Resource Quantity and Quality in Cross-Ecosystem Food Web Subsidies

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

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Steven Merkley

August 2016

Dissertation Committee:
Dr. William Walton, Chairperson
Dr. Kurt Anderson
Dr. James Sickman
The Dissertation of Steven Merkley is approved:

____________________________________

____________________________________

____________________________________

Committee Chairperson

University of California, Riverside
Acknowledgments

I’d like to acknowledge my advisor, Bill Walton, for his mentoring, advice, and expertise. Thank you to the members of my dissertation and oral exam committees, Kurt Anderson, Jim Sickman, Jonah Piovia-Scott, Matt Daugherty, Darrel Jennerette, and David Reznick for all their input in developing, editing, and refining my experimental design. I’d also like to thank the following people for their help and support on various aspects of the experiments, editing the manuscript, statistical analysis, and access to field sites and organisms: Dagne Duguma, Nate McConnell, David Popko, Tom Tao, Adena Why, Margaret Wirth, Eric Huynh, Kevin Wu, Anh Dao, Gabriela Griffin, Scott Nygren, Bonnie Johnson, Crystal Chaw, Mark DeGuzman, Ashkaan Fahimipour, Delores Lucero, Keenan Morrison, Daniel Quintana, Jim Starrett, Heather Hulton VanTassel, Frank Whiteman, Jim Jenson, Eric Duggan, Stephen Brown, Adriana Medina, Rick Vetter, and the Dahanukar and Trumble labs. I’d like to thank those that sent me data for the meta-analysis: Art Woods, Angelica Gonzalez, Wyatt Cross, Jim Elser, Knut Mehler, Donald Yee, and Sue Bertram. Many of the materials and isotope samples were funded by a Graduate Dean’s Dissertation Research Grant from the University of California-Riverside.
To my lovely wife, Elizabeth, and her never-ending support and love.
Prey moving from donor to recipient ecosystems (e.g. freshwater to terrestrial) are known as cross-ecosystem food web subsidies. These prey can affect the distribution and abundance of subsidized predators. Most investigators have focused on how the quantity of prey (body size and biomass) affect predators, often assuming that resource quantity is an accurate proxy for resource quality. In the present dissertation, I used meta-analysis, lab, and field experiments to show the importance of measuring resource quality and how shifting quality of prey subsidies (insects) can influence a subsidized predator (*Tetragnatha* sp. spiders). Specifically, I define resource quality as a function of body nutrient
content (%N) and carbon:nitrogen (C:N) ratio. In chapter two using data from 30 studies and ~250 effect sizes, I performed meta-analyses and demonstrated that increases in metrics of resource quantity (body size and dry weight) are not synonymous with increases in metrics of resource quality. In fact, there was a negative correlation between body phosphorus content (%P) and resource quantity. In chapter three, I tested how terrestrial spiders (*Tetragnatha nitens*) responded to different levels of prey quality by rearing them on three different prey: midges (*Chironomus dilutus*), mosquitoes (*Culex quinquefasciatus*), and fruit flies (*Drosophila melanogaster*). Mosquitoes were the lowest quality resource and lowered the nutrient content and stoichiometry of spider predators compared to the other treatments. Spiders fed on mosquitoes also suffered delays in growth and development due to being fed on the lower quality resource. Lastly, in chapter four, I tested how terrestrial spiders (*Tetragnatha* sp.) were affected by an aquatic nutrient enrichment in experimental field mesocosms. Abundance, body size (tibia + patella length (TPL) of first walking leg) (mm), biomass (mg), or nutrient stoichiometry (C:N ratio) of the subsidized *Tetragnatha* sp. spider did not differ significantly between enriched and non-enriched pools. However, significantly higher nitrogen content (% body N) was
in *Tetragnatha* sp. spiders from the Santa Ana River inflow in a constructed
treatment wetland compared to a site farther along this aquatic nutrient gradient
during July 2015. Nutrient enrichment of the aquatic ecosystem due to
anthropogenic runoff during a summer monsoon event may explain this pattern.
Contents

List of Figures xii
List of Tables xiii

1 Introduction

1.1 Food Web Subsidies ................................. 1
1.2 A Brief Summary of the Dissertation ............... 6

2 Is there a correlation between resource quality and resource quality in cross-ecosystem food web subsidies? 10

2.1 Abstract .............................................. 10
2.2 Introduction ......................................... 12
2.3 Materials and Methods ............................... 14
    2.3.1 Statistical Analyses ............................. 15
2.4 Results .............................................. 17
    2.4.1 Nitrogen ........................................ 17
    2.4.2 Phosphorus ..................................... 17
    2.4.3 Carbon .......................................... 18
    2.4.4 Carbon:Nitrogen Ratio .......................... 18
Prey quality affects the growth rate of a subsidized terrestrial predator (*Tetragnatha nitens*)

3.1 Abstract ................................. 31

3.2 Introduction .............................. 32

3.3 Materials and Methods ............................. 37

  3.3.1 Statistical Analyses ............................ 39

3.4 Results ........................................ 40

  3.4.1 Prey Quality ............................... 40

  3.4.2 Predator Survivorship ........................... 41

  3.4.3 Predator Body Size, Biomass, & Stoichiometry ........... 41

  3.4.4 Predator Growth Rate .......................... 42

  3.4.5 Number of Instars and Intermolt Interval .............. 43

  3.4.6 Stable Isotopes .............................. 43

3.5 Discussion .................................... 44
Do nutrient enrichments have cascading effects across ecosystems?

4.1 Abstract ................................................................. 56
4.2 Introduction ............................................................ 58
4.3 Materials and Methods .............................................. 62
4.4 Results ................................................................. 66
  4.4.1 Benthic Insects ................................................. 66
  4.4.2 Emergent Insects ............................................... 67
  4.4.3 Spider Abundance ............................................... 68
  4.4.4 Spider Size and Web Size ...................................... 69
  4.4.5 Spider Nutrients and Stoichiometry ......................... 69
  4.4.6 Spider Stable Isotopes ........................................... 70
  4.4.7 Nutrients and Stable Isotopes in Field-collected Spiders 70
4.5 Discussion ........................................................... 72

5 Conclusions ............................................................ 94
  5.1 Conclusions .......................................................... 94

Bibliography .............................................................. 96
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Forest plot of nitrogen against dry weight/body mass</td>
<td>23</td>
</tr>
<tr>
<td>2.2</td>
<td>Forest plot of phosphorus against dry weight/body mass</td>
<td>24</td>
</tr>
<tr>
<td>2.3</td>
<td>Forest plot of carbon against dry weight/body mass</td>
<td>25</td>
</tr>
<tr>
<td>2.4</td>
<td>Forest plot of C:N ratio against dry weight/body mass</td>
<td>26</td>
</tr>
<tr>
<td>2.5</td>
<td>Forest plot of C:P ratio against dry weight/body mass</td>
<td>27</td>
</tr>
<tr>
<td>2.6</td>
<td>Forest plot of N:P ratio against dry weight/body mass</td>
<td>28</td>
</tr>
<tr>
<td>2.7</td>
<td>Scatterplot C:N ratio against TPL (mm)</td>
<td>29</td>
</tr>
<tr>
<td>2.8</td>
<td>Scatterplot nitrogen content against TPL (mm)</td>
<td>30</td>
</tr>
<tr>
<td>3.1</td>
<td>Nitrogen content of prey species</td>
<td>49</td>
</tr>
<tr>
<td>3.2</td>
<td>C:N ratio of prey species</td>
<td>50</td>
</tr>
<tr>
<td>3.3</td>
<td>Effect of treatments on biomass (mg) of spiders</td>
<td>51</td>
</tr>
<tr>
<td>3.4</td>
<td>Nitrogen content of spiders</td>
<td>52</td>
</tr>
<tr>
<td>3.5</td>
<td>C:N ratio of spiders</td>
<td>53</td>
</tr>
<tr>
<td>3.6</td>
<td>Effect of treatments on TPL (mm) of spiders</td>
<td>54</td>
</tr>
<tr>
<td>3.7</td>
<td>Stable isotopes of prey and spiders</td>
<td>55</td>
</tr>
<tr>
<td>4.1</td>
<td>Photo of mesocosm at study site</td>
<td>84</td>
</tr>
<tr>
<td>4.2</td>
<td>Nitrate concentration (mg/L) at Prado</td>
<td>85</td>
</tr>
<tr>
<td>4.3</td>
<td>Schematic of Prado Wetlands</td>
<td>86</td>
</tr>
<tr>
<td>4.4</td>
<td>Wing length (mm) of midges from mesocosms</td>
<td>87</td>
</tr>
<tr>
<td>4.5</td>
<td>Emergent insect $\delta^{15}$N ($%$) from mesocosms</td>
<td>88</td>
</tr>
<tr>
<td>4.6</td>
<td>Nitrogen content of spiders from mesocosms</td>
<td>89</td>
</tr>
<tr>
<td>4.7</td>
<td>Spider $\delta^{15}$N ($%$) from mesocosms</td>
<td>90</td>
</tr>
<tr>
<td>4.8</td>
<td>Proportion of <em>Tetragnatha</em> diet from different sources</td>
<td>91</td>
</tr>
<tr>
<td>4.9</td>
<td>Spider biomass (mg) and TPL (mm) from Prado wetlands</td>
<td>92</td>
</tr>
<tr>
<td>4.10</td>
<td>Spider nitrogen content from Prado wetlands</td>
<td>93</td>
</tr>
</tbody>
</table>
List of Tables

4.1 Number of benthic invertebrates in mesocosms ............. 80
4.2 Number of emergent insects in mesocosms ................. 82
4.3 Web radius (cm) and size range (cm) of spider webs ....... 83
Chapter 1

Introduction

1.1 Food Web Subsidies

Almost every natural process is linked by patterns of energy transfer. The earth and its atmosphere are often considered components of the only truly closed natural system, however, this system also receives vital inputs of sunlight and less important meteorites and dust from the solar system. Forces outside of the earth also affect natural processes, such as the moon’s gravitational pull affecting ocean tides (Brosche and Shuh 1998). An excellent way of illustrating the openness of the ecosystems on earth is by placing an empty bowl of purified
water outside. Within a short time span, the bowl will be colonized by a host of bacteria, plants, and even aquatic insects. Organisms do not live in a vacuum.

In ecological networks, a food web or resource subsidy refers to a flux of materials and/or energy from a donor ecosystem that causes a population or community response in a recipient ecosystem. Subsidies can be manifest in many forms: nutrients, detritus, actively dispersing organisms, carrion, waste products, and even habitat. Consumers can receive additional energy from food sources outside of their ecosystem by active or passive transport across an ecosystem boundary (Puth and Wilson 2001). Subsidies from donor food webs are ubiquitous, provide energy, and can structure community composition and populations of consumers in recipient food webs (e.g. Polis et al. 1997). A common example is riparian vegetation falling into a stream and providing allochthonous (external) nutrients, which can be broken down by bacteria and consumed by aquatic invertebrate consumers, known as “shredders”, such as caddisfly larvae and mayfly nymphs (e.g. Vannote et al. 1980). Energy can also actively flux across an ecosystem boundary, such as emergent aquatic insects providing nutrients and energy to terrestrial food webs (e.g. Nakano and Murakami 2001).
Although Summerhayes and Elton (1923) and others hinted at the potential importance of spatially and temporally connected food webs from adjacent ecosystems, scientists departed from studying food webs at the landscape or ecosystem scale (Power 2001). In the 1970’s and 1980’s, the study of food webs moved to a theoretical focus by attempting to find patterns in the structure and stability of food webs (e.g. May 1972, Pimm 1982). In his landmark book, Food Webs (1982), Stuart Pimm outlined 12 causes governing the structure and patterns of food webs, based on theoretical modeling of empirical food webs. Polis (1991) argued that most of these causes structuring food webs were built upon assumptions that were unrealistic, not based upon complex food webs in nature, and lacked the spatiotemporal complexity necessary to assess the stability and feasibility of a food web in nature. On the other hand, landscape and ecosystem ecologists were studying nutrient flows between ecosystems; however, they were not addressing how these flows might alter local population dynamics and community structure (Likens and Bormann 1974, Forman and Godron 1981).

