of total richness precluded determining whether differences were significant. Mean richness at littoral sites was 17 compared to 15 in open-water sites (Table 1.1). Mean richness was 16 in northern sites, 18 in central sites, and 14 in southern sites (Table 1.1). The interaction between habitat and region was non-significant ($p = 0.052$), but the magnitude of mean richness in the central littoral sites appeared substantially higher (21) than in any other habitat-region combination (Table 1.1). There was no effect of month on mean total richness. Within the central region of the lake, mean richness in littoral habitat was significantly higher than in trench and open-water habitats (Table 1.4). There was no effect of month on richness and no interaction between habitat and month within the central region (Table 1.3). As with density, measures of richness indicated no interactions between habitat × month, region × month, or habitat × region × month, hence they are not displayed in Table 1.2.

Richness of the three dominant taxa (oligochaetes, chironomids and leeches) varied in relation to different factors (Table 1.2). Habitat and region each appeared to have effects on mean oligochaete richness; however, there was an interaction between these two factors. Mean oligochaete richness was spatially complex. It was higher in littoral sites than in open-water sites, and higher at northern and central sites compared to southern sites (Table 1.1). Mean oligochaete richness was higher in central littoral sites compared to elsewhere in the lake. Additionally, oligochaete richness in southern open-water sites was lower than in northern open-water or littoral sites, as well as central littoral sites. As with total richness, there was no effect of month on oligochaete richness. Chironomid and leech richness did not differ with habitat or region; however, both differed between months (Table 1.2). Mean chironomid richness was higher in July than in May and June (Table 1.1). In contrast, mean leech richness was higher in June and July than in May.

*Sediment composition*

Sediment composition was generally similar throughout the lake. On average, substrates were equal parts sand (48 ±19%, n=42) and silt (48 ±18%, n=42) with a small amount of clay (4 ±2%, n=42). However, three sites (LN03, LC02, and TR02) differed substantially from the other sites (Figure 1.2). Sediment composition variability was highest both within and among trench sites (27.7 standard deviation units (s.d.u.) ±12.9, n=6) compared to littoral sites (26.4 s.d.u ±7.8, n=18). Variability in sediment composition both within and particularly among open-water sites was low (21.4 s.d.u. ±1.2, n=18). Littoral sites had the highest mean organic matter content, as well as the most variable percent organic matter of the sampled habitats (18 ±11%, n=18). Trench sites were characterized by the lowest mean percent organic matter (9 ±6%, n=6) and

open-water sites had moderate sediment organic matter with lowest variability (13 ±2%, n=18).

Regions within UKL also had variable sediment composition. Similar to lake-wide averages, northern sites (n=12) were on average equal parts sand (48 ±16%) and silt (48 ±15%), with 4 ±1% clay. Central sites (n=12, excluding trench sites) were 51 ±22% sand, 45 ±21% silt and 4% ±2 clay and southern sites (n=12) were 36 ±10% sand, 59 ±9% silt, and 5 ±1% clay. Trench sites (n=6) were 61 ±25% sand, 36 ±23% silt and 4 ±2% clay. Organic matter was similar in northern (18 ±10%, n=12) and southern sites (18 ±14%, n=12) but much lower at central sites (11 ±5%, n=12).

*Water-quality*

Water-quality parameters measured near the sediment surface varied both spatially (Table 1.5) and temporally (Figure 1.3). Mean summer temperature was similar among habitats. C*onductivity* tended to increase as site depth increased (from littoral to trench). In contrast, benthic chlorophyll, pH and dissolved oxygen tended to decrease as site depth increased. No regional differences in temperature and pH were observed. Conductivity increased from north to south, while dissolved oxygen decreased. Benthic chlorophyll was similar in northern and central regions but was lower in the southern region. Average lake-wide water-column temperature near the lakebed increased by nearly 9°C from May to July. Mean pH increased from slightly alkaline (7.9) in May to strongly alkaline (9.4) in July. Mean conductivity also increased from May (0.14 mS $cm^{-1}$) to July (0.22 mS $cm^{-1}$), while mean benthic chlorophyll more than doubled from 3.8 $\mu$g $cm^{-2}$ in May to 7.9 $\mu$g $cm^{-2}$ in June. Benthic chlorophyll measurements were not available in July. Mean dissolved oxygen saturation increased from May to June, (78% to 92%) but then decreased in July to 40% (Figure 1.3).

## DISCUSSION

Much of our understanding of limnology has been based on the study of deep lakes (Beklioğlu et al. 2016). As such, our general concept of how a typical lake functions is related to seasonal changes in temperature, which drives mixing/stratification and consequent changes in water chemistry. Stratification events lead to appreciable differences between shallow (littoral) zones and deep (profundal) zones and strongly influence phytoplankton, zooplankton and benthic invertebrate distributions.

Although there is no single, accepted definition of shallow in comparison to deep lakes, shallow lakes such as UKL typically lack the common lake processes related to stratification. For example, water-column mixing in shallow lakes, including UKL, is typically wind-driven, and leads to almost continuously well-mixed systems, although temporally short stratification evens can occur (Wood et al. 2013). As a result, plankton and benthic invertebrate distributions tend to be less stratified. Carbon sources for the benthos also tend to be more uniformly distributed in shallow lakes than in deep lakes (Kuwabara et al. 2016). Because the depth of overlying water is shallow, benthic fauna in shallow lakes have potentially greater influence on water-column processes than they do in deep lakes (Beklioğlu et al. 2016). Even though shallow lakes may lack some of the distinct habitat differences found in deep lakes, differences in habitats exist. Regional differences, which are often related to lake inflow and outflow, influence benthic

invertebrate distributions, as do differences between open-water and littoral habitats. Further, in both shallow and deep lakes, benthic invertebrate distributions in littoral habitats tend to vary greatly as a function of substrate composition, which can range from fine-grain, soft substrates to larger pebble and cobble substrates. The occurrence and composition of macrophytes can also be a major factor in structuring invertebrate assemblages in littoral habitats (Free et al. 2009).

UKL has high densities of benthic invertebrates that influence many aspects of lake function via activities such as bioturbation, organic matter processing, solute trophic transfer and metabolic nutrient release. Two issues of particular interest in UKL are their contribution to benthic nutrient release (and consequent water quality), and their importance as a food source for benthic-feeding vertebrates, particularly two endangered suckers (Catostomidae). Estimates of densities of benthic invertebrates - and their spatial and temporal variation - are necessary for accurately describing lake function.

Upper Klamath Lake is unusual in the western United States for being a large, shallow, natural lake, so comparisons of our data with similar lakes is challenging. Results from the present study, however, fall within the large range of total benthic invertebrate densities found in eutrophic and hypereutrophic shallow lakes in the western USA and other areas of the world. One example is Utah Lake, a large (380 $km^2$), shallow (4.3 m), eutrophic lake in Utah, USA that has substrates ranging from rocky to clay-silt. Barnes and Toole (1981) reviewed a number of studies on the benthos of Utah Lake. They found that in fine sediment habitats, similar to those sampled in the present study, two taxa dominated: oligochaetes and chironomids. Oligochaete densities ranged from 8643 – 26,192 individuals $m^{-2}$. Chironomids were less abundant and ranged from 237 – 7167 individuals $m^{-2}$. Unlike UKL, leeches were not reported from fine sediment samples; however, leech (*Helobdella stagnalis*) densities of 150 individuals $m^{-2}$ were reported from rubble-type substrate. Similar to Utah Lake, oligochaetes were the dominant benthic taxon in UKL with densities (based on the mean of three Ekman grabs) that ranged from 489 – 16,044 individuals $m^{-2}$. Also similar to Utah Lake, chironomids were subdominant and ranged from 163-30,074 individuals $m^{-2}$ – somewhat higher than found in Utah Lake. In contrast to the relatively low leech densities found in Utah Lake, UKL has a rich and abundant fauna of leeches, with leech densities ranging from 0-12,993 individuals $m^{-2}$.

Clear Lake is a shallow, natural, eutrophic lake located in the coastal range of north-central California. Suchanek et al. (1995) sampled 36 sites distributed throughout the lake for benthic invertebrates using an Ekman grab. Common taxa included oligochaetes, leeches, chironomids, and the phantom midge, *Chaoborus astictopus*. Oligochaete densities averaged 3,300 (range=258-8,668) individuals $m^{-2}$. The most abundant chironomids had a mean density of 507 (0-4,155) individuals $m^{-2}$ and were represented by *Chironomus* spp. (439, 0-2,476) and *Procladius bellus* (368, 0-1,679). Leeches averaged 60 (0-839) individuals $m^{-2}$. These densities are substantially lower than densities in UKL. Unlike UKL, Clear Lake is somewhat contaminated with mercury; however, Suchanek et al. (1995) only identified a minor link between the level of contamination and benthic densities.

Estimates of benthic invertebrate densities by previous studies in UKL have varied. Kuwabara et al. (2012) found a mean density of 9,515 ±6,185 individuals $m^{-2}$ among four open-lake sites in the central-northern regions, which is similar to our lake-

wide open-lake density (10,081 ±6,377 individuals $m^{-2}$). Wood et al. (2013) studied sites confined to the northern portion of the lake and determined a mean density of 20,625 ±11,671 individuals $m^{-2}$, similar to densities reported herein for the northern region (17,256 ±8,452 individuals $m^{-2}$; Table 1.1). Hazel (1969) studied benthic invertebrates within UKL in the mid-1960s. A total of 107 Ekman grabs were collected from sites throughout the lake during 1964 - 1965. Sampling sites from Hazel (1969) were located throughout the lake, their exact locations were not provided. Although site locations and processing methods differed between our study and Hazel (1969), mean total abundance and the abundance of each major taxon were remarkably similar (Table 1.6).

Regional differences within lakes often influence the distribution of benthic invertebrates. The northern region of UKL, which is the location of most tributary input, had much higher benthic invertebrate densities and richness than the central or southern regions (Table 1.1). Pamplin et al. (2006) and Devine and Vanni (2002) found similar results when they compared benthic invertebrate densities between the upstream and downstream portions of their studied reservoirs. Both studies concluded that higher densities were likely related to higher amounts of dissolved oxygen (DO) in the upstream portions of their reservoirs. Low DO is known to stress lake fauna (Bortleson and Fretwell 1993, Cao et al. 2012). Although mean DO in UKL trended from higher in the upstream north to lower in the downstream south (Table 1.5), regional differences were not statistically different. However, UKL experiences suboxic conditions as a result of AFA senescence during mid-summer (Wood et al. 2013). Therefore, minimum % DO saturation, which decreased from 24.9 in the northern inflow region to only 3.3 in the southern dam region during 2013, likely stresses benthic populations and limits faunal distributions (Cao et al. 2012).

Total density and densities of the three main taxa in UKL were higher in littoral compared to open-water habitat. Pamplin and Rocha (2007) sampled benthic invertebrates from 90 randomly selected sites in an oligotrophic Brazilian reservoir and found that oligochaetes and chironomids, although occurring at low densities, preferentially inhabited the littoral areas. Littoral and open-water habitat differed in a number of physical characteristics. For example, they found that depth was the main predictor of benthic invertebrate distributions, but the effects of temperature, dissolved oxygen and organic matter were also important. Lassen et al. (1997) found that light was a limiting factor for microbenthic photosynthesis in a shallow eutrophic lake. In UKL, the relationship between depth and light was apparent, with shallow littoral habitat having higher benthic chlorophyll than deeper open-water (and trench) habitat (Table 1.5). Increased photosynthesis by phytobenthos increases the food available to benthic invertebrates (Gullberg et al. 1997). Thus, in shallow littoral areas of UKL the high level of benthic chlorophyll (food availability) was likely one of the factors influencing the high density of some invertebrates.

Comparing taxonomic richness among studies is challenging because the taxonomic levels to which organisms are resolved often vary among studies, and richness is frequently estimated at a mixed taxonomic level of resolution in aquatic studies of invertebrates (Carter and Resh 2001). Cognizant of such differences in methods, useful comparisons can be made and richness-habitat relationships can be explored. Peralta et al. (2002) reviewed a number of lake studies, and determined that oligochaete richness varied between 10 and 41 per lake. Pamplin and Rocha (2007) identified 10 oligochaete

species and 21 chironomid taxa in an oligotrophic reservoir. Hazel (1969) distinguished 10 chironomid genera, some of which represented more than one species, hence the number of chironomid taxa he found was likely similar to that identified in the present study. Ji et al. (2011) collected only two leech species from three sites in a shallow eutrophic lake in China. In comparison to the above studies, we identified 24 oligochaete taxa, 14 chironomid taxa and at least 9 resident leech species. Variation among studies may also represent differences in lake trophic level.

There is a well-established relationship between species richness and habitat heterogeneity (Tews et al. 2004), and littoral habitat is often heterogeneous in comparison with open-lake habitat (Árva et al. 2014). However, no single set of parameters is universally used to quantify habitat heterogeneity in lakes. Studies have focused on the variability in the abundance of macrophytes (Free et al. 2009), sediment particle size (Heino 2000), organic matter content (Árva et al. 2014), flow regulated movements (Stendara and Johnson 2005), or variation in lake water level (Rose and Mesa 2013). Heino (2000) studied 21 lentic systems in Finland and found that habitat heterogeneity, operationally defined by an index based on a number of different variables (*e.g.*, particle size, organic matter content, macrophytes and water quality) was positively correlated to the number of invertebrate species. Verberk et al. (2006) measured multiple physical and chemical variables as well as vegetation composition and found that more heterogeneous bog pools had higher macroinvertebrate species diversity than uniform bog pools. Littoral habitat in UKL was characterized by higher variability in sediment particle sizes and organic matter content both within and among sites than open-water habitat. Littoral habitat also had higher species richness than open-water habitat, which suggests a positive correlation between habitat heterogeneity and species richness.

Temporal changes in benthic invertebrate densities are influenced by a number of factors, such as species-specific life-histories, environmental conditions, competition and predation. We found no significant month effect on total invertebrate density, which apparently was a result of differences in the timing and growth of populations within and among the three major taxa. Oligochaete densities decreased from early to late summer (May to July), a trend found in a variety of other lakes as well. For example, Lindegaard and Jonasson (1979), who studied a volcanic shallow lake in Iceland, and Yildiz et al. (2015), who studied a shallow hypereutrophic lake in Turkey, both also found that *Tubifex* populations tended to decrease from spring through summer. In contrast, Devine and Vanni (2002) sampled benthic invertebrates from June to November and found that tubificid densities peaked around the beginning of July and then decreased until around late August. In UKL, oligochaete densities are dominated by species of *Ilyodrilus*, *Limnodrilus*, and *Varichaetadrilus*, all of which had population declines over the three-month study period from May to July.

Chironomid populations in UKL varied over the course of the study but were lowest overall in June, likely a result of the spring emergence of several of the more abundant taxa such as *Cryptotendipes* and *Cladotanytarsus*. However, *Procladius*, the most abundant midge, actually had its maximum recorded densities in June. Yamagishi and Fukuhara (1971) studied *Chironomus plumosus,* a large and common species that decreased in density from May through July in UKL, and found that its density was also highest between May and June in shallow (mean depth 4.1 m) eutrophic Lake Suwa, Japan, but varied among years. Barnes and Toole (1981) report similar population cycles

of chironomids from the fine sediments in Utah Lake as we found in UKL. Consistent patterns of maximum chironomid density among studies, as well as within UKL, are likely the result of similar emergence times by the related chironomid taxa.

Leech density increased substantially over the course of the present study (Tables 1.1 and 1.4), an observation also made by Hilsenhoff (1967) in eutrophic Lake Winnebago, Wisconsin. The most abundant leeches in UKL, in the genus *Helobdella*, brood their young. In eutrophic Utah Lake, overwintering adult leeches had two broods of young, one in May and another in June (Tillman and Barnes 1973). Consistent with Tillman and Barnes' observations, *Helobdella* species in UKL appeared to have one to two broods with the highest abundance of brooding leeches occurring in May and decreasing through July. As juveniles detached from their parents, overall leech density increased. The increase in leech density partially offset the decrease in oligochaete density over the study period. As a result of the different temporal changes in the densities of the dominant taxa, no significant month effect on total invertebrate density was observed (Table 1.1).

Numerous studies have shown that autochthonous sources of nutrients can contribute substantially to nutrient loading in lakes (*e.g.* Sondergaard et al. 2001, Steinman et al. 2006, Biswas et al. 2009). Kuwabara et al. (2012) quantified diffusive benthic flux of dissolved solutes from three sites in UKL over six sampling periods in summer and fall and indicated that: (1) average benthic flux of Soluble Reactive Phosphorus (SRP) was 3.53 ±1.82 mg $m^{-2}$ $day^{-1}$ and (2) internal loading of SRP to the water column of UKL from benthic flux was comparable to or greater than the riverine load to the lake.

The benthic flux of nutrients can increase as a result of excretion by benthic invertebrates (Devine and Vanni 2002, Henry and Santos 2008, Wood et al. 2013). Gardner et al. (1981) estimated release rates of inorganic phosphorus from a lumbriculid (*Stylodrilus heringianus*) and a chironomid (*Chironomus* spp.), taxa similar to those found in UKL, and calculated that these two taxa accounted for 20% and 13%, respectively, of the total phosphorus (P) released from sediments. Based on the percentage of benthic invertebrate biomass that oligochaetes and chironomids represented, Gardner et al. (1981) estimated that all benthic invertebrate excretion accounted for most of the phosphorus released from the aerobic sediment of the study area. Wood et al. (2013) measured SRP released by dominant benthic invertebrates in UKL. When measured SRP release rates were scaled to mean densities of chironomids and tubificids from the northern region of UKL, they estimated that excretion by benthic invertebrates was 3.8 mg $m^{-2}$ $day^{-1}$. They concluded that benthic invertebrates were contributing approximately the same amount of SRP to the water column as diffusive flux (Kuwabara et al. 2012).

Benthic invertebrates are important food resources for bottom feeding fishes, including the endangered Lost River and Shortnose Suckers found in UKL (Markle and Clausen 2006). Although adult chironomids are the important surface prey for larval suckers, larval chironomids are one of the main food resources for benthic-feeding juveniles of both endangered species (Markle and Clausen 2006). Leeches have been found to be significant food sources for other fish species occurring in UKL, such as the invasive yellow perch (Clady 1974) and brown bullhead (Keast and Webb 1966), although they have not been identified in juvenile sucker guts (Markle and Clausen

2006). Even though leech densities appear extremely high in UKL, there is no evidence that they parasitize the endangered suckers (Burdick et al. 2015). Our study found that oligochaetes, chironomids and leeches were represented 97% of the benthic invertebrate assemblage in UKL. Whether benthic invertebrates represent a limiting resource to fish in UKL requires further study.

Knowledge of the factors affecting the spatial and temporal distributions of benthic invertebrates in lakes is fundamental to basic lake ecology. Quantitative information on the function of benthic invertebrates in lakes, whether as a food resource for fishes or their contribution to nutrient cycling, is especially important since lakes and their fauna are becoming more impaired because of increased eutrophication (Ji et al. 2011, Lima et al. 2013, Nelson and Steinman 2013). The legacy of multi-decadal nutrient accumulation in lakes can last for hundreds or thousands of years after allochthonous nutrient loading has been curbed (Carpenter 2005) due to persistent internal solute cycling. Therefore, incorporating processes influenced by resident benthic invertebrates into lake nutrient models (*e.g.*, total maximum daily load models) seems a necessary aspect of lake management.

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**Table 1.1.** Summary statistics (n, mean, and standard deviation) of the density and richness of all benthic invertebrates and the three main taxa (oligochaetes, chironomids and leeches) in open-lake and littoral habitats partitioned by habitat, region and month. Data from trench sites are listed in Table 1.4. Units for density values are individuals $m^{-2}$ and units for richness values are number of taxa per sample.

| | | Habitat | | | Region | | | Month | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Lake-wide | Littoral | Open-water | North | Central | South | May | June | July |
| Density | N | 54 | 27 | 27 | 18 | 18 | 18 | 18 | 18 | 18 |
| Total | Mean | 12,745 | 15,408 | 10,081 | 17,256 | 9,257 | 11,721 | 11,848 | 13,205 | 13,181 |
| | SD | 7,935 | 8,540 | 6,377 | 8,452 | 4,731 | 8,181 | 7,160 | 7,853 | 9,059 |
| Oligochaetes | Mean | 6,424 | 7,852 | 4,996 | 8,199 | 5,551 | 5,523 | 7,155 | 6,540 | 5,579 |
| | SD | 3,476 | 3,134 | 3,252 | 2,380 | 4,243 | 3,001 | 3,317 | 3,542 | 3,577 |
| Chironomids | Mean | 4,026 | 4,617 | 3,436 | 6,291 | 1,381 | 4,407 | 3,827 | 3,449 | 4,803 |
| | SD | 5,784 | 6,658 | 4,812 | 6,955 | 1,345 | 6,414 | 6,092 | 3,681 | 7,254 |
| Leeches | Mean | 1,926 | 2,363 | 1,489 | 2,612 | 1,486 | 1,681 | 556 | 2,719 | 2,503 |
| | SD | 2,191 | 2,631 | 1,570 | 2,991 | 1,423 | 1,794 | 402 | 2,985 | 1,697 |
| Richness | N | 54 | 27 | 27 | 18 | 18 | 18 | 18 | 18 | 18 |
| Total | Mean | 16 | 17 | 15 | 16 | 18 | 14 | 15 | 17 | 16 |
| | SD | 4 | 4 | 2 | 3 | 4 | 3 | 3 | 4 | 4 |
| Oligochaetes | Mean | 7 | 8 | 7 | 8 | 9 | 6 | 7 | 8 | 7 |
| | SD | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 |
| Chironomids | Mean | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 5 |
| | SD | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | 2 |
| Leeches | Mean | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 |
| | SD | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

**Table 1.2.** Results from three-way repeated measures ANOVA with the factors habitat (littoral and open-water), region (north, central and south) and month (May, June and July). The trench sites were analyzed separately (Table 4) because they only occur in the central region of Upper Klamath Lake, Oregon. Significant *P* values (<0.05) are indicated in bold. Factors and interactions with no significant values are excluded from the table.

| | Habitat | | Region | | Month | | Habitat X Region | |
|---|---|---|---|---|---|---|---|---|
| | F | p | F | p | F | p | F | p |
| Density | | | | | | | | |
| Total | 6.99 | **0.021** | 4.67 | **0.032** | 0.23 | 0.794 | 0.22 | 0.808 |
| Oligochaete | 7.72 | **0.017** | 2.87 | 0.096 | 5.06 | **0.015** | 3.05 | 0.085 |
| Chironomid | 0.68 | 0.426 | 10.97 | **0.002** | 0.05 | 0.955 | 5.40 | **0.021** |
| Leech | 0.78 | 0.395 | 0.85 | 0.452 | 6.00 | **0.008** | 2.03 | 0.174 |
| Richness | | | | | | | | |
| Total | 9.86 | **0.009** | 9.65 | **0.003** | 2.21 | 0.132 | 3.82 | 0.052 |
| Oligochaete | 7.85 | **0.016** | 5.70 | **0.018** | 0.94 | 0.404 | 5.89 | **0.017** |
| Chironomid | 0.37 | 0.552 | 1.94 | 0.187 | 4.05 | **0.030** | 0.46 | 0.642 |
| Leech | 0.22 | 0.647 | 0.13 | 0.879 | 7.95 | **0.002** | 1.99 | 0.179 |

**Table 1.3.** Results from two-way repeated measures ANOVA with the factors habitat (littoral, open-water and trench) and month (May, June and July). Significant *P* values (<0.05) are indicated in bold. Factors and interactions with no significant values are excluded from the table.

| | Habitat | |
|---|---|---|
| | F | p |
| Density | | |
| Total | 6.00 | **0.037** |
| Oligochaete | 16.76 | **0.003** |
| Chironomid | 7.14 | **0.026** |
| Leech | 0.43 | 0.666 |
| Richness | | |
| Total | 29.55 | **0.001** |
| Oligochaete | 15.27 | **0.004** |
| Chironomid | 0.08 | 0.923 |
| Leech | 1.21 | 0.362 |

**Table 1.4.** Summary statistics (n, mean, and standard deviation) of the density and richness of benthic invertebrates and of the three main taxa: oligochaetes, chironomids and leeches within the central region of the lake by factor (habitat and month). Units for density values are individuals $m^{-2}$ and units for richness values are number of taxa per sample.

| | | Habitat | | | Month | | |
|---|---|---|---|---|---|---|---|
| | | Littoral | Open-water | Trench | May | June | July |
| Density | N | 9 | 9 | 9 | 9 | 9 | 9 |
| Total | Mean | 12,166 | 6,347 | 11,850 | 9,342 | 10,685 | 10,338 |
| | SD | 4,901 | 2,120 | 4,327 | 4,753 | 4,417 | 5,304 |
| Oligochaetes | Mean | 8,767 | 2,336 | 8,459 | 6,795 | 6,703 | 6,064 |
| | SD | 3,791 | 784 | 3,631 | 4,388 | 4,063 | 4,654 |
| Chironomids | Mean | 594 | 2,168 | 1,765 | 1,561 | 1,340 | 1,626 |
| | SD | 497 | 1,484 | 590 | 796 | 875 | 1,689 |
| Leeches | Mean | 1,366 | 1,605 | 1,572 | 433 | 1,929 | 2,181 |
| | SD | 1,267 | 1,633 | 2,838 | 307 | 1,741 | 2,688 |
| Richness | N | 9 | 9 | 9 | 9 | 9 | 9 |
| Total | Mean | 21 | 16 | 13 | 16 | 17 | 16 |
| | SD | 4 | 1 | 2 | 4 | 5 | 4 |
| Oligochaetes | Mean | 10 | 7 | 7 | 8 | 8 | 8 |
| | SD | 2 | 1 | 1 | 2 | 3 | 2 |
| Chironomids | Mean | 3 | 4 | 4 | 4 | 3 | 4 |
| | SD | 1 | 1 | 1 | 1 | 1 | 1 |
| Leeches | Mean | 3 | 3 | 2 | 2 | 3 | 3 |
| | SD | 2 | 1 | 1 | 1 | 1 | 2 |

**Table 1.5.** Water quality parameter summary statistics (mean and SD). Units for temperature are °C, units for specific conductivity are mS $cm^{-1}$, units for benthic chlorophyll are $\mu$g $cm^{-2}$ and dissolved oxygen, as well as minimum dissolved oxygen, are given as percentages. All values were measured at the maximum depth of sites and central sites do not include trench site values.

| | Littoral | Open-water | Trench | North | Central | South |
|---|---|---|---|---|---|---|
| N | 27 | 27 | 9 | 18 | 18 | 18 |
| Temperature ($^{o}$C) | 15 | 15 | 15.4 | 13 | 12.7 | 12.8 |
| S.D | 2.3 | 2.3 | 2.3 | 0.4 | 0.4 | 0.4 |
| pH | 8.3 | 7.9 | 7.4 | 7.7 | 8 | 8 |
| S.D | 0.5 | 0.7 | 0.7 | 0.1 | 0.2 | 0.4 |
| Conductivity (mS $cm^{-1}$) | 0.13 | 0.15 | 0.21 | 0.11 | 0.13 | 0.19 |
| S.D | 0.04 | 0.07 | 0.15 | 0 | 0.04 | 0.07 |
| Benthic Chl-a ($\mu$g $cm^{-2}$) | 7.4 | 3.7 | 1.2 | 5.2 | 5.2 | 2.2 |
| S.D | 2.6 | 5.8 | 0.5 | 3.2 | 4 | 1.3 |
| Dissolved Oxygen (%) | 83 | 70.4 | 56.6 | 80.8 | 75.7 | 71.3 |
| S.D | 30.5 | 31.5 | 30.6 | 28.8 | 28.5 | 39.3 |
| Minimum Dissolved Oxygen (%) | 4.8 | 3.3 | 2.8 | 24.9 | 14.3 | 3.3 |

**Table 1.6.** A comparison of benthic invertebrate densities (individuals $m^{-2}$) in Upper Klamath Lake from Hazel's (1969) study and the present study.

| Year | 1965 | 2013 |
|---|---|---|
| Oligochaetes | 5,341 | 6,715 |
| Chironomids | 1,264 | 3,703 |
| Leeches | 1,552 | 1,875 |
| Ttl Density | 11,303 | 12,617 |

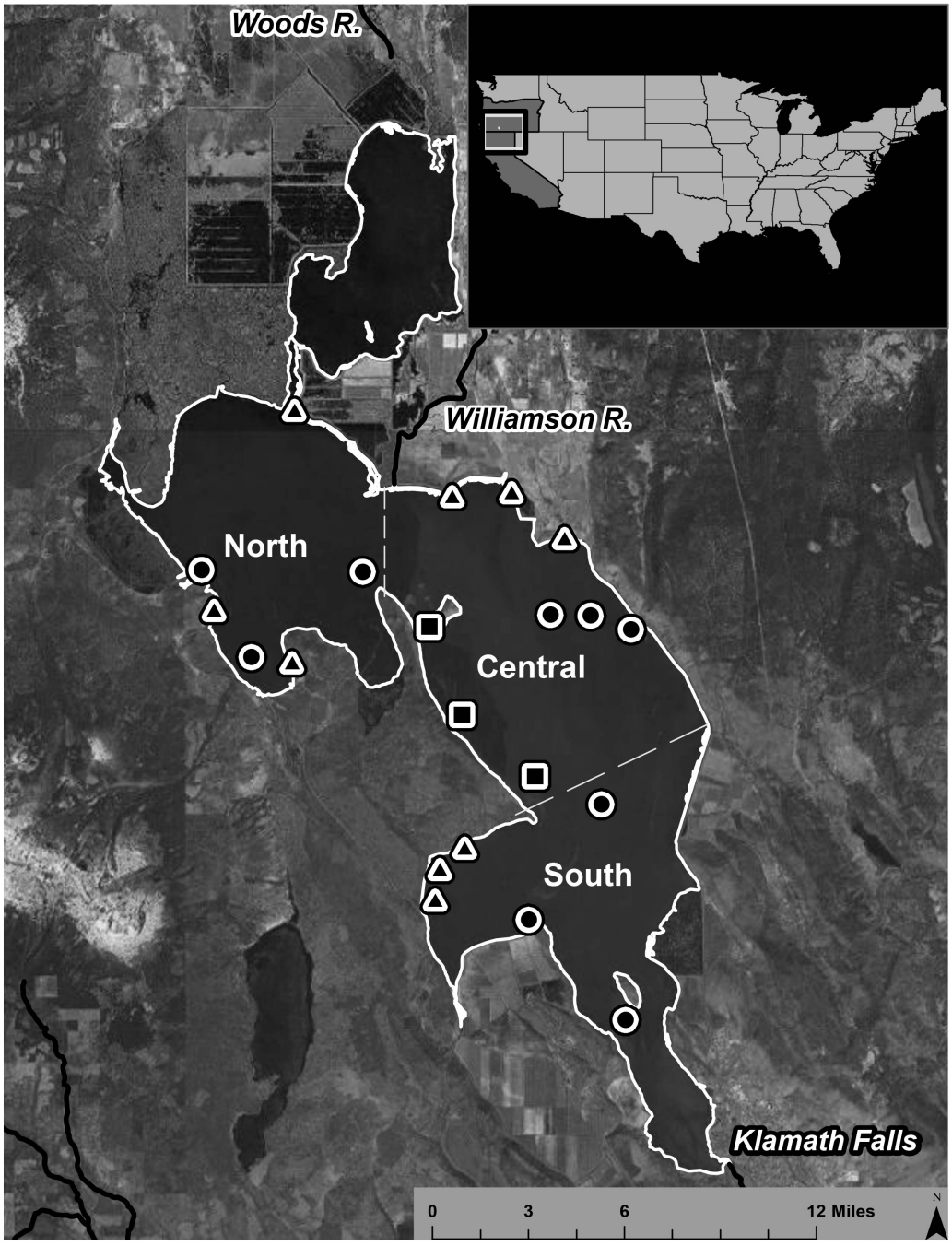


**Figure 1.1.** Map of Upper Klamath Lake, Oregon with 21 sampling sites. Factors (region and habitat) are also indicated. Sampling habitat is indicated by squares (trench, n=3), circles (open water, n=9), and triangles (littoral, n =9). Lake border is highlighted in white.

