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Spatial and temporal linkage of stream-riparian food webs by seasonal migration of mayfly
Ephemerella maculata

by

Hiromi Uno

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

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California Berkeley

Committee in charge

Professor Wayne P. Sousa

Professor Jonathon Stillman

Professor Stephanie M. Carlson

Spring 2016

Spatial and temporal linkage of stream-riparian food webs by seasonal migration of mayfly
Ephemerella maculata

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Abstract

Spatial and temporal linkage of stream-riparian food webs by seasonal migration of mayfly
Ephemerella maculata

by

Hiromi Uno

Doctor of Philosophy in Integrative Biology

University of California Berkeley

Professor Wayne P. Sousa, Chair

Stream environments are spatially and temporally heterogeneous. Mainstem rivers are often wide, sunlit, warm and productive, while tributaries are shaded by riparian trees, unproductive, and remain cool in summer. Within mainstem rivers themselves there is substantial spatial heterogeneity in habitat structure and physical conditions, such as water temperature. River environments also change dramatically with season. Organisms that live in the riverine environment respond to and take advantage of such heterogeneous environments by moving between microhabitats or shifting their phenology. I studied the life cycle of a riverine mayfly, *Ephemerella maculata* (Ephemerellidae), in a northern California river system, its responses to spatial and temporal heterogeneity, and how its movements connect stream and riparian food webs in space and time.

I discovered that *E. maculata* migrates between the mainstem and tributaries of rivers during its life cycle, thereby linking food webs in these two habitats, and enhancing predator growth in unproductive tributaries. The resource subsidy from productive but warm rivers to cool, unproductive tributaries associated with the mayfly migration increase the growth of stenothermic predators like juvenile salmonids in otherwise food-limited, cool thermal refuges, and increase their resilience to future warming. Furthermore, I examined the resilience of *E. maculata* to changes in water temperature using field surveys and lab rearing experiments. I discovered that different life stages of *E. maculata* have different thermal responses, and they shift their phenology depending on the water temperature, allowing each life stage to occur in the most desirable thermal condition. Therefore, as long as the natural seasonal pattern of the water temperature is sustained, *E. maculata* can resist temperature changes by shifting their phenology. Finally, I have shown that thermal spatial heterogeneity of rivers desynchronizes mayfly emergence timing, prolonging the subsidy period to riparian predators, and changing the predators' responses to this subsidy.

For the beautiful Eel River

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Introduction

Natural environments are spatially and temporally heterogeneous. Many organisms respond to such heterogeneity by moving or migrating among habitats to enhance their growth, reproduction, and survival. Such movement of organisms can link spatially distant communities (Lundberg & Moberg 2003). In stream and riparian communities, fluxes of adult aquatic insects from rivers are known to be an important food source for riparian predators (Nakano & Murakami 2001; Sabo & Power 2002). In this dissertation, I show that adult aquatic insects, which emerge from productive rivers, can trophically support not only riparian predators of the river, but also predators in adjacent tributaries by their migrations from productive mainstems to less productive tributaries.

In my first summer at Angelo Coast Range Reserve in 2010, before embarking on Ph.D. research, I encountered massive numbers of dead adult aquatic insects in a small shaded tributary, Fox Creek. The numbers of adult aquatic insects I observed in the creek far exceeded the expected production of insects from such a small tributary. The observation made me suspect that the adult mayflies originated in other parts of the river. Careful inspection of this mysterious eruption of adult mayflies in the small creek revealed that they were all one species, *Ephemerella maculata* (Ephemerellidae), and that their nymphs only occur in the adjacent productive mainstem river, indicating a migratory life cycle.

In the Angelo Coast Range Reserve, there had been a number of novel previous studies on resource movements in the landscape; Sabo and Power (2002) had shown the importance of emerging adult aquatic insects from mainstem river to riparian lizard predators. Atlas *et al*., (2013) had shown the importance of aerial inputs in the stream food webs in small tributaries. The observation of the *E.maculata* migration from the productive mainstem to adjacent tributaries and associated movement of resources spatially linked those previous studies. It became apparent to me that the migration of adult *E. maculata* transported a trophic resource from the mainstem river to both the peripheral riparian food webs, and the food webs in adjacent tributaries.

Therefore, in my dissertation, I identified *E. maculata* as a key species of the ecosystem, and studied details of its life cycle, with special attention to its spatial and temporal distributions, and how their movements connect food webs in space and time. In the first chapter, I describe the life history of *E. maculate* in detail, with a focus on their migratory life cycle and its fitness advantages. In the second chapter, I document ecological consequence of the *E. maculata* migration, and experimentally demonstrated that trophic subsidy from the productive mainstem to less productive tributaries triples the growth of juvenile steelhead trout, which rear in cool but food-limited tributaries. In third chapter, I examined temporal aspects of the *E. maculata* life cycle, and showed how *E. maculata* is adapted to the thermally varying seasonal environment. In fourth chapter, I studied ecological consequences of the *E.maculata* life cycle's distribution in space and time, and showed that spatial heterogeneity with in the source habitat of their migration, the mainstem river, leads to asynchronous emergence of *E.maculata* and thereby prolongs the resource subsidy to tributaries, altering consumer responses.

Organisms move in space and shift their life cycle timing in response to the spatial and temporal heterogeneity in the environment. Such a life cycle can link food webs and communities in space and time. This dissertation presents a novel example of this phenomenon in an aquatic ecosystem.

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Foremost, I would like to thank the two great advisors, Mary Power and Wayne Sousa who I had chance to be mentored at Berkeley. It is the biggest regret of my life that I cannot have Mary's signature on this dissertation as an advisor, but at same time, I am really grateful that a chance was given me to be also mentored by Wayne. I was very fortunate that I had chance to be mentored both by Mary and by Wayne. Mary taught me excitement and joys of research. I had never shared such excitement and love for rivers, and really enjoyed hopping around and talking about rivers with her. Mary has been and will remain to be my great role model as a stream ecologist. On the other hand, Wayne was always a source of calm and stability for me, and was always very big for me. Sometimes he was my blanket, while other times, he was the last strong hold. Wayne also taught me how to analyze data, and logically write scientific papers, which was extremely valuable for me to become an independent scientist. However, the most important thing that I learned from Wayne probably was his rigorous philosophy and attitude toward science, students, and his colleagues. I respect Wayne's dedication from the bottom of my heart. Wayne is my very big role model as a mentor.

Interactions with amazing scientists with various backgrounds allowed me to develop my curiosity and explore around various aspects of the natural science. Stephanie Carlson taught me about salmonid fish, their life histories and how to study fish. Jonathon Stillman taught me physiology of organisms, and also engineering for aquarium experiments. Vincent Resh taught me various aspects of aquatic insects and freshwater sciences, and more importantly provided me insights for my future career with global view. Bill Dietrich taught me geomorphology and how to read the landscape. Learning geomorphology from Bill totally changed my view of the world and made a strong basis as a stream ecologist. Todd Dawson taught me various isotope analysis.

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Publication

[2] Hiromi Uno, Mary E. Power (2015) Mainstem-tributary linkages by mayfly migration help sustain salmonids in warming river networks, *Ecology Letters*, **18**, 1012-1020.

[1] George Poinar Jr., Larissa Walder, Hiromi Uno (2015) *Anomalomermis ephemerophagis* n.g., n.sp. (Nematoda: Mermithidae) parasitic in the mayfly *Ephemerella maculata* Traver (Ephemeroptera: Ephemerellidae) in California, USA, *Syst Parasitol*, **90,** 231-236.

[#] Hiromi Uno (*in review*) Stream thermal heterogeneity prolongs an aquatic-to-terrestrial subsidy and enhances riparian spider growth and reproduction.

[#] Hiromi Uno, Jonathon Stillman (*in prep*) Whole life cycle response of an annual mayfly *Ephemerella maculata* to varying seasonal environment.

[#] Hiromi Uno (*in prep*) Migratory life cycle of *Ephemerella maculata* (Ephemeroptera; Ephemerellidae).

[#] Hiromi Uno, Kanehiro Kitayama, David Dudgeon (*in prep*) The hierarchical spatial structure of the river network supports high biological diversity in headwater streams.

Awards, Grants and Fellowships

- Doctoral Dissertation Improvement Grant, NSF (2015-2016): $16380
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[15] <u>Hiromi Uno</u>, Mary Power (2015) "Trophic subsidy from the mainstem to tributaries by migratory mayflies is strengthened by mainstem thermal variations" Californian Chapter of the Society for Freshwater Sciennce 2014 (Oral)
[*] Shelley Pneh, <u>Hiromi Uno</u> (2015) "Emergence timing of aquatic insects in the South Fork Eel River" Californian Chapter of the Society for Freshwater Sciennce 2014 (Poster)
[14] <u>Hiromi Uno</u>, Mary E. Power (2014) "Trophic connections of productive mainstems and unproductive tributaries in river networks by migratory adult aquatic insects" Ecological Society of America 99th annual meeting (Oral)
[*] Larissa B. Walder, <u>Hiromi Uno</u>, Mary E. Power (2014) "Life history of Mermithid nematode parasites of the migratory mayfly, *Ephemerella maculata*" Ecological Society of America 99th annual meeting (Poster)
[13] <u>Hiromi Uno</u>, Mary Power (2014) "Mainstem-Tributary Food Web Interactions in River Networks Mediated by Migratory Mayflies and Anadromous Fish" AAAS Pacific Division meeting (Oral)
[12] <u>Hiromi Uno</u>, Mary E. Power (2014) "Resource subsidy from productive mainstems to unproductive tributaries by migratory mayflies sustain salmonid juveniles rearing in cool tributaries" Joint Aquatic Sciences Meeting (Oral)
[11] <u>Hiromi Uno</u>, Mary E. Power (2014) "Mayfly migration from mainstem to tributaries mediates biological backflows that sustain juvenile steelhead in warming river networks" Ecological Society of Japan annual meeting (Oral)
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[10] <u>Hiromi Uno</u>, Mary E. Power (2013) "Spatiotemporal matching of an unusual mayfly migration with steelhead trout growth in tributaries: mainstem-to-tributary subsidies sustain rearing salmonids in a Northern Californian river network." 11th International Congress of Ecology (Oral)

[9] <u>Hiromi Uno</u>, Mary E. Power (2013) "Unusual mayfly life cycle connects mainstem and tributary food webs" Society of Freshwater Science 2013 Annual Meeting (Oral)

[8] <u>Hiromi Uno</u>, Kanehiro Kitayama, David Dudgeon (2012) "The hierarchical spatial structure of the river network supports high biodiversity in headwater streams" Ecological Society of America Annual Meeting (Oral)

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[6] <u>Hiromi Uno</u>, Yasuhiro Takemon, Kanehiro Kitayama, Noboru Okuda (2011) "Intra-reach heterogeneity of macroinvertebrate trophic origin in a tropical montane stream" University Consortium on Aquatic Sciences in Hong Kong (Oral)

[*] Mary E. Power, David Moreno-Mateos, <u>Hiromi Uno</u>, Collin Bode, William E. Rainey (2010) "Seasonal and spatial variation of bug flux in a northern California drainage network under a Mediterranean climate: implications for reciprocal subsidies between coupled ecosystems" American Geophysical Union 2010 Fall meeting

[5] <u>Hiromi Uno</u>, Yasuhiro Takemon, Kanehiro Kitayama, Noboru Okuda (2010) "Heterogeneous resource use by benthic assemblages in a tropical mountain stream ~approach from habitat scale SI data~ " International Symposium on Isotope Ecology 2010 in Kyoto (Poster)

[4] <u>Hiromi Uno</u>, Yasuhiro Takemon, Kanehiro Kitayama, Noboru Okuda (2010) "Heterogeneous use of resources by benthic assemblages in a reach of tropical montane stream" The 95th Ecological Soceity of America Annual Meeting (Poster)

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[3] <u>Hiromi Uno</u>, Yasuhiro Takemon, Kanehiro Kitayama, Noboru Okuda (2010) "Heterogeneous food web structure and food source in tropical montane stream" The 57th Ecological Society of Japan (Oral; in Japanese)

[2] <u>Hiromi Uno</u>, Yasuhiro Takemon, Kanehiro Kitayama, Noboru Okuda (2009) "Benthic foodweb structures in a tropical montane stream" The 74th Annual meeting of Japanese Society of Limnology (poster; in Japanese)

[1] <u>Hiromi Uno</u>, Rota Wagai, Yasuto Fujiki, Kanehiro Kitayama (2009) "The effect of nutrient supply on the carbon dynamics focusing on top soil of the forest" Japanese Society of Pedology (Poster; in Japanese)

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- Graduate Student Instructor "*General Biology*" Biology 1B, UC Berkeley (2012 -2013)
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- Exhibition of "stream and riparian food webs" booth at "Water Day", a local citizen event in the Eel River, collaboration with the Eel River Recovery Project (2013)
- Invited talk at Oregon Department of Fish and Wildlife (Aug 2014) "Mainstem-Tributary Food Web Interactions in River Networks Mediated by Migratory Mayflies and Anadromous Fish"
- Invited lecture at the Renaissance International School "Biodiversity" and field excursion to backyard creek (Sept 2014)
- Invited talk at Salmon River Restoration Council (July 2015) "Food webs in river networks"

Chapter 1

Migratory life cycle of *Ephemerella maculata* (Ephemerellidae)

Abstract

Ephemerella maculata is a common mayfly widely distributed in California, USA. Details of their migratory life cycle within the mainstem and tributaries of the upper drainage of the South Fork Eel River in Northern California were documented, and the fitness advantages of this life history were examined by transplant experiments. *E. maculata* nymphs grow in sunny mainstem rivers, emerge, then the female adults fly into adjacent dark tributaries, oviposit eggs and die. The eggs diapause over summer and hatch in fall in tributaries, then small nymphs drift down back to the mainstem before the subsequent spring growing season. Transplant experiments and diet analysis showed that *E. maculata* nymphs rely on the abundant algae that grow in the sunny mainstem river for food. In contrast, eggs survived and hatched equally well in mainstem and tributaries, indicating no fitness advantage of their migration to the tributaries in the studied system. Their migration to canyon tributaries may be a fixed species trait that evolved in arid environments common to other parts of California, where many alluvial mainstem rivers dry up in summer. The adult migration of *E. maculata* from alluvial mainstem rivers to adjacent canyon creeks that sustain more stable water in the beginning of dry season may help them to avoid desiccation in dry summer conditions.

Introduction

Migration is a key component of the life cycles of many animal species, and it occurs over a range of spatial and temporal scales. Some animals migrate over extremely long distance (e.g. Arctic tern, Egevang *et al.* 2010; whales, McKintosh 1965; monarch butterflies, Calvert *et al*. 1979), while others migrate regionally across landscapes (e.g. altitudinal migration of butterflies, Shapiro 1973). Animal migrations can have significant ecological consequences by linking distinct habitats for consumers (e.g. snow geese, Jefferies *et al.* 2004), resources (e.g. salmon carcasses from the ocean to rivers, Wipfli *et al.* 1998) or vectors of diseases (Altizer *et al.* 2011). Despite their significance in ecological function, some migrations, particularly of small organisms, are cryptic and not well-studied.

Migratory behaviors can be advantageous (or obligate) in various cases: tracking predictable but temporally restricted resources (e.g. ungulates, Fryxell *et al.* 2005); specific habitats needed for certain life stage (e.g. breeding migration for sea turtle; Diamond 1976, moult migration of waterfowl; Finn 1968); escape from predators (e.g. diel vertical migration by zooplankton; Loose & Dawidowicz, 1994) or parasites (Poulin *et al.* 2012). While some organisms exhibit migratory behaviors only under certain conditions (e.g. three-spine stickleback, Bell &Foster 1994), other species have evolved to migrate as a fixed species trait, and inherently all individuals of the species migrate (e.g. Chinook salmon, Quinn 2005).

Environments within streams dramatically change with their drainage area. Small creeks are narrow, shaded by trees, cool and typically have low productivity, while rivers with larger drainage area are typically wide, sunlit, warm and often have high productivity (Vannote *et al*., 1980). At confluences where small tributaries meet large rivers, adjacency of the two habitats enables mobile organisms to take advantage of the contrasting environments. Many freshwater fish grow in productive mainstems and migrate to tributaries to spawn (Fausch *et al.* 2002). In contrast, the foothill yellow-legged frog, *Rana boylii*, migrates to sunny mainstem rivers to breed, their tadpoles grow in the mainstem consuming algae and metamorphose, then adult frogs return to peripheral

tributaries or riparian forests (Kupferberg 1996). The mayfly *Leptophlebia cupida* (Leptophlebiidae), distributed in Canada and the northeastern United States, also grow in mainstem rivers, then as nymphs, they migrate up the smaller tributaries in early spring to avoid flooding in the mainstem following the ice-melt (Hayden & Clifford 1974, Clifford *et al.,* 1979).

The migratory life cycle of *Ephemerella maculata* (Ephemerellidae) (Fig. 1), a mayfly indigenous to California, was recently discovered (Uno & Power 2015), and is described in detail in this paper, The particular migratory pattern found in *E. maculata*, which grows in the mainstem river as nymphs, then, after emerging as adults, migrates into tributaries for oviposition, had not been previously documented for any aquatic insect species. Uno & Power (2015) demonstrated that the migration of adult *E. maculata* from sunny mainstem river to adjacent shaded tributaries provides an important resource subsidy to food-limited predators in tributaries (Fig. 2). A detailed understanding and description of the migratory life cycle of *E. maculata* would contribute to our understanding of this important trophic pathway, and may propel the search for other aquatic insect species that exhibit similar migratory life cycles of ecological significance.

In this paper, I first describe details of the life history of *E. maculata* derived from field observations, and supported by the findings of stable isotope analysis. Then, I report the results of field transplant experiments that evaluated the advantages of their migratory life cycle.

Methods

Study System
E.maculata is widely distributed in California (Fig. 3; Allen et al. 1968, Meyer and McCafferty, 2008), a region characterized by a very diverse environment and climate, ranging from the desert in the south to redwood forests in the north. Many rivers in California, especially in southern California are intermittent and dry up in summer, while rivers in the North and East (in the Sierra mountain range) typically exhibit some flow throughout the year.

Most of the studies reported here were conducted in the Eel River, a major river in Northern California (Fig. 3b). The Eel River and its major tributaries such as the Van Duzen River, the South Fork Eel River, the North Fork Eel River, the Middle Fork Eel River are low gradient, wide and sunny alluvial rivers, with many small tributaries that are high gradient, narrow and often forested creeks flowing into the mainstem rivers. Experiencing Mediterranean seasonality, the Eel River watershed typically has wet winters and dry summers. As the discharge decreases in summer the water in the rivers exceeds 25°C while smaller forested tributaries sustain cooler water (historic record of the stream water temperature at the Angelo Coast Range Reserve gage).

Life cycle description
The life cycle of *E. maculata* was described for the upper basin of the South Fork Eel River, in or near the University of California Angelo Coast Range Reserve (39° 44' 17.7" N, 123° 37' 48.8" W) (Fig. 3c). To describe habitat use by each life stage, distributions of adult and nymph *E. macualta* were surveyed in ten tributaries and ten adjacent reaches of the mainstem South Fork Eel River. Additionally, to investigate the distribution of *E. maculata* in larger landscape context in the river network, broad distribution of *E. maculata* nymphs in the entire Eel River basin

(drainage area 9500 km^2) was examined by field surveys at 48 sites distributed throughout the whole watershed (Fig. 1b). The presence/absence of *E. maculata* nymphs at each site was determined by exhaustive survey with kick nets for at least 30 minutes by at least three trained aquatic biologists. The presence/absence of *E. maculata* adults was determined by a combination of sticky traps (>20 traps, one square meter area in total) and sweeping with butterfly nets for over 30 minutes before and after the dusk for at least three days during the flight period. The phenology of *E. maculata* was described by daily observations from late May thru August during the period of their adult migration, and by monthly visits for the rest of the year.

For the nymph stage, density, body condition, sex-ratio, and their emergence behaviors were investigated. Densities of *E. maculata* nymphs were estimated with a Surber net sampler, which covers 0.09m^2 of the stream bottom at a time. Their external morphology and conditions as well as the presence/absence of parasites in their bodies were examined under 10X dissecting scope. Sex of *E. maculata* nymphs in the South Fork Eel River was examined by rearing field collected nymphs to adults. Their emergence behavior was observed in the field in the South Fork Eel River. Final instar nymphs were collected from the South Fork Eel River, and kept in captivity to examine the longevity of subimago and imago stages following the emergence.

For the adult stage, sex, body size distribution across the flight season, as well as the prevalence of nematode parasite were examined. The amount of *E. maculata* captured in drift nets (dry mass (g)) were compared with air temperature records from the Angelo Reserve weather station. Adult *E. maculata* were collected from Fox Creek tributary. Their sex, was determined from examination of external morphology such as their reproductive organs and compound eyes. The body size (length mm) was measured on July 9, July 16, and July 22, 2011. Nematode, *A. ephemerophagis* emerges from the adult *E. maculata* when they are submerged in the water. Therefore, I examined their prevalence by placing the adult female *E. maculata* in freshwater for one hour, and counting nematodes that emerged from the mayflies. Linear regression analysis was applied for the relationship of the season and the adult body size. Previous research has shown that *E. maculata* that oviposit in tributaries provide resource for predators in tributaries, such as juvenile steelhead trout (Uno & Power, 2015). To examine which body parts of *E. maculata* are consumed by predators in tributaries, I conducted gut content analysis of 55 individual juvenile steelhead trout from Fox Creek. I used non-lethal gastric lavage to remove the gut contents. The fish gut contents were then separated into categories; *E. maculata* adults, *E. maculata* egg mass, *A. ephemerophagis*, and other materials, and then counted.

