# PROJECT SUMMARY

## Overview

Freshwater streams are intimately connected with the terrestrial landscape and depend upon terrestrial subsidies ranging from nutrients in runoff that fuel aquatic primary production to terrestrial leaves and insects that fuel secondary production. Reciprocally, streams provide energy and nutrients in the form of emergent aquatic insects to predators in the riparian zone. Emergent aquatic insects likely constitute a high quality food source for riparian predators because they contain ***highly unsaturated fatty acids*** (HUFA), which limit secondary production rates and affect a host of physiological processes. To date, most major studies on reciprocal aquatic-terrestrial subsidies have been conducted in relatively undisturbed forested landscapes. However, aquatic subsidies to riparian predators may actually be less important in pristine, but low productivity forested streams than in highly productive streams in agricultural landscapes that provide large quantities of high quality emergent insects to predators in riparian food webs.

The proposed research will examine: 1) the effects of human land use on aquatic and terrestrial-derived food quantity and quality for a representative avian riparian predator, and 2) the effects of food quantity and quality on secondary production rates on this riparian predator.

## Intellectual Merit

Riparian zones constitute a middle ground between terrestrial and aquatic systems and are influenced by human impacts to both. Previous research has demonstrated that nutrients and energy move between streams and the riparian zone as ***reciprocal subsidies***. Most studies in this area have focused solely on the quantity of aquatic versus terrestrial-derived resources moving between streams and the riparian zone, while ignoring differences in the quality of those resources. However, aquatic-derived food resources for riparian predators are likely higher quality than terrestrial food resources, especially in terms of their HUFA content. As a consequence, streams may subsidize riparian food webs in terms of both food quality and quantity. This research will be the first to document aquatic-derived HUFA fluxes from streams to a representative riparian predator using compound-specific stable isotopes. In addition, human land uses, such as agriculture, likely impact the quantity and quality of reciprocal subsidies. This study will examine the effect of agricultural land use on both the quantity and quality of both aquatic and terrestrial-derived resources. This research will be the first to document: 1) the effects of human land use on aquatic and terrestrial-derived food quantity and quality, and 2) the effects of food quantity and quality on an avian riparian predator through a combination of observational and experimental approaches.

## Broader Impacts

The co-PI has demonstrated a commitment to collaboration with environmental managers, science outreach, and undergraduate mentoring. The co-PI will collaborate with the X Land Trust, the Y Natural Areas Program, and Z Agricultural Experiment Station Program as well as local private landowners while finalizing study-site selection and conducting research. A summary of results will be shared with all landowners and data will be shared upon request. At publicly accessible study sites, the co-PI will continue to install informational signs with contact information explaining the research. In addition, the co-PI will continue to work with the Sciencenter to develop kid-friendly “Showtime!” presentations based on the research themes. Finally, the co-PI will continue to mentor undergraduates both through the proposed research as well as outside the research setting.

## PROJECT DESCRIPTION

For years ecologists were puzzled by the paradox of high secondary production in ecosystems with low primary production or nutrient availability (e.g. Allen 1951). Research on nutrient subsidies has revealed that more productive or nutrient-rich donor ecosystems can subsidize less productive or nutrient-poor recipient systems through the movement of energy and nutrients, often in the form of migratory animals (Polis et al. 1997). For example, anadromous fishes move marine-derived nutrients hundreds of miles increasing both primary and secondary production in freshwater streams and lakes (Flecker et al. 2010). Nutrient subsidies can also be more local, such as the seasonal fluxes terrestrial insects and leaf litter that subsidize many forested stream food webs (Nakano and Murakami 2001).

Past work on nutrient subsidies has focused on the movement of elemental nutrients and energy through food webs (e.g. Polis et al. 1997; Flecker et al. 2010). However, unlike autotrophs, animals and other secondary producers have more limited biosynthesis pathways and require organic substances in their diets to support energetic and physiological demands (Iverson 2009). As a consequence, animal consumers may rely not only upon subsidies of inorganic elemental nutrients and energy, but also upon subsidies of complex organic compounds, such as vitamins and fatty acids (Anderson and Pond 2000; Frost et al. 2005).

**Highly unsaturated fatty acids** (HUFAs: long-chain ω-3 fatty acids) are especially important organic compounds that can increase rates of secondary production and appear likely to subsidize animal consumers (Persson et al. 2008). Most animals cannot synthesize HUFAs *de novo* and so must obtain them or their molecular precursors directly from diet in order to survive and avoid growth limitation (Brenna et al. 2009). As a consequence, animal demand for HUFAs is likely to guide nutrient movement within (Danielsdottir et al. 2007; Gladyshev et al. 2012) and across ecosystems where major disparities in HUFA availability occur (Gladyshev et al. 2013).

