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Benthic Secondary Production In A Mesotrophic Lake And Its Implications For Terrestrial Consumer Energetics

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by

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ABSTRACT

Historically, limnological studies have focused on understanding the pelagic habitats in lakes. However, recent research suggests that benthic habitats are very productive and can make significant contributions to whole lake productivity. There are a variety of benthic invertebrates that emerge from the lake into adult form providing resources for the surrounding terrestrial habitat. Terrestrial consumers have been shown to have greater densities near water bodies, likely due to high productivity of aquatic emergent invertebrates. The transfer of organic matter across aquatic and terrestrial ecosystems allows consumers in either ecosystem to shift diets depending on seasonal invertebrate production fluxes in their respective ecosystems. Understanding the temporal aspect of invertebrate production is important when assessing resource competition between aquatic and terrestrial ecosystems. Lake ecosystems are often manipulated for management purposes through fish stocking (e.g. recreation, clarity), which may impact the availability of emerging aquatic invertebrates due to fisheries reliance on littoral-benthic habitats. The impact of stocked fish on aquatic invertebrate emergence may alter the foraging behavior of terrestrial consumers, such as bats, that forage on aquatic emergent invertebrates to some degree.

The goals of this study were to understand benthic invertebrate production and aquatic and terrestrial prey availability for higher consumers (e.g. bats, fishes). The first chapter provides an introduction to the project. The objectives of the second chapter were to 1) determine seasonal and spatial patterns of benthic invertebrate production in a mesotrophic lake and 2) estimate the emergence of invertebrates from the lake in order to determine potential availability for terrestrial consumers. Annual, seasonal, and whole-lake benthic secondary production was estimated using the size-frequency method for the dominant taxa. Emergence-to-production ratios were examined during all seasons. The results suggest that while there were abundant benthic invertebrates in mesotrophic Castle Lake, there was a reduction in aquatic invertebrate emergence possibly due to consumption by stocked fish or natural mortality. The objectives of the third chapter were to 1) review the feeding behavior of bats and classify fatty acids as aquatic or terrestrially derived and 2) examine the relationship between fatty acid and isotopic composition of potential aquatic and terrestrial prey sources and bat consumers based on seasonal variations in the relative abundance of aquatic and terrestrial prey. Aquatic emergent and terrestrial invertebrate densities and biomass, carbon stable isotopes, and fatty acids were analyzed to determine aquatic and terrestrial invertebrate contributions to bat energetics. Isotope analyses indicated the relative percentages of aquatic versus terrestrial invertebrates in secondary consumer diets. Bats relied 100 % on aquatic invertebrates in fall, yet shifted to a terrestrial invertebrate dominant diet in summer and fall. Fatty acids were separated into distinct terrestrial and aquatic functional groups through the use of a principal component analysis and a synthesis of current fatty acid literature. Insectivorous bats received 20 % of their total fatty acids from both aquatic and terrestrial ecosystems. Chapter 3 contains the conclusions from the research.

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1.0 Chapter 1

Introduction to project

Limnological research has occurred on Castle Lake, CA since 1959, creating the longest sub-alpine lake monitoring program in North and South America. Researchers from the University of California-Davis, and more recently the University of Nevada-Reno, have mostly studied the chemistry, physics, and biology of the open water habitat. Estimates of primary production, water chemistry, zooplankton composition, physical parameters, and water clarity have occurred weekly on the lake during ice-free months (typically June through September) and in spring (May) and fall (October). Historically, limnological studies have focused on understanding pelagic habitats. However, benthic habitats are very productive and contribute to whole lake productivity as well. Benthic habitats not only collect detritus, they often contain periphyton and macrophytes and provide habitat for invertebrates. Invertebrates living above, or in, lake soils assist in cycling nutrients and serve as prey for aquatic consumers, such as predaceous invertebrates and fish. Therefore, benthic invertebrates act as trophic linkages between benthic and pelagic habitats.

There are a variety of benthic invertebrates that emerge from the lake into adult form. The flux of resources emerging from the lake may provide resources for the surrounding terrestrial habitat. Terrestrial consumers have been shown to have greater densities near water bodies, likely due to high productivity of aquatic emergent invertebrates. Another important aspect of aquatic contributions to terrestrial ecosystems is the seasonal changes in resource fluxes. The transfer of organic matter across aquatic and terrestrial ecosystems allows consumers in either ecosystem to shift diets depending on seasonal invertebrate production fluxes in their respective ecosystems. Understanding the temporal aspect of invertebrate production is important when assessing resource competition between aquatic and terrestrial ecosystems.

Current research investigating energy flow linkages across aquatic and terrestrial ecosystems have typically focused on riverine contributions to forests or ocean to island contributions, yet lake secondary production may contribute substantially to terrestrial consumer energetics in the landscape. Furthermore, lake ecosystems are often manipulated for management purposes through fish stocking (e.g. recreation, clarity), which may impact the availability of emerging aquatic invertebrates due to fisheries reliance on littoral-benthic habitats. For example, fish stocking has had a negative impact on native mountain yellow-legged frogs due to resource competition and predation. Therefore, the impact of stocked fish on aquatic invertebrate emergence may alter the foraging behavior of terrestrial consumers, such as bats, that forage on aquatic emergent invertebrates to some degree.

The goals of this study were to understand benthic invertebrate production and aquatic and terrestrial prey availability for higher consumers (e.g. bats, fishes). The objectives were to 1) determine seasonal and spatial patterns of benthic invertebrate production and emergence from the lake (Chapter 1), 2) examine the relationship between fatty acid and isotopic composition of potential aquatic and terrestrial prey sources and bat consumers, based on seasonal variations in the relative abundance of aquatic and terrestrial prey (Chapter 2) and 3) review the feeding behavior of bats and classify fatty acids as aquatic or terrestrially derived (Chapter 2).

2.0 Chapter 2

Seasonal and spatial patterns of secondary benthic production in a sub-alpine lake (Castle Lake, California)

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Abstract. Aquatic invertebrate secondary production plays an important role in ecosystem food webs by contributing energy to lake and terrestrial consumers. To determine seasonal and spatial patterns in aquatic energy contributions to aquatic and terrestrial consumers, benthic and emergent invertebrate densities were measured in a mesotrophic sub-alpine lake. Benthic invertebrate densities and diversity were highest in sub-littoral habitats. Benthic secondary production was estimated using the size-frequency method. Annual benthic secondary production was highest for Odonata and lowest for Ephemeroptera, with 36.85 g m^{-2} y^{-1} and 0.63 g m^{-2} y^{-1}, respectively. Secondary production varied seasonally and by depth and whole-lake benthic secondary production was estimated at 22.68 g m^{-2} y^{-1}. The dominant emergent invertebrate, Chironomidae, reached peak densities (45/m^2) in the eulittoral and sub-littoral depths from late spring to mid-summer. Emergent Ephemeroptera and Odonata were both most abundant at eulittoral depths, with Ephemeroptera densities peaking (0.8/m^2) in late summer and Odonata densities peaking (1.4/m^2) in late spring. Ephemeroptera had the highest emergence-to-production ratio during all seasons, with a high of 47.5 % emergence from the lake from late summer to early fall. Odonata had 20.4 % emergence from late spring to midsummer, and dropped to < 1 % emergence from late summer to early fall. Chironomidae had 5.1-6.7 % emergence from late spring to late summer and 1 % emergence from late summer to early fall in eulittoral depths. Chironomidae emergence was 1 % or less during all seasons in sublittoral and profundal depths. This suggests that while there were abundant benthic invertebrates in Castle Lake, aquatic invertebrates did not enter the terrestrial landscape at their maximum potential possibly due to consumption by stocked fish, or to natural mortality.

Key words: secondary benthic production, emergence, whole-lake productivity, cross-habitat subsidies, Castle Lake

2.1 Introduction

Limnological studies have historically focused on pelagic waters, yet recently there has been increased focus on benthic production and its impact on whole lake productivity (Vadeboncoeur et al. 2002, Vadeboncoeur et al. 2003, Vander Zanden et al. 2006). Benthic invertebrates couple pelagic and benthic waters by assisting in the cycling of nutrients (Devine and Vanni 2002) and serving as a dominant prey source for fishes. Vander Zanden et al. (2006) found that fish populations relied heavily (63 %) on benthic production as an energy source. Benthic invertebrates that survive fish predation emerge in adult form from aquatic habitats, where they may potentially serve as a prey source for terrestrial consumers. Therefore, benthic invertebrates not only connect pelagic and benthic habitats, but also provide habitat coupling between aquatic and terrestrial systems through emergence.

Similarly, terrestrial secondary production serves as a trophic input to aquatic habitats in a reciprocal subsidy. Fish depend on both terrestrial and benthic productivity (Vander Zanden et al. 2006, Pope et al. 2009), and there is increasing evidence that terrestrial consumers such as birds, bats and amphibians also rely on aquatic and terrestrial secondary production for energy (Nakano and Murakami 2001, Fukui et al. 2006, Finlay and Vrendenburg 2007). Nakano and Murakami (2001) found that birds received 25.6 % of their annual total energy budget from emergent benthic invertebrates, while fish received 44.0 % of their annual total energy budget from terrestrial invertebrates falling into streams. Consumer diets may shift because of seasonal changes in aquatic and terrestrial invertebrate production, resulting in temporal subsidies between aquatic and terrestrial systems. Understanding these interactions and the temporal aspect of production is important when assessing resource competition between aquatic and terrestrial ecosystems.

The number of studies on stream contributions to terrestrial ecosystems is increasing (Jackson and Fisher 1986, Nakano and Murakami 2001, Sabo and Power 2002, Bastow et al. 2002, Baxter et al. 2005), yet there are few studies examining aquatic to terrestrial energy transfers in lake basin ecosystems (Knight et al. 2005, Gratton et al. 2008, Gratton and Vander Zanden 2009). Lake secondary production estimates are limited as well (Babler et al. 2008, Northington et al. 2010), in contrast to greater numbers of stream production estimates (Benke 1984, Jackson and Fisher 1986, Stead et al. 2005). Rivers tend to be more productive than lakes because of proportionally more littoral habitat (Wetzel 2001); however, stream width tends to be smaller than lake radius, so emergent invertebrate flux to land may be greater from lakes (Gratton and Vander Zanden 2009). Lake benthic invertebrate densities differ by depth; therefore, production estimates can convey spatial differences and may provide explanations for consumer habitat preferences.

The objectives of this study were to expand the knowledge of benthic contributions to the landscape at Castle Lake, a sub-alpine lake with a traditional emphasis on pelagic studies. Specifically, our goals were to 1) determine spatial and temporal benthic secondary production dynamics and 2) estimate benthic emergence-to-production ratios to provide insight to potential aquatic subsidies to terrestrial consumers.

2.2 Methods

Study site

Castle Lake is located in the Siskiyou mountain range of northern California (Figure 1). The sub-alpine, cirque lake has a maximum depth of 35 m, a mean depth of 11.4 m, and a surface area of 20.1 ha (Vander Zanden et al. 2006). For the last 50 years, limnological monitoring has occurred on the lake during ice-free months (from the end of May until November). Researchers from the University of California, Davis and the University of Nevada, Reno have recorded primary productivity, water chemistry, and physical measurements. The littoral zone of Castle Lake includes ~54 % of the lake's surface area and is dominated by benthic algae (Axler and Reuter, 1996). Littoral zones are very productive, containing sediments high in organic matter (Axler and Reuter, 1996). These shallow habitats are occupied by a variety of aquatic macroinvertebrates. Previous research demonstrated that benthic invertebrate production contributed significantly to aquatic consumer energetics (Vander Zanden et al. 2006). Prior to this study, no research had been conducted to determine the efficiency of emergence or the importance of aquatic secondary production for terrestrial environments.

Invertebrate collection

Lake benthos

Benthic macroinvertebrates were collected four times in 2008 during late spring, mid-summer, late summer, and early fall. In 2009, benthic macroinvertebrates were collected in spring and late summer. Seasonal differences were determined by changes in the depth of the lake's thermocline. As the lake warmed throughout the seasons, the thermocline became deeper. The shifting thermocline depth represented a change in season and each season lasted slightly over a month. Samples were retrieved from nine locations, including both deep and near-shore habitats (Figure 1). Samples were collected at eulittoral (0.1, 0.5, 2 m), sub-littoral (5, 10, 15 m), and profundal (20 and 30 m) habitats. A composite of three Ekman dredge pulls, with an area of 0.023 m², were used to collect bottom sediment samples for all but two sites. These two sites, with depths of 0.5 m, had rocky substrate and a dredge was not effective in collecting invertebrates. Therefore, a bottomless 0.045 m² 18.9-liter bucket was placed at the collection site to a depth of 5 cm, where rocks and sediment were transferred from the sample site to another collection bucket. In the field, samples were sieved, and macroinvertebrates were identified to Order and preserved in 70 % ethanol. In the laboratory, invertebrates were identified further to Family when possible using an appropriate key (Merritt and Cummins, 1996) and counted.

Emergence

Aquatic emergent invertebrates were collected in 2008 and 2009 four days a week from June to August and twice a week during September. Emergent traps were anchored at eight locations for 24 hours a day. The traps were placed along a depth gradient at 0.1, 0.5, 2 m (eulittoral), 5, 10, 15 m (sub-littoral), and 20 and 30 m (profundal) to capture both near-shore and deep-water habitats. The 0.315 m^2 traps were emptied every morning to observe daily invertebrate flux. All invertebrates were collected and stored in 70 % ethanol in the field. In the laboratory, all invertebrates were counted and identified to Family when possible (Merritt and Cummins, 1996). The three dominant taxa were counted, dried at 60 °C for 24-48 hours, and weighed on a microbalance to the nearest 0.1 mg.

Invertebrate analysis

Lake benthos

Benthic secondary production was quantified in terms of presence, abundance, and distribution of taxa (Brower and Zar, 1977). Invertebrate community diversity was calculated for various depths and by season using the Shannon Diversity Index ($H' = -\Sigma(p_i \ln p_i)$)(Brower and Zar, 1977). The relative abundance of each taxa is represented by p_i. Index values range from 0 to 4.6, with higher values signifying high biological diversity and evenness. Benthic invertebrate abundance data were quantified and converted to density for each taxa. Dominant benthic invertebrate densities were log (x +1) transformed to normalize data and one-way ANOVAs with interaction effects were used to determine whether invertebrate densities were significantly different among habitats and/or seasons. A post-hoc Tukey's comparison was computed for significant results. While there were many benthic taxa observed, the dominant taxa that were able to emerge from the lake and therefore potentially contribute to terrestrial consumer diets were Chironomidae, Ephemeroptera, and Odonata. Other emergent taxa were not dominant; therefore, emergent densities were calculated for Chironomidae, Ephemeroptera, and Odonata seasonally and by depth.

Five dominant benthic invertebrates were measured for productivity using the size-frequency method (Benke 1984). Invertebrate body lengths were measured to the nearest 0.1 mm using a micrometer, excluding antennae and cerci. Taxa were dried at 60 °C for 24-48 hours, and weighed on a microbalance to the nearest 0.01 mg to obtain dry mass. Taxa were divided into size classes by depth and season. Published cohort production interval (CPI) values were used to correct for non-univoltine taxa (Babler et al. 2008). Whole-lake benthic secondary production was determined by calculating the contributing area of production from a hypsographic curve created by a bathymetric map of Castle Lake. Annual production estimates for 2008 were based on the 150 days that the lake was free of ice (Vander Zanden et al. 2006) and was estimated by fitting depth-specific secondary production values to the areas of each depth class (Northington et al. 2010). To compare Castle Lake whole-lake secondary production estimates from Vander Zanden et al. (2006) and this study, annual production estimates were converted to carbon units by assuming that invertebrate dry biomass was 45 % carbon (Strayer and Likens 1986, Wetzel 2001, Vander Zanden et al. 2006).