Polis noticed that there was a high density of spiders around ocean coasts, especially on island ecosystems (e.g. Polis and Hurd 1995). This observation led
him to begin thinking about how spatial food web subsidies could lead to increased population size and structure biotic communities. His research demonstrated that the two main sources of external energy to desert island ecosystems came in the form of detritus (e.g. algal wrack and marine carrion) and nutrients (via deposition from seabird guano) from the sea (Polis and Hurd 1996), and that these allochthonous inputs affected the distribution and abundance of a number of terrestrial producers and consumers (Rose and Polis 1998, Sanchez-Piñero and Polis 2000, Stapp and Polis 2003, Barrett et al. 2005). Polis and others synthesized the fields of landscape and food web ecology and outlined major types of energy flux between spatially separated food webs in a highly influential review, which has been cited over 1,000 times, as of June 2016 (Polis et al. 1997).

Shigeru Nakano, who was known for his attention to detail, devised large-scale manipulation experiments to determine the importance of spatial food web subsidies to both aquatic and terrestrial ecosystems (Nakano et al. 1999, Nakano and Murakami 2001). He echoed the message of previous researchers by showing that forests feed the stream (through leaf fall and terrestrial invertebrates), but also, in a reciprocal way, streams feed the forest through aquatic insect
emergence. He also showed the importance of spatiotemporal dynamics by demonstrating that riparian insectivores depend on aquatic insect emergence more heavily during the fall and winter (Nakano and Murakami 2001).

Movement of nutrients between ecosystems may happen via natural processes, such as upwelling on coastal shores (Palumbi 2003, Witman et al. 2010). Animals travelling or migrating across ecosystem boundaries can also be vectors for transporting nutrients, usually through feces or guano (Vanni 2002, Ellis 2005). Allochthonous detritus most often subsidizes primary consumers, allowing an alternative source of energy at the base of the food web, especially if in-situ primary productivity is low (Moore et al. 2004). Both plant and animal detritus can passively flow between adjacent ecosystems, providing energy to a wide variety of aquatic, terrestrial, and marine organisms (Hall et al. 2001, Reys et al. 2009, Piovia-Scott et al. 2011). Lastly, predators can be subsidized by mobile prey actively moving across ecosystem boundaries, such as emerging aquatic insects moving from streams and lakes to provide food for terrestrial predators, such as birds, bats, and lizards (Murakami and Nakano 2002, Sabo and Power 2002, Gratton et al. 2008, Wesner 2012).
When considering the flow of resources in food webs, many ecologists have overlooked the abundant resources fluxing from adjacent ecosystems and what impact they may have on the abundance and distribution of organisms (Power 2001). Dependence on resource subsidies is strong in many systems and there is recent evidence that removal of external inputs of prey and detritus from donor ecosystems can cause species decline in recipient ecosystems (e.g. Bishop et al. 2010). Resource subsidies can modulate many important ecological processes (competition, predation, etc.) within ecosystems and provide additional energy to consumers from adjacent ecosystems, which may help better predict the distribution and abundance of organisms on the landscape.

1.2 A Brief Summary of the Dissertation

Researchers need to combine the quantity and quality of resources to measure how organisms respond to an increase or decrease in food web subsidies. Marcarelli and others (2011) have stated, “The greatest insight into the importance of subsidies is gained when information on quality and quantity are combined.” It is only by combining the food quality to the consumer and the overall amount of food available (quantity) that ecologists can understand how
much a consumer in a recipient ecosystem depends on a food web subsidy from a
donor ecosystem. Many studies have measured the quantity of resource subsidies
(Marczak et al. 2007), but to we need more information to measure the quality of
subsidies. Resource quality can be judged by caloric, nutrient, and lipid content,
or by the measurement of certain chemical characteristics (Lau et al. 2008, Brett
et al. 2009). It can also be measured by feeding plasticity of the consumer,
spatial and temporal availability of the resource, or handling time of the
consumer (e.g. Wipfli and Baxter 2010, Marcarelli et al. 2011). We need to
understand quantity, quality, behavioral responses, spatial and temporal
availability of resources, and availability of in-situ resources before we can
understand what impact food web subsidies may have on any organism in the
recipient ecosystem. This can only be achieved through a framework of
collaboration and using multiple methods (experimental, observational,
theoretical, etc.) to achieve a broad consensus.

In the present dissertation, I combine meta-analysis with lab and field
experiments to show how changing the quality of food web subsidies can affect
predators in a subsidized ecosystem. In chapter two, I use meta-analysis to show
that an increase in resource quantity is not synonymous with an increase in
resource quality. Although an increase in quantity of prey subsidies can affect subsidized predators (Polis et al. 1997), the magnitude and direction of prey quality is not easily predicted by body size or dry weight. Specifically, there is no significant correlation between nitrogen content and dry weight of prey and a negative correlation between phosphorus content and dry weight, showing a decrease in nutrient content with increase in size.

In chapter three, I use a lab experiment to test how differing food quality will affect the growth and development of a subsidized spider, *Tetragnatha nitens*. The lowest quality prey, southern house mosquito (*Culex quinquefasciatus*), caused a delay in growth of spiders compared to the higher quality midge (*Chironomus dilutus*) or fruit fly (*Drosophila melanogaster*). Spiders fed on lower quality prey also had a lower overall nitrogen content (% body nitrogen) and higher C:N ratio than spiders in other treatments.

In chapter four, I use a field mesocosm experiment to test how aquatic nutrient enrichment might affect the quantity and quality of aquatic insect prey and in turn the terrestrial spider predators that depend on them. Nutrient enrichment had a positive effect on the body size of chironomid midges, but did not significantly affect abundance, nutrients, or stoichiometry of aquatic insects.
or spider predators. Long-term anthropogenic nitrogen increased the body size and dry weight of spider predators, as well as nitrogen content, due to agricultural run-off after a large rain event.

Although many different patterns have been expressed between prey quality and prey quantity (Fagan et al. 2002, Bertram et al. 2008, Gonzalez et al. 2011, Back and King 2013), in this dissertation, I show that increase in prey quantity does not correspond to an increase in prey quality. It is unclear how nutrient enrichment will modulate the quality of resource subsidies and affect the abundance of subsidized predators, although it has been shown to change the community composition of emerging aquatic insects (Davis et al. 2011).

However, differing the quality of prey moving across ecosystem boundaries may have an affect on the growth, development, and reproductive fitness of subsidized predators.
Chapter 2

Is there a correlation between resource quality and resource quantity in cross-ecosystem food web subsidies?

2.1 Abstract

Resource quantity is often used as a proxy for resource quality in the study of food web subsidies. Meta-analysis from 30 studies and over 250 effect sizes is used to show that an increase in resource quantity is not synonymous with an increase in resource quality. Although an increase in quantity of prey subsidies
can affect subsidized predators (Polis et al. 1997), the magnitude and direction of prey quality is not easily predicted by body size or dry weight. Specifically, there is no significant correlation between nitrogen content and dry weight of prey and a negative correlation between phosphorus content and dry weight, showing a decrease in nutrient content with increase in size. There was no correlation between nitrogen content (%N) or C:N ratio and dry weight biomass (mg) of *Tetragnatha* sp. spiders in either the lab study (Chapter 3) or the mesocosm study (Chapter 4). However, there was a significantly positive correlation between C:N ratio and TPL (mm) in spiders from lab study, but a significantly negative correlation between C:N ratio and TPL (mm) in spiders from the mesocosm study. Also, there was a significantly negative correlation between nitrogen content (%N) and TPL (mm) in spiders from lab study, but a significantly positive correlation between nitrogen content (%N) and TPL (mm) in spiders from the mesocosm study. These patterns further show the inaccuracy of using nutrient quantity as a consistent proxy for nutrient quality.
2.2 Introduction

While plants and other primary producers have a wide range of nutrient content and stoichiometry, it is often assumed that herbivores and their predators maintain elemental homeostasis (Sterner and Elser 2002). However, more recent evidence has shown that there can be significant variation in nutrient stoichiometry within and between predators and their prey (Fagan et al. 2002, Cross et al. 2003, Liess and Hillebrand 2005, Evans-White et al. 2005, Back and King 2013). This variation in nutrient content and stoichiometry (resource quality) has led many investigators to analyze the allometric relationship of the variation in resource quality.

There have been many different expressed relationships between prey quality and prey quantity in natural ecosystems. Fagan et al. (2002) have shown body length (mm) of insect predators to be positively correlated to N content. Evidence has shown an inverse relationship between P content and biomass (Woods et al. 2004, Liess and Hillebrand 2005, Kay et al. 2006, Hambäck et al. 2009, Schneider et al. 2010, Kendrick and Benstead 2013, Back and King 2013). Bertram et al. (2008) also found a decrease in N and P and an increase in C as biomass increased in crickets. Other investigators have found no significant

In the study of food web subsidies, ecologists have assumed that resource quantity (size, mass, abundance) and resource quality (nutrient content, nutrient stoichiometry, etc.) are positively correlated (Polis et al. 2004, Marcarelli et al. 2011) and therefore quantity metrics are often used as a proxy for resource quality. However, disturbances causing changes in community composition can lead to a decrease in resource quality of food web subsidies, even when the resource quantity is increased (e.g. Davis et al. 2011).

If quality increases with size, predators would most likely always prefer the larger, more nutritious prey. However, if there is an inverse relationship between prey quality and quantity, predators may preferentially feed on smaller, more nutritious prey. Understanding this general relationship can help ecologists make predictions about predator-prey dynamics in natural systems, especially in communities experiencing significant disturbance. Although this relationship may be context-dependent, I predict that there will be no significant correlation between resource quantity and resource quality across taxa.
2.3 Materials and Methods

I searched the literature on invertebrates (mostly insects) and used a meta-analysis to show if there is a correlation between invertebrate prey quantity and prey quality. Using the ISI Web of Science database, I used the following search terms to find literature in May 2016: ("biomass" OR "body size") AND ("nutrient content" OR "stoichiometr*") AND ("insect" OR "invertebrate")). I also found relevant papers by checking the reference lists of articles obtained through this search and from well-known review articles in this topic. I only included articles in the analysis that contained data on both resource quantity and resource quality. Metrics of prey quantity included dry weight, biomass, and body size of individual organisms or groups of organisms. Metrics of prey quality included nutrient content (%C, %N, and %P) and nutrient stoichiometry (C:N, N:P, and C:P).