Freshwater and terrestrial primary producers in natural ecosystems differ greatly in their HUFA content (Napolitano et al. 1994; Napolitano et al. 1996; Volk and Kiffney 2012), creating the potential for freshwaters to subsidize animals in terrestrial food webs. Few terrestrial plants contain any detectable HUFAs (Simopoulos et al. 2004) while a number of major freshwater primary producers contain high levels of HUFAs (Ahlgren et al. 1992; Brett and Müller-Navarra 1997; Gushina and Harwood 2009). Laboratory studies suggest that animals consuming HUFA-poor foods grow less efficiently (McDonald 1984; Sargent et al. 2002) and serve as lower quality food for the next trophic level (Torres-Ruiz et al. 2010). In contrast, animals consuming high quality freshwater primary producers grow more efficiently and serve as higher quality food for their predators (Hanson et al. 1985; Hessen et al. 2006; Koussoroplis et al. 2013).

**My primary dissertation research focuses on how watershed land use affects terrestrial to aquatic subsidies and food quantity and quality for stream consumers.** Stream food webs range from being almost entirely fueled by subsidies of terrestrial resources in forested landscapes with high canopy cover and low nutrients to relying primarily on in-stream primary producers in agricultural landscapes with open canopies and high nutrients (Vannote et al. 1980; Twining unpublished). However, most previous studies on subsidies in streams have focused on forested streams where consumers rely on inputs of HUFA-poor terrestrial detritus and heterotrophic stream microbes instead of open high nutrient agricultural streams with an abundance of HUFA-rich freshwater primary producers likely to provide freshwater consumers with higher quality food (Boechat et al. 2011; Larson et al. 2013).

Here, I propose to expand upon my dissertation to examine how land use affects food quality for riparian (i.e. streamside ecotone) predators. Although riparian predators, such as spiders and insectivorous birds and bats, live in the terrestrial realm, they are often highly reliant on aquatic resource fluxes (Muehlbauer et al. 2014). Consequently, the effects of land use on stream food quantity and quality have the potential to reverberate through the food web to affect aquatic subsidies to riparian predators.

I have conducted preliminary research examining the availability of terrestrial versus aquatic resources across a forested to agricultural landscape gradient and the extent to which riparian predators rely upon terrestrial versus freshwater resources based on multiple bulk stable isotopes ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$). I now propose to examine: **1) whether riparian predators in natural systems obtain their HUFAs from terrestrial molecular precursors or are subsidized by HUFAs produced *de novo* by aquatic primary producers and how this varies with land use**, and **2) if increased dietary HUFA levels from aquatic to terrestrial subsidies increase secondary production rates (growth rates) in riparian predators under both natural and experimental conditions**.

## Background

Freshwater streams are intimately connected with the terrestrial landscape and depend upon terrestrial subsidies ranging from elemental nutrients in runoff to fuel aquatic primary production to terrestrial leaves to fuel secondary production by heterotrophic stream microbes and invertebrates (Vannote et al. 1980; Nakano et al. 1999; Kawaguchi et al. 2003). Reciprocally, streams provide an important subsidy of energy and nutrients in the form of emergent aquatic insects to the animals in the surrounding riparian zone including spiders, birds, and bats (Nakano and Murakami 2001; Baxter et al. 2005; Clare et al. 2011). For example, insectivorous birds, such as flycatchers, concentrate their feeding around streams (Iwata et al. 2005; Uesugi and Murakami 2007). In forested landscapes, emergent stream insects are especially important for riparian birds in the early spring before leaf-out when terrestrial insects are scarce and stream production is high (Uesugi and Murakami 2007).

To date, most major studies on reciprocal aquatic-terrestrial subsidies have been conducted in relatively undisturbed forested landscapes (but see Alberts et al. 2013 and Rowse et al. 2014 for work on contaminant fluxes in urban systems). However, aquatic subsidies to riparian predators may actually be less important in undisturbed forested landscapes where stream production rates decline after leaf-out than in highly modified agricultural landscapes (Twining unpublished; Figures 1-2). Highly productive streams in agricultural landscapes with high light and nutrient levels allow emergent insect biomass to remain high and continue to provide prey to riparian predators throughout the season (Twining unpublished; Figures 1-2). Land use effects on aquatic and terrestrial insect quantity appear likely to influence where riparian predators obtain their food (Twining unpublished).

In addition to affecting food quantity for riparian predators, land use may also affect food composition and quality. Animals, unlike plants, require organic compounds, such as vitamins, amino acids, and fatty acids, in addition to elemental nutrients, such as nitrogen and phosphorus, to grow, develop, and complete their life cycles. Highly unsaturated fatty acids (HUFAs), which include the omega-3 polyunsaturated fatty acids DHA and EPA, are especially important organic compounds for most animals and affect a range of important physiological processes from hormonal regulation to cardiac function (Lands et al. 1992; Arts and Kohler 2009). Animals must either consume HUFAs directly from diet or consume their molecular precursors short chain n-3 PUFAs, and then elongate them into HUFAs. The ability to elongate short-chain n-3 PUFAs into HUFAs varies greatly across taxa: strict carnivores, such as cats (MacDonald 1984), and animals from environments rich in HUFAs, including most marine fish (Sargent et al. 2002), have lost the ability to elongate short-chain n-3 PUFAs into HUFAs and must obtain HUFAs directly from diet while terrestrial herbivores appear to be relatively efficient (Blomquist et al. 1991; Jakobsson et al. 2006). The ability of riparian predators to elongate short-chain n-3s remains untested, but as carnivores they are likely candidates for high dietary HUFA needs.