Emergence

Quantification of aquatic invertebrate emergence was characterized by presence, abundance, and distribution of taxa (Brower and Zar, 1977). Emergent invertebrate abundance data were quantified and converted to density for each taxa. The three dominant emergent taxa (Diptera, Ephemeroptera, and Odonata) were compared seasonally and by habitat (eulittoral, sub-littoral, and profundal). The dominant taxa were the most common and the most accessible for fish consumption as they passed through the water column to emerge. Emergent invertebrate densities were log transformed to normalize the data and averaged for the seasons of sampling. One-way ANOVAs with interaction effects were used for log (x +1) transformed data to determine whether invertebrate densities depended on season and/or habitat. A post-hoc Tukey's comparison was calculated for significant results. Invertebrates were dried at 60 °C for 24-48 hours and weighed to the nearest 0.01 mg to obtain dry mass for biomass calculations. Using the mean daily emergence and production (g dry weight m^{-2} d^{-1}) of the three dominant emergent invertebrates in Castle Lake, emergence-to-production ratios (E/P) were calculated as percent emergence. The percent of benthic invertebrates that emerged (%) from the lake was estimated for late spring, midsummer, late summer and fall.

Fish collection and analysis

Fish were collected in late spring, mid-summer, and fall of 2009 using gill nets placed at two near-shore sites. Length measurements, sex, diet, and weight were recorded for all fish collected. Gut contents were preserved in 70 % ethanol and invertebrate parts were identified to Order when possible (Merritt and Cummins, 1996). Invertebrates were counted, dried at 60 °C for 24-48 hours, and weighed. Each diet item was calculated as a percentage by dry weight that contributed to total fish diet.

2.3 Results

Benthic invertebrate diversity, densities, and production

Twenty taxa of benthic invertebrates were collected in Castle Lake in 2008 and 2009, with the dominant taxa consisting of Diptera, Ephemeroptera, Odonata, Oligochaeta, Pisidae, and Trichoptera (Table 1). The greatest diversity of taxa was observed in the eulittoral depths and declined in the profundal depths. The Shannon Diversity Index H` values decreased with depth and as the seasons progressed, with a H` value of 1.47 in late spring at 2 m and a H` value of 0 at 30 m (no invertebrates found at this depth) (Figure 2). A peak in diversity occurred at 15 m after an initial decline in diversity at 10 m.

Benthic Chironomidae and Ephemeroptera densities peaked in late summer (2,683/m^2 and 678/m^2, respectively), while Odonata reached peak density (332/m^2) in early fall (Figure 3). Chironomidae densities did not differ significantly by habitat (p =

0.06) or season ($p = 0.65$), but densities were highest in sub-littoral depths in all seasons (Figure 3). Ephemeroptera densities did not differ significantly by habitat ($p = 0.09$) or by season ($p = 0.09$), but highest densities shifted from eulittoral depths early in the season to sub-littoral depths later in the season (Figure 3). Odonata densities did not differ significantly by habitat ($p = 0.21$) or by season ($p = 0.99$), but densities increased consistently from spring to fall with highest densities in sub-littoral depths (Figure 3).

Annual benthic secondary production (P) was highest for Odonata: Corduliidae and lowest for Ephemeroptera: Caenidae, with 36.85 g dry weight m^{-2} y^{-1} and 0.63 g dry weight m^{-2} y^{-1}, respectively (Table 2). Production-to-biomass ratios (P/B) were greatest for Ephemeroptera: Baetidae and for profundal Diptera: Chironomidae, indicating that these taxa have high biomass turnover rates in comparison to the other taxa (Table 2). Secondary production varied seasonally, with Odonata: Corduliidae and Diptera: Chironomidae having greatest production from late summer to early fall (Table 3). Ephemeroptera maintained consistent production over the course of the field season, but different Families were dominant in different seasons (Table 3). Taxa that had a production value of < 0.0001 g dry weight m^{-2} d^{-1} were not represented in the sample, and therefore it may be presumed production was very low. There are no production estimates for Odonata and Ephemeroptera in sub-littoral and profundal depths because production was minimal or non-existent in these habitats (Tables 2 and 3). Whole-lake benthic secondary production was estimated at 22.68 g dry weight m^{-2} y^{-1} (Figure 4) or 10.206 g C m^{-2} y^{-1}.

Emergent invertebrate densities, biomasss, and E/P ratios

The dominant emergent invertebrate, Chironomidae, reached peak densities ($45/m^2$) in the eulittoral and sub-littoral depths (Figure 5). Emergent Ephemeroptera and Odonata were both most abundant at eulittoral depths. Odonata densities peaked ($1.4/m^2$) in late spring and Ephemeroptera peaked ($0.8/m^2$) in late summer (Figure 5). Emergent Chironomidae and Odonata densities were significantly different among seasons ($p < 0.0001$; $p = 0.01$, respectively) and among habitats ($p < 0.0001$; $p = 0.0006$, respectively). A Tukey's post-hoc comparison demonstrated that eulittoral and sub-littoral Chironomidae were significantly greater than those in profundal depths ($p < 0.0001$). Chironomidae densities were significantly lower in early fall than in late spring ($p < 0.0001$), early fall < mid-summer ($p < 0.0001$), late spring > late summer ($p < 0.0001$), and late spring > mid-summer ($p < 0.0001$). Eulittoral Odonata densities were significantly greater than sublittoral depths ($p = 0.0006$), and lower in early fall than in late spring ($p = 0.01$). Ephemeroptera densities were significantly different among habitats ($p = 0.002$), but not among seasons ($p = 0.27$). A Tukey's post-hoc comparison showed that eulittoral Ephemeroptera densities were significantly greater than profundal depths($p = 0.002$).

Ephemeroptera had the highest emergence-to-production ratio during all seasons, with a high of 47.5 % emergence from the lake in late summer and early fall (Table 4). Odonata had 20.4 % emergence in late spring and midsummer, and dropped to < 1 % emergence in late summer and early fall. Chironomidae (Diptera) had 5.1-6.7 % emergence in late spring and late summer and 1 % emergence in late summer and early fall in eulittoral depths. Chironomidae emergence was 1 % or less during all seasons in sublittoral and profundal depths. Ephemeroptera emergence produced 0.012 g dry weight m^{-2} in eulittoral depths in late spring and decreased to 0 g m^{-2} by early fall; Odonata emergence produced 0.056 g m^{-2} in eulittoral depths in late spring and decreased to 0 g m^{-2} by early fall; Chironomidae emergence produced 0.0005 g m^{-2} in profundal depths in late spring and decreased to 0 g m^{-2} by early fall (Appendix 1B). Therefore, Odonata produced the greatest amount of biomass and Chironomidae produced the lowest amount of biomass available to terrestrial consumers (Appendix 1B).

Fish diet

During the three sampling sessions, non-native rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) were caught (n = 30). Golden shiners (*Notemigonus crysoleucas*) were caught as well, but we were not able to analyze gut contents. In late spring, trout diet consisted of, on average, 10 % Chironomidae, 5.5 % Ephemeroptera, 36 % Odonata, and 17 % Trichoptera by dry weight (Figure 6). In mid-summer, trout diet consisted of, on average, 8.3 % Chironomidae, 42.7 % Ephemeroptera, 21.5 % Odonata, and 1.2 % Trichoptera (Figure 5). In fall, trout diet consisted of, on average, 0.8 % Chironomidae, 24 % Ephemeroptera, 13.5 % Odonata, and 7.3 % Trichoptera (Figure 6).

2.4 Discussion

Benthic secondary production

The dominant taxa in Castle Lake consisted of Chironomidae, Oligochaeta, and Pisidae, which are all considered taxa that are tolerant of most water conditions (Lenat 1993). Trichoptera, Ephemeroptera, Odonata, and Hirudinea were also represented in the lake in varying amounts, adding to the lake's diverse invertebrate community. The benthic invertebrate taxa found in Castle Lake are typical of small sub-alpine lakes (Knapp et al. 2001, Pope et al. 2009).

Benthic invertebrate diversity was greatest in littoral depths, possibly due to wave action disturbance, and high light penetration and nutrient concentrations. Castle Lake consists of ~54 % littoral habitat (shallow depths with high light availability), potentially accounting for a large proportion of the whole-lake benthic secondary production. The deep chlorophyll maximum at Castle Lake (Jassby et al. 1990) refers to the high concentrations of chlorophyll-a found in deeper waters, and may explain the secondary peak in invertebrate diversity at 15 m. Deep chlorophyll maximum depths accumulate settling particulate matter and generate some phytoplankton growth, which may produce heightened trophic activity (Ortner 1977). This suggests that with a peak in energy resources available at approximately 15 m due to the deep chlorophyll maximum at Castle Lake (Jassby et al. 1990), there may be a concomitant secondary peak in benthic invertebrate diversity. In order to be certain that benthic production is related to the deep chlorophyll maximum, we recommend evaluating periphyton and settling rates of seston at this depth of the lake in the future.

Benthic invertebrate densities increased from late spring to late summer, possibly due to an increase in primary productivity and phytoplankton biomass and an influx of particles settling through the water column over the productive season (Goldman et al. 1973). This suggests that benthic invertebrates may rely directly or indirectly on pelagic primary production and algal biomass settling to the lake bottom (Chandra et al. 2005). Ephemeroptera and Chironomidae densities peaked earlier in the season than Odonata densities. Benthic Chironomidae were the most abundant taxa in Castle Lake, which supports previous findings that Chironomidae are typically the dominant taxa in aquatic ecosystems (Merritt and Cummins 1996, Wetzel 2001). Benthic Odonata reached peak densities later in the season than other taxa, possibly due to hatching of new generations after the large emergence of parent cohorts in spring and summer.

The eulittoral and sub-littoral depth zones, where light penetration occurred, contained the highest densities of invertebrate taxa. Chironomidae and Odonata had higher densities in the sub-littoral depths than in the eultittoral depths. Profundal depths did not have high densities of any invertebrate taxa, despite potential high concentrations of settled particles. Castle Lake sediments from the profundal zone often become anoxic from late summer through winter (Chandra, unpublished data), and likely act as a moderating disturbance for the invertebrate community. While there were no statistically significant differences between habitat and benthic invertebrate densities, trends were observed between density means (Figure 3). There was quite a bit of variability among benthic invertebrate densities, which explains the non-significant findings. Invertebrates may occur in patchy distribution across lake bottoms because of substrata heterogeneity

(Brinkhurst 1974, Wetzel 2001) and therefore invertebrate densities may be highly variable between locations that were sampled.

While benthic invertebrate densities provide an understanding of abundance and distribution, secondary production estimates further provide a rate of benthic turnover and an indication of its importance for whole-lake productivity. Annual benthic secondary production in Castle Lake was highest for Odonata and lowest for Ephemeroptera. Odonata tend to have high production estimates (Babler et al. 2008, Runck and Blinn 1993), perhaps due to their predaceous feeding behavior. Strayer and Likens (1986) demonstrated that predation by invertebrates accounted for 80 % of the fate of benthic production, while fish predation accounted for just 15 % of benthic mortality in Mirror Lake. Odonata are known to consume Chironomidae and Ephemeroptera, among other invertebrates (Benke 1978, Merritt and Cummins 1996, Benke et al. 2001). Using stable isotope measurements, Vander Zanden et al. (2006) determined the benthic contributions to fish production from Castle Lake. Odonata had similar carbon isotope values and higher nitrogen isotope values compared to Chironomidae and Ephemeroptera, suggesting that Odonata consumed Chironomidae and Ephemeroptera in Castle Lake (Vander Zanden et al. 2006). Odonata production may also be greater than other taxa in Castle Lake because of their large individual biomass and the number of size classes they encompass. Due to high Odonata production and low prey invertebrate (Chironomidae and Ephemeroptera) production, it appears as if the Allen paradox may occur at Castle Lake. According to Allen’s paradox, there must be high prey invertebrate biomass turnover rates (P/B) to support high predator production (Benke 1978, Benke 1984). Ephemeroptera: Baetidae and Chironomidae (the average P/B of

Chironomidae from all habitats) had higher turnover rates than Odonata (9.63, 5.98, and 4.36 respectively), which may explain high Odonata production at Castle Lake. Odonata may also be consuming non-emergent taxa, which were not included in production calculations. Chironomidae were found in all depth zones, and therefore three production estimates were calculated. Highest Chironomidae production occurred in sublittoral depths, followed closely by profundal depths, possibly due to the abundance and larger size of larvae in deeper depths. Ephemeroptera had the lowest amount of production, possibly because they were not as abundant as Chironomidae, nor were they as large as Odonata. Therefore, production estimates demonstrated that Odonata had high individual biomass and moderate densities, Chironomidae had low individual biomass and high densities, and Ephemeroptera had moderate individual biomass and low densities.

While seasonal secondary production was generally high for Odonata and generally low for Ephemeroptera, benthic production varied by season. Both Odonata and Chironomidae production increased from late spring to early fall, with greatest production occurring between late summer and early fall. This may be attributed to settling detritus and plankton reaching Chironomidae later in the season. Ephemeroptera maintained fairly consistent production throughout the seasons, but different Families were dominant in different seasons, likely due to various life cycles and temperature requirements for different Families (Flannagan and Lawler 1972, Wetzel 2001). Seasonal shifts in secondary production are likely to affect fish production and fish reliance on seasonal subsidies from aquatic versus terrestrial ecosystems.

Comparison to other lakes

Data presented in Northington et al. 2010 demonstrates a range of whole-lake secondary production estimates for oligotrophic and eutrophic lakes around the world. Secondary production values range from 2-11 g m^{-2} y^{-1} of dry biomass for oligotrophic lakes and 9-3000 g m^{-2} y^{-1} for eutrophic lakes; Castle Lake produces 22.68 g m^{-2} y^{-1} as a mesotrophic lake. This estimate for a mesotrophic lake is between the estimates for oligotrophic and eutrophic lakes. Therefore, this estimate supports the observation that with increasing trophic status, there is a greater amount of secondary production (Wetzel 2001).

Previous research on secondary production in Castle Lake suggested that benthic secondary production was 0.360 g C m^{-2} y^{-1} (Vander Zanden et al. 2006) in comparison to this study's estimate of 10.206 g C m^{-2} y^{-1}. The Vander Zanden et al. (2006) estimate was based on P/B ratios and the amount of carbon associated with invertebrate production. This study, using the size-frequency method (Benke 1984), and assuming the same proportion of carbon in dry mass, suggests that there is an order of magnitude greater annual secondary production in Castle Lake than was previously estimated. Further, our estimate for whole-lake annual benthic secondary production at Castle Lake is an underestimate because only dominant taxa that were capable of emerging out of the lake were included in production calculations. It is presumed that other, non-emergent taxa further increase the secondary production in Castle Lake.

Emergence

Ephemeroptera were the dominant invertebrates to emerge from Castle Lake from late spring until early fall, with close to 50 % of benthic Ephemeroptera emerging. These

Ephemeroptera potentially served as an aquatic contribution to the surrounding terrestrial system. While Odonata emergence was high in the early season, they did not emerge in great proportions later in the season. Up to 6 % of Chironomidae emerged from eulittoral depths and less than 1 % emerged from sublittoral and profundal depths. There are a few possible explanations for the medium to low proportions of invertebrate emergence from Castle Lake. Fish diet data suggested that annually stocked trout consumed a fair amount of Ephemeroptera in mid-summer, which may explain the decrease from 45 % emergence in late spring to 31 % emergence in mid-summer (Table 4 and Figure 6). Another explanation for high Ephemeroptera emergence may be their high production to biomass ratio (P/B). Odonata emergence may have been low during all seasons because trout consumed Odonata at high quantities (Figure 6). Odonata have experienced high mortality from hatching to just before emergence, with 80-95 % mortality, due to fish predation (Benke and Benke 1975). Odonata emergence may also be underestimated by the trap samples because some species of Odonata, such as Corduliidae, crawl to the water's edge and emerge on rocks and vegetation, rather than from open water. Fish predation does not adequately explain the low Chironomidae emergence because Chironomidae were not heavily represented in trout diet (Figure 6). Perhaps Chironomidae were not observed in fish diets due to faster digestion rates of soft-bodied organisms in fish guts (Hess and Rainwater 1939, Pandian 1967, James et al. 1989). Chironomidae may have a high natural mortality rate in Castle Lake or more likely, they were consumed by Odonata, either in the larval state or when trapped in the emergent traps (thus not adequately represented in the trap sample). Chironomidae body parts were found in the gut contents of Odonata at Castle Lake (J. D. Brownstein and A. Caires, *personal observation*). Benke (1976) observed that, in laboratory experiments, Odonata larvae can consume at least 30 % of their body weight in a day, thus it is probable that Castle Lake Odonata consumed a large proportion of benthic Chironomidae before emergence occurred. In summary, a decrease in invertebrate emergence due to fish predation may not affect terrestrial consumers if the consumers rely on Ephemeropterans, rather than Odonates and Chironomids.