In addition to data from the meta-analysis, I also collected data on the quantity and quality of Tetragnatha sp. spiders during chapters 3 and 4 of this dissertation. For the lab study (Ch. 3), quality and quantity metrics were recorded from spiders (Tetragnatha nitens) fed on midges (Chironomus dilutus) and mosquitoes (Culex quinquefasciatus), because survival of spiders fed on fruit
flies (*Drosophila melanogaster*) was extremely low. For the mesocosm study (Ch. 4), quality and quantity metrics were recorded from *Tetragnatha* sp. spiders collected at the end of the 8-week study from the mesocosms. After spiders were collected, they were measured for prey quantity metrics: dry weight biomass (mg) and first tibia-patella length or TPL (mm), as a proxy for body size. Spiders were then kept in a freezer (-20 °C), until they could be dried up and ground into powder for nutrient analysis. Prey quality metrics were measured using the mass spectrometer: nutrient content (C and N) and nutrient stoichiometry (C:N ratio). These metrics were used to determine if there was a significant correlation between resource quantity and quality.

2.3.1 Statistical Analyses

After searching using the criteria above, I included ~250 effect sizes from 30 studies between 1992-2015. After collecting correlational data (Pearson’s r correlation coefficient and sample size) from each study, I analyzed the data using the packages ‘metafor’ (Viechtbauer 2010) and ‘robumeta’ (Fisher and Tipton 2015) for R statistical software (R development core team 2015; sensu Quintana 2015). Separate meta-analyses were run for phosphorus content (%P),
nitrogen content (%N), carbon content (%C), carbon:nitrogen ratio (C:N),
carbon:phosphorus ratio (C:P), and nitrogen:phosphorus ratio (N:P). Each
analysis had a different number of studies and effect sizes [%N (24 studies, 86
effect sizes); %P (18 studies, 75 effect sizes); %C (10 studies, 44 effect sizes), C:N
ratio (7 studies, 33 effect sizes), C:P ratio (7 studies, 13 effect sizes), N:P ratio (9
studies, 16 effect sizes)]. Each meta-analysis was checked for publication bias by
using a funnel plot in R and testing for funnel plot asymmetry using the rank
correlation test for funnel plot asymmetry (‘ranktest’ in R) and the regression
test for funnel plot asymmetry (‘regtest’ in R). If publication bias was detected,
the trim and fill method (‘trimfill’ function in R) was used to impute missing
data and meta-analysis was re-run to account for publication bias. To correct for
multiple effect sizes within some studies (e.g. Gonzalez et al. 2011), a hierarchical
effects model with small-sample corrections (robust variation estimation analyses;
RVE) was used (sensu Quintana 2015).

Resource quantity and quality of *Tetragnatha* sp. spiders from
experiments in chapters 3 and 4 was compared using a linear regression model
(‘lm’) in R statistical software (R development core team 2015). All response
variables were checked for normality and appropriate transformations were made before analysis.

2.4 Results

2.4.1 Nitrogen

The overall correlation between nitrogen content and dry weight or body size for the 24 studies included was non-significant ($r = -0.034 \pm 0.114$ (SE), $t_{6.74} = -0.3$, $p = 0.773$). The forest plot shows effect sizes with 95% confidence intervals for each study, as well as the overall effect size for the meta-analysis (Fig. 2.1).

2.4.2 Phosphorus

The overall correlation between phosphorus content and dry weight or body size for the 18 studies included was significantly negative ($r = -0.746 \pm 0.097$ (SE), $z = -7.72$, $p < 0.0001$). The forest plot shows effect sizes with 95% confidence intervals for each study, as well as the overall effect size for the meta-analysis (Fig. 2.2).
2.4.3 Carbon

The overall correlation between carbon content and dry weight or body size for the 10 studies included was non-significant \( (r = 0.122 \pm 0.073 \text{ (SE)}, z = 1.66, p = 0.096) \). The forest plot shows effect sizes with 95% confidence intervals for each study, as well as the overall effect size for the meta-analysis (Fig. 2.3).

2.4.4 Carbon:Nitrogen Ratio

The overall correlation between C:N ratio and dry weight or body size for the 7 studies included was significantly positive \( (r = 0.289 \pm 0.091 \text{ (SE)}, z = 3.18, p = 0.0015) \). The forest plot shows effect sizes with 95% confidence intervals for each study, as well as the overall effect size for the meta-analysis (Fig. 2.4).

2.4.5 Carbon:Phosphorus Ratio

The overall correlation between C:P ratio and dry weight or body size for the 7 studies included was non-significant \( (r = 0.14 \pm 0.118 \text{ (SE)}, t_{5.26} = 1.18, p = 0.288) \). The forest plot shows effect sizes with 95% confidence intervals for each study, as well as the overall effect size for the meta-analysis (Fig. 2.5).
2.4.6 Nitrogen:Phosphorus Ratio

The overall correlation between N:P ratio and dry weight or body size for the 9 studies included was non-significant ($r = 0.118 \pm 0.162$ (SE), $z = 0.732$, $p = 0.465$). The forest plot shows effect sizes with 95% confidence intervals for each study, as well as the overall effect size for the meta-analysis (Fig. 2.6).

2.4.7 *Tetragnatha* spiders

For spiders (*Tetragnatha nitens*) collected during lab experiment (Ch. 3), there was no relationship between C:N ratio and dry weight biomass (mg) ($F_{1,28} = 1.86$, $p = 0.184$) or between nitrogen content (%N) and dry weight biomass (mg) ($F_{1,28} = 1.738$, $p = 0.198$). However, there was a significantly positive correlation between C:N ratio and TPL (mm) (Fig. 2.7a; $F_{1,28} = 5.567$, $p = 0.0255$). There was also a significantly negative correlation between nitrogen content (%N) and TPL (mm) (Fig. 2.8a; $F_{1,28} = 6.011$, $p = 0.0207$).

For spiders (*Tetragnatha* sp.) collected at the end of the mesocosm study (Ch. 4), there was no relationship between C:N ratio and dry weight biomass (mg) ($F_{1,31} = 2.584$, $p = 0.118$) or between nitrogen content (%N) and dry weight biomass (mg) ($F_{1,31} = 1.557$, $p = 0.221$). However, there was a significantly
negative correlation between C:N ratio and TPL (mm) (Fig. 2.7b; $F_{1,31} = 10.44$, $p = 0.00292$). There was also a significantly positive correlation between nitrogen content (%N) and TPL (mm) (Fig. 2.8b; $F_{1,31} = 6.852$, $p = 0.0136$).

### 2.5 Discussion

In the meta-analyses performed, most of the nutrients (%C, %N) and nutrient ratios (C:P ratio, N:P ratio) of invertebrates were uncorrelated with metrics of resource quantity (dry weight and body size). This pattern shows the importance of providing metrics of resource quality in the study of cross-ecosystem food web subsidies, especially as it relates to invertebrates (Marcarelli et al. 2011). However, there were a few significant results that arose from the meta-analyses.

First, there was a significant negative correlation between phosphorus content (%P) and metrics of resource quantity (dry weight and body size). This can be best explained by the growth-rate hypothesis and repeated measures within the same individuals (Elser et al. 1996, Sterner and Elser 2002). The growth-rate hypothesis posits that as organisms increase in size during development, they are producing an abundance of mRNA and rRNA, which is
rich in phosphorus. As the growth of the organism slows down, the percentage of phosphorus contained in the body decreases. Since this is a common result reported across many studies (Elser et al. 2000, Woods et al. 2004, Liess and Hillebrand 2005, Bertram et al. 2006, Kay et al. 2006, Bertram et al. 2008, Hambäck et al. 2009, Schneider et al. 2010, Visanuvimol and Bertram 2010, Kendrick and Benstead 2013, Back and King 2013, Danger et al. 2013), that is reflected in the results of the meta-analysis.

Second, there was a significant positive correlation between C:N ratio and metrics of resource quantity (dry weight and body size). Higher C:N ratio actually correlates to a lower quality resource (Sterner and Elser 2002) for predators, so the pattern here is a negative relationship between resource quantity and resource quality.

An interesting pattern was seen in the *Tetragnatha* sp. spiders measured during the lab and mesocosm experiments (Fig. 2.7, Fig. 2.8). While there were significant correlations between TPL (mm) and C:N ratio and nitrogen content (%N), the direction of this relationship shifted depending on the context. Spiders during the lab study were only fed on specific prey and showed a negative relationship between quantity and quality, while spiders collected during the
mesocosm experiment in a more natural setting showed a positive relationship between quantity and quality. Invertebrates in the same ecosystem may also show differing directions in the correlation between resource quantity and quality (e.g. Gonzalez et al. 2011).

There was no evidence of a positive relationship between resource quantity and resource quality across the meta-analyses and conflicting evidence of this relationship depending on context during the experiments in this present dissertation. Overall, it is unreliable to make predictions of the direction and magnitude of the relationship between resource quantity and quality without collecting data on the species of interest in the context in which its measured.
Figure 2.1. Forest plot from meta-analysis of nitrogen against dry weight/body size. Dot represents mean of the correlation (Pearson’s r) between variables in each study. Error bars represent 95% confidence intervals.
Figure 2.2. Forest plot from meta-analysis of phosphorus against dry weight/body size. Dot represents mean of the correlation (Pearson’s r) between variables in each study. Error bars represent 95% confidence intervals.
Figure 2.3. Forest plot from meta-analysis of carbon against dry weight/body size. Dot represents mean of the correlation (Pearson’s r) between variables in each study. Error bars represent 95% confidence intervals.
Figure 2.4. Forest plot from meta-analysis of carbon:nitrogen ratio against dry weight/body size. Dot represents mean of the correlation (Pearson’s r) between variables in each study. Error bars represent 95% confidence intervals.
Figure 2.5. Forest plot from meta-analysis of carbon:phosphorus ratio against dry weight/body size. Dot represents mean of the correlation (Pearson’s r) between variables in each study. Error bars represent 95% confidence intervals.
Figure 2.6. Forest plot from meta-analysis of nitrogen:phosphorus ratio against dry weight/body size. Dot represents mean of the correlation (Pearson’s r) between variables in each study. Error bars represent 95% confidence intervals.
Figure 2.7. Scatterplot with linear regression of carbon:nitrogen ratio with first tibia-patella length (TPL) (mm) of *Tetragnatha* sp. spiders from (A) lab study (Chapter 3) and (B) mesocosm study (Chapter 4).
Figure 2.8. Scatterplot with linear regression of nitrogen content (%N) with first tibia-patella length (TPL) (mm) of *Tetragnatha* sp. spiders from (A) lab study (Chapter 3) and (B) mesocosm study (Chapter 4).
Chapter 3

Prey quality affects the growth rate of a subsidized terrestrial predator (*Tetragnatha nitens*)

3.1 Abstract

Interspecific differences of nutrient stoichiometry of prey species can create imbalances in predator body stoichiometry that affect the growth and development of subsidized consumers. Predators suffering stoichiometric imbalances exhibit reduced growth rates and lengthened development times, and may differentially excrete or store excess carbon (C) or nutrients in order to maintain body stoichiometry. For this experiment, I tested how diets of prey (*Culex quinquefasciatus*, *Chironomus dilutus*, and *Drosophila melanogaster*) with varying nutrient quality affected the growth and development of predatory
terrestrial spiders (*Tetragnatha nitens*). The prey with the lowest body nitrogen (7.52±0.10 %) and highest C:N ratio (6.18±0.09) were mosquitoes (*C. quinquefasciatus*). Spiders fed on mosquitoes had significantly lower body nitrogen (N) and higher C:N ratio than spiders fed on midges (*C. dilutus*) or fruit flies (*D. melanogaster*). Although spiders fed on mosquitoes had lower growth rate (mm•d⁻¹) during early instars, growth rate increased during later instars compared to spiders fed on other diets. Spiders fed mosquitoes had significantly lower δ¹³C ‰ than mosquitoes, spiders fed on fruit flies had significantly higher δ¹³C ‰ than fruit flies, while spiders fed on midges did not differ significantly in δ¹³C ‰ from their prey.