Recent studies suggest aquatic foods from algae to predatory fishes contain more HUFAs than terrestrial foods (Gladyshev et al. 2009; Volk and Kiffney 2009). Several taxa of aquatic primary producers, such as diatoms and some flagellates (Ahlgren et al. 1992; Brett and Müller-Navarra 1997; Gushina and Harwood 2009), contain HUFAs and provide a pathway for stream consumers to get HUFAs directly from diet. In contrast, terrestrial plants generally lack HUFAs and contain only their molecular precursors (Simopoulos et al. 2004), short-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs), which consumers must use energy to elongate. Differences in fatty acid composition and HUFA abundance between terrestrial and aquatic-derived food items appear to persist up food chains to even the highest trophic levels (Koussoroplis et al. 2008) because the fatty acid composition of animals is highly dependent upon the fatty acid composition of their food sources (Torres-Ruiz et al. 2010; Bayes et al. 2014). For example, grazing mayflies and their fish predators contain more HUFAs than detritivorous crayfish (Twining unpublished). Emergent insects from streams appear to contain more HUFAs (Hanson et al. 1985; Torres-Ruiz et al. 2007; Torres-Ruiz et al. 2010) than terrestrial insects and are therefore a higher quality food. As a consequence, riparian predators in agricultural landscapes where HUFA-rich emergent aquatic insects are abundant throughout the growing season have access to higher quality food than those in forested landscapes where aquatic insects are relatively scarce throughout much of the summer.

In addition, land use may also affect the quality of emergent aquatic insects by altering HUFA abundance at the base of stream food webs. Some aquatic primary producers, including many cyanobacteria contain few detectable short-chain n-3 PUFAs, much less HUFAs and are a poor quality food source while others, such as many green algae, contain only short-chain n-3 PUFAs (Ahlgren et al. 1992). The relative abundance of different quality aquatic primary producers varies across landscapes driven by light, nutrients, and temperature (Hill et al. 2011; Cashman et al. 2013). Light, temperature, and nutrient availability can also influence the fatty acid composition of individual aquatic primary producer taxa (Hill et al. 2011; Piepho et al. 2012; Cashman et al. 2013). For example, high light and temperature decrease HUFAs (Piepho et al. 2012), while high nutrient levels may increase HUFAs (Hill et al. 2011; Piepho et al. 2012). However, even aquatic primary producers at the base of stream food webs in agricultural landscapes appear to provide a higher quality food source for invertebrates than detritus and heterotrophic microbes at the base of food webs in forested landscapes (Boechat et al. 2011; Larson et al. 2013). Invertebrate species composition also varies with land use and its effect on basal resources (Cummins and Klug 1979; Vannote et al. 1980). For example, algae-grazing mayflies are more abundant in open high nutrient agricultural streams with high aquatic primary producer biomass while detrivorous caddisflies are more abundant in forested streams where terrestrial material is the dominant energy source (Cummins and Klug 1979). Agricultural stream conditions not only support higher quality basal resources, but they also favor the herbivores that consume and move these resources into the riparian zone. Overall, highly productive streams in agricultural landscapes with high light, temperature, and nutrient levels likely serve as HUFA sources by exporting large quantities of high quality HUFA-rich emergent aquatic insect prey to the riparian zone.

## Research Objectives

**Objective One**: determine how land use affects HUFA levels and sources in a representative riparian predator in natural systems using bulk stable isotope, fatty acid composition, and compound-specific stable isotope techniques. **Hypothesis One**: Riparian predators in forested landscapes have lower HUFA levels and obtain most HUFAs from short-chain n-3 PUFAs in terrestrial plants because aquatic subsidies are small and low quality; riparian predators in agricultural landscapes have higher HUFA levels and obtain most HUFAs synthesized *de novo* by aquatic primary producers because aquatic subsidies are large and high quality.

**Objective Two**: determine if dietary HUFA levels increase secondary production rates in a representative riparian predator using natural and experimental approaches to quantity the effects of food quantity and quality on growth rates. **Hypothesis Two**: Riparian predators feeding primarily on high quality emergent aquatic insects/high HUFA foods will grow faster than those feeding primarily on lower quality terrestrial insects/low foods regardless of food quantity.

## Study System

I will conduct my research along a forested-to-agricultural landscape gradient in around Ithaca, New York. The Ithaca area is an ideal system in which to ask questions about the effects of land use because it contains both a highly heterogeneous terrestrial landscape of deciduous forests and working farms (Smith et al. 1993) and numerous mid-sized streams. I will survey a common representative avian predator, the Eastern Phoebe (*Sayornis phoebe*), in the riparian zone around three forested streams and three agricultural streams.