For the egg stage, their density, external characters, number of eggs per female adult were investigated. The density of eggs was estimated with 48 Surber net samplings, where each sampling covered 0.09m^2. Numbers of eggs per female adults were counted for adult females in Fox Creek. External characters of eggs as well as 1st instar nymphs that just hatched out of eggs were examined investigated under 30X dissecting scope, and their size (mm) were measured.

Isotope analysis
Carbon and nitrogen stable isotope analyses were carried out to estimate the growing habitat of *E. maculata* adults that fly in tributaries. *E. maculata* female adults were collected from the air with nets at three tributaries of the South Fork Eel River where they oviposit (Fox Creek, Elder Creek and McKinley Creek) four to six times between May 26 and June 24, 2015 and four times between

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July 2 and July 20, 2016. *E. maculata* nymphs were collected on May 30, 2015 at 20 sites within a six km segment of the South Fork Eel River where Fox Creek, Elder Creek and McKinley Creek flow into the mainstem of the Eel. Nymph samples were kept alive in freshwater for 24 hours prior to the analysis to clear their gut contents. At each sampling event, more than five individuals of *E. maculata* nymphs or adults were collected, dried, and ground together for analysis. Additionally, for comparison, I collected other aquatic insect species emerging from two habitats in the South Fork Eel River and two habitats in Fox Creek with non-chemical emergence traps on July 18-20, 2015. I sorted the samples at order level (Ephemeroptera, Trichoptera, Plecoptera, Diptera), then dried and ground the pooled sample of each order from a particular habitat prior to the analysis.

All samples were oven dried at 60°C overnight before grinding, then their carbon and nitrogen stable isotope ratios were analyzed. The analysis was conducted at the Center for Stable isotope biogeochemistry at University of California Berkeley using a CHNOS Elemental Analyzer interfaced to an IsoPrime100 mass spectrometer in 2012, and at the Stable Isotope Facility at University of California Davis using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) in 2015.

Diet analysis
To examine the diet of nymphs, five nymphs were placed in a bucket with three cobbles from the South Fork Eel River and another five nymphs in a separate bucket with three cobbles from Fox Creek. The buckets were kept in a lab with clean water at ambient temperature. After 48 hours, the nymphs were removed and immediately dissected, then their guts were removed intact and preserved in 10% formalin for later examination. To quantify the algae provided to nymphs in each treatment, epiphytes in a 5cm by 5cm area were scraped off the surface of each cobble with a toothbrush, and preserved in 10% formalin.

Later in the lab, the gut contents and algae on the cobbles were examined with a 200X compound microscope. The gut contents were extruded onto slide glass and dispersed, then total numbers of algal cells in each gut were counted. In addition, representative fields were examined for the relative abundance of intact diatoms, green algae, cyanobacteria, and detritus using a Whipple grid. We counted and identified material observed at grid intersections, with at least 50 points counted for each gut sample.

Algal samples from each cobble surface was homogenized, and subsample of each sample was taken and examined with Sedgewick rafter chamber slide and Whipple grid under a 200X compound microscope. 6.0 % subsample of each algal sample from Fox Creek and 0.019% subsample of each algal sample from the South Fork Eel River were identified and counted.

Transplant experiments of nymphs and eggs
To examine advantages of the migratory life cycle, I conducted field reciprocal transplant experiments of nymphs and eggs between the nymph habitat, the mainstem South Fork Eel River, and their egg habitat, Fox Creek.

In the nymph transplant experiment, the location and the origin of the food supply were crossed to discern the effect of both the habitat and available food. Nymphs were obtained from the South Fork Eel River. Nymphs were placed in flow-through buckets that had 1mm mesh on the sides to

allow natural water exchange and flow (ten nymphs per bucket).). Three algae covered cobbles (~10 cm diameter) collected from the treatment site, either the South Fork Eel River or Fox Creek, were placed in each bucket, and replaced once a week. A 10cm by 10 cm sticky trap was suspended over the water in each bucket to trap emerged adults, and the trap was replaced and examined once a week. The top of the buckets were covered by 1mm mesh to prevent the dispersal of emerging individuals and allow air exchange. Five buckets with cobbles from South Fork Eel River and five buckets with cobbles from Fox Creek were placed in each of the South Fork Eel River and Fox Creek sites. The experiment was initiated on June 11, 2014 and continued until all nymphs emerged or died on July 7, 2014. The successful emergence rate and average emergence date were calculated for each bucket, and they were compared among treatments. The arcsine transformation was conducted applied for the successful emergence rate. Then the transformed values of the successful emergence rate and the date of the emergence were analyzed with two-way ANOVA to compare the effect of the location and the food.

The effect of the environment on egg hatching success was examined in protected flow through containers. Eggs were obtained from Fox Creek on June 28, 2014, and immediately placed in 50ml plastic containers that had no-see-um mesh on the side to allow water exchange. Three containers were placed in each of the South Fork Eel River and Fox Creek sites. Containers were tied on rocks and placed in riffles. The containers were removed on November 15, 2014 and the proportion of empty egg shells that indicate successful hatch, dead eggs, and unhatched alive eggs (embryos) were immediately counted under a dissecting scope. For comparison, three wild egg masses collected from Fox Creek on the same day, November 15, 2014, were also examined using the same procedures.

Finally, the effect of predators on *E.maculata* eggs were quantified with a transplant experiment in which the eggs were left unprotected. Egg masses collected from Fox Creek were bound on white 5cm by 5cm tiles with thin rubber bands (one egg mass per tile). Six plates were placed in each of the South Fork Eel River and Fox Creek from July to September 2012. Whether the eggs on the plate remained or disappeared were examined at the end of the experiment.

Results
Life cycle of *E. maculata*
Exhaustive surveys in the upper basin of the South Fork Eel River found late instar nymphs (>5mm) of *E. maculata* only in the mainstem of the South Fork Eel River. *E. maculata* nymphs were distributed in sunny sections of the South Fork Eel River, and they were not found in upstream sections of the South Fork Eel River, where channels were narrow and shaded by forest canopy. The nymphs were found starting from early to mid-May. The late instar nymphs occurred at high density on patches of small pebbles (~2-5cm diameter), and often were locally the most dominant species, at up to 85 individuals per square meter. The bodies of *E. maculata* nymphs were often covered with epiphytes, including diatoms and *Vorticella spp*. The final instar nymphs had darkened wing pads, making them distinguishable from younger instar nymphs. The emergence of *E. maculata* were not strictly synchronized by time of day, but more emerged in late afternoon (Uno, personal observation). At emergence, the final instar nymphs swam up to the surface of the water, floated, then eclosed at the water surface and emerged out of the water. The emerged *E. maculata* subimago (pre-matured winged stage common in Ephemeroptera) flew up

high, and were not found on riparian vegetation below 3 meters tall. In captivity, the subimagoes molted to adults (imagoes) two days after the emergence, and the adults died within 2-3 days after the molt. Fifty-two percent of individuals (219 out of 419) that were collected from the South Fork Eel River as nymphs, then raised to adults in captivity were female, indicating a 50:50 adult sex ratio.

In contrast to the nymph distribution, adults were found only in four tributaries of the South Fork Eel River that flow into the mainstem reaches where nymphs were present (Table 1). The adults found in the tributaries were >99% females. No adult males were found except a few individuals that were part of the female reproductive swarm; it is unknown where the majority of males go after emergence or where mating occurs. Reproductive swarms of female adults were found in the downstream reaches of tributaries from near the confluence with the river mainstem to approximately one kilometer upstream. The adults were observed July 7th –July 22nd in 2011 (the migration probably started earlier this year, July 7th was the first day of sampling), June 18th – July 15th in 2012, June 2nd – July 5th in 2013, June 1st – July 6th in 2014 and May 18th – June 24th in 2015. The body size of the female adult *E. maculata* declined as flight season proceeded (R^2 = 0.21, $P < 0.001$), 7.4 ± 0.26 mm length and 1.5 ± 0.13 mg dry weight on July 9, 2011, 7.3 ± 0.27 mm length and 1.03 ± 0.08 mg dry weigh on July 16, 2011, then 6.9 ± 0.24 mm length and 0.83 ± 0.05 mg dry weight on July 22, 2011). The swarms formed at dusk for only about 30 minutes each day at each creek, and the swarms occurred about 30 minutes earlier in smaller creeks that had higher canopy coverage and became dark earlier than larger creeks with more prolonged light. The swarms formed continuous bands over channels, and the swarms were particularly dense over riffles where >50 individuals of adults could be captured in one swing of a butterfly net. In the swarm, each individual female adult slowly flew upstream or downstream along the current, hovered a few seconds over riffles, then jumped into riffles. Each female in swarms had an egg mass at the posterior end of her abdomen, or if not, a nematode (occasionally 2 or 3 nematodes), *Anomalomermis ephemerophagis* (Mermithidae) (Poinar, Walder and Uno, 2015) came out of the mayfly abdominal coelom when submerged in water. The proportion of female *E. maculata* that were parasitized by *A. ephemerophagis* varied by season and location, on average 38±3 %, ranging from 0 to 78% (Walder & Uno, unpublished). As female adults jumped into riffles, they get trapped on the water surface by the surface tension, shivered for a while and died (Uno, pers. obs.). Once adults fell on the water, the egg masses detached from the adult body within a second and sank to the bottom of the creek, or nematodes came out of the adult body within a few seconds breaking through the posterior end of the adult mayfly abdomen (Uno, pers. obs.). In the guts of 55 juvenile steelhead sampled in *E. maculata* reproductive habitats and during their flight period in June 2014, I found 1163 adult *E. maculata*, two *E. maculata* egg masses, and 48 *A. ephemerophagis* along with other prey such as terrestrial invertebrates.

Once the egg mass detached from the female's body, they sank to the bottom of creeks and attached on substrates. Then, they absorbed water and turned from clear to dark brown. The egg masses often formed a crust on the surface of tributaries because of their high densities: 8229 ± 6139 egg masses on average and sometimes > 20000 egg masses/m². The number of eggs per female adult averaged 302 ± 36 SE, ranging from 5 to 875. The eggs hatched between late October and mid-November. First instar nymphs were 0.64 ± 0.01 SE mm long, and were observed crawling around the egg shells in tributaries.

Most years, the first flood of the winter rainy season occurred after the eggs hatched, and the egg shells were washed out of the tributaries into the mainstem. On January 19, 2012, in the first flood of the winter, empty egg shells were captured in a drift net placed at the mouth of Fox Creek. No young instar nymphs of *E. maculata* were found in the same drift net, indicating that the young nymphs either floated down to the mainstem earlier or remained in the creeks under the substrate. No nymphs were found either in tributaries or in the South Fork Eel River until late instar nymphs (> 5mm long) were observed in the mainstem of the South Fork Eel River in April.

Isotope analysis
Carbon and nitrogen stable isotope ratios of *E. maculata* adults and nymphs as well as other aquatic insects emerging from Fox Creek and the South Fork Eel River are shown in Fig. 4. *E. maculata* nymphs and other emerging aquatic insects from the South Fork Eel River exhibited higher $\delta^{13}C$ and lower $\delta^{15}N$ than other emerging aquatic insects from a tributary, Fox Creek, corresponding to the previously reported difference in $\delta^{13}C$ and $\delta^{15}N$ in algae (food source of many aquatic insects) in the South Fork Eel River and Fox Creek (Finlay *et al.,* 1999). Adult *E. maculata* collected from tributaries had $\delta^{13}C$ and $\delta^{15}N$ values more similar to emerging aquatic insects from the South Fork Eel River than to those in Fox Creek. They overlapped in $\delta^{13}C$ and $\delta^{15}N$ values with those of *E. maculata* nymphs in the South Fork Eel River, indicating that *E. maculata* adults flying in tributaries fed, grew, and emerged from the South Fork Eel River.

Nymph distribution in the watershed
E. maculata nymphs were found in 18 out of 48 sites. The presence/absence of *E. macualta* nymphs was associated with the drainage area (Fig. 5a; logistic regression analysis R^2=0.48), and occurred in river segments where drainage area was over 35km^2. The canopy opened up at about the same threshold of drainage area that *E. macualta* started to occur (Fig. 5b; logistic regression $R^2 = 0.81$), and the water temperature also increased as drainage area increased (Fig. 5c).

Nymphal diet
Algae cell count revealed that mainstem cobbles had 6241 ± 1779 cells/cm^2 diatoms and 3429 ± 2086 cells/cm^2 cyanobacteria, while cobbles from Fox Creek only had 12.3 ± 1.6 cells/cm^2 diatoms, 23.3 ± 10.8 cells/cm^2 green algae, and 6.7 ± 6.7 cells/cm^2 cyanobacteria (Table 2a). The gut contents analysis revealed that nymphs with mainstem cobbles ingested 55 ±18% diatoms and 44 ± 18 % detritus, while nymphs with tributary cobbles ingested 1.3 ± 0.6% diatoms, 1.7 ± 1.7% green algae, and 97.0 ±2.1% detritus (Table 2b). Total number of algal cells per individual gut was 2757 ± 1003 cells diatoms with mainstem cobbles, and 5.3 ± 2.9 cells diatoms and 17 ± 9.5 cells green algae with Fox Creek cobbles (Table 2c). Most of the diatoms ingested by *E.maculata* nymphs with cobbles from the South Fork Eel River were *Epithemia sp*., a diatom that are known to be a nitrogen fixer and rich in nutrients (Round *et al.,* 1991).

Transplant experiment
The emergence rate of nymphs varied by habitat and food. The nymphs reared with mainstem cobbles successfully emerged at a higher rate than ones reared with tributary cobbles (Fig. 6a; $F_{1, 15} = 5.5$, $P = 0.03$), while the rearing habitat did not have a significant effect on the emergence rate ($F_{1, 15} = 1.6$, $P = 0.23$). The interaction of the treatments was not significant ($F_{1, 15} = 4.5$, $P = 0.052$) (Fig. 6a). The nymphs emerged earlier when reared in mainstem than when reared in the tributary ($F_{1, 97} =18.1$, $P <0.001$), and earlier when reared with mainstem cobbles than reared with cobbles

from the tributary ($F_{1, 97} = 5.8$, $P = 0.018$). The interaction of the treatments were not significant ($F_{1, 97} = 2.2$, $P = 0.15$) (Fig. 6a).

In contrast, the eggs placed in the South Fork Eel River and Fox Creek both successfully hatched at a high rate. The hatching rate in the experiment were 96.1 ± 1.3 % in Fox Creek and 99.1 ± 0.4 % in the South Fork Eel River, while the wild egg masses in the Fox Creek hatched at 99.1 ± 0.6 % (Fig. 6b). Furthermore, I could not detect any sign of predation on unprotected eggs in the transplant experiment. All 12 egg masses placed in Fox Creek and the South Fork Eel River remained intact on the tiles for over two months during the experiment.

Discussion
Field observations, sampling, and isotope analysis confirmed the migratory life cycle of *E. maculata* in the South Fork Eel River, which has been found to provide an important resource subsidy from the productive mainstem habitats to less productive tributaries (Uno & Power 2015). The fact that not a single individual late instar nymph was found in the tributaries, and no adults were found in the mainstem despite intense field observations and searches over five years, indicates that all individuals of *E. maculata* exhibit migration, at least in the studied area. While upstream flight by adult aquatic insects has been shown to compensate for downstream drift movement of larval aquatic insects in the colonization cycles of various aquatic insects (Müller, 1982), the migration of *E.maculata* from mainstem to tributaries is unique in that they migrate between two distinct habitats.

The transplant experiments of the nymphs and eggs showed no evidence of a fitness advantage to the migratory life cycles in the studied area. Nymphs required rich algae that grow in sunny environments for their food, and they exhibited higher performance when reared with the algae from the South Fork Eel River in the South Fork Eel River, their natural habitat. Given the nymphs could eat very limited amount of algae on cobbles from Fox Creek, the emergence rate would had been much lower or zero if they were reared with tributary food since when they were smaller. In contrast, eggs that naturally occur only in tributaries performed equally well both in a tributary, Fox Creek, and in the South Fork Eel River. While the distribution of nymphs is constrained to sunny mainstem rivers by their food source, there seems to be no particular advantage to ovipositing eggs in tributaries. There remains a possibility that reproduction in tributaries is advantageous for adults for reasons such as a reduction in the per capita risk of predation to the aggregation. However, this seems unlikely, as many adult aquatic insects of other species synchronously fly in the mainstem, and per capita risk of predation did not appear to be high in either habitat.

An alternative hypothesis to explain their fixed migration pattern is that it is a species trait that evolved elsewhere in the species' historic range. It may confer an important advantage in other parts of the species' distribution. *E. maculata* is widely distributed in California, and most of that range is in more arid environments than the Angelo Reserve. Many rivers where *E. maculata* nymphs are reported in Southern California are intermittent seasonal rivers (M. Bogan personal communications). In fact, in the Eel River the highest density of the *E. maculata* nymphs were observed in intermittent reaches of the sunny mainstem rivers. Sunny wide mainstem rivers, where algae grow and *E. maculata* nymphs would dominate, often flow alluvial valley. In dry season, the

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water often flows under sediments and surface water disappears from such rivers. In contrast, steep canyon tributaries with white water riffles where *E. maculata* adults would oviposit are more erosive and the water flow over bedrock, and maintain permanent flow over summer. The migration of adult *E. maculata* to small steep tributaries may be an adaptation to avoid the desiccation in an arid landscape that is typical over most of their distribution.

The migration of *E. maculata* is extremely cryptic because their migratory movements are largely invisible to a casual human observer. Adult *E. maculata* fly from mainstem to tributaries high in the air, and the young instar nymphs that drift down to the mainstem are very small. Therefore, species level identification of adult aquatic insects and their larval aquatic stages, along with rigorous comparison of their distributions is required to verify such migrations. When unusually large numbers of adult aquatic insects are encountered in unproductive small tributaries, their origin should be examined carefully, in collaboration with taxonomist, to rigorously evaluate their migratory patterns. Only when this is done, can we determine if the migration pattern of *E. maculate* is truly unique.

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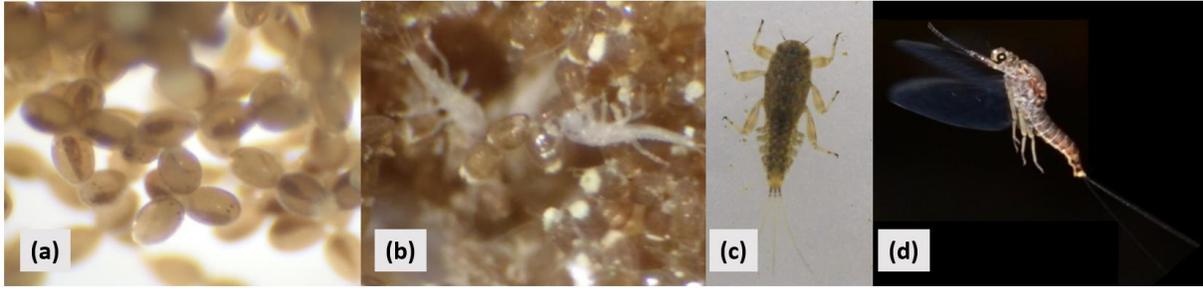
Figure 1. *Ephemerella maculata* in different life stages. (a) embryo (egg), (b) 1st instar nymphs, (c) late instar nymphs, (d) adult female.