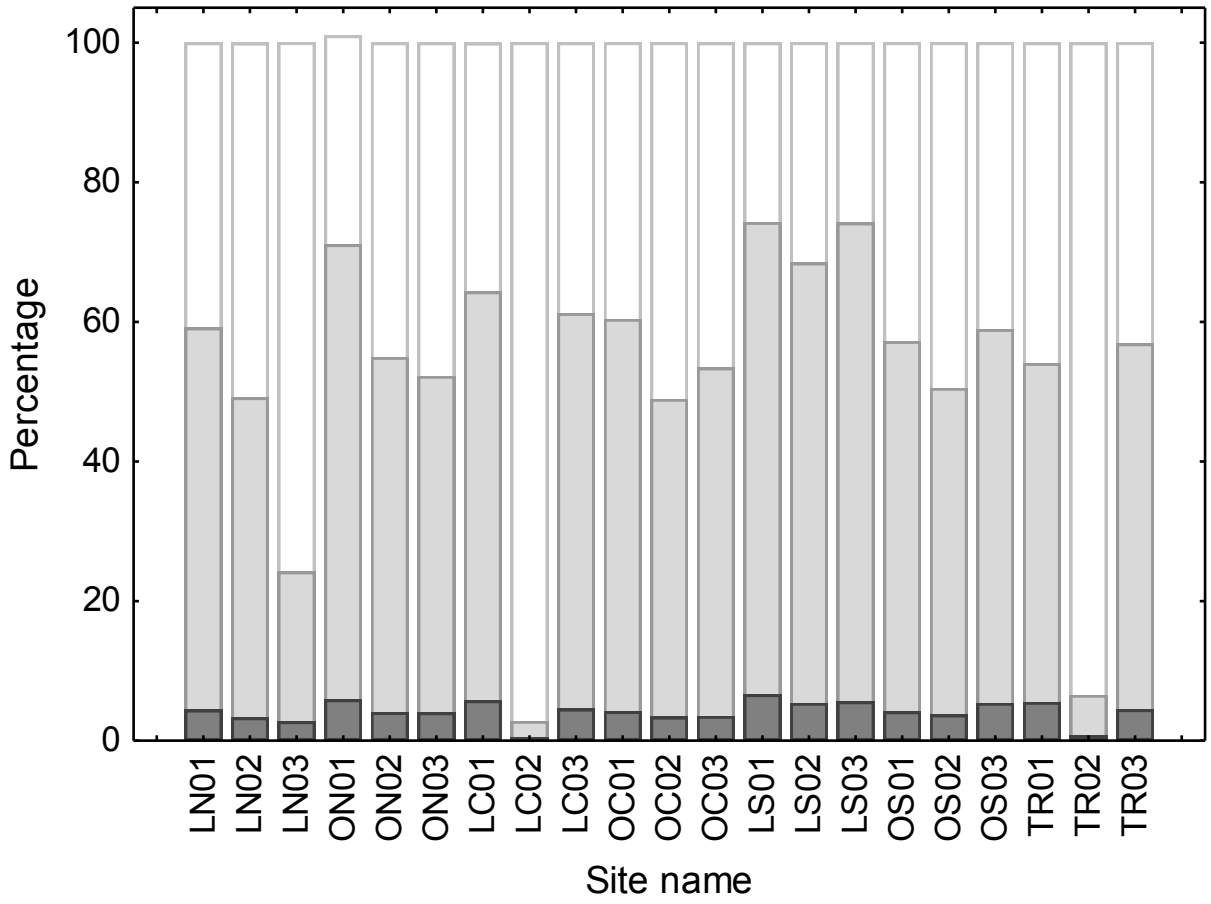


**Figure 1.2.** Sediment composition (mean percentage sand, silt and clay) at each of the 21 sampling sites. Percentage sand, silt, and clay are represented by white, light-grey and dark-grey bars, respectively (N=2 per site).

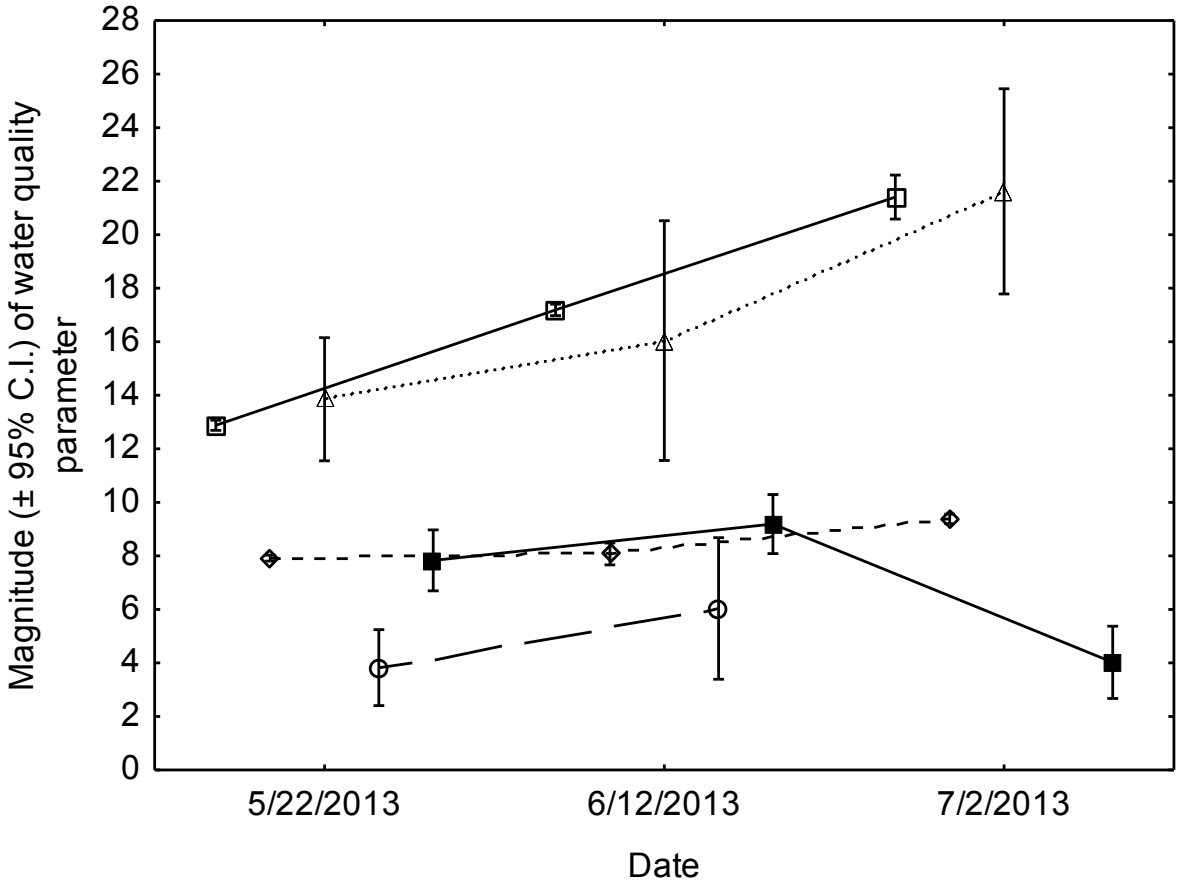


**Figure 1.3.** Selected water quality parameters per sampling date: average bottom temperature (ºC), average bottom pH, average bottom specific conductivity (mS $cm^{-1}$ multiplied by 100 to adjust magnitude for figure clarity), average benthic chlorophyll ($\mu$g $cm^{-2}$) and average percentage saturation of dissolved oxygen (% divided by 10 for figure clarity) in Upper Klamath Lake, Oregon. Temperature = open square, pH = open diamond, conductivity = open pyramid, benthic chlorophyll = open circle and dissolved oxygen = solid square.

# CHAPTER 2

## Temporal patterns of genetic diversity in *Baetis tricaudatus* (Ephemeroptera: Baetidae) from the Russian River, Northern California

**Temporal patterns of genetic diversity in *Baetis tricaudatus* (Ephemeroptera: Baetidae) from the Russian River, Northern California**


## ABSTRACT

The mayfly *Baetis tricaudatus* is an abundant, widespread, and ecologically important multivoltine benthic macroinvertebrate that is found throughout most of North America. *Baetis tricaudatus* belongs to the *Baetis rhodani* species group, which is known to have cryptic species. Some investigators have found that *B. tricaudatus* morphospecies have cytochrome oxidase I (COI) diversity >20%. However, no investigators have examined whether this diversity is structured temporally, with some haplotypes being more common in certain years or seasons than others. We sequenced COI from 371 *B. rhodani* specimens. The 371 *rhodani* species group sequences generated fell into 2 well-supported clades, one with 38 *Baetis adonis* specimens and another with 333 *B. tricaudatus* specimens, which were the focus of our study. We examined the temporal and spatial dynamics of genetic diversity in *B. tricaudatus* populations from northern California using COI haplotype networks. The maximum genetic diversity among *B. tricaudatus* specimens was 1.7% and was found at a single site (Austin Creek). The same 2 dominant haplotypes of *B. tricaudatus* were consistent through years, sites, and seasons, and $\Phi_{ST}$ values were correspondingly low. In 2 intensive sampling events, each with >40 individuals examined, intrapopulational divergence was 1.2 to 1.4%. This result suggests that most of the genetic diversity for this species in this system could be captured in 1 high-effort sampling event rather than in smaller, long-term monitoring events. Our results suggest that, based on the sites examined, Russian River populations of *B. tricaudatus* constitute a single species with no evidence of cryptic diversity.



**Key words:** Ephemeroptera, *Baetis tricaudatus*, COI gene region, genetic diversity, temporal population genetic structuring, biodiversity, California


## INTRODUCTION

Biodiversity is an important aspect of the natural world and a cornerstone of resilience in ecosystems (Folke et al. 2004). Despite its importance, some investigators examining the genetic diversity of organisms have found that species concepts based on morphology have underestimated the actual biodiversity of systems (Witt et al. 2006, Saunders 2008, Kieneke et al. 2012, Gebiola et al. 2012). Underestimation of biodiversity has occurred in many taxa, including the mayfly genus *Baetis* (Williams et al. 2006, Ståhls and Savolainen 2008, Lucentini et al. 2011, Jackson et al. 2014). Studies on the genetic diversity of organisms have contributed to a variety of scientific discussions, including those on species concepts and species delimitation (e.g., Agapow et al. 2004, Sites and Marshall 2004, DeSalle et al. 2005, Pons et al. 2006, De Queiroz 2007, White et al. 2014), concerns about the accurate estimation of biodiversity (e.g., Isaac et al. 2004, Zachos et al. 2013), and the relationships between diversity and geography (e.g., Hughes et al. 2003b, Szpiech 2008, Spitzer 2014). Nevertheless, much remains to be learned about the patterns and dynamics of genetic diversity.

An understanding of the dynamics of genetic diversity in freshwater ecosystems is especially important because of recent biodiversity losses in these environments (Jenkins 2003, Dudgeon et al. 2006, Moyle et al. 2011). Increased genetic structure, which is one aspect of population diversity, can contribute to a stabilizing portfolio effect (Carlson and Satterthwaite 2011). A portfolio effect describes the increased production and resiliency of a population that comprises spatially or temporally segregated subpopulations with diverse adaptations (Carlson and Satterthwaite 2011). The portfolio effect can be an important component in the stability and survival of freshwater species, such as Sockeye Salmon (Schindler et al. 2010). Other investigators examining the genetic diversity of freshwater organisms have found evidence for cryptic species, which are morphologically similar but, genetically, appear to be separately evolving lineages when described by methods such as an arbitrary threshold of genetic divergence (De Queiroz 2007). Investigators have found evidence for cryptic species in freshwater taxa, such as Ephemeroptera (e.g., Sweeney and Funk 1991, Ståhls and Savolainen 2008, Zhou et al. 2010, Lucentini et al. 2011, Webb et al. 2012, Jackson et al. 2014), Trichoptera (e.g., Jackson and Resh 1992, 1998, Pauls et al. 2010, Zhou et al. 2011, Harvey et al. 2012), Plecoptera (e.g., Mynott et al. 2011), Diptera (e.g., Smith et al. 2006b, Kim et al. 2012, Renaud et al. 2012), and other groups (e.g., Monaghan et al. 2005, Larson et al. 2012).

Genetic methods are particularly useful for examining biodiversity of freshwater invertebrates for many reasons (Mynott et al. 2011, Webb et al. 2012, Stein et al. 2014). For example, species-level identifications generally require adult specimens rather than the aquatic larval forms that are the life stage collected in many bioassesment and biomonitoring programs to assess the quality of freshwater habitats (Carter and Resh 2013). Furthermore, morphological identification of freshwater invertebrates, particularly the immature stages, is exceptionally difficult because many are small or have numerous instars, dynamic life histories, and systematic uncertainties (McCafferty et al. 2008). Many *Baetis* species, including *B. tricaudatus*, cannot be accurately and consistently identified using traditional morphological methods. Separating *B. tricaudatus* larvae from other congeneric taxa living in the same stream is currently impossible, so Jacobus et al. (2014) recommended identifying these taxa to the *rhodani* group. These taxonomic challenges can be mitigated and potentially solved by integrating molecular and morphological methods (Webb et al. 2012).

Many investigators using molecular methods to study genetic diversity in mayflies, including in the genus *Baetis*, have focused on spatial patterns, such as isolation by distance, and have found mixed results. Some investigators have found increases in genetic diversity or population structure with increasing geographic distance between populations (e.g., Smith et al. 2006a, Alexander 2007, Watanabe et al. 2010, Baggiano et al. 2011). Others have not found increasing genetic diversity or population structure with increasing distance between populations (e.g., Schmidt et al. 1995, Bunn and Hughes 1997, Hughes et al. 2003a, Peckarsky et al. 2005, Rebora et al. 2005, Zickovich and Bohonak 2007, Múrria et al. 2014). Population structure also can vary between closely related mayfly species (Peckarsky et al. 2005) and species that occupy the same geographic region (Monaghan et al. 2001, Baggiano et al. 2011).

Temporal variation in the genetic diversity of mayfly populations is an aspect of biodiversity that has received less study than spatial issues (Lucentini et al. 2011). Genetic diversity, whether spatially or temporally structured, is part of the population

diversity that gives rise to the portfolio effect found in salmon populations (Carlson and Satterthwaite 2011, Schindler et al. 2010). Like salmon, mayflies respond to unpredictable and dynamic environmental conditions, which might select for unique adaptations in certain cohorts or populations. Over time, these adaptations might accumulate to create a diverse portfolio of co-occurring populations with substantial genetic structure or even cryptic species, such as Lucentini et al. (2011) found in *B. rhodani.* Lucentini et al. (2011) studied populations from Italy and the UK and found evidence for 3 sympatric cryptic species that were temporally segregated. Adults of each putative species emerged at different times of year and took advantage of unique resources (Lucentini et al. 2011). Temporally structured portfolio effects might be especially important in mayfly species given the increase in disturbance of natural systems (Lucentini et al. 2011). Despite its importance, temporal population structuring of mayfly populations has received little study (Lucentini et al. 2011).

*Baetis tricaudatus* is thought to be the most widespread *Baetis* species in North America and occurs throughout the continent, except in the extreme southeast (Morihara and McCafferty 1979, McCafferty et al. 2010). Larvae of this species are collected frequently in bioassessment and biomonitoring programs, and *B. tricaudatus* is one the most abundant mayfly species in some systems, including a brown-water stream in Alberta, Canada (Clifford 1978) and the San Bernardino mountains in southern California (Spitzer 2014). Larvae play key roles in freshwater ecosystems as consumers of periphyton and particulate matter (Culp and Scrimgeour 1993) and as components in stream drift (Ciborowski 1983). *Baetis tricaudatus* populations also have dynamic life histories. For example, bivoltine *B. tricaudatus* populations have been observed in the northern part of its range in Saskatchewan (Webb 2002), whereas multivoltine *B. tricaudatus* populations have been observed in more central and southern parts of its range, including Idaho (Robinson et al. 1992).

We examined the genetic diversity of the COI gene region in *B. tricaudatus* collected from several sites in the Russian River drainage in northern California. Our objectives were to: 1) assess whether population structuring was present among years, seasons, or presumed cohorts; 2) assess whether population structuring was present among sites, streams, and watersheds; and 3) search for evidence to support cryptic diversity of *B. tricaudatus* at sites and times examined.

## METHODS

### *Study area*

The Russian River watershed in northern California drains ~3800 $km^2$ of Sonoma and Mendocino Counties and flows into the Pacific Ocean. The region has a Mediterranean climate, with 93% of precipitation occurring as rain during winter (NOAA 2009). The watershed is mostly rural–residential and agricultural, and grapes are the most notable and common crop (NOAA 2009).

The Russian River-mainstem collection site 1 (Figure 2.1) was in a wide, sunny channel with thick riparian vegetation and mostly silt and gravel sediment. The mainstem is part of a managed system in which water flow is regulated by upstream dams, but at least one scouring event occurred each winter during the study period and probably affected the benthic fauna (Resh et al. 1988). Collection site 2 is ~11 km from site 1

(Table 2.1, Figure 2.1) and is on Austin Creek, an unregulated tributary of the Russian River. Sediment at this site ranges from mostly large gravel in riffles to silt in pools. We chose sites 1 and 2 because high density of *B. tricaudatus* was expected year-round. We sampled 3 more sites in the Russian River watershed and 3 sites in the Sacramento River watershed (Table 2.1, Figure 2.1). We selected these sites on the basis of the presence of *B. tricaudatus* and to provide ecological contrast (e.g., land use and elevation) to the Russian River sites.

*Taxon sampling and identification*

We made monthly collections at sites 1 and 2, and we sampled the other 6 sites at least once (Table 2.2). We made monthly collections at sites 1 and 2 from September 2012 to September 2015 (total = 37 events). Sampling events were ~1 month apart to ensure collection of *B. tricaudatus* cohorts and to capture the natural variation of populations of *B. tricaudatus* within and between years. Two monthly collections at site 1 were more intensive than the rest to capture the genetic diversity within populations. One intensive collection was made before the rains in autumn 2014 (26 November 2014) and the other followed the rains in spring 2015 (15 May 2015). These 2 collection events are referred to as autumn and spring collections, respectively.

We collected larvae by disturbing the sediment in a variety of habitats, including riffles and pools, and catching suspended material with a D-frame net. We also used a D-net with a smaller mesh bag (500-µm) in an effort to capture early instars. To ensure consistent sampling effort among sites and dates, we collected for 20 min/sampling event, except for the 2 intensive sampling events, which were 40 min long.

We preserved specimens in 95% ethanol immediately after collection and transported them to the laboratory at University of California (UC) Berkeley for identification. All specimens were identified to the *rhodani* species group based on diagnostic characters outlined by Jacobus et al. (2014). Specimens were identified to species group because diagnostic characters for *B. tricaudatus* are sometimes indistinguishable and inconsistent (Jacobus et al. 2014). The *rhodani* group includes 4 species known from northern California: *B. tricaudatus, B. adonis*, *Baetis palisade***,** and *Baetis piscatoris* (Meyer and McCafferty 2008). For consistency, all identifications were made by a single taxonomist (NSO) and more difficult identifications were confirmed by other experienced taxonomists (e.g., L. Jacobus from Indiana University-Purdue University of Columbus and J. Webb from Rhithron Associates Inc.).

*DNA extraction, amplification, and sequencing*

Following identification, we chose *rhodani* species group specimens for DNA extraction. The number of specimens selected from each monthly collection at sites 1 and 2 ranged from 0 to 14 individuals, based on the number of specimens collected. We sequenced 45 and 44 specimens, respectively, from the November 2014 and May 2015 intensive sampling events (Table 2.2) and extracted DNA from a total of 1 to 178 specimens from each of the 8 sites.

We used reagents from a Qiagen DNeasy DNA extraction kit (Qiagen, Alameda, California) to extract DNA with slight modifications to the manufacturer's protocol. We removed specimens from 95% ethanol and dried them on a clean tissue before putting them into microcentrifuge tubes, to which we added proteinase K and AL buffer. We

lysed samples for ≥5 h at 56°C. After lysing, we placed samples in 95% ethanol and stored them as vouchers at the UC Berkeley Essig Museum of Entomology (EMEC numbers 1173400–1173692). We followed manufacturer's instructions for the rest of the protocol.

We amplified and sequenced the mitochondrial gene region cytochrome oxidase I (COI) because it is variable at the intraspecific level (Hebert et al. 2003a, b, Hajibabaei et al. 2006, Webb et al. 2012). Sequence divergence <2% is regarded as the amount of variation expected within populations or among individuals of the same species (Hebert et al. 2003b, Zhou et al. 2009, White et al. 2014), but the cutoff to differentiate species varies with taxon and study (DeWalt 2011). The COI gene region has been used effectively to identify *Baetis* species in previous studies (Webb et al. 2012, White et al. 2014). We used universal primers LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994) to amplify a 658 base pair (bp) fragment of COI. Polymerase chain reaction (PCR) for COI was done with the following ratio of reagents: 17.5 µL sterile $H_2O$, 2.5µL iTaq (Biorad) buffer, 2.5 µL $MgCl_2$ (25 µM), 0.5 µL Deoxynucleotide (dNTPs) (10 µM), 2.5 µL of each primer (10 µM), 0.25 µL iTaq polymerase, and 1 to 2 µL of the extracted template DNA. For amplification, the following protocol was used: 5 min initial denaturing at 94°C, 15 cycles of 30 s at 94°C, 30 s at 45°C, and 45 s at 72°C, 20 cycles of 30 s at 94°C, 30 s at 55°C, and 45 s at 72°C, and a final extension step of 72°C for 5 min.

We cleaned PCR products with Exonuclease I - Shrimp Alkaline Phosphatase (ExoSAP) following the manufacturer's protocol (Thermo Fisher Scientific, Waltham, Massachusetts). We incubated samples at 37°C for 15 min, then 80°C for another 15 min. Once cleaned, we sent PCR products to the UC Berkeley DNA Sequencing Facility.

*Sequence editing and phylogenetics*

We used the program Geneious Pro (version 6.1.4; Kearse et al. 2012) to create and edit contigs of individual sequence reads, build an alignment, generate basic sequence statistics, and conduct phylogenetic analyses. Contigs were edited individually and most had 99 to 100% high-quality (HQ) bases. We discarded contigs with <80% HQ bases because base-pair calls were not reliable. We also discarded sequences with <80% overlap between forward and reverse reads to ensure that sequences used in analyses were effectively proofread by overlapping strands. Overall, 37 of 408 contigs were removed because of low-quality reads or insufficient overlap. Contigs were translated into amino acids to check for stop codons and shifts in reading frame that could indicate the presence of nuclear-mitochondrial copies (numts), but none were detected.

After editing, we made an alignment with the MAFFT Geneious Pro plugin (version 7.017; Katoh and Standley 2013) and uploaded it to Figshare (available from: https://figshare.com/s/c7f7b1c33f4600256586). We uploaded all sequences to the Barcode of Life Database (BOLD; samples: NAT1–NAT827) and GenBank (accession numbers: KY580901- KY581193). We used the Basic Local Alignment Search Tool (BLAST) to compare generated sequences against GenBank sequences from laboratories with well-established Ephemeroptera taxonomists.

We created phylogenetic trees to explore species identifications and to evaluate relationships among taxa sampled from northern California. jModelTest 2 (version 01.10;

Darriba et al. 2012) indicated that the HKY85 substitution model best fit our data, so we used it to infer maximum likelihood trees with the Geneious Pro plugin PhyML (version 2.2.0; Guindon and Gascuel 2003). We assessed support for relationships by running 100 bootstrap replicates (Felsenstein 1985) and on the basis of selected GenBank sequences for the *B. tricaudatus* and *B. adonis* clades generated.

*Population genetics analyses*

For temporal analyses, we grouped genetic sequences from sites within the Russian River watershed and from all sites according to year, presumed cohort (January–April, May–August, September–December), and season (winter: December–February; spring: March–May; summer: June–August; and autumn: September–November) (Table 2.2). The presumed cohort grouping used may not represent actual cohort and emergence timing, which were not measured. However, cohorts were identified based on our field observations in the study area and provided an approximate estimate that is useful for comparison. We chose multiple temporal groupings to reflect a variety of life-history and temporal categories. For spatial analyses, we grouped sequences from sites 1 and 2 and compared them with: 1) sequences grouped from other sites within the watershed, and 2) sequences grouped from sites outside the watershed (Table 2.2) to reflect different scales of geographic distance.

Sample sizes in the temporal and spatial groups were unequal, so we performed analyses on: 1) all sequences and 2) standardized sample sizes (Table 2.2) based on rarification (Szpiech et al. 2008). We removed sequences from 2012 from the rarefied comparison because only 4 sequences from 2012 existed. For the spatial analysis, we randomly chose 8 sequences from the 2 main sites and compared them to 8 sequences from other sites within the Russian River watershed and 8 sequences collected from the Sacramento River watershed. We compared analyses based on all sequences to analyses based on standardized sample sizes to understand the effect of sample size on observed genetic diversity and population genetic statistics.

We exported sequence alignments from Geneious Pro (version 6.1.4; Kearse et al. 2012) for further analyses. We imported nexus files to POPART (Population Analysis with Reticulate Trees) (Leigh and Bryant 2015) for analysis and converted fasta files were converted to Arlequin project files (.arp) in PGDSpider (Lischer and Excoffier 2012) for analysis in Arlequin (version 3.5.1.2; Excoffier and Lischer 2010). We used these programs to describe intraspecific genetic variation and to create haplotype networks. Results were largely congruent, so our subsequent focus was on population genetic statistics generated in POPART, which were: $\Phi_{ST}$ (Table 2.3), number of haplotypes ($H_N$), and number of unique haplotypes ($H_U$; haplotypes that did not occur at any other site/time). We calculated $\Phi_{ST}$ with analysis of molecular variance (AMOVA; 1000 permutations) (Leigh and Bryant 2015). We grouped sequences based on collection times or locations as described above and calculated population genetic statistics and haplotype networks based on these groups. We used POPART to create median-joining haplotype networks to reconstruct intraspecific relationships and to identify temporal relationships among haplotypes.

## RESULTS

*Phylogenetic trees and clades*

A total of 371 sequences identified as belonging to the *rhodani* species group were collected. Phylogenetic analyses indicated 2 well-supported clades: *B. adonis* clade with 38 sequences (93% support) and *B. tricaudatus* clade with 333 sequences (100% support). Based on the well-supported *B. adonis* and *B. tricaudatus* clades and the much larger number of *B. tricaudatus* sequences, further analyses focused on sequences within the *B. tricaudatus* clade.

Of the 333 sequences that fell into the *B. tricaudatus* clade, 178 were from site 1, 128 were from site 2, 19 were from other sites within the Russian River watershed, and 8 were from sites outside the watershed (Table 2.2). The *B. tricaudatus* sequences were generated from 29 of the monthly collections from 2012–2015 (Table 2.2). Of the 38 sequences that fell into the *B. adonis* clade, 24 were from site 1, 6 were from site 2, 6 were from other sites within the Russian River watershed, and 2 were from sites outside of the watershed. *Baetis adonis* sequences were collected on 17 sampling dates. Genetic divergence between the *B. tricaudatus* and *B. adonis* clades was ≥8.2%. Sequences within the *B. adonis* clade had 2% within group-diversity and sequences within the *B. tricaudatus* clade had 1.7% within group diversity (Table 2.2).

Species identifications based on BLAST results were generally consistent with our own identifications. For both *B. tricaudatus* and *B. adonis* clades, many of our sequences were most similar to those from Southern California Coastal Water Research Project (SCCWRP) and Webb et al. (2012). For example, sequences in our *B. tricaudatus* clade were similar to *B. tricaudatus* sequences with accession numbers HQ938581 (from SCCWRP) and JQ663270 (Webb et al. 2012). Sequences within our *B. adonis* clade were similar to *B. adonis* sequences with accession numbers HQ941363 (from SCCWRP) and JQ661573 (from Webb et al. 2012).

*Temporal dynamics of genetic diversity*

We analyzed data in several ways to assess whether genetic diversity or population structure varied from year to year, between presumed cohorts, or from season to season. Analyses were done with all sequences generated for a given treatment (Appendix 2.1) and with rarefied sampling to control for unequal sampling between events (see below). Overall, genetic diversity in temporal samples varied by <0.5% (1.1–1.6%) across the rarefied annual, cohort, and seasonal comparisons within the Russian River watershed (Table 2.2). Population structuring was nonsignificant or very weak in the 3 temporal groupings, as indicated by the low $\Phi_{ST}$ values (Table 2.3). However, for presumed cohorts and season, *p*-values were >0.05, so accurate comparisons could not be made among the temporal groupings. Regardless, population structuring by time appeared very weak at best.

***Annual comparisons*** Annual variation in genetic diversity in rarefied comparisons varied by only 0.3%, from a low of 1.1% in 2014 to a high of 1.4% in 2013 (Table 2.2). Sample year 2013 had the lowest number of haplotypes ($H_N$ = 12) and unique haplotypes ($H_U$ = 4) in the rarefied samples (Table 2.2). The years 2014 and 2015 shared the highest $H_N$ (14) while 2015 had the highest $H_U$ (7) (Table 2.2). Two major haplotypes, A ($N$ = 91 individuals, 37%) and B ($N$ = 98 individuals, 40%), were present across the 3 y included in rarefied analyses (Figure 2.2). Other minor haplotypes, most of which differed by only a single base-pair change from either Haplotype A or B also were present (Figure 2.2).

The total number of base-pair changes separating haplotypes ranged from 1 to 9 (Figures 2.2–2.7), with Haplotypes A and B differentiated by 2 base-pair changes (Figures 2.2–2.7).

***Cohort comparisons*** The presumed spring cohort had the lowest genetic diversity (1.3%) and the lowest number of haplotypes (9), but not the lowest number of unique haplotypes (the summer cohort had only 3; Table 2.2). The presumed autumn cohort had the highest genetic diversity (1.6%) and correspondingly high $H_N$ (16) and $H_U$ (8) (Table 2.2). Figure 2.4 shows the haplotype network for the presumed cohort comparisons. Haplotype A ($N$ = 62 individuals, 32%) and Haplotype B ($N$ = 85 individuals, 44%) were represented in similar proportions in each presumed cohort, revealing temporally well-mixed populations with no cryptic diversity.