### 3.2 Introduction

In ecological networks, a resource subsidy refers to a flux of materials and/or energy from a donor ecosystem that causes a population or community response in a recipient ecosystem. Subsidies from donor food webs are ubiquitous, provide energy, and can structure community composition and populations of consumers in recipient food webs (Polis et al. 1997, 2004).
Resource quantity is often used as an erroneous proxy for resource quality in the study of food web subsidies (Marczak et al. 2007). For example, terrestrial leaf fall is often pointed to as an important terrestrial subsidy to aquatic systems (e.g. Minshall 1967). In its raw form, however, terrestrial leaf litter is much more difficult to consume by aquatic invertebrates than aquatic algal growth, because bacteria and fungi must first break down cellulose and lignin before terrestrial inputs can be utilized by aquatic consumers (Hieber and Gessner 2002). In contrast, small amounts of terrestrial invertebrate inputs can have a relatively large impact on freshwater fish because of high nutritional quality and susceptibility to predators (Wipfli 1997).

Resource quality can be judged by caloric, nutrient, and lipid content or by the measurement of certain chemical characteristics (Lau et al. 2008, Brett et al. 2009). It can also be measured by feeding plasticity of the consumer, spatial and temporal availability of the resource, or handling time of the consumer (Wipfli and Baxter 2010, Marcarelli et al. 2011). Resource subsidies that are nutritionally rich in nitrogen (N) or phosphorus (P) may increase the productivity of producers or consumers in recipient ecosystems (e.g. Fagan et al. 2002). Quality of a resource may be relative, depending on the traits of the
consumer or predator: ontogeny, life history traits, allocation of biomolecules in the body, and so on (Cross et al. 2003, Raubenheimer et al. 2009). For the purposes of this study, however, I will use body nutrient content (N and P) and stoichiometry (C:N, N:P, and C:P ratios) to determine high and low quality prey resources.

Prey quantity is an important factor in limiting predator growth (Paradise and Stamp 1991, Wilder 2011), but prey quality may also limit the growth of subsidized predators (Mayntz and Toft 2001). Quality can be measured by the nutrient imbalance between the predator and prey (Stelzer and Lamberti 2002). There is significant interspecific variation of nutrient stoichiometry in the prey species that invertebrate predators consume (Fagan et al. 2002, Liess and Hillebrand 2005, Back and King 2013). This variation may create an imbalance between the nutrient stoichiometry of primary prey and predator body stoichiometry (Sterner and Elser 2002, Malzahn et al. 2007). In fact, insect predators have significantly higher N content (Fagan et al. 2002), but not higher P content (Woods et al. 2004) than their prey. Due to this imbalance, predators may: a) differentially feed on prey of a higher quality (Cowles et al. 1988), b) suffer losses of growth (Witt et al. 1984, Groeger et al. 1991), and/or c)
differentially excrete or store excess C or nutrients in order to maintain body stoichiometry (Elser and Urabe 1999, Mayntz et al. 2005, Boersma and Elser 2006).

Spiders commonly feed on adult aquatic insects emerging from aquatic into terrestrial ecosystems (e.g. Williams et al. 1995). While some species actively feed on insects as they emerge (fishing spiders, Dolomedes sp.), many spiders are sit-and-wait predators, consuming insects that become trapped in their silken webs. Long-jawed spiders (Family: Tetragnathidae) are often used in food web subsidy research, because of their dependence on adult aquatic insects through their construction of horizontal orb webs near water sources and the relative ease of capturing and measuring them (Nakano and Murakami 2001, Kato et al. 2003, Baxter et al. 2004). Seasonal pulses of aquatic insects can increase the quantity of prey available to subsidized consumers, such as spiders (e.g. Henschel et al. 2001). Marczak and Richardson (2008) examined the impact that varying the quantity and timing of prey subsidies had on the life history of Tetragnatha versicolor. In a laboratory manipulation, they found that large fluxes of fruit flies later in the development of T. versicolor helped individual spiders reach reproductive age in the same or better body condition as
individuals either fed a constant food source or given subsidies earlier in development.

The purpose of this study was to test how varying the resource quality of adult aquatic insects (Culex quinquefasciatus, Chironomus dilutus) and terrestrial insects (Drosophila melanogaster) would affect the growth and development of terrestrial spiders (Tetragnatha nitens). Each of these species was chosen because of their ease in lab rearing and to reduce intraspecific variation of prey nutrient stoichiometry within treatments. Significant differences in nutrient content and stoichiometry were expected between these groups because of difference in habitat and growth patterns (Cross et al. 2003, Liess and Hillebrand 2005, Malzahn et al. 2007, Hambäck et al. 2009). Furthermore, an increase in dietary N availability should lead to an increase in growth and shortened development time for predatory spiders (Furrer and Ward 1995, Toft 1999). Prey nutrient quality may be specifically important for terrestrial arthropod predators because they are often nutrient limited in nature (e.g. Fagan et al. 2002). Specifically, spiders fed on higher-quality prey were predicted to have higher growth rates, faster development, and a higher nutrient content than spiders fed on lower-quality prey.
3.3 Materials and Methods

Gravid female spiders (*Tetragnatha nitens*) were captured from the Prado wetlands in Corona, CA, USA (33.918643°N, 117.619592° W). Adult spiders were kept in separate containers in the lab, until egg sacs were collected from eight wild-caught adult females and juveniles were randomized among treatments. Both wild-caught and spiderlings born in the lab were reared in small Perspex containers (8 cm height × 6 cm diameter) with a small wooden stick for web connections, a lid with a hole to add in prey, and moist Kimwipe® to avoid drying out. Spiders were raised under conditions that mimic a natural environment (27 °C and 16:8 light: dark cycle with 1 hour of dawn/dusk). After juveniles emerged from the egg sacs (2nd instar), individual spiders were separated into individual containers. Juveniles in the 2nd instar were fed on *D. melanogaster* until reaching 3rd instar, after which they were assigned an experimental treatment.

Laboratory-raised juveniles (N=60) were fed one of three diets after they survived to the third instar. Juveniles from different mothers were randomized among treatments to control for any maternal effects. The three experimental treatment diets were *Culex quinquefasciatus* mosquitoes, *Chironomus dilutus*
midges, and *Drosophila melanogaster* fruit flies. Prey species were assessed for quality (nutrient content and nutrient stoichiometry) and quantity (biomass) to determine significant differences between prey types. Since the prey taxa had different average adult dry mass (*D. melanogaster* = 0.29 ± 0.09 mg, *C. quinquefasciatus* = 0.87 ± 0.13 mg, *C. dilutus* = 3.26 ± 0.61 mg; mean ± standard error), biomass was standardized within treatments by feeding spiders ~1 mg of prey per day. Prey were fed on different lab media: mosquitoes fed a media consisting of 75% ground dog food, 25% yeast, midges fed a media containing ground Tetramin® fish food, and fruit flies fed on a standard lab media consisting of yeast, cornmeal, and agar. Prey were frozen before presenting to spiders and thus spiders generally fed on them on the bottom of the cup instead of building webs to catch flying insects.

Feeding experiments were run for 60 days. After each molt, individual spiders were sedated in a CO$_2$ chamber. A Sartorius® digital analytical laboratory scale (Model 1712 MP8 Silver Edition) was used to measure wet mass (mg). Body size (mm) was estimated using TPL (first walking leg tibia + patella length), which is a standard method for measuring orb-weaving spiders (e.g. Marczak and Richardson 2008). High-quality photographs were taken of each
spider after each molt using a Canon® EOS Rebel T4i DSLR camera attached to a dissecting microscope. Photographs were taken on a 1 mm grid background and TPL (mm) was determined using ImageJ (Schneider et al. 2012). Number of molts and intermolt period were also measured. After the experiment ended, all spiders were dried at 50 °C for 24 hours and then ground into powder using a pestle and mortar. Powder samples were weighed and deposited into tins for nutrient and stable isotope analysis.

Total carbon and nitrogen of individual spiders were measured on the basis of mass using an isotope ratio mass spectrometer to account for dietary differences between treatments. Stable isotopes (δ¹³C and δ¹⁵N) were also measured in spiders at the conclusion of the study and compared with prey δ¹³C and δ¹⁵N to determine how spiders were differentially excreting or storing nutrients of varying prey.

3.3.1 Statistical Analyses

A repeated measures linear mixed effects model ANOVA using library ‘nlme’ in R version 3.3.3 (2015) was used to test for differences in biomass (mg), body size TPL (mm), and growth rate (mm\cdot d⁻¹) of spiders. Each of these factors
was analyzed independently. To meet assumptions of normality and equal variance, biomass (mg) was ln (x+1) transformed, body size TPL (mm) was 5th-root transformed, and growth rate (mm•d⁻¹) was square root transformed. A generalized linear model (GLM) was performed to compare survivorship of spiders among the treatments. A Kruskal-Wallis rank sum test and Wilcoxon rank sum test with Bonferroni adjustment for pairwise comparisons were used to test for differences in number of molts, intermolt period, total carbon and nitrogen of spiders among diets. Analysis of variance (ANOVA) with Tukey HSD pairwise comparisons were used to test for differences in δ¹³C and δ¹⁵N (DDF) between prey types and spider predators. These data were also transformed when appropriate to meet assumptions of normality and equal variance. All analyses were performed using R version 3.3.3 (2015).

3.4 Results

3.4.1 Prey Quality

Mosquitoes (*C. quinquefasciatus*) were the lowest quality resource of the three prey species. Mosquitoes had significantly lower percent body nitrogen than both midges (*C. dilutus*) and fruit flies (*D. melanogaster*) (Fig. 3.1; $\chi^2 =$
15.17, d.f. = 2, p < 0.001). *Chironomus dilutus* had slightly higher nitrogen content than fruit flies (p = 0.015). Mosquitoes also had a significantly higher C:N ratio than either prey type (Fig. 3.2; $\chi^2 = 15.16$, d.f. = 2, p < 0.001).

### 3.4.2 Predator Survivorship

Spiders fed on fruit flies had significantly lower survivorship than spiders fed on the other two diets ($z = -4.62$, p < 0.001). Only 9% of the spiders fed on fruit flies survived to the end of the 60-day study compared to 75% and 84% for spiders fed the midge and mosquito diets, respectively.

### 3.4.3 Predator Body Size, Biomass, & Stoichiometry

There was no significant effect of treatment on body size ($F_{2,69} = 2.56$, p = 0.085) or the interaction between treatment and time ($F_{2,146} = 0.740$, p = 0.479) on body size of spiders, as measured by TPL (mm). However, there was a significant effect of treatment on biomass (mg) of spiders (Fig. 3.3; $F_{2,69} = 3.46$, p = 0.037) and the interaction between treatment and time (Fig. 3.3; $F_{2,146} = 7.46$, p < 0.001). The average biomass of spiders fed on mosquitoes across all dates was significantly lower than for spiders fed on other diets ($t_{69} = -2.72$, p = 0.008).
The biomass of spiders from later instars fed on fruit flies, however, was significantly lower than spiders fed on mosquitoes or midges ($t_{146} = -3.24, p = 0.001$). Spiders fed on mosquitoes had significantly less body nitrogen (Fig. 3.4; $p < 0.001$) and higher C:N ratio than spiders fed on midges or fruit flies (Fig. 3.5; $p < 0.001$). There was no significant difference in body N ($p = 0.222$) or C:N ratio ($p = 0.182$) between spiders fed on midges and spiders fed on fruit flies.