Figure 2. Massive flight of *E. maculata* in a small shaded tributary, Fox Creek. (a, b) Dead adult female *E. maculata* floating on the water surface. (c) Ovipositing female adult *E. maculata* over a riffle. (d) Egg masses of *E. maculata* laid on substrate surface.
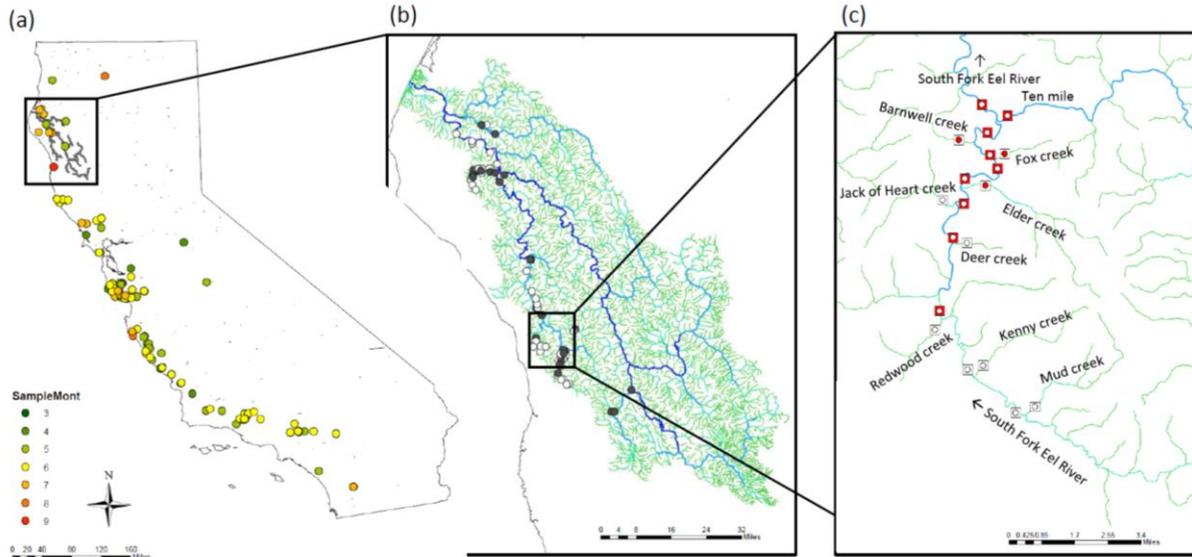
Figure 3. Distribution of *E. maculata* (a) nymph records from California Environmental Data Exchange Network (CEDEN) in 2001-2011. The colors in the circle reflect the latest month of the year when the nymphs were recorded at each location. (b) Presence (●) and absence (○) of *E. maculata* nymphs in the Eel River Basin as results of the field surveys. (c) Presence and absence of *E. maculata* adults and nymphs in upper South Fork Eel River basin around Angelo Coast Range Reserve. ■ and □ outside represents the presence and absence of nymphs, while ● and ○ inside represents the presence and absence of adults respectively.
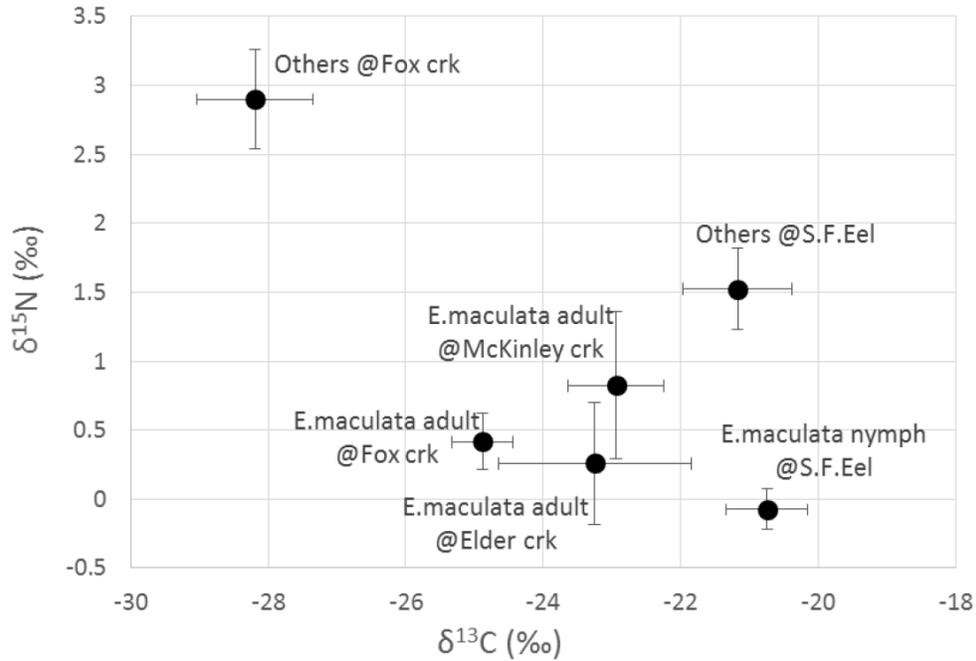
Figure 4. Carbon and nitrogen stable isotope ratios of *E. maculata* nymphs and adults collected in their rearing habitats (S.F.Eel River) and oviposition habitats (Fox Creek, Elder Creek, McKinley Creek) as well as other aquatic insects emerging from South Fork Eel River and Fox Creek for comparison. Carbon and nitrogen stable isotope ratio of adult *E. maculata* flying in tributaries were close to other aquatic insects emerging from South Fork Eel River and *E. maculata* nymphs rearing in South Fork Eel River, indicating *E. maculata* adults flying in tributaries are coming from mainstem South Fork Eel River.
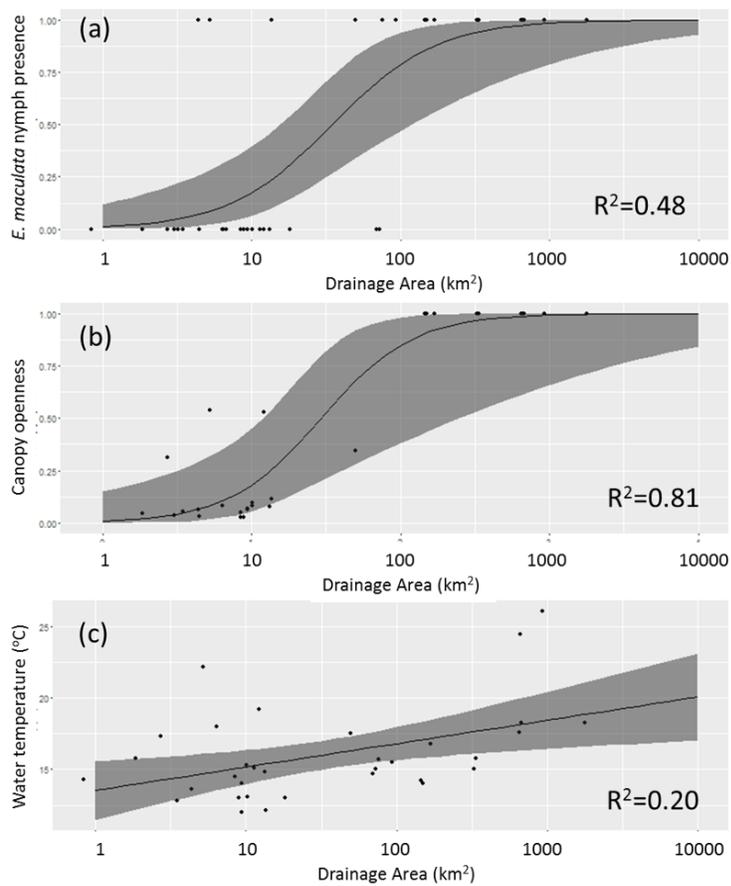
Figure 5. (a) The presence/absence of *E. maculata* nymphs along with the drainage area gradient. (b) The relationship of canopy openness and drainage area (c) The maximum water temperature and the drainage area.
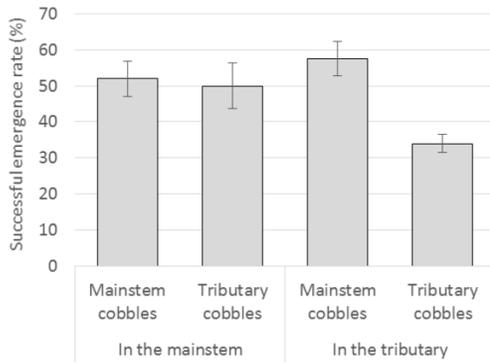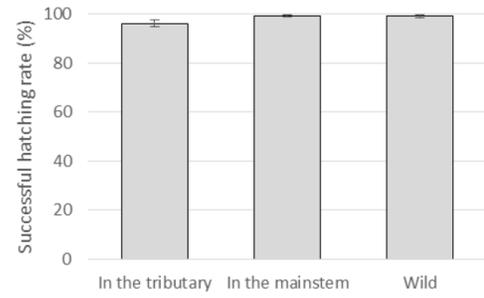
Figure 6. The results of the transplant experiment with late instar nymphs (a) and with eggs (b). Bars represent the successful emergence rate of the late instar nymphs (a) and the successful hatching rate (b).

Table 1. Description of ten tributaries and their adjacent mainstem reaches in the South Fork Eel River basin where *E. maculata* nymphs and adults presence (+) / absence (-) were examined.

Tributary name	Tributary DA (km²)	Adult in tributary	Mainstem DA (km2)	Nymph in mainstem
Mud Creek	13.3	-	40.0	-
Kenny Creek	9.1	-	72.6	-
Redwood Creek	8.1	-	83.9	+
Deer Creek	2.9	-	114.4	+
Jack of Heart Creek	10.2	-	125.9	+
Skunk Creek	0.5	-	124.9	+
Elder Creek	17.0	+	125.6	+
McKinley Creek	0.6	+	130.0	+
Fox Creek	2.7	+	149.9	+
Barnwell Creek	1.9	+	152.6	+

Table 2. (a) Algae compositions and densities on cobbles from mainstem South Fork Eel River and Fox Creek. (b) Gut content composition of *E. maculata* nymphs reared with cobbles from mainstem South Fork Eel River and Fox Creek. (c) Average number of algal cells found in gut of one *E. maculata* nymph.

(a)

Number of algal cells on cobbles (#/ cm²)	Fox Creek		S.F.Eel River	
	Average	(SE)	Average	(SE)
Diatom				
Epithemia adnata	1.6	0.6	1440	119
Epithemia sorex	0.9	0.4	1989	754
Rhoicosphenia	0.9	0.4	1646	661
Gomphonima	1.1	0.6	274	181
Cocconeis	7.8	1.7	343	247
Synedra	0	0	69	69
Encyonema	0	0	480	480
Green algae				
Green filamentous algae (cells)	23.3	10.8	0	0
Blue green algae (cyanobacteria)				
Oscillatoria	6.7	6.7	3429	2086
TOTAL	42.3	10.1	9671	2984

(b)

Proportion in gut contents (%)	Fox Creek		S.F. Eel River	
	average	(SE)	average	(SE)
Diatom	1.3	0.6	55.9	18.0
Green algae	1.7	1.7	0.0	0.0
Detritus	97.0	2.1	44.1	18.0

(c)

Number of cells in a gut	Fox Creek		S.F. Eel River	
	average	(SE)	average	(SE)
Diatom	5.3	2.9	2756.7	1003.8
Green algae	17.0	9.5	0.0	0.0

Chapter 2

Mainstem-tributary linkages by mayfly migration help sustain salmonids in a warming river network

Abstract
Animal migrations can link ecosystems across space. We discovered an aquatic insect that migrates between a river mainstem and its tributaries, and provides an important trophic subsidy for tributary predators. A mayfly, *Ephemerella maculata*, rears in a warm, sunlit productive river mainstem, then migrates as adults to cool, shaded unproductive tributaries where they oviposit and die. This migration tripled insect flux into a tributary for one month in summer. A manipulative field experiment showed that this *E.maculata* subsidy nearly tripled the growth of young of the year steelhead trout (*Oncorhynchus mykiss*) in the recipient tributary over the summer months, and was more important than terrestrial invertebrate subsidies, which have been considered the primary food source for predators in small, forested creeks. By delivering food subsidies from productive but warming river mainstems to cool but food-limited tributaries, aquatic insect migrations could enhance resilience of cool-water predators in warming river networks.

Introduction
Animal migrations can link distinct ecosystems over large landscape scales (e.g. salmon from the ocean to rivers (Wipfli *et al.* 1998), continental migrations of snow geese (Jefferies *et al.* 2004) or monarch butterflies (Calvert *et al*. 1979). Seasonal or life history migrations can concentrate many individual migrants into specific habitats, with large impacts on local communities, either as consumers (e.g. snow geese that migrate continentally, Jefferies *et al*. 2004), vectors of resources (e.g. salmon carcasses from the ocean to rivers, Wipfli *et al.* 1998) or diseases (Altizer *et al*. 2011). Habitat coupling by migratory animals may destabilize ecosystems (Jefferies *et al*. 2004), or enhance their resilience (Lundberg & Moberg 2003). Effective conservation and land management requires understanding natural migratory patterns of animals and their ecological significance.

Fluxes of adult aquatic insects from productive rivers to riparian predators have attracted the attention of ecologists (Gray 1993; Nakano & Murakami 2001; Sabo & Power 2002). River to forest subsidies primarily occur near land-water margins, with fluxes declining exponentially with distance from the shore (Power *et al*. 2004; Muehlbauer *et al.* 2014), as adult aquatic insects stay near water for oviposition (Huryn & Wallace 2000). Adult aquatic insects, in fact, often actively fly along river corridors to aquatic oviposition sites. Müller (1982) hypothesized a "colonization cycle" for riverine insects, in which upstream flight of adults compensated for downstream drift and active movement of larvae. Movement of adult aquatic insects along river corridors has been well documented (Williams & Williams 1993, Macneale *et al*. 2005). However, the effect of their longitudinal movements along channels on food webs is less well studied.

In river networks, many small tributary streams flow into larger mainstem channels. Large mainstem channels are generally wide, sunlit, and warm, and typically have high biological productivity. Smaller tributaries are narrow, shaded and cool, and typically have low productivity (Vannote *et al*. 1980). While small tributaries have received less public and political attention and protection, they make up a large part of the river network, and provide distinct habitats and ecological functions (Benda *et al*. 2004). Many cold-adapted stenothermic animals like juvenile salmonids and salamanders live in small shaded headwater tributaries that remain relatively cool through summer. However, shading by riparian vegetation often lowers productivity, so predators in tributaries are often food-limited, and rely largely on the allochthonous resource input such as

terrestrial invertebrates and organic matter for food (Nakano & Murakami 2001; Baxter *et al*. 2005; Atlas *et al*. 2013).

At confluences where small shaded tributaries meet mainstems, the adjacency of contrasting environments enable some animals to exploit favorable habitats that may change for a given season, diel time period or life stage. For example, some amphibians (Kupferberg 1996; Wheeler *et al*. 2008) and fish (Fausch *et al*. 2002) are known to migrate across confluences between mainstems and tributaries in river networks. Movements of such species link mainstems and tributary communities. Similar migrations by aquatic insects (but see Hayden & Clifford 1974), which play critical roles in stream and riparian food webs, are poorly known. Here we document a previously unknown migratory pattern in the life history of the riverine mayfly (Fig.1), *Ephemerella maculata* (Ephemerellidae), which is widely distributed across California (Allen 1968; Meyer & McCafferty 2008).

We hypothesized that adult migration of this mayfly from productive mainstems to unproductive tributaries could support consumers in tributaries. Recipient consumers include juvenile salmonids, which are increasingly thermally restricted to these cool refugia as mainstem habitats warm due to factors like drought, water withdrawals, and deforestation. This biological backflow could therefore prolong the persistence of salmonids and other cold-adapted stenothermic predators in warming river networks.

Materials and Methods
Study system
We studied mayfly life history and its ecological consequences in the upper basin of the South Fork Eel River, in and around the Angelo Coast Range Reserve in Mendocino County, California (39°44'17.7" N, 123°37'48.8" W). Under Mediterranean seasonality, the South Fork Eel River watershed typically has wet winters and dry summers. Wide mainstem reaches of the South Fork Eel River (that drain >80km^2) are sunlit, and in summer, water column temperatures warm to 25°C (Fig. 2a,c). In contrast, smaller tributaries of the South Fork Eel River are shaded by riparian forest, and remain cool (Fig. 2b,c). Mainstems support productive blooms of attached algae (Power *et al*., 2008), which in turn support abundant aquatic insect larvae that feed not only aquatic predators (Finlay *et al*. 2002), but also terrestrial insectivores (spiders, lizards, and bats) via emergence (Power *et al*. 2004, Sabo & Power, 2002). In contrast, in small tributaries of the South Fork Eel, where primary productivity is light-limited, algal accrual is limited and almost entirely suppressed by armored grazers (*Glossosoma spp*.), which are largely avoided as prey by the dominant predators (McNeeley *et al*. 2007). Therefore the predators in the small tributaries largely depend on allochthonous subsidies (Atlas *et al*. 2013) for their food.

The South Fork Eel River joins the Eel River, which flows to the Pacific Ocean. Anadromous steelhead trout (*Oncorhynchus mykiss*) spawn in the mainstem and tributaries of the South Fork Eel River. Juvenile steelhead rear in the river for two years before they migrate to the ocean. *O. mykiss* is the only fish found in small tributaries, co-occurring there with the Pacific giant salamander (*Dicamptodon tenebrosus*). The density of juvenile steelhead varies from year to year, with estimates for Fox Creek ranging from 0.17-1.5 individuals m^{-2}. In the South Fork mainstem, in contrast, juvenile steelhead are less dense, and outnumbered by other fishes, such as California

roach (*Lavinia (Hesperoleucus) symmetricus*), three-spined stickleback (*Gasterosteus aculeatus*), and Sacramento pikeminnow (*Ptychocheilus grandis*) (Fig. 2d).

In this study, we investigated the distribution of adult and nymphal *Ephemerella maculata* in a 25-kilometer reach of the South Fork Eel River mainstem and ten tributaries (Fig. 3). We observed and quantified the mayfly subsidy flux and conducted a field experiment to evaluate its impacts on consumers in Fox Creek, a tributary of the South Fork Eel River. Fox Creek drains 2.6 km^2, and during summer base flow is 0.5-2m wide with pool depths ranging from 25-60 cm.

Life history description of *Ephemerella maculata*
In Fox Creek, floating accumulations of dead adult mayflies that cover large portions of pool surfaces have been observed by researchers during early summer for over 20 years. Mayflies were identified as *Ephemerella maculata* (Ephemerellidae) (Allen 1968) in 2010. To investigate how the life history of *E. maculata* led to massive accumulations of dead adult females in unproductive tributaries, we carried out intensive visual field observations in the South Fork Eel River and ten of its tributaries including Fox Creek from 2012-2014.

Predator density and subsidy flux estimation
Areal rates of input of terrestrial and adult aquatic insects into Fox Creek were measured with pan traps and drift nets. To measure terrestrial insect subsidies, five transparent circular pan traps (30cm diameter) were set on the water surface of Fox Creek for 24 or 48 hours on five occasions from June through July 2012. Because *E. maculata* adults and many other adult aquatic insects selectively oviposit and get drawn into stepped riffles, their flux was not measurable with pan traps. Therefore, we set drift nets across the channel downstream of riffles, and measured mayfly input to each riffle, corrected for the capture efficiency of drift nets (Fig. S1). In June and July 2014, six drift nets were set out 9 times at 3-7 days intervals for 1-2 hour periods at dusk, when most of adult aquatic insects including *E. maculata* plunge into the water. Color-marked Douglas fir katkins (floating tree flowers similar in size and buoyancy to spent adult *E. maculata*) were used to estimate capture efficiency of drift nets in each riffle where the subsidy was measured. We distributed 50 color marked katkins evenly over the riffle just upstream of the driftnet, and in the next two riffles upstream, with distinct color for each riffle. After 1-2 hours we counted how many of the released katkins were captured in the drift net. Very few katkins released in two most upstream riffles were captured in drift nets, so only inputs to the adjacent upstream riffle were considered as a source to the drift nets. The capture efficiency from the nearest riffle varied from 34-92% among riffles. Biomass of *E. maculata* and adult aquatic insects captured in drift nets was divided by the capture efficiency estimated for the given riffle to estimate total input to the riffle, which was then divided by the area of the riffle to estimate area-specific input. Finally, the estimated mayfly input per riffle area was converted to the biomass per channel area by multiplying the input per riffle by the proportion of the total wetted channel area made up of riffles (46%) over the surveyed reach.

The densities of fish and salamanders in Fox Creek and the South Fork Eel River were estimated by visual counts from the bank at night and snorkeling surveys in July 2013 following the methods described in Hankins & Reeves (1988). In Fox Creek, 24 riffles and pools over a 300 m reach were mapped, surveyed and all the fish and salamanders were counted. In 2013, Fox Creek steelhead fell almost entirely into two age classes: young-of-the-year (age 0, hereafter YOY) and juveniles

of more than one year of age (hereafter 1+). In the South Fork Eel River, 7 riffles and 7 pools in an 1130-m reach near the Fox Creek confluence were surveyed and all the fish and salamanders were counted. Densities were estimated as counts per water surface area.

Gut contents analysis
Juvenile (1+) steelhead diet composition was quantified in three creeks (Fox Creek, Elder Creek, Barnwell Creek: Fig. 3) where *E. maculata* adults oviposit. Sixty-eight juvenile steelhead (47 in Fox Creek, 11 in Elder Creek, and 10 in Barnwell Creek) were captured by night-time dip-netting in each creek over one or two days. We used non-lethal gastric lavage, which limited our diet samples to larger (1+) individuals. The sampled 1+ steelhead were 105.9 ± 2.5 mm standard length, and 14.4 ± 1.1 g live weight. The gut contents of each individual were filtered through 0.3 mm mesh nylon netting in the field, and frozen within 3 hours. Fish gut contents were separated into categories (i.e., *E. maculata*, other aquatic insects, terrestrial insects, fish and unknown) under a dissecting microscope. Each category of food from each individual fish was separately oven dried at 60°C for 24 hours and weighed. The proportion of the gut contents in each creek was calculated by summing each category of gut contents weight of all individual fish in the creek, and dividing by the total gut contents weight of all individual fish in the creek.

Field manipulative experiment
A manipulative field experiment was conducted in Fox Creek to evaluate the impact of the *E. maculata* subsidy relative to that from other terrestrial insects on growth of YOY steelhead in Fox Creek, we manipulated both subsidies experimentally. After four weeks, we compared the growth of YOY steelhead trout among treatments: with and without terrestrial subsidy, and with and without the *E. maculata* subsidy (Fig. 4).