***Seasonal comparisons*** Seasonal comparisons of genetic diversity showed the greatest variation (0.5%) among the temporal comparisons examined, ranging from a low of 1.1% in winter and spring samples to a high of 1.6% in autumn (Table 2.2). Absolute and unique haplotype numbers were correlated, with winter having the lowest $H_N$ (7) and $H_U$ (2), and autumn having the highest $H_N$ (11) and $H_U$ (5) (Table 2.2). As with the previous 2 comparisons, Haplotype A ($N$ = 49 individuals, 34%) and Haplotype B (N = 55 individuals, 38%) were again represented in similar proportions among seasons. The haplotype network for seasonal comparisons showed no structuring or cryptic diversity (Figure 2.4).

***Intensive sampling events*** The spring (2015) intensive sampling event at the Russian River mainstem site yielded 1.2% genetic diversity, or 71% of the genetic diversity collected within the Russian River watershed over the entire study period. The autumn (2014) intensive sampling event captured 1.4% genetic diversity, or 82% of the genetic diversity collected within the Russian River watershed over the entire study period. The autumn intensive sampling event also revealed a higher $H_N$ (14) than did the spring intensive sampling event (10) (Table 2.2). Haplotype networks for the 2 intensive sampling events in November 2014 (Figure 2.5) and May 2015 (Figure 2.6) showed the higher diversity in autumn than in spring samples. However, autumn samples had a higher $H_N$ (14) than spring (10) (Figures 2.5, 2.6). Haplotypes A and B were captured in both events.

*Spatial distribution of sampled haplotypes*

Spatial analyses were done with all sequences generated for a given treatment (Appendix 2.2) and with rarefied sampling to control for unequal sampling among events (see below). Rarefied analyses of sequences grouped spatially indicated that diversity at sites 1 and 2 was lower (0.7%) than diversity captured at sites within the Russian River watershed (1.0%), which was lower than diversity captured at sites outside the watershed (1.2%) (Table 2.2). The low $\Phi_{ST}$ value indicated a well-mixed population with minor structuring spatially and no cryptic diversity (Table 2.3). The $p$-value associated with the rarefied spatial $\Phi_{ST}$ was <0.05. The small amount of population structuring spatially probably was the result of the presence of 2 unique haplotypes at Putah Creek site that

were not collected at other locations. The haplotype network confirmed a population with little structuring (Figure 2.7).

## DISCUSSION

The COI gene region of sampled *B. tricaudatus* populations showed genetic diversity indicative of a single species with little or no temporal or spatial population structuring. The *rhodani* group species collected in our study, *B. tricaudatus* and *B. adonis*, formed distinct clades that had genetic differences expected between 2 congeneric species (Ball et al. 2005). Morphological differentiation between these species can be difficult or impossible, but our genetic approach supports the existence of 2 discrete species.

We found little evidence for cryptic diversity within *B. tricaudatus* from the Russian River, a result that differs from those of previous studies done on various ranges of this taxon (Webb et al. 2012, Spitzer et al. 2014). A number of factors, including sample sizes, differing geographic coverage, and environmental factors may explain the conflicting results observed between our and earlier studies. For example, Webb et al. (2012) examined a large number of sites across most of North America, whereas populations were sampled from 2 neighboring watersheds in our study. Cryptic diversity is more likely to be detected over a broad than over a narrow geographic range. Spitzer et al. (2014) sampled populations throughout the mountains of southern California across a geographic range similar in size to our study, but the drier intervening habitats in southern California may have limited dispersal among streams, increasing population structuring. Populations subject to the more extreme droughts in southern California may have increased genetic diversity as a result of genetic drift. For example, if Haplotypes A and B occurred at sites 1 and 2 but drought resulted in occurrence of Haplotype A only at site 1 and Haplotype B only at site 2, populations might appear more diverse than they really are.

Authors of studies based on DNA barcoding approaches often interpret cryptic diversity at different levels of sequence divergence. Maximum % divergence in our study was 1.7%. Some authors have recommended a conservative cutoff of ≥3% for cryptic species (Hebert et al. 2003a, Sweeney et al. 2011). Others consider populations diverging by only 1–2% as distinct species (Hebert et al. 2003b, Zhou et al. 2009, White et al. 2014). Based on the less conservative cutoff of 1–2%, cryptic species might have been suspected in our study, but the patterns of population structure based on haplotype networks provided no evidence of cryptic diversity. In comparison, authors of studies of *B. tricaudatus* populations (Jackson et al. 2014, Stein et al. 2014) have suggested that a 1% divergence in the COI gene region is sufficient diversity to support cryptic species. However, these authors did not report how the diversity they found was structured, e.g., via haplotype networks.

The lack of temporal population structuring of *B. tricaudatus* in our study differs from results found by Lucentini et al. (2011) in *B. rhodani*. Their molecular analyses of western European populations of B. *rhodani* supported the existence of 3 co-occurring, temporally segregated cryptic species (Lucentini et al. 2011). However, we found no evidence for temporal structuring in northern California populations of *B. tricaudatus*, in spite of the similar life-history characteristics between these 2 taxa; e.g., both species can

be multivoltine and have dynamic life histories with differing voltinism in different habitats (Humpesch 1979, Brittain 1982, Robinson et al. 1992, Webb 2002).

Lucentini et al. (2011) hypothesized that temporal segregation of cryptic species within the *B. rhodani* species group arose from different emergence times of adult cohorts. We have limited information on emergence times, but we have no genetic evidence of temporally segregated cohorts in this system. Our data indicate that interbreeding does occur among cohorts, and *B. tricaudatus* emergence probably is less synchronous in the Russian River watershed than emergence of *B. rhodani* cohorts sampled by Lucentini et al. (2011) in western Europe. Our analyses support previous reports that larval *B. tricaudatus* populations often consist of multiple cohorts without discrete cohort emergence (Robinson et al. 1992, Webb 2002, Spitzer 2014).

Lucentini et al. (2011) suggested that cryptic diversity in *B. rhodani* was advantageous for resource partitioning and conferred a type of portfolio effect. A portfolio effect, or temporal structuring, did not appear to be the case for *B. tricaudatus* in the Russian River watershed because we did not find evidence for either cryptic species or temporal structuring of genetic diversity. Resource partitioning could be advantageous if sites were variable temporally, which is not the case for the Russian River mainstem site, which is in a managed river with relatively consistent flow throughout spring and summer. The lack of temporal genetic structuring in our study might be explained in part by phenotypic plasticity within the sampled populations. For example, Peckarsky et al. (2005) found that traits associated with local adaptations in streams with and without fish were plastic and not captured by analysis of COI diversity.

Our results also indicate that populations of *B. tricaudatus* are well-mixed within and between watersheds, a finding that has been reported for other species within *Baetis* (Monaghan et al. 2001, Hughes et al. 2003b, Peckarsky et al. 2005). Highly mobile larvae , such as those of *B. bicaudatus* (Peckarsky 1996), probably maintain mixing within watersheds, whereas dispersal by adults probably maintains mixing among watersheds, as has been reported for other *Baetis* species (Peckarsky et al. 2005).

Flight ability affects population structure. Jackson and Resh (1989) examined dispersal of adult aquatic insects in the Russian River watershed and found that species richness, number of individuals, and biomass decreased as distance from the stream increased. Petersen et al. (2004) caught >90% of mayflies within 60 m of their natal stream. Limited adult dispersal could partially explain the relationship between genetic diversity and geographic distance and the unique haplotypes found outside the watershed. The slight increase in genetic diversity (0.7% at sites 1 and 2; 1.0% at other sites within the watershed; and 1.2% at sites outside the watershed) that corresponded with increased distances between sites might indicate the amount of gene flow that occurs at varying spatial scales. Nevertheless, gene flow certainly occurs between sampled *B. tricaudatus* populations, as indicated by the similar proportions of the same 2 dominant haplotypes across sampling sites and watersheds and the consistently low $\Phi_{ST}$ values.

Patterns in genetic structure can be the result of historical events and may not necessarily indicate current dispersal patterns. A population bottleneck in the mayfly *Ephemerella inconstans* was hypothesized as the cause of low levels of polymorphism across a large (200 km) and diverse (forested, agricultural, and residential) study area (Alexander and Lamp 2008). Our study had a similar range, so an historical event, such as a population bottleneck, could explain the low genetic diversity in our study.

The higher % diversity at Austin Creek (Site 2) than over the entire study could indicate patchy recruitment by a small number of females. Patchy recruitment has been suggested in other studies of mayflies (Schmidt et al. 1995, Rebora et al. 2005). However, the 1.7% diversity in our study was spread out over time, as indicated in the haplotypes networks, so we think patchy recruitment from only a few egg masses is unlikely. The higher diversity at site 2 could be a result of larger numbers of individuals, but additional research is needed to test this hypothesis. Our results indicate that gene flow between streams and watersheds in the study area is sufficient to keep populations temporally and spatially well-mixed.

Our autumn and summer intensive sampling results suggest that, for *B. tricaudatus* in the Russian River, most diversity within a watershed can be captured in a single intensive collection event. Bergsten et al. (2012), who studied *Agabini* diving beetles in the family Dytiscidae, found that a sample size of 70 individuals was necessary to capture 95% of intraspecific diversity, a finding in agreement with ours. However, results from studies in which evidence of cryptic diversity was found within a single watershed or neighboring watersheds (e.g., White et al. 2014, Jackson et al. 2014) suggest the need for more robust sampling schemes in order to fully capture the genetic diversity of a taxon.

Extensive cryptic diversity has been found in freshwater organisms (e.g., Funk et al. 1988, Lucentini et al. 2011, Webb et al. 2012) and has contributed to the ideas that freshwater biodiversity is underestimated and that molecular methods can help identify the true biodiversity of freshwater systems (Sweeney et al. 2011, Jackson et al. 2014). Our data support separation of *B. adonis* and *B. tricaudatus* as 2 species, despite the morphological similarity of their larvae. Interpretation of results, such as the value for % genetic divergence used to differentiate putative species, can change conclusions. The *B. tricaudatus* populations we sampled do not differ genetically more than expected for a single species and have little or no genetic structure at the COI gene region. We acknowledge limitations associated with use of a single mitochondrial gene region and suggest continued research on temporal and spatial patterns of genetic diversity. An improved understanding of genetic diversity will be needed to conserve biodiversity and manage natural systems.

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# APPENDICES

**Appendix 2.1**. Nonrarefied temporal dynamics of genetic diversity.

When all sequences were compared, % genetic diversity varied ≤0.8% (0.9–1.7%) among years, approximate generations, and seasons in the Russian River watershed. Diversity captured in 2012 (which had only 4 sequences) was the least among the years (0.9%) and corresponded with the lowest $H_N$ (3) and $H_U$ (0) among years. 2014 and 2015 both contained 1.5% genetic diversity, but 2014 had the highest $H_N$ (20) and $H_U$ (10) (Table 2.2). The presumed spring cohort contained the lowest % diversity (1.2%), $H_N$ (13), and $H_U$ (7) of the presumed cohorts. The presumed summer cohort had the highest % diversity (1.7%), $H_N$ (19), and $H_U$ (10) of the presumed cohorts. Winter samples had the lowest % diversity (1.1%) and $H_N$ (7). Summer and autumn had the highest % diversity (1.6%) of the seasons. However, summer also had the lowest $H_U$ (1) among seasons, whereas spring had the highest $H_N$ (24) and $H_U$ (15) (Table 2.2).

**Appendix 2.2.** Non-rarefied spatial distribution of sampled haplotypes

When all sequences were considered, the maximum diversity captured within the Russian River watershed (1.7%) was present at site 2 (Table 2.2). In comparison, diversity among sequences collected from outside of the watershed was 1.2%. Spatial population structuring was very weak, as indicated by the low $\Phi_{ST}$ values (Table 2.3). When all sequences were compared, sites outside the Russian River watershed had the lowest $H_N$ (5) and sites within the Russian River watershed (excluding sites 1 and 2) had the lowest $H_U$ (0). Site 1 had the highest $H_N$ (26) and $H_U$ (16) (Table 2.2).

**Table 2.1.** Global positioning system coordinates for all sites where *Baetis tricaudatus* samples were collected and successfully sequenced.

| Site | Latitude (N) | Longitude (W) | Elevation (m) |
|---|---|---|---|
| Russian River watershed | | | |
| Russian River mainstem | 38.504 | –122.93 | 9 |
| Austin Creek | 38.511 | –123.075 | 46 |
| Salmon Creek | 38.356 | –123.004 | 30 |
| Austin Creek Site 2 | 38.506 | –123.07 | 20 |
| Dutch Bill Creek | 38.453 | –122.984 | 53 |
| Sacramento River watershed | | | |
| Capell Creek | 38.495 | –122.243 | 156 |
| Putah Creek | 38.492 | –122.027 | 45 |
| Schneider Creek | 39.917 | –121.065 | 1253 |

**Table 2.2.** Number of sequences, number of haplotypes ($H_N$), number of unique haplotypes ($H_U$), and % genetic diversity present in each temporal and spatial grouping based on all data and rarefied data. For temporal groups, numbers are from collections made within the Russian River watershed. Numbers in parentheses are calculated based on specimens collected from all sites.

| | All data | | | | Rarefied data | | | |
|---|---|---|---|---|---|---|---|---|
| Site/time | Sequences | $H_N$ | $H_U$ | % diversity | Sequences | $H_N$ | $H_U$ | % diversity |
| 2012 | 4 | 3 | 0 | 0.9 | – | – | – | – |
| 2013 | 82 (89) | 12 (14) | 3 (5) | 1.4 (1.5) | 82 | 12 | 4 | 1.4 |
| 2014 | 113 | 20 | 10 | 1.5 | 82 | 14 | 5 | 1.1 |
| 2015 | 126 (127) | 18 (19) | 9 (10) | 1.5 (1.5) | 82 | 14 | 7 | 1.3 |
| Spring cohort | 113 | 13 | 7 | 1.2 | 65 | 9 | 4 | 1.3 |
| Summer cohort | 147 (148) | 19 (20) | 10 (10) | 1.7 (1.7) | 65 | 11 | 3 | 1.4 |
| Autumn cohort | 65 (72) | 16 (18) | 5 (7) | 1.6 (1.7) | 65 | 16 | 8 | 1.6 |
| Spring | 178 | 24 | 15 | 1.4 | 36 | 8 | 3 | 1.1 |
| Summer | 49 (50) | 8 (9) | 1 (1) | 1.6 (1.6) | 36 | 8 | | 1.2 |
| Autumn | 62 (69) | 16 (18) | 5 (7) | 1.6 (1.7) | 36 | 11 | 5 | 1.6 |
| Winter | 36 | 7 | 2 | 1.1 | 36 | 7 | 2 | 1.1 |
| Site 1 | 178 | 26 | 16 | 1.6 | 8 | 4 | 2 | 0.7 |
| Site 2 | 128 | 18 | 9 | 1.7 | | | | |
| Russian River watershed | 19 | 6 | 0 | 1.7 | 8 | 3 | 0 | 1.0 |
| Sacramento River watershed | 8 | 5 | 2 | 1.2 | 8 | 5 | 2 | 1.2 |
| Autumn intensive | 45 | 14 | – | 1.4 | 44 | 14 | – | 1.4 |
| Spring intensive | 44 | 10 | – | 1.2 | 44 | 10 | – | 1.2 |

**Table 2.3.** Population genetics statistics for each temporal and spatial grouping based on all data and rarefied data. ns = not significant, $p > 0.05$.

| | All data | | Rarefied data | |
|---|---|---|---|---|
| Grouping | $\phi_{ST}$ | $p$ | $\phi_{ST}$ | $p$ |
| Year | ns | <0.001 | ns | <0.001 |
| Presumed cohort | 0.003 | 0.707 | 0.007 | 0.177 |
| Season | 0.003 | 0.263 | 0.019 | 0.071 |
| Russian River watershed | ns | <0.001 | ns | <0.001 |
| All sites | 0.008 | 0.05 | 0.098 | 0.049 |

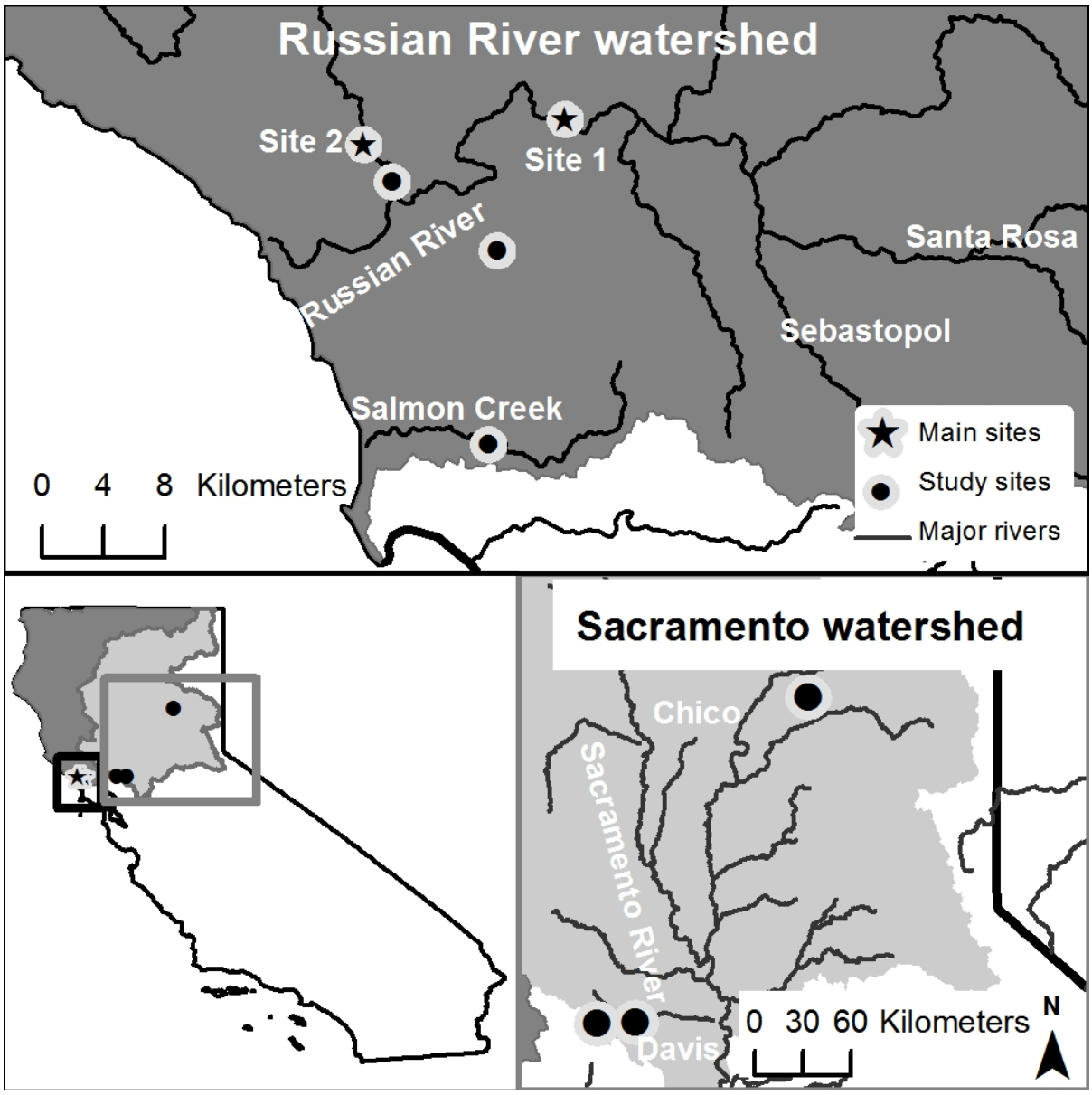


**Figure 2.1.** Map of collection sites. In the map of California (lower left), watersheds are delineated by hydrologic unit code (HUC) 8. Sites 1 and 2 are indicated with stars and the other 6 collecting sites are marked with circles.

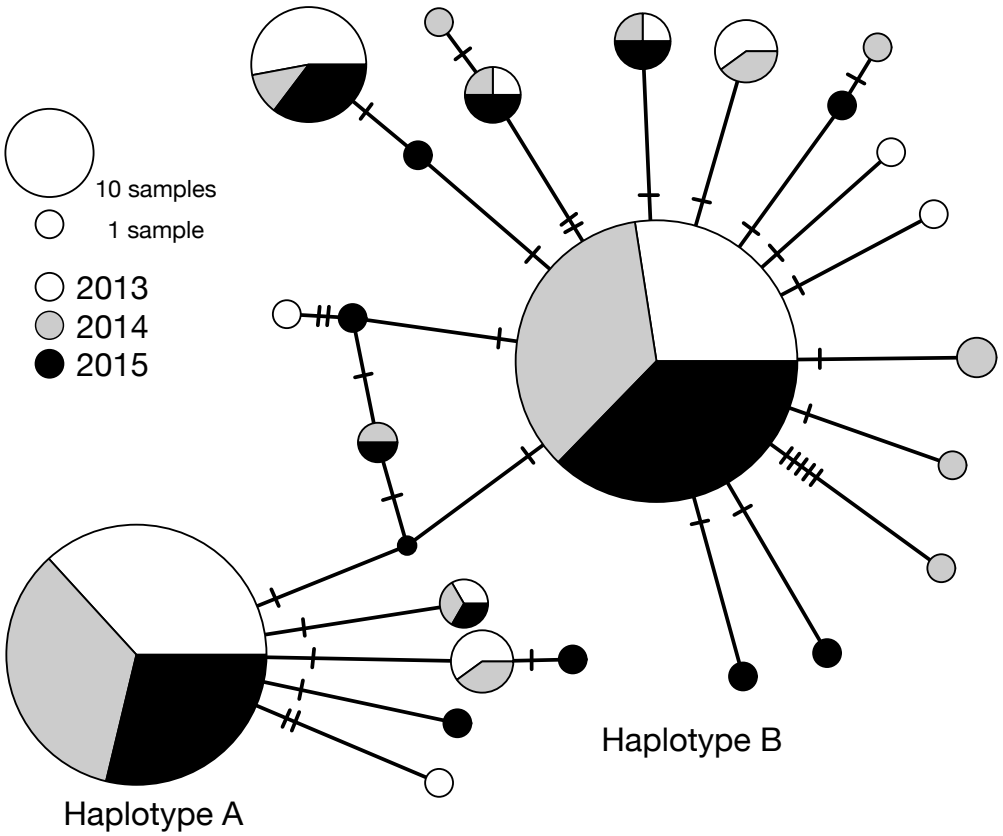


**Figure 2.2.** Median-joining haplotype network with rarefied data that indicates years that haplotypes were present. Circles represent haplotypes, and circle sizes represent the number of individuals with that haplotype. Shading corresponds to collection year, so solid circles represent haplotypes that were present in only 1 year. The small hash marks on lines connecting the different haplotypes represent base-pair changes, which are additive on either side of a circle.

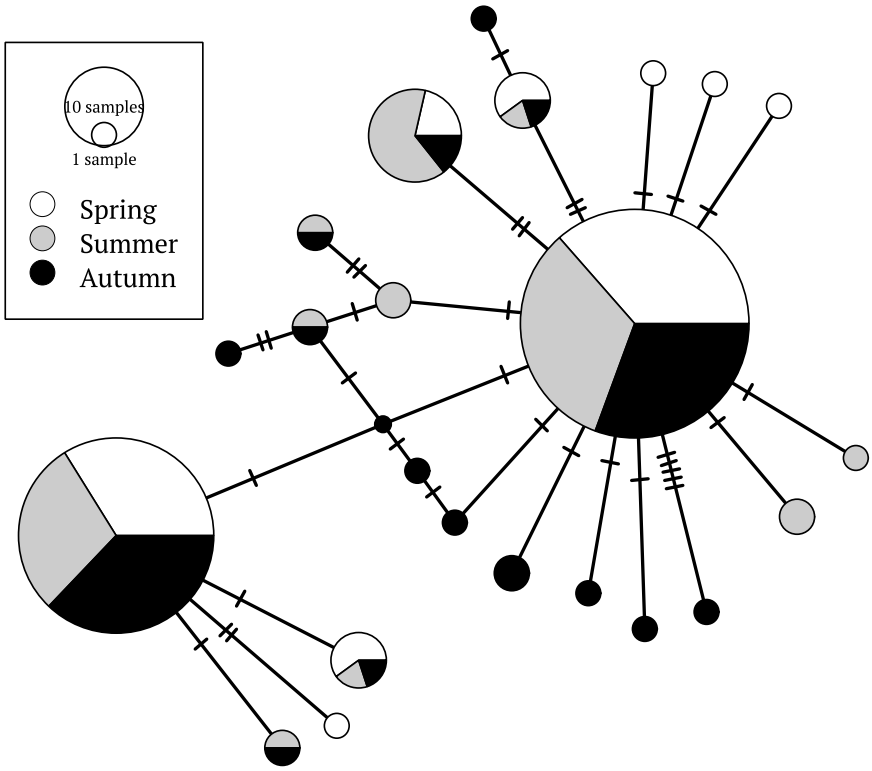


**Figure 2.3.** Median-joining haplotype network with rarefied data that indicates presumed cohorts in which haplotypes were present (spring: January–April, summer: May–August, autumn: September–December). See Fig. 2.2 for explanation of figure.

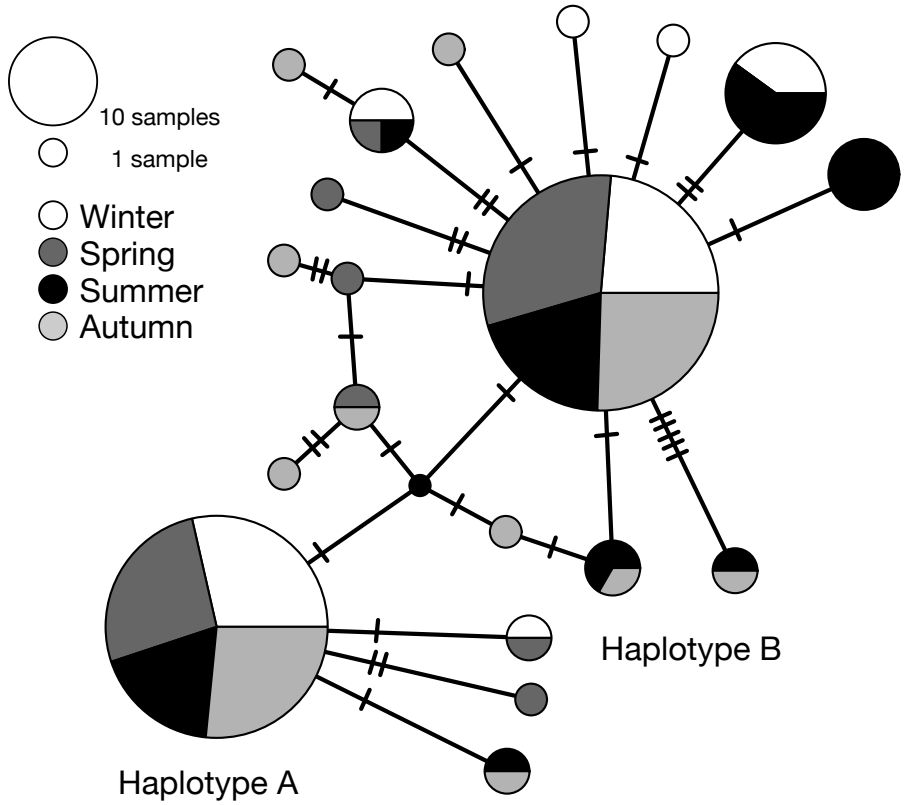


**Figure 2.4.** Median-joining haplotype network with rarefied data that indicates seasons when haplotypes were present (winter: December–February, spring: March–May, summer: June–August, autumn: September–November). See Fig. 2.2 for explanation of figure.

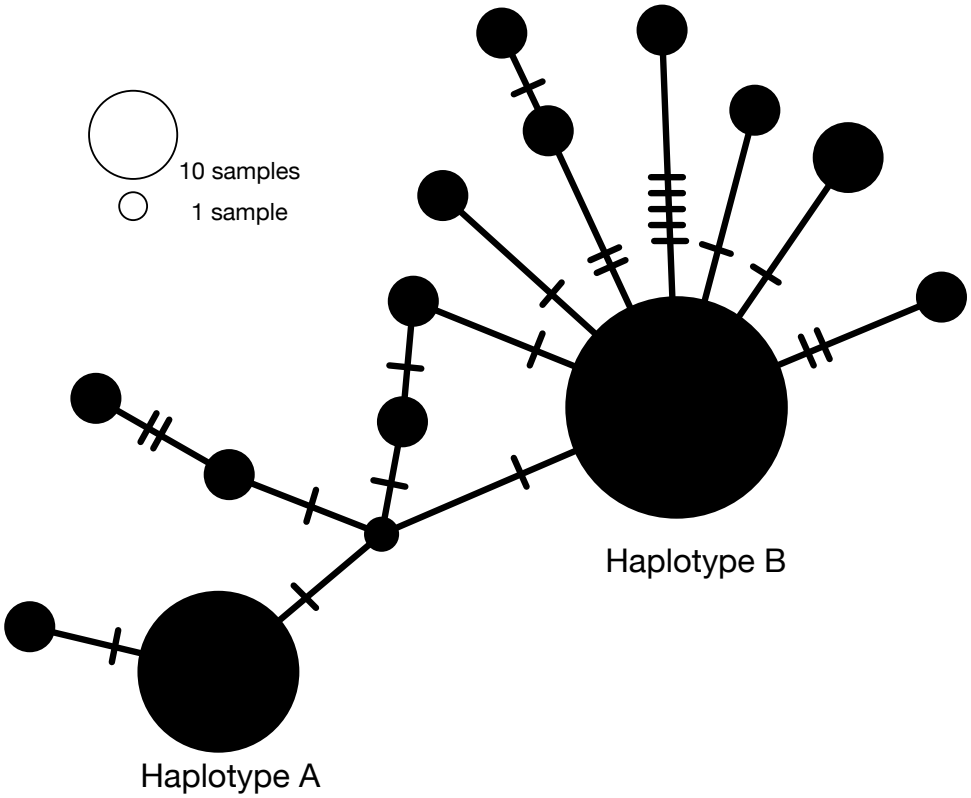


**Figure 2.5.** Median-joining haplotype network for intensive autumn (November 2014) sampling. See Fig. 2.2 for explanation of figure.