### 3.4.4 Predator Growth Rate

There was a significant effect of treatment (Fig. 3.6; $F_{2,69} = 3.25, p = 0.045$) and the interaction between treatment and time (Fig. 3.6; $F_{2,146} = 5.14, p = 0.007$) on growth rate (mm·d⁻¹) of *T. nitens*. Spiders fed on mosquitoes had a lower overall growth rate than spiders fed on midges ($t_{69} = -3.28, p = 0.002$). Spiders fed on mosquitoes grew significantly slower in earlier instars and then significantly faster later in development compared to spiders fed on midges or fruit flies (Fig. 3.6; $t_{146} = 2.48, p = 0.014$).
3.4.5 Number of Instars and Intermolt Interval

Although some individuals survived to the end of the 60-day study, none of the spiders fed on fruit flies matured to become adults. Although spiders fed on mosquitoes took more time to mature (~41 days) than spiders fed on midges (~38 days), this effect was not statistically significant ($t_{29.9} = -0.99$, $p = 0.33$). A greater percentage of spiders fed on lower-quality mosquitoes reached maturity at the 9th instar (40%), compared to spiders fed on higher quality midges (7%).

The intermolt interval of spiders fed on fruit flies (5.65 ± 0.47 days) was significantly shorter than for spiders fed on midges (6.96±0.49 days; $p = 0.046$) and mosquitoes (7.42 ± 0.39 days; $p < 0.001$). Incomplete development of spiders fed on fruit flies may explain this pattern on intermolt interval. There was no significant difference in intermolt interval between spiders fed on midges or mosquitoes ($p = 0.1063$).

3.4.6 Stable Isotopes

Spider $\delta^{15}N$ ‰ was enriched compared to each prey type (Fig. 3.7; $p < 0.001$). Spiders fed on midges closely mirrored the isotopic composition of their prey in $\delta^{13}C$ ‰ ($p = 0.83$). Spiders fed on mosquitoes had significantly lower
\[ \delta^{13}C \%o \] than their mosquito prey \((p < 0.001)\). In contrast, spiders fed on fruit flies had significantly higher \(\delta^{13}C \%o\) than their fruit fly prey \((\text{Fig 3.7}; p < 0.001)\).

### 3.5 Discussion

Quality of a food web subsidy can change with prey type and can have a significant effect on the growth and development of a subsidized consumer. In this study, a lower quality resource \((C. quinquefasciatus)\) had a significant impact on body nutrient content, nutrient stoichiometry and growth rate of a terrestrial predator \((Tetragnatha nitens)\) that feeds mainly on resources from aquatic ecosystems.

Spiders in all treatments mirrored the nutrient body composition \(\%N\) and body stoichiometry \((C:N\) ratio) of their prey. This is especially interesting when considering the nutrients in the lower quality prey. Although there is wide variation in nutrient stoichiometry of primary producers, the stoichiometry of herbivores and predators are generally constrained \((\text{Sterner and Elser 2002})\). However, recent evidence has shown variation in body stoichiometry of consumers reared on different quality prey resources \((\text{e.g. Wilder et al. 2010})\). Web and trap-building spiders are sit-and-wait predators, so they may not be
able to actively incorporate higher quality prey into their diet (but see Mayntz et al. 2009 and Blamires et al. 2011). Thus, orb-weaving spiders like *T. nitens* might have to respond by adjusting internal body stoichiometry to match the prey they catch in their webs (Mayntz et al. 2003). Plasticity in nutrient stoichiometry and nutrient body content may explain why spiders mirrored the nutrient composition and body stoichiometry of their prey. Even spiders with similar total biomass can have different levels of total body nitrogen, suggesting an ability to store nitrogen (Toft et al. 2010, Wilder 2011).

In studies of plasticity of growth rates, Higgins and Rankins (1996) showed that individuals of orb-weaving spiders (*Nephila clavipes*) with faster growth rates go through more instars and grow to a larger size before maturing. A similar pattern was observed in *T. nitens*, where spiders fed on lower quality diets (mosquitoes) grew at faster rates near the end of development. This faster growth rate caused the spiders to go through more instars before maturity than spiders with slower growth rates.

Tradeoffs between age and size at maturation could explain why lower quality prey caused later maturity (Stearns 1992, Tenhumberg et al. 2000, Mayntz et al. 2003). Spiders fed on lower quality diets take longer to mature
(both in days and number of instars). This can lead to lower female fecundity and increased susceptibility to predation during juvenile stages (Higgins 1992). Future studies should attempt to determine how decreased prey nutrient quality affects female fecundity and the fitness of offspring.

Diet discrimination factor (DDF) of $\delta^{15}$N‰ between spider predators and prey was positive, but varied depending on prey and did not reflect the pattern (increase by 2.4‰) predicted by the literature (e.g. Hansson et al. 1997). While spiders fed on fruit flies (mean = 2.96‰ $\delta^{15}$N) and spiders fed on mosquitoes (mean = 3.23‰ $\delta^{15}$N) were close to the observed pattern, spiders fed on the higher-quality midges (mean = 0.69‰ $\delta^{15}$N) had a much lower level of enrichment. In addition, $\delta^{13}$C was significantly lower in spider predators fed on lower-quality mosquito prey, relative to mosquito $\delta^{13}$C (Fig. 3.7; p < 0.001). DDF of $\delta^{13}$C for spiders fed mosquitoes was approximately -5‰, compared to 0.5‰ for spiders fed on midges and 2‰ for spiders fed on fruit flies. This rare pattern of $\delta^{13}$C depletion relative to diet was also shown with a lower-quality maize diet fed to locusts (Webb et al. 1998). When prey is unbalanced compared to predator body stoichiometry, predators may try to overcompensate by over-ingesting nutrients and/or egesting excess nutrients to maintain body
stoichiometry, as shown with grass-feeding and polyphagous locusts (Raubenheimer and Simpson 1999).

The effect of prey type on body size (TPL) was not significant and the effect of prey type on wet biomass was only slightly significant. Differences in prey nutrient stoichiometry have no effect on total increase of body size (carapace length) and dry mass of two species of wolf spiders (Jensen et al. 2011). However, prey quality can significantly affect body size and growth rate of spiders (Mayntz and Toft 2001, Mayntz et al. 2003, Jensen et al. 2011). For example, wolf spiders (Pardosa amentata) fed fruit flies (D. melanogaster) of varying nutrient quality grew at different rates (Mayntz and Toft 2001). In addition to active hunters like wolf spiders, sit-and-wait predators (orb-weaving spider, Zygiella x-notata) also showed decreased juvenile survivorship and a reduced number of instars when fed on lower quality prey (Mayntz et al. 2003). However, the number of instars increased in Tetragnatha spiders (also orb-web sit-and-wait predators) fed a low quality diet (Culex mosquitoes).

Prey quality had a significant impact on the growth rate of subsidized predators. There is evidence that there is enough variation in intraspecific prey quality to affect the life history of spider predators (Wilder et al. 2010).
Intraspecific prey quality may change by season or habitat due to shifting resources at the base of the aquatic food web for emerging aquatic insects. Quality may also shift when community composition changes seasonally and spiders are relying on much different resources for energy (e.g. Nakano and Murakami 2001). Seasonal or permanent changes (due to climate change) of prey nutrient composition may affect the long-term survivorship and growth rate of subsidized predators, such as spiders (Jonsson et al. 2015). Negative effects on aquatic systems may cascade across into terrestrial ecosystems, affecting animals that feed on subsidized predators, such as spiders like *T. nitens* (e.g. Gunnarsson 2007). During seasons of low food availability, nutrient content of prey should have an even larger impact on growth, reproduction, and survival of spiders due to decreased selectivity of high quality prey (Mayntz et al. 2003, Wilder 2011).

Studies have also shown that wolf spiders are extremely prey-limited in the wild (Edgar 1969, Nyffeler and Breene 1990). Future studies should investigate the link between fluctuating resource quality and condition of predator populations.
Figure 3.1. Mean percent body nitrogen of three prey species: Fruit fly (*Drosophila melanogaster*), Midge (*Chironomus dilutus*), and Mosquito (*Culex quinquefasciatus*). Error bars represent 1 standard error of the mean.
Figure 3.2. Mean Carbon:Nitrogen ratio of three prey species: Fruit fly \((Drosophila melanogaster)\), Midge \((Chironomus dilutus)\), and Mosquito \((Culex quinquefasciatus)\). Error bars represent 1 standard error of the mean.
Figure 3.3. The effect of the 3 experimental diets on the biomass (mg) of *Tetragnatha nitens* over the course of the 60-day study. Curves represent LOESS fit regressions.
Figure 3.4. Mean percent body nitrogen of *Tetragnatha nitens* spiders fed on three prey species: fruit fly (*Drosophila melanogaster*), midge (*Chironomus dilutus*), and mosquito (*Culex quinquefasciatus*). Error bars represent 1 standard error of the mean.
Figure 3.5. Mean Carbon:nitrogen ratio of *Tetragnatha nitens* spiders fed on three prey species: fruit fly (*Drosophila melanogaster*), midge (*Chironomus dilutus*), and mosquito (*Culex quinquefasciatus*). Error bars represent 1 standard error of the mean.
Figure 3.6. The effect of the three experimental diets on the growth rate of tibia-patella length (mm•d⁻¹) of Tetragnatha nitens over the course of the 60-day study. Curves represent LOESS fit regressions.
Figure 3.7. The $\delta^{13}$C and $\delta^{15}$N values of spider predators (*Tetragnatha nitens*) and the prey they were fed on: Fruit Fly (*Drosophila melanogaster*), Midge (*Chironomus dilutus*), and Mosquito (*Culex quinquefasciatus*). Confidence level of ellipses signify 0.95 of data within each group.
Chapter 4

Do nutrient enrichments have cascading effects across ecosystems?

4.1 Abstract

Anthropogenic nitrogen becomes incorporated in many natural terrestrial and aquatic ecosystems and has the potential to influence ecological interactions by shifting the abundance, distribution, nutrient content and stoichiometry, and community composition of organisms. Changes in the nutrient stoichiometry of aquatic-derived organisms may influence the prey quality consumed by subsidized terrestrial predators. The effects of nitrogen (N) enrichment on the abundance, body size, % body N, nutrient stoichiometry (carbon:nitrogen ratio), and isotopic
composition of emergent aquatic insects and a subsidized spider predator, *Tetragnatha* sp., were studied over 8 weeks during the summer of 2015. Although larger midges (Chironomidae) emerged from enriched pools, there was no significant difference in abundance, nutrient stoichiometry (C:N ratio), or % body N of emergent aquatic insects between enriched and non-enriched pool mesocosms. For the subsidized *Tetragnatha* sp. spider, abundance, body size (tibia + patella length (TPL) of first walking leg) (mm), biomass (mg), or nutrient stoichiometry (C:N ratio) did not differ significantly between enriched and non-enriched pools. However, % body N of spiders from the non-enriched treatment was higher than in spiders from the enriched treatment, which may be due to the shift to a higher percentage of terrestrial prey in the diets of spiders from the non-enriched treatment. Both emergent aquatic insects and spiders differed in $\delta^{15}$N between enriched and non-enriched pools, which may be explained by the isotopic composition of the nutrient enrichment. Significantly higher nitrogen content (% body N) was in *Tetragnatha* sp. spiders from the Santa Ana River inflow in a constructed treatment wetland compared to a site farther along this aquatic nutrient gradient during July 2015. Nutrient
enrichment of the aquatic ecosystem due to anthropogenic runoff during a summer monsoon event may explain this pattern.