Sixteen reaches of Fox Creek, each containing one upstream riffle and one downstream pool, were fenced with 5mm mesh Vexar® plastic fence to enclose YOY steelhead for the experiment. The sixteen reaches spanned 300 m of Fox Creek, and were spaced more than 2 m apart. The area of the enclosed reaches ranged from 5.1 to 22.9 m^2, and maximum depth varied from 25-60 cm. The 16 reaches were divided into four blocks of four reaches from upstream to downstream, and within a block, each experimental reach was randomly assigned one of four treatments: *E. maculata* subsidy present and terrestrial subsidy present, *E. maculata* subsidy absent and terrestrial subsidy absent, *E. maculata* subsidy present and terrestrial subsidy absent, and *E. maculata* subsidy absent and terrestrial subsidy present (Fig. 4b).

We used past field estimates to determine our experimental stocking densities. Juvenile steelhead densities in Fox Creek varied from year to year. In 2013, juvenile steelhead, were present at densities of 0.071 individuals m^{-2} (YOY) and 0.096 individuals m^{-2} (1+). In 2009, densities of YOY and 1+ steelhead were 1.25 individuals m^{-2} and 0.26 individuals m^{-2}, respectively. We chose an average of these YOY estimates for our experimental stocking density of 0.6 individuals m^{-2} of YOY. 1+ and larger steelhead trout were removed from the enclosures to avoid the risk of predation on YOY steelhead, as *O. mykiss* is threatened in California. All YOY steelhead for the experiment were collected from the experimental reaches of Fox Creek, and randomly assigned and redistributed to enclosures one week prior to the beginning of the experiment. We standardized the density and size of YOY steelhead among all four treatments, so that at the onset of the experiment, there were no statistical differences in initial size among treatments (Table S1).

To control the migratory subsidy, we used driftnets and dipnets to remove *E. maculata* adults floating on Fox Creek after oviposition every day from early to late June during their flight period. These mayflies were frozen. We began the enclosure experiment as soon as their flight period ended in early July by adding defrosted *E. maculata* to mainstem subsidy-present treatments every dusk until the experiment ended early August. We standardized this input of the *E. maculata* at average levels measured during their flight period, adjusting *E. maculata* input to juvenile steelhead density, so that each individual received 1.5 g in wet mass, equivalent to 0.23 g in dry mass of *E. maculata* adults per day for one month. Based on the previous estimate, the mean *E. maculata* adult flux into Fox Creek was 0.152 g $m^{-2}d^{-1}$. As the density of fish in the creek is 0.6 individuals m^{-2} on average, the *E. maculata* adult subsidy that each fish received was estimated as 0.25 g d^{-1} (0.152 g $m^{-2}d^{-1}$ divided by 0.6 individuals m^{-2}). Therefore, the amount added in the experiment was slightly less than the natural input of *E. maculata* adults into the creek available per capita to rearing steelhead. The terrestrial subsidy was excluded from terrestrial-free treatments with roofs of transparent plastic and screen (Fig. 4a) after the method of Atlas *et al.* (2013). Roofs had no effect on temperature, water flow, or chlorophyll-a in benthic algae in enclosed pools (Table. S2, S3).

All steelhead were individually tagged (Fig. S2) and their body sizes (wet mass and standard length) measured at the beginning and the end of the experiment. Visible Implant Elastomer Tags (Northwest Marine Technology, Inc.) were injected under the skin of all YOY steelhead, and individuals were distinguished with distinct combinations of color and position on the body. Initial average standard length and body weight of YOY steelhead used in the experiment was 46.0 ± 1.3 mm and 1.96 ± 0.14 g.

To examine indirect effects of subsidies to predators on the other community members, we measured chlorophyll-a and the benthic invertebrate density in each site before and after the experiment (Table S2). Algae were sampled from three cobbles in each enclosed pool: $25cm^2$ of the cobble surface was scrubbed to collect algae and biofilm, and acetone extraction and fluorometry were used to estimate the mean chlorophyll-a per area on each sampled area. All benthic invertebrates >1mm long were counted on three randomly selected benthic cobbles 10-20 cm in median diameter.

Changes in standard length and weight of the YOY steelhead in each site were analyzed using a two-way randomized block design ANOVA, with longitudinal (upstream-downstream) block treated as a random factor. The analyses were performed on average growth values of the individual fish in length (mm) and weight (g) within each enclosed pool. Cochran's tests confirmed that variances were homogeneous among treatments, so the analyses were conducted on untransformed data. Preliminary tests found no evidence of interactions between the two treatments and blocks (P > 0.8 in all cases), so sums-of-squares for these interactions were pooled into the error mean square prior to testing main effects.

Seasonality of growth of free-swimming fish

The standard length of YOY steelhead trout was measured five times throughout the year from 2013-2014 (Fig. S1). In each sampling event, ten or more YOY steelhead trout were captured,

measured, and released outside the enclosures in the same reach of Fox Creek where *E. maculata* subsidies occur.

Results
Life cycle of *Ephemerella maculata*
Intensive field observations (2012-2014) in the upper South Fork Eel River revealed that different life stages of the mayfly *E. maculata* occupy different habitats, suggesting a migratory life cycle. Large (5mm-10mm) late-stage nymphs occur only in sunlit rivers from May to July, where they sometimes dominate benthic invertebrate assemblages. Ovipositing adult female *E. maculata* were only found flying above small tributaries (in four of ten surveyed, Fig. 3), up to 1 km upstream the confluences with mainstem reaches where *E. maculata* nymphs occurred. Adult females emerge from the mainstem reaches, mate, then fly into tributaries every evening for about 30 minutes at dusk for a month between June and July. Ovipositing females plunge into white water in riffles, drop their egg masses, and die on the water surface, to float downstream and collect in pools occupied by juvenile steelhead. *E. maculata* eggs sink and stick to the riverbed, remaining in the tributaries in a resting state until December, when they hatch and the small first instar nymphs drift down tributaries into the mainstem (Fig. 1). The timing of the life cycles varies across rivers, years, and locations: dates of observations of *E. maculata* nymphs in rivers throughout California have ranged from March to August (Meyer & McCafferty 2008).

Resource subsidy to tributary predators
In Fox Creek where *E. maculata* adults were observed from early June until early July in 2014, the estimated input of *E. maculata* from the confluence with the mainstem to 1 km upstream was 0.15 ± 0.06 SE g dry mass $m^{-2}d^{-1}$ (86.2 individuals $m^{-2}d^{-1}$). In contrast, the average input of terrestrial insects and other adult aquatic insects into Fox Creek during this same period was 0.075 ± 0.031g dry mass $m^{-2}d^{-1}$ and 0.0015 ± 0.0007g dry mass $m^{-2}d^{-1}$ respectively.

Stomachs of juvenile steelhead in recipient tributaries were distended during the mayfly flight period, and gastric lavage showed that their stomachs were full of dead *E. maculata* adults (Fig. 3). In the creeks where *E. maculata* migrated, adult mayflies were the dominant food item in the guts of 1+ steelhead trout, making up 14-47% (average proportion) of gut contents. Few gut contents of YOY steelhead trout were examined, as they were generally too small for non-lethal gastric lavage. We observed, however, that YOY steelhead trout also foraged on *E. maculata* adults. We also observed active foraging on adult *E. maculata* by other predators in tributaries: pacific giant salamanders (*Dicamptodon tenebrosus*), foothill yellow-legged frogs (*Rana boylii*), water striders (*Gerris remigis*), spiders (*e.g. Tetragnatha spp.*), and bats (*Myotis spp.*)

Effect of the subsidy on YOY steelhead growth
In enclosed segments of the creek where YOY steelhead received the *E. maculata* subsidy, they grew nearly three times more than without the *E. maculata* subsidy, while exclusion of the terrestrial subsidy had no significant effect (Fig. 5, Table S1). YOY steelhead that received the *E. maculata* subsidy grew 6.6 ± 0.7 mm (14.3%) in length, and gained 0.68 ± 0.07 g (34.7%) in live weight over the month-long experiment, while the steelhead without the *E. maculata* subsidy grew 2.8 ± 0.7 mm (6.1%) and had little or no weight change (-0.08 ± 0.11 g; -4.1%). During this one-month period (which this year was after the flight period for mayflies), the *E. maculata* subsidy

increased YOY steelhead growth three-fold over the average growth in length and weight (1.5 ± 0.5 mm (3.3%) and 0.42 ± 0.09 g (21.4%)) of free-swimming YOY steelhead in the same creek reaches outside the enclosures. Potential indirect impacts of subsidies on algae and benthic invertebrates were also examined, but no effects were detected (Table S2).

Seasonal growth patterns of YOY steelhead trout in Fox Creek showed that free-swimming YOY steelhead trout grow very rapidly when *E. maculata* migrate into tributaries (Fig. 6). The eggs of steelhead trout are laid in February and March, and the YOY fry (20-30mm standard length) emerge from the stream bed in late May. They grow rapidly until August, with little growth over the subsequent winter.

Discussion
This newly discovered adult mayfly migration represents a cryptic but potentially important trophic pathway that can support food-limited predators in headwaters and small tributaries. Our observations and experiment show that the *E. maculata* subsidy can support and enhance growth of YOY steelhead trout rearing in small tributaries during a critical growth period. While terrestrial subsidies have been considered the primary food source for juvenile salmonids rearing in small creeks, the effects of terrestrial insects on YOY steelhead growth were insignificant in our experiment. Mainstem-to-tributary subsidies should be further considered, in addition to subsidies from watersheds and from the ocean, as potential trophic linkages that can fuel food webs and add resilience in river networks.

The *E. maculata* subsidy lasts for only one month, but it occurs in summer when tributary water temperatures result in high rates of YOY steelhead growth. YOY steelhead trout in Fox Creek grew rapidly when the *E. maculata* subsidy occurred, but added little length over the rest of the season (Fig. 6). At favorable temperatures, the food demand of juvenile salmonids increases, along with their growth rates if food levels are sufficient (McCullough *et al.* 2009). Our experiment showed that summer growth of YOY steelhead is food-limited without the *E. maculata* subsidy. The *E. maculata* subsidy is also likely stored as lipid to fuel annual growth, as has been shown for other salmonids exploiting resource pulses (Armstrong & Bond 2013; Armstrong & Schindler 2011). Increased summer growth and lipid stores should also increase overwinter survivorship (Biro *et al*. 2004). Although the terrestrial subsidy flux measured in this study (0.075 g m^{-2}d^{-1}) is similar to the flux of terrestrial subsidy Nakano and Murakami (2000) reported in summer in Japan (0.050 to 0.130 g m^{-2}d^{-1}) as being a significant food source for aquatic predators, its contribution to YOY steelhead growth was small when compared to the *E. maculata* subsidy, and not statistically detectable in our month-long experiment. Lower-level but more prolonged terrestrial subsidies likely contribute to fish production over the rest of the year, but the *E. maculata* subsidy is more important for the summer growth of YOY steelhead in our system.

Synchrony of the subsidy both in time of the day (~30 minutes at dusk) and season of the year (one month from June to July) distinguishes the *E. maculata* subsidy from other subsidies in the nature. *E. maculata* adults accumulated in pools or stranded along shores are indicative of predator satiation or handling time limitation. The stomach of many (both YOY and 1+) steelhead were distended and full of *E.maculata* adults when subsidized. The pulsed nature of the subsidy appeared to reduce or eliminate agonistic interactions of juvenile steelhead in Fox Creek, within

and across cohorts. At very high levels of food abundance, agonistic behaviors to defend food resources is often abandoned, as there is little benefit from interference when all foragers are satiated (Davies & Houston 1984). In the experiment, we only used YOY steelhead trout to avoid the risk of the cannibalism. However, we believe the benefit to YOY from the *E.maculata* subsidy would not change in the presence of 1+ steelhead trout, given that the 1+ steelhead trout would also be satiated by the *E.maculata* subsidy. Although not examined in this study, it seems likely that the *E.maculata* subsidy would decrease predation by 1+ steelhead on YOY, and therefore increase YOY survivorship.

A trophic subsidy to shaded cool tributaries would be particularly important for cold-adapted stenothermic predators like salmonid juveniles, which are concentrated in these thermal refugia in warming rivers (Fig. 1d). As summer river temperatures warm above 20° C, juvenile salmonids become increasingly physiologically stressed (McCullough *et al*. 2009, Richter & Kolmes 2005), and also more susceptible to parasites and diseases (McCullough *et al*. 2009; Marcogliese 2001), as well as competition or predation from invasive warm-water fishes (Reese & Harvey 2002). Therefore, small tributaries that sustain cooler water over summer would be critical refuges for stenothermic predators like juvenile salmonids and many salamanders. The density of juvenile steelhead in Fox Creek was five times higher than in the river mainstem (Fig. 2d). The *E. maculata* subsidy occurred only in the downstream reaches of tributaries; however, these locations are particularly important habitat for anadromous salmonid juveniles whose distribution is limited by adult upstream migration. In Fox Creek in 2012, steelhead in the *E. maculata* subsidy recipient reach were twice as abundant as those occupying reaches more than 1 km upstream of the confluence (0.32 ± 0.09 individuals m^{-2} compared to 0.17 ± 0.08 individuals m^{-2}). By redistributing resources from the productive but stressfully warm mainstems river to cool but food-limited tributaries, the *E. maculata* migration could help sustain cold-adapted stenothermic predators in river networks.

Prey movements that support stenothermic predators may be of increasing importance in warming rivers and other habitats. With deforestation, water withdrawals, and climate warming, temperatures of river mainstems are increasing (Webb *et al.* 2008). Shaded tributaries that remain cool through the summer rearing period are increasingly important thermal refugia for juvenile steelhead and other cold-water native species. California is at the southern limit of the geographic range of coho salmon (*Oncorhynchus kisutch)*, chinook salmon (*Oncorhynchus tshawytscha*), and steelhead trout. The *E. maculata* migration that delivers food resources from productive warm mainstem rivers to adjacent cool, otherwise food-limited tributaries, could help rearing salmonids persist in warming river networks in California.

Migrations of adult aquatic insects from mainstems to tributaries, though not yet well-known, may be widespread. Another mayfly *Leptophlebia cupida* (Hayden & Clifford 1974) also migrates between mainstem and tributaries, but the nymphs migrate from mainstems to tributaries, in the opposite direction to *E. maculata*. Longitudinal flights of adult aquatic insects have been widely observed (Müller 1982, Williams & Williams 1993, Macneale *et al*. 2005), but the extent to which these flights traverse confluences is not yet known. Movements of adult aquatic insects are cryptic. It is far more difficult to observe aerial adult aquatic insects than the movements of their larvae in water (Macneale *et al*. 2004). Flight and mating activity can occur high above the ground, and in many species, including *E. maculata*, reproductive migrations are tightly synchronized within

narrow seasonal and diel time windows. The lack of taxonomic expertise to associate adult and juvenile life stages of potential migrants may also challenge field biologists attempting to study whole life cycles of aquatic insects. More collaborative research on life histories of aquatic insects by field biologists and taxonomists, along with new technologies such as isotope analysis (Macneale *et al*. 2005) and genetic tools (Valentini *et al*. 2008) may help reveal whether similar migrations in other insect taxa convey undetected subsidies from productive mainstems to predators in tributaries.

The importance of trophic subsidies across habitat boundaries have long been recognized by ecologists (Summerhayes & Elton 1923). Subsidies delivered by directed migrations of small organisms, however, are more difficult to detect than chronic cross-habitat fluxes of multiple resource species over relatively short distance (e.g. seaweed stranded on the coast (Polis & Hurd 1995), or aquatic insects dispersing into forests (Power *et al.* 2004)). While animal migration can transport massive amounts of resource over long distances, the short duration of these synchronized population life history events may make them harder to observe. Long distance movements of migrants from their source to recipient habitats may also make it harder to recognize the subsidy fluxes they contribute. While migrations are commonly known for large animals like mammals, birds and fish, migratory patterns of small animals like insects are understudied, except for a few species like locust, monarch butterflies or milkweed bugs (Dingle 2014). Further study of insect migrations may reveal additional cryptic trophic linkages over landscapes with large potential ecosystem impacts. In river networks, the importance of habitat connectivity for migratory fish, especially for salmonids, is well known. Much remains to be learned, however, about how salmonid persistence in freshwater rearing habitat is influenced by migrations of their food organisms, and the habitat features required by these invertebrate river migrants. Given the strong impact of a single migratory species, *E. maculata,* in tributaries where it was detected, we suggest that occurrence and impacts of other such migrations in river drainages be further examined, so that the environmental features and types of connectivity that support such services can be maintained in watersheds.

Rivers and many other natural environments exhibit surprising degrees of thermal heterogeneity at small to large scales (Nielsen *et al*. 1994; Helmuth & Hofmann 2001). With global warming, habitat shifts of cold-adapted stenothermic animals to cooler local habitats could allow their survival and persistence (Scheffers *et al*. 2014; Moritz & Agudo 2013, Kearney *et al.* 2009), but if productivity in cool refugia is light- or temperature-limited, taking refuge may come at the cost of lowering their energy intake. Migration-mediated subsidies and other fluxes of prey or food resources from warm, productive habitats to cool refugia could help sustain cold-adapted consumers in a warming world. Conservation of environmental features that connect habitats is needed, not only for consumers, but also, in some cases, for their important mobile prey.