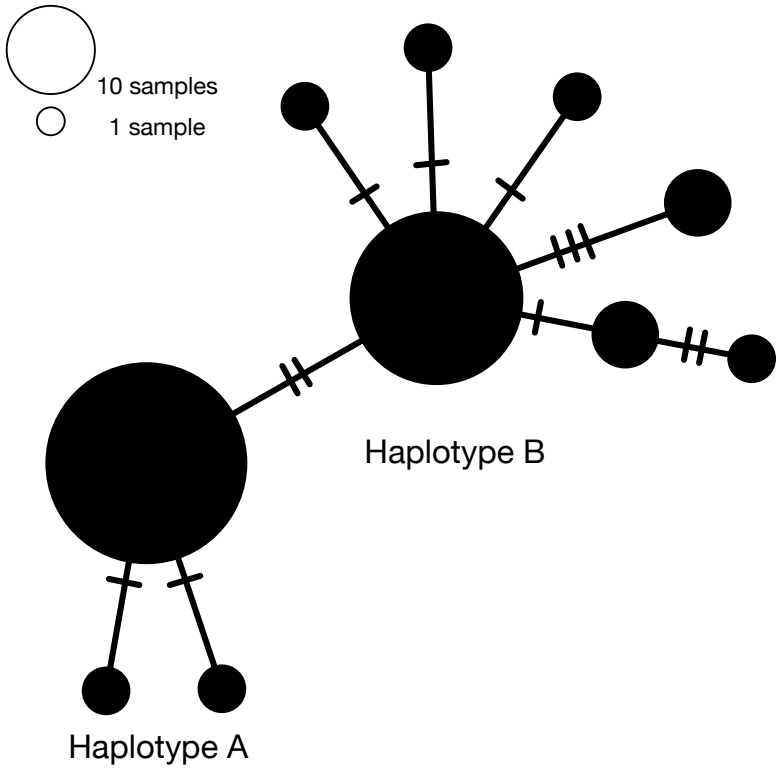


**Figure 2.6.** Median-joining haplotype network for intensive spring (May 2015) sampling. See Figure 2.2 for explanation of figure.

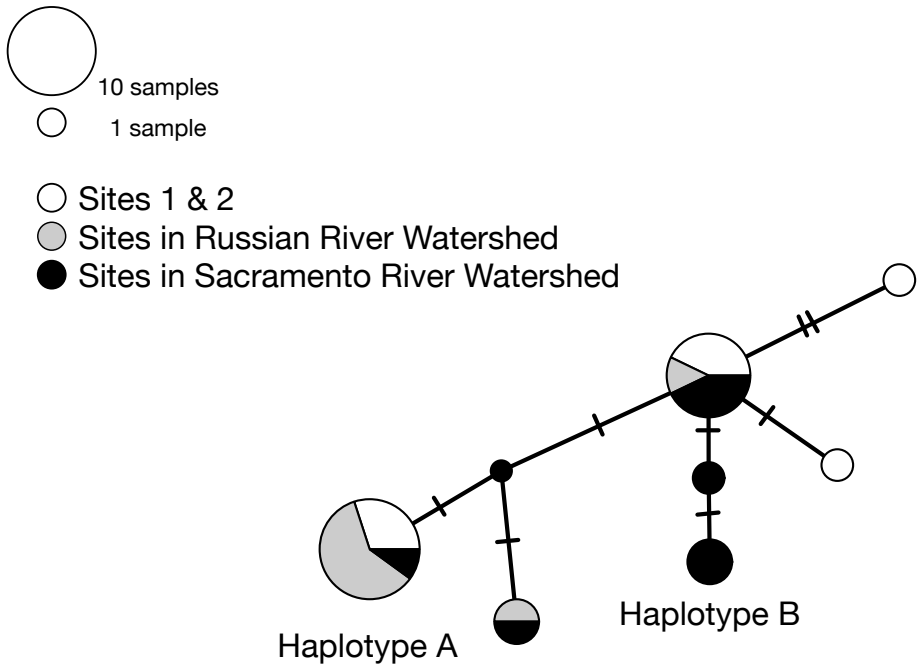


**Figure 2.7.** Median-joining haplotype network with rarefied data that indicates sites where haplotypes were collected. See Figure 2.2 for explanation of figure.

# CHAPTER 3

## Invertebrate Biota in Upper Klamath Lake, Oregon: Assemblage Composition and Correlation with Environmental Variables

**Invertebrate Biota in Upper Klamath Lake, Oregon: Assemblage Composition and Correlation with Environmental Variables**


## ABSTRACT

In this study we describe benthic invertebrate taxa collected from Upper Klamath Lake (UKL), Oregon and compare the assemblages found in different habitats, regions, and summer months. Like many lakes, excessive nutrient loading and anthropogenic disturbance have led to increasingly poor water quality in UKL. Large blooms of the cyanobacteria *Aphanizomenon flos-aquae* (AFA) occur in UKL every summer as a result of nutrient loading and contribute to areas of suboxia or temporary anoxia. Benthic invertebrates increase autochthonous nutrient cycling in lakes, which can contribute to poor water quality. To better understand the role that benthic invertebrates play in benthic nutrient flux and their relationships to environmental variables that might influence their distribution, sixteen abiotic parameters were measured and DISTLM tests were run in PERMANOVA+. We found that benthic invertebrate assemblages were comprised of 73 distinct taxa and differed among habitats, regions and months. The environmental variables most correlated to fauna at sites excluding the trench habitat were bottom temperature, depth, percent sand, percent silt, and percent clay. In the central region of the lake, which was analyzed separately because trench habitat was only present in the central region, benthic invertebrate assemblages differed among habitats but not months. Percent silt, depth, and pH were the environmental variables that explained the most variance in invertebrate assemblages in the central region of UKL.



**Key words:** benthic invertebrates, spatial dynamics, temporal dynamics, hypereutrophic lake


## INTRODUCTION

Over the past century, water quality has severely declined in freshwater ecosystems globally (Ji *et al.*, 2011; Nelson & Steinman, 2013). As a result, biodiversity has suffered and many freshwater organisms have gone extinct or are threatened with extinction (Moyle, Katz & Quiñones, 2011). Algal blooms that degrade water-quality pose one of the most pressing threats to freshwater biodiversity worldwide (Brooks et al. 2016). Algal blooms occur naturally in freshwater ecosystems; however, increased nutrient input and changes in water management such as diversions for human use have created conditions favorable to dense, harmful algal blooms (Brönmark & Hansson, 2002; Bradbury, Colman & Dean, 2004a; Reynolds *et al.*, 2004).

As a result of threats to both biodiversity and human health, many efforts are being made to understand and improve water-quality in lakes such as Upper Klamath Lake (UKL) in south-central Oregon. For example, total maximum daily load (TMDL) models have been designed to test and set limits on nutrient inputs into UKL (Walker, 2001). However, in many cases, once a hypereutrophic state is reached in a lake, nutrients can be recycled and it can take decades, centuries and potentially even millennia for restoration efforts to improve water quality (Carpenter, 2005). In UKL, internal (autochthonous) input from benthic processes contributes substantially to nutrient cycling

(Kuwabara *et al.*, 2007, 2009, 2012; Wood *et al.*, 2013). Therefore, autochthonous nutrient sources need to be considered in models seeking to understand and improve water-quality (*e.g*. Walker 2001).

Benthic invertebrates contribute to nutrient cycling in lakes through metabolic processes (Gardner et al, 1981) and bioturbation activities that affect diffusive and/or advective flux (Nogaro *et al.*, 2009). Bioturbation, the reworking and mixing of the sediment by biota living on or beneath the sediment-water interface, encompasses a wide range of species-specific behaviors that contribute in different ways to nutrient cycling (Biswas *et al.*, 2009; Wood *et al.*, 2013; Hood *et al.*, 2014). Metabolic and feeding activities are also species-specific, which results in substantial differences among species in nutrient efflux (Kuwabara *et al.*, 2016). Despite the contribution that benthic invertebrates likely make to nutrient loads and poor water quality in lakes, much remains to be learned about the composition of benthic invertebrate assemblages and what environmental variables are related to their distributions.

Upper Klamath Lake is an example of a freshwater ecosystem that has degraded water-quality, decreases in biodiversity, harmful algal blooms (Wood *et al*., 2013), and high benthic invertebrate densities (Kuwabara *et al*., 2016). UKL has become increasingly hypereutrophic over the past century, which is likely a result of watershed development (Bradbury, Colman & Reynolds, 2004b). Studies seeking to improve accuracy of the TMDL model proposed in 2001 (Walker, 2001) have found that benthic sources of soluble reactive phosphorus (SRP) contribute more P than external sources that are generally included in a TMDL model (Kuwabara *et al*., 2016). Phosphorus loading in UKL has created conditions that allow for dense blooms of the nitrogen-fixing cyanobacterium, *Aphanizomenon flos– aquae* (AFA*)* to occur every summer (Wood *et al.*, 2013). When near-monoculture blooms of the buoyant AFA senesce, they sink to the lakebed and cause large areas of UKL to become suboxic or anoxic (Kuwabara *et al*., 2009). Areas of hypoxia stress lake fauna, including zooplankton, benthic invertebrates, and fishes, including populations of the two endangered sucker species, the shortnose sucker (*Chasmistes brevirostris*) and Lost River sucker (*Deltistes luxatus*).

In order to effectively manage water-quality in UKL and protect lake fauna, more information is needed about autochthonous nutrient sources and the contribution of benthic invertebrates to nutrient loading. More specifically, knowledge of the relationships between benthic nutrient flux and benthic invertebrates is necessary because of their potential contribution to autochthonous nutrient loading in UKL. In this study, we seek to fill this knowledge gap and improve our understanding of the role that benthic macroinvertebrates play in benthic nutrient flux and nutrient cycling in UKL and what environmental variables influence their distributions. In particular, we focus on 1) describing the benthic invertebrate fauna found in UKL, 2) determining whether benthic invertebrate assemblage composition differ among regions and habitats of the lake over three summer sampling events, and 3) exploring what environmental variables, including benthic nutrient flux, correlate with and potentially explain changes in benthic invertebrate assemblages.

# METHODS

*Study location*

Upper Klamath Lake (UKL) in south-central Oregon is one of the largest water bodies in the western United States (Figure 3.1). UKL has a surface area of approximately 260 $km^2$ and a mean depth of approximately 2.8 m. UKL is mostly shallow with a deep (15 m) trench that runs along the western edge of the central part of UKL. Water currents in the lake are mostly wind-induced and normally mix the lake from the surface to the bottom. The main tributaries of the lake, the Williamson, Sprague, and Wood Rivers, drain into the northern portion of the lake. Water from UKL discharges into Link River through Link Dam located at the southern part of the lake and eventually drains into the Klamath River, which winds through southern Oregon and northern California before reaching the ocean (Figure 3.1).

Upper Klamath Lake, a remnant of a large pluvial lake that receded approximately 10,000 years ago, was historically surrounded by extensive marshes and wetlands and was naturally eutrophic prior to development within the watershed (Bradbury *et al.*, 2004b; Colman *et al.*, 2004). However, over the past century, many wetlands have been filled and the watershed is now largely cattle ranches or farms. Reclamation and development have likely contributed to UKL becoming hypereutrophic (Bradbury *et al.*, 2004b).

*Sampling design and methods*

The 21sampling sites in UKL were chosen using a stratified random design (Figure 1). The lake was partitioned into 3 geographic locations (north, central, and south) to reflect that most water enters UKL from tributaries in the north and exits through a dam in the south. Three sites were randomly selected from both littoral and open-water habitats within each geographic location. Littoral habitat was located near shore at a depth of approximately 2 m. Open-water habitat was between 2 and 8 m in depth. Three sites were also randomly selected from trench habitat, located in the central portion of the lake. Trench habitat was >8 m in depth.

A reconnaissance trip was made on May 1, 2013 to test sampling equipment and protocols. Site markers were also placed in the lake to maximize constancy of site locations between the three sampling events. All 21 sites were sampled on May 23, June 13, and July 2, 2013, a time period that encompassed the development of the first AFA bloom in UKL (Morace, 2007).

*Collection and Processing of Benthic Invertebrates*

Benthic invertebrates were collected at each site using a tall Ekman grab modified to limit its collection depth of the soft UKL substrate to 10 cm. The volume sampled per grab was approximately 15.2 x 15.2 x 10 cm. Three replicate grabs were collected from each of the 21 sites during each of the 3 sampling events for a total of 189 grabs. Grabs were individually sieved in the field with a 500 μm mesh sieve to remove excess sediment. All material retained on the sieve was preserved with 10% buffered formalin and then transferred to 70% ethanol within a week of the collection event. Results from the three replicate grabs were averaged to provide single site-time samples.

In the laboratory, benthic invertebrates were sorted from each grab using a microscope at 7-10 × magnification. Less than 1% of the grabs were subsampled; however, when necessary the procedures described in Moulton *et al*. (2000) were used. Initially, representative taxa of chironomid midges and oligochaete worms were identified from slide-mounted specimens using standard keys to create a reference collection. Based on this collection, all invertebrates were identified to species or the lowest possible taxonomic level in the laboratory (Tables 3.2 and 3.2A). Operational taxonomic units were created when necessary for consistency of identification. Keys and information in Andersen et al. (2013) were used to identify chironomids, while Kathman & Brinkhurst (1998) was used to identify oligochaetes, and Davies & Govedich (2001) was used to identify leeches. Non-benthic microcrustaceans were not included in this study because they represented contamination from the sieving process. Polychaetes such as *Manayunkia speciosa* and nematodes were also not included in this study because they are inadequately sampled with 500 μm mesh.

*Collection and Processing of Physical and Chemical Parameters*

Environmental parameters were chosen and collected to help determine what variables influence benthic invertebrate distributions and also to inform whether there were correlations between benthic nutrient fluxes and benthic invertebrate assemblages. Water-column parameters including depth, temperature, specific conductivity, pH, and dissolved oxygen (Table 3.3) were measured during each sampling event using a YSI multiparameter sonde. Data was recorded by the sonde at 15-second intervals during vertical deployments.

Nutrient samples were collected from nonmetallic pore-water profilers (U.S. Patent 8,051,727 B1) designed and fabricated for previous studies of UKL (Kuwabara et al., 2009). Six independent sampling circuits per profiler were used to characterize dissolved-solute vertical gradients. Each profiler was set up to collect two samples from just above the sediment water interface, two from 1 cm below that interface, and two from 2 cm below the interface. The design allowed for two measurements of solute benthic flux per profiler. After collection, nutrient samples immediately refrigerated in darkness without acidification. Concentrations were determined for dissolved (0.2-μm filtered) nitrate-nitrite (EPA method 353.1), ammonia (EPA method 350.1), orthophosphate (SRP; EPA method 365.2), and silica (EPA method 370.1) by batch automated spectrophotometry (Aquakem 250, Thermo Scientific). Concentration units for dissolved nutrients are in terms of the element rather than the associated molecule, with the exception of silica as SiO2. For example, the concentration unit for dissolved ammonia is milligrams of nitrogen per liter (mg-N $L^{-1}$). Certified standards for all nutrients were purchased from Microgenics Corporation (Fremont, California), and a new calibration curve was established for each analyte on each analytical day as described in Kuwabara *et al.* (2016). For further quality assurance and quality control (QA/QC), certified standards and blanks were also analyzed every 10–20 samples. Method detection limits (MDL) for each analyte were determined and established for all samples reported. For nitrate and nitrite, the MDL was 0.01 mg-N $L^{-1}$. For ammonia, the MDL was 0.007 mg-N $L^{-1}$. For orthophosphate (SRP), the MDL was 0.002 mg-P $L^{-1}$. For silica, the MDL was 0.1 mg-SiO2 $L^{-1}$. For additional methods, see Kuwabra *et al.,* (2016).

Dissolved organic carbon (DOC) samples were collected in duplicate in baked 60-mL glass bottles with acid-washed fluoroethylene-polymer caps and filtered (0.7-µm baked glass-fiber filter) for analysis by high-temperature catalytic combustion (Vandenbruwane *et al*., 2007). Potassium phthalate was used as the standard. Low-DOC water (blanks less than 40 µg-C $L^{-1}$) was generated from a double-deionization unit with additional ultraviolet treatment (Milli-Q Gradient, Millipore Corporation). A new calibration curve was established on each analytical day (Kuwabra *et al.,* 2016). For further QA/QC, certified standards and blanks were also analyzed every 10–20 samples. For DOC, the MDL was 0.1 mg-C $L^{-1}$

Sediment was collected on June 13 and July 2 from each site (42 samples total) using a modified Ekman grab. For benthic chlorophyll *a* analysis, surficial sediment was collected from the top centimeter of bed material collected by fresh Ekman grabs. Following collection, samples were immediately placed in a plastic Petri dish within a sealed plastic bag and refrigerated. Each dish was subsampled in triplicate for benthic chlorophyll *a*. The surficial sediment for each replicate (0.785 $cm^2$) was collected on a glass-fiber filter and buffered with 1 mL of a supersaturated magnesium carbonate suspension (10 g $L^{-1}$). Water was removed from the buffered samples by vacuum at less than 0.35 $Kg/cm^2$ to avoid cell lysis. Samples were then frozen on dry ice and later at −80 °C and held in darkness for preservation until analyzed within 3 months of sampling (Thompson and others, 1981; Franson, 1985). Additional details can be found in Kuwaraba *et al.* (2016).

For physical and chemical analyses, sediment cores were collected from Ekman grabs. A modified Ekman grab was used to retrieve sediment and a core (5 cm diameter × 10 cm long) was removed from the center of the grab. Core depths ranged from 2 cm to 10 cm. Following collection, sediment samples were put in plastic jars and stored in a cooler for transportation to the laboratory.

For particle size analysis, sediment subsamples (2 per site) were dried at 105°C for 48 hours, finely ground, and sieved with a 2 mm sieve to remove gravel, roots and coarse organic debris. Dried samples were then rewetted with deionized water, mixed with 30% hydrogen peroxide ($H_2O_2$) to remove organic matter and left for approximately 6 hours. Next, samples were centrifuged and the supernatant was decanted. Samples were re-suspended with 20 mL water and analyzed for particle size using a LISST-PORTABLE|XR (Version 1.00). Results for sediment grain size were reported as percentage clay (<2 µm), silt (2 – <50 µm), and sand (50 – 2,000 µm). Organic content of the sediment samples was measured as loss-on-ignition (Boyle, 2004). Ten to 12 grams of wet sediment samples were weighed, dried at 105°C for 12 hours, ignited in an oven at 550°C for 2 hours, and then reweighed.

*Statistical Analyses*

Statistical tests were conducted using permutational multivariate analysis of variance (PERMANOVA) +1.0.1 (Clarke *et al*., 2014; Clarke & Gorley, 2015). PERMANOVA partitions variation in a data cloud as described by a resemblance matrix according to an ANOVA model. Three-way repeated measures PERMANOVAs using Bray-Curtis (dis)similarities based on $\log_{10}(x+1)$ densities were used to determine whether differences in species composition existed among habitats (littoral and open-water), regions (north, central, and south), and months (May, June, and July). Statistical

significance (α=0.05) among factors was determined using 999 permutations (Table 3.4). Two-way repeated measures PERMANOVAs using Bray-Curtis (dis)similarities based on $\log_{10}$ (x+1) densities were used to compare central sites and determine whether significant differences in species composition could be detected among habitats (littoral, open-water, and trench) and months (May, June, and July). Statistical significance (α=0.05) was again determined using 999 permutations (Table 3.3). Principal Coordinates Analysis (PCO) plots were then created to examine assemblage composition in two-dimensional space (Figures 3.2 and 3.2A).

Before running PERMANOVA analyses, PERMDISP was used to test for of the homogeneity of multivariate dispersions among groups of individual factors. Non-significant PERMDISP results indicate equally dispersed distances to centroids within each factor, meaning that significant PERMANOVA tests are not the result of unequal variances among factors. In addition, PERMDISP was used to compare the variances of the differences between months to ensure equality of variances across treatments for the repeated measures month factor (Table S3.1).

Taxa that contributed most to the dissimilarities between habitats, regions and months were identified using similarity percentages (SIMPER) analyses (Clarke *et al*., 2014) (Tables S3.2 and S3.3).

The DISTLM (DISTance Based Linear Models) approach was then used to analyze and model the relationship between the multivariate species composition data matrix and environmental variables (Clarke & Gorley, 2015). DISTLM identifies which of the measured environmental variables explain the most variance in community composition by partitioning variance according to a multiple regression model. Sediment data was only collected in June and July, so values for May were approximated as the average of June and July values, which were very similar. Several environmental parameters had missing data from individual site/times and benthic chlorophyll data from July was not available because of a laboratory equipment failure. To estimate missing value the "missing" tool in PRIMER was used. Before analyses, environmental variables were examined using draftsman plots and individually transformed when necessary. Variables were also normalized. DISTLM was run using the BEST model selection procedure and the Akaike Information Criterion corrected for small sample sizes (AICc) selection criterion. In each DISTLM run, marginal tests were also done to test for a significant correlation between the biota and each of the environmental variables on its own. Distance-based redundancy analysis (dbRDA) biplots were also created to visualize the resulting models from each DISTLM run (Figures 3.3 and 3.3A). Trench sites, which only occur in the central region of UKL, were only compared to littoral and open-water sites within the central region.

In order to determine what environmental variables influenced the distribution of benthic invertebrates, DISTLM was run using the ten physical parameters: bottom temperature, depth, conductivity, benthic chlorophyll *a*, dissolved oxygen, percent organic matter, percent clay, percent silt, and percent sand (Figure 3.3, Table S3.4). Collected parameters were chosen to quantify a wide variety of habitat characteristics that might influence benthic invertebrate distributions. To determine whether there were correlations between benthic nutrient fluxes and benthic invertebrate assemblages, DISTLM was also run using the six benthic flux parameters (Table S3.4). Both of these DISTLM runs were also done on central sites only (Figure 3.3A, Table S3.4).

## RESULTS

*Species composition*

The 53,653 benthic invertebrates included in the analyses comprised 73 distinct taxa from 32 different families. Of the 73 taxa, 24 were identified to species (or equivalent operational taxonomic unit), 30 to genus and 19 to taxonomic levels above genus (such as tribe, subfamily, or family). Overall, *Ilyodrilus frantzi*, *Varichaetadrilus pacificus*, *Procladius* sp. 1, cf. (confer, a species that closely matches another species) *Limnodrilus hoffmeisteri*, and *Cladotanytarsus,* respectively, were the most abundant taxa (Table 3.2). *Ilyodrilus frantzi* was one of the five most abundant taxa in every habitat, region and month. *Varichaetadrilus pacificus, Procladius* sp. 1, and cf. *Limnodrilus hoffmeisteri* were also among the five most abundant species in many groupings.

*Benthic assemblages*

For the analyses that did not include trench habitat, PERMDISP results were all non-significant, which lends support to the accuracy of the significant PERMANOVA tests, i.e. they are not just the result of unequal variances among factors (Table 3S.1). However, the PERMDISP result for the pairwise comparison of trench and open-water habitat was significant. A significant PERMDISP result indicates that the significant PERMANOVA test could be the result of unequal variances among factors. Such unequal variance is visible in Figure 3.2A.

The PERMANOVA analyses indicated that invertebrate composition differed among habitats, regions and months, although the greatest amount of variation was residual (Table 3.5). There was also a significant interaction between the factors region and habitat (Table 3.5). By far the largest component of variation that characterized benthic assemblages was residual, followed by region x habitat, region, month, and then habitat (Table 3.5). A PCO plot showed separation between the various factors, particularly between regions and habitats (Figure 3.2).

Within the central region of the lake, PERMANOVA indicated that invertebrate composition differed among habitats, although again the greatest amount of variation was residual (Table 3.5). Differences did not occur among months and there were no significant interactions between factors. The largest component of variation that characterized benthic assemblages in the central region of UKL was residual, followed by habitat and month components (Table 3.5). A PCO plot also showed that sites within the same habitat clustered together, with trench sites having the most within-group variance and open-water sites having the least (Figure 3.2A).

The role that various species contribute to differences (dissimilarity) among habitats, regions and months, based on similarity percentage procedure (SIMPER) analyses, indicated that differences in assemblages were in part the result of different compositions and abundances of the dominant taxa (Table S3.2). The species that contributed most to differentiation between littoral and open water habitats were the taxa Tanytarsini, *Cladotanytarsus*, and cf. *Chironomus* e.i. (early instar) (Table S3.2). Small Tubificidae with chaetae, Sphaeriidae (a family of bivalve mollusks), and *Fluminicola* (freshwater snails) contributed most to differentiation between both central and northern

regions of the lake as well as the central and southern regions. North and south were most differentiated by *Cladotanytarsus*, Tanytarsini, and *Varichaetadrilus* nr. (near) *pacificus.* Among months, May and June were most differentiated by *Helobdella* hatchlings, *Cladotanytarsus*, and small Tubificidae with chaetae. May and July were most differentiated by cf. (confer, a species that closely matches another species) *Chironomus* e.i., Tanytarsini, and *Cladotanytarsus.* June and July were most differentiated by undetermined Chironomini, cf. *Chironomus* e.i., and Tanytarsini (Table S3.2). Although the species mentioned above contributed the most to differences in benthic invertebrate assemblages collected from different regions, habitats and months, in each case their densities were similar between the compared regions, habitats and months. For example, the average densities of Tanytarsini, *Cladotanytarsus*, and cf. *Chironomus* e.i. were similar between littoral and open water habitats (Table 3.2), although they contributed the most to differences in assemblage composition between these two habitats.

Within the central region of the lake, *Aulodrilus pigueti, Limnodrilus silvani,* and *Cryptotendipes* contributed most to the difference in assemblages found in littoral and open water habitats. Although the average densities of *Aulodrilus pigueti* and *Limnodrilus silvani* appeared higher in littoral compared to open water habitat and *Cryptotendipes* density appeared higher in open water compared to littoral habitat, in each case the standard deviations were high so the densities could not be differentiated (Table 3.2A). Littoral and trench assemblages were most differentiated by *Aulodrilus pigueti,* Sphaeriidae, and *Quistadrilus multisetosus*. Open water and trench assemblages were most differentiated by small Tubificidae with chaetae, *Helobdella stagnalis*, and *Fluminicola.* May and June were most differentiated by *Helobdella* hatchlings, small Tubificidae with chaetae and *Cladotanytarsus.* Assemblages from May and July were most differentiated by cf. *Chironomus* e.i., *Cladotanytarsus* and small Tubificidae with chaetae. June and July were most differentiated by cf. *Chironomus* e.i., undertermined Chironomini, and small Tubificidae with chaetae (Table S3.2). In each case mentioned above, standard deviation values were high enough that differences in density values between the compared regions, locations or habitats could not be detected (Table 3.2A).

*Correlations to environmental variables*

Marginal tests from the DISTLM run that included physical variables as described above found that bottom temperature, bottom pH, benthic chlorophyll, water depth, percent clay, percent silt, and percent sand each had a significant correlation with invertebrate composition (Table S3.4). The step-wise selection procedure found that the best model included bottom temperature, percent silt, depth, percent clay and percent sand and explained 33% of the cumulative variation, (Figure 3.3). Marginal tests from the DISTLM run that included benthic flux parameters found that only dissolved organic carbon had a significant correlation with invertebrate composition (Table S3.4). The step-wise selection procedure found that the best model included just dissolved organic carbon and explained 4% of the cumulative variation.

Within the central region of UKL, marginal tests from the DISTLM run that included physical variables found that bottom pH, benthic chlorophyll, water depth, percent clay, percent silt, percent sand and percent organic matter each had a significant correlation with invertebrate composition (Table S3.4). The step-wise selection procedure found that the best model included water depth, percent silt, and bottom pH explained

37% of the cumulative variation, (Figure 3.3A). Marginal tests from the DISTLM run that included benthic flux parameters from central sites found that benthic flux of ammonium (NH4), iron (Fe) and manganese (Mn), as well as dissolved organic carbon had significant correlations with invertebrate composition (Table S3.4). The step-wise selection procedure found that the best model included just dissolved organic carbon and explained 16% of the cumulative variation.

*Environmental Variables*

The 21 environmental variables included in this study are discussed in detail in Stauffer-Olsen, Carter & Fend, (2017) and Kuwabara *et al.,* (2016). Generally, sediment composition was similar throughout the lake. On average, substrates consisted of equal parts sand (48 ±19%, n=42) and silt (48 ±18%, n=42) with a small amount of clay (4 ±2%, n=42). Three sites (LN03, LC02, and TR02), however, differed substantially from the other sites. Littoral sites had the highest mean organic matter of the habitats, while trench had the lowest.

Temperature was generally consistent throughout the lake spatially. However, mean temperature increased from 12.8 °C in May to 21.5 °C in July (Table 3.3). Mean pH showed a similar pattern and increased from 7.9 in May to 9.4 in July. Benthic chlorophyll and dissolved oxygen tended to decrease as site depth increased from shallow littoral sites to deep trench sites (Table 3.3). Average benthic chlorophyll also more than doubled from May to June. July samples of benthic chlorophyll were not included because of a freezer failure in the laboratory. Conductivity did not appear to follow any patterns spatially, but its average value did appear to increase from June to July (Table 3.5). Benthic fluxes of SRP and silica were consistently positive and did not appear to follow any consistent patterns according to the factors in our study. Benthic flux of ammonia was also consistently positive and appeared to increase with depth and through time to July. Benthic flux of dissolved organic carbon (DOC) was positive in shallow littoral habitat, but became increasingly negative with increased depth (Table 3.3). Conversely, DOC flux increased from negative to slightly positive during the sampling period from May to July. Diffusive benthic flux of both dissolved iron (Fe) and manganese (Mn) were consistently positive. Benthic flux of iron appeared to decrease as depth increased, a pattern that manganese also followed when values from littoral and trench sites were compared.

## DISCUSSION

*Species Composition*

Of the five most abundant taxa in UKL, several have been found to be abundant in other eutrophic lakes (Fischer & Beeton, 1975; Spencer & Denton, 2003), indicating that the main benthic taxa of UKL are not unusual for nutrient rich systems. However, it can be difficult to compare taxa between studies because identifications of many distinct taxa are approximate and at mixed taxonomic levels. For example, cf. *Limnodrilus hoffmeisteri* and *Varichaetadrilus* nr. *pacificus*, two of the five most common benthic invertebrates analyzed in this study, are likely currently undescribed species. Even with current identification keys and well-trained taxonomists, many taxa in UKL are not described species. The occurrence of numerous undescribed species could be a result of poor dispersers in an isolated system adapting to local habitats (Cohen & Johnston,

1987). The lack of species descriptions and identification keys for many of the collected taxa highlights the need for additional research on lake benthic fauna. Given limitations, however, useful comparisons between the fauna found in UKL with other systems can still be made.

*Ilyodrilus frantzi*, the most abundant taxon in UKL, has also been found to be common in a wide variety of other lentic systems. For example, *I. frantzi* was also the most abundant oligochaete species in Utah and Bear lakes in Utah (Spencer & Denton, 2003). *Ilyodrilus frantzi* is an annelid endemic to western North America that is known to survive well in eutrophic waters, such as Utah Lake, which our results support, as well as under oligotrophic conditions such as Bear Lake, Pyramid Lake, and Lake Tahoe (Spencer & Denton, 2003). Dumnicka & Pasternak (1978) showed that *I. frantzi* is not habitat selective, a finding supported by (Yildiz, 2016) and the present study, which found that *I. frantzi* was the only taxon among the five most abundant that was found in every habitat, region, and month.