Terrestrial consumers may subsidize their terrestrial diet with aquatic prey if aquatic emergent production is high during seasons of low terrestrial invertebrate production (Nakano and Murakami 2001, Fukui et al. 2006). Spring and mid-summer generate the greatest biomass of emergent invertebrates at Castle Lake (Appendix 1B). Insectivorous bats may benefit from the early season abundance of emergent aquatic invertebrates since they are often opportunistic feeders (Barclay and Brigham 1994) and have been known to consume Trichoptera, Chironomidae, and Ephemeroptera (Anthony and Kunz 1977, Barclay et al. 1991, Brigham et al. 1992). Bats tend to have high metabolism and may consume more than their body weight in prey in a single night during times of high energy demand (Barclay et al. 1991). Therefore, bats (and other terrestrial consumers such as frogs, birds and spiders) may subsidize their diet with aquatic emergent invertebrates, such as Chironomidae and Ephemeroptera, in spring and early summer and return to a more terrestrial diet once emergence decreases in late summer.

Conclusions

This study suggests that Castle Lake had a diverse benthic invertebrate community which varied seasonally and by depth. The lake generated a moderate amount of benthic secondary production for a mesotrophic lake and at a notably higher rate than previously estimated. While there was greater emergent invertebrate densities and biomass in late spring and early summer than in late summer and fall, there was a reduced amount of dominant benthic invertebrates that emerged from the lake. Therefore, this study suggests that while there was abundant benthic secondary production in Castle Lake, the emergence of invertebrates may have been reduced, potentially due to non-native fish consumption of invertebrate resources.

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2.6 Tables

TABLE 1. Benthic invertebrates found in Castle Lake, CA in 2008 and 2009.

	Proportion of total capture (%)	
Taxon	**2008**	**2009**
Coleoptera		
Psephenidae	<1	<1
Dytiscidae	<1	
Curculionidae	<1	
Diptera		
Ceratopogonidae	1	
Chironomidae	27	34
Ephemeroptera		5[a]
Baetidae	<1	
Caenidae	3	
Leptophlebiidae	1	
Gastropoda		
Physidae	<1	1
Hirudinea	4	<1
Megaloptera		
Sialidae	1	<1
Odonata		
Aeshnidae	<1	
Coenagrionidae	<1	<1
Corduliidae	2	<1
Oligochaeta	37	35
Pisidae		
Pelecypodidae	14	13
Trichoptera		7[a]
Limnephilidae	<1	
Leptoceridae	<1	
Lepidostomatidae	<1	
Sericostomatidae	3	

[a] Taxa were identified to Order, not Family

TABLE 2. Annual benthic standing stock biomass (B), secondary production (P), and production-to-biomass ratios (P/B) for the dominant taxa in Castle Lake in 2008 (Note: 0.1-5 m, eulittoral; 6-19 m, sublittoral; 20-35 m, profundal).

Taxa	**Depth (m)**	**B (g m^{-2})**	**P (g m^{-2} y^{-1})**	**P/B**
Odonata				
Corduliidae	0.1-5	8.4	36.85	4.36
Ephemeroptera				
Baetidae	0.1-5	0.1	0.99	9.63
Caenidae	0.1-5	0.18	0.63	3.53
Leptophlebiidae	0.1-5	0.21	0.99	4.68
Diptera				
Chironomidae	0.1-5	0.32	1.34	4.15
	6-19	1.02	5.35	5.2
	20-35	0.53	4.55	8.57

TABLE 3. Daily benthic secondary production (P) between seasons for dominant taxa in Castle Lake from late spring to midsummer, midsummer to late summer, and late summer to early fall 2008 (Note: 0.1-5 m, eulittoral; 6-19 m, sublittoral; 20-35 m, profundal).

		P (g m^{-2} d^{-1})		
Taxa	**Depth (m)**	**Late spring-midsummer**	**Midsummer-late summer**	**Late summer-early fall**
Odonata				
Corduliidae	0.1-5	0.24	0.58	0.94
Ephemeroptera				
Baetidae	0.1-5	0.02	<0.0001	<0.0001
Caenidae	0.1-5	<0.0001	<0.0001	0.01
Leptophlebiidae	0.1-5	<0.0001	0.02	<0.0001
Diptera				
Chironomidae	0.1-5	0.01	0.007	0.03
	6-19	0.03	0.03	0.08
	20-35	<0.0001	0.09	<0.0001

TABLE 4. Percentages of emergence-to-production (E/P) ratios for selected aquatic invertebrates (Note: 0.1-5 m, eulittoral; 6-19 m, sublittoral; 20-35 m, profundal).

		E/P (%)		
Taxa	**Depth (m)**	**Late spring-midsummer**	**midsummer-late summer**	**late summer-early fall**
Odonata	0.1-5	20.4	7.5	<1
Ephemeroptera	0.1-5	45	31.1	47.5
Diptera	0.1-5	5.1	6.7	1
	6-19	1	<1	<1
	20-35	<1	<1	<1

Figure legends

FIGURE 1. Map of Castle Lake, CA. Benthic and emergent invertebrate sampling locations are marked with an “x”. Isobars show depth.

FIGURE 2. Shannon diversity index for A) total diversity of benthic invertebrates found at various depths in Castle Lake in 2008 and B) seasonal shifts in benthic diversity in 2008.

FIGURE 3. Benthic invertebrate densities in Castle Lake in 2008 with standard error bars for A) Diptera: Chironomidae, B) Ephemeroptera, and C) Odonata. Late summer sub-littoral upper error bar for Ephemeroptera is cut off to preserve scale.

FIGURE 4. Whole-lake secondary production in mesotrophic Castle Lake compared to oligotrophic and eutrophic whole-lake secondary production estimates from data in Northington et al. 2010. Note the discontinuity in the y-scale.

FIGURE 5. Emergent invertebrate densities in Castle Lake in 2008 with standard error bars for A) Diptera: Chironomidae, B) Ephemeroptera, and C) Odonata. Significant differences between habitats and seasons are represented by “a” and “b” and “*” and “^”, respectively.

FIGURE 6. Average percent (%) of prey items by dry weight in Castle Lake trout diet (n = 30).

2.7 Figures

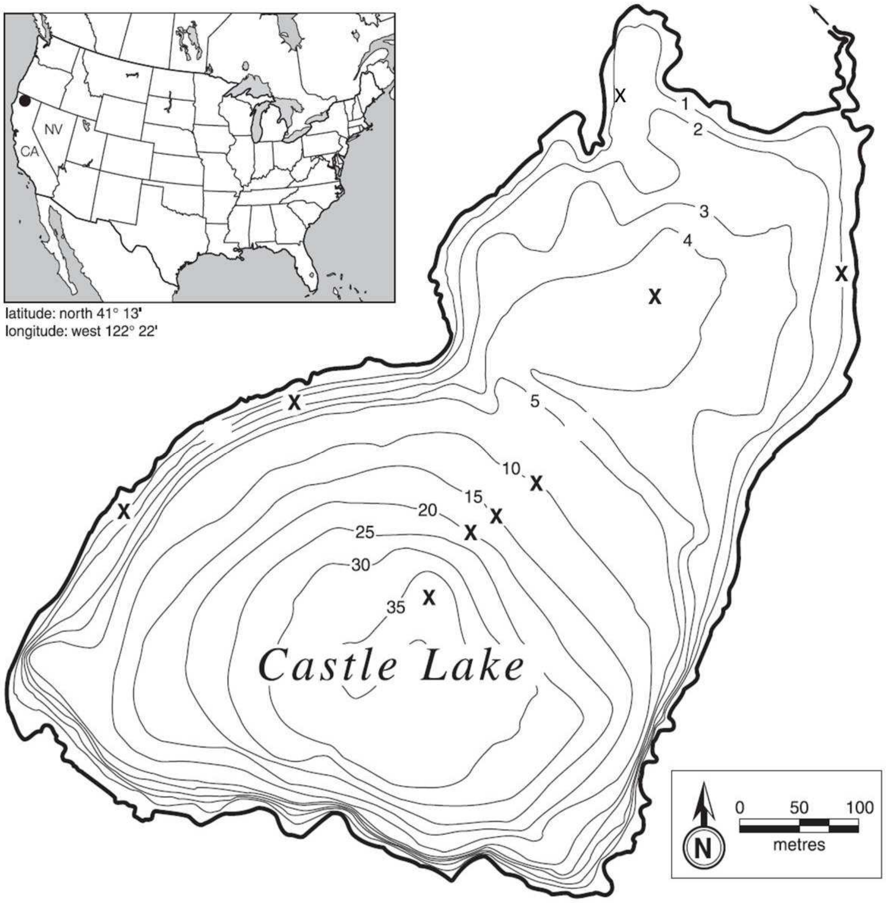

FIGURE 1.

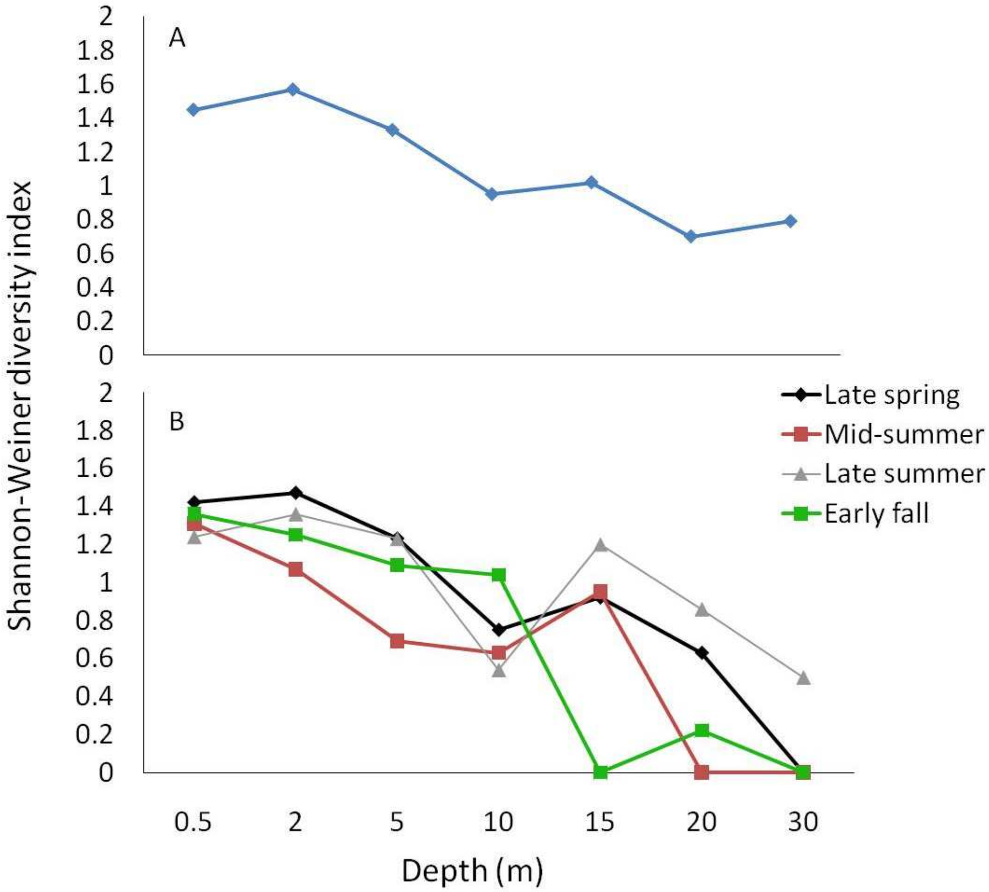
A
B
Shannon-Weiner diversity index
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Late spring
Mid-summer
Late summer
Early fall
0.5
2
5
10
15
20
30
Depth (m)

FIGURE 2.

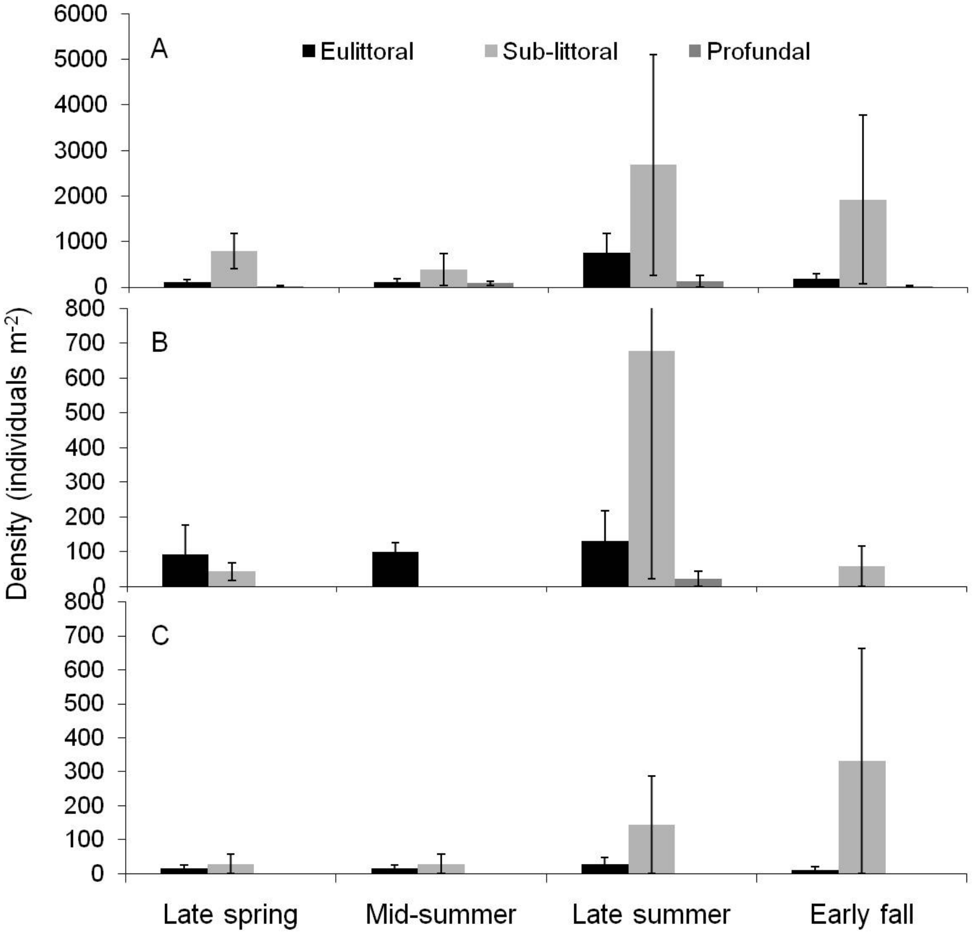
A
B
C
■ Eulittoral
■ Sub-littoral
■ Profundal
Density (individuals m-2)
6000
5000
4000
3000
2000
1000
0
800
700
600
500
400
300
200
100
0
Late spring
Mid-summer
Late summer
Early fall

FIGURE 3.