I will work with Eastern Phoebes as a representative riparian predator. Phoebes are widespread and abundant in both forested and agricultural landscapes and thus represent overall patterns likely to occur in other riparian avian predators with similar dietary niches, but more restrictive habitat preferences. Like many riparian passerines, phoebes are aerial insectivores that often forage for emerging insects directly over streams (Troy and Bacchus 2009; Twining personal obs.). They are also highly territorial and forage locally while nesting (Beheler et al. 2003) so that their diet is likely to reflect local food quantity and quality. In addition, phoebes prefer nesting on manmade structures such as low bridges and barns (Weeks et al. 1978) that are easy to access and monitor.

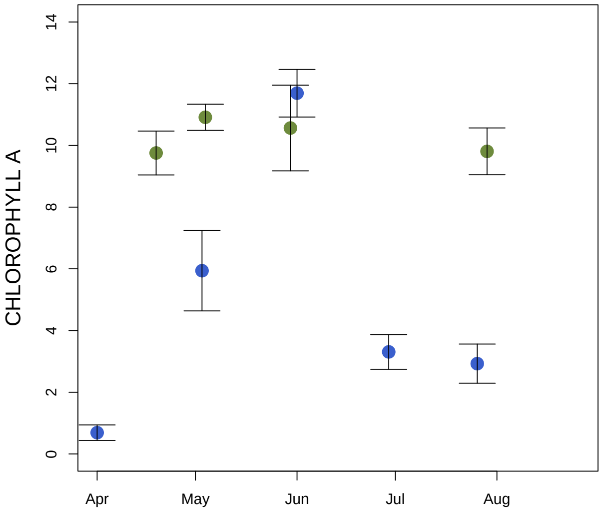

Figure 1: Chlorophyll *a* in µg/L in a forested stream (blue) and an agricultural stream (green).

## Preliminary Data

I have already completed preliminary research on stream and riparian food webs in several streams in my study system. I have been I surveying one forested stream and two agricultural streams since before leaf-out. My preliminary data suggest that streams in forested landscapes in the area have significantly lower temperatures, and light and nutrient levels (total nitrogen, total and soluble reactive phosphorus, and the ratio of nitrogen to phosphorus), than streams in agricultural areas. My data also suggest that agricultural streams have significantly higher aquatic primary producer biomass (chlorophyll a; Figure 1), primary production rates, and aquatic insect emergence rates (Figure 2) than forested streams throughout the season. Riparian predators likely have access to more high quality aquatic-derived prey items than those in forested landscapes. In addition, primary producer abundance (Figure 1), primary production rates, and emergent aquatic insect biomass in my forested site peaked in late spring prior to leaf out and then returned to early spring (post-ice out) levels. At my agricultural sites, primary production and primary producer and emergent insect biomass continued to increase until mid-summer. This suggests that not only do riparian predators have more aquatic-derived prey items in agricultural landscapes, but that these high quality resources continue to remain abundant throughout the late spring and early summer, when food demands for offspring are greatest.

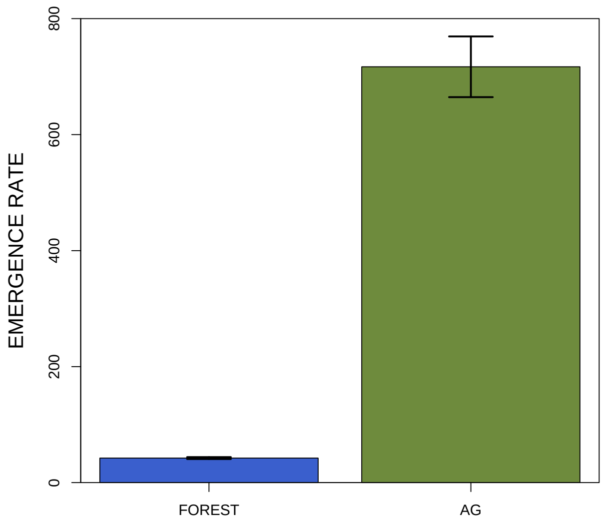

Figure 2: Mid-summer aquatic insect emergence rate from a forested and an agricultural stream. Units are emergent insects • $m^{-2}$ • $day^{-1}$.

I have also conducted preliminary stable isotope research in my study system. Aquatic and terrestrial resources have significantly different $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ signatures and separate isotopically from the level of basal resources (aquatic and terrestrial primary producers) to primary and secondary consumers. For example, terrestrial and emergent aquatic insect prey items at both forested and agricultural sites have distinctive isotopic signatures (Figures 3a and 3b), making them ideal for use in stable isotope mixing models for diet estimation (Parnell et al. 2009; Phillips et al. 2014).