The taxon identified as cf. *Limnodrilus hoffmeisteri,* in addition to closely matching the morphology of the species *L. hoffmeisteri*, also exhibited some of the ecological characteristics of this cosmopolitan species. For example, cf. *L. hoffmeisteri* was one of the five most abundant taxa in UKL, and *L. hoffmeisteri* is thought to be the most abundant aquatic oligochaete in the world (Kennedy, 1965). *Limnodrilus hoffmeisteri* has also long been known to be a eutrophic and pollution tolerant oligochaete (Fischer & Beeton, 1975) that can utilize anaerobic metabolic pathways in order to survive in hypoxic conditions for limited time periods (Volpers & Neumann, 2005). The present study seems to support such findings because cf. L. *hoffmeisteri* was one of the most common taxa in trench habitat, which was characterized by the lowest amount of DO among habitats within UKL. In addition to being abundant in UKL, *L. hoffmeisteri* is abundant in many other lakes including Green Bay, Lake Michigan (Fischer & Beeton, 1975), the shallow and hypereutrophic Lake Taihu in China (Cai, Gong & Qin, 2011) and the hypereutrophic Americana Reservoir in Brazil (Pamplin, 2006). Such evidence supports our findings regarding the habitat location of cf. *L. hoffmeisteri* and suggests that DO is a factor influencing the distribution of benthic invertebrates in UKL.

Comparing the abundances and distributions of the other three most abundant taxa in UKL to other systems is more difficult for a variety of reasons. *Varichaetadrilus pacificus*, for example, was first described by Brinkhurst (1981) and, in addition to not being well-studied, the taxon found in UKL is only near the described species. The genus *Varichaetadrilus,* however, has been found in shallow, eutrophic Clear Lake in California (Lamphere *et al.*, 1999) but was not identified from eutrophic Utah Lake (Spencer & Denton, 2003). The abundance of *V.* nr. (near) *pacificus* in UKL suggests that, like other species from the genus *Varichaetadrilus* found in Clear Lake (Spencer & Denton, 2003), the taxon found in UKL thrives in nutrient rich systems. *Cladotanytarsus,* overall very abundant in UKL, was less abundant in the deep trench sites, which supports results that found this genus to be common in the large and shallow Lake Balaton in Hungary (Árva *et al.*, 2014) as well as shallow eutrophic waters in The Netherlands (Vos *et al.*, 2004). Although *Procladius* sp. 1, the third most abundant taxon in UKL, cannot be compared since its identification is uncertain, the genus *Procladius* is common in eutrophic systems worldwide, including shallow Lake Suwa in Japan (Yamagishi & Fukuhara, 1971), Lake

Figueira located in a coastal lagoon system in Brazil (Lima & Lanzer, 2013), and shallow Lake Pawnee in Nebraska (Popp & Hoagland, 1995).

*Benthic Invertebrate Assemblages*

The present study found that benthic invertebrate assemblages differed among lake regions, habitats, and time periods, results that support previous work examining benthic invertebrate assemblages in lakes (e.g. Barnes & Toole, 1981; Devine & Vanni, 2002; Pamplin, 2006, Pamplin & Rocha, 2007; Yildiz *et al.*, 2015; Hu *et al.*, 2016) . Previous work on UKL found that benthic invertebrate density and richness differed spatially and temporally (Stauffer-Olsen, Fend & Carter, 2017), which are aspects that contribute to differences in assemblage composition. The present study examined how the abundance and percent composition of individual taxa contributed to the dissimilarities in assemblages among habitats, regions and months. Examining the ecology of taxa that contributed the most to dissimilarity among sites can help determine the effects of environmental parameters on benthic invertebrate assemblages.

Tanytarsini, the taxon that contributed most to the differentiation in assemblage composition between littoral and open water habitats, are generally detritivores (Vos *et al.*, 2004). In UKL, Tanytarsini were much more abundant in littoral compared to open water habitat, perhaps a result of the higher percent organic matter that is characteristic of littoral compared to open water habitat (Table 3.5). *Cladotanytarsus,* another significant contributor to differences in assemblage composition between littoral and open water habitat, is also generally considered detrivivorous (Vos *et al.*, 2004), however, it was more abundant in open water compared to littoral habitat, suggesting that environmental factors other than food availability were potentially influencing its distribution. Distributions of *Chironomus* have been found to be influenced by water temperature and DO concentration (Forsyth, 1986) as well as depth (Cao *et al.*, 2012). However, *Chironomus* posseses hemoglobin, which allows it to survive in low-oxygen conditions (Little & Smol, 2000). Thus, the preference of *Chironomus* larvae for littoral habitat compared to open water habitat in UKL remains unclear.

Within the central region of UKL, *Aulodrilus pigueti* was much more abundant in littoral compared to open water or trench habitats and was one of the main contributors to the differing assemblages between these two habitats. A likely reason for this pattern is that *Aulodrilus pigueti* prefers habitats with abundant organic detritus in bottom sediments (Marchese, 1987; Zilli & Marchese, 2011), and littoral habitat had the highest mean percent organic matter of the habitats in the central region of UKL. The oligochaete *Quistadrilus multisetosus,* one of the three main contributors to the difference between assemblages in littoral and trench habitat, was much less common in trench habitat. *Quistadrilus multisetosus* is known to prefer both clay sediment and profundal zones (Nadushan *et al.*, 2010). In UKL, trench sites were not characterized by more clay sediment than littoral sites, but they were in the profundal zone, which suggests that depth might have a stronger influence on *Q. multisetosus* distribution than sediment particle size.

Benthic invertebrate assemblages in the central region of UKL were differentiated from the north and south regions by Sphaeriidae, *Fluminicola,* and small Tubificidae with chaetae, all of which are difficult to compare with other systems as a result of their level of identification. The *Fluminicola* genus is likely the Klamath pebblesnail, an

undescribed sensitive species endemic to UKL (Klamath Project, 2007). Previous research has found that Sphaeriidae prefer shallower habitats (Carter, Nalepa & Rediske, 2006), however, the average depth among regions is remarkably similar. Site LC02 was characterized by sandy sediment and high abundances of Sphaeriids. Thus, Sphaeriidae comparably high abundance in central compared to northern or southern sites might have been in part a result of the sandy sediment in LC02.

Differences in benthic invertebrate composition among months were likely the result of a variety of influences including life-histories, environmental conditions, competition, and predation (Moss & Timms, 1989; Brönmark & Hansson, 2002; Devine & Vanni, 2002). Overall, much remains to be learned about the specific habitat preferences of many of the taxa differentiating benthic invertebrate assemblages among habitats and regions in UKL. In general, many taxa in lakes remain undescribed (Brönmark & Hansson, 2002). As such, additional research is required to fully understand the causes of the differing distributions of specific taxa.

*Correlations to Environmental Variables*

Benthic invertebrate assemblage composition is related to habitat conditions (Weatherhead & James, 2001). Previous work has found correlations between benthic invertebrate assemblages and a wide variety of environmental variables including sediment quality, turbidity, contaminants, biotic factors, oxygen conditions, chlorophyll *a* concentrations, anthropogenic disturbances, and macrophytes (Phipps, Mattson & Ankley, 1995; Dinsmore & Prepas, 1997; Weatherhead & James, 2001; Kagalou *et al.*, 2006; Zbikowski & Kobak, 2007; Cai *et al.*, 2011; Hu *et al.*, 2016). However, untangling the relationships between multiple species and multiple environmental factors can be difficult. García-Criado *et al.* (2005), for example, found no significant correlations between five different benthic invertebrate indices, including abundance, and measured environmental variables.

In the present study, temperature most significantly influenced assemblage composition, which is indicated by a gradual shift in benthic invertebrate composition from May-July (Figure 3.3). Although there was virtually no change in benthic invertebrate density (Stauffer-Olsen, Fend & Carter, 2017), temperature did have an effect on composition, especially in July when temperature was warmest. Temperature was also one of the environmental variables that had the greatest influence on benthic invertebrates assemblages in similar lake systems sampled throughout the year, such as shallow, hypereutrophic lakes in Turkey (Yildiz *et al.*, 2015) and in Greece (Kagalou *et al.*, 2006). Temperature and depth were also major predictors of benthic invertebrate distribution in the deep (10.5 m average) oligotrophic Brazilian reservoir studied by Pamplin & Rocha (2007). Depth, another environmental parameter included in the multiple regression model that explained the most variance in assemblage composition (both lake wide without trench sites and in the central region with trench sites), also had a large influence on the distribution of benthic invertebrates and assemblage composition in hypereutrophic Lake Golcuk (Yildiz *et al.*, 2015) as well as nine oligotrophic lakes in New Zealand (Weatherhead & James, 2001). Within the central region of UKL, pH was another environmental parameter that correlated with benthic fauna, a result also found by Yildiz *et al.* (2015).

Many studies have found that sediment characteristics influence benthic invertebrates (Weatherhead & James, 2001; Peeters, Gylstra & Vos, 2004; Free *et al.*, 2009), results that our findings support. Weatherhead & James, (2001) found that chironomids and oligochaetes, two of the most abundant invertebrate groups in their study, showed a significant relationship with substrate size in multiple regression analyses and were more common in fine substrates compared to coarse substrates. Our results align well with this study since chironomids and oligochaetes were also abundant in UKL and, particle size influenced benthic invertebrate assemblage composition (when trench sites were not included, percent silt, sand, and clay were all correlated with environmental parameters and when trench sites were included in central region DISTLM analyses, percent silt was one of the most influential environmental parameters measured). Similarly, fine sediment fraction was also a major factor influencing invertebrate distribution in the reservoir studied by Pamplin & Rocha (2007).

When benthic flux measurements were analyzed using DISTLM, the best model to explain variance in assemblages included just dissolved organic carbon, although DOC explained 4% of variance when trench sites were excluded and 16% in the central region that included trench sites. Such results suggest that DOC flux might be affected by invertebrate assemblages. Benthic invertebrates can contribute nutrients directly and/or facilitate nutrient cycling in lakes through bioturbation, organic matter processing, and metabolic nutrient release (Herringshaw & Solan, 2008; Wood *et al.*, 2013; Holker, 2015). Thus, it is possible that species composition of benthic invertebrates might have contributed to the positive DOC flux in the central region of UKL. However, the contribution of nutrients by benthic invertebrates can also vary based on the mechanism and environment (Gardner et al, 1981) and average DOC flux was negative in central and southern regions of the lake. As a result, additional research is required to fully understand the relationship between benthic invertebrate assemblage composition and benthic nutrient flux.

Upper Klamath Lake is a unique ecosystem that also offers important natural resources. Over the past several decades, poor water quality in UKL (and the Klamath River downstream) has become of increasing concern, which has led to a large number of studies, including the present study, and restoration projects. Interest in understanding and improving the lake's water quality will likely only increase with the recent agreement to decommission the four hydroelectric dams on the Klamath River and restore salmonids to spawning areas previously blocked by dams. As such, an understanding of benthic invertebrate assemblage composition, spatial and temporal variation, and relationships to environmental variables are necessary to accurately describe the lake function and understand the drivers of poor water quality.

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**Table 3.1** Sampling site locations and descriptions within Upper Klamath Lake, Oregon.

| Site | Description | Latitude | Longitude |
|---|---|---|---|
| **Lake Sites** | | **WGS 84 Datum** | |
| LN01 | Littoral north 1 | N42.4901 | W122.0023 |
| LN02 | Littoral north 2 | N42.4294 | W122.0387 |
| LN03 | Littoral north 3 | N42.4057 | W122.0034 |
| ON01 | Open-lake north 1 | N42.4051 | W122.0206 |
| ON02 | Open-lake north 2 | N42.4362 | W121.9715 |
| ON03 | Open-lake north 3 | N42.4368 | W122.0444 |
| LC01 | Littoral central 1 | N42.4613 | W121.9309 |
| LC02 | Littoral central 2 | N42.4472 | W121.8802 |
| LC03 | Littoral central 3 | N42.4626 | W121.9041 |
| OC01 | Open-lake central 1 | N42.4219 | W121.8866 |
| OC02 | Open-lake central 2 | N42.4170 | W121.8503 |
| OC03 | Open-lake central 3 | N42.4217 | W121.8684 |
| LS01 | Littoral south 1 | N42.3368 | W121.9367 |
| LS02 | Littoral south 2 | N42.3323 | W121.9368 |
| LS03 | Littoral south 3 | N42.3439 | W121.9254 |
| OS01 | Open-lake south 1 | N42.2864 | W121.8526 |
| OS02 | Open-lake south 2 | N42.3586 | W121.8635 |
| OS03 | Open-lake south 3 | N42.3200 | W121.8964 |
| TR01 | Trench 1 | N42.3679 | W121.8937 |
| TR02 | Trench 2 | N42.3918 | W121.9350 |
| TR03 | Trench 3 | N42.4179 | W121.9414 |

**Table 3.2** Mean density and standard deviation of benthic invertebrates $m^{-2}$ from 54 samples collected from Upper Klamath Lake, Oregon during 2013. Trench sites are excluded. Sample density was estimated as the mean of 3 Ekman grabs. The five most abundant taxa in each group are indicated in bold.
(spp., species (plural); cf., confer (a species that closely matches another species); nr., near (taxonomically); sp., species; undet., undetermined; e.i., early instar)

| Taxon | | Lake-wide | | Littoral | | Open-water | |
|---|---|---|---|---|---|---|---|
| | N | 54 | 54 | 27 | 27 | 27 | 27 |
| | Family | Mean | SD | Mean | SD | Mean | SD |
| Hydra | Hydridae | 8 | 51 | 11 | 56 | 8 | 55 |
| Dugesia | Planariidae | 0 | 6 | 1 | 10 | 0 | 0 |
| microturbellarians undet. | Turbellaria | 0 | 5 | 0 | 0 | 1 | 7 |
| mermithids | Mermithidae | 21 | 101 | 43 | 151 | 6 | 22 |
| Enchytraeidae | Enchytraeidae | 0 | 3 | 1 | 5 | 0 | 0 |
| Arcteonais lomondi | Naididae | 3 | 11 | 4 | 13 | 2 | 11 |
| Dero spp. | Naididae | 65 | 195 | 84 | 265 | 65 | 131 |
| Nais spp. | Naididae | 2 | 11 | 4 | 16 | 0 | 0 |
| Slavina appendiculata | Naididae | 1 | 7 | 2 | 11 | 0 | 0 |
| Stylaria | Naididae | 0 | 3 | 1 | 5 | 0 | 0 |
| Naidinae undet. | Naididae | 19 | 162 | 44 | 246 | 0 | 0 |
| Aulodrilus pigueti | Tubificidae | 252 | 710 | 527 | 1,002 | 60 | 208 |
| Ilyodrilus frantzi | Tubificidae | **1,770** | 1,527 | **1,512** | 1,230 | **1,886** | 1,817 |
| cf. Limnodrilus hoffmeisteri | Tubificidae | **886** | 1,457 | **1,155** | 1,583 | 412 | 559 |
| Limnodrilus silvani | Tubificidae | 41 | 144 | 44 | 121 | 0 | 0 |
| Quistadrilus multisetosus | Tubificidae | 56 | 195 | 123 | 284 | 8 | 30 |
| Rhyacodrilus | Tubificidae | 6 | 23 | 8 | 27 | 0 | 0 |
| Spirosperma ferox | Tubificidae | 9 | 53 | 22 | 80 | 0 | 0 |
| Spirosperma nikolskyi | Tubificidae | 1 | 7 | 2 | 11 | 0 | 0 |

| Taxon | | Lake-wide | | Littoral | | Open-water | |
|---|---|---|---|---|---|---|---|
| | N | 54 | 54 | 27 | 27 | 27 | 27 |
| | Family | Mean | SD | Mean | SD | Mean | SD |
| Varichaetadrilus pacificus | Tubificidae | 259 | 738 | 242 | 587 | 183 | 612 |
| Varichaetadrilus nr. pacificus | Tubificidae | **1,513** | 1,906 | **1,708** | 1,962 | **1,091** | 1,324 |
| Tubificidae with chaetae small | Tubificidae | 570 | 876 | 561 | 902 | 522 | 858 |
| Bothrioneurum veidovskyanum | Tubificidae | 6 | 46 | 13 | 69 | 0 | 0 |
| Tubificidae without chaetae small | Tubificidae | 736 | 1,179 | 1,067 | 1,553 | 334 | 393 |
| Tubificidae very small | Tubificidae | 91 | 191 | 122 | 221 | 60 | 156 |
| Altmanella freidris | Lumbiculidae | 170 | 258 | 193 | 282 | 201 | 258 |
| Lumbriculus | Lumbiculidae | 0 | 5 | 1 | 7 | 0 | 0 |
| Rhynchelmis | Lumbiculidae | 260 | 310 | 414 | 392 | 173 | 157 |
| Glossiphonia complanata | Glossiphoniidae | 1 | 8 | 2 | 12 | 0 | 0 |
| Helobdella nr. californica | Glossiphoniidae | 686 | 1,030 | 916 | 1,371 | 537 | 628 |
| Helobdella nr. robusta | Glossiphoniidae | 281 | 704 | 509 | 1,024 | 119 | 136 |
| Helobdella stagnalis | Glossiphoniidae | 74 | 214 | 26 | 94 | 144 | 300 |
| Helobdella hatchlings undet. | Glossiphoniidae | 805 | 1,915 | 857 | 2,215 | **682** | 1,386 |
| Theromyzon sp. | Glossiphoniidae | 0 | 3 | 0 | 0 | 0 | 0 |
| Mooreobdella microstoma | Erpobdellidae | 12 | 48 | 24 | 69 | 3 | 15 |
| Erpobdellidae undet. | Erpobdellidae | 2 | 17 | 1 | 7 | 0 | 0 |
| Piscicolidae | Piscicolidae | 14 | 56 | 27 | 83 | 4 | 14 |
| Caecidotea cf. occidentalis | Asellidae | 4 | 31 | 10 | 46 | 1 | 5 |
| Hyalella | Hyalellidae | 3 | 15 | 5 | 23 | 1 | 5 |
| Caenis | Caenidae | 12 | 60 | 28 | 89 | 0 | 0 |
| Coenagrionidae undet. | Coenagrionidae | 0 | 5 | 1 | 7 | 0 | 0 |
| Isoperla | Perlodidae | 0 | 3 | 1 | 5 | 0 | 0 |
| Sialis | Sialidae | 0 | 3 | 1 | 5 | 0 | 0 |
| Hydroptila | Hydroptilidae | 0 | 3 | 1 | 5 | 0 | 0 |

| Taxon | | Lake-wide | | Littoral | | Open-water | |
|---|---|---|---|---|---|---|---|
| | N | 54 | 54 | 27 | 27 | 27 | 27 |
| | Family | Mean | SD | Mean | SD | Mean | SD |
| Limnephilus | Limnephilidae | 0 | 5 | 1 | 7 | 0 | 0 |
| Mystacides | Leptoceridae | 1 | 9 | 3 | 13 | 1 | 5 |
| Oecetis | Leptoceridae | 3 | 13 | 0 | 0 | 7 | 19 |
| Brychius | Haliplidae | 0 | 3 | 1 | 5 | 0 | 0 |
| Dytiscidae | Dytscidae | 0 | 3 | 1 | 5 | 0 | 0 |
| Dubiraphia | Elmidae | 1 | 8 | 2 | 12 | 0 | 0 |
| Bezzia | Ceratopogonidae | 22 | 64 | 47 | 91 | 4 | 19 |
| Procladius sp. 1 | Tanypodinae | **1,352** | 1,209 | **1,333** | 1,473 | **1,396** | 968 |
| Orthocladiinae | Orthocladiinae | 1 | 8 | 3 | 13 | 0 | 0 |
| Chironomus cf. plumosus | Chironomidae | 47 | 144 | 35 | 167 | 35 | 89 |
| Chironomus cf. utahensis | Chironomidae | 28 | 85 | 30 | 91 | 21 | 71 |
| cf. Chironomus e.i. | Chironomidae | 508 | 2,456 | 819 | 3,586 | 311 | 1,050 |
| Cryptochironomus | Chironomidae | 35 | 103 | 46 | 146 | 27 | 52 |
| Cryptotendipes | Chironomidae | 169 | 312 | 41 | 65 | 325 | 419 |
| Dicrotentipes | Chironomidae | 2 | 20 | 4 | 30 | 0 | 0 |
| Glyptotendipes sp. 1 | Chironomidae | 18 | 158 | 42 | 239 | 1 | 5 |
| Parachironomus | Chironomidae | 8 | 32 | 10 | 44 | 4 | 13 |
| Chironomini "Harnischia comp." e.i. | Chironomidae | 1 | 6 | 2 | 8 | 0 | 0 |
| Chironomini undet | Chironomidae | 91 | 422 | 112 | 391 | 98 | 512 |
| Cladotanytarsus | Chironomidae | **850** | 4,139 | 865 | 3,903 | **1,112** | 4,971 |
| Tanytarsini | Chironomidae | 595 | 2,359 | **1,275** | 3,485 | 107 | 335 |
| cf. Hygrobates | Eriophyidae | 3 | 16 | 3 | 14 | 4 | 19 |
| cf. Piona | Eriophyidae | 1 | 6 | 1 | 7 | 1 | 5 |
| Mite undet. | Eriophyidae | 4 | 17 | 4 | 13 | 4 | 22 |
| Valvata | Valvatidae | 15 | 67 | 33 | 100 | 1 | 7 |

| Taxon | | Lake-wide | | Littoral | | Open-water | |
|---|---|---|---|---|---|---|---|
| | N | 54 | 54 | 27 | 27 | 27 | 27 |
| | Family | Mean | SD | Mean | SD | Mean | SD |
| Fluminicola | Hydrobiidae | 51 | 114 | 36 | 75 | 83 | 151 |
| Pyrgulopsis | Hydrobiidae | 6 | 37 | 14 | 56 | 0 | 0 |
| Planorbidae spp. | Planorbidae | 6 | 25 | 7 | 25 | 3 | 15 |
| Sphaeriidae undet. | Spheriidae | 159 | 569 | 324 | 837 | 35 | 97 |

Table 3.2. Continued

| Taxon | | North | | Central | | South | | May | | June | | July | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| | Family | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Hydra | Hydridae | 2 | 10 | 15 | 68 | 12 | 67 | 13 | 67 | 15 | 68 | 0 | 0 |
| Dugesia | Planariidae | 2 | 12 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| microturbellarians undet. | Turbellaria | 0 | 0 | 0 | 0 | 2 | 8 | 1 | 6 | 0 | 6 | 1 | 6 |
| mermithids | Mermithidae | 58 | 183 | 10 | 24 | 6 | 19 | 10 | 19 | 46 | 19 | 16 | 87 |
| Enchytraeidae | Enchytraeidae | 0 | 0 | 1 | 6 | 0 | 0 | 1 | 6 | 0 | 6 | 0 | 0 |
| Arcteonais lomondi | Naididae | 3 | 15 | 6 | 15 | 0 | 0 | 4 | 15 | 3 | 13 | 2 | 8 |
| Dero spp. | Naididae | 33 | 62 | 176 | 332 | 15 | 50 | 34 | 96 | 63 | 96 | 119 | 316 |
| Nais spp. | Naididae | 2 | 12 | 3 | 15 | 1 | 6 | 2 | 0 | 3 | 0 | 1 | 6 |
| Slavina appendiculata | Naididae | 0 | 0 | 2 | 13 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 6 |
| Stylaria | Naididae | 0 | 0 | 1 | 6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Naidinae undet. | Naididae | 0 | 0 | 67 | 300 | 0 | 0 | 3 | 15 | 2 | 15 | 61 | 301 |
| Aulodrilus pigueti | Tubificidae | 296 | 643 | 581 | 1,079 | 2 | 13 | 229 | 611 | 291 | 611 | 358 | 949 |
| Ilyodrilus frantzi | Tubificidae | **2,315** | 1,292 | **987** | 1,165 | **1,796** | 1,854 | **2,022** | 1,863 | **1,556** | 1,863 | **1,480** | 1,286 |
| cf. Limnodrilus hoffmeisteri | Tubificidae | 1,044 | 1,616 | **923** | 1,188 | 384 | 636 | **919** | 1,484 | 813 | 1,484 | 522 | 785 |
| Limnodrilus silvani | Tubificidae | 4 | 25 | 62 | 143 | 0 | 0 | 14 | 51 | 40 | 51 | 12 | 35 |

| Taxon | | North | | Central | | South | | May | | June | | July | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| | Family | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Quistadrilus multisetosus | Tubificidae | 8 | 30 | 180 | 334 | 8 | 31 | 39 | 102 | 63 | 102 | 90 | 278 |
| Rhyacodrilus | Tubificidae | 2 | 12 | 10 | 31 | 1 | 6 | 2 | 18 | 5 | 18 | 5 | 19 |
| Spirosperma ferox | Tubificidae | 0 | 0 | 32 | 96 | 1 | 6 | 8 | 39 | 20 | 39 | 5 | 36 |
| Spirosperma nikolskyi | Tubificidae | 2 | 12 | 1 | 6 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 |
| Varichaetadrilus pacificus | Tubificidae | 362 | 739 | 275 | 685 | 1 | 6 | 120 | 478 | 302 | 478 | 138 | 386 |
| Varichaetadrilus nr. pacificus | Tubificidae | **1,602** | 1,611 | 467 | 553 | **2,128** | 2,093 | **1,881** | 2,096 | **1,442** | 2,096 | **1,476** | 1,588 |
| Tubificidae with chaetae small | Tubificidae | 948 | 1,106 | 300 | 716 | 376 | 589 | 312 | 527 | 703 | 527 | 658 | 866 |
| Bothrioneurum veidovskyanum | Tubificidae | 0 | 0 | 20 | 84 | 0 | 0 | 1 | 6 | 17 | 6 | 2 | 12 |
| Tubificidae without chaetae small | Tubificidae | 1,053 | 1,237 | **814** | 1,507 | 235 | 351 | 783 | 1,228 | 793 | 1,228 | 362 | 483 |
| Tubificidae very small | Tubificidae | 155 | 240 | 81 | 214 | 36 | 53 | 108 | 214 | 47 | 214 | 101 | 233 |
| Altmanella freidris | Lumbiculidae | 156 | 282 | 94 | 170 | 341 | 280 | 228 | 292 | 193 | 292 | 166 | 251 |
| Lumbriculus | Lumbiculidae | 0 | 0 | 2 | 8 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 6 |
| Rhynchelmis | Lumbiculidae | 215 | 246 | 467 | 429 | 198 | 153 | 353 | 404 | 275 | 404 | 202 | 198 |
| Glossiphonia complanata | Glossiphoniidae | 2 | 12 | 2 | 8 | 0 | 0 | 1 | 6 | 0 | 6 | 2 | 13 |
| Helobdella nr. californica | Glossiphoniidae | 1,116 | 1,512 | **732** | 899 | 333 | 369 | 388 | 227 | **735** | 227 | **1,102** | 990 |
| Helobdella nr. robusta | Glossiphoniidae | 226 | 658 | 74 | 116 | 642 | 1,051 | 175 | 188 | 156 | 188 | 643 | 1,222 |
| Helobdella stagnalis | Glossiphoniidae | 26 | 113 | 133 | 256 | 96 | 275 | 28 | 34 | 97 | 34 | 129 | 284 |
| Helobdella hatchlings undet. | Glossiphoniidae | **1,215** | 2,769 | 520 | 1,075 | 574 | 1,098 | 584 | 333 | **1,163** | 333 | 970 | 1,027 |
| Theromyzon sp. | Glossiphoniidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Mooreobdella microstoma | Erpobdellidae | 22 | 77 | 19 | 41 | 0 | 0 | 7 | 16 | 20 | 16 | 14 | 39 |
| Erpobdellidae undet. | Erpobdellidae | 0 | 0 | 1 | 6 | 1 | 6 | 0 | 0 | 2 | 0 | 0 | 0 |
| Piscicolidae | Piscicolidae | 6 | 19 | 5 | 17 | 35 | 99 | 4 | 15 | 3 | 15 | 40 | 99 |

| Taxon | | North | | Central | | South | | May | | June | | July | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| | Family | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Caecidotea cf. occidentalis | Asellidae | 15 | 56 | 0 | 0 | 1 | 6 | 2 | 6 | 8 | 6 | 5 | 27 |
| Hyalella | Hyalellidae | 0 | 0 | 6 | 26 | 3 | 12 | 1 | 6 | 7 | 6 | 2 | 8 |
| Caenis | Caenidae | 12 | 54 | 30 | 95 | 0 | 0 | 12 | 42 | 25 | 42 | 6 | 26 |
| Coenagrionidae undet. | Coenagrionidae | 0 | 0 | 2 | 8 | 0 | 0 | 2 | 8 | 0 | 8 | 0 | 0 |
| Isoperla | Perlodidae | 0 | 0 | 0 | 0 | 1 | 6 | 0 | 0 | 1 | 0 | 0 | 0 |
| Sialis | Sialidae | 0 | 0 | 1 | 6 | 0 | 0 | 1 | 6 | 0 | 6 | 0 | 0 |
| Hydroptila | Hydroptilidae | 0 | 0 | 1 | 6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Limnephilus | Limnephilidae | 1 | 6 | 1 | 6 | 0 | 0 | 1 | 8 | 0 | 8 | 0 | 0 |
| Mystacides | Leptoceridae | 0 | 0 | 5 | 17 | 0 | 0 | 2 | 8 | 2 | 8 | 1 | 6 |
| Oecetis | Leptoceridae | 0 | 0 | 7 | 21 | 2 | 10 | 5 | 17 | 4 | 17 | 1 | 6 |
| Brychius | Haliplidae | 0 | 0 | 1 | 6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Dytiscidae | Dytscidae | 0 | 0 | 1 | 6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Dubiraphia | Elmidae | 0 | 0 | 3 | 15 | 0 | 0 | 2 | 13 | 0 | 13 | 1 | 6 |
| Bezzia | Ceratopogonidae | 16 | 75 | 60 | 82 | 1 | 6 | 28 | 42 | 26 | 42 | 21 | 62 |
| Procladius sp. 1 | Tanypodinae | **2,317** | 1,480 | **586** | 644 | **1,192** | 717 | **1,300** | 993 | **1,756** | 993 | 962 | 831 |
| Orthocladiinae | Orthocladiinae | 0 | 0 | 2 | 13 | 2 | 8 | 0 | 0 | 1 | 0 | 3 | 15 |
| Chironomus cf. plumosus | Chironomidae | 76 | 212 | 4 | 13 | 24 | 79 | 28 | 97 | 47 | 97 | 18 | 60 |
| Chironomus cf. utahensis | Chironomidae | 35 | 103 | 2 | 10 | 38 | 92 | 15 | 74 | 15 | 74 | 43 | 112 |
| cf. Chironomus e.i. | Chironomidae | **1,286** | 4,344 | 220 | 1,223 | 188 | 336 | 9 | 6 | 86 | 6 | **1,607** | 4,428 |
| Cryptochironomus | Chironomidae | 3 | 15 | 83 | 174 | 23 | 51 | 61 | 137 | 37 | 137 | 16 | 64 |
| Cryptotendipes | Chironomidae | 153 | 214 | 170 | 280 | 226 | 454 | 263 | 450 | 211 | 450 | 66 | 113 |
| Dicrotentipes | Chironomidae | 6 | 37 | 0 | 0 | 1 | 6 | 5 | 6 | 0 | 6 | 1 | 6 |
| Glyptotendipes sp. 1 | Chironomidae | 50 | 289 | 12 | 53 | 2 | 8 | 3 | 15 | 11 | 15 | 50 | 289 |
| Parachironomus | Chironomidae | 11 | 50 | 7 | 24 | 4 | 13 | 0 | 0 | 7 | 0 | 15 | 51 |

| Taxon | | North | | Central | | South | | May | | June | | July | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 |
| | Family | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Chironomini "Harnischia comp." e.i. | Chironomidae | 0 | 0 | 2 | 10 | 0 | 0 | 1 | 6 | 0 | 6 | 2 | 8 |
| Chironomini undet | Chironomidae | 173 | 471 | 116 | 622 | 26 | 79 | 5 | 26 | 7 | 26 | 302 | 752 |
| Cladotanytarsus | Chironomidae | 1,040 | 4,766 | 156 | 371 | **1,769** | 6,014 | **2,268** | 6,156 | 85 | 6,156 | 212 | 536 |
| Tanytarsini | Chironomidae | 1,141 | 3,641 | 19 | 41 | **913** | 2,359 | 31 | 114 | 514 | 114 | **1,516** | 3,938 |
| cf. Hygrobates | Eriophyidae | 2 | 12 | 7 | 22 | 2 | 13 | 7 | 23 | 2 | 23 | 2 | 13 |
| cf. Piona | Eriophyidae | 1 | 6 | 1 | 6 | 1 | 6 | 1 | 6 | 2 | 6 | 0 | 0 |
| Mite undet. | Eriophyidae | 0 | 0 | 10 | 29 | 2 | 8 | 4 | 25 | 4 | 25 | 3 | 15 |
| Valvata | Valvatidae | 0 | 0 | 50 | 119 | 1 | 6 | 16 | 68 | 13 | 68 | 21 | 88 |
| Fluminicola | Hydrobiidae | 13 | 44 | 95 | 111 | 70 | 163 | 53 | 104 | 66 | 104 | 58 | 142 |
| Pyrgulopsis | Hydrobiidae | 1 | 6 | 20 | 68 | 0 | 0 | 9 | 55 | 4 | 55 | 7 | 40 |
| Planorbidae spp. | Planorbidae | 7 | 28 | 7 | 20 | 2 | 8 | 4 | 16 | 10 | 16 | 2 | 8 |
| Sphaeriidae undet. | Spheriidae | 25 | 65 | 507 | 983 | 7 | 23 | 153 | 592 | 240 | 592 | 143 | 385 |