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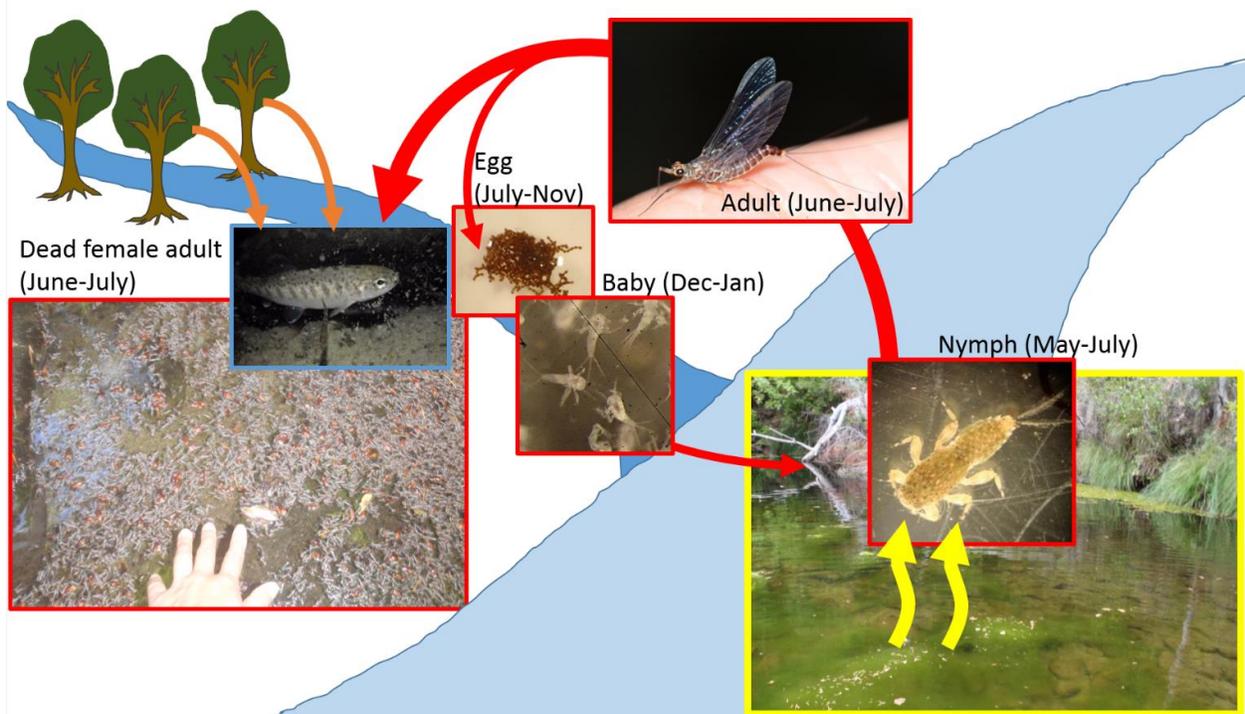
Figure 1. Life cycle of *Ephemerella maculata* and associated energy flow. *E. maculata* nymphs feed on algae in productive mainstem, then emerge as adults and mate. Female then fly into small tributaries, oviposit and die. The dead female adults subsidize predators rearing in cool unproductive tributaries including juvenile steelhead.
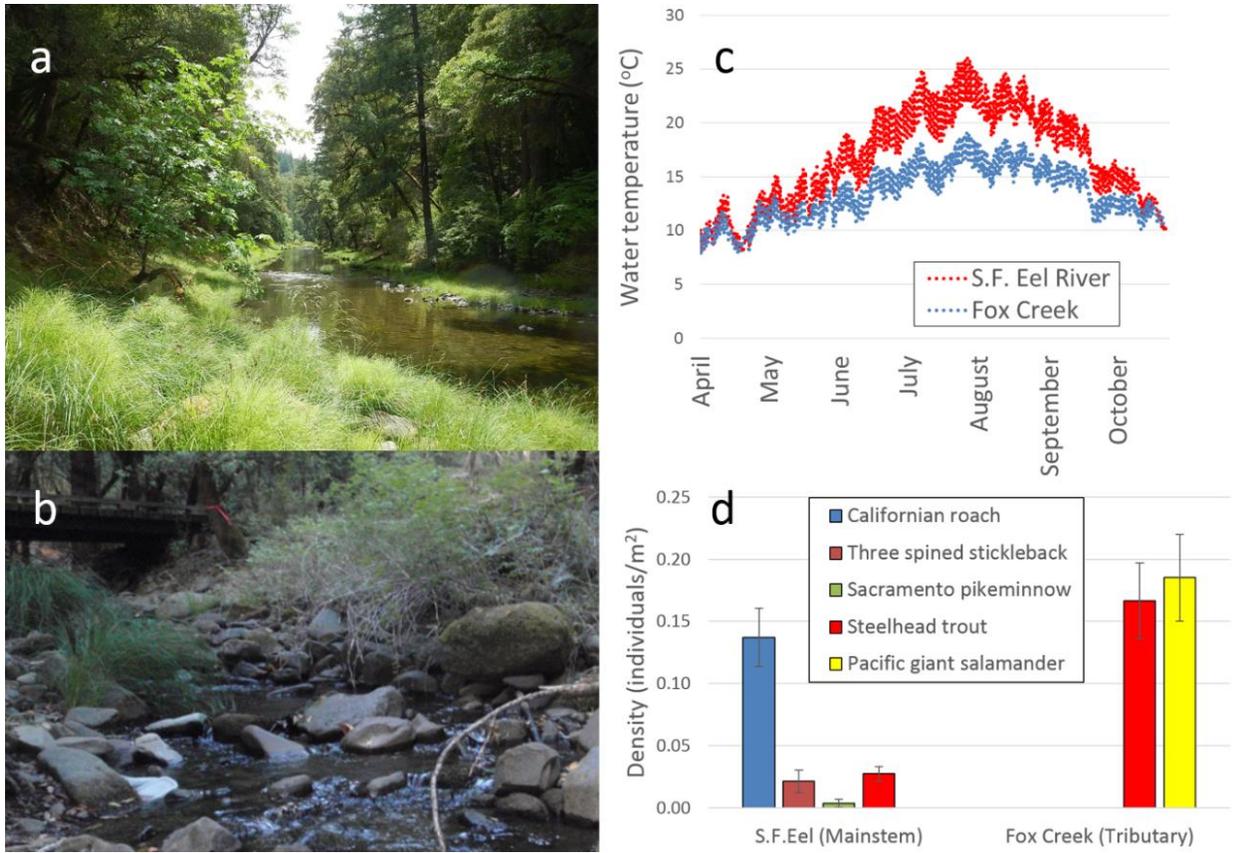
Figure 2. **Environment and predator distributions in mainstem and tributary.** (**a**) Mainstem of the South Fork Eel River. (**b**) Fox Creek, a tributary of the South Fork Eel River. (**c**) Water temperature in the South Fork Eel River and Fox Creek. (**d**) Major vertebrate predator densities in the South Fork Eel River and Fox Creek in 2013. Error bars show mean ± SE.
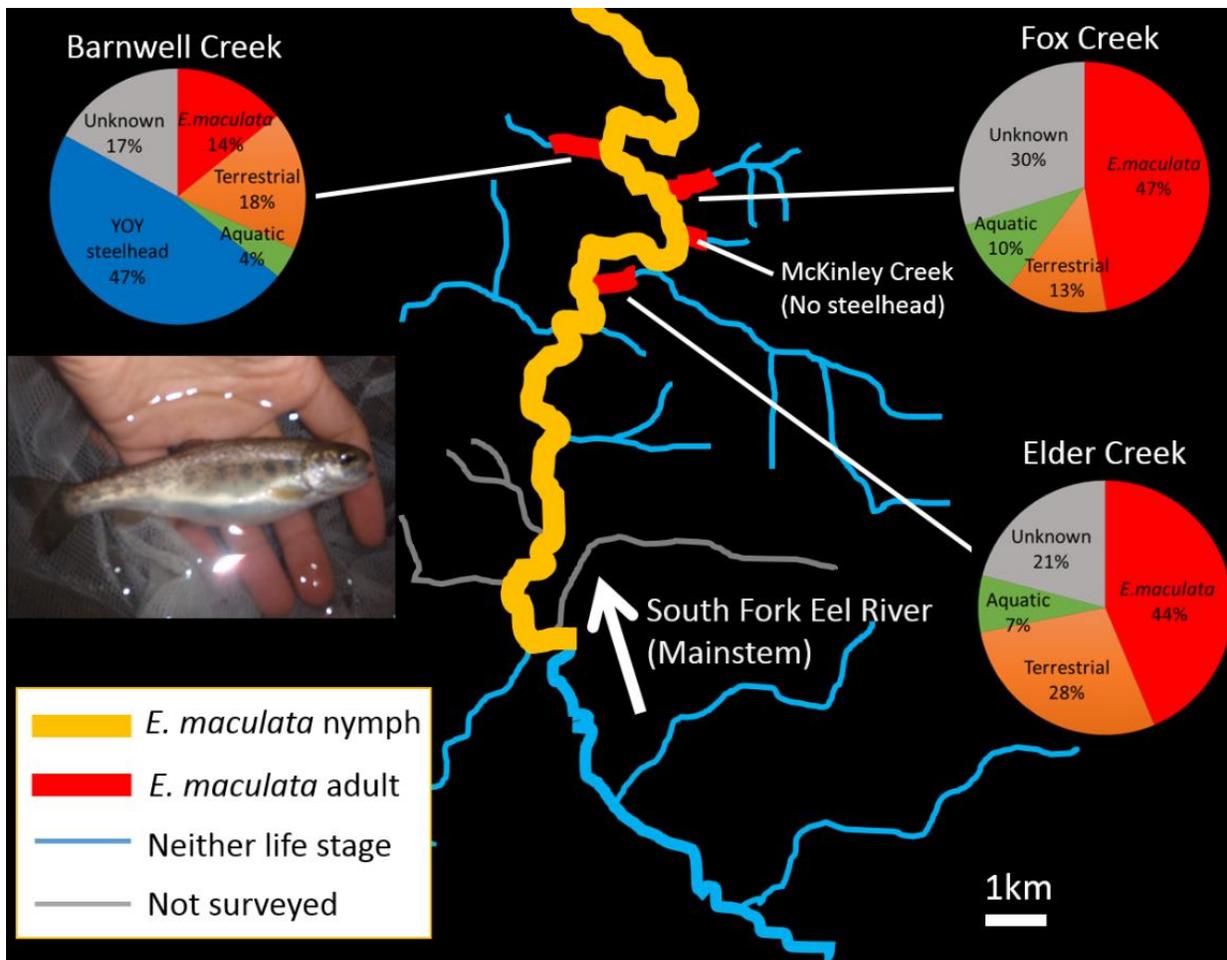
Figure 3. Migratory pattern of *E. maculata* in the headwater South Fork Eel River, and juvenile steelhead gut contents in *E. maculata* recipient tributaries. Red lines indicate reaches *E. maculata* adults but no nymphs were found; yellow lines indicate reaches where *E. maculata* nymphs but no adults were found; blue lines are reaches where neither adults nor nymphs *E. maculata* were found; gray lines are non-surveyed reaches. The pie graphs show the proportion of gut contents in 1+ steelhead trout in three creeks in June and July 2014. Inset, lower left: typical 1+ steelhead in Fox Creek with its stomach distended with *E. maculata* adults.

Figure 4. Design of the field experiment. (a) Experimental manipulation of mayfly subsidy and terrestrial subsidy (b) The distribution of field sites in Fox Creek. The thin lines are 1m contour lines, and the thick lines are 5m contour lines. The bold line shows the border of Fox creek watershed.
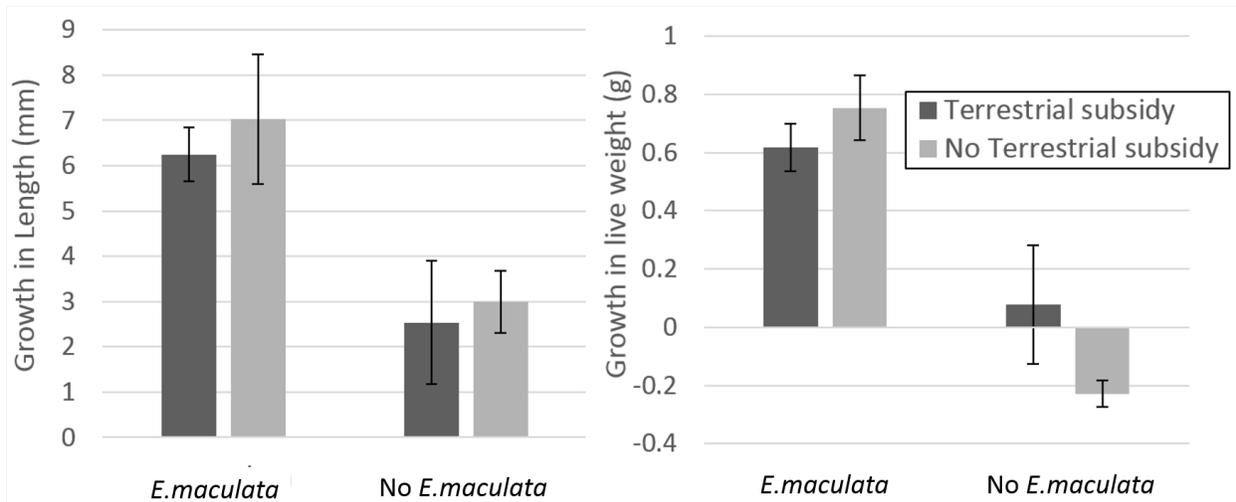
Figure 5. Growth of juvenile steelhead in standard length (a) and live weight (b). The *E. maculata* subsidy significantly increased the growth of the juvenile steelhead (standard length: $F_{1,9} = 12.5$, $P = 0.006$, weight: $F_{1,9} = 41.7$, $P = 0.0012$), while the terrestrial subsidy did not (standard length: $F_{1,9} = 0.32$, $P = 0.6$, weight: $F_{1,9} = 0.55$, $P = 0.5$). The interaction of the treatments were not significant (standard length: $F_{1,9} = 0.03$, $P = 0.88$, weight: $F_{1,9} = 3.5$, $P = 0.095$). Error bars show mean ± SE.
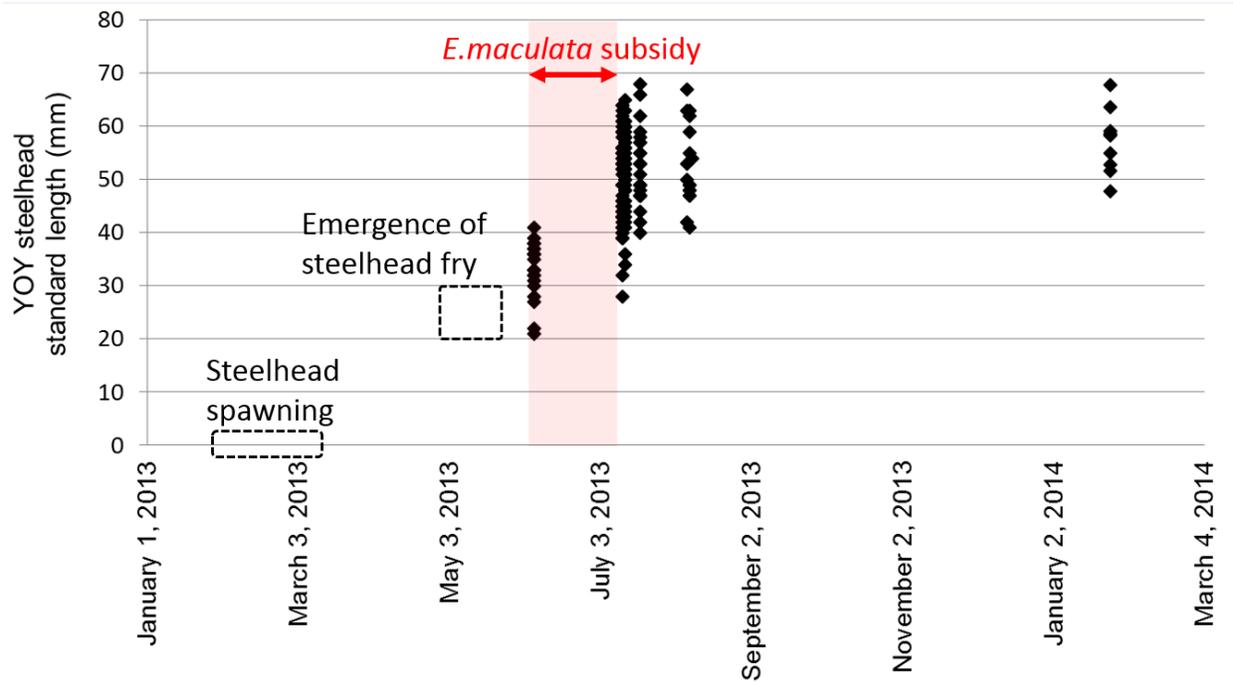
Figure 6. Annual growth of YOY steelhead in Fox Creek. The eggs of steelhead trout were deposited between February and March 2013, and the first steelhead fry (20-30mm standard length) emerged from the nest to the water column on May 14th in 2013. The juvenile steelhead grow rapidly until mid-August, then maintain their body size until the next spring.

Chapter 3

Whole life cycle response of an annual mayfly *Ephemerella maculata* to thermally varying seasonal environment

Abstract
Organisms that live in temperate regions are exposed to wide range of temperatures due to seasonal variation, inter-annual differences in weather, and variation among different habitats. Annual organisms with synchronous life cycles are exposed to distinct thermal environment at each life stage in different season. To predict the impacts of climate change on organisms in seasonal environments, it is thus important to understand how their whole life cycle responds to thermal variation. Here we investigate, with lab experiments and field observations, whole life cycle responses of an annual mayfly *Ephemrella maculata* to seasonal variation in the thermal environment. We test hypotheses that thermal optima and tolerance range in *E. maculata* shifts across life stages corresponding to the seasonal variation in the water temperature, and that timing of life stage transitions plastically shift in response to the thermal regimes of their habitats, matching the thermal range of the exhibiting life stage and the exposed temperature.

The lab experiments confirmed that the thermal optima and tolerance range of *E. maculata* varied across life stages corresponding to the temperature that each life stage naturally experiences in its habitat within a particular season. Furthermore, timing of life stage transitions was controlled by the water temperature, allowing them to seasonally match the optimal temperature range with the seasonal temperature fluctuation. Despite the relatively narrow thermal range of each life stage, the shift in thermal range across life stages and plastic seasonal adjustment of life stage transition timing allows them to live in a thermally variable seasonal environment. Field observations confirmed that plastic adjustment of life stage transition timing in response to thermal conditions predicted from the lab experiments occur naturally, corresponding to the spatial and year-to-year variation in thermal regimes. Early emergence of *E. macualta* nymphs from warm rivers and years allow them to avoid exposure to high temperature as heat sensitive nymphs. Organisms with annual life cycles that have distinct thermal range across life stages and flexible phenology matching the prevailing temperature regime may be more resilient to the climate change than would be predicted from a single life stage.

Introduction
Temperature is an important environmental factor that affects the performance of organisms (Hochachka & Somero, 2002). Most regions of the earth exhibit seasonally varying thermal environments, so resident organisms experience a wide range of temperatures through their lives. A synchronous pattern of phenology (the timing of life history events), often observed in seasonal environments is not only advantageous ecologically (e.g. taking advantage of seasonal resources; Hatchinson 1961, finding a mate or pollinators: Faegri and Van Der Pijl 1971, avoiding predators: Sweeney and Vannote 1982), but also important physiologically to cope with the seasonally varying thermal environment.

Some organisms respond to a varying thermal environment by having a wide range of temperature tolerance through their lives (Somero *et al.* 1996). However, maintaining tolerance to a wide range of temperatures can come at a cost to performance in moderate temperatures (Kristensen *et al.,* 2008; Willett 2010). The different life stages of organisms with complex life cycles often live in thermally distinct habitats, and their optimal temperature and tolerance range varies matching with the characteristic thermal regime in their respective microhabitats (Kingsolver *et al*., 2011; Miller *et al*., 2013; Pincebourde & Casas, 2015) As annual organisms with complex life cycles are also

exposed to distinct temperature regimes for each life stage, they may also develop life stage specific thermal range as an adaptation to the seasonally varying thermal environment.

For organisms adapted to the seasonal environment, timing of life stage transitions (phenology) is a critically important part of their thermal response. Phenological stage transitions of organisms are often cued by specific physical conditions, such as temperature, photoperiod, or water availability. Some organisms exhibit local adaptation in their phenology, and their life stages shift at appropriate timing for each local habitat (Bradshaw *et al.,* 2004, Ragland and Kingsolver, 2008). However, the environment also varies from year to year both as natural variation and as a result of the climate change. Plastic responses to environmental variation in addition to such evolutional responses would enable organisms to thrive in such varying environment (Charmantier *et al.,* 2008). Phenological shifts corresponding to climate change have been reported in various taxa (e.g. plant: Parmesan & Yohe, 2003, Cleland *et al.,* 2007, Korner & Basler, 2010, insect; Hodgson *et al*., 2011; birds: Winkler *et al.*, 2002). While such phenological shifts may alter community structures and negatively affect some organisms (Stenseth & Mysterud 2002), they may reflect a plastic and/or adaptive response of an organism or population that insures persistence in a variable thermal environment. For plants, phenological shifts in response to climate change can affect their population positively (e.g. prolonged growing season; Menzel & Fabian 1999) or negatively (e.g. exposure to wrong seasonal environment; Lianhong *et al.,* 2008). Mechanistic and functional roles of phenological shift for the species would help to better predict the species' responses to the climate change (Chevin, Lande and Mace, 2010).

We hypothesized that optimal temperatures and ranges of temperature tolerance of each life stage was specialized to the thermal range that each life stage would naturally experience in the season of the occurrence (Fig. 1a). Furthermore, we hypothesized that they respond to different seasonal thermal regimes by shifting the life stage transition timing; shifting phenology early and late in fall in warm conditions (Fig. 1b). This would allow each life stage to occur at appropriate time of year corresponding to local temperature regimes for the specific year, and the species can live in the thermally varying seasonal environment with relatively narrow thermal range at each life stage. To test those hypotheses, we investigated the whole life cycle responses of an annual mayfly, *Ephemerella maculata* (Ephemerellidae) to a seasonally fluctuating thermal environment. We conducted lab-rearing experiments with three distinct life stages of the mayfly to characterize optimal temperatures, thermal tolerance ranges, and the effect of temperatures on the life stage transition timing. Patterns in the adult emergence timing in response to the water temperature were also investigated in the field data to examine if the pattern observed in the lab rearing experiment also occurs in the nature corresponding to the spatial and year-to-year thermal variations.

Methods
Study system
E. maculata is a stream dwelling mayfly, widely distributed in California (Allen et al. 1968, Meyer & McCafferty, 2008). Uno & Power (2015) documented that *E. maculata* has an annual migratory life cycle within a watershed (Fig. 2). Late instar nymphs in warm mainstem rivers, emerge and mate in early summer, and female adults fly to adjacent cool tributaries, oviposit eggs, and die. Eggs hatch in fall and young instar nymphs drift down to the mainstem river over the fall to winter period.

Individuals of *E. maculata* were collected from the watershed of the South Fork Eel River, in and around the Angelo Coast Range Reserve in Mendocino County, California (39°44′17.7″ N, 123°37′ 48.8″ W). Late instar nymphs were collected from their rearing habitat, the South Fork Eel River (drainage area ~100km^2), while eggs and 1st instar nymphs were collected from an adjacent tributary, Fox Creek (drainage area 2.6km^2), one of their oviposition sites.

Lab rearing experiments

Optimal temperatures and life stage transition timing

Effects of temperature on survivorship and development time of *E. maculata* in three distinct life stages (eggs, 1st instar nymphs and late instar nymphs) were measured in the laboratory. Treatment temperatures (6 or 7 levels) from 3°C to 25°C, were achieved using water baths regulated by aquarium heaters and chillers with temperature controllers (STC-1000, AGPtek, China), and were maintained within ±1 °C of constant temperature through the experimental period. The water in each water-bath was constantly mixed with pumps, and the temperature was homogeneous throughout the tank. Inside each water-bath, we set three replicate containers (17cm diameter, 15cm high), each with one liter of aerated COMBO, a defined freshwater culture medium (Kilham *et al.,* 1998). Several individuals of each life stage were placed in each container as described below, and the average data for each container was used as a data for statistical analysis. Temperature in each replicate container was monitored with an iButton (Dallas Semiconductor, Dallas, TX, USA).