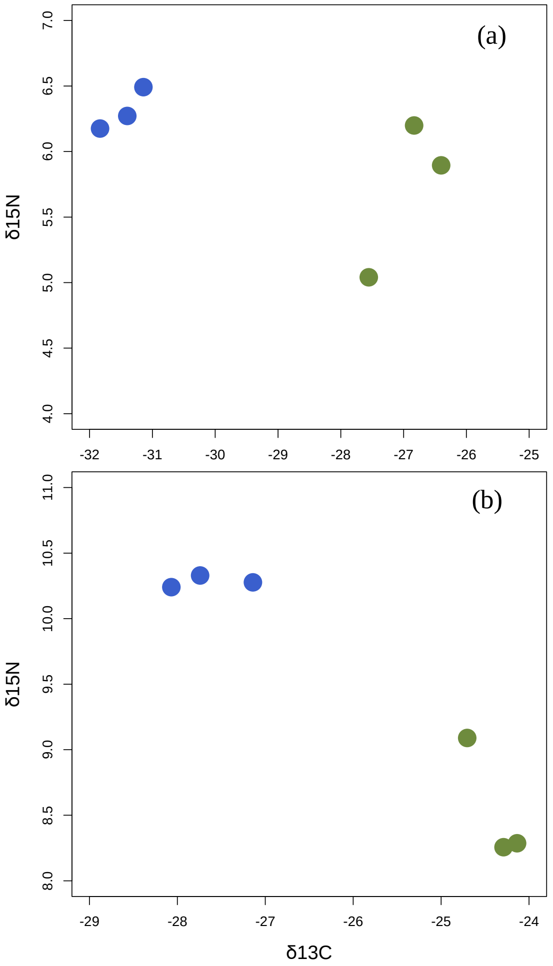

Figure 3: $\delta^{13}C$ and $\delta^{15}N$ signatures of aquatic (blue) and terrestrial (green) insect prey at a forested (a) and an agricultural (b) stream. Units are ‰.

## Proposed Research

***Objective One**: Determine how land use affects HUFA levels in Eastern phoebes and where Eastern phoebes obtain their HUFAs in natural systems.*

I will survey Eastern Phoebe chicks and their aquatic and terrestrial food resources in the riparian areas along three agricultural streams and three forested streams. First, I will determine the degree to which chicks' diets are dominated by aquatic or terrestrial resources using stable isotope analyses. Second, I will determine the HUFA levels of chicks and their potential prey items using fatty acid composition analyses. Finally, I will determine if chicks obtain HUFAs through aquatic pathways (HUFAs synthesized *de novo* by aquatic primary producers and consumed by emergent aquatic insects) or terrestrial pathways (terrestrial-derived short-chain n-3 PUFA precursors elongated by terrestrial insects into HUFAs) using compound-specific stable isotope analyses.

***Bulk Stable Isotopes – Energy Sources:*** Bulk stable isotope analyses will allow me to estimate the proportion of aquatic and terrestrial prey items in the diets of Eastern Phoebe chick. At each site, I will collect aquatic and terrestrial prey items once and blood samples twice (days 3 and 9 of developement) from Eastern Phoebe chicks for bulk stable isotope analyses ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$). All samples will be stored at -20ºC until being processed for analysis. I will equilibrate samples for $\delta^{2}H$ with the local $\delta^{2}H$ of my lab and the Stable Isotope Lab for over six weeks prior to processing and analysis. Bulk stable isotope analyses will be conducted at the Stable Isotope Lab on a Finnigan MAT Delta Plus IRMS for $\delta^{13}C$ and $\delta^{15}N$ analyses and a Thermo Delta V Advantage IRMS for $\delta^{2}H$ analyses. I will then create Bayesian mixing models in the R package SIAR to determine the degree to which Eastern Phoebe chicks rely on aquatic and terrestrial energy sources (Parnell et al. 2009: Phillips et al. 2014).

***Fatty Acid Composition – HUFA Levels:*** Fatty acid composition analyses will tell me the relative abundance of HUFAs and their molecular precursors in chicks and their food sources across systems. At each site, I will collect aquatic and terrestrial prey items and blood samples from Eastern Phoebe chicks for fatty acid composition analyses. All samples for compound-specific stable isotope analyses will be stored at -80ºC before and after being processed prior to analysis to prevent HUFA degradation throughout all steps of the process. Samples will be processed in the Department of Nutrition using the one-step method (Garces and Mancha 1993). I will determine sample composition using an HP5890 series II GC-FID and fatty acid percentages using a Varian Saturn 2000 ion trap GCMS and a Varian Star 3400 GCMS with the assistance of expert technicians in the Brenna Lab. I will determine fatty acid composition from chromatogram data using the program PeakSimple. In particular, I will focus on percentages of DHA (22:6n-3), the HUFA most likely to limit secondary production (Gladyshev et al. 2009), and its molecular precursor ALA (18:3n-3) in samples.