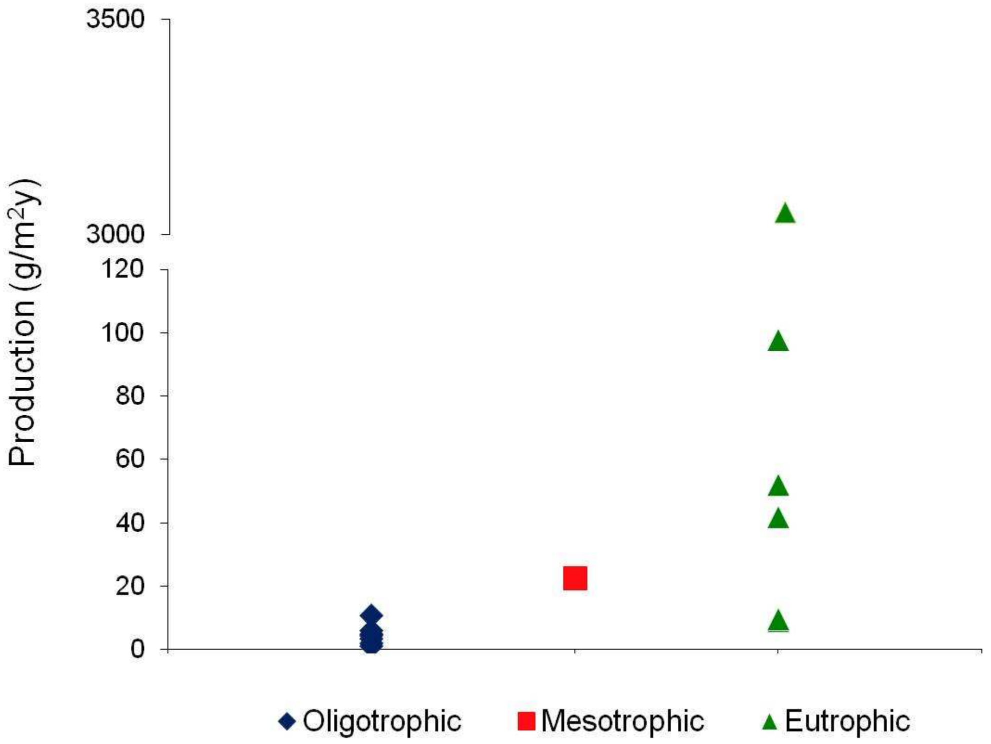
3500
3000
120
100
80
60
40
20
0
Production (g/m^{2}y)
◆ Oligotrophic
■ Mesotrophic
▲ Eutrophic

FIGURE 4.

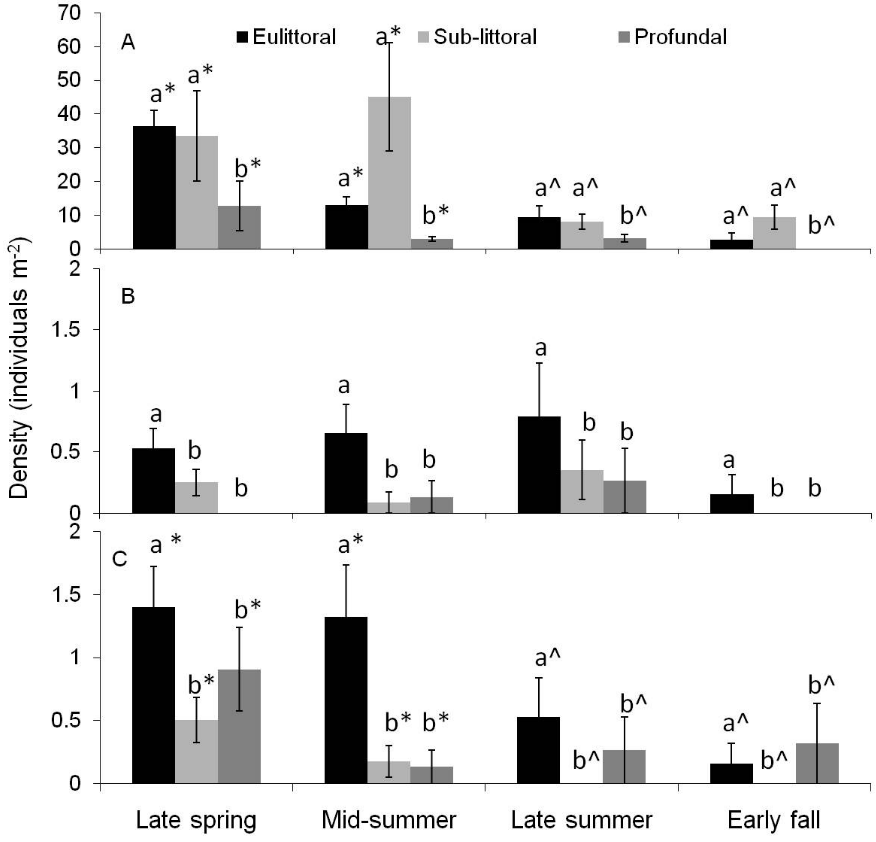
A
Eulittoral
Sub-littoral
Profundal
70
60
50
40
30
20
10
0
a*
a*
b*
a*
a*
b*
a^
a^
b^
a^
a^
b^
Density (individuals m-2)
B
2
1.5
1
0.5
0
a
b
b
a
b
b
a
b
b
a
b
b
C
2
1.5
1
0.5
0
a *
b*
b*
a*
b*
b*
a^
b^
b^
a^
b^
b^
Late spring
Mid-summer
Late summer
Early fall

FIGURE 5.

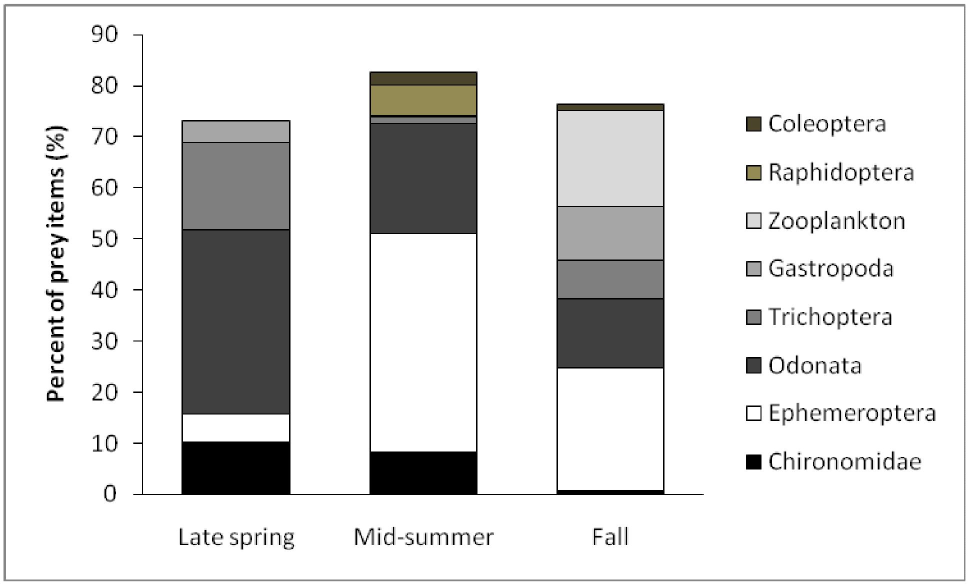
Percent of prey items (%)
0
10
20
30
40
50
60
70
80
90
Late spring
Mid-summer
Fall
Coleoptera
Raphidoptera
Zooplankton
Gastropoda
Trichoptera
Odonata
Ephemeroptera
Chironomidae

FIGURE 6.

2.8 Appendix

Appendix 1A. Biomass (g m^{-2}) and densities (individuals m^{-2}) with standard errors for the dominant benthic invertebrates in Castle Lake.

Taxa	Season	Habitat	Biomass (g m^{-2})	SE	Density (ind m^{-2})	SE
Odonata	Late spring	Eulittoral	0.5307	0.164	1.3988	0.324
	Mid-summer	Eulittoral	1.2519	0.01	1.3232	0.41
	Late summer	Eulittoral	0.6717	0.392	0.5293	0.312
	Early fall	Eulittoral	0	0	0.1588	0.159
	Late spring	Sub-littoral	0.0415	0.004	0.5041	0.179
	Mid-summer	Sub-littoral	0.1447	5E-04	0.1764	0.123
	Late summer	Sub-littoral	0.2837	0.129	0	0
	Early fall	Sub-littoral	0.1604	0.057	0	0
	Late spring	Profundal	0	0	0.9073	0.33
	Mid-summer	Profundal	0	0	0.1323	0.132
	Late summer	Profundal	0	0	0.2646	0.265
	Early fall	Profundal	0	0	0.3176	0.318
Ephemeroptera	Late spring	Eulittoral	0.0038	3E-04	0.5293	0.169
	Mid-summer	Eulittoral	0.0089	0.003	0.6616	0.231
	Late summer	Eulittoral	0.0115	0.002	0.7939	0.438
	Early fall	Eulittoral	0	0	0.1588	0.159
	Late spring	Sub-littoral	0.0099	0.004	0.252	0.109
	Mid-summer	Sub-littoral	0	0	0.0882	0.088
	Late summer	Sub-littoral	0.0021	1E-04	0.3528	0.242
	Early fall	Sub-littoral	0.0044	3E-04	0	0
	Late spring	Profundal	0	0	0	0
	Mid-summer	Profundal	0	0	0.1323	0.132
	Late summer	Profundal	0.0014	0	0.2646	0.265
	Early fall	Profundal	0	0	0	0
Chironomidae	Late spring	Eulittoral	0.0058	0.001	36.406	4.644
	Mid-summer	Eulittoral	0.0021	6E-04	12.901	2.597
	Late summer	Eulittoral	0.0067	0.001	9.3945	3.464
	Early fall	Eulittoral	0.0078	0.002	2.6993	1.918
	Late spring	Sub-littoral	0.0041	9E-04	33.571	13.39
	Mid-summer	Sub-littoral	0.0029	5E-04	45.076	16.12
	Late summer	Sub-littoral	0.0023	1E-04	8.1155	2.197
	Early fall	Sub-littoral	0.0031	2E-04	9.3151	3.567

	Late spring	Profundal	0.019	0	12.778	7.274
	Mid-summer	Profundal	0.0348	0.013	2.911	0.713
	Late summer	Profundal	0.0227	0.009	3.1756	1.173
	Early fall	Profundal	0.0445	0	0	0

Appendix 1B. Biomass (g m^{-2}) and densities (individuals m^{-2}) with standard errors for the dominant emergent invertebrates from Castle Lake.

Taxa	Season	Habitat	Biomass (g m^{-2})	SE	Density (ind m^{-2})	SE
Odonata	Late spring	Eulittoral	0.0563	0.0076	16.35	10.38
	Mid-summer	Eulittoral	0.0451	0.0057	16.35	10.38
	Late summer	Eulittoral	0	0	27.17	20.47
	Early fall	Eulittoral	0	0	10.82	10.82
	Late spring	Sub-littoral	0.0335	0.0065	28.86	28.86
	Mid-summer	Sub-littoral	0.0169	0	28.86	28.86
	Late summer	Sub-littoral	0	0	144.3	144.3
	Early fall	Sub-littoral	0	0	331.8	331.8
	Late spring	Profundal	0.0474	0.009	0	0
	Mid-summer	Profundal	0.0336	0.0018	0	0
	Late summer	Profundal	0	0	0	0
	Early fall	Profundal	0	0	0	0
Ephemeroptera	Late spring	Eulittoral	0.0117	0.0024	92.09	84.88
	Mid-summer	Eulittoral	0.0069	0.0012	98.55	26.19
	Late summer	Eulittoral	0.0048	0	130.5	87.74
	Early fall	Eulittoral	0	0	0	0
	Late spring	Sub-littoral	0.0067	0.0013	43.28	24.99
	Mid-summer	Sub-littoral	0.0033	0.0003	0	0
	Late summer	Sub-littoral	0	0	678.1	656.6
	Early fall	Sub-littoral	0	0	57.71	57.71
	Late spring	Profundal	0	0	0	0
	Mid-summer	Profundal	0.0053	0.002	0	0
	Late summer	Profundal	0	0	21.64	21.64
	Early fall	Profundal	0	0	0	0
Chironomidae	Late spring	Eulittoral	0.0005	0.0003	103.1	68.01
	Mid-summer	Eulittoral	0.0005	0.0002	108.7	81.89
	Late summer	Eulittoral	0.0003	2E-05	746.9	431.8
	Early fall	Eulittoral	0	0	178.9	115.9
	Late spring	Sub-littoral	0.0003	0.0002	793.5	392.2
	Mid-summer	Sub-littoral	0.0002	0.0002	389.5	347.2
	Late summer	Sub-littoral	0.0004	3E-05	2684	2424
	Early fall	Sub-littoral	0	0	1919	1854
	Late spring	Profundal	0.0007	0.0003	21.64	21.64
	Mid-summer	Profundal	0.0005	0.0002	86.57	43.28

	Late summer	Profundal	0	0	129.8	129.8
	Early fall	Profundal	0	0	21.64	21.64

3.0 Chapter 3

A comparison of novel tracers to determine aquatic and terrestrial contributions to terrestrial consumer energetics

By:

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Abstract

Aquatic emergent invertebrates act as a trophic link between aquatic and terrestrial ecosystems by contributing energy to terrestrial consumers. Aquatic emergent and terrestrial invertebrate densities and biomass, carbon stable isotopes and fatty acids were analyzed to determine aquatic and terrestrial invertebrate contributions to bat energetics in Castle Lake, a sub-alpine lake in Northern California. Aquatic emergent and terrestrial invertebrate densities (# of individuals m^{-2} d^{-1}) were 380 and 54 in late spring, 55 and 18 in summer, and 17 and 4 in fall, respectively. Aquatic and terrestrial invertebrate biomass (g dry weight m^{-2} d^{-1}) was 0.05 and 0.2 in late spring, 0.04 and 0.11 in summer, and 0.002 and 0.08 in fall, respectively. Carbon stable isotope ($\delta^{13}C$) analyses indicated that the relative percentages of aquatic versus terrestrial invertebrates in secondary consumer diets were 100 % and 0 % in late spring, 20 % and 80 % in summer, and 64 % and 36 % in fall, respectively. Fatty acids were separated into distinct terrestrial and aquatic

functional groups through the use of a principal component analysis and a synthesis of current fatty acid literature. Insectivorous bats (*Myotis* spp., *Lasionycteris noctivagans, Eptesicus fuscus, Lasiurus cinereus,* and *Corynorhinus townsendii*) received 20 % of their total fatty acids from both aquatic and terrestrial ecosystems and were not significantly different between seasons. Differences between biomarker analysis results suggest bat consumers assimilated organic carbon through different pathways.

3.1 Introduction

There is an increasing amount of research suggesting that trophic links between aquatic and terrestrial ecosystems are important for determining secondary consumer energetics. Many fish populations rely heavily on both benthic and terrestrial invertebrates, while terrestrial consumers (e.g. birds, lizards, spiders, and bats) utilize emergent aquatic invertebrates (Vander Zanden et al. 2006, Nakano and Murakami 2001, Sabo and Power 2002, Paetzold et al. 2005, Fukui et al. 2006). Insectivorous bats may serve as important species for understanding linkages across ecosystems due to high deposition of guano in tree hollows close to water bodies (Gellman and Zielinski 1996) and their ability to consume their body weight in prey each night during periods of high energy demand (Barclay et al. 1991). Certain insectivorous bat species forage for invertebrates over still or slow moving water bodies and in riparian zones rather than upland habitats (Grindal et al. 1999, Barclay 1991), indicating the importance of aquatic ecosystems in supporting bat productivity. In addition, bat foraging activity often is greatest during peaks in aquatic invertebrate emergence (Rainey et al. 2006). Nakano and Murakami (2001) found similar feeding strategies for birds that shifted their diets based on seasonal availability of prey in aquatic and terrestrial habitats. Therefore, insectivorous bats may rely seasonally on different ecosystems by following patterns in aquatic invertebrate emergence availability in the terrestrial ecosystem. One method to demonstrate cross-ecosystem energy flows is through an understanding of diet selectivity and assimilation of food resources. Typically, ecologists use diet selectivity information (stomach or fecal analysis) or tracers (e.g. stable isotopes) to determine the flow of energy within an ecosystem, with recent studies focusing on contributions across ecosystems (Sanzone et al. 2003, Finlay and Vredenburg 2007).

Bat diets have been analyzed through the use of fecal pellets (Anthony and Kunz 1977, Brigham et al. 1992) and identification of culled invertebrate parts at roosts (Johnston and Fenton 2001). Fecal analysis has demonstrated that some *Myotis* species acquired anywhere from 50 % to 90 % of their diet from aquatically derived invertebrates, including trichopterans, chironomids, and ephemeropterans (Anthony and Kunz 1977, Barclay 1991, Brigham et al. 1992). However, fecal pellet identification and collection of invertebrate parts at roosts may not identify all consumed prey due to decomposition of soft bodied invertebrates. Novel diet tracers may provide more descriptive information on consumer diet preferences.