We conducted experiments on egg development and survival in 2013 and 2014. We collected eggs from Fox Creek, a tributary of the South Fork Eel River, on September 20, 2013 and June 28, 2014, and they were transported at the collection temperature in an insulated cooler to the lab on the University of California Berkeley campus (37°52′20.2″ N, 122°15′32.8″ W) within five hours. The experiment was conducted in completely dark conditions in 2013, and with natural light in 2014. We placed three egg masses in each of the three containers at each temperature. Using a dissecting scope at 10-30X magnification, we examined the eggs 20 days prior to their expected hatch (based on previous years' observations), then at 5 day intervals during the hatching season until all eggs hatched or died (March 7, 2014 and November 7, 2014). We counted the number of dead eggs, unhatched alive eggs (embryos), and empty egg shells. Then for each container, we calculated the proportion of successful hatching to evaluate the optimal temperature for hatching, and average date of the hatch as development time.

To assess the temperature responses of early nymph instars we collected 1st instar nymphs from Fox Creek on October 26, 2013 and October 13, 2015, and transported them at the collection temperature in an insulated cooler to the lab on the University of California Berkeley campus within five hours. We reared ten individuals in each of three replicate containers per temperature treatment for ten days. For each container, survivorship of the nymphs was calculated as optimal temperature for the early instar nymph.

We tested temperature responses of late instar nymph in 2014. Late instar nymphs were collected from the South Fork Eel River on June 22, 2014, and transported them at the collection temperature to a lab at Angelo Coast Range Reserve within an hour. We placed five individuals in each of three

replicate containers. As late instar nymphs forage on algae growing on cobbles (H. Uno personal observation), we provided three periphyton covered cobbles (~10cm diameter, also from South Fork Eel River) in each container as food; they were replaced with fresh cobbles every five days. We assessed survival at five day intervals until July 30, 2014 when all nymphs had emerged as adults or died. Then, for each container, we calculated the successful emergence rate as an indicator of optimal temperature for emergence, and the average days before they emerged as a development time.

For each life stage, survivorship or successful transition to next life stages of each container (3 replicates for each temperature) was compared with the water temperature. To estimate the range of temperature with >50% survivorship as optimal temperature, we fitted second order polynomial regression or logistic regression, depending on the data pattern. The influence of the water temperature on the development times (egg hatching time and nymph emergence time) was examined with linear regression analysis. Levene's test was applied prior to each analysis to confirm the homogeneity of the variances.

Lethal high temperature
We tested the lethal high temperatures of late instar nymphs and eggs on July 1, 2014. Late instar nymphs and eggs were collected from South Fork Eel River and its tributary, and were held at ~20°C (ambient temperature) for 48 hours prior to exposing them to one of three treatments (29, 33, and 37 °C). These temperatures mimic the extreme warm water temperatures observed in the natural rivers in the species' distribution range (H. Uno personal observation). We placed three late instar nymphs and two egg masses in each of three replicate containers (17cm diameter, 15cm high) per treatment. Survival of the eggs and nymphs were examined after two hours in the warm water conditions. The death of a nymph was diagnosed by the observation that its gill tissues had ceased moving. Egg mass viability was assessed by respiration rate, measured using a Loligo Systems SDR Sensor Dish Reader (PreSens, Germany). Eggs were defined as dead if their respiration rates were less than 50 % compared with eggs that were not exposed to the high temperatures

Respiration rate
We measured the respiration rates of eggs, 1st, and late instar nymphs at six temperatures in the range that naturally occur in the South Fork Eel River (5, 9, 13, 17, 21, 25°C; Respiration rate of 1st instar nymphs was not measured at 25°C, as they all died). They were acclimated at the temperature of the measurement for more than two weeks prior to respiration measurement. Measurements for each of these stages were made on 1) ten 1st instar nymphs placed in a 200 µl sealed cell, 2) 0.2-0.5 mg of egg masses in a 200 µl sealed cell, and 3) one individual late instar nymph in a 750 µl sealed cell. At each temperature for each life stage, we measured the respiration rate of nine replicate cells that contained randomly selected specimens and two blank cells (with the same water, but no specimens). These rates were measured simultaneously with a Loligo Systems SDR Sensor Dish Reader under dark conditions. We measured respiration rate over 15 minutes after their respiration stabilized. The lowest dissolved oxygen achieved in the measurement was 65%. After drying them in an oven at 60°C for > 10 hours, we weighed individual late instar nymphs and egg masses to the nearest milligram and then calculated the mass specific respiration rate for each life stage. The effects of water temperature and life stage on the mass specific respiration rate were tested with two way-ANOVA followed by a Tukey HSD post-

hoc test. To examine in detail the effect of the water temperature on the respiration rate for each life stage, linear regression analysis of mass specific respiration rate against water temperature was conducted for each life stage.

Natural variations in life stage transition timing

Geographic variation of *E. maculata* life stage transition timing

We examined the broad geographical distribution and timing of *E. maculata* nymph occurrence with data base queries and field surveys. To examine the phenology of *E. maculata*, we analyzed data from the California Environmental Data Exchange Network (CEDEN; http://www.ceden.org/) (73 sites), museum collection records listed in Meyer and McCafferty (2008) (63 sites), and data we collected at 29 sites in the Eel River watershed. For each site, we defined emergence timing as the latest month in which *E. maculata* nymphs were found, and examined the relationship between emergence timing and mean summer temperature. The phenology data of *E. maculata* in California from CEDEN and museum data were compared with the mean August air temperature from PRISM high-resolution spatial climate data for the United States (National Center for Atmospheric Research Staff, 2015), and their relationship was evaluated with linear regression. The phenology data of *E. maculata* in the Eel River watershed, and the predicted August mean water temperature of each site (Isaak *et al*., 2014) were compared, a linear regression was applied and its significance was tested.

Year-to-year variation of *E. maculata* flight period

Flight periods of adult *E. maculata* in Fox Creek were recorded from 2011 through 2015 based on daily field observations. The water temperatures and the discharge of the South Fork Eel River during that period was recorded at retired USGS Gage 11475500 (South Fork Eel River at Branscomb), which is located about 5km upstream of the study sites near Fox Creek. In summer, the water temperature was 1-2 °C warmer around the study site near Fox Creek than at the gage (H.Uno, personal observations). The average summer water temperature of each year was compared with the *E. maculata* flight period.

Results

Thermal sensitivity of survivorship to next life stage

The optimal temperature range for survival varied among life stages (Fig. 3). The survivorship of eggs (Fig. 3a) and 1st instar nymphs (Fig. 3b) were higher at lower temperature in the range of temperature the experiment was conducted (3-25 °C). In contrast, the survivorship of late instar nymphs was highest 21°C and declined both at higher and lower temperatures. Therefore, to estimate the temperature range of the 50% survivorship, logistic curve was fit for the survivorship of eggs and 1st instar nymphs, and second order polynomial regression was fit for the late instar nymphs (Fig. 3). Estimated temperature range of >50% survivorship was <18 °C for eggs, < 14 °C for 1st instar nymphs, and 12-27 °C for late instar nymphs.

Effect of temperature on life stage transition

The timing of life stage transitions also varied with the rearing temperature (Fig. 4). Eggs hatched earlier at cooler temperatures (Fig. 4a), but the relationship between development time and temperature was best described by a two-segment regression ($R^2 = 0.95$, $P < 0.001$): development

time was the same at 5, 9, and 13 °C (slope = 0.014), then rose linearly with temperature from 13 – 19 °C (slope = 17.6), with the estimated break between line segments at 11.9 °C. On average, eggs hatched on October 25th at 5 °C and January 27th at 19 °C. In contrast, nymphs emerged to adults earlier at warmer temperatures (Fig. 4b; R^2=0.47, $P < 0.01$), on average 19 days at 9 °C and 10 days at 25 °C after the experiment started on June 22nd (Fig. 4b).

Lethal high temperature
The lethal high temperature was higher for eggs than for late instar nymphs. The late instar nymphs all survived at 29 °C, but all died at 33 °C and 37 °C suggesting their lethal high temperature was between 29-33 °C. In contrast, none of the eggs died after exposure to temperatures as high as 37 °C.

Respiration rate
Mass specific respiration rate varied across life stages ($F_{2, 107}$= 43.0, $P < 0.001$), and there was significant interaction between the life stages and the temperature effects on the mass specific respiration rate ($F_{2, 107}$ = 3.7, $P < 0.05$) (Fig. 5). The mass specific respiration rate was higher for 1st instar nymphs than late instar nymphs, and higher for late instar nymphs than eggs (Tukey HSD, $P < 0.05$). There was positive correlation between the temperature and the mass specific respiration rate of late instar nymphs (R^2 =0.49, $P < 0.001$) and 1st instar nymphs (R^2 = 0.097, $P = 0.068$), but the mass specific respiration rate of the eggs did not co-vary with the water temperature (R^2=0.044, $P = 0.14$).

Spatial variation of *E. maculata* emergence timing
The emergence timing of *E. maculata* varied between March and October within the species' distributional range. The emergence was generally later in the North, and at sites located closer to the Pacific Ocean, where the air temperature is cooler under the influence of coastal fog. Within the Eel River watershed, the emergence timing of *E. maculata* was earlier in downstream habitat where the water temperature is usually warmer, and later in cooler tributaries (Fig. 6a). In the geographical scale, the emergence timing was negatively correlated with the mean August air temperature (Fig, 6b). The emergence timing also varied by the landscape positions within a watershed, and the emergence timing was negatively correlated with the mean August water temperature (Fig. 6c).

Year-to-year variation of *E. maculata* emergence timing
The summer water temperature gradually increased in the South Fork Eel River from 2011 to 2015, mainly due to drought in California in 2014 and 2015 (Fig. 7a). Correspondingly, the emergence season of the *E. maculata* from the South Fork Eel River shifted early from 2011 to 2015; The reproductive swarms of female adult *E. maculata* were observed July 7th (or earlier)–July 22nd in 2011, June 18th – July 15th in 2012, June 2nd – July 5th in 2013, June 1st – July 6th in 2014 and May 18th – June 24th in 2015 (Fig. 7b).

Discussion
Our experiments and observations demonstrate that the life cycle of *E. maculata* is well adapted to the thermally varying seasonal environment in temperate rivers. While the optimal thermal range for late instar nymphs is relatively narrow, an egg stage that can survive high temperatures

and a young instar nymph stage that performs well at cold temperatures alternately emerge in summer and winter, and allow this species to live in the thermally varying seasonal environment. Furthermore, their temperature–based phenology allows them to transition between life stages in tune with local conditions: in warm years and places, they spend longer periods as heat-tolerant eggs, shorter periods as cool-tolerant young instar nymphs, and the late instar nymphs that prefer intermediate temperatures occur earlier in spring. Therefore, their whole life cycle responses to thermal regime of the habitat enables *E. maculata* to cope with year-to-year variation in thermal environment, and also exploit and tolerate wider range of thermal habitats represented by streams of varying size within a dendritic network in wide geographical range.

Shifts in thermal tolerances and performance optima across life stages that are synchronous with seasonal fluctuation in temperature represent an important thermal adaptation to the seasonal environment. It represents an alternative strategy to each life stage maintaining a wide range of thermal tolerance across seasons. Maintaining thermal tolerance to a wide range of temperatures at one time is energetically expensive, and would come at cost of lowered performance at moderate temperature (Kristensen *et al.,* 2008; Willett 2010). Tight seasonal matching of the thermal tolerance range and the change in the water temperature as a life cycle strategy found in this study should allow organisms to live in the seasonal environment with minimum energetic cost. Organisms with complex life cycles are often exposed to various microclimates across life stages as a result of their ontogenetic habitat shifts, and corresponding shifts in the optimal thermal ranges are known in a few insect taxa (e.g. leaf mining moth, Pincebourde & Casas 2015). This study demonstrates that shifts in thermal ranges through a life cycle can occur not only in response to the habitat shifts but also to the seasonal changes in the environment, and that allow organisms with complex life cycles to live in wide range of temperatures.

Plasticity in the timings of life stage shifts in response to the temperature allow organisms to inhabit various environments with considerable thermal variation from year to year, and may buffer the effects of climate change on this annual insect to a greater extent than would be expected by the narrower range of thermal optima of each life stage. The metamorphosis from nymphs to adults was accelerated in warmer conditions in spring, whereas the egg hatching was delayed in warmer conditions in fall. As a result, each life stage expands or contracts in response to temporal and spatial variation in temperature regimes, and the transition to next life stage occurs when the thermal environment is appropriate for the next life stage in each environment. *E. maculata* was distributed in rivers all around California over a wide range of thermal regimes, and their transition timings from nymphs to adults varied among rivers and by years corresponding to their thermal regimes. Early emergence of aquatic insects in warmer water has been commonly known in various species of aquatic insects, and our observations confirm that general pattern (Sweeney & Vannote 1978, Vannote & Sweeney 1980). While some organisms use other environmental cues such as photoperiod for their life stage shifts, and adapt their phenology to the local environment over generations, they cannot adjust their phenology to the year-to-year variation in thermal regime (Ragland & Kingsolver 2008). The plastic phenological shift observed in this study allows organisms to adjust their life cycles to the environment of each year, and would be particularly advantageous for organisms that are exposed to various thermal regimes from a generation to next. Stream water temperature varies both in space and time, as they are affected not only by the air temperature but also with precipitation and its landscape positions (Webb *et al.*, 2008). This study showed emergence timing of the stream insect varied not only in geographic scale, but also varied

by the landscape positions within a watershed due to the landscape scale variations in the water temperature. Uno (in prep) has also documented that emergence timing of *E. maculata* also varies within a few hundred meters due to fine scale spatial variation of water temperature in rivers. Furthermore, the water temperature of the same river varied year to year, altering emergence timing of the mayfly. Droughts decrease the discharge of rivers, slow the flow velocity, and warm up the stream water by prolonged exposure to the sun. Plastic thermal adaptation maybe particularly beneficial and important for stream aquatic insects. While stream water temperatures around the globe is increasing due to the climate warming, drought, water-withdrawal and change in land-use (Webb *et al.,* 2008), annual stream aquatic insects such as *E.maculata* may be able to survive by shifting their phenology.

Elevated lethal high temperature of eggs and their early hatching from cooler water as well as long egg period with low metabolic rate, which are critical for this observed life cycle response to the seasonal environment, is assumed to be achieved by special physiological state, diapause. Diapause is a widespread form of dormancy among insects characterized by a special physiological state (Tauber & Tauber 1976). While diapause of mayfly eggs has not been previously recognized, a prolonged egg development period has been documented in other univoltine mayfly species, indicating a similar physiological adjustment (Elliott 1978). Low metabolic rate during diapause, as was observed in this study, limits carbon loss during the long dormant state when they cope with the stressful environment. While various environmental factors such as photoperiod and physical stimuli are known to affect the termination of diapause (Vinogradova 2007, Denlinger, 2002), temperature dependent terminations of diapause observed in this study help organisms to cope with seasonal thermal stresses by plastically adjusting diapause duration.

Life cycle responses to temperatures provide resilience against changes in temperature, but they may increase vulnerability to alteration of the seasonal pattern in temperature (i.e. gradual temperature rise in spring and decline in fall). As their thermal adaptations are based on the predictable seasonal pattern of temperature changes, unusual deviations in temperature such as cold days in summer or hot days in winter would misalign the thermal range of the life stage with the ambient temperature. Atypical temperature changes and/or modification of natural thermal regimes rather than a smooth rise in temperature might be a serious problem for annual organisms that exhibit life cycle response to temperatures. In streams, seasonal regulations of water release from dams can alter the seasonal pattern of the water temperature, and summer cooling and winter warming of the water is common downstream of dams (Stanford et.al. 1996, Olden & Naiman 2010). More attention to the natural seasonal pattern of water temperature along with the discharge pattern (Poff *et al.,* 1997) in designing restoration efforts may be important to help sustain organisms in rivers.

Furthermore, it should be noted that phenological shifts, which allow organisms to withstand thermal variations, may lead to mismatches of important species interactions (match-mismatch hypothesis; Cushing 1974, Stenseth & Mysterud 2002). The phenological shift may disrupt the synchrony between resource availability and demand for food by consumers. Predator populations maybe be degraded if the prey phenology shifts (Visser & Both 2005; Bewick *et al.* 2016). Uno & Power (2015) have described that migration of *E. maculata* adults from mainstems to tributaries provides a large resource subsidy to consumers from productive mainstem to unproductive tributaries from June to July when insectivores such as juvenile steelhead rearing in tributaries are

food limited. The influx of *E. maculata* increases their summer growth rate three times above fish not receiving the subsidy. However, it is highly possible that a shift in emergence timing due to warmer or cooler water temperatures could cause the subsidy to occur too early or too late for predators such as juvenile steelhead to exploit them, and lead to a temporal mismatch of the trophic interaction. In fact, following the unusually warm winter and spring of California's historic drought the emergence of *E. maculata* occurred as early as mid-May in 2015 when young of year (age 0) steelhead trout had not yet grown large enough to forage on *E. maculata* adults. As the stream water temperature is often changed due to change in the land use, dams, and climate change, such mismatch of the trophic linkages between emerging aquatic insects and their predator might be increasingly widespread.

Organisms in temperate regions are exposed to various temperatures seasonally, and can resist to some spatial and year-to-year variations that naturally occur. To predict impacts of the climate change on organisms, it is important to understand the sensitivity and resistance of organisms against the temperature variance across all life cycle stages. The present study shows that organisms in seasonal environment can exhibit whole life cycle responses to the temperature variance, and are more resistant to the temperature variation than would be predicted from the response of a single life stage. A whole life cycle perspective should be accounted to understand the ultimate fitness consequences of an organism's response and to predict the impact of climate change.