***Compound-Specific Stable Isotopes – HUFA Sources:*** Compound-specific stable isotope analyses will allow me to determine if chicks rely primarily on DHA synthesized *de novo* by aquatic primary producers or DHA elongated from ALA (Bec et al. 2011). I will use the same aquatic and terrestrial prey items and blood samples from Eastern Phoebe chicks collected and processed for fatty acid composition analyses for compound-specific stable isotope analyses ($\delta^{13}C_{DHA}$ and $\delta^{13}C_{ALA}$). All samples for compound-specific stable isotope analyses will be stored at -80ºC before and after being processed prior to analysis to prevent HUFA degradation throughout all steps of the process. I will run compound-specific stable isotope analyses on an Agilent 6890 GC coupled to a Thermo Finnigan MAT 253 IRMS equipped with a Thermo Finnigan Conflo III with the help of expert technicians. I will then create Bayesian mixing models in the R package SIAR to determine the degree to if chicks obtain their DHA primarily from **aquatic pathways** from DHA synthesized *de novo* by aquatic primary and consumed by emergent aquatic insects or **terrestrial pathways** from terrestrial-derived ALA elongated by terrestrial insects (Bec et al. 2011).

***Objective Two****: Determine if dietary HUFA levels increase secondary production rates in Eastern phoebe chicks.*

***Objective 2A: Natural observation of effects of food quantity and quality***

I will monitor Eastern Phoebe chick growth rate and body condition in a natural setting as a measure of riparian secondary production in three agricultural and three forested streams. While taking blood samples for isotopic analyses as part of objective one, I will measure chick head and tarsus length and weight on days 3, 6, 9, and 12 after hatch. I will then calculate growth rate as: (ln(weight on day n) – ln(weight on day n - 3)) / 3 and body condition as: 1) weight / tarsus length and 2) weight / head length.

I will estimate differences in food quantity for chicks among sites by installing small waterproof Go Pro ® video cameras above nests to monitor feeding rate. I will estimate food quality of prey items, in terms of DHA content, in chick diets based on the following equation:

Food quality = $H_a \times D_a + H_t \times D_t$

$H_a$ = average DHA content of emergent aquatic insect prey items

$H_t$ = average DHA content of terrestrial insect prey items

$D_a$ = percentage emergent aquatic insect prey items in diet estimated from SIAR

$D_t$ = percentage terrestrial insect prey items in diet estimated from SIAR

I will relate growth rate and body condition to estimated food quantity and quality and to chick DHA levels measured in objective one.

***Objective 2B: Experimental manipulation of effects of food quantity and quality***

**Design:** I will experimentally manipulate food quantity and quality for Eastern Phoebe chicks under controlled conditions and measure growth rates from day 0 to day 5, the period of most rapid growth for aerial insectivores (Zach and Mayoh 1982). I will manipulate food quantity as calories and food quality as DHA content in a fully factorial design, which will enable me to disentangle the potentially confounding effects of food quantity and quality that I expect to observe in natural systems. I will collect 40 eggs from sites with active nests monitored by a collaborator. Eggs will be incubated until hatch then fed hourly from 6am to 9pm (Lanyon 1979; Kroodsma and Konishi 1991). I will raise chicks under standard rearing conditions at the Cornell Experimental Ponds Facility, a university approved animal facility run by my collaborator. All chicks will be sacrificed at the end of the experiment for analysis.

**Food quantity and quality:** After hatch, ten chicks will receive a low calorie, low HUFA diet; ten chicks will receive a low calorie, high HUFA diet; ten chicks will receive a high calorie, low HUFA diet; and ten chicks will receive a high calorie, high HUFA diet. Diets based on a modified Lanyon diet (Lanyon 1979; Kroodsma and Konishi 1991) will be iso-nitrogeneous and high and low HUFA diets will be iso-caloric. High HUFA diets will contain DHA content equivalent to a 100% emergent aquatic insect diet based average emergent aquatic insect DHA levels measured across sites in objective two. Low HUFA diets will contain DHA content equivalent to a 100% terrestrial insect diet based average terrestrial insect DHA levels measured across sites in objective two.

**Sampling and Analysis:** I will measure chick head and tarsus length and weight daily for calculations of growth rate and body condition. At the end of the experiment, I will take blood samples from 5 chicks per feeding treatment group prior to sacrifice for fatty acid composition analysis. Fatty acid composition analyses will be conducted following methods described in objective one. I will then relate growth rate and body condition to known food quantity and quality and to measured chick DHA levels.

## Feasibility

I have experience with bulk stable isotope analysis, a tool that I have used from my undergraduate thesis work to the present. I also have experience conducting fatty acid composition analysis, a skill that I have learned and honed with the help of my committee member and his lab. Members of his lab will also help me become proficient in compound-specific stable isotope analyses. My collaborator and members of his lab have extensive experience sampling, raising, and monitoring aerial insectivore chicks. This past summer lab members trained me in the collection of blood samples from and care of Eastern Phoebes and they will continue provide me with expertise. I also have experience creating animal feeds with manipulated HUFA levels.