Recently, researchers have measured chemical tracers in bats to determine the incorporation of energy from ecosystem sources (Herrera et al. 2001, Painter et al. 2009). In the last two decades, ecologists have employed the use of stable isotope tracers to determine assimilated food sources from different habitats and ecosystems to higher level consumers. Due to contributions of organic matter to consumer diet and retention of isotopic carbon across trophic levels, the specific habitat from which a consumer derives its energy may be determined (Sanzone et al. 2003, Hecky and Hesslein 1995). Even though bats have high metabolic rates, their wing tissue has a low carbon isotope turnover rate, and should therefore represent diet over a long period of time (Voight et al. 2003).

In the last decade lipid biomarkers, such as essential fatty acids, have been employed as trophic biomarkers. Their use has been limited, yet fatty acid analysis is believed to be an emerging tracer in determining energy flow within and across ecosystems (Brett et al. 2006, Sushchik et al. 2006, Kuusipalo and Kakela 2000). Similar to isotopes, fatty acid measurements may provide information on the foraging behavior of difficult-to-observe predators by quantitatively estimating diet based on fatty acid signatures of predator and prey (Budge et al. 2006). While the lipid biomarker approach has not been utilized to determine bat diet specifically, fatty acids have provided information on diets of other consumers, such as tadpoles, seabirds, echidnas, and carnivorous mammals (Whiles et al. 2010, Hebert et al. 2009, Falkenstein et al. 2001, Koussoroplis et al. 2008). Certain fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have served as indicators of high food quality and when transferred to higher trophic levels, suggest high quality prey in consumer diet (Brett et al. 2006). Along with DHA and EPA, saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs), arachidonic acid (ARA), and C_{18} $\omega6$ and C_{18} $\omega3$ polyunsaturated fatty acids (PUFAs) have been used to represent key fatty acid functional groups (Goedkoop et al. 2000, Brett et al. 2006, Koussoroplis et al. 2008). Fatty acid analyses may therefore be useful for revealing cross ecosystem resource utilization, demonstrating the importance of aquatic subsidies for terrestrial consumers (e.g. bats) as well as seasonal variation in aquatic versus terrestrial prey preferences.

Typically, fatty acids are not synthesized *de novo* in mammals and need to be obtained through diet (Budge et al. 2006). Lipids accumulate over time in the tissues of organisms; therefore fatty acid signatures may provide evidence of resource intake over long periods of time (Budge et al. 2006). In some species, certain lipids, or lipid ratios, have been associated with specific ecosystems and thus may be used to differentiate between aquatic and terrestrial derived energy sources. For example, aquatic invertebrates tend to have greater amounts of PUFAs than terrestrial invertebrates (Uscian and Stanley-Samuelson 1994, Howard and Stanley-Samuelson 1996). Koussoroplis et al. (2008) demonstrated that ratios of DHA/linoleic acid (LNA) decreased as small carnivorous mammals' dependence on aquatic prey decreased. Insectivorous bat fatty acid composition and ratios may reflect fatty acid composition of their invertebrate prey, indicating the relative contribution of aquatic and terrestrial invertebrates to bat diet. Similarly, as some bats have been documented as opportunistic foragers (Barclay 1985, Barclay and Brigham 1994) seasonally reliant on cross habitat subsidies, the fatty acid composition of bats may change seasonally, as the relative abundance of aquatic versus terrestrial prey varies.

This study was designed to determine the feeding behavior and resource reliance of a terrestrial consumer, bats, on aquatic and terrestrial resources. Specifically, we wanted to determine whether carbon stable isotopes and lipid biomarkers could be used to infer long-term energy utilization by bat consumers. The objectives of this study were to 1) examine seasonal variation of aquatic and terrestrial prey sources, 2) investigate the potential to measure the relative contribution of those prey to the diets of higher level consumers using carbon isotope and fatty acid signals in both bats and prey, and 3) synthesize current literature that has measured fatty acids from terrestrial and aquatic invertebrate forage resources to determine if there is a conservative fatty acid biomarker that is representative of aquatic and terrestrial ecosystems. We hypothesized that bats consumed higher quantities of aquatic invertebrates than terrestrial invertebrates, especially in spring when aquatic invertebrate emergence was highest. This study demonstrates the importance of resource exchanges across lake and terrestrial ecosystems.

3.2 Methods

Study site

Sub-alpine Castle Lake is located in northern California in the Shasta-Trinity National Forest. The Shasta-Trinity National Forest has a diverse landscape ranging from 304.8 m to 4316.5 m. The snowmelt-fed lake (1,657 m) is typically covered in ice from November through June and drains to Lake Siskiyou in the headwaters of the Sacramento River basin. The surrounding watershed is composed of mixed conifer forests, including ponderosa pine (*Pinus ponderosa*), red fir (*Abies magnifica*), white fir (*Abies concolor*), lodgepole pine (*Pinus contorta*), and incense cedar (*Libocedrus decurrens*) among others. Terrestrial predators in the forest include ospreys (*Pandion haliaetus*), bald eagles (*Haliaeetus leucocephalus*), and mountain lions (*Puma concolor*). Castle Lake is an ideal research setting due to ongoing limnological monitoring since 1959 by researchers from the University of California, Davis and the University of Nevada, Reno. Due to the long-term monitoring program, Castle Lake is a suitable location to study cross-ecosystem exchanges and foraging behavior of sensitive forest species. The littoral zone of Castle Lake, which includes ~54 % of the lake's surface area, is very productive, containing sediments high in organic matter (Axler and Reuter, 1996). These productive sediments contain a diversity of aquatic invertebrates, many of which emerge from the lake (Brownstein et al., in review).

Invertebrate collection

Aquatic emergent invertebrates were collected four days a week from May through August and twice a week during September in 2009. Mesh emergent traps (Rainey et al. 2006) were anchored at eight locations for 24 hours a day in both deep and near shore habitats (Figure 1). The traps were placed along a depth gradient at 0.1, 0.5, 2, 5, 10, 15, and 20 m to capture both near-shore and deep-water habitats. The 0.315 m^2 traps were emptied each morning to observe daily invertebrate flux. A subset of invertebrates were collected for lipid analysis and thus immediately stored at -80 °C or preserved in 70 % ethanol to determine the biomass of emergence. Terrestrial invertebrates were collected weekly from three Malaise traps (Bioquip, Rancho Dominguez, CA) placed around the lake (Fukui et al. 2006). The Malaise traps (1.69 m^2 in area and 2 m in height) were set up at the same time as the emergent traps. A ultra-violet light trap (Bioquip, Rancho Dominguez, CA) was set out concomitant with bat sampling (Painter et al. 2009). The light trap was placed in the riparian habitat one hour before sunset and turned off at the end of the four hour bat capture period. In order to minimize interference with the sampling, the light trap was located at least 100 m away from mist nets. Invertebrates were identified to order or family if possible, using an appropriate key guide (Merritt and Cummins 1996, Marshall 2006).

In order to determine the resource availability from terrestrial and aquatic ecosystems, terrestrial and emergent invertebrate densities (# individuals m^{-2} day^{-1}) and biomass (g dry weight m^{-2} day^{-1}) were estimated for the lake and watershed using the method in Brower and Zar (1977). Aquatic emergent invertebrate densities and biomass were calculated for three depth zones (0-5 m, 6-19 m, and 20-35 m) and applied to the area of the lake corresponding to those depths. As a result, the daily mean lake-wide density and biomass of aquatic emergent invertebrates were calculated for late spring, summer, and fall. Riparian terrestrial invertebrate densities and biomass were calculated for the above seasons as well. Aquatic and terrestrial invertebrate densities and biomass were compared for seasonal differences using a one-way ANOVA, followed by a Tukey's post hoc comparison if significance was determined. Densities and biomass were compared using a student's *t*-test.

Bat capture

Bat capture occurred once a month, for two nights, from June to September in 2009 by the US Forest Service. Mist nets were placed over the lake and at selected riparian sites (Figure 1). Net sites and sizes (6.1 m, 9.1 m, and 12.8 m in width) varied by sampling date to maximize bat capture. Additionally, high nets (12.8 m in width and 11 m in height) were erected in a small, isolated wetland at the north end of the lake, and mid-water nets were designed and installed above open water locations to capture high flying and water grazing bats. Habitat descriptions were recorded at each capture location. For all bats, species identification, sex and reproductive condition, age (adult vs. juvenile), forearm length, weight, and canine tooth wear were determined and recorded. A small tissue sample was collected from the bat's wing using a sterile biopsy punch and preserved in liquid nitrogen before storage at -80 °C.

Additionally, a synthesis of bat foraging behavior literature was conducted to determine whether insectivorous bats were opportunistic or selective foragers.

Stable isotopes

Bat wing tissue punches from *Myotis* species, *Lasionycteris noctivagans,* and *Eptesicus fuscus* and the dominant aquatic and terrestrial invertebrates were dried at 60 °C for 24 hours and placed in 5x9 mm tin cups in preparation for carbon stable isotope analysis at the University of Nevada, Reno's Stable Isotope Laboratory. Invertebrate and bat samples from late spring, summer and fall were analyzed. A Eurovector (Milan, Italy) elemental analyzer interfaced to a Micromass (Manchester, UK) IsoPrime continuous-flow stable-isotope ratio mass spectrometer was used for isotope analysis. Mean δ^{13} C (‰) values for secondary consumers (bats), aquatic and terrestrial invertebrates were separated by season and used in a two-source mixing model using the following equation from Phillips and Gregg (2001):

$$f_A = \frac{\bar{\delta}_{TC} - \bar{\delta}_T}{\bar{\delta}_A - \bar{\delta}_T} \tag{1}$$

Terrestrial and aquatic invertebrates were used as the two end-members in the equation to determine terrestrial consumer diet. Mean δ^{13} C (‰) values were used for terrestrial consumers ($\bar{\delta}_{TC}$), aquatic invertebrates ($\bar{\delta}_A$), and terrestrial invertebrates ($\bar{\delta}_T$). The proportion of aquatic invertebrates represented in terrestrial consumer diet was calculated as f_A. To determine terrestrial invertebrate representation in terrestrial consumer diet, the $\bar{\delta}_T$ and $\bar{\delta}_A$ in the equation were switched. To determine the variance for $\bar{\delta}_T$ and $\bar{\delta}_A$, the following equation was applied:

$$\sigma_{fA}^2 = \frac{1}{(\bar{\delta}_A - \bar{\delta}_T)^2}\left[\sigma_{\bar{\delta}_{TC}}^2 + f_A^2\sigma_{\bar{\delta}_A}^2 + (1-f_A)^2\sigma_{\bar{\delta}_T}^2\right] \tag{2}$$

where the variances of the mean carbon isotopic values were represented by $\sigma_{\bar{\delta}_{TC}}^2, \sigma_{\bar{\delta}_A}^2$, and $\sigma_{\bar{\delta}_B}^2$. The variance for f_B was estimated by replacing the A and T subscripts in the above equation. Mixing model results identified the proportion (and variance) of consumer dependence on aquatic and terrestrial resources in late spring, summer, and fall. Significant differences between carbon isotope values and taxonomic group were determined using an ANOVA, followed by a Tukey's post hoc comparison when significance was found.

Biochemical analysis

Fatty acid samples were stored in airtight containers at -80 °C until extractions occurred. After 5-10 µl of 19:0 (10 mg/10 ml) was added to samples as an internal standard, samples were extracted and methylated using the Kattner and Fricke (1986) method. Fatty acid composition was then analyzed with a gas chromatograph (Hewlett Packard 6890) equipped with a programmable temperature vaporizer injector, a fused silica capillary column (Supelco, Bellefonte, PA) and a flame ionization detector (Park et al. 2002, Mueller-Navarra et al. 2004) at the University of California, Davis' Tahoe

Environmental Research Center. Chromatogram peaks of the fatty acid methyl esters (FAME) were identified by comparison to known fatty acid standards (Supelco, Bellefonte, PA).

Peak areas were used to calculate fatty acid absolute concentrations (µg FA/mg) and total percentage (%) of fatty acids. The percentages of total fatty acids were arcsine square root transformed for normality. To examine seasonal trends, fatty acids were separated into functional groups: saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs), arachidonic acid (ARA), eicosapentaenoic acid and docosahexaenoic acid (EPA and DHA), 18ω6 and 18ω3 polyunsaturated fatty acids (PUFAs). One-way ANOVAs, followed by a Tukey's post hoc comparison when appropriate, were used to determine significant differences between seasons and the above fatty acid functional groups. The following ratios of fatty acids were calculated to determine the proportion of aquatic and terrestrially derived fatty acids in Castle Lake taxa: ω3/ω6, DHA/LNA, ARA/EPA+DHA, and C24/C14. A principal component analysis (PCA) was used to separate invertebrate fatty acids into distinct aquatic and terrestrial groups.

A synthesis of current fatty acid literature was conducted to determine if aquatic and terrestrial invertebrate lipid signals were specific to their respective ecosystems. If fatty acids were found in both ecosystems, regardless of proportions, the fatty acids were not designated as an exclusively aquatic or terrestrially derived fatty acid. Distinct aquatic and terrestrial fatty acids derived from the literature, along with the PCA-determined fatty acid groups, were included in a discriminant analysis to determine if aquatic invertebrates, terrestrial invertebrates, and bats differed by the pre-defined fatty acids. Percent (%) of total bat fatty acids were plotted in conjunction with aquatic and terrestrial invertebrate fatty acids.

3.3 Results

Aquatic and terrestrial invertebrate abundance

Aquatic emergent and terrestrial invertebrate densities (# of individuals m^{-2} d^{-1}) were 380 and 54 in late spring, 55 and 18 in summer, and 17 and 4 in fall, respectively (Figure 2A). Aquatic and terrestrial invertebrate biomass (g dry weight m^{-2} d^{-1}) was 0.05 and 0.2 in late spring, 0.04 and 0.11 in summer, and 0.002 and 0.08 in fall, respectively (Figure 2B). Densities and biomass decreased (380 to 17 individuals m^{-2} and 0.05 to 0.002 g m^{-2}, respectively) as the season progressed from late spring through fall for aquatic emergent invertebrates (Figure 2A) and for terrestrial invertebrates (54 to 4 individuals m^{-2} and 0.2 to 0.08 g m^{-2}, respectively)(Figure 2B). The disparity between aquatic and terrestrial invertebrate densities and biomass was greatest in late spring, yet densities and biomass were not significantly different throughout the seasons ($p = 0.283$). While aquatic and terrestrial invertebrate densities were significantly different from each other ($p = 0.007$), biomass estimates were not significantly different between aquatic and terrestrial invertebrates ($p = 0.132$). The biomass of aquatic emergent invertebrates were significantly different depending on season ($p = 0.007$) because late spring and summer produced greater biomass than fall, while densities were not significantly different by season ($p = 0.209$). Biomass and densities did not differ significantly by season in terrestrial invertebrates ($p = 0.662$ and $p = 0.142$, respectively).

A synthesis of bat foraging behavior literature demonstrated that insectivorous bats have been documented as both opportunistic and selective foragers (Table 1).

Stable isotopes

Mean $\delta^{13}C$ (‰) values and standard error for secondary consumers (bats), aquatic emergent invertebrates, and terrestrial primary consumers were -23.6 ± 0.2, -23.9 ± 0.4, and -26.7 ± 2.8 in late spring, -23.9 ± 0.7, -20.3 ± 0.1, and -24.8 ± 0.3 in summer, -22.6 ± 0.2, -22.2 ± 0, and -23.3 ± 0.3 in fall, respectively (Figure 3). The two-source mixing model with terrestrial and aquatic invertebrates as end-members indicated that the relative percentages of aquatic versus terrestrial invertebrates in secondary consumer diets were 100 ± 4 % and 0 ± 4 % in late spring, 20 ± 3 % and 80 ± 3 % in summer, 64 ± 8 % and 36 ± 8 % in fall, respectively. However, when bat species other than *Myotis* were excluded from the analysis in fall (*Myotis* species were found in all three seasons, while other bats were not), aquatic invertebrates represented 18 ± 6 % of secondary consumer diet, while terrestrial primary consumers represented 82 ± 6 % of secondary consumer diet. In spring and fall, $\delta^{13}C$ values did not significantly differ between taxa groups ($p = 0.123$ and $p = 0.137$, respectively). In summer, $\delta^{13}C$ values differed significantly between taxa groups ($p = 0.0001$), with aquatic invertebrate values greater than bats and terrestrial invertebrates.