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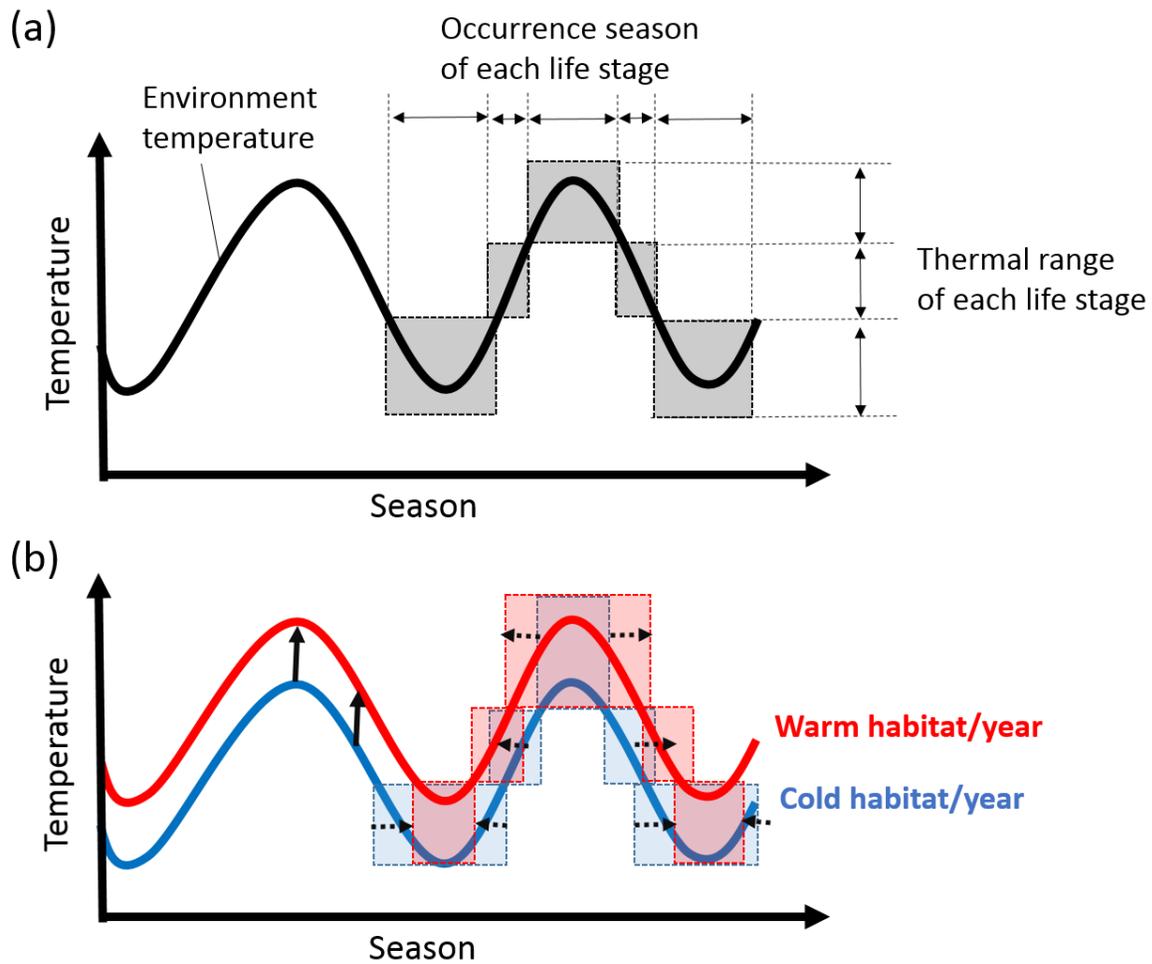
Figure 1. (a) Hypothetical whole life history response to seasonal thermal environment by an organism with complex life cycle. The thermal range shift with life stages corresponding to the temperature that each life stage experiences. (b) Hypothetical response of organisms to varying thermal regimes by shift in life stage transition timing.
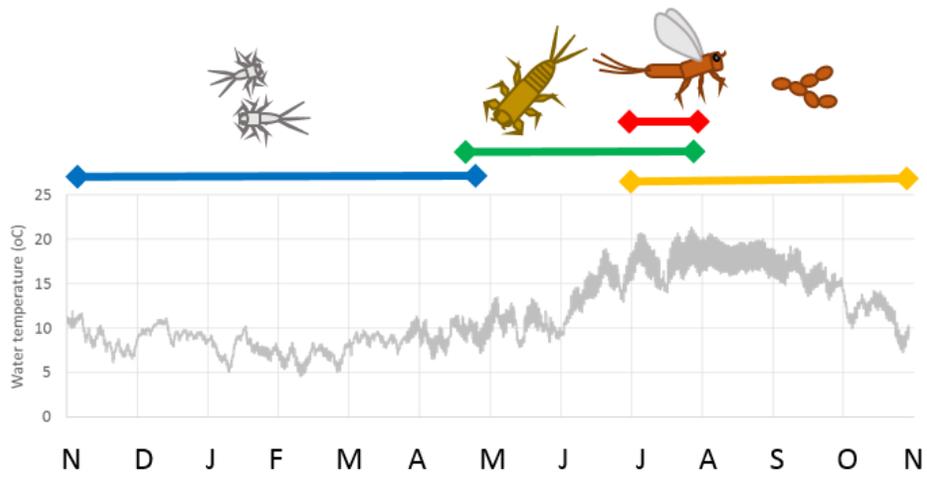
Figure 2. Seasonal variation in the water temperature and the occurrence timing of four distinct life stages of *E. maculata* in the South Fork Eel River in 2011.
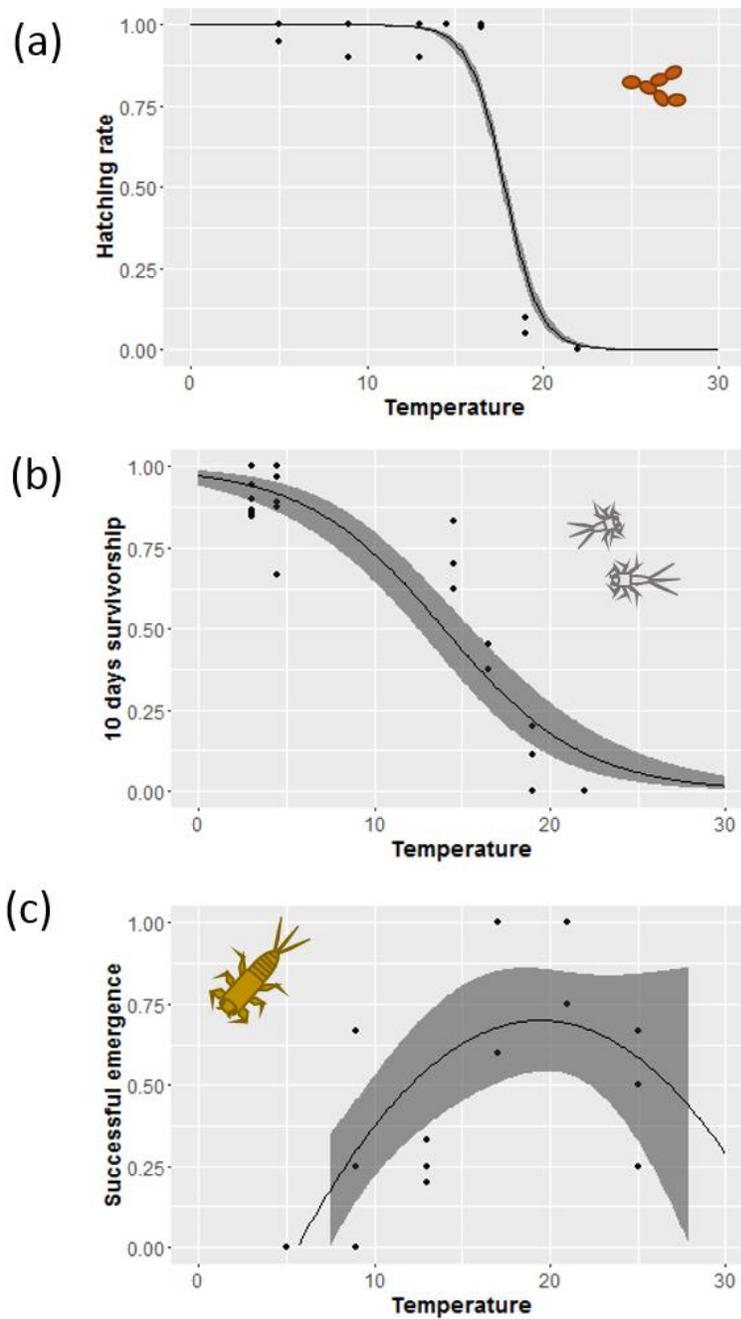
Figure 3. Thermal response of *E. maculata* in three distinct life stages (a) Successful hatching rate of the eggs (b) 10 days survivorship of 1st instar nymphs (c) successful emergence rate of late instar nymphs. The gray area indicate 95 percent confidence intervals.
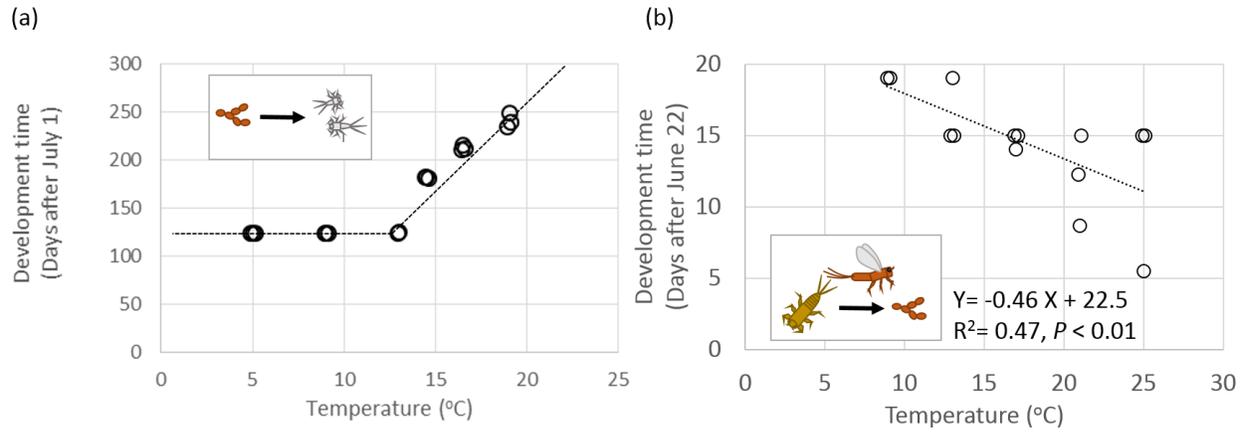
(a)

(b)

Figure 4. The relationships of the water temperature and the timing of life stage shifts. (a) Number of days until eggs hatched after July 1st. (b) Number of days that nymphs were reared at the set temperatures before they emerged. Late instar nymphs were collected from the South Fork Eel River on June 22nd, then incubated under controlled temperature. The error bars represent standard errors.
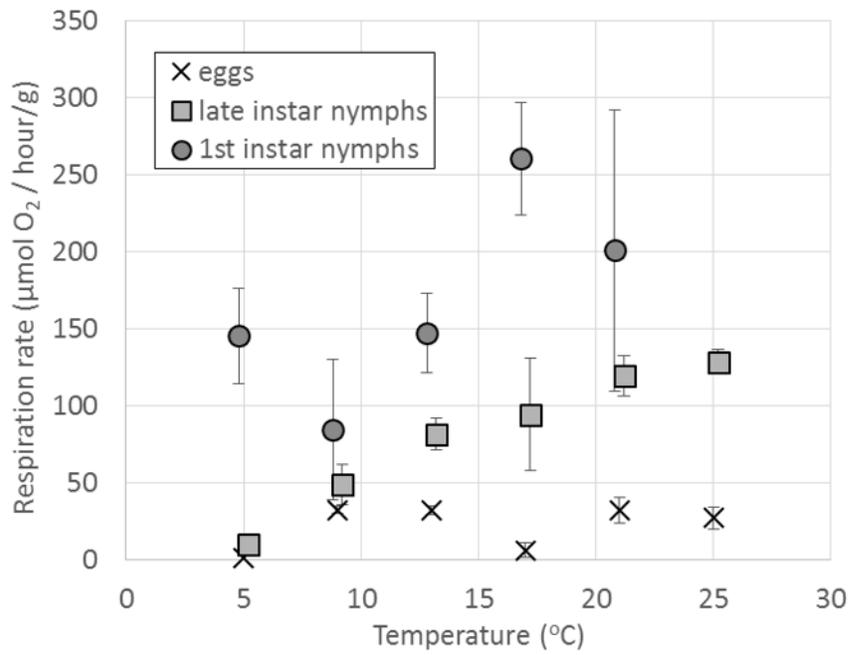
Figure 5. Mass specific respiration rate of *E. maculata* at three life stages; embryos (eggs), 1st instar nymphs, and late instar nymphs. Error bars represent Standard error.
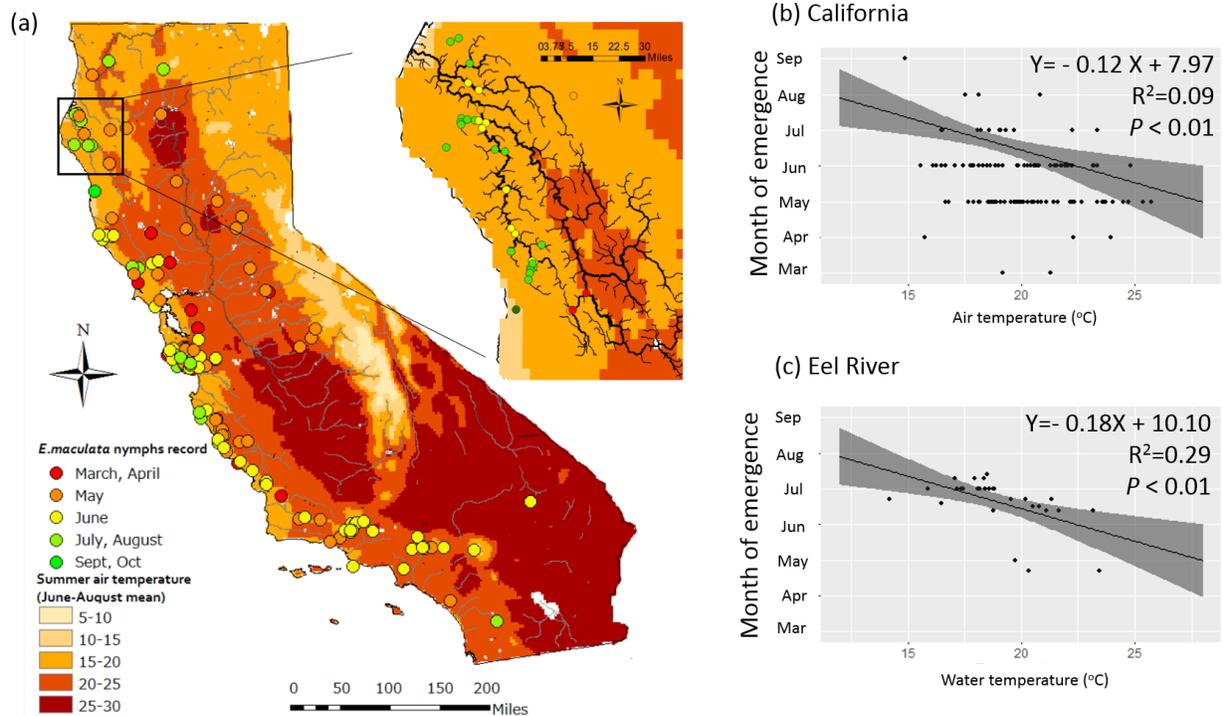
Figure 6. Spatial variation in the emergence timing of *E. maculata*. (a) Broad geographical distribution and the timing of *E. maculata* nymph occurrence based on the California Environmental Data Exchange Network (CEDEN) (73 sites), museum collection record listed in Meyer and McCafferty (2008) (63 sites), and field surveys in the Eel River watershed (Top-right; 29 sites). The circles represent locations of *E. maculata* nymphs records. The color inside the circles represent the latest month of the occurrence at the site. The background colors represent the mean air temperature from June –August in 1992-2008 based on the PRISM data (Parameter elevation Regression on Independent Slopes Model). (b) Significant linear regression of *E. maculata* nymph occurrence timing in California from CEDEN and museum records vs. August air temperature. (c) Significant linear regression of *E. maculata* nymph occurrence timing in Eel River watershed based on field survey vs. August water temperature. The gray zone in figures indicate 95 % confidence interval of the data.
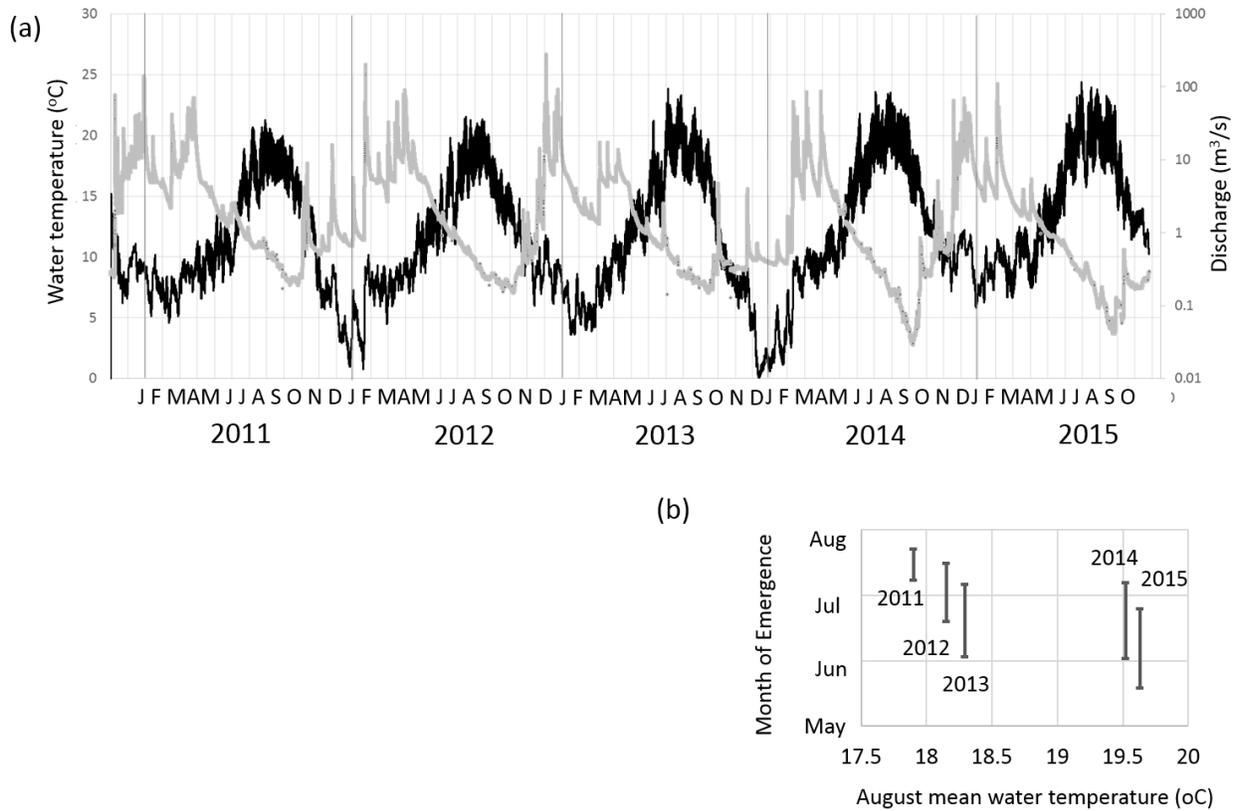
Figure 7. Year-to-year variation in the stream environment and emergence season of *E. maculata* in the South Fork Eel River. (a) Water temperature (black line) and discharge (gray line) of the South Fork Eel River from 2011 to 2015. (b) Emergence season of *E. maculata* in the South Fork Eel River plotted against the mean August water temperature of each year.

Chapter 4

Stream thermal heterogeneity prolongs aquatic-terrestrial subsidy and enhances riparian spider growth and reproduction

Abstract

Emerging aquatic insects from streams are important food source for riparian predators, yet their availability is seasonally limited. Spatial heterogeneity in stream water temperature was found to spatially desynchronize the emergence timing of aquatic insects, and prolong their flight period, enhancing consumer growth. While a mayfly *Ephemerella maculata* emergence lasted for 12-22 days across study sites along a river, mayflies emerged 19 days earlier from warmer than cooler sites. Therefore, the overall emergence of *E. maculata* from the river lasted for 37 days, and adult swarms were observed over that same period in an adjacent reproductive habitat. A feeding experiment with the riparian spider *Tetragnatha versicolor* showed that a prolonged subsidy, as would occur in a heterogeneous river, led to higher juvenile growth than a synchronous pulsed subsidy of equal total biomass, as would typify a more homogeneous river. Since larger female adult spiders produce more eggs, spiders that received prolonged subsidy as juveniles should achieve higher fecundity. Restoring spatial heterogeneity in streams may benefit not only stream communities but also riparian predators.

Introduction

Life history transitions and associated movements of organisms often provide transient pulses of resource for predators (e.g. salmon: Gende *et al.* 2002, cicada outbreak: Yang 2004, aquatic insect emergence: Nakano & Murakami 2001, plant litter fall: Richardson 1991). As consumers have limited consumption and assimilation abilities, they are often unable to take full advantage of resource subsidies if pulsed over a short period of time (Gende *et al.* 2002, Yang 2004). Therefore, the duration of the resource subsidy influences the efficiency of trophic transfer and its benefit to consumers (Wright *et al.* 2013, Marczak & Richardson 2008), and may alter the response of recipient communities (Yang 2008).

While, asynchronous prey phenology prolongs the period in which prey are available to predators, prey in turn often have life history strategies that synchronize their phenology as a means of lowering the per capita risk of predation (periodical cicada; Karban 1982, mayfly emergence: Sweeney 1982). However, as the phenology of organisms is often determined by environmental cues such as temperature and/or photoperiod, spatial heterogeneity in environmental conditions can desynchronize the phenology of organisms and prolong the resource supply to predators.

Emerging aquatic insects from rivers provide a pulse of resource to riparian predators such as birds, lizards, and spiders, and support their rapid growth and reproduction in the summer growing season (Nakano & Murakami 2001, Sabo & Power 2002, Marczak & Richardson 2007). Emergence of aquatic insects is often seasonally synchronized in early summer, and Sweeney & Vannote (1982) have shown that the high densities of prey resulting from this synchrony can satiate predators seasonally, reducing the per capita risk of predation. However, as emergence timing is water temperature-dependent (Sweeney & Vannote 1982), spatial heterogeneity of the water temperature, which is often observed in natural rivers in the summer dry season (Arscott *et al.* 2001, Webb *et al.* 2008), can spatially desynchronize the emergence timing of aquatic insects. In this paper, I test the hypothesis that spatial heterogeneity in the stream water temperature broadens the timing of aquatic insect emergence at the whole-stream scale and prolongs the period of adult flight, thus, providing a more extended period of resource subsidy to riparian predators that enhances their growth, reproduction, and survival.

This hypothesis was tested in a system where the emergence of a single species of aquatic insect is known to exert a strong impact on consumers of the subsidy. *Ephemerella maculata* is a mayfly widely distributed in California, which migrates from the sunny and productive mainstem habitat to shaded tributaries as adults. This migration of adults represents a considerable trophic subsidy for food-limited predators in shaded tributaries. Uno & Power (2015) demonstrated that the growth of juvenile steelhead, rearing in the tributaries, was tripled by the subsidy of *E. maculata* from the mainstem habitat. With field observations and feeding experiments, I examined the effect of spatial heterogeneity in temperature on the emergence timing of *E. maculata*, the duration of adult *E. maculata* subsidy to riparian spiders, and its effect on spider vital rates.

Methods
Study system
The field components of the study were conducted in the upper basin of the South Fork Eel River, in and around the UC Angelo Coast Range Reserve in Mendocino County, California (39˚44017.7" N, 123˚37048.8" W). The mean gradient of the river is 0.005, and the channel is 20-50 meter wide and sunlit. Under Mediterranean seasonality, the South Fork Eel River watershed typically has wet winters and dry summers. In the summer low flow period, flow slows and the wet channel width narrows as the discharge decreases. At this time of year, various topographic features of the river emerge from the water, creating considerable spatial heterogeneity in the river.