**Table 3.2A.** Mean density and standard deviation of benthic invertebrates m-2 from 18 samples collected from the central region of Upper Klamath Lake, Oregon during 2013. Samples density was estimated as the mean of 3 Ekman grabs. . The five most abundant taxa in each group are indicated in bold.
(spp., species (plural); cf., confer (a species that closely matches another species); nr., near (taxonomically); sp., species; undet., undetermined; e.i., early instar)

| Taxon | | Littoral | | Open-water | | Trench | | May | | June | | July | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| | Family | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Hydra | Hydridae | 30 | 95 | 0 | 0 | 0 | 0 | 10 | 94 | 3 | 17 | 0 | 0 |
| Dugesia | Planariidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| microturbellarians undet. | Turbellaria | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| mermithids | Mermithidae | 20 | 31 | 0 | 0 | 3 | 17 | 10 | 24 | 8 | 23 | 6 | 19 |
| Enchytraeidae | Enchytraeidae | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| Arcteonais lomondi | Naididae | 10 | 19 | 2 | 9 | 0 | 0 | 8 | 12 | 2 | 14 | 2 | 12 |
| Dero spp. | Naididae | 216 | 430 | 137 | 190 | 8 | 21 | 74 | 129 | 123 | 179 | 183 | 427 |
| Nais spp. | Naididae | 7 | 20 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 19 | 2 | 9 |
| Slavina appendiculata | Naididae | 5 | 19 | 0 | 0 | 0 | 0 | 3 | 0 | 2 | 17 | 0 | 9 |
| Stylaria | Naididae | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 9 | 0 | 0 |
| Naidinae undet. | Naididae | 133 | 417 | 0 | 0 | 0 | 0 | 5 | 20 | 5 | 19 | 137 | 420 |
| Aulodrilus pigueti | Tubificidae | **1,160** | 1,294 | 2 | 9 | 2 | 9 | 258 | 442 | 443 | 832 | 407 | 1,277 |
| Ilyodrilus frantzi | Tubificidae | **1,119** | 1,531 | **854** | 620 | **2,194** | 1,266 | **1,291** | 933 | **1,452** | 1,495 | **1,559** | 1,505 |
| cf. Limnodrilus hoffmeisteri | Tubificidae | **1,588** | 1,379 | 257 | 227 | **1,500** | 2,316 | **1,542** | 2,431 | 838 | 1,069 | **656** | 1,001 |
| Limnodrilus silvani | Tubificidae | 123 | 184 | 0 | 0 | 155 | 292 | 137 | 279 | 87 | 196 | 54 | 119 |
| Quistadrilus multisetosus | Tubificidae | 337 | 416 | 23 | 48 | 0 | 0 | 130 | 135 | 89 | 288 | 115 | 378 |
| Rhyacodrilus | Tubificidae | 20 | 41 | 0 | 0 | 15 | 35 | 10 | 39 | 10 | 31 | 11 | 26 |
| Spirosperma ferox | Tubificidae | 64 | 129 | 0 | 0 | 0 | 0 | 41 | 54 | 10 | 117 | 0 | 51 |
| Spirosperma nikolskyi | Tubificidae | 2 | 9 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 9 | 0 | 0 |

| Taxon | | Littoral | | Open-water | | Trench | | May | | June | | July | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| | Family | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Varichaetadrilus pacificus | Tubificidae | 550 | 894 | 0 | 0 | 538 | 1,275 | 275 | 621 | 329 | 825 | 520 | 1,244 |
| Varichaetadrilus nr. pacificus | Tubificidae | 545 | 703 | 390 | 343 | **2,194** | 2,804 | **1,091** | 1,810 | **1,184** | 2,212 | **941** | 1,522 |
| Tubificinae with chaetae small | Tubificidae | 584 | 936 | 16 | 43 | 742 | 860 | 188 | 500 | 481 | 742 | 698 | 1,021 |
| Bothrioneurum veidovskyanum | Tubificidae | 40 | 117 | 0 | 0 | 2 | 9 | 2 | 9 | 36 | 116 | 4 | 17 |
| Tubificinae without chaetae small | Tubificidae | **1,419** | 1,946 | 209 | 287 | 950 | 1,125 | **981** | 1,521 | **991** | 1,736 | 413 | 469 |
| Tubificinae very small | Tubificidae | 158 | 284 | 5 | 14 | 89 | 181 | 74 | 91 | 58 | 110 | 122 | 318 |
| Altmanella freidris | Lumbiculidae | 13 | 46 | 174 | 208 | 10 | 23 | 100 | 201 | 67 | 132 | 33 | 62 |
| Lumbriculus | Lumbiculidae | 3 | 12 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 9 | 0 | 9 |
| Rhynchelmis | Lumbiculidae | 667 | 506 | 267 | 189 | 61 | 70 | 415 | 528 | 295 | 373 | 163 | 229 |
| Glossiphonia complanata | Glossiphoniidae | 3 | 12 | 0 | 0 | 2 | 9 | 3 | 12 | 0 | 0 | 2 | 9 |
| Helobdella nr. californica | Glossiphoniidae | **951** | 1,070 | **512** | 636 | 440 | 609 | 333 | 185 | 693 | 647 | **957** | 1,108 |
| Helobdella nr. robusta | Glossiphoniidae | 16 | 35 | 132 | 140 | 82 | 141 | 81 | 128 | 77 | 123 | 81 | 127 |
| Helobdella stagnalis | Glossiphoniidae | 16 | 33 | 250 | 323 | 7 | 20 | 21 | 33 | 161 | 333 | 102 | 150 |
| Helobdella hatchlings undet. | Glossiphoniidae | 344 | 426 | **696** | 1,452 | **1,019** | 2,322 | 171 | 279 | **999** | 1,475 | **980** | 2,274 |
| Theromyzon sp. | Glossiphoniidae | 0 | 0 | 0 | 0 | 2 | 9 | 0 | 0 | 0 | 0 | 2 | 9 |
| Mooreobdella microstoma | Erpobdellidae | 28 | 51 | 10 | 26 | 5 | 14 | 8 | 20 | 13 | 26 | 20 | 50 |
| Erpobdellidae undet. | Erpobdellidae | 2 | 9 | 0 | 0 | 12 | 42 | 0 | 0 | 13 | 42 | 0 | 0 |
| Piscicolidae | Piscicolidae | 5 | 14 | 5 | 19 | 5 | 14 | 3 | 9 | 2 | 9 | 11 | 23 |
| Caecidotea cf. occidentalis | Asellidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hyalella | Hyalellidae | 12 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 36 | 2 | 9 |
| Caenis | Caenidae | 59 | 129 | 0 | 0 | 0 | 0 | 13 | 58 | 35 | 123 | 4 | 14 |
| Coenagrionidae undet. | Coenagrionidae | 3 | 12 | 0 | 0 | 0 | 0 | 2 | 12 | 0 | 0 | 0 | 0 |

| Taxon | | Littoral | | Open-water | | Trench | | May | | June | | July | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| | Family | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Isoperla | Perlodidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sialis | Sialidae | 2 | 9 | 0 | 0 | 0 | 0 | 2 | 9 | 0 | 0 | 0 | 0 |
| Hydroptila | Hydroptilidae | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 9 | 0 | 0 |
| Limnephilus | Limnephilidae | 2 | 9 | 0 | 0 | 0 | 0 | 2 | 9 | 0 | 0 | 0 | 0 |
| Mystacides | Leptoceridae | 8 | 21 | 2 | 9 | 0 | 0 | 0 | 12 | 5 | 19 | 2 | 9 |
| Oecetis | Leptoceridae | 0 | 0 | 15 | 28 | 0 | 0 | 7 | 20 | 7 | 20 | 2 | 9 |
| Brychius | Haliplidae | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 9 | 0 | 0 |
| Dytiscidae | Dytscidae | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 9 | 0 | 0 |
| Dubiraphia | Elmidae | 7 | 20 | 0 | 0 | 0 | 0 | 2 | 19 | 0 | 0 | 2 | 9 |
| Bezzia | Ceratopogonidae | 109 | 87 | 12 | 32 | 2 | 9 | 40 | 56 | 54 | 78 | 22 | 81 |
| Procladius sp. 1 | Tanypodinae | 212 | 232 | **960** | 707 | **1,274** | 998 | **831** | 707 | **978** | 972 | 674 | 807 |
| Orthocladiinae | Orthocladiinae | 5 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 19 |
| Chironomus cf. plumosus | Chironomidae | 5 | 14 | 3 | 12 | 119 | 183 | 82 | 169 | 21 | 67 | 26 | 87 |
| Chironomus cf. utahensis | Chironomidae | 3 | 12 | 2 | 9 | 46 | 106 | 28 | 99 | 2 | 9 | 24 | 51 |
| cf. Chironomus e.i. | Chironomidae | 23 | 78 | **416** | 1,722 | 166 | 335 | 2 | 9 | 10 | 26 | 669 | 1,710 |
| Cryptochironomus | Chironomidae | 132 | 233 | 35 | 53 | 23 | 43 | 61 | 179 | 72 | 154 | 28 | 88 |
| Cryptotendipes | Chironomidae | 23 | 51 | 316 | 335 | 84 | 121 | 194 | 235 | 181 | 316 | 41 | 84 |
| Dicrotentipes | Chironomidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Glyptotendipes sp. 1 | Chironomidae | 25 | 73 | 0 | 0 | 0 | 0 | 3 | 19 | 20 | 72 | 0 | 0 |
| Parachironomus | Chironomidae | 12 | 32 | 3 | 12 | 10 | 28 | 8 | 27 | 8 | 28 | 9 | 21 |
| Chironomini "Harnischia comp." e.i. | Chironomidae | 5 | 14 | 0 | 0 | 0 | 0 | 0 | 9 | 2 | 0 | 2 | 12 |
| Chironomini undet | Chironomidae | 25 | 47 | 207 | 878 | 7 | 20 | 0 | 35 | 8 | 12 | 250 | 874 |
| Cladotanytarsus | Chironomidae | 100 | 133 | 212 | 507 | 16 | 35 | 242 | 497 | 30 | 69 | 41 | 102 |
| Tanytarsini | Chironomidae | 25 | 50 | 13 | 30 | 20 | 54 | 15 | 42 | 7 | 18 | 41 | 62 |

| Taxon | | Littoral | | Open-water | | Trench | | May | | June | | July | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| | Family | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| cf. Hygrobates | Eriophyidae | 7 | 16 | 8 | 28 | 0 | 0 | 8 | 28 | 5 | 14 | 2 | 9 |
| cf. Piona | Eriophyidae | 2 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 9 | 0 | 0 |
| Mite undet. | Eriophyidae | 10 | 19 | 10 | 38 | 2 | 9 | 12 | 35 | 5 | 16 | 6 | 20 |
| Valvata | Valvatidae | 97 | 155 | 3 | 12 | 0 | 0 | 35 | 94 | 43 | 83 | 2 | 122 |
| Fluminicola | Hydrobiidae | 81 | 101 | 110 | 120 | 3 | 12 | 66 | 73 | 74 | 103 | 46 | 122 |
| Pyrgulopsis | Hydrobiidae | 40 | 93 | 0 | 0 | 0 | 0 | 7 | 77 | 16 | 20 | 0 | 56 |
| Planorbidae spp. | Planorbidae | 10 | 23 | 3 | 17 | 10 | 43 | 8 | 20 | 13 | 46 | 2 | 9 |
| Sphaeriidae undet. | Spheriidae | 938 | 1,249 | 76 | 156 | 35 | 85 | 239 | 815 | 482 | 1,089 | 237 | 512 |

**Table 3.3.** Water quality parameter summary statistics (mean and SD). Units for temperature are °C, units for specific conductivity are mS $cm^{-1}$, units for benthic chlorophyll are $\mu$g $cm^{-2}$ and dissolved oxygen, as well as minimum dissolved oxygen, are given as percentages. SRP benthic flux values are given as mg-P $m^{-2}$ $d^{-1}$, NH4 flux unit is mg-N $m^{-2}$ $d^{-1}$, $SiO_2$ flux units are mg-$SiO_2$ $L^{-1}$, DOC benthic flux values are given as mg-C $m^{-2}$ $d^{-1}$, while benthic flus of manganese is $\mu$g-Mn $m^{-2}$ $d^{-1}$, and iron is $\mu$g-Fe $m^{-2}$ $d^{-1}$.All values were measured at the maximum depth of sites and central sites do not include trench site values. Dissolved (0.2-micron filtered) macronutrient benthic fluxes from profiler deployments.

| | Littoral | Open-water | Trench | North | Central | South | May | June | July |
|---|---|---|---|---|---|---|---|---|---|
| Temperature | 17.40 | 16.90 | 17.30 | 17.20 | 16.60 | 17.40 | 12.80 | 17.50 | 21.50 |
| S.D | 4.10 | 3.50 | 3.30 | 3.70 | 3.40 | 4.40 | 0.40 | 0.50 | 1.80 |
| Depth | 1.70 | 2.80 | 9.40 | 2.30 | 2.20 | 2.20 | 2.30 | 2.30 | 2.10 |
| S.D. | 0.20 | 0.60 | 3.50 | 0.60 | 0.60 | 0.80 | 0.70 | 0.70 | 0.70 |
| pH | 8.60 | 8.40 | 8.10 | 8.60 | 8.50 | 8.50 | 7.90 | 8.30 | 9.40 |
| S.D | 0.70 | 0.90 | 1.10 | 0.80 | 1.00 | 0.70 | 0.30 | 0.80 | 0.40 |
| Conductivity | 0.17 | 0.17 | 0.18 | 0.16 | 0.17 | 0.19 | 0.14 | 0.14 | 0.23 |
| S.D | 0.07 | 0.08 | 0.12 | 0.07 | 0.07 | 0.09 | 0.05 | 0.06 | 0.08 |
| Benthic Chl-*a* | 8.10 | 5.10 | 1.20 | 5.60 | 5.10 | 9.20 | 4.20 | 9.10 | N/A |
| S.D | 4.80 | 12.00 | 0.48 | 2.80 | 3.70 | 15.20 | 3.20 | 12.20 | N/A |
| Dissolved Oxygen | 84.30 | 70.40 | 56.60 | 80.80 | 77.80 | 71.30 | 82.20 | 93.10 | 48.30 |
| S.D | 30.50 | 31.50 | 30.60 | 28.80 | 29.20 | 39.30 | 25.00 | 25.90 | 28.70 |
| % Organic matter | 18.30 | 13.20 | 8.80 | 17.50 | 11.40 | 18.20 | N/A | 14.30 | 17.20 |
| S.D | 14.10 | 2.80 | 5.60 | 10.00 | 4.70 | 13.60 | N/A | 6.50 | 13.10 |
| %clay | 4.30 | 4.30 | 3.60 | 4.10 | 3.60 | 5.20 | N/A | 4.20 | 4.20 |
| S.D | 1.90 | 0.90 | 2.20 | 1.10 | 1.80 | 1.20 | N/A | 1.60 | 1.70 |
| %silt | 48.80 | 52.20 | 35.60 | 47.70 | 44.80 | 58.80 | N/A | 48.70 | 48.00 |
| S.D | 22.30 | 6.30 | 23.30 | 14.70 | 20.60 | 9.00 | N/A | 17.80 | 18.30 |
| %sand | 46.90 | 43.70 | 60.80 | 48.40 | 51.50 | 36.10 | N/A | 47.10 | 48.00 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| S.D | 24.10 | 6.80 | 25.50 | 15.50 | 22.20 | 10.00 | N/A | 19.30 | 19.70 |
| SRP Benthic Flux | 5.20 | 3.70 | 4.20 | 3.90 | 3.70 | 5.60 | 3.40 | 2.70 | 7.20 |
| S.D | 6.70 | 3.00 | 3.90 | 4.00 | 3.40 | 7.40 | 2.90 | 2.50 | 7.50 |
| NH4 Benthic Flux | 8.80 | 10.10 | 16.50 | 9.60 | 7.80 | 11.20 | 8.70 | 7.50 | 12.20 |
| S.D. | 7.30 | 8.00 | 9.70 | 9.10 | 4.50 | 8.60 | 4.50 | 5.20 | 11.10 |
| SiO2 Benthic Flux | 0.12 | 0.14 | 0.10 | 0.15 | 0.12 | 0.12 | 0.11 | 0.11 | 0.16 |
| S.D | 0.08 | 0.09 | 0.05 | 0.11 | 0.08 | 0.07 | 0.06 | 0.07 | 0.11 |
| DOC Benthic Flux | 0.19 | -0.63 | -2.20 | -0.62 | 0.10 | -0.21 | -0.52 | -0.20 | 0.03 |
| S.D | 0.87 | 0.98 | 1.20 | 0.92 | 0.90 | 1.12 | 0.82 | 1.07 | 1.10 |
| Fe Benthic Flux | 4,495.00 | 2,628.00 | 1,924.00 | 3,132.00 | 4,223.00 | 3,180.00 | 4,358.00 | 2,216.00 | 3,953.00 |
| S.D | 4,364.00 | 2,043.00 | 1,998.00 | 2,885.00 | 4,168.00 | 3,250.00 | 4,572.00 | 2,085.00 | 2,973.00 |
| Mn Benthic Flux | 1,066.00 | 1,135.00 | 534.00 | 890.00 | 1,248.00 | 1,251.00 | 1,079.00 | 1,322.00 | 1,050.00 |
| S.D | 764.00 | 890.00 | 491.00 | 738.00 | 852.00 | 1,079.00 | 829.00 | 1,265.00 | 781.00 |

Table **3.4**. PERMANOVA results indicating whether differences in species composition exist among habitats "Ha" (littoral and open-water), regions "Re" (north, central, and south), and months "Mo" (May, June, and July). Statistical significance among factors was determined using 999 permutations. P values <0.05 were considered significant. Df is degrees of freedom. SS is the sum of squares. MS is the mean sum of squares.

| Source | df | SS | MS | Pseudo-F | P(perm) | perms |
|---|---|---|---|---|---|---|
| Ha | 1 | 3851.5 | 3851.5 | **5.971** | **0.001** | 999 |
| Re | 2 | 9194.7 | 4597.4 | **7.1273** | **0.001** | 998 |
| Mo | 2 | 5657.2 | 2828.6 | **4.3851** | **0.001** | 997 |
| MoxRe | 4 | 854.74 | 213.68 | 0.33127 | 1 | 999 |
| MoxHa | 2 | 842.69 | 421.35 | 0.65321 | 0.847 | 999 |
| RexHa | 2 | 5596.5 | 2798.2 | **4.3381** | **0.001** | 999 |
| MoxRexHa | 4 | 690.86 | 172.72 | 0.26776 | 1 | 998 |
| Res | 36 | 23221 | 645.04 | | | |
| Total | 53 | 49910 | | | | |
| Central Region | | | | | | |
| Mo | 2 | 2922 | 1461 | 2.0646 | 0.015 | 999 |
| Ha | 2 | 10445 | 5222.3 | **7.3801** | **0.001** | 998 |
| MoxHa | 4 | 1141.4 | 285.35 | 0.40325 | 0.999 | 997 |
| Res | 18 | 12737 | 707.62 | | | |
| Total | 26 | 27245 | | | | |

Table **3.5.** Estimates of the components of variation that characterized benthic invertebrate assemblages. Mo=month, Re=region, Ha=habitat.

| Source | Estimate | Sq.root |
|---|---|---|
| S(Mo) | 121.31 | 11.014 |
| S(Re) | 219.57 | 14.818 |
| S(Ha) | 118.76 | 10.898 |
| S(MoxRe) | -71.893 | -8.479 |
| S(MoxHa) | -24.855 | -4.9855 |
| S(RexHa) | 239.24 | 15.467 |
| S(MoxRexHa) | -157.44 | -12.548 |
| V(Res) | 645.04 | 25.398 |
| Central | | |
| S(Mo) | 83.707 | 9.1491 |
| S(Ha) | 501.63 | 22.397 |
| S(MoxHa) | -140.76 | -11.864 |
| V(Res) | 707.62 | 26.601 |

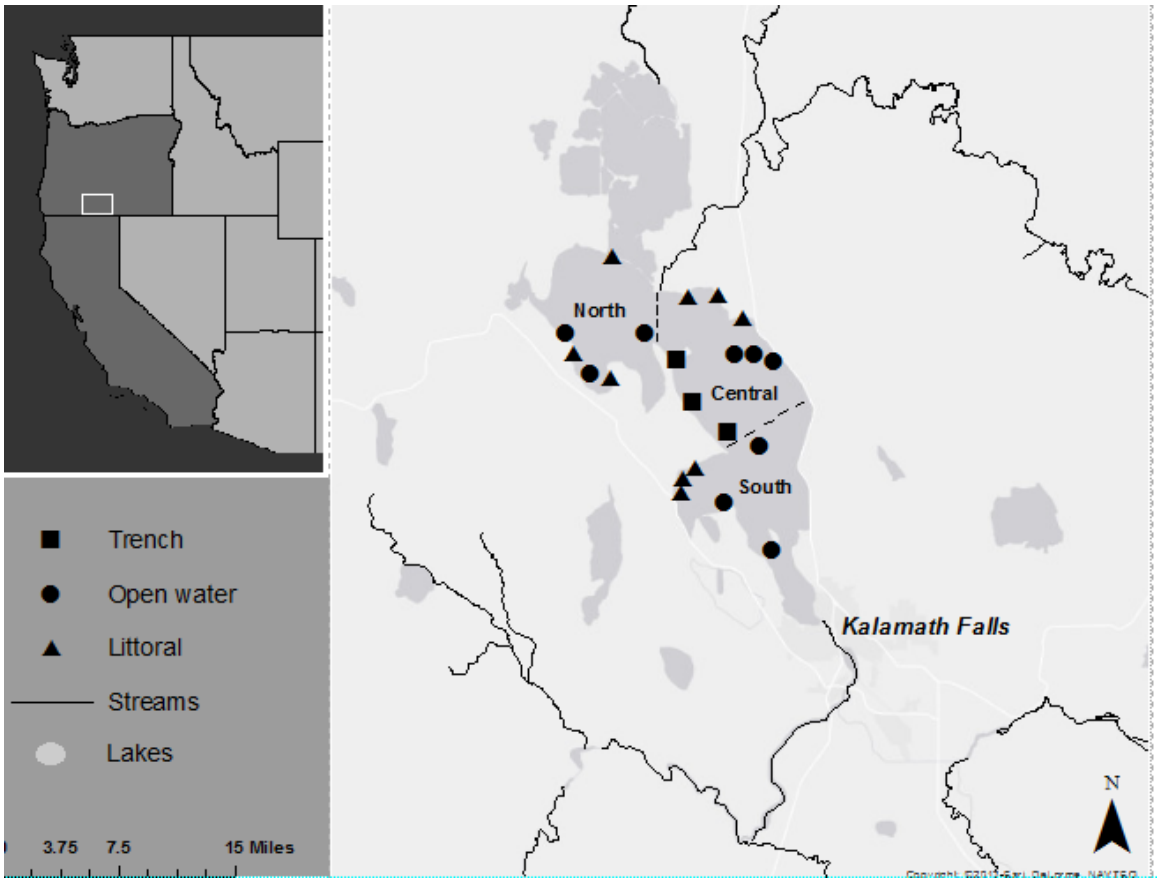


**Figure 3.1**. Map of Upper Klamath Lake, Oregon with 21 sampling sites. Factors (region and habitat) are also indicated. Sampling habitat is indicated by squares (trench, n=3), circles (open water, n=9), and triangles (littoral, n=9).

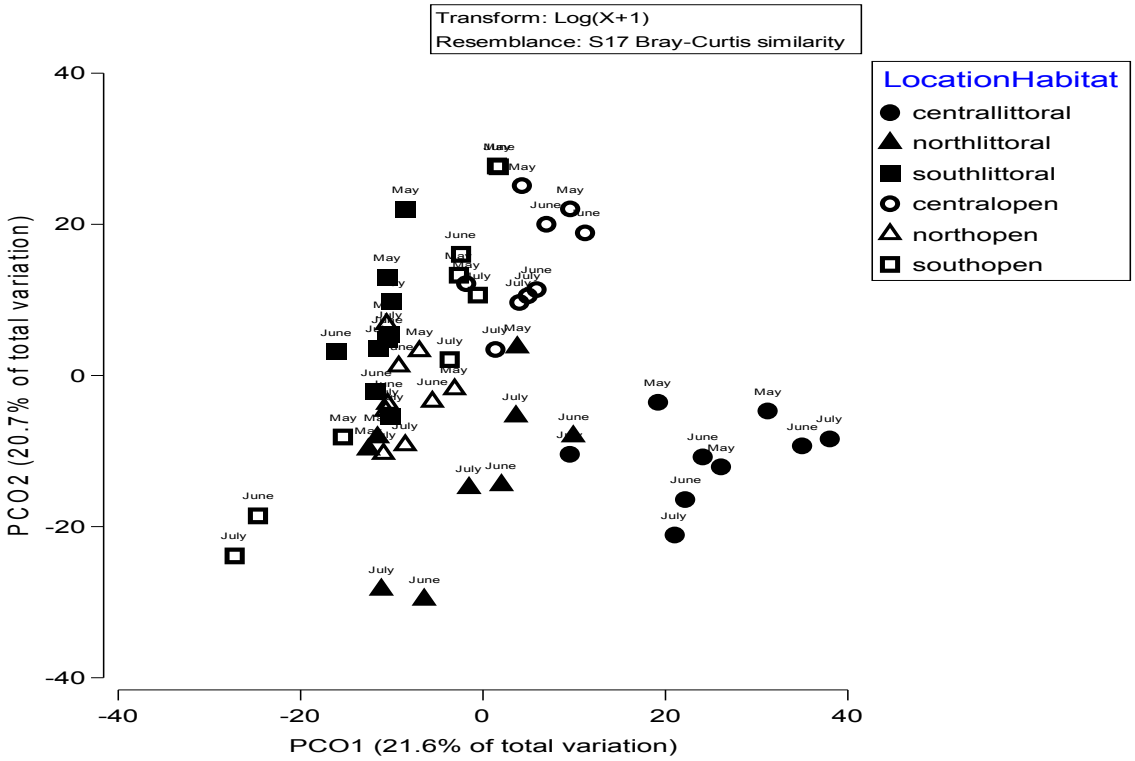


**Figure 3.2**. Principal coordinates analysis ordination of all samples (excluding those from trench sites) collected during the study using Bray-Curtis dissimilarities based on $\log_{10}(x+1)$ benthic invertebrate densities. Principal coordinates analysis axes 1 and 2 (PCO1, PCO2) are centered and represent the distance between sites in Bray-Curtis resemblance space.