Fatty acids

The dominant fatty acids found in bat tissue were 16:0, 16:1, 18:0, 18:1ω9, 18:2ω6, 20:0, 18:3ω6, 18:3ω3, 22:0 and 24:0. Seasonal differences were observed in some of the dominant bat fatty acids. Of the dominant fatty acids found in bat tissue, 18:1ω9, 18:2ω6, 18:3ω6, 22:0, and ω6 PUFAs made up higher proportions of total fatty acids in June than in September ($p = 0.045$, 0.007, 0.009, 0.055, and 0.0012, respectively). As for functional fatty acid groups, SAFAs were greatest in bats and made up 65 % of their total fatty acids in June and 58 % in September 2009. Aquatic emergent invertebrates had 32 % SAFAs in June and 29 % in September, while terrestrial invertebrates had 18 % SAFAs in June and 17 % in September. SAFAs in taxa groups were significantly different from each other ($p = 0.000$), where bats had a greater % of total SAFAs than aquatic invertebrates, and aquatic invertebrates were greater than terrestrial invertebrates. MUFA content differed in bats (17 % June, 13 % in September), aquatic invertebrates (28 % June, 37 % September), and terrestrial invertebrates (36 % June, 25 % September). Aquatic and terrestrial invertebrates had significantly higher amounts of MUFAs than bats ($p = 0.000$). Neither aquatic nor terrestrial invertebrates had ARA, and ARA in bats only made up 2 % of total fatty acids in June and 1.5 % in September. Aquatic invertebrates had the most EPA and DHA, with 17 % in June and 8 % in September and were significantly different from terrestrial invertebrates and bats ($p = 0.000$). Terrestrial invertebrates contained very little EPA and DHA, with 0.27 % and 1 %, in June and September, respectively. Bats had 0.13 % EPA and DHA in September. Terrestrial invertebrates had significantly higher amounts of 18ω3 PUFAs than aquatic invertebrates and bats ($p = 0.000$), with 30 % in June and 44 % in September. While aquatic invertebrates had 4 % 18ω3 PUFAs in June and 11 % in September, bats did not have any 18ω3 PUFAs. Bats did have 8 % 18ω6 PUFAs in June and 5 % in September, while aquatic invertebrates had 12 % in June and 10 % in September. Terrestrial invertebrates had 13 % 18ω6 PUFAs in June and 9 % in September.

The % total and absolute concentrations of fatty acids for individual taxa are reported in Tables 2 and 3, respectively. There were no differences in ω3/ω6 ratios between aquatic and terrestrial taxa (Table 2). There were also no differences between taxa using DHA/LNA and ARA/EPA+DHA ratios (Table 2). Invertebrates had very low ratios of C24/C14 (Table 2).

PCA suggested that terrestrial invertebrates differed from aquatic invertebrates because of high proportions of 18:3ω3, 18:1ω9, and 22:0 and aquatic invertebrates differed from terrestrial invertebrates because of 16:0, 16:1, and 20:5ω3. The PCA suggested two distinct groups of fatty acids: terrestrial fatty acids and aquatic fatty acids. The first three principal components of the PCA explained 65 %, 15 %, and 5 % of the variance, respectively. The first and second PCs were associated with 16:1 and 18:3ω3, while the third PC was associated with 18:2ω6.

A synthesis of current fatty acid literature (aquatic and terrestrial source data available from 11 out of the 17 articles reviewed) revealed that 16:2ω4, 16:3, 18:4ω3, 22:4ω6, 22:5ω6, 22:5ω3, 22:6ω3, and 24:1ω9 were found only in aquatic taxa and 15:1 and 19:0 were found only in terrestrial taxa (Table 4). 22:6ω3 and 24:1ω9 (fatty acids found in this study from the above list) were added to the PCA-determined aquatic fatty acid group. Additionally, 12:0, 14:1, 21:0, 22:0, 23:0, and 24:0 were present in Castle Lake aquatic taxa. Defined aquatic fatty acids (16:0, 16:1, 20:5ω3, 22:6ω3 and 24:1ω9) and PCA-determined terrestrial fatty acids (18:3ω3, 18:1ω9, and 22:0), along with bat fatty acids, were used in a discriminant analysis. Aquatic and terrestrial invertebrates and bat fatty acids were significantly different from each other (Wilks' Lambda test; $p < 0.0001$). The first factor in the discriminant analysis accounted for 86.4 % of the variance, while the second factor accounted for 13.6 % of the variance. The two factors accounted for 100 % of the variance overall. Terrestrial and aquatic fatty acid proportions were not significantly different between June and September ($p = 0.815$ and $p = 0.059$, respectively). Bat fatty acids did not overlap with either the terrestrial or aquatic invertebrate fatty acids, but rather had low proportions (about 20 % of total fatty acids) of both terrestrial and aquatic fatty acids (Figure 4).

3.4 Discussion

In this study, we utilized two different biomarkers (carbon stable isotopes and fatty acids) to determine aquatic and terrestrial energy assimilation by terrestrially-oriented consumers (*Myotis* spp., *Lasionycteris noctivagans*, and *Eptesicus fuscus*). To our knowledge, this is the first time that this has been conducted for bats, a keystone species in many ecosystems, and rarely has a combined approached been utilized for higher level consumers (Hebert et al. 2008, Hebert et al. 2009). Both biomarker methods suggest that bats consumed both terrestrial and aquatic emergent invertebrates over the course of the summer, with some seasonal variation. The significantly greater densities of aquatic emergent invertebrates throughout the seasons, especially in late spring, suggest that there were an abundance of aquatic resources available for terrestrial consumers early in the season, yet aquatic and terrestrial invertebrate biomass did not differ significantly throughout the seasons. Since bat carbon isotope signals demonstrated a stronger aquatic signal in late spring, it may be that invertebrate density rather than biomass was driving prey selection in these terrestrial consumers.

Carbon isotope analysis suggests that terrestrial consumers assimilated energy from aquatic ecosystems in late spring and to a lesser extent in fall while relying on terrestrial prey in summer, which may be attributed to seasonal subsidies in invertebrate prey. *Myotis* diet specifically appears to have been subsidized by the high densities of aquatic emergent invertebrates in late spring due to mixing model results demonstrating 100 % consumer reliance on aquatic resources. In summer and fall, however, mixing model results demonstrated that *Myotis* may have relied on terrestrial invertebrates (80 % and 82 %, respectively) due to low densities and biomass of aquatic invertebrates during those seasons. The higher biomass relative to density in terrestrial invertebrates was likely a function of their large size, and it may be that when aquatic invertebrate densities decreased in summer, bats focused on the larger terrestrial prey (Anthony and Kunz 1977). In addition, aquatic invertebrate $\delta^{13}C$ values were significantly higher than bats and terrestrial invertebrates in summer, suggesting that bats consumed terrestrial invertebrates in summer. The stable isotope mixing model also suggested that all bat taxa consumed aquatic invertebrates in late spring and to a lesser extent in fall (64% in comparison to late spring's 100%). Perhaps migratory bats that were captured in fall consumed aquatic invertebrates from other locations and relied less on seasonal fluxes of invertebrate prey than resident *Myotis* species (Fleming and Eby 2003). To determine if migratory bats consumed aquatic invertebrates from other locations, and whether they had different carbon isotope values than Castle Lake invertebrates, we suggest further invertebrate analyses from other locations.

While bats may have had significantly higher proportions of some fatty acids in June as a function of their diet being dominated by aquatic invertebrates, than in September where terrestrial invertebrates made up the larger portion of their diet, overall % of total bat fatty acids did not differ by season. Aquatic and terrestrial invertebrate fatty acids also did not differ by seasons. Since bats exhibited similar percentages of aquatic (20%) and terrestrially (20%) associated fatty acids throughout the summer, this may suggest that prey from both ecosystems were available during bat foraging, despite higher consumption of aquatic invertebrates during late spring. The fatty acid composition of Castle Lake bats also did not correspond to any single sampled invertebrate taxa, suggesting that bats exhibited dietary plasticity, consuming a variety of taxa. Whiles et al. (2010) demonstrated that pond-dwelling tadpoles, similarly, had different degrees of omnivory depending on resource availability. Further, bat foraging literature demonstrates both selective and opportunistic feeding strategies for various insectivorous bat taxa, as well as variation in the proportions of aquatic versus terrestrial invertebrates in cases where both were present in bat diets (Anthony and Kunz 1977, Barclay 1985, Brigham et al. 1992) (Table 1). Sushchik et al. (2006) demonstrated that even though riverine benthic invertebrates showed seasonal variations in PUFAs, the fish that consumed these invertebrates did not have significant seasonal variations in PUFA content. A similar phenomenon may explain the lack of seasonal variation in bat fatty acid composition as well.

Another explanation for the lack of differences between seasons in consumer fatty acid composition may be the conversion of fatty acids within bat tissues. Budge et al. (2006) noted that birds and mammals typically only synthesized fatty acids *de novo* if consumer diets were low in fat and high in carbohydrates. Since ARA was not found in Castle Lake terrestrial or aquatic invertebrate fatty acid composition, bats may have obtained ARA from 18:2ω6, a precursor to ARA (Stanley-Samuelson et al. 1987), rather than directly from their diet. Similarly, 18:3ω3 is a precursor to EPA (Stanley-Samuelson et al. 1987) and bats may be able to convert 18:3ω3 obtained from terrestrial invertebrates into EPA. Insectivorous hibernating mammals may not need high proportions of PUFAs in their diets because they are able to metabolize high proportions of MUFAs instead. Echidnas relied on high proportions of MUFAs, rather than PUFAs, before entering hibernation (Falkenstein et al. 2001). Additionally, during a food selection experiment in a laboratory, ground squirrels were found to intake the minimum level of PUFAs required for hibernation (Frank et al. 1998). Likewise, insectivorous bats may not need high levels of PUFAs in their diet if high proportions of MUFAs are available.

Previously documented biologically significant fatty acid ratios identified during our literature synthesis, including ω3/ω6, DHA/LNA, ARA/EPA+DHA, and C24/C14 did not differentiate aquatic and terrestrial sources in this study. Typically, high ω3/ω6 ratios (> 1) are present in aquatic taxa and low ratios (< 1) are found in terrestrial taxa (Olsen 1999). We were not, however, able to use the ω3/ω6 ratio to distinguish between aquatic and terrestrially derived fatty acids in consumer diet. We found the ω3/ω6 ratio to be low in bats but high in aquatic invertebrates as well as in the dominant terrestrial invertebrate Lepidoptera (Table 2). Castle Lake terrestrial invertebrates also had high amounts of 18:3ω3, which are typically considered an aquatically derived fatty acid (Napolitano 1999), suggesting that the ω3/ω6 ratio did not appropriately distinguish between aquatic and terrestrial signals in Castle Lake prey. DHA/LNA and ARA/EPA+DHA ratios resulted in mostly “0” ratios due to most taxa not containing ARA or DHA (Table 2) and therefore did not differentiate between ecosystems either. C24/C14 (allochthonous versus autochthonous source) ratios were not used to interpret this study because, while bats had high ratios of C24/C14 which would have suggested a terrestrial diet, terrestrial invertebrates had very low ratios (C24/C14 = 0) when they are typically represented by much higher ratios (Table 2). Terrestrial sources should have higher C24/C14 ratios if used to differentiate between aquatic and terrestrial ecosystems (Napolitano 1999). Therefore, no previously exiting fatty acid ratios were able to classify terrestrial consumer energetics as derived solely from either aquatic or terrestrial ecosystems.

The synthesis of current fatty acid literature demonstrated that fatty acids that have been characterized as aquatic or terrestrially derived, nonetheless occur in both ecosystems, even if only present in low proportions (Table 4). Since most fatty acids in our study were found in both ecosystems, this likely explains why predefined ratios did not determine differences between aquatic or terrestrially derived resources. Because predetermined ratios did not define differences between aquatic and terrestrial ecosystem signals, alternate fatty acids were deemed important for this study. While 16:0, 16:1, 20:5ω3, 22:6ω3 and 24:1ω9 were found in Castle Lake aquatic invertebrates, 18:3ω3, 18:1ω9 and 22:0 were found in terrestrial invertebrates. Additionally, 12:0, 14:1, 21:0, 22:0, 23:0, and 24:0 were also present in Castle Lake aquatic taxa, despite not being associated with aquatic taxa in previous studies.

Findings from this study suggest that subsidies occurred across ecosystems throughout the seasons, and were perhaps more prominent in spring due to high aquatic invertebrate emergence. While both biomarker methods suggested terrestrial consumer reliance on both aquatic and terrestrial ecosystems, only carbon isotope analysis demonstrated seasonal variation in consumer energetics. Perhaps aquatic invertebrate fatty acids did not dominate bat fatty acid composition because of the previously mentioned fatty acid transformations. It is important to consider the scale of analysis because, although the two biomarker methods did not correspond on a seasonal scale, both methods did demonstrate consumer reliance on aquatic and terrestrial ecosystems when all seasons were combined. Kharlamenko et al. (2001) found that while carbon stable isotope analysis showed eelgrass carbon as a dominant food resource for consumers, lipid analysis showed that none of the consumers directly assimilated eelgrass carbon. The difference between biomarker methods may suggest that assimilation of organic carbon occurred through a variety of pathways in bat consumers. We suggest further lipid analyses from laboratory experiments to determine whether biosynthesis of fatty acids occurs within the tissues of hibernating mammals.

Conclusions

Trophic exchanges between aquatic and terrestrial ecosystems were demonstrated through the use of both fatty acids and carbon stable isotope analyses. While aquatic emergent invertebrate densities were greater than terrestrial invertebrate densities across seasons, terrestrial invertebrate biomass was greater than aquatic emergent invertebrate biomass across seasons. Carbon stable isotope analysis demonstrated that bats consumed greater proportions of aquatic emergent invertebrates than terrestrial invertebrates in late spring and to a lesser extent in fall, while terrestrial prey dominated their diet in summer. Fatty acid analysis demonstrated that bats received resources from both terrestrial and aquatic ecosystems but did not exhibit variability across seasons. An aquatic specific fatty acid signal, as defined in previous studies, was not detectable in bats from Castle Lake in any of the three seasons, perhaps because of biosynthesis of fatty acids. The difference between the two biomarker results suggests that assimilation of organic carbon occurred through a variety of pathways in bat consumers and at different scales.

Acknowledgements

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Figure Legends

FIGURE 1. Map of Castle Lake, CA. Aquatic emergent invertebrate sampling locations are marked with an “x” and bat capture sites are marked with an “O”.

FIGURE 2. Densities (A) and biomass (B) of aquatic and terrestrial invertebrates emerging from and surrounding Castle Lake in late spring, summer, and fall in 2009.

FIGURE 3. Mean carbon isotope values and standard errors for secondary consumers (bats), terrestrial primary consumers, and aquatic emergent invertebrates during late spring, summer, and fall. *Aquatic emergent invertebrates were significantly different from terrestrial invertebrates and bats in summer ($p = 0.001$).

FIGURE 4. Plot of total aquatic versus total terrestrial fatty acids (as percentage of total fatty acids) in the tissues of aquatic emergent invertebrates, terrestrial primary consumers, and a variety of bat taxa. Bat taxa include sample size (n) in the legend.