The nymphal stage of *E. maculata* grows in sunlit mainstem of the South Fork Eel River. After metamorphosis, winged adult *E. maculata* emerge from water surface, mate, then female adults fly to adjacent tributaries of the South Fork Eel River within a few days, oviposit in creeks, and then die on the water surface. The *E. maculata* nymphs emerge earlier from warmer water than cooler water, as confirmed by a laboratory experiment (H. Uno & J. Stillman, unpublished data). The response of a common riparian spider, *Tetragnatha versicolor* (Tetragnathidae) to the subsidy was examined in this study. *T. versicolor* is the most abundant orb-web spider along creeks in the study region (Levi 1981), and mainly consume adult aquatic insects (Gillespie 1987). *T. versicolor* has a one year life cycle; adults lay their eggs throughout the summer months and this is also the period of spiderling growth (Williams *et al.* 1995, Aiken and Coyle, 2000). Juvenile *T.versicolor* molt as they grow, but cease to molt and grow once they reach sexual maturity. Juveniles grow first summer, overwinter, and then reproduce during their second summer (Williams *et al.* 1995, Aiken and Coyle, 2000). Adult female *T. versicolor* reproduce multiple times over the summer depending on the conditions. (Gillespie 1987)

Spatial variation in the emergence timing of *E. maculate*
The emergence timings of *E. maculata* from six sites representing habitat and longitudinal thermal variations were investigated (Fig.1). At each site, 100 individual *E. maculata* nymphs were incubated in 10 flow-through buckets with 1mm mesh cover, and their emergence timing was monitored with sticky traps suspended in the buckets every five days. The nymphs for the experiment were collected from the vicinity of each site at the beginning of the experiment. As *E. maculata* nymphs forage on algae growing on cobbles (H. Uno unpublished), I provided two periphyton covered cobbles (~10cm diameter) in each bucket as food; they were replaced with fresh cobbles every five days. The water temperature was monitored with DS1920 iButton

temperature loggers (Maxim integrated, Dallas) at 30 minutes intervals inside and outside the flow-through buckets at each site. The experiment was initiated on May 31, 2014, and continued until July 11, 2014, when all *E. maculata* in the buckets had either emerged or died.

Adult arrival in tributaries
The timing and duration of *E. maculata* adult swarm that provide the resource subsidy to consumers was recorded in Fox Creek in 2014 concurrently with the measurement of their emergence timing from the river. The presence and absence of an *E. maculata* swarm was determined by a combination of sticky traps and exhaustive searching with butterfly nets for 30 minutes at dusk each night from June 1, 2015 to July 31, 2015.

Field observations and measurements for the feeding experiment
As a prelude to the feeding experiment, population-level information on tetragnathid spiders and their responses to the *E.maculata* subsidy were investigated in Fox Creek. The densities of spiders were estimated by visual count along eight 30-meter-long transects along Fox Creek at night (9pm-12am) on June 25, 2015 and August 5, 2015. Additionally, the diameters of the spider webs were measured for 48 adult females and 38 juveniles at night on June 25, 2015.

To estimate the amount of *E.maculata* each spider captured, the flux of *E. maculata* over Fox Creek was measured with sticky traps in 2012. Sticky traps were constructed of transparent plastic sheets (928cm^2 in area) covered with non-attractive Tanglefoot insect trap coating (Tanglefoot, Michigan, USA). Five sticky traps were set over the current (~1m high) in Fox Creek for five times (24 or 48 hours each time) between June 20, 2012 and July 24, 2012. Numbers of *E. maculata* adults on each trap were counted, and the flux of *E. maculata* per day per trap area was estimated.

Tetragnathid spider feeding experiment
To examine the effect of the subsidy and its temporal duration on the tetragnathid spiders, I conducted a feeding experiment with juvenile and female adult spiders, offering surrogate food in the form of wildtype fruitflies, *Drosophila melanogaster*, that corresponded to unsubsidized and subsidized field resource levels. Fruitflies were fed to spiders with four different feeding regimes: No subsidy (n=11, 12 for adults and juveniles, respectively; same notation is used hereafter), 12 days subsidy (n=9, 12), 24 days subsidy (n=5, 12), and 48 days subsidy (n=9, 12). The experiment ran for 48 days, starting on June 22, and ending on August 7, 2015.

A baseline level of food supply, simulating the biomass of terrestrial insects that spiders would capture in the absence of an aquatic insect subsidy (The "No subsidy" food level) was provided to spiders in all treatments: 120 (to adult female spiders) or 48 flies (to juvenile spiders), delivered at rates of 2.5 and 1 flies per day, respectively, for 48 days. Spiders in the subsidy treatments were provided an additional 120 (adult female spiders) or 48 flies (juvenile spiders) over the 12, 24, or 48-day period. The timing of the subsidy was randomized within the 48-day experimental period. The subsidies of 120 and 48 flies provided to the two spider life stages were equivalent in biomass to 18 and 7.2 mayflies, respectively, as the average dry weights of *E. maculata* and *D. melanogaster* are 1mg and 0.15mg. These subsidy rates fall near the lower end of the estimated range in the numbers of adult mayfies caught per spider under field conditions over the 36-day flight period (see details below): 21-682 *E. maculata* for female adult spiders and 8-270 *E. maculata* for juvenile spiders.

For juvenile spiders, growth and mortality were measured over the 48 days experimental period. Each spider was maintained in its own container, and their body size (weight) was measured at the beginning and end of the experiment. For female adults, the mortality, egg production, and web building frequency were measured. The presence/absence of a spider web in each enclosure was monitored for 12 nights (9-11pm) evenly spread over the experimental period. For mating, a male spider was introduced to each enclosure over-night, once a week. The combination of male and female adults was randomized each time. On some occasions, females ate the males (10.4% of pairings) but the frequency of these events did not differ among the feeding treatments (X^2 =5.2, df = 3). The egg sacs were removed and weighed to the nearest milligram at the mid-point (24 days) and end of the experiment (48 days).

Spiders for the experiment were collected in Fox Creek early June 2015. Adult female spiders were individually housed in cylindrical laundry hampers (50cm height and 45 cm diameter) with 1mm mesh, and juvenile and adult male spiders were individually housed in 500 milliliter clear plastic cups with fine nylon netting for air exchange. A small slit was created on the lid of each container, as a means of supplying food. A wet cotton ball and sticks for climbing were put in each container. All containers were placed in a screened cage house at the Field Station for the Study of Behavior, Ecology and Reproduction (37˚88078.2" N, 122˚24303.6" W). The cage floor was kept covered with ~10cm of standing water, and all containers were hung on ropes over the water to maintain a humid environment similar to that along a natural creek.

Statistical Analyses
The results of the feeding experiment were compared between the no subsidy treatment and all other subsidy treatments, then among the three subsidy treatments of different duration. ANCOVA was employed in the analyses of juvenile growth, adult female egg production, and adult female web building, with initial spider mass as a covariate. In none of the analyses did the influence of the covariate differ with treatment (i.e. regression slopes of dependent variable against initial weight were equal for all treatments, $P > 0.1$ in all cases). Post-hoc comparisons of treatment means were made with Tukey-HSD tests. Rates of juvenile and adult female mortality among treatments were compared with Chi-square tests.

Results
Asynchronous emergence of *E. maculata* and prolonged subsidy
The emergence timing of *E. maculata* from the South Fork Eel River was asynchronous in space, corresponding to spatial heterogeneity in water temperature. The mean water temperature was higher at the downstream site F, and cooler at the upstream site E (Fig.1). Among the four sites within a 300 meter segment A-D, the mean water temperature was cooler in site A located upstream of the deep pool, where the water is warmed. The mean water temperature was similar in sites B, C, and D, but site B, which was located in a slow flowing dead-arm had higher daily variance in the water temperature compared to sites C and D. The emergence of *E. maculata* occurred earlier from the warm downstream site F and from the dead arm site with high temperature fluctuation B, than the four sites with lower water temperatures (Fig. 2). In turn, emergence from intermediate temperature sites C and D, was earlier that from the cooler site upstream of the deep pool A, with the latest emergence occurring from the coolest upstream site

E. Within a site, *E. maculata* emerged over 12-22 days, (excluding the first and last individuals to emerge at each site from the analysis as outliers), but overall emergence of *E. maculata* from the South Fork Eel River lasted for 37 days, as the date of median emergence differed by 19 days from the earliest site to the latest site. The 37 day period that I observed emergence of *E. maculata* from the South Fork Eel River (June 4th-July 11th, 2014) closely overlapped with the period that female adult swarms were observed at an oviposition site, Fox Creek (June 1st-July 6th, 2014). Observations in 2015 in three other tributaries of the South Fork Eel River showed similar durations of emergence (H. Uno, personal observation).

Field observations of Tetragnathid spiders
Number of juvenile spiders along Fox Creek significantly increased from 0.08 ± 0.03 webs meter[-1] SE to 0.61 ± 0.09 webs meter[-1] SE between June 25th and August 5th, 2015, indicating active reproduction in July, while the density of female adults decreased from 0.1 ± 0.05 webs meter[-1] SE to 0.06 ± 0.04 webs meter[-1] SE. The web sizes that female adults created averaged 25.6 ± 1.0 cm SE in diameter and 530 ± 37 cm^2 SE in area, and were larger than webs of juvenile spiders; 16.6 ± 1.3 cm SE diameter and 210 ± 26 SE cm^2 area. The *E. maculata* flux was spatially heterogeneous in Fox Creek, and ranged 11.2–357.3 individuals m^{-2} day^{-1}. Therefore, based on the *E. maculata* flux and spider web diameter, female adult spiders and juvenile spiders were estimated to catch between 21-663 *E. maculata* and 8-263 *E. maculata* during the entire subsidy period, respectively.

Response of tetragnathid spiders to resource subsidy of differing temporal duration
Juvenile growth co-varied with the initial body weight of individual spiders: smaller spiders grew more in the experiment (Fig 3a; $F_{1, 37}$=68.9, $P < 0.01$). Adjusting for this covariate, the growth of juvenile spiders was higher with subsidy than without subsidy ($F_{1, 37}$=12.6, $P < 0.01$). When subsidized, the weight gained by juvenile spiders varied with the temporal duration of the subsidy ($F_{2, 25}$=4.5, $P < 0.05$): they gained more weight when subsidized over 48 days than 12 days (Tukey HSD; $p < 0.05$; none of the other pairwise comparisons was significant). Using the no subsidy treatment as a baseline, a 48 days subsidy enhanced the growth of juveniles 2.2-fold compared to a 12 days subsidy (from 3.4 mg at two weeks to 7.5 mg at eight weeks). Mortality of juvenile spiders during the experiment was 10% on average, and did not significantly differ among treatments ($X^2 = 2.5$, $df = 3$, $p = 0.5$).

Egg production by adult females co-varied with their initial body weight: larger individuals produced more eggs (Fig 3b; $F_{1, 33}$=10.0, $P < 0.01$). Adjusting for this covariate, the egg production of adult females were both significantly higher with subsidy than without subsidy (on average by 19 times; $F_{1, 33}$=9.8, $P < 0.01$), but did not significantly vary with the temporal duration of the subsidy ($F_{2, 21}$=1, $P =0.38$). Web building frequency of adult females was significantly higher with subsidy than without subsidy (on average by 1.4 times; $F_{1, 33}$=4.7, $P < 0.05$), but was not significantly different by the duration of the temporal duration of the subsidy ($F_{2, 21}$=0.90, $P =0.42$). Mortality of adult female spiders during the experiment was 44% on average, and the mortality rate was lower with the subsidy (30%) than without the subsidy (73%) ($X^2 = 3.8$, $df = 1$, $p = 0.051$). When subsidized, the adult female mortality was not significantly different with the duration of the subsidy ($X^2 = 1.4$, $df = 2$, $p = 0.5$).

Discussion
This study demonstrated that spatial heterogeneity in source habitats can desynchronize timings of resource movements and prolong resource subsidies, enhancing effects of resource subsidies. Spatial variation in water temperature within the Eel River spatially desynchronized the emergence timing of aquatic insects, thereby prolonging the subsidy to riparian predators. Growth of juvenile spiders and egg production by adult female spiders increased with elevated food supply from the subsidy, and enhancement of juvenile growth was greater when the subsidy was provided over prolonged period as would occur in a thermally heterogeneous river. The direct effect of the subsidy duration on the adult egg production was not significant, but the enhanced growth of juvenile spiders with the prolonged subsidy would lead to higher reproductive output in the following year, because larger female spiders produced more eggs in the experiment.

Both longitudinal and habitat scale spatial heterogeneity in stream water temperature contributed to the prolonged *E. maculata* subsidy to riparian spiders. Longitudinally, the daily mean stream water temperature increased as the channel widened and received more sunlight, which led to earlier insect emergence downstream. In contrast, the daily fluctuation in stream water temperature varied among nearby sites, depending on the rate of water exchange, and *E. maculata* emerged earlier from sites with greater thermal daily fluctuation. As emergence timing of many aquatic insects are affected by mean water temperature (Sweeney & Vannote 1982, Uno & Stillman *in prep*), as well as daily temperature fluctuation (Sweeney & Schnack 1977), spatially asynchrony in emergence timing should be widespread for many aquatic insects that emerge from natural rivers. As winged adult aquatic insects have higher mobility than aquatic insect larvae, the adult aquatic insects emerging from various stream habitats are spatially mixed in the air and form reproductive swarms (Jackson 1988). Therefore, spatial heterogeneity in the timing of emergence is translated into temporal variation in the length of time that adults are flying.

Enhanced growth of spiders in response to prolonged subsidy of equal amount in this study indicates that assimilation rate of the resource by spiders were higher when they were fed over a prolonged period than when the food was provided as a pulse. As higher assimilation rate with lower ration is observed in various taxa (Brett *et al*. 1969; Navarro & Winter 1982; Wiegert & Petersen 1983), enhanced growth of consumers in response to prolonged resource subsidy that is observed in this study should be also wide spread. (Marczak & Richardson 2008; Skalski *et al*. 2005). Furthermore, theory predicts that duration of resource supply can alter food web structure (Holt 2008, Yang *et al*. 2010), although that was not measured in this study.

The importance of the trophic subsidy afforded by emerging aquatic insects to riparian predators, such as spiders (Marczak & Richardson 2007), birds (Nakano & Murakami 2001), lizards (Sabo & Power 2002), and bats (Power *et al.* 2004) is well documented. The result of this study shows that the thermal spatial heterogeneity in rivers where aquatic insects grow enhances the effect of this subsidy, and support more riparian predators. The importance of spatial environmental heterogeneity in river ecosystems has been discussed with regard to its effect on biodiversity (Pringle *et al* 1988) and species interactions (Bellmore *et al*. 2015). The result of the present study shows that the spatial heterogeneity in rivers can also affect the stream-riparian food web linkages. Spatial heterogeneity of natural river environments is threatened by anthropogenic habitat alterations such as channelization, damming, and alteration of sediment supply (Ward & Stanford

1995). Preserving geomorphological heterogeneity that sustains spatial heterogeneity in water temperatures may also play a role in maintaining stream-riparian food web linkages.

Spatial heterogeneity in environment such as temperature, light availability, precipitation, or elevation can produce spatial asynchrony in resource blooms across natural landscapes. It is known that some mobile predators can track resource blooms and take advantage of a spatially asynchronous resource (e.g. Serengeti grazers following flushes of food plants; Fryxell *et al.* 2005, bears tracking salmon among tributaries, Schindler *et al.* 2013). However, spatial mixing of a resource due to the movement of prey organisms, as reported in this study, has been understudied as a process that can mediate the availability of food to consumer populations. Cross-ecosystem subsidy is often associated with movements of resources or mobile prey (Polis *et al.* 1997), and spatial mixing of resources delivered from various source habitats maybe common in cross-ecosystem subsidy. Thus, preservation of natural heterogeneity can be important not only for the organisms in the system, but also for consumers that receive resource subsidies from the system.

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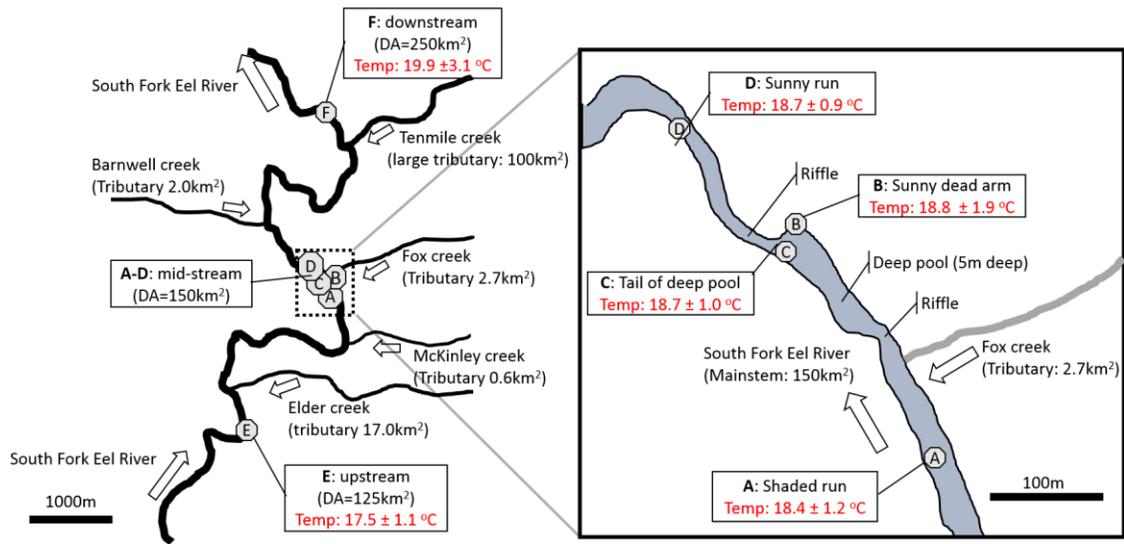
Figure 1. Map of study sites where *E. maculata* nymph emergence were observed (A – F), and creeks where *E. maculata* adult flight were observed (Fox Creek, Barnwell Creek, Elder Creek, McKinley Creek). *E. maculata* nymphs grow in mainstem of South Fork Eel River and Ten Mile Creek and emerge, then migrate to adjacent tributaries as adults, oviposit, and die. The temperatures in boxes show mean and daily standard division of the water temperature from June 1-June 31, 2015.
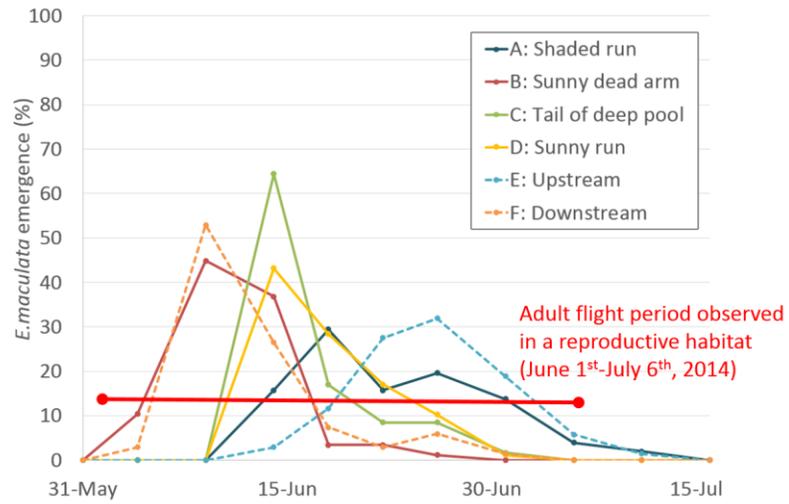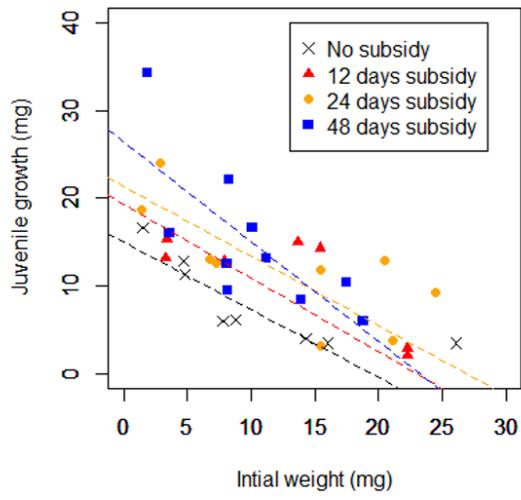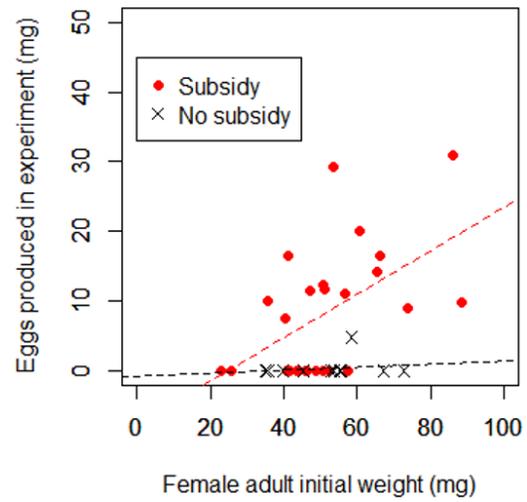
Figure 2. Emergence timing of *E. maculata* from six sites in the South Fork Eel River and flight period of *E. maculata* adults in tributaries.

(a)   (b)

Figure 3. Growth of juvenile spiders (a) and egg reproduction of adult female spiders (b) during the feeding experiment as a function of initial weight of the individual spider at the beginning of the experiment. Least-squares linear regression lines are shown for each treatment. Spiders in subsidy treatments received twice as much food as spiders in No subsidy treatment. Spiders in 12-24-48 day subsidy treatments received in total same amount of food over different durations.