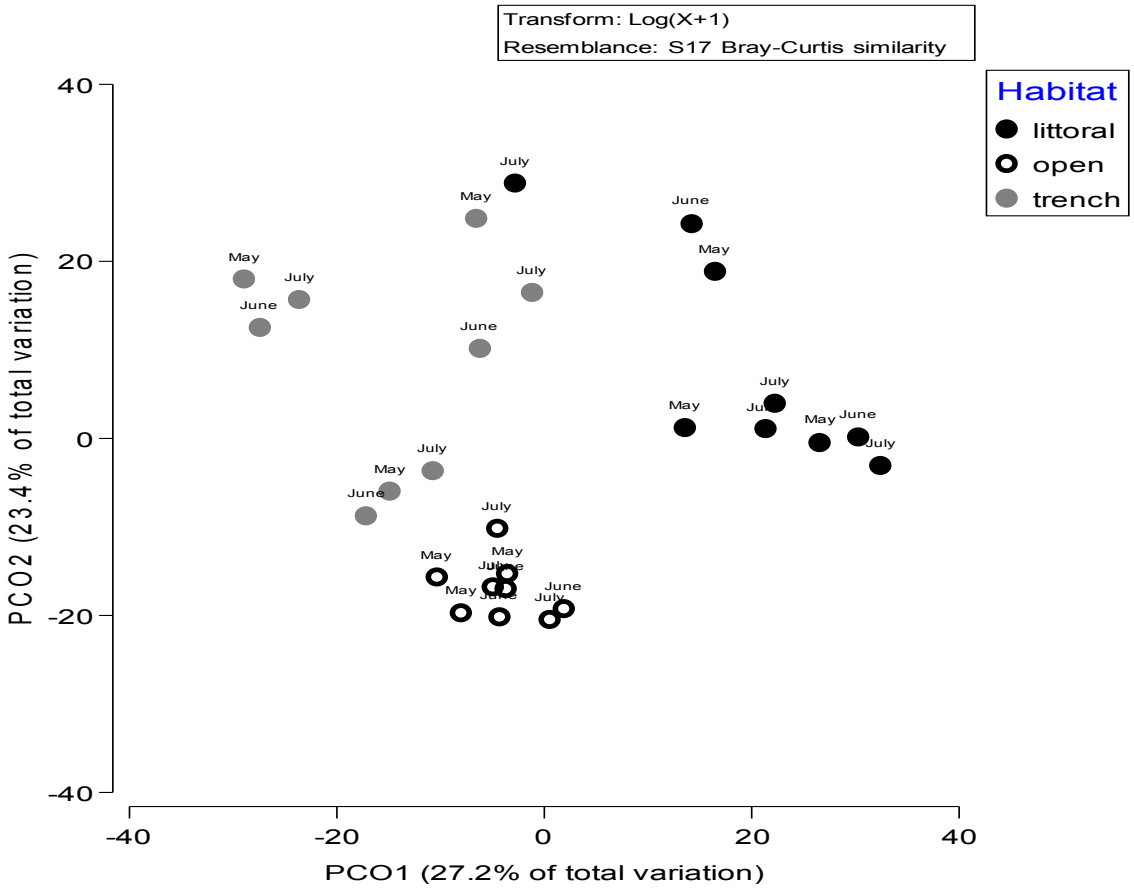


**Figure 3.2A**. Principal coordinates analysis ordination of samples collected from the central region.

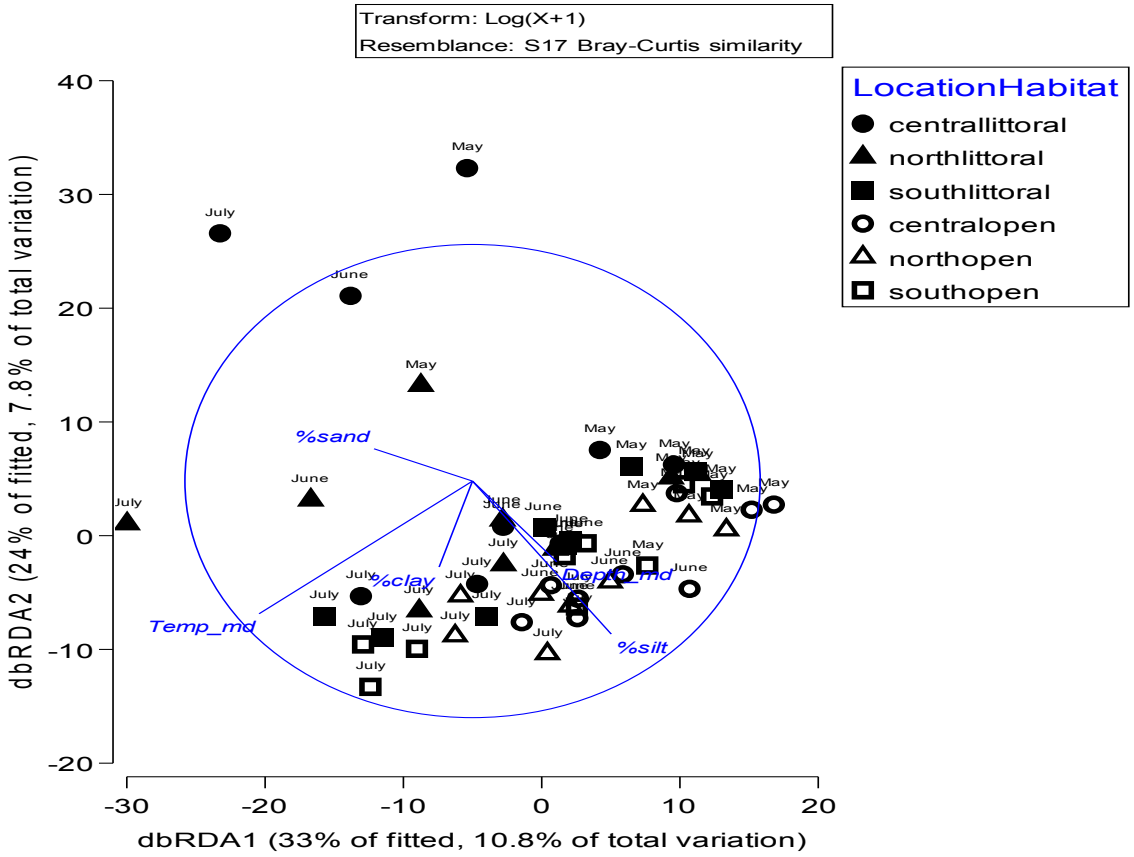


**Figure 3.3**. Distance-based Redundancy Analysis (dbRDA) biplot from the DISTLM runs that utilized Akaike's Information Criterion for small sample sizes (AICc) model of benthic invertebrate data constrained by environmental variables. Samples are grouped by region x habitat. Vectors indicate the 2 environmental variables that explained most of the variability in the invertebrate data set. Environmental variables included in analyses were physical parameters that could influence benthic invertebrate distribution: average bottom temperature (°C), average bottom pH, bottom depth, average bottom specific conductivity (mS $cm^{-1}$ multiplied by 100 to adjust magnitude for figure clarity), average benthic chlorophyll ($\mu$g $cm^{-2}$), percent dissolved oxygen, percent organic matter, percent clay, percent silt, and percent sand.

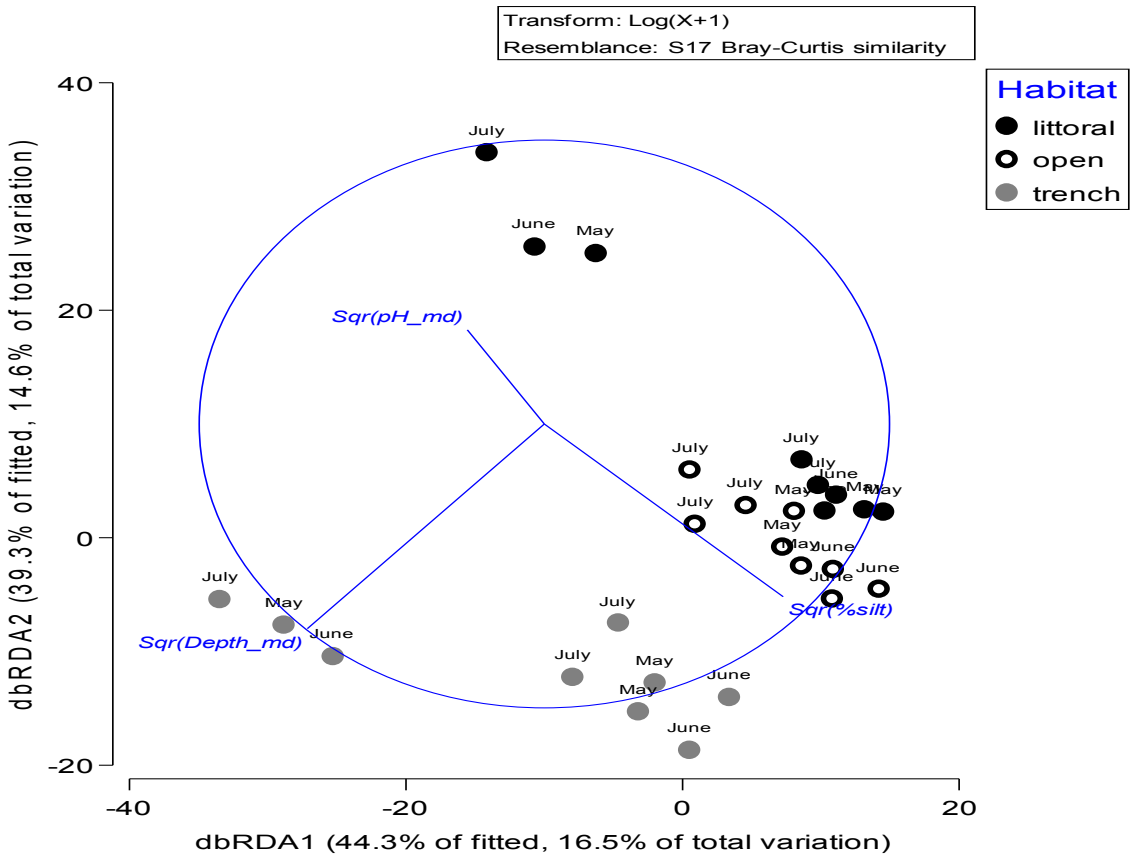


**Figure 3.3A**. Distance-based Redundancy Analysis (dbRDA) biplot, described in detail in Figure 3.3, for the central region of Upper Klamath Lake.

# CHAPTER 4

## Comparing Population Structure in Baetis (Ephemeroptera: Baetidae) Species from Northern and Southern California

**Comparing Population Structure in Baetis (Ephemeroptera: Baetidae) Species from Northern and Southern California**


## ABSTRACT

The mayfly genus *Baetis* is geographically widespread, occurring from the subarctic to tropical areas around the world. Many of the 20 described *Baetis* species in North America are known to have cryptic species diversity; however, studies that have examined morphology and genetic diversity have found mixed results for cryptic species, most notably in California. We used Bayesian analyses, intra- and interspecific genetic diversity values, and median-joining haplotype networks to compare cytochrome oxidase I (COI) genetic sequences from *Baetis* specimens from northern and southern California (n=742). Using a combination of genetic and morphological methods, we found evidence for two putative *Baetis* species in northern California and five putative *Baetis* species in southern California. The putative species morphologically and genetically identified as *Baetis tricaudatus* was the only taxon that occurred in both regions and no haplotypes were shared between regions. Intraspecific diversity in putative species from northern California was >1%. In contrast, intraspecific diversity in species from southern California was always <1%. Such discrepancies highlight the need for locally derived reference libraries in using next generation sequencing or environmental DNA to examine genetic diversity.



**Key words:** genetic diversity, population genetic structuring, biodiversity, California


## INTRODUCTION

The description and categorization of biodiversity has been a major focus in biology since before the time of Linnaeus (Godfray, 2007). Significant technological advances in molecular biology over the past 30 years, such as polymerase chain reaction (PCR) and novel DNA sequencing techniques, has made it easier for non-experts in traditional morphology to classify species based on DNA sequences (Hebert et al., 2003a; Hebert & Gregory, 2005). Therefore, the focus of many taxonomists has shifted away from a traditional morphological concept of species and toward a more holistic view that includes both morphology and genetic characteristics (Padial et al., 2010; Schlick-Steiner et al., 2010). This shift has fundamentally altered how biodiversity is defined and studied (Will et al., 2005; De Queiroz, 2007; Puillandre et al., 2012; Taberlet et al., 2012). Although these integrated taxonomic methods for species delimitation are quite robust (Padial et al., 2010; Pfrender et al., 2010; Schlick-Steiner et al., 2010; Webb et al., 2012), the criteria used to determine patterns of genetic diversity and reach conclusions about biodiversity are often inconsistent. For example, some authors recommend a cutoff of ≥3% sequence divergence for cryptic species (Hebert et al., 2003a; Sweeney et al., 2011) while others suggest divergences of 1–2% are evidence of cryptic species diversity (Hebert et al., 2003b; Zhou et al., 2009; White et al. 2014).

The COI mitochondrial gene region has been suggested as an effective and efficient way to identify (*e.g.,* Hebert et al., 2003a; b), delimit (*e.g.* White et al., 2014),

and help describe (*e.g.,* Gill et al., 2014) species including benthic invertebrates (*e.g.,* Ståhls & Savolainen, 2008; Zhou et al., 2010; Lucentini et al., 2011; Mynott et al., 2011; Renaud et al., 2012; Webb et al., 2012; Jackson et al., 2014). While this genetic tool and has been the focus of many studies (Webb et al., 2012; Hughes et al., 2014), there is also much debate in the community about the application of genetic methods to species delineation (Will et al., 2005; DeWalt, 2011). Large databases such as GenBank and the Barcode of Life Database (BOLD) (Ratnasingham & Hebert, 2007) provide COI gene segments and their associated data to scientists and managers worldwide and these are useful tools in the assessment of biodiversity.

The use of next generation sequencing techniques to perform amplicon-based studies that are able to characterize entire communities is an exciting application for biomonitoring and bioassessment programs (Pfrender et al., 2010; Sweeney et al., 2011; Jackson et al., 2014; Uyaguari-Diaz et al., 2016)). Freshwater biomonitoring programs that collect biodiversity information such as richness and species composition to describe or determine the health of ecosystems are used globally (Carter & Resh, 2013). Such efforts require clear and consistent criteria for species identification in order to be accurate and effective (Carter & Resh, 2013). However, the taxonomic resolution used by many monitoring programs is coarse, typically at the genus-or even family-levels, even though species-level identification is necessary to optimize the diagnostic abilities of collected data (Lenat & Resh, 2001; Hawkins, 2006; Jones, 2008; Pfrender et al., 2010). Methods such as DNA barcoding could be useful in performing bioassessments more quickly and accurately (Pfrender et al., 2010; Sweeney et al., 2011; Webb et al., 2012; Stein et al., 2014). However, accurate links between the DNA barcode and the taxonomic identity of the sample is necessary in order to leverage the power of a barcoding approach (De Queiroz, 2007; White et al., 2014).

Observed patterns of genetic diversity can differ between closely related taxa or even within populations of the same taxon, which makes it difficult to conceptualize and delimit variation of populations or species. For example, some studies examining species within the mayfly genus *Baetis,* have found well-mixed populations both within and between watersheds (Monaghan et al., 2001; Hughes et al., 2003b; Peckarsky et al., 2005), while others have found significant structuring at both small (Monaghan et al., 2002; Lucentini et al. 2011) and large scales (Williams et al., 2006; Webb et al. 2012). Similarly, a number of studies have found evidence for cryptic genetic diversity (Webb et al., 2012; Spitzer et al., 2014), while others have not (Stauffer-Olsen et al., 2017). Different study areas, geographic ranges, temporal scales, and arbitrary species delimitation cutoffs are all potential causes of the variety of research results and conclusions. Most studies exploring the structure and diversity of the COI gene region in freshwater insects focus on broad-scale parameters such as percent diversity (Sweeney et al., 2011) or presence of a "barcoding gap," reflecting a discontinuity in gene flow between species (Puillandre et al., 2012; Macher et al., 2016). Some have made the concerted effort to link together morphological and genetic diversity and to compare the two (*e.g*., Sweeney et al., 2011; Stein et al., 2014). Fewer studies (*e.g*. Finn et al., 2007) have examined the arrangement of genetic diversity of benthic invertebrates through methods such as haplotype networks.

The family Baetidae (Ephemeoptera) is represented by 35 species in California, 10 of which belong to the genus *Baetis* Leach, 1815 (Meyer & McCafferty, 2008). *Baetis*

is cosmopolitan and can be found from the tropics to subarctic regions (Gattolliat & Nieto, 2009). Because many species are difficult to identify morphologically, this genus has been divided into a number of subgroups containing closely related species (Jacobus, et al., 2014). The *Baetis rhodani* species group (Meyer & McCafferty, 2008) includes four species from California: *B. tricaudatus, B. adonis*, *B. palisadi* and *B. piscatoris*. Two of these species, *B. tricaudatus* and *B. adonis*, are widely dispersed and abundant in California. The other two *Baetis* species have also been found in California and *B. palisadi* has in fact never been found outside of California. However, they are also less common than *B. tricaudatus* and *B. adonis* (Meyer & McCafferty, 2008). *Baetis tricaudatus* is closely related to *B. adonis* and cannot be morphologically differentiated for most larval stages (Jacobus et al., 2014). Furthermore, studies have found inconsistent evidence for cryptic genetic diversity in these two taxa (Spitzer, 2014; Stein et al., 2014, Stauffer-Olsen et al., 2017). As such, a reliable molecular taxonomy necessary to quickly identify biodiversity using genetic methods has yet to be defined for *B. tricaudatus* and *B. adonis*.

Previous work done on *Baetis* populations in northern California (Stauffer-Olsen et al., 2017) found different results than studies done on the same taxa in southern California (White et al., 2014; Spitzer, 2014; Stein et al., 2014; Jackson et al., 2014). Northern California populations of *B. tricaudatus* were found to be well mixed within and between watersheds with no evidence for cryptic genetic diversity (Stauffer-Olsen et al., 2017). In that study, intra-specific genetic diversity was 1.7% at the COI gene region, which could be interpreted as evidence for cryptic diversity if the 1% divergence value used to differentiate *Baetis* species (*e.g.*, Stein et al., 2014; Jackson et al., 2014) was applied. However, examining the organization of genetic diversity with haplotype networks demonstrated that sampled populations were panmictic (Stauffer-Olsen et al., 2017). Studies in southern California populations, in contrast, used different methods to conceptualize and delimit genetic diversity (White et al., 2014; Stein et al., 2014; Jackson et al., 2014) and found evidence for cryptic genetic diversity within *B. tricaudatus* and within the morphologically similar *B. adonis*.

We examined COI sequences from *rhodani* group species collected in northern and southern California. Our objectives were to 1) create a phylogenetic tree to infer relationships between *Baetis* species, 2) describe the genetic diversity within the *rhodani* group species in California using haplotype networks, and 3) compare patterns of genetic diversity between the *rhodani* group species collected in northern and southern California.

## METHODS

### *Study sites*

*Baetis* specimens (n=371) were collected from eight sites in northern California that were located in two watersheds (Fig. 4.1). Five sites were located within the Russian River watershed and three sites were located in the neighboring Sacramento River watershed. Both watersheds have a Mediterranean climate with most precipitation occurring as rain during winter months (NOAA, 2009). Collection sites encompassed a large river (the Russian River-mainstem collection site), perennial streams (the Austin Creek, Cappell, Putah and Schneider Creek sites), and intermittent streams (the Salmon and Dutch Bill Creek sites). Sites occurred on both regulated and unregulated waterways

and represented a range of other habitat parameters including sediment, shade cover, flow, elevation, and land-use (Table 4.1).

*Taxon sampling and identification*

Collections from three sites within the Russian River watershed (Russian River mainstem site, Austin Creek site and Salmon Creek site) were made monthly from September 2012 to September 2015. These multiple sampling events were designed to maximize collection of the natural variation of *Baetis* populations. Other collections from northern California sites were made only during the summer months (June-August). Specimens were collected by disturbing benthic sediment in a variety of localized habitats such as riffles and pools and catching suspended material in a D-frame net placed just downstream of the disturbed area.

Immediately following collection, northern California samples were stored in 95% ethanol and transported to the laboratory at UC Berkeley for identification. The key provided in Jacobus et al. (2014) was used for morphological identifications. Some specimens were identified to species group because diagnostic characters are absent or inconsistent during early larval instars for *B. tricaudatus* and *B. adonis* (Jacobus et al., 2014). For consistency, all specimens collected in northern California were identified by a single taxonomist (NSO) with some identifications confirmed by other experienced taxonomists (*e.g.* L. Jacobus, J. Webb).

*DNA extraction, amplification & sequencing*

DNA was extracted from identified northern California specimens using Qiagen DNeasy DNA extraction kit (Qiagen, Inc., Alameda, United States) with slight modifications to the manufacturer's protocol. Specimens were removed from 95% ethanol and dried on a clean kimwipe. Proteinase K and AL buffer were then added and samples were lysed for at least 5 hours at 56°C. After lysis, samples were placed in 95% ethanol and stored as vouchers at the University of California, Berkeley Essig Museum of Entomology (EMEC numbers 1173400-1173692). The remainder of the protocol followed the manufacturer's instructions.

The a region of the mitochondrial gene cytochrome oxidase I (COI), which has been shown to be variable at the intraspecific level, was amplified and sequenced (Hebert et al., 2003b, 2003c; Burns et al., 2007; Webb et al., 2012). Generally, sequence divergence of less than 2% is considered to conform to the amount of variation observed within a species (Hebert et al., 2003c; Burns et al., 2007; Webb et al., 2012), although the arbitrary cutoff chosen to differentiate species varies with taxa and study (DeWalt, 2011). The COI gene region has been effective at identifying baetid species in previous studies (Webb et al., 2012; White et al., 2014). Universal primers LCO 1490 (5' - GGT CAA CAA ATC ATA AAG ATA TTG G- 3') and HCO 2198 (5' - TAA ACT TCA GGG TGA CCA AAA AAT CA - 3') (Folmer et al., 1994) were used to amplify a 658 base pair fragment of COI. PCR for COI was performed using the following ratio of reagents: 17.5 µl sterile $H_2O$, 2.5µl iTaq (Biorad) buffer, 2.5 µl $MgCl_2$ (25 µM), 0.5 µl dNTPs (10 µM), 2.5 µl of each primer (10 µM), 0.25 µl iTaq polymerase and 1-2 µl of the extracted template DNA. For amplification, the following protocol was used: 5 min initial denaturing at 94°C, 15 cycles of 30 s at 94°C, 30 s at 45°C and 45 s at 72°C, 20 cycles of 30 s at 94°C, 30 s at 55°C, and 45 s at 72°C, and a final extension step of 72°C for 5 min.

PCR products were cleaned using Exonuclease I - Shrimp Alkaline Phosphatase (ExoSAP) following the manufacturer's protocol (Thermo Fisher Scientific, Waltham, Massachusetts). Samples were then incubated at 37°C for 15 min, then 80°C for another 15 min. Once cleaned, PCR products were sent to the University of California, Berkeley DNA Sequencing Facility for sequencing.

*Sequence editing*

The program Geneious Pro (Version 6.1.4) (Kearse et al., 2012) was used to create and edit contigs of individual sequences reads, build alignments, generate basic sequence statistics, and perform phylogenetic analyses. Contigs were edited individually and most had 99%-100% high quality (HQ) bases. Contigs with less than 80% HQ bases were discarded because base pair calls were not reliable. Sequences with less than 80% overlap between forward and reverse reads were also discarded in order to ensure that sequences used in analyses were effectively proofread by overlapping strands. Overall, 42 out of 511 contigs were removed because of low quality reads or insufficient overlap. Contigs were also checked for shifts in reading frame that could indicate the presence of nuclear-mitochondrial copies (numts) and translated into amino acids to check for stop codons, neither of which were detected.

The Basic Local Alignment Search Tool (BLAST) was used to compare generated sequences against all GenBank sequences. For comparisons, we only focused on sequences that were identified to the species level by an expert and for which voucher material existed.

*Database sequences*

Southern California COI *Baetis* sequences (n = 836) generated by Stein et al. (2014) were obtained from BOLD (project codes CFWIA through CFWIH). As described by Stein et al. (2014), samples were collected from five sites on three streams in the Los Angeles, California area. Southern California is substantially drier than northern California and gets approximately one half the precipitation. Sites were located on both regulated and unregulated waterways and represented a range of land uses (Table 4.1) and habitats (Stein et al., 2014). Morphological identifications were done by expert taxonomists of the Ephemeroptera, as described in Stein et al. (2014). Taxonomists identified three distinct species based on morphology: *B. tricaudatus*, *B. adonis*, and an unknown *Baetis* sp. CA1. COI sequences from collected *Baetis* samples were generated as described by Stein et al. (2014).

*Phylogenetics and population genetic analyses*

Phylogenetic analyses were carried out on XSEDE using the CIPRES Science Gateway (Miller et al., 2010) to explore species identifications and to determine relationships between taxa. Analyses on two different input files were completed. One analysis examined the phylogenetic relationships between all of the unique sequences from both northern and southern California (n=479). The other analysis examined population genetic patterns with the rarefied sequences described below (n=742). jModelTest 2 (Darriba et al., 2012), running on the Cipres Science Gateway, was used to determine the appropriate model of evolution for subsequent analyses. Bayesian analyses were run using the HKY85 model with two independent runs of four Monte Carlo–

Markov Chains for 2.5 million generations, 25% generation burn-in in MrBayes (version 3.2.2; Ronquist et al., 2012). The output trees were then imported to Geneious and both the phylogenetic and population genetic analyses were found to be similar, so a single tree that represented both analyses was edited in FigTree (Version 1.4.3) (Rambaut, 2016) and Adobe Illustrator (Version 19.2.1) (Fig. 4.2).

Sample sizes from northern (n=371) and southern (n=836) California were uneven, so 371 sequences from southern California were randomly chosen in order to make accurate population genetics comparisons (Szpiech et al., 2008). Alignments of northern and southern California sequences (n=742) were then made using the MAFFT Geneious Pro plugin (Version 7.017) (Katoh and Standley 2013). All sequences in the alignment were trimmed to the same length: 577 base pairs. The final alignment used for analyses was uploaded to Figshare (available at https://figshare.com/articles/Stauffer Olsen_Baetis_SoCal_vs_NorCal_rarefied/4828672). Sequences generated from northern California were uploaded to BOLD (sample IDs: NAT1-NAT827) and GenBank (accession numbers KY580901- KY581193). Geneious Pro (Version 6.1.4) (Kearse et al. 2012) was used to calculate the percent diversity between each of the sequences in the final alignment (Table 2).

For additional analyses, the rarefied population genetic sequence alignment (n=742) was exported from Geneious Pro (Version 6.1.4) (Kearse et al., 2012) as a nexus file into TextEdit for manipulation of the file for compatibility with POPART (Population Analysis with Reticulate Trees) (Leigh & Bryant, 2015). POPART was used to create haplotype networks to describe and visualize the genetic variation of sampled populations. Haplotype networks were built using the median-joining inference method (Bandelt et al., 1999).

## RESULTS

### *Morphological versus genetic diversity*

A total of three species were morphologically identified from northern and southern California. Two of these, *B. tricaudatus* and *B. adonis*, are previously described species and could be identified mostly on the basis of morphology, although some northern California taxa were initially identified as "*B. rhodani* species group" and further identified to *B. adonis* using a combination of BLAST and phylogenetic methods (Stauffer-Olsen et al., 2017). A third species, *Baetis* sp. CA1, was also identified (Stein et al., 2014).

Sequences that were morphologically identified as belonging to the *B. rhodani* species group appeared to consist of two distinct genetic clusters. One cluster consisted of 333 sequences most similar to *B. tricaudatus* sequences uploaded by well-established laboratories to GenBank. Maximum divergence within this group was 1.7%. The other cluster, which consisted of 38 sequences that were at least 8.2% different than the *B. tricaudatus* group, had a maximum intra-group divergence of 2.0% and was most similar to *B. adonis* sequences uploaded by experts and for which voucher material existed. For clarity, these groups will subsequently be referred to as *B. tricaudatus* and *B. adonis* NCA.

### *Phylogenetic and population genetic analyses*

There was little difference in topology between phylogenetic analyses performed on all unique sequences from northern and southern California and rarified samples where the number of sequences was equal between regions. Focus was placed on phylogenetic results of rarified analyses in order to be more comparable with population genetic analyses.

Within the rarefied *rhodani* group sequence alignment (n=742), 337 sequences were identical and 405 were unique. Population genetic analyses indicated that specimens fell into eight lineages or clades/grades (Fig. 4.2, Table 4.2). Clade A (Fig. 4.2, blue, PP=1.0) consists of 466 species with a maximum sequence divergence (msd) of 2.3%. Morphologically, these species all identify as *B. tricaudatus*. Bayesian analyses (Fig. 4.2) grouped the majority of sequences from northern and southern California separately, although two sequences from northern California were more similar to sequences from southern California. *Baetis tricaudatus* in northern California (Fig. 4.3) were represented by 30 haplotypes, one of which was very common (n=260), while the others were more rare (n=1-22). The northern California sequences had a maximum within-group diversity of 1.7%. Southern California *B. tricaudatus* (Fig. 4.3) had a maximum within group diversity of 1.0%, and were represented by 11 haplotypes, one of which was most common (n=74), while two others were less common (n=19, n=20), and the rest were rare (n=1-5). The two individuals collected in northern California that grouped with the southern California haplotypes were unique and not the same as the other southern California *B. tricaudatus* samples. These sequences did not change the value of msd from southern California when they were included in calculations.

Lineage B (Fig. 4.2, pink) is a paraphyletic grade of 174 *B. adonis* individuals collected in Southern California (Stein et al., 2014). The msd within this grade is 0.9%. The haplotype network of *B. adonis* sequences from southern California (Fig. 4.3) indicated 16 haplotypes, two of which were common. This group was at least six base pairs different from the *B. tricaudatus* samples from northern and southern California (Fig. 4.3). While most *B. adonis* individuals collected in southern California are placed in this grade, one individual was quite divergent (Fig. 4.2, Lineage E). Clade A and Grade B together form a monophyletic group with strong support (PP=1.0).

Clade C (PP=1.0) consists of 38 *B. adonis* individuals collected in northern California (Fig. 4.2, red). The msd within this group is quite high, roughly 2.4% (Table 4.2). The haplotype network for clade C (Fig. 4.4) indicated three haplotypes, one of which was separated by 13 base pair changes. When a 2% divergence cutoff was applied to this group, two putative species were indicated, as represented by the two boxes that separate the putative species in Fig. 4.4. One putative species consisted of 31 sequences differing by 0.2% (represented by the two haplotypes separated by a single base pair change in Fig. 4.4), and the other consisted of seven identical sequences, represented by a single haplotype. Lineages A-C form a well-supported monophyletic group (PP=1.0).

Clade D is comprised of 46 *B. tricaudatus* sequences from southern California with an msd of 0.7% (Fig. 4.2, orange). While these samples were identified as *B. tricaudatus*, they are over 16% different from the *B. tricaudatus* sequences in clade A (Fig. 4.2, blue). There are seven haplotypes in this group, three of which each occur in a single specimen (Table 4.2). A maximum of four base pair changes occur between haplotypes in these southern California *B. tricaudatus* sequences (Fig. 4.5). The nearest relative (PP=1.0) of Clade D is a single *B. adonis* individual from southern California

(Lineage E). The haplotype network identified 16 base pair changes between Clade D and Lineage E (Fig. 4.5). The *rhodani* species group is composed of all members of lineages A-E. This clade is weakly supported, with a posterior probability of 0.78.

The closest relative of the *rhodani* group (PP=0.99) is an exemplar of *B. bicaudatus* from Utah (Lineage F, Fig. 4.2). The sister lineage of the *rhodani-bicaudatus* clade is a clade comprised of two distinct lineages. The first, which we label as Clade G, is 17 sequences from an undescribed *Baetis* species (Stein et al. 2014) from southern California (*Baetis sp. CA1*; Fig. 4.2, yellow). Clade G is a well-supported lineage (PP=1.0) with 0.7% msd. There is a single dominant haplotype (n=8) and eight less common haplotypes (n=1-2) with a maximum of four base pair changes between haplotypes within Clade G (Fig. 4.6; Table 4.2). The sister taxon of clade G is a single representative of *Diphetor hageni* from northern California that is placed in Lineage H (Fig. 4.2).

## DISCUSSION

The COI gene region suggests that there are at least six putative species within the genus *Baetis* in California, two occurring in northern California and five in southern California. Some of these conform to the traditional morphological definitions of *B. adonis* and *B. tricaudatus*. Phylogenetic and population genetic analyses, however, demonstrate that there is actually a large sequence divergence within each of these morphological species that conforms to a number of cryptic species (*Baetis* sp. CA1, *B. adonis* sp. 1, *B. tricaudatus* sp. 1, *B. adonis* NCA, *B. adonis* SCA, *B. tricaudatus*).

There are two clades on the Bayesian phylogeny that include taxa identified as *B. tricaudatus* (Fig. 4.2, A and D). Clade A contains individuals from both northern and southern California while Clade D includes individuals from only southern California. Sequences included in these two clades are at least 16.6% different from one another, suggesting they represent at least two distinct species.