3.6 Figures

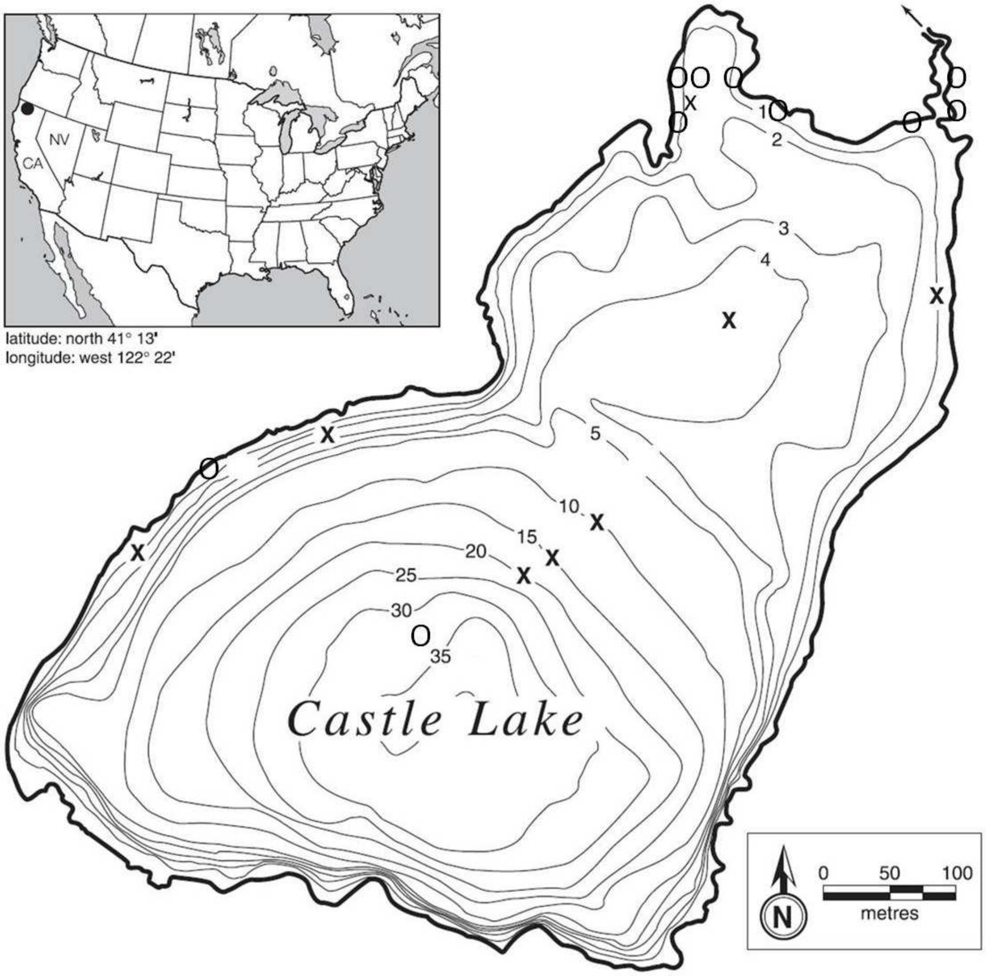

FIGURE 1.

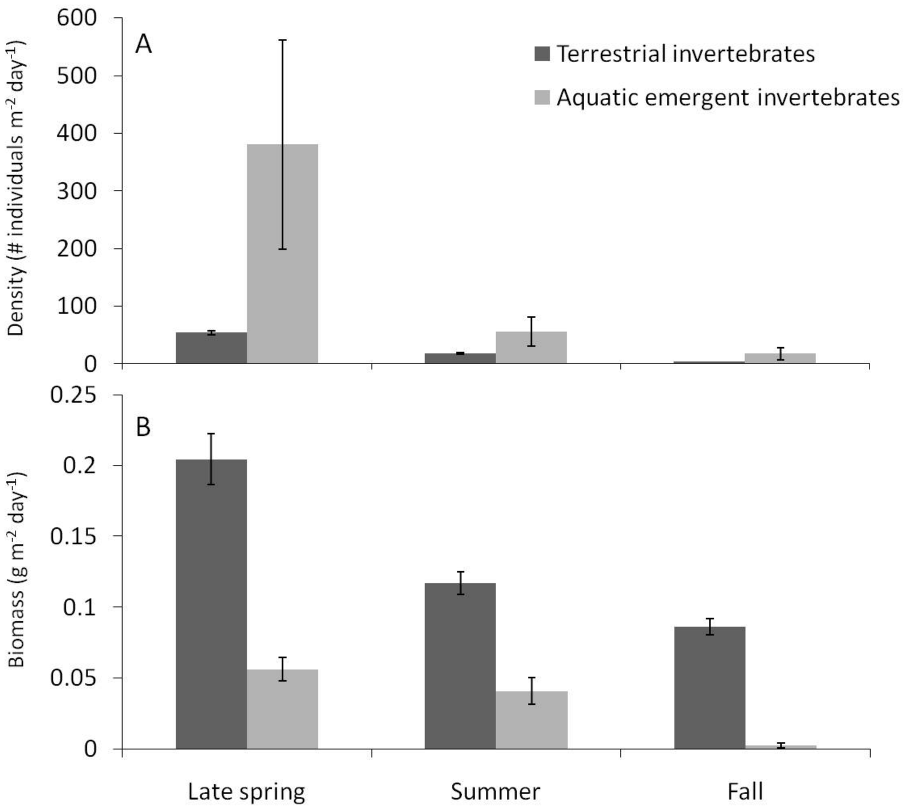
A
B
Terrestrial invertebrates
Aquatic emergent invertebrates
Density (# individuals m-2 day-1)
0
100
200
300
400
500
600
Biomass (g m-2 day-1)
0
0.05
0.1
0.15
0.2
0.25
Late spring
Summer
Fall

FIGURE 2.

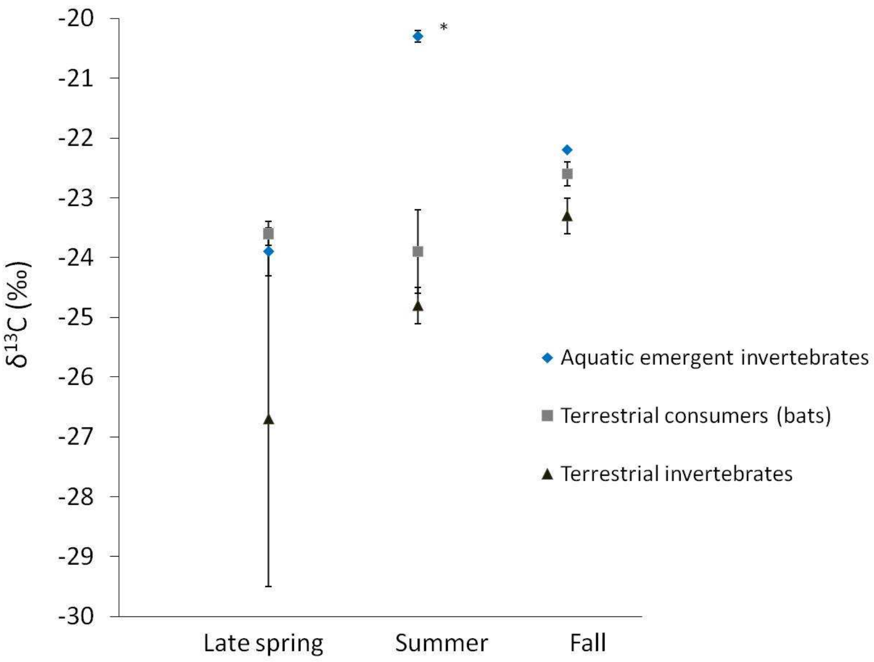
-20
-21
-22
-23
-24
-25
-26
-27
-28
-29
-30
δ13C (‰)
*
Late spring
Summer
Fall
◆ Aquatic emergent invertebrates
■ Terrestrial consumers (bats)
▲ Terrestrial invertebrates

FIGURE 3.

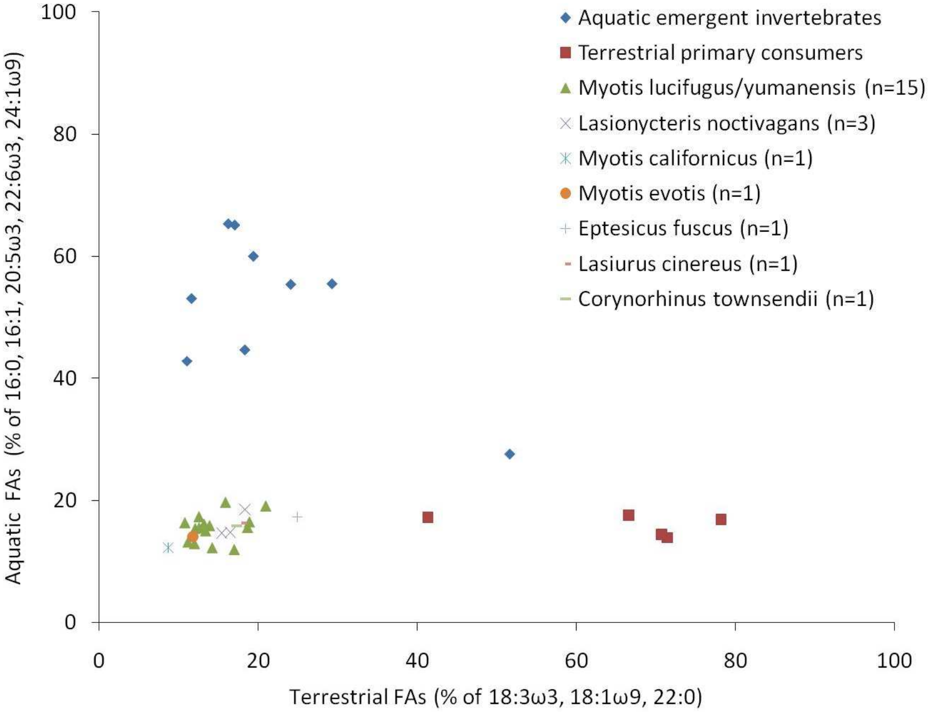
100
80
60
40
20
0
Aquatic FAs (% of 16:0, 16:1, 20:5ω3, 22:6ω3, 24:1ω9)
0
20
40
60
80
100
Terrestrial FAs (% of 18:3ω3, 18:1ω9, 22:0)
Aquatic emergent invertebrates
Terrestrial primary consumers
Myotis lucifugus/yumanensis (n=15)
Lasionycteris noctivagans (n=3)
Myotis californicus (n=1)
Myotis evotis (n=1)
Eptesicus fuscus (n=1)
Lasiurus cinereus (n=1)
Corynorhinus townsendii (n=1)

FIGURE 4.

3.7 Tables

TABLE 1. Feeding strategies of various insectivorous bat taxa. Data from: (1) Buchler 1976, (2) Fenton and Morris 1976, (3) Anthony and Kunz 1977, (4) Belwood and Fullard 1984, (5) Brack and La Val 1985, (6) Barclay 1985, (7) Jones 1990, (8) Brigham et al. 1992, (9) Barclay and Brigham 1994, (10) Johnston and Fenton 2001, (11) Agosta et al. 2003.

Bat taxa	Opportunist	Selective	Diet (Aquatic and/or Terrestrial)	Reference
Myotis (*yumanensis, lucifugus, sodalis*)	x	x	A: 20-90 %, T: 20-90 %	1,2,3,5,8,9
Pallid (*Antrozous pallidus)*	x		T	10
Greater horseshoe (*Rhinolophus ferrumequinum*)		x	T	7
Hawaiian hoary (*Lasiurus cinereus semotus*)		x	T	4
Silver-haired (*Lasionycteris noctivagans*)	x		A: 3-70 %, T: 23-90 %	6
Hoary (*Lasiurus cinereus*)	x		A: 1-46 %, T: 50-80 %	6
Big brown (*Eptesicus fuscus*)		x	A: 0.3 %, T: 99.7 %	11

Table 2. Total fatty acids (% of total) and standard deviation of the dominant aquatic emergent invertebrates, terrestrial invertebrates, and terrestrial consumers (bats) in Castle Lake in 2009.

Fatty acid	Chironomidae (n=3)	Trichoptera (n=2)	Ephemeroptera (n=4)	Diptera (Terrestrial) (n=1)	Lepidoptera (Terrestrial) (n=4)	*Eptesicus fuscus* (n=1)	*Lasiurus cinereus* (n=1)	*Myotis* spp. (n=17)	*Lasionycteris noctivagans* (n=3)	*Corynorhinus townsendii* (n=1)
11:0	0	0	0	0	0	0	0.29	0.02 ± 0.08	0.08 ± 0.14	0
12:0	0	0	0.24 ± 0.25	0.17	0.007 ± 0.01	0	0.53	0.17 ± 0.23	0.26 ± 0.04	0
13:0	0	0	0.007 ± 0.01	0	0	0	1.6	0.11 ± 0.23	0.32 ± 0.28	0
14:0	0.79 ± 0.11	0.28 ± 0.15	3.15 ± 1.03	2.4	0.08 ± 0.10	1.3	3.2	1.63 ± 0.67	1.86 ± 0.47	1.4
14:1	0	0.07 ± 0.10	0	0.93	0.03 ± 0.06	0	0	0	0	0
15:0	0.77 ± 0.38	0.09 ± 0.007	0.71 ± 0.26	0.28	0.11 ± 0.21	1	3.6	1.0 ± 0.48	1.53 ± 0.65	1.2
16:0	16.16 ± 2.59	14.25 ± 2.61	25.17 ± 2.54	11	13.1 ± 2.42	14.3	16	12.89 ± 2.53	13.83 ± 1.66	14.5
16:1	10.66 ± 4.07	19.5 ± 23.75	26.72 ± 5.18	6.2	1.95 ± 2.30	1.7	0	1.10 ± 0.33	1.30 ± 0.54	0.31
17:0	1.6 ± 0.26	0.715 ± 0.04	0.68 ± 0.41	0.37	0.32 ± 0.27	1.3	0.43	0.86 ± 0.41	1.12 ± 0.51	1.2
18:0	7.56 ± 0.49	3.55 ± 2.19	3.87 ± 0.35	4.4	3.72 ± 1.38	13.8	13	14.44 ± 2.66	12.23 ± 1.04	11.8
18:1ω9t	0	1.45 ± 2.05	0	0	0	1.9	2.3	1.82 ± 1.37	0.53 ± 0.92	0
18:1ω9c	8.7 ± 1.80	21.1 ± 4.52	12.55 ± 2.12	14.9	35.72 ± 10.61	20.5	13.3	10.68 ± 3.19	12.93 ± 1.66	11.4
18:2ω6t	0	0.18 ± 0.18	0	0	0	0	0	0	0	0
18:2ω6c	18.96 ± 4.06	7.9 ± 4.52	5.95 ± 1.34	32	6.75 ± 3.46	8.2	4.5	5.63 ± 1.87	7.53 ± 1.83	5.5
20:0	0.75 ± 0.17	0.25 ± 0.35	0.265 ± 0.09	0.59	0.60 ± 0.65	1.5	2	2.12 ± 0.53	1.33 ± 0.11	3.8
18:3ω6	0.59 ± 0.60	0.215 ± 0.30	0.57 ± 0.04	0	0.07 ± 0.08	0	0	0.13 ± 0.23	1.4 ± 1.31	0
20:1ω9	0.24 ± 0.31	0.045 ± 0.06	0	0	0.06 ± 0.07	0	0	0.68 ± 0.38	0.58 ± 0.52	0
18:3ω3	4.44 ± 4.69	18.5 ± 10.60	6.125 ± 1.97	26.1	35.12 ± 6.55	0	1.3	0.20 ± 0.31	0.14 ± 0.12	0.51
21:0	0.07 ± 0.08	0.055 ± 0.07	0.025 ± 0.03	0	0.17 ± 0.34	0.25	0.21	0.49 ± 0.40	0.43 ± 0.13	1.3
20:2	0.80 ± 0.44	0.29 ± 0.12	1.57 ± 0.46	0	0.06 ± 0.07	0	0	0.5 ± 1.11	0	0
22:0	0.51 ± 0.02	0.83 ± 0.66	0.50 ± 0.15	0.34	0.87 ± 0.65	4.4	3.3	3.11 ± 0.72	3.66 ± 1.09	5.4
20:3ω6	0.08 ± 0.10	0.05 ± 0.07	0.05 ± 0.03	0	0	0.42	0	0.13 ± 0.25	0.71 ± 0.63	0
22:1ω9	0	0	0	0	0.01 ± 0.03	0.21	0	0.29 ± 0.23	0.06 ± 0.10	0