## Intellectual Merit

Riparian zones constitute a middle ground between terrestrial and aquatic systems and are influenced by human impacts to both (Sweeney et al. 2004). In the United States the Conservation Reserve Program provides incentives to encourage riparian buffer zones through payments to farmers who leave vegetation buffers around streams (Natural Resources Conservation Service 2010; USDA 2013). As a result, riparian zones occur even in highly human-dominated landscapes. Most riparian buffer zone legislation focuses on preventing contaminants and high nutrient loads associated with intensive urban and agricultural land use from reaching streams. The effect of the riparian zone on preventing contaminants and eutrophication from reaching stream food webs is already an area of active research (Sweeney and Newbold 2014). However, the effects of contaminants and nutrients on the riparian zone itself have yet to be studied in great detail. Recent studies suggest that contaminants that reach streams can return to riparian zones as reciprocal contaminant subsidies (Alberts et al. 2013; Rowse et al. 2014). Increased nutrient and light levels associated with intensive human land use that influence food quantity and quality in stream food webs are likely to have reciprocal effects on riparian food webs as well. My research will document the effects of a change in aquatic subsidy quantity and quality on growth rates in a widespread riparian consumer. In addition, my research will document the movement of aquatic-derived HUFAs to riparian consumers.

## Broader Impacts

Throughout my previous research efforts, I have demonstrated a commitment to collaboration with environmental managers, science outreach, and undergraduate mentoring (see Biosketch). I will continue these efforts as part of my proposed DDIG research. I will collaborate with the X Land Trust and the Y Natural Areas Program as well as local private landowners while finalizing study-site selection and conducting research. I will also share a summary of my results with all landowners and share data upon request. At publicly accessible sites I will continue to put out signs explaining my research. In addition, I will continue to work with the

Sciencenter to develop presentations based on my research themes. I will also continue my broader science education and communication efforts through Expanding Your Horizons, Enviromentors, and as an officer of my campus Sigma Xi chapter (see Biosketch). Finally, I will continue to mentor undergraduates, including the minority female Environmental Science major whom I have mentored since her freshman year.

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# CONTEXT FOR IMPROVEMENT

## Relevance to Broader Dissertation Work

The goal of my PhD dissertation is to understand how watershed land use affects food quantity and quality for stream consumers. In particular, my research looks at how watershed land use affects stream food quality in terms of ***highly unsaturated fatty acid*** (HUFA) levels, which affect the growth, development, and health of fishes. I have already conducted feeding research demonstrating that HUFA availability increases growth rates in Eastern blacknose dace (*Rhinichthys atralutus*), a common North American stream fish. I have also conducted research documenting how HUFA availability varies seasonally with changes in resource abundance in a forested headwater stream. I am currently conducting research on in-stream energy and nutrient fluxes in agricultural and forested streams.

DDIG funding would allow me to expand upon my primary dissertation research to examine how land use affects aquatic and terrestrial-derived food quantity and quality for riparian predators. I have conducted preliminary research examining: the quantity of terrestrial versus aquatic resources across a forested to agricultural landscape gradient and the extent to which riparian predators rely upon terrestrial versus aquatic resources based on bulk stable isotopes. DDIG funding would allow me to use compound-specific stable isotope analyses to determine whether riparian predators in natural systems obtain HUFAs from their molecular precursors or from *de novo* synthesis by aquatic primary producers. In addition, my proposed DDIG project will allow me to determine if HUFA levels increases growth rates in riparian predators in the same way that they affect growth rates for stream fishes.

## Significance of Funding and Relation to PI's Lab Research

My advisor has research interests and experience focused on resource subsidies in freshwaters. Previous work in the lab has examined elemental nutrient movement and recycling by stream fishes and invertebrates. My work builds on this theme by examining the movement of limiting organic compounds, HUFAs, within streams and from streams to the riparian zone. I am the first in the lab to collaborate with members of the Department of Nutrition and the first to use fatty acid composition as a metric of food quality. I will also be the first in the lab to use compound-specific stable isotope analyses as an additional food web tracer. Although my advisor and collaborators are able to provide me with intellectual and methodological support for this proposal, they are not able to provide me with financial support. To this point, my research has been entirely self-funded.

Currently, I have secured funding for preliminary fieldwork (see preliminary research) and preliminary bulk stable isotope analyses. However, this funding is not sufficient to run fatty acid or compound-specific stable isotope analyses. DDIG funding will allow me to use these tools to answer questions about food quality. My current funding is also insufficient to monitor and conduct analyses on a greater number of study sites. DDIG funding will allow me to increase the number of study sites to determine if my preliminary research results are replicated across the greater regional landscape or are simply site-specific phenomena.

## Budget Justification

I am requesting funds for to examine the effects of land use on aquatic-derived nutrient fluxes to riparian predators. I am requesting funds for an undergraduate field and lab assistant, waterproof video cameras, and stable isotope and fatty acid lab analyses. Fieldwork and a feeding experiment will take place during year one. Bulk stable isotope and fatty acid composition analyses will take place during year one. Compound-specific stable isotope analyses will take place during year two.