4.2 Introduction

Optimal foraging theory states that organisms will generally feed on prey that has the largest body size per amount of energy expended for capture (Charnov 1976). This may lead to the expectation that predators select comparatively larger prey, especially predators such as orb-weaving spiders, which are sit-and-wait predators (Venner and Casas 2005, Blackledge 2011).

However, organisms may also respond behaviorally or physiologically to increased resource quality in natural food webs. One potential issue is that there may be a correlation between ecosystem quality and prey quantity (abundance and/or biomass). For example, ecosystem enrichment led to larger insects at emergence (increased quantity) compared to insects in non-enriched treatments, but changed the community composition of insect prey (more caddisfly adults) and as a result, terrestrial spiders were unable to utilize some aquatic food web subsidies (decreased quality) (Davis et al. 2011).
Anthropogenic nutrient enrichment has the potential to shift the dynamics of natural ecosystems and change the nutrient content and stoichiometry of organisms within the ecosystem (Smith et al. 1999). For example, Cross et al. (2003) showed that invertebrates in enriched streams had significantly higher % body phosphorus (P) and lower carbon:phosphorus (C:P) ratio and nitrogen:phosphorus (N:P) ratios than did similar-sized taxa in reference streams. Nutrient enrichment can also increase body size, increase growth, and enhance survival of herbivores (Huberty and Denno 2006). Enrichment effects can cascade up the food web. For example, predators fed on higher quality prey consume less prey to maintain body nutrient stoichiometry (e.g. Couture et al. 2010). Nutrient enrichment in detritus-based aquatic systems leads to an overall increase in invertebrate production and increased prey flux to predators from 5.8 to 9.2 g•m²•yr⁻¹ (Cross et al. 2007). Although nutrient enrichment has a positive effect on aquatic primary producers, other researchers have found little to no effect of enrichment on the nutrient stoichiometry of benthic macroinvertebrates in mountain streams (Bowman et al. 2005) and shallow lakes (Ventura et al. 2008).
If nutrient enrichment increases abundance of in-situ prey, predators in subsidized ecosystems may congregate and have increased fitness around ecosystem boundaries (e.g. Polis et al. 2004). On the other hand, nutrient enrichment may lead to a subsidy-stress response, where consumer response to enrichment becomes reduced at high levels of enrichment (King and Richardson 2007). For example, high soil nutrient concentrations (N and P) can reduce growth, survival, and reproduction of insect herbivores due to maintenance costs associated with excreting excess nutrients (e.g. Zehnder and Hunter 2009). One assumption is that large quantity resource subsidies must have a significant positive impact on consumers in recipient ecosystems. However, if prey quality of resource subsidies are lower compared to in-situ resources, consumers may have increased fitness when feeding on prey within their own ecosystem compared to subsidized prey. To accurately predict the effect that aquatic nutrient enrichment will have on terrestrial predators, it is important to understand the nutrient imbalance-balance threshold of the predator (Bowman et al. 2005).

I predicted that enriched treatments would contain greater abundance and biomass of larval and adult aquatic insects. I also predicted that adult aquatic
insects from the enriched treatment would have lower C:N ratio than adult aquatic insects from the non-enriched treatment and thus will be a higher quality resource subsidy. As a result, terrestrial spiders (Family: Tetragnathidae) would have increased nutrient content (N) and/or increased abundance in nutrient enriched treatments. In accordance with Davis and others (2011), diet type of spiders would differ significantly between enriched and non-enriched treatments. As a result, there would be a significant difference in stable isotopes (δ^{13}C and δ^{15}N) of aquatic insects and terrestrial Tetragnatha sp. spiders between treatments.

Alternatively, spiders may switch to terrestrial prey if enrichments cause larger insects (harder to catch by Tetragnathid spiders) to emerge from enriched treatment pools (Davis et al. 2011). Switching to terrestrial prey will cause nutrient content (N) to increase in spiders from enriched treatments, because terrestrial insects have higher nutrient content that aquatic insects (Cross et al. 2003, Frost et al. 2003, Evans-White et al. 2005).
4.3 Materials and Methods

Twelve plastic wading pools (~20 cm height × 1 m diameter) were divided between two treatments (enriched nitrogen and non-enriched) at the Prado Wetlands in Corona, CA, USA (33.918643° N 117.619592° W) to determine how increasing donor ecosystem quality can affect consumers in the recipient ecosystem. Each treatment contained equal amounts of water (~440 L) from a diversion channel from the Santa Ana River. Five wooden stakes (height ~1m) were placed around the perimeter and two stakes were placed in the interior of each pool to provide structure for web-building spiders. Twine was tied between stakes to allow attachment points for web-building spiders (Fig. 4.1). Nitrogen additions of ~25 mg/pool of (NH$_4$)$_2$SO$_4$ (ammonium sulfate) were made weekly to the enriched mesocosms starting two weeks before the study began and throughout the 8-week study. Water levels were maintained in each pool by adding water from wetlands weekly to compensate for evaporative losses.

Secondary production (benthic and emergent insects) and consumer (*Tetragnatha* sp. spiders) response in both treatments were quantified. Larval and immature insects in each replicate pool were surveyed with a benthic tube sampler (~25 L of water) three times during peak emergence for
aquatic insects (June-Aug). Adult aquatic insects emerging from each replicate mesocosm were captured using floating aquatic emergence traps (0.25 m²). Traps were set biweekly for a three-day period and all aquatic insects were identified to Family level (sensu Baxter et al. 2004) in the laboratory. Dry weight (mg), wing length (mm), nitrogen content (% body N), carbon content (% body C), carbon:nitrogen ration, δ¹³C, and δ¹⁵N of adult aquatic insects were measured using the same protocol outlined in Chapter 3 of the dissertation. Wing length (mm) is an appropriate surrogate for body size for adult insects (Xue and Ali 1994).

Occupied spider webs, as well as free-roaming spiders, were counted above each treatment (2x/week) throughout the study. Spiders were counted using headlamps between 15 minutes before and after sunset to account for times of highest activity and web spinning (Lesar and Unzicker 1978). *Tetragnatha sp.* spiders were identified and separated from other orbweaving spiders (Family: Araneidae). Web size and orientation (horizontal, diagonal, or vertical) was also recorded. Because the main prey of *Tetragnatha* spiders is aquatic insects, they tend to build mostly horizontal webs above the water (Williams et al. 1995). Along with recording the abundance of spiders throughout the study, dry weight
(mg), first walking leg tibia + patella length (referred to as TPL throughout) (mm), nutrient content (% N and % C), and stable isotopes ($\delta^{13}$C and $\delta^{15}$N) of spiders ($N = 42$) caught at the end of the experiment in mid-August 2015 were measured.

A repeated measures mixed model ANOVA using R version 3.1.3 (2015) was used to test for the effect of the enriched treatment on biomass, body size, abundance, web size, nutrient content (C and N), nutrient stoichiometry (C:N ratio), and stable isotopes ($\delta^{13}$C and $\delta^{15}$N) of aquatic insect prey and terrestrial consumers (Tetragnatha sp. spiders) across the 8-week experiment. Data were transformed to meet assumptions of normality and equal variance. For the abundance of spiders and abundance of specific taxonomic groups of benthic insects, the ‘glmmADMB’ package in R was used to run a repeated measures mixed model with a negative binomial distribution because of the zero-heavy data. For spider body size throughout the study, the ‘lme’ package in R was utilized to perform a linear mixed effects model with a ln ($x+1$) transformation and a Tukey-Kramer adjustment was run for all pairwise comparisons. For Tetragnatha sp. dry weight (mg) and TPL (mm) at the end of the study, a t-test was used with ln ($x+1$) transformation to account for normality. For
*Tetragnatha sp.* nutrients (C and N), stoichiometry (C:N ratio), and stable isotopes ($\delta^{13}$C and $\delta^{15}$N), the data were analyzed using a *t*-test in R. In order to determine the contribution of aquatic insects vs. terrestrial insects in the diets of spiders in the mesocosms, a stable isotope mixing model was run and data was analyzed using the package ‘SIAR’ in R statistical software (Parnell and Jackson 2013).

In addition to the mesocosm experiment, *Tetragnatha sp.* spiders were collected in the Prado Wetlands, a 186-ha manmade constructed treatment wetland in Corona, CA, US. Water nitrate concentrations at the Santa Ana River inflow are significantly higher than further into the wetland complex (unpublished data from Orange County Water District, Fig. 4.2). *Tetragnatha* sp. spiders were collected on four dates (March, May, July, and September 2015) at the inflow of the Santa Ana River (sites E1 and E4) and at the site W6 within the Prado treatment wetlands (Fig. 4.3). Dry weight (mg) was measured using a Sartorius® digital analytical laboratory scale (Model 1712 MP8 Silver Edition). TPL (mm) was measured using ImageJ software (Schneider et al. 2012) after digital photography. Nutrient content (C and N), nutrient stoichiometry (C:N ratio), and stable isotopes ($\delta^{13}$C and $\delta^{15}$N) were determined by drying spiders,
grinding into powder, and analyzed using an isotope ratio mass spectrometer. A mixed model ANOVA in R was used to test for the effect of site on spider biomass, TPL, and nutrient content, nutrient stoichiometry, and stable isotopes. A stable isotope mixing model was performed using ‘SIAR’ in R statistical software (Parnell and Jackson 2013) to determine contributions of aquatic and terrestrial insects to spiders in wetlands.

4.4 Results

4.4.1 Benthic Insects

The abundance of aquatic invertebrates did not differ between treatments ($F_{1,10} = 0.99$, $p = 0.34$; date*treatment interaction: $F_{2,52} = 0.79$, $p = 0.46$). The dominant insects in both treatments were midge larvae (Family: Chironomidae) which constituted over 70% of the individual aquatic insects (Table 4.1). The abundance of chironomid midge larvae did not differ significantly between enriched and non-enriched treatments ($z = -0.63$, $p = 0.53$). There was also no significant date*treatment interaction between any sampling dates for chironomid midges ($p > 0.05$).
There was no significant difference in the number of dragonfly nymphs (Order: Odonata) between enriched and non-enriched mesocosms throughout the study \((z = 0.82, p = 0.41)\). There was also no significant date*treatment interaction between any sampling dates for dragonfly nymphs \((p > 0.05)\).

**4.4.2 Emergent Insects**

The average number of insects \((\text{individuals} \cdot \text{m}^{-2} \cdot \text{d}^{-1})\) emerging out of each pool varied by date \((F_{3,30} = 11.29, p < 0.0001; \text{Table 4.2})\); however, there was no significant effect of treatment on the abundance of aquatic insects emerging from the mesocosms \((F_{1,10} = 0.096, p = 0.76)\). Although there was no difference in abundance of insects, body size of the chironomid midges emerging from enriched pools was significantly larger compared to insects emerging non-enriched pools \((F_{1,10} = 6.29, p = 0.03, \text{Fig. 4.4})\). However, the average biomass per individual midge was not significantly different between treatments \((F_{1,10} = 0.27, p = 0.61)\).