*Baetis adonis* is represented in three distinct lineages in the Bayesian analyses (Fig. 4.2, B, C, E). Clade C is from Southern California and is at least 8.6% different than other sequences morphologically identified as *B. adonis* (Fig. 4.2, B and E). Genetic analyses suggest that, with a 2% cutoff, there may be two species present in Clade C, one with 31 sequences differing from one another by 0.2%, and another with seven identical sequences that are over 2% different. Overall, there are at least three (Fig. 4.2, B, C, E), and possibly four (two within Clade C; Fig. 4.4), genetically distinct species within the morphological limits of what we currently understand as *B. adonis*. Such results support results from Jackson et al. (2014), which found evidence for two cryptic species morphologically identified as *B. adonis* from southern California. *Baetis* sp. CA1 was represented by a single clade (Fig. 4.2, G) that was at least 17.5% different from other collected *rhodan*i group sequences.

Haplotype networks showed how genetic diversity was geographically arranged and that the sequences identified as *B. adonis* and *B. tricaudatus* were separated by at least six base pair changes (Fig. 4.3). However, the small inter-specific divergences between *B. adonis* and *B. tricaudatus* illustrate how the methods used to delimit species and estimate biodiversity are subject to inconsistent interpretations. Our results suggest that genetic diversity at the COI gene region in populations from northern California

supports the diversity indicated by morphology. However, populations in southern California exhibit more genetic diversity than indicated by morphology, which suggests cryptic species diversity.

When the same number of sequences from northern and southern California were compared, there was more genetic diversity in populations from southern California, despite the wider spatial coverage of sampling in northern California (Fig. 4.2). For example, specimens morphologically identified as *B. adonis* or *B. tricaudatus* were supported by genetic analyses to be two species in northern California (Fig. 4.2). However, the same analyses suggested the same two species were in fact four species in southern California, a result supported by Stein et al. (2014) and Jackson et al. (2014). Conclusions regarding species diversity can be difficult to make based only using thresholds between the intra- and interspecific genetic distances. Furthermore, when arbitrary cut-offs between ≥3% (Hebert et al., 2003a; Sweeney et al., 2011) and 1% (Jackson et al., 2014; Stein et al. 2014) are used to differentiate cryptic species, interpretations of diversity become further complicated. The use of both phylogenetic trees and haplotype networks helped tease apart the relationships between cryptic species, and in each case cryptic putative species were supported by all of the genetic analyses completed in the present study.

*Diversity in northern versus southern California*

The difference in cryptic species diversity between northern and southern California could be the result of a number of processes, outlined in Table 4.3. One explanatory hypothesis is that the comparably dry and warm climate of southern California may have limited adult dispersal and recolonization of sampled species among streams. Limited adult dispersal could have then increased the potential for isolation by distance and the resulting cryptic species diversity present in southern California. The relationship between genetic population structure or cryptic diversity and isolation by distance (*i.e.,* distance between populations) has been examined in numerous studies (*e.g*., Hughes et al., 2003; Zickovich & Bohonak 2007, Smith et al., 2006; Spitzer, 2014), and contrasting results have been found. However, our results suggest that the warmer and drier climate of southern California compared to northern California might limit adult dispersal and result in higher genetic divergence between populations (Table 4.3).

In addition to distance between wet habitat (streams and rivers), the type of land cover among the streams and rivers examined in the present study could also have impacted adult dispersal of sampled *Baetis* species. For example, Alexander & Lamp (2008) studied mayfly populations in forested and deforested streams during two years of drought conditions followed by two years of recovery conditions. Although there was no difference in mayfly density or the level of population decline over the course of the drought, they did find that one year after the drought, mayfly densities were higher in forested than deforested areas and that forested streams were more likely to be recolonized. Such results suggest that in southern California, which is significantly less forested than the northern part of the state, mayfly populations might have more difficulty recovering and recolonizing than their northern California counterparts (Table 4.3). Such difficulty in recovering and recolonizing might result in more significant bottleneck effects and locally adapted populations that genetically differ from other populations, a finding our results seem to support.

The Patchy Recruitment Hypothesis (PRH) (Bunn & Hughes, 1997), which suggests that individuals at a particular location might be the products of only a small number of successful matings, might have worked in unison with decreased adult dispersal to create the pattern of more cryptic diversity in southern than northern California. Results from Rebora et al. (2005) supported patchy recruitment of *B. rhodani* populations in Italian streams. Similarly, Spitzer (2014) studied microsatellite markers in *B. tricaudatus* specimens from the San Bernardino mountains in southern California and obtained results that supported the PRH. Our results suggest that the genetic effects of patchy recruitment are more prevalent in southern California *Baetis* populations than in northern California *Baetis* populations (Table 4.3).

Another hypothesis that could explain the higher level of cryptic diversity in southern California compared to northern California is that higher levels of precipitation, and subsequent higher flows in river and streams, might increase gene flow through increased larval drift (Table 4.3). Poff & Ward (1991) studied the drift responses of several lotic benthic invertebrates, including a *Baetis* spp., to experimental changes in flow. Results indicated that *Baetis* spp. consistently had a greater drift rate in high-flow versus low-flow riffle habitat. This result would support the explanation of higher flows in northern California increasing gene flow and thus contributing to the different patterns of cryptic diversity between northern and southern California in the present study. In addition, Hughes et al. (2003a) tested a similar hypothesis, whether the genetic variation among pools in a stream was greater after dry periods than after wet periods, using the Baetid mayfly *Bungona narilla* in Queensland, Australia. However, mitochondrial DNA results from their study only partially supported this hypothesis and allozyme data indicated very low variation among pools after both dry and wet periods (Hughes et al., 2003a).

Genetic drift resulting from the more extreme drought events in southern California might also have increased cryptic diversity in sampled *Baetis* populations. For example, if two different haplotypes occur at two different sites, but drought causes the local extinction of one haplotype in each site, over time this sort of localized differentiation can result in speciation (Nosil & Feder, 2012). Results from Alexander & Lamp (2008), who found that densities of a baetid mayfly in deforested areas were lower than forested areas after a drought event, suggest that genetic drift impacts might be especially important after disturbance events that cause densities to decrease and potential bottleneck effects to impact mayfly populations (Table 4.3). The higher number of duplicate gene sequences in southern California compared to northern California suggests that many alleles are fixed, evidence that adds support to the hypothesis that genetic drift could be a factor impacting the patterns present in our study.

Previous studies seem to support the hypotheses that the higher amount of cryptic diversity in southern California compared to northern California could be the result of decreased adult dispersal or larval drift, or patchy recruitment or genetic drift. However, previous genetic studies on freshwater insects in the Russian River watershed, where most of the northern California specimens from the present study were collected, found evidence of cryptic species diversity. Jackson & Resh (1992) used allozyme electrophoresis to study the caddisfly *Helicopsyche borealis* within three streams and found evidence of cryptic diversity, unlike findings from northern California in the present study. Similarly, allozyme studies on a different caddisfly species in the genus

*Gumaga* found evidence for sympatric cryptic species at sites within the Russian River watershed (Jackson & Resh, 1998). Such findings contradict those of the present study and are likely explained by drifting behavior characteristic of *Baetis* species (Fontaine et al., 1990).

*Diversity thresholds*

The differing results obtained between northern and southern California *Baetis* are complicated by the different thresholds between the intra- and interspecific genetic distances. For example, the two putative species in northern California (*B. tricaudatus* and *B. adonis* NCA) both had greater than 1% intraspecific diversity (Table 4.2). In contrast, the five putative species in southern California each had ≤1% intraspecific diversity (Table 4.2). Interspecific diversity also had a wide range and was less than 2% between *B. adonis* NCA and *B. tricaudatu*s. Stein et al. (2014) and Jackson et al. (2014) both used the same southern California dataset, from which we analyzed a subset of in the present study, and used a 1% cutoff to delimit *Baetis* species, a method which our results from southern California samples support. A 1% distance threshold, however, would differentiate single *Baetis* species in northern California and would lead to conflicting results (Stauffer-Olsen et al., 2017). However, a 2% distance threshold to delimit species would similarly underestimate diversity in southern California because it would not differentiate between *B. adonis* SCA and *B. tricaudatus*, which are only 1.4% divergent. Such differing patterns support the idea that species delimitation cutoffs might need to be different for different species, or even the same species within different regions. Another method to achieve accurate results would be to use specific measurements such as those employed in the preset study instead of simple arbitrary cutoffs to delimit species, especially closely related taxa.

*Implications for bioassessment/biomonitoring*

Our results have important implications for biomonitoring or bioassessment programs. *Baetis* are commonly encountered in biomonitoring studies (White et al., 2014). In our study, only one species (*B. tricaudatus*) occurred in both northern and southern California. This result suggests that biomonitoring programs that use morphologic or genetic data will need local type specimens or sequences to compare against in order to have accurate results. Biomonitoring programs using genetic methods to identify biodiversity have garnered substantial interest because of their potential to both improve accuracy and potentially limit time investments (Sweeney et al., 2011; Webb et al. 2012; Macher et al., 2016). In order for biomonitoring programs to use next-generation sequencing (NGS) techniques to estimate the health of freshwater ecosystems as detailed by Baird et al. (2012) or (Hajibabaei et al., 2011) and have reliable and repeatable results, they need to be compared to an accurate reference library (Pfrender et al., 2010). Our study highlights the necessity of having local reference libraries to compare collected sequences to.

As the methods used to identify and quantify biodiversity continue to evolve, it is important to continually compare results and understand the reasons for different findings between species and regions. By looking at phylogenetic relationships and haplotype networks based on COI gene region barcodes, the relationships between sequences and

the arrangement of diversity is visible so more accurate estimations of biodiversity can be defined.

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**Table 4.1.** Global positioning system coordinate, approximate elevation, and predominant land use for all sites where samples were collected and successfully sequenced.

| Site | N | W | Elevation (m) | Predominant land use |
|---|---|---|---|---|
| Northern California | | | | |
| Russian River mainstem | 38.504 | -122.93 | 9 | Agriculture+forest |
| Austin Creek | 38.511 | -123.075 | 46 | Forest |
| Salmon Creek | 38.356 | -123.004 | 30 | Forest |
| Austin Creek Site 2 | 38.506 | -123.07 | 20 | Forest |
| Dutch Bill Creek | 38.453 | -122.984 | 53 | Forest |
| Capell Creek | 38.495 | -122.243 | 156 | Agriculture |
| Putah Creek | 38.492 | -122.027 | 45 | Agriculture |
| Schneider Creek | 39.917 | -121.065 | 1,253 | Forest |
| Southern California | | | | |
| W. Fork San Gabriel River | 34.241 | -117.869 | 496 | Forest |
| E. Fork San Gabriel River | 34.23 | -117.78 | 536 | Forest |
| Big Tujunga | 34.274 | -118.315 | 396 | Mixed scrub-shrub + urban |
| Arroyo Seco | 34.205 | -118.166 | 348 | Mixed forest + urban |
| Conejo Creek | 34.201 | -119.001 | 31 | Agriculture |

**Table 4.2.** Number of sequences, number of haplotypes ($H_N$), and % genetic diversity present in each putative species based on rarefied data.

| Putative species | Northern California | | | Southern California | | | % Divergence * |
|---|---|---|---|---|---|---|---|
| | No. sequences | $H_N$ | % Diversity | No. sequences | $H_N$ | % Diversity | |
| *Baetis* sp. CA1 | 0 | 0 | 0 | 17 | 9 | 0.7 | 17.5 |
| *B. adonis* sp. 1 | 0 | 0 | 0 | 1 | 1 | N/A | 2.9 |
| *B. tricaudatus* sp. 1 | 0 | 0 | 0 | 46 | 7 | 0.7 | 2.9 |
| *B. adonis* NCA | 38 | 3 | 2.4 | 0 | 0 | 0 | 8.6 |
| *B. adonis* SCA | 0 | 0 | 0 | 174 | 16 | 0.9 | 1.4 |
| *B. tricaudatus* | 333 | 30 | 1.7 | 133 | 11 | 1 | 1.4 |

* Divergence from sister group

Table **4.3**. Hypotheses for differing genetic results between northern and southern California *B. rhodai* group populations.

| Hypothesis | Northern California | Southern California |
|---|---|---|
| Differing climate | Cooler/wetter-more dispersal | Warmer/drier-less dispersal |
| Differing land cover | More forested-more recovering and recolonizing | Less forested-less recovering and recolonizing |
| Patchy Recruitment (PRH) | PRH effects less prevalent | PRH effects more prevalent |
| Differing larval drift | More water flow and larval drift | Less water flow and larval drift |
| Differing genetic drift | Less pronounced effect because fewer drought events | More pronounced effect because more drought events |

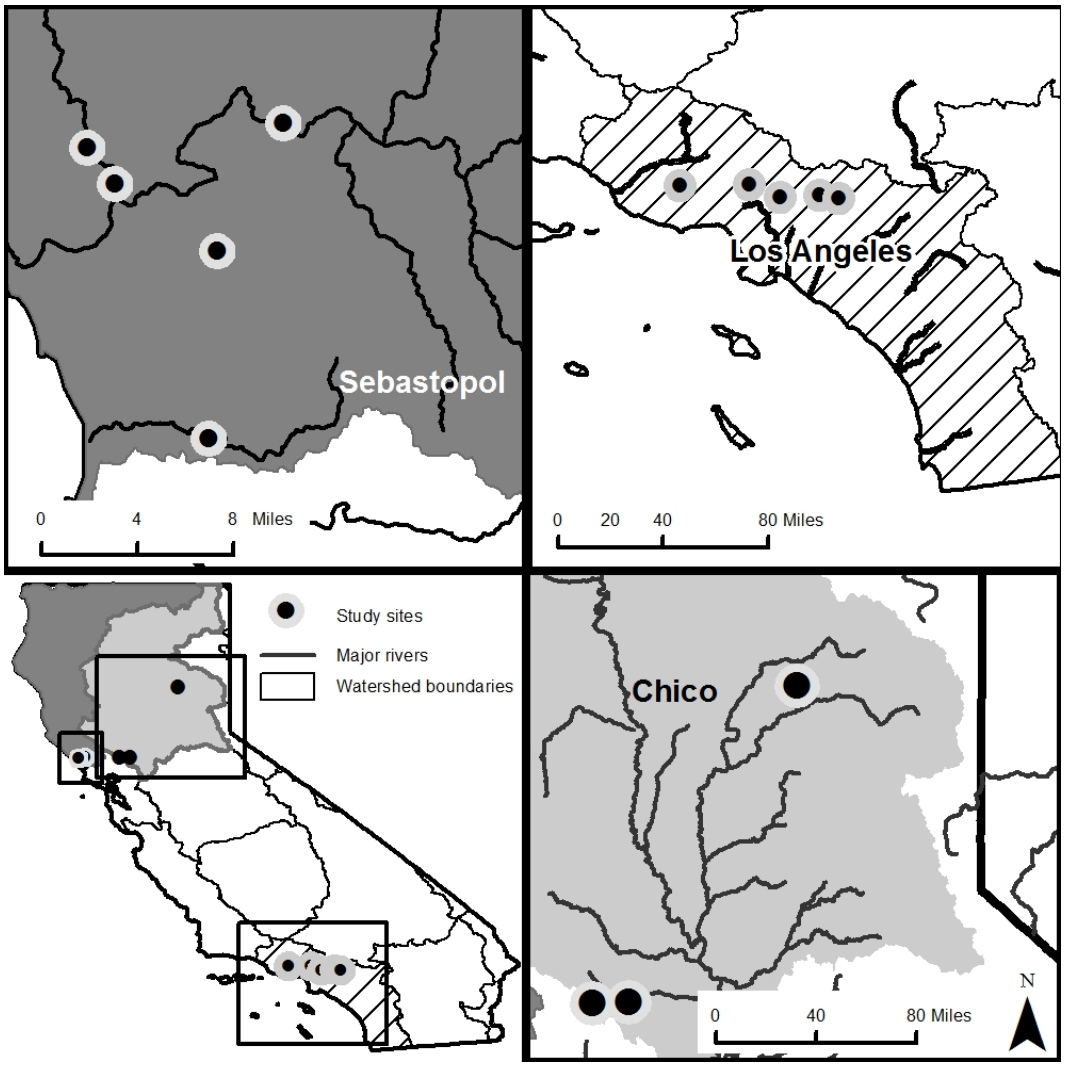


**Figure 4.1.** Map of collection sites. In the map of California (lower left), watersheds are delineated by hydrologic unit code (HUC) 6.

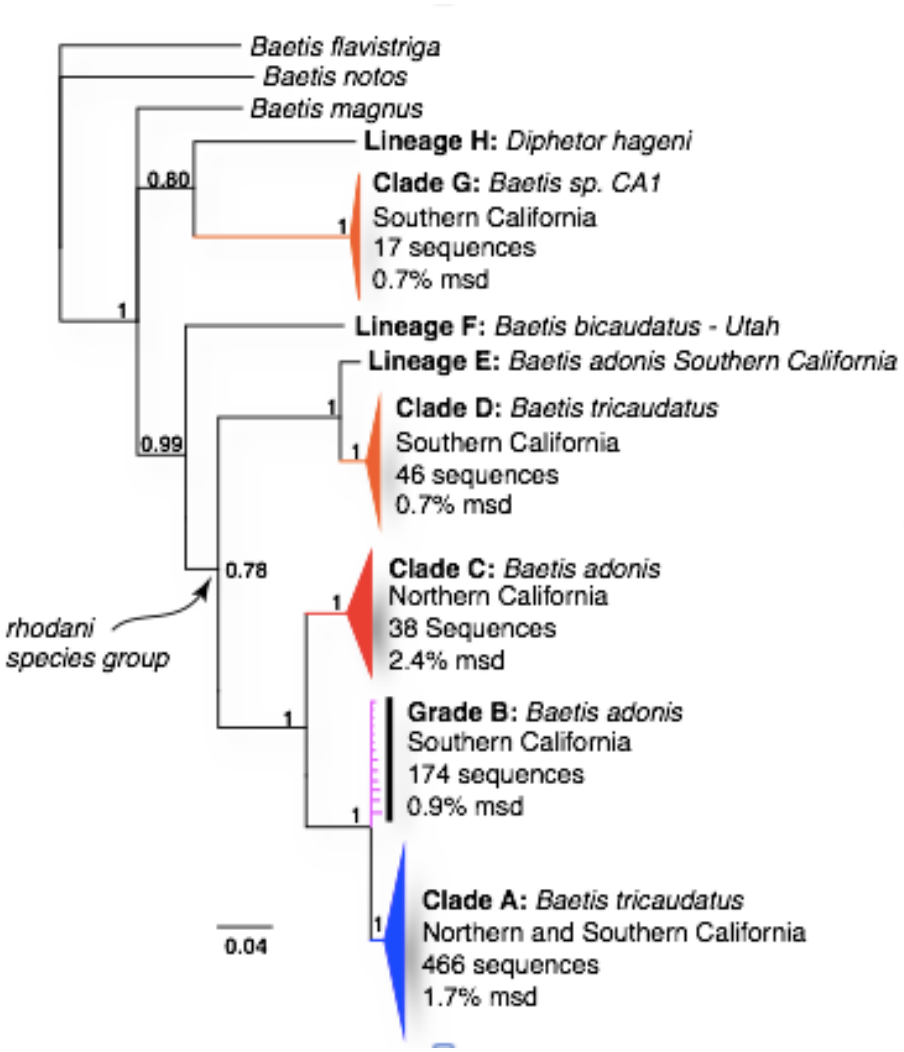


**Figure 4.2.** Consensus tree summarizing Bayesian analysis of 742 sequences from northern and southern California, several other *Baetis* species found in California (*B. notos*, *B. magnus*, *B. bicaudatus*, and B. flavistriga) and *Diphetor hageni*, which was specified as the outgroup. Posterior probabilities (PP) of each highlighted clade, lineage, or grade are written above branches at the nodes.

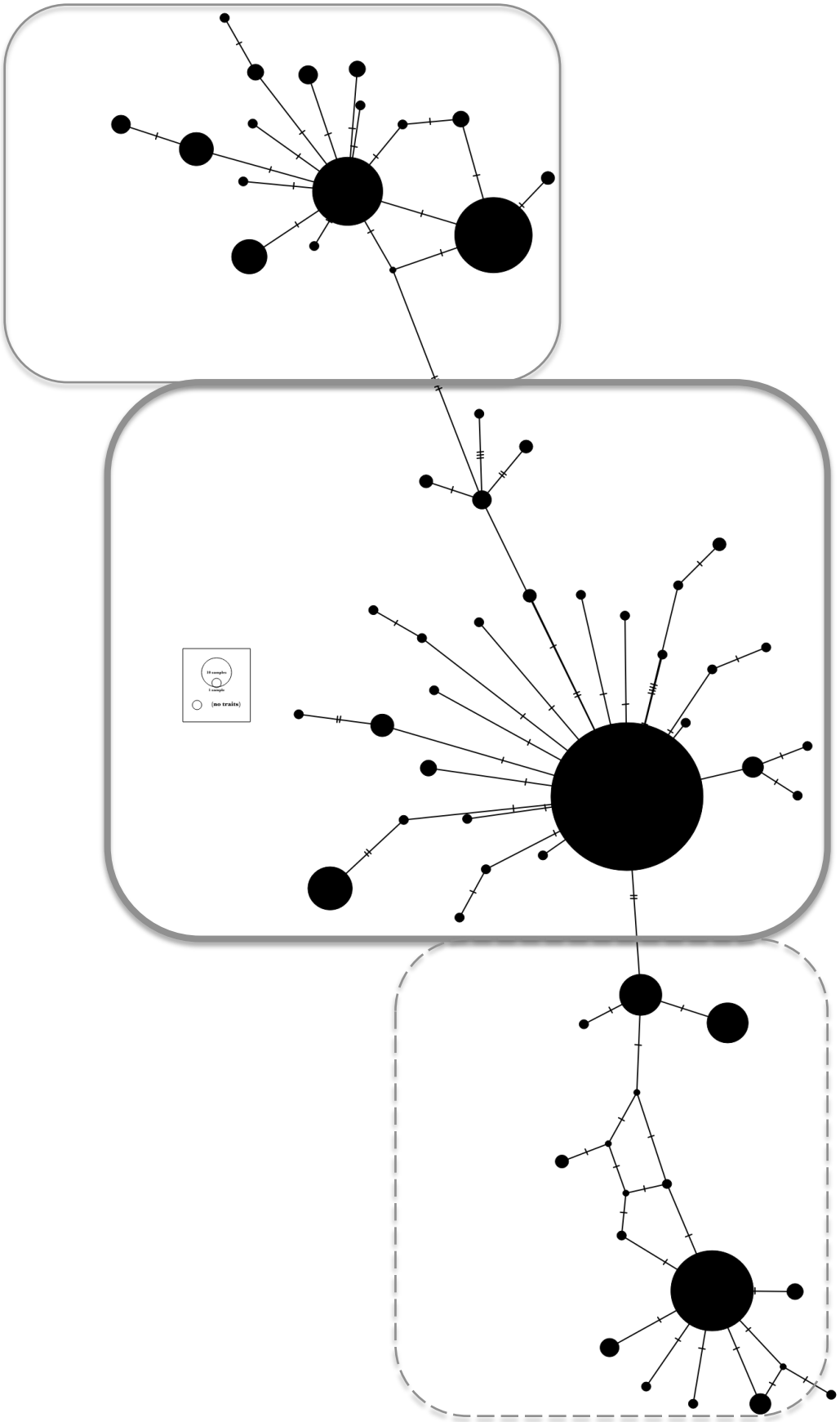
10 samples
1 sample
(no traits)

**Figure 4.3.** Median-joining haplotype network showing the arrangement of genetic diversity within *B. adonis* species collected from southern California, indicated by the box with a thin grey outline; *B. tricaudatus* sequences from northern California, indicated by the box with a thick grey outline; and *B. tricaudatus* from southern California, indicated by the box with a dashed grey outline. Circles represent haplotypes, and circle sizes represent the number of individuals with that haplotype. The small hash marks on lines connecting the different haplotypes represent base-pair changes, which are additive on either side of a circle.

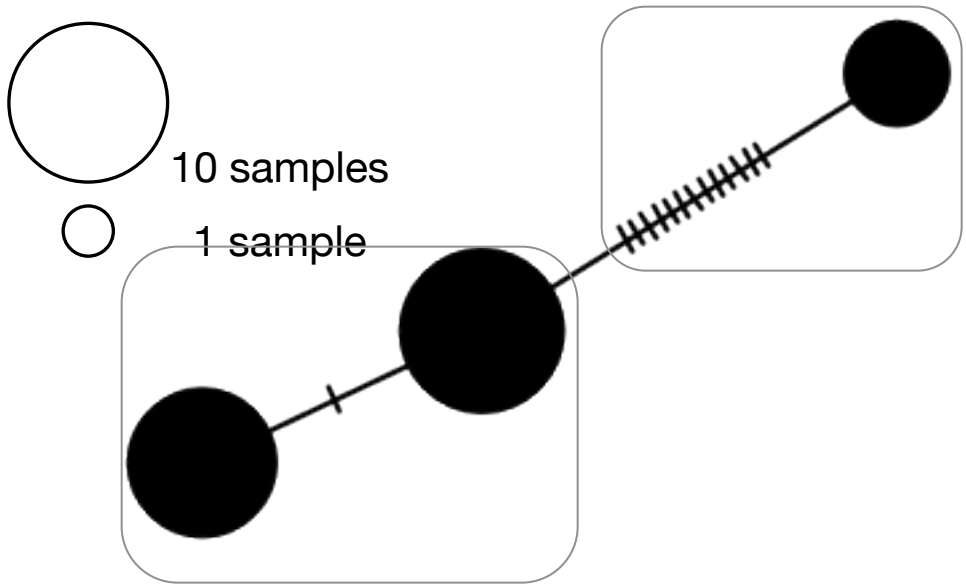


**Figure 4.4.** Median-joining haplotype network showing the arrangement of genetic diversity within *B. adonis* species collected from northern California. Circles represent haplotypes, and circle sizes represent the number of individuals with that haplotype. The small hash marks on lines connecting the different haplotypes represent base-pair changes, which are additive on either side of a circle.

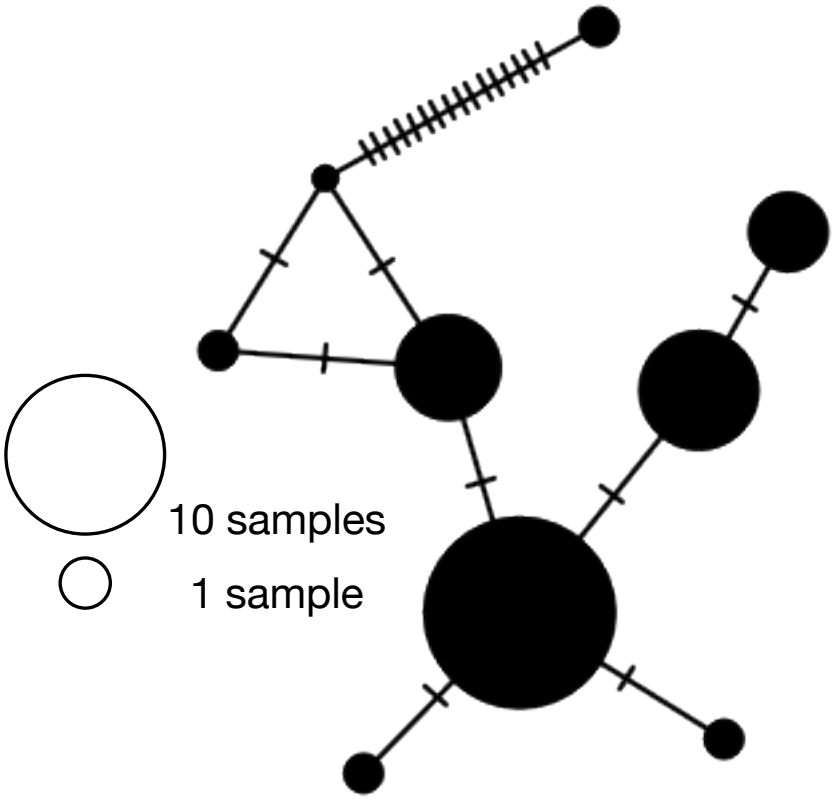


**Figure 4.5.** Median-joining haplotype network showing the arrangement of genetic diversity within *B. tricaudatus* sp. 2 (Clade D in Fig. 2). Another potential cryptic species is also present as indicated by the circle separated from the network by 16 hash marks (base pair changes). Circles represent haplotypes, and circle sizes represent the number of individuals with that haplotype. The small hash marks on lines connecting the different haplotypes represent base-pair changes, which are additive on either side of a circle.

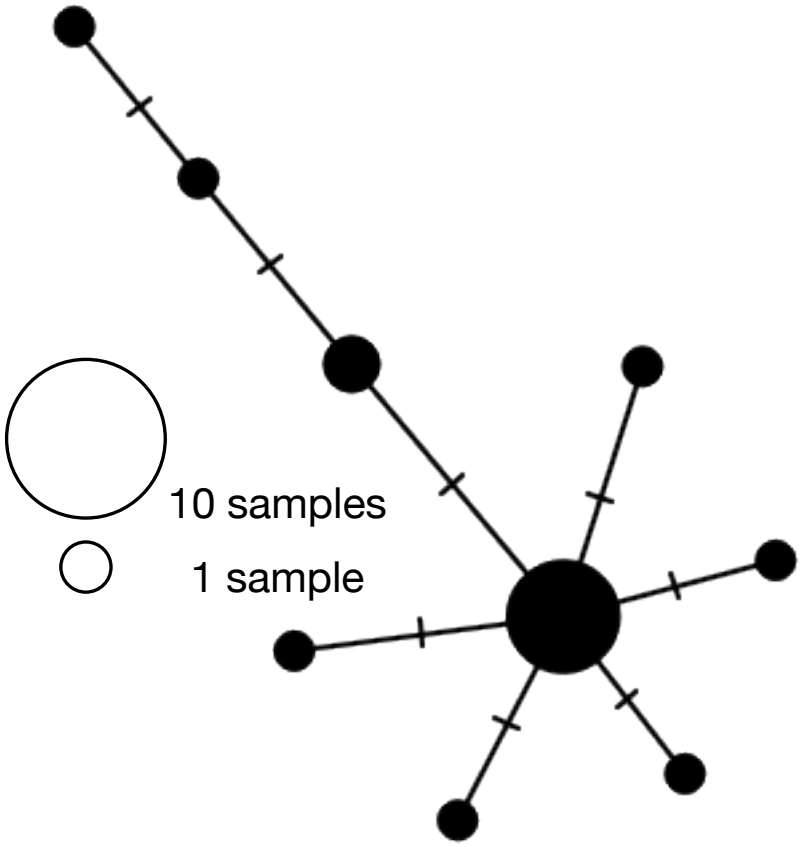


**Figure 4.6.** Median-joining haplotype network showing the arrangement of genetic diversity within *Baetis* sp. CA1 (Clade G in Fig. 3.2). Circles represent haplotypes, and circle sizes represent the number of individuals with that haplotype. The small hash marks on lines connecting the different haplotypes represent base-pair changes, which are additive on either side of a circle.