### *Direct Costs*

**Personnel, Undergraduate Student,** will provide assistance in the field and with feeding experiments. I am requesting funding for undergraduate student support. I anticipate the student will devote approximately 30 hours per week for 6 weeks at a rate of $10 per hour.

| | Year 1 | Year 2 |
|---|---|---|
| Undergraduate Student ($10/hour, 184 hours) | $1840 | $0 |

### Materials and Supplies

I am requesting funds for 6 Go-Pro ® waterproof video cameras to record Eastern Phoebe feeding rates in the field.

| | Year 1 | Year 2 |
|---|---|---|
| Go-Pro ® Hero 3 White Edition ($200/camera, 6 cameras) | $1200 | $0 |

I am requesting funds for consumable supplies necessary to conduct the proposed research. These expenses include, but are not limited to:

| | Year 1 | Year 2 |
|---|---|---|
| Costech silver capsules ($69/pack, 2 packs) | $138 | $0 |
| Costech aluminum capsules ($26/pack, 2 packs) | $52 | $0 |
| Other consumables | $42 | |

### Other

I am requesting funds for bulk stable isotope analyses at the Stable Isotope Laboratory and fatty acid composition analyses and compound-specific stable isotope analyses in the Department of Nutrition.

| | Year 1 | Year 2 |
|---|---|---|
| Bulk Stable Isotope Analyses ($18/sample, 96 samples) | $1728 | $0 |
| Fatty Acid ($35/sample, 106 samples) | $3710 | $0 |
| Compound-Specific Stable Isotope Analyses ($65/sample, 66 samples) | | $4290 |

## Data Management Plan

### Description

The raw data generated by this project will be of five types:

1) Stable isotope data (.csv)
2) Fatty acid chromatogram data (.chr)
3) Compound-specific stable isotope data (.csv)
4) Video recording files (.mpg)
5) Chick head length, tarsus length, and weight (growth rate) data (.csv)

### Content and format

Stable isotope data, processed fatty acid chromatogram data, compound-specific stable isotope data, and chick growth rate data will all be stored as .csv and .pdf files for long-term accessibility and compatibility. Video recording data will be stored as .mpg files for long-term accessibility and compatibility. Files will be named according to standard formats that include project name, data type, and date of collection or analysis. Metadata files describing data will also be stored as .csv and .pdf files. Metadata will be compiled according to Ecological Metadata Language specifications.

### Protection and Privacy

All data and results of analyses will be backed up daily using Mozy Home, an online data server.

No aspect of this project involves the use of human subjects and there are no privacy or confidentiality concerns. All research participants will be trained in Responsible Conduct of Research through the Office of Research Integrity and Assurance and in Animal Care through the Institutional Animal Care and Use Committee and the Center for Animal Resources and Education.

### Access

Upon publication in a peer-reviewed journal or within two years following collection, the PI will make all csv files from this project available. Data will be made available online as supplementary material to a peer-reviewed publication or in an online open-access data archive, such as DRYAD. Video recording data on Eastern Phoebe feeding rates will be donated at the Lab of Ornithology.

### Preservation and Transfer of Responsibility

Original field and lab notebooks will be stored in the PI's lab in addition to being entered in Microsoft Excel and saved as .csv and .pdf files as described. Data files will be stored on the Mozy Home server and as supplementary material to peer-reviewed publications or in an online open-access data archive.

## Facilities, Equipment, and Other Resources

### Laboratory

PI's Laboratory, Department of Ecology and Evolutionary Biology

- The lab has a balance that the co-PI will use to weigh out samples for stable isotope, fatty acid, and compound-specific stable isotope analyses.

Stable Isotope Laboratory, Department of Ecology and Evolutionary Biology

- The Stable Isotope Laboratory has a Finnigan MAT Delta Plus IRMS for running $\delta^{13}C$ and $\delta^{15}N$ analyses and a Thermo Delta V Advantage IRMS with a Conflo III interface for running $\delta^{2}H$ analyses.

Collaborator's Laboratory, Department of Nutritional Sciences

- The collaborator's lab houses a Varian Saturn 2000 ion trap GCMS, a Varian Star 3400 GCMS with Varian Saturn 5.5.1 software, an HP5890 series II GC-FID with PeakSimple software, an Agilent 6890 GC coupled to a Thermo Finnigan MAT 253 IRMS equipped with a Thermo Finnigan Conflo III for $CO_2$ gas calibration introduction with ISODAT 3.0 software for data acquisition and analysis, and two -80ºC freezers for sample storage. This laboratory provides all resources needed for completion of this study.

### Computers

- The PI and co-PI each have both Windows and Mac computers with software for statistical analyses, word processing, and graphics programs.
- The co-PI has R for running the package SIAR (Bayesian mixing model package) on her Mac and PeakSimple chromatography software on her Windows for analyzing fatty acid chromatograms. Her Mac (primary computer) has MozyHome, an online storage service that automatically backs up files daily.
- The Department of Ecology and Evolutionary Biology has a computer support specialist, who takes care of computers and backups.

### Offices

- The PI and co-PI each have offices in the Department of Ecology and Evolutionary Biology.

### Other

- The co-PI has a designated unit for sample storage in the Department of Ecology and Evolutionary Biology shared -80ºC freezer in Corson Hall.
- The co-PI has received funding for fieldwork and preliminary laboratory analyses (field collection, bulk stable isotopes, and stoichiometry) from Sigma Xi and the Department of Ecology and Evolutionary Biology.