Nutrient \((\text{C and N})\) content and stoichiometry \((\text{C:N ratio})\) of emergent aquatic insects did not differ significantly between the treatments \((\text{carbon } [F_{1,10} = 0.47, p = 0.51]; \text{nitrogen } [F_{1,10} = 0.551, p = 0.48]; \text{C:N ratio } [F_{1,10} = 0.616, p = 0.45])\). There were no significant date or date by treatment interaction effects for
emergent insect nutrients or stoichiometry (C:N ratio) between enriched and non-enriched pools (p > 0.05 for C, N, and C:N ratio).

Emergent aquatic insects from non-enriched pools had a $\delta^{15}$N ‰ value higher than insects emerging from enriched pools ($F_{1,10} = 22.57$, $p < 0.0001$; Fig. 4.5). However, there was no significant difference in $\delta^{13}$C ‰ of emergent insects between treatments ($F_{1,10} = 0.91$, $p = 0.36$). There was a significant difference in both $\delta^{15}$N ‰ ($F_{3,34} = 80.71$, $p < 0.0001$) and $\delta^{13}$C ‰ ($F_{3,34} = 13.20$, $p < 0.0001$) of emergent insects across date, but no significant date*treatment interactions ($\delta^{15}$N ‰ [$F_{3,34} = 0.18$, $p = 0.91$]; $\delta^{13}$C ‰ [$F_{3,34} = 0.40$, $p = 0.75$]).

### 4.4.3 Spider Abundance

There was no significant difference between the number of spiders above mesocosms in the enriched vs. non-enriched treatments ($z = 0.36$, $p = 0.72$), although there was a significant effect of date on spiders in both treatments ($z = 1.97$, $p = 0.04$), which is due to higher abundance of spiders above mesocosms in both treatments during weeks 3-6 of the study compared to other weeks. Excluding all other spiders, *Tetragnatha* spiders with and without webs ($z = 0.83$, $p = 0.40$) and *Tetragnatha* spiders with webs ($z = 0.85$, $p = 0.40$) did not
vary significantly between treatment or have a significant date by treatment interaction.

4.4.4 Spider Size and Web Size

Spider body size (mm) did not differ significantly between enriched and non-enriched treatments throughout the study ($t_{10} = 0.49$, $p = 0.63$).

*Tetragnatha* spiders above non-enriched pools were marginally larger ($t_{536} = 1.87$, $p = 0.06$) than *Tetragnatha* spiders above enriched pools on only one sampling date (June 29). With the spiders collected at the end of the experiment ($N = 42$) in mid-August 2015, there was no difference between dry weight (mg) ($t_{34.2} = 0.94$, $p = 0.35$) and TPL (mm) ($t_{33.1} = 0.79$, $p = 0.43$) of *Tetragnatha* spiders between the treatments. There was also no significant difference in the size of spider webs between treatments with all spiders ($F_{1,10} = 0.28$, $p = 0.61$; Table 4.3) and with *Tetragnatha* spiders alone ($F_{1,10} = 1.08$, $p = 0.32$; Table 4.3).

4.4.5 Spider Nutrients and Stoichiometry

Spiders removed at the end of the study ($N = 33$) in mid-August 2015 were not significantly different in % body C ($t_{18.6} = 0.38$, $p = 0.71$) or C:N ratio
(t_{17.53} = 1.33, p = 0.20) between the enriched and non-enriched treatments.

However, spiders from non-enriched treatments had significantly higher % body N compared to spiders from enriched treatments (t_{24.9} = -2.03, p = 0.05, Fig. 4.6).

4.4.6 Spider Stable Isotopes

Tetragnatha spiders from non-enriched treatments had a higher δ^{15}N ‰ compared to spiders from enriched treatments (t_{27.36} = -2.13, p = 0.042; Fig. 4.7).

There was no significant difference in δ^{13}C ‰ between spiders in the two treatments (t_{29.35} = 0.69, p = 0.49).

Tetragnatha sp. spiders in both treatments rely on terrestrial insects as the main part of their diet and less so on emergent aquatic insects. Spiders are mainly feeding on flies (Order: Diptera) from the Dolichopodidae family (Fig. 4.8). Only ~20% of the diet of Tetragnatha spiders sampled at the end of the study originated from aquatic insects (Chironomidae and Culicidae).

4.4.7 Nutrients and Stable Isotopes in Field-collected Spiders

While the nutrient content of Tetragnatha spiders did not increase due to enrichment in the artificial mesocosms, seasonal nutrient variation from the
natural environment did cause a response in the spiders. *Tetragnatha sp.* spiders collected from the inflow site were significantly heavier (mg) and larger (TPL (mm)) than those collected at a site near the terminus of the wetlands (biomass \[ t_{79} = -2.35, p = 0.013\]; TPL \[ t_{79} = -3.58, p > 0.001\]; Fig. 4.9). While there was no significant correlation between spider dry weight (mg) and C:N ratio (\( F_{1,31} = 2.58, p = .12, r^2 = .047 \)), TPL (mm) of spiders was negatively correlated with C:N ratio (\( F_{1,31} = 10.44, p = 0.0029, r^2 = 0.23 \)) and positively correlated with % body nitrogen (\( F_{1,31} = 6.85, p = 0.014, r^2 = 0.15 \)). Therefore, the spiders from the inflow may have been higher in nutrients, such as nitrogen, due to larger body size, but this link is inconclusive due to the non-significant correlation to dry weight.

Nutrient content of *Tetragnatha* spiders did not differ between the two wetland sites on 3 of the 4 dates; however, spiders at the Santa Ana River inflow site had significantly higher % body N compared to those at the W6 interior site \( (t_{79} = -6.15, p > 0.0001; \text{Fig. } 4.10) \) in July. The \( \delta^{15}N \) \% of *Tetragnatha* was significantly higher in W6 interior site compared to Santa Ana River inflow site \( (F_{79} = 54.91, p < 0.0001) \), but no significant date*site interaction. On the other hand, \( \delta^{13}C \) \% of *Tetragnatha* was significantly lower at W6 interior site
compared to the Santa Ana River inflow site ($F_{79} = 9.58, p = 0.0027$), as well as a significant date by site interaction ($F_{79} = 7.78, p = 0.0001$).

4.5 Discussion

Although other studies have shown that addition of nitrogen enrichments increase the abundance of benthic insects (e.g. Cross et al. 2006), there is no evidence that these nitrogen enrichments led to an increase of benthic aquatic invertebrates. However, it is possible that nutrient enrichments are causing an increase in algal biomass (primary production) without nutrients being transferred to higher levels of the food web, such as aquatic insects or subsidized terrestrial predators (e.g. Rabalais et al. 2002). Addition of aquatic predators, such as dragonfly nymphs (Order: Odonata) might reduce the total amount of aquatic insect emergence available and thus may be disrupting the flow of energy from aquatic to terrestrial ecosystems to terrestrial predators (Johnson 1985, Johansson 1993). However, there was no evidence that dragonfly nymphs were more abundant in enriched treatments.

Although there was no significant difference in abundance of emergent insects, chironomid midges emerging from enriched pools were larger, but not
heavier, than midges emerging from non-enriched pools. However, other studies have shown that nutrient enrichments can lead to an increase in biomass of emergent insects (e.g. Haddad et al. 2000). Enrichments applied over a longer time period (~2 years) in a natural stream ecosystem have caused a significantly higher growth rate in larval chironomid midges compared to control streams (Cross et al. 2005). If the time of the experiment was extended, it is possible that much larger chironomids would emerge, as well as an overall increase in emergent insect biomass. There was no significant difference in nitrogen content (% body nitrogen) and C:N ratio between emergent insects from enriched and non-enriched pools. Another enrichment study also showed no correlation in nutrients of aquatic invertebrates between enriched and non-enriched streams (Cross et al. 2003).

There was no significant difference between the number of spiders and number of *Tetragnatha* spiders between enriched and non-enriched treatments. The most plausible reason why spiders did not respond numerically to the nutrient enrichment is that adult spiders are moving too often to detect differences in prey, such as larger chironomid midges, due to nitrogen enrichment in the mesocosms. To my knowledge, no research has been performed
demonstrating the range or dispersal ability of spiders from the family Tetragnathidae, although dispersal of other orb-weaving spiders has been studied (Lubin and Henschel 1996, McNett and Rypstra 2000). Counterintuitively, spiders may not be moving at all because they are sated and thus they are not seeking out higher quality prey. Because there is no difference between the quantity of insects emerging from enriched vs. non-enriched pools, the spiders may not have a need to move.

Based on the emergent insect biomass data, there is an average of ~15 mg•d⁻¹ emerging from each mesocosm, in addition to the aquatic and terrestrial insects coming from the natural ecosystem. In chapter 3, spiders (Tetragnatha nitens) were shown to subsist on ~1 mg of prey per day. Since multiple prey items were observed in webs during study (S. Merkley, personal observation), it is not likely that spiders in this system are prey-limited. Thus, this may be a case where the spiders are only searching for suitable structure to build their web and may not move between locations, unless they become prey-limited. The density of orb-weaving spiders, such as Argiope trifasciata, is correlated with habitat complexity, but not other quantity measures, such as insect activity, prey capture, or prey consumption (McNett and Rypstra 2000). Given more time and
resources, I would conduct this experiment in a greenhouse or some other closed system where I could test how spiders respond to a prey-limited system (Baxter et al. 2004).

There was no significant difference of the size of spiders throughout the study or TPL (mm) and biomass (mg) at the end of the study between enriched and non-enriched treatments. Average size decreases with the increase of different ages and stages of spiders, with spiderlings causing the average size to decrease. This may not be an accurate reflection of how spiders are reacting to treatments, but a measure of the recruitment of the populations in each mesocosm. The smaller average body size in enriched treatments may actually be reflecting increased recruitment of young on enriched pools, rather than reduced size of adults.

The nitrogen content (%N) of the (NH$_4$)$_2$SO$_4$ (ammonium sulfate) enrichment was quite high (21%) and may have led to higher %N values of spiders in enriched treatments. However, surprisingly, spiders in non-enriched treatments have significantly higher % body N than spiders in enriched treatments. There are few possible explanations to explain this pattern. First, since spiders were only sampled once at the end of the study, they may not be
responding to the enrichment, but instead are just random samples of spiders that happened to be over the pools at the time of sampling. In addition, although there was nitrogen added to the enriched treatments, it is possible most of that excess nitrogen was used up by algae in the mesocosms, leading to less nitrogen availability at higher levels of the food web, such as benthic insects and terrestrial spider predators.