20:3ω3	0	0.03 ± 0.04	0.02 ± 0.02	0	0.19 ± 0.04	0	0	0.05 ± 0.22	0	0
20:4ω6	0	0	0	0	0.007 ± 0.01	1.6	1.2	1.50 ± 0.46	2.06 ± 0.40	2.5
23:0	6.63 ± 2.23	1.78 ± 1.15	1.72 ± 0.99	0	0	2.8	2.3	2.72 ± 0.90	2.13 ± 0.57	4.3
22:2	0.02 ± 0.04	0	0.12 ± 0.01	0	0	0	0	0	0	0
24:0	0.12 ± 0.11	0.04 ± 0.05	0.04 ± 0.08	0	0.12 ± 0.15	23.1	28.7	19.35 ± 4.98	25.43 ± 4.85	33
20:5ω3	19.93 ± 11.23	7.75 ± 6.57	9.55 ± 3.32	0	0.63 ± 0.50	0	0	0.11 ± 0.26	0	0
24:1ω9	0	0.035 ± 0.04	0	0	0	1.3	0.33	1.01 ± 0.57	0.83 ± 0.14	1
22:4ω6	0	0	0	0	0	3.1	2.4	3.72 ± 2.97	4.36 ± 0.51	4
Unidentified 1	0	0.08 ± 0.11	0.0175 ± 0.035	0	0.0475 ± 0.095	16.6	20.7	13.77 ± 4.46	14.23 ± 1.18	22.1
Unidentified 2	0	0	0.0625 ± 0.125	0	0	1.2	16.8	4.59 ± 5.09	7.68 ± 9.35	2.1
22:6ω3	0.09 ± 0.16	0	0	0	0	0	0	0.12 ± 0.53	0	0
SAFA	34.99 ± 2.39	21.84 ± 0.81	36.42 ± 3.15	19.55	19.12 ± 1.24	63.75	75.16	58.96 ± 7.96	64.25 ± 7.88	77.9
MUFA	19.60 ± 2.52	42.20 ± 21.40	39.27 ± 3.80	22.03	37.79 ± 8.30	25.61	15.93	15.61 ± 4.22	16.25 ± 1	12.71
18ω3 PUFA	4.44 ± 4.69	18.5 ± 10.60	6.12 ± 1.97	26.1	35.12 ± 6.55	0	1.3	0.20 ± 0.31	0.14 ± 0.12	0.51
EPA & DHA	20.03 ± 11.39	7.75 ± 6.57	9.55 ± 3.32	0	0.63 ± 0.50	0	0	0.24 ± 0.69	0	0
18ω6 PUFA	19.55 ± 4.18	8.29 ± 4.03	6.52 ± 1.31	32	6.82 ± 3.44	8.2	4.5	5.77 ± 1.98	8.93 ± 1.06	5.5
ω3/ω6	1.27 ± 0.66	2.90 ± 0.57	1.86 ± 0.24	0.816	6.92 ± 4.94	0	0.2281	0.07 ± 0.12	0.01 ± 0.01	0.06375
DHA/LNA	0.006 ± 0.01	0	0	0	0	0	0	0.03 ± 0.13	0	0
ARA/ DHA+EPA	0	0	0		0.007 ± 0.01	0	0	0	0	0
C24/C14	0.15 ± 0.14	0.44	0.01 ± 0.02	0	0	18.76923	9.0719	0	14.60 ± 4.52	24.2857

Table 3. Absolute concentrations of fatty acids (μg FA/mg) and standard deviation of the dominant aquatic emergent invertebrates, terrestrial invertebrates, and terrestrial consumers (bats) in Castle Lake in 2009.

Fatty acid	Chironomidae (n=3)	Trichoptera (n=2)	Ephemeroptera (n=4)	Diptera (Terrestrial) (n=1)	Lepidoptera (Terrestrial) (n=4)	*Eptesicus fuscus* (n=1)	*Lasiurus cinereus* (n=1)	*Myotis* spp. (n=17)	*Lasionycteris noctivagans* (n=3)	*Corynorhinus townsendii* (n=1)
11:0	0	0	0	0	0	0	0.13	0.01 ± 0.05	0.04 ± 0.08	0
12:0	0	0	0.44 ± 0.33	0.09	0.002 ± 0.004	0	0.24	0.09 ± 0.12	0.13 ± 0.04	0
13:0	0	0	0.01 ± 0.03	0	0	0	0.44	0.06 ± 0.14	0.17 ± 0.16	0
14:0	0.54 ± 0.17	0.08 ± 0.05	5.98 ± 2.83	1.33	0.12 ± 0.17	0.3	0.88	0.80 ± 0.39	0.75 ± 0.19	0.44
14:1	0	0.02 ± 0.03	0	0.5	0.01 ± 0.01	0	0	0	0	0
15:0	0.54 ± 0.31	0.02 ± 0.004	1.34 ± 0.52	0.15	0.04 ± 0.05	0.23	1.01	0.49 ± 0.26	0.61 ± 0.21	0.38
16:0	11.17 ± 3.76	4.01 ± 1.07	45.71 ± 14.25	6.05	18.51 ± 28.87	3.23	4.42	5.98 ± 1.28	5.58 ± 0.66	4.42
16:1	7.55 ± 3.75	5.73 ± 7.09	49.31 ± 17.93	3.41	0.70 ± 0.62	0.38	0	0.53 ± 0.19	0.57 ± 0.19	0.13
17:0	1.08 ± 0.10	0.20 ± 0.03	1.18 ± 0.54	0.2	0.22 ± 0.32	0.28	0.2	0.41 ± 0.14	0.50 ± 0.24	0.37
18:0	5.20 ± 1.25	0.97 ± 0.53	6.86 ± 1.32	2.41	2.25 ± 2.74	3.11	3.61	6.64 ± 1.06	4.97 ± 0.73	3.6
18:1ω9t	0	0.43 ± 0.62	0	0	0	0.43	0.63	0.90 ± 0.80	0.20 ± 0.34	0
18:1ω9c	5.80 ± 0.70	5.84 ± 0.75	21.65 ± 2.61	8.14	49.59 ± 78.11	4.61	3.68	5.01 ± 1.77	5.26 ± 0.93	3.48
18:2ω6t	0	0.05 ± 0.05	0	0	0	0	0	0	0	0
18:2ω6c	12.99 ± 4.08	2.17 ± 1.06	10.19 ± 0.97	17.52	3.31 ± 3.38	1.85	1.26	2.64 ± 0.46	3.02 ± 0.56	1.67
20:0	0.52 ± 0.16	0.07 ± 0.10	0.45 ± 0.09	0.32	0.21 ± 0.25	0.34	0.57	0.97 ± 0.22	0.55 ± 0.12	1.15
18:3ω6	0.46 ± 0.49	0.06 ± 0.09	1.03 ± 0.27	0	0.17 ± 0.27	0	0	0.06 ± 0.10	0.58 ± 0.51	0
20:1ω9	0.18 ± 0.24	0.01 ± 0.01	0	0	0.15 ± 0.26	0	0	0.32 ± 0.16	0.24 ± 0.21	0
18:3ω3	3.39 ± 3.81	5.04 ± 2.52	10.45 ± 2.14	14.26	39.54 ± 58.27	0	0.36	0.10 ± 0.15	0.07 ± 0.07	0.21
21:0	0.05 ± 0.06	0.01 ± 0.02	0.04 ± 0.05	0	0.01 ± 0.01	0.07	0.1	0.23 ± 0.15	0.21 ± 0.05	0.41
20:2	0.58 ± 0.42	0.08 ± 0.02	2.84 ± 0.87	0	0.05 ± 0.08	0	0	0.21 ± 0.49	0	0
22:0	0.35 ± 0.07	0.23 ± 0.17	0.85 ± 0.09	0.19	0.23 ± 0.14	0.99	0.92	1.43 ± 0.29	1.46 ± 0.29	1.65
20:3ω6	0.04 ± 0.05	0.01 ± 0.01	0.10 ± 0.06	0	0	0.12	0	0.07 ± 0.13	0.29 ± 0.25	0

22:1ω9	0	0	0	0	0.004 ± 0.007	0.06	0	0.14 ± 0.11	0.02 ± 0.05	0
20:3ω3	0	0.01 ± 0.01	0.05 ± 0.05	0	0.25 ± 0.40	0	0	0.03 ± 0.13	0	0
20:4ω6	0	0	0	0	0.002 ± 0.004	0.35	0.34	0.68 ± 0.17	0.83 ± 0.08	0.76
23:0	4.40 ± 1.33	0.49 ± 0.29	2.99 ± 1.31	0	0	0.63	0.64	1.25 ± 0.36	0.87 ± 0.21	1.3
22:2	0.02 ± 0.03	0	0.22 ± 0.03	0	0	0	0	0	0	0
24:0	0.09 ± 0.08	0.01 ± 0.01	0.04 ± 0.08	0	0.02 ± 0.03	5.2	7.93	8.86 ± 2.16	10.14 ± 0.51	10.04
20:5ω3	12.58 ± 4.42	2.09 ± 1.63	16.14 ± 2.70	0	0.14 ± 0.08	0	0	0.05 ± 0.12	0	0
24:1ω9	0	0.01 ± 0.01	0	0	0	0.29	0.15	0.50 ± 0.30	0.37 ± 0.02	0.3
22:4ω6	0	0	0	0	0	0.89	1.11	1.85 ± 1.43	2.22 ± 0.07	1.69
Unidentified 1	0	0.02 ± 0.03	0.02 ± 0.03	0	0.01 ± 0.02	4.74	9.49	6.90 ± 2.30	7.31 ± 0.69	9.33
Unidentified 2	0	0	0.14 ± 0.25	0	0	0.34	7.72	2.48 ± 3.11	4.33 ± 5.59	0.87
22:6ω3	0.05 ± 0.09	0	0	0	0	0	0	0.05 ± 0.24	0	0
SAFA	23.98 ± 5.66	6.13 ± 0.26	65.95 ± 18.90	10.77	21.63 ± 32.46	14.42	21.14	27.29 ± 3.34	26.06 ± 0.80	23.82
MUFA	13.55 ± 4.08	12.05 ± 6.96	70.96 ± 20.26	11.55	50.45 ± 78.71	5.79	4.48	7.43 ± 2.62	6.69 ± 0.70	3.92
18ω3 PUFA	3.39 ± 3.81	5.04 ± 2.52	10.45 ± 2.14	14.26	39.54 ± 58.27	0	0.36	0.10 ± 0.15	0.07 ±0.07	0.21
EPA & DHA	12.64 ± 4.51	2.09 ± 1.63	16.14 ± 2.70	0	0.14 ± 0.08	0	0	0.11 ± 0.31	0	0
18ω6 PUFA	13.45 ± 4.37	2.28 ± 0.92	11.22 ± 1.19	17.52	3.48 ± 3.66	1.85	1.26	2.70 ± 0.50	3.60 ± 0.14	1.67
ω3/ω6	1.27 ± 0.66	2.88 ± 0.58	1.85 ± 0.21	0.81	6.94 ± 4.34	0	0.22	0.07 ± 0.11	0.01 ± 0.01	0.08
DHA/LNA	0.006 ± 0.01	0	0	0	0	0	0	0.03 ± 0.13	0	0

TABLE 4. Fatty acids found in aquatic and/or terrestrial taxa. High or low designations represent low (0-1 %), medium (2-9 %), or high (>10 %) percentages of total fatty acids (%) across taxa. Data from: (1) Uscian and Stanley-Samuelson 1994, (2) Howard and Stanley-Samuelson 1996, (3) Kuusipalo and Kakela 2000, (4) Budge et al. 2002, (5) Brett et al. 2006, (6) Caramujo et al. 2008, (7) Goedkoop et al. 2000, (8) Bashan et al. 2002, (9) Nor Aliza et al. 1999, (10) Chamberlain and Black 2005 and (11) Falkenstein et al. 2001.

FA	Aquatic	Terrestrial	Aquatic-High or Low	Terrestrial-High or Low	Reference
12:0	x	x	L	L	1,9,11, this study
14:0	x	x	M	L	1,2,3,4,5,7,8,9,10,11
14:1	x	x	L	L	1, this study
15:0	x	x	L	L	1,2,3,5,8,9,10
15:1		x		L	10
16:0	x	x	H	M-H	1,2,3,4,5,6,7,8,9,10,11
16:1	x	x	L-M	L-H	1,2,3,4,5,6,7,8,9,10,11
16:2ω4	x		L		3,6
16:3	x		L-M		6
17:0	x	x	L	M	1,2,3,5,7,8,9,10,11
17:1	x	x	L	L	1,2,3,9,11
18:0	x	x	M	H	1,2,3,4,5,6,7,8,9,10,11
18:1	x	x	M	H	1,2,3,4,5,6,7,8,9,10,11
18:2ω6	x	x	M	H	1,2,3,4,5,6,7,8,9,10,11
19:0		x		L	2,10
18:3ω6	x	x	L	M	1,2,5,8,9,11
18:3ω3	x	x	L	M	1,2,3,5,6,7,8,9,10
18:4ω3	x		L		3,4,5,7
21:0	x	x	L	M	1, this study
20:0	x	x	L	L	2,3,4,5,7,9,10,11
20:1	x	x	L	L	1,2,3,4,5,7,8,9,10
20:2	x	x	L	L	1,2,3,5,8,9
20:3ω6	x	x	L	L	2,3,8,10
20:4ω6	x	x	M	M	1,2,3,4,5,6,7,8,9,10,11
20:3ω3	x	x	L	L	2,3
20:5ω3	x	x	M-H	M	1,2,3,4,5,6,7,8,9,10
22:0	x	x	L	L	2,9, this study
22:1	x	x	L-M	L	2,4,11
22:4ω6	x		L		3
22:5ω6	x		M		3
22:5ω3	x		M		3,4
22:6ω3	x		M		3,4,5,6
23:0	x	x	L-M	L	2, this study

24:0	x	x	L	L	2, this study
24:1ω9	x		L		3,4
SAFA	x	x	L	H	1,2,3,4,5,6,7,8,9,10,11
MUFA	x	x	H	H	1,2,3,4,5,6,7,8,9,10,11
EPA & DHA	x		H	L	3,4,5,6
18ω3 PUFAs	x	x	H	L	1,2,3,4,5,6,7,8,9,10,11
18ω6 PUFAs	x	x	L	L	1,2,3,4,5,6,7,8,9,10,11

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Conclusions from the project

This study suggests that Castle Lake had a diverse benthic invertebrate community which varied seasonally and by depth. The lake generated a moderate amount of benthic secondary production for a mesotrophic lake, in comparison to eutrophic and oligotrophic ecosystems, and at a notably higher rate than previously estimated. While there was greater emergent invertebrate densities and biomass in late spring and early summer than in late summer and fall, there was a reduction in dominant benthic invertebrates emerging from the lake due to predation. Therefore, this study suggests that while there was abundant benthic secondary production in Castle Lake, the emergence of invertebrates contributing substantially to terrestrial ecosystems may have been reduced, potentially due to non-native fish consumption of invertebrate resources.

Trophic exchanges between aquatic and terrestrial ecosystems were demonstrated through the use of both fatty acids and carbon stable isotope analyses. While aquatic emergent invertebrate densities were greater than terrestrial invertebrate densities across seasons, terrestrial invertebrate biomass was greater than aquatic emergent invertebrate biomass across seasons. Carbon stable isotope analysis demonstrated that bats consumed greater proportions of aquatic emergent invertebrates than terrestrial invertebrates in late spring and to a lesser extent in fall, while terrestrial prey dominated their diet in summer. Fatty acid analysis demonstrated that bats received resources from both terrestrial and aquatic ecosystems but did not exhibit variability across seasons. An aquatic specific fatty acid signal, as defined in previous studies, was not detectable in bats from Castle Lake in any of the three seasons, perhaps because of intra conversion of fatty acids. The difference between the two biomarker results suggests that assimilation of organic carbon occurred through a variety of pathways in bat consumers and at different scales. Fatty acid analysis is a novel technique and is still in its infancy. We suggest that further lipid research is necessary to determine whether biosynthesis of fatty acids occur within the tissues of hibernating mammals. Feeding experiments in a laboratory setting may shed light on aquatic versus terrestrially derived fatty acid resources.

This study demonstrated the importance of energy fluxes from lake ecosystems, particularly benthic secondary production and emergence to surrounding terrestrial ecosystems and consumers. Management of one ecosystem (i.e. fish stocking) may impact surrounding ecosystems (i.e. terrestrial consumers). We suggest that aquatic and terrestrial ecosystems be managed in conjunction with one another due to these across-ecosystem resource exchanges, rather than managed as separate ecosystems.