Although *Tetragnatha* spiders feed on mostly aquatic insect prey because of their horizontal web building behavior (Lesar and Unzicker 1978, Kato et al. 2003), spiders in this study seemed to derive a large percentage of their prey from dolichopodid flies, which may be semi-aquatic as larvae, but usually only inhabit damp to saturated substrate, not fully-wetted pools (Courtney and Merritt 2008). No dolichopodid flies were found in benthic samples throughout the study. Dolichopodid flies made up the majority of the spiders’ diet, even though there was an abundance of chironomid midges emerging from the pools. This result is unexpected, but may be due to the habitat that mesocosms were placed in. As the summer progressed, more and more terrestrial vegetation grew in and around experimental mesocosm pools (S. Merkley 2015, personal observation). This growth may have lead to an increase of small-bodied predatory flies (Family:
Dolichopodidae), which can easily be captured in spider webs. However, spiders from non-enriched pools had a higher level of $\delta^{15}N$ ‰, suggesting that they are feeding a greater proportion of terrestrial prey than spiders from enriched pools (Cross et al. 2003, Frost et al. 2003, Evans-White et al. 2005). Emergent insects and spiders may have had lower $\delta^{15}N$ ‰ in enriched treatments because ammonium sulfate added to the enriched treatments had a lower $\delta^{15}N$ ‰ value than water from diversion channel of the Santa Ana River ($\sim 29\%o\ \delta^{15}N$ in Santa Ana River compared to $\sim 21\%o\ \delta^{15}N$ for ammonium sulfate). However, it is possible that many potential prey sources were not sampled in the traps, which may have caused different results in the stable isotope mixing model. Since the isotopes of spiders may change seasonally, future studies should collect spiders for isotopic analysis throughout the course of study to determine what impact shifting community composition of aquatic and terrestrial prey might have on subsidized predators.

Spiders from the Santa Ana River inflow had significant higher nitrogen content (% body nitrogen) than spiders in the interior of the wetlands in July 2015, but not on the other sampling dates. A seasonal monsoon that happened in July 18-20 might explain the increase in nutrients in the bodies of spiders at
this sampling date (Ebbert and Wagner 1987). An average of 3.6 cm of rain fell on the region during these three days, leading to an increase in the baseline river flow from ~1.7 m to a maximum of 2.4 m on July 20 (NOAA 2015, USGS 2015). Agricultural and residential runoff, due to summer rain events in Southern California, can lead to a significant increase of nitrates in the water (Jacobs and Gilliam 1985, Brauer et al. 2015). Nitrate levels are especially noticeable at the Santa Ana River inflow leading into the Prado Wetlands compared to farther into the wetland complex (Fig. 4.2). Since this effect of increased nitrogen was only noticed in the spiders from July, this suggests that there may be a rapid turnover of nutrients in the bodies of spiders responding to an influx of nutrients from storm runoff just one week prior to measuring nutrients of spiders. Another monsoon occurred on September 15-16, dropping approximately 3.9 cm of rain on the region, but no difference in nitrogen level of spiders was measured between inflow and interior sites two weeks after the rain event on September 30. Increase in nitrates also seemed to have an effect on the isotopic composition of the spiders with *Tetragnatha* spiders at the Santa Ana River inflow having a higher $\delta^{15}$N ‰ and lower $\delta^{13}$C ‰ than spiders in the interior of the Prado wetlands.
Although *Tetragnatha* sp. spiders are often studied because of their dependence on aquatic sources of energy, this study showed that these spiders can also utilize a large percentage of terrestrial flies in their diet when the structure to build their webs is not completely surrounded by water. Other studies have shown that as distance from water increases, the percentage of aquatic prey decreases and the reliance on aquatic subsidies by terrestrial predators also decreases (Baxter et al. 2005, Gratton and Vander Zanden 2009). Although there was no increase in abundance, size, or nutrients of spiders due to nitrogen enrichment, stable isotopes did differ between treatments. It is possible that distance from the water for experimental mesocosms increased the amount of terrestrial insects in the diets of the spiders, which minimized the effect of aquatic nitrogen enrichment. Spiders from the Santa Ana River inflow had significantly higher nitrogen content (% body nitrogen) compared to the site in the interior of Prado wetlands. Future studies should attempt to manipulate a larger scale ecosystem and control for the influence of terrestrial prey on the diets of terrestrial consumers subsidized by aquatic prey.
Table 4.1. Mean number of benthic invertebrates\textsuperscript{a} in each mesocosm across dates and treatments during the 8-week experimental study.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Enriched</td>
<td>Chironomid larvae</td>
<td>29.8 ± 11.7</td>
<td>444.3 ± 105.4</td>
<td>380.8 ± 59.3</td>
</tr>
<tr>
<td>Non-enriched</td>
<td>Chironomid larvae</td>
<td>21.3 ± 8.1</td>
<td>470.6 ± 106.5</td>
<td>317.3 ± 85.6</td>
</tr>
<tr>
<td>Enriched</td>
<td>Culex larvae</td>
<td>1.5 ± 1.0</td>
<td>0.08 ± 0.08</td>
<td>30.1 ± 23.0</td>
</tr>
<tr>
<td>Non-enriched</td>
<td>Culex larvae</td>
<td>0.08 ± 0.08</td>
<td>0</td>
<td>35.4 ± 23.9</td>
</tr>
<tr>
<td>Enriched</td>
<td>Culex pupae</td>
<td>3.2 ± 1.7</td>
<td>0</td>
<td>0.08 ± 0.08</td>
</tr>
<tr>
<td>Non-enriched</td>
<td>Culex pupae</td>
<td>2.8 ± 1.6</td>
<td>0</td>
<td>2.6 ± 2.2</td>
</tr>
<tr>
<td>Enriched</td>
<td>Ephydrid larvae</td>
<td>4.9 ± 1.2</td>
<td>1.8 ± 0.7</td>
<td>1.7 ± 1.3</td>
</tr>
<tr>
<td>Non-enriched</td>
<td>Ephydrid larvae</td>
<td>3.3 ± 0.9</td>
<td>0.4 ± 0.3</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Enriched</td>
<td>Ephydrid pupae</td>
<td>2.8 ± 0.9</td>
<td>3.1 ± 1.2</td>
<td>2.0 ± 0.9</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Number of individuals per replicate mesocosm.
<table>
<thead>
<tr>
<th></th>
<th>Ephydrid pupae</th>
<th>Ephemeroptera</th>
<th>Coleoptera larvae</th>
<th>Coleoptera adult</th>
<th>Odonata nymph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-enriched</td>
<td>0.8 ± 0.3</td>
<td>1.1 ± 0.6</td>
<td>0.3 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enriched</td>
<td>0.08 ± 0.08</td>
<td>0.9 ± 0.4</td>
<td>0.08 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-enriched</td>
<td>0.08 ± 0.08</td>
<td>0.2 ± 0.1</td>
<td>0.08 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enriched</td>
<td>1.1 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-enriched</td>
<td>1.3 ± 0.6</td>
<td>0</td>
<td>0.08 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enriched</td>
<td>2.9 ± 1.1</td>
<td>1.1 ± 0.5</td>
<td>0.7 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-enriched</td>
<td>3.3 ± 1.1</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enriched</td>
<td>0.6 ± 0.4</td>
<td>6.3 ± 1.9</td>
<td>3.2 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-enriched</td>
<td>1.0 ± 0.7</td>
<td>4.2 ± 1.7</td>
<td>2.9 ± 0.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Other groups include Diptera (Psychodidae, Ceratopogonidae), Plecoptera, and Hemiptera*
Table 4.2. Mean number of emergent insects (individuals\(\cdot\)m\(^{-2}\)\(\cdot\)d\(^{-1}\)) ± SE from each mesocosm across dates and treatments during the 8-week experimental study.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Enriched</td>
<td>72.33 ± 31.54</td>
<td>392 ± 117.87</td>
<td>61.33 ± 24.82</td>
<td>97 ± 34.02</td>
</tr>
<tr>
<td>Non-enriched</td>
<td>103 ± 46.02</td>
<td>376.67 ± 113.50</td>
<td>54.33 ± 10.49</td>
<td>79.95 ± 32.64</td>
</tr>
</tbody>
</table>
Table 4.3. Mean web radius (cm) ± SE and range of spider webs above mesocosms during 8-week experimental study.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Web radius (cm)</th>
<th>Range (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All spiders</td>
<td>6.06 ± 0.15</td>
<td>1.0 – 30.0</td>
</tr>
<tr>
<td><em>Tetragnatha</em> sp.</td>
<td>5.40 ± 0.14</td>
<td>1.0 – 18.0</td>
</tr>
<tr>
<td>Other spiders</td>
<td>8.39 ± 0.38</td>
<td>1.5 – 30.0</td>
</tr>
</tbody>
</table>
Figure 4.1. Mesocosm at study site at Prado Wetlands in Corona, CA in July 2015.
Figure 4.2. Nitrate concentration (mg/L) in the water during spring and summer 2013 at Prado Wetlands in Corona, CA. Data courtesy of Orange County Water District. Error bars represent 1 standard error of the mean.
Figure 4.3. Schematic of the Prado Wetlands in Corona, CA, USA (33.918643° N 117.619592° W). Figure courtesy of Orange County Water District.
Figure 4.4. Wing length of aquatic midges (Family: Chironomidae) across dates emerging from enriched and non-enriched mesocosms.
Figure 4.5. Emergent insect $\delta^{15}$N (‰) from experimental mesocosms at the Prado Wetlands.
Figure 4.6. Mean nitrogen content (% body nitrogen) of *Tetragnatha* sp. collected above mesocosms subjected to two enrichment treatments at the end of the 8-week study in August 2015 at the Prado Wetlands. Error bars represent 1 standard error of the mean.
Figure 4.7. Mean *Tetragnatha* sp. $\delta^{15}$N (‰) from experimental mesocosms at the end of the 8-week study in August 2015 at Prado Wetlands. Error bars represent 1 standard error of the mean.
Figure 4.8. Proportion of *Tetragnatha* sp. diet from different prey sources from enriched treatment of experimental mesocosms. Differences in shading reflect standard deviations of the mean.
Figure 4.9. *Tetragnatha* spider biomass (mg) and body size (TPL: mm) at two sites (Inflow and W6) in the Prado Wetlands on four sampling dates (Mar, May, July, and Sep 2015).
Figure 4.10. *Tetragnatha* spider % body nitrogen at two sites (Inflow and W6) in the Prado Wetlands on four sampling dates (Mar, May, July, and Sep 2015).
Chapter 5

Conclusions

5.1 Conclusions

Cross-ecosystem food web subsidies have the ability to structure the distribution, abundance, and structure of natural communities (Polis et al. 1997). Variation in the quantity and quality of resources fluxing from donor to recipient ecosystems may affect growth, survival, and reproduction of subsidized consumers (Marcarelli et al. 2011). In this dissertation, I have investigated the importance of measuring resource quality, rather than just resource quantity in the study of food web subsidies. In addition, I have isolated the effect of varying prey quality across the life cycle of a subsidized consumer and how aquatic
nutrient enrichment affects a subsidized terrestrial predator. Global anthropogenic additions of nitrogen and phosphorus in aquatic ecosystems may not only increase productivity of ecosystems, but also shift the nutrient content and stoichiometry of consumers (Smith et al. 1999). The studies outlined in this dissertation show how this increase of nutrients might shift the quality of prey and growth, development, and abundance of subsidized predators which rely on these prey.

The meta-analysis and experiments within this dissertation show the importance of including metrics of resource quality in the study of food web subsidies. In chapter two, I demonstrated through meta-analysis and empirical data that resource quantity is not a proper proxy for resource quality. With my third chapter, I employed a lab study to show how a subsidized predator (Tetragnatha nitens) suffered developmental delays due to feeding on lower quality prey. Lastly, in chapter four, I showed how variation in environmental nitrogen can affect the abundance and nutrient content of subsidized spider predators. Future work may build on these studies to show how individual fitness and populations might respond to long-term shifts in nutrient availability and increase or decrease in prey quality of cross-ecosystem food web subsidies.
Bibliography


National Oceanic and Atmospheric Administration. 2015. Climate Data Online: Dataset Discovery.


