EFFECTS OF PREDATORS ON THE CARBON DIOXIDE DYNAMICS OF FRESHWATER ECOSYSTEMS

by

Trisha Brooke Atwood

B.A., University of Hawaii at Hilo, 2007
M.Sc., University of Hawaii at Hilo, 2009

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

The Faculty of Graduate Studies

(Forestry)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

July 2013

ABSTRACT

Freshwater ecosystems are important natural emitters of the greenhouse gas CO_2. The magnitude and direction of the exchange of CO_2 between freshwaters and the atmosphere, or flux, is influenced by the concentration of CO_2 in the water. Every organism within a freshwater ecosystem influences the net CO_2 balance of that ecosystem either through respiration, photosynthesis or both. Thus, large changes in populations due to natural or anthropogenic stressors and the underlying food web structure of the ecosystem have the potential to alter CO_2 fluxes of aquatic ecosystems. To evaluate the influences of species loss on food web structure and CO_2 fluxes of aquatic ecosystems, I experimentally manipulated species from different consumer trophic levels (predator, grazer, or detritivore) and tested the effects of these losses on CO_2 fluxes of experimental streams, ponds and bromeliads. In streams, I found that influences on CO_2 emissions were most sensitive to the loss of a predatory insect compared to other trophic levels, including a tadpole grazer and an insect detritivore. Similarly, the removal of a fish predator to ponds or an insect predator to bromeliads resulted in trophic cascades that significantly influenced the CO_2 flux of the ecosystem. Both the identity of the predator and interspecific competition among predatory insects influenced the strengths of cascading effects of predators on CO_2 emissions from bromeliads. However, across all three ecosystems (streams, ponds, and bromeliads) predators, via trophic cascades, had surprisingly consistent effects on the CO_2 flux of the ecosystem.

Finally, as alterations to predator abundance often occurs in concert with increasing water temperatures and nutrient loading, I determined the individual and interactive effects of these stressors on pond communities. I found that nutrients often increased top-down control of predators on CO_2 fluxes, but the individual effect of warming and its combined effects with nutrients had negative effects on both consumers and primary producers making predictions about CO_2 fluxes complicated. My results provide novel insights into the influence of predators and food web structure on CO_2 fluxes and the potential for predator loss to markedly alter CO_2 fluxes of freshwaters.

PREFACE

Chapter 2: Effects of trophic level on the CO_2 fluxes of experimental stream ecosystems

Authors: TB Atwood, E Hammill, JS Richardson.

Status: In preparation (anticipated submission date: 22/07/2013)

Comments: This study was conducted, analyzed and written by TBA under the supervision of JSR. EH provided field assistance, statistical assistance, and feedback.

Ethics Approval: This research was approved by the University of British Columbia Animal Ethics Committee (animal care permit A12-0247) in accordance with the Canadian Council on Animal Care. Wildlife Act permit SU13-84946.

Chapter 3: Predator-induced reduction of freshwater carbon dioxide emissions

Authors: TB Atwood, E Hammill, HS Greig, P Kratina, JS Shurin, DS Srivastava, JS Richardson.

Journal: Nature Geoscience 2013 6: 191-194.

Status: Accepted 17/01/2013

Comments: This study was conducted, analyzed and written by TBA under supervision of JSR. EH, HSG, and PK provided field assistance, statistical assistance, and feedback. JSS and DSS provided insight on experimental design and interpretations.

Ethics Approval: This research was approved by the University of British Columbia Animal Ethics Committee (animal care permit A08-0220) in accordance with the Canadian Council on Animal Care.

Chapter 4: Antagonist interactions between predators alters top-down effects on CO_2 emissions from a freshwater ecosystem

Authors: TB Atwood, E Hammill, DS Srivastava, JS Richardson.

Status: In preparation (submission date: 02/07/2013)

Comments: This study was conducted, analyzed and written by TBA under the supervision of DSS and JSR. EH provided field assistance, statistical assistance, and feedback.

Chapter 5: Warming, predators, and eutrophication alter community dynamics and CO_2 fluxes of freshwater ponds

Authors: TB Atwood, E Hammill, HS Greig, P Kratina, JS Shurin, JS Richardson.

Status: In preparation (anticipated submission date: 20/08/2013)

Comments: This study was conducted, analyzed and written by TBA under the supervision of JSS and JSR. EH, HSG, and PK provided field assistance, statistical assistance, and feedback provided field assistance, statistical assistance, and feedback.

Ethics Approval: This research was approved by the University of British Columbia Animal Ethics Committee (animal care permit A08-0220) in accordance with the Canadian Council on Animal Care

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

DEDICATION

This thesis is dedicated to my brilliant and wonderful husband, Edd. Without his knowledge, patience, unconditional love and sheer wizardry with R this thesis would have never been possible. I would also like to dedicate this thesis to my parents Duke and Tami Atwood and sister Taunya Atwood who have always provided me with love and support on all my endeavors.

CHAPTER 1: INTRODUCTION

1.1 Global carbon cycle

Atmospheric carbon dioxide (CO_2) is one of the largest contributors to recent trends in global climate change. Average atmospheric concentrations of CO_2 have risen to 390 ppmv (ICPP 2007), nearly 100 ppmv higher than the previous 420,000 years before the industrial revolution (Crowley *et al.* 2000). With continued dependence on fossil fuels and alterations to land use by humans, CO_2 concentrations are predicted to more than double by the year 2100 (ICPP 2001). This may increase global temperatures by as much as 4° C, and forever change Earth's ecosystems. Even if global emissions of CO_2 had been stabilized in the year 2000, we would still be committed to at least half a degree increase in global temperature by the end of the 21st century (Meehl *et al.* 2005). With the imminent threat these changes will have on the world's ecosystems, national and international research initiatives have focused on understanding the global carbon (C) cycle to help better predict future climate change.

The global C cycle quantifies exchanges of C within and among four major reservoirs: geological deposits, the atmosphere, continental, and oceans. These reservoirs can be further divided into two categories based on their controlling processes; geological (geological deposits) and physical/biological (atmosphere, continental, and ocean). Although the geological portion of the C cycle is vastly important for understanding long-term CO_2 fluxes, these exchanges occur over millions of years as opposed to those in the physical/biological category, which occur on the order of seconds to centuries. As a result, the most currently accepted models of the global C cycle show three main active reservoirs: atmosphere, ocean, and continental (IPCC 2001, IPCC 2007).

Carbon exchange among and within the three active reservoirs is important to global climate change. The rate at which atmospheric concentrations of CO_2 change is determined by the ratio of the rate at which reservoirs sequester atmospheric CO_2 and the rate at which CO_2 is emitted (Falkowski *et al.* 2000). Gas exchange between the continental-ocean-atmosphere systems is governed by chemical, physical, and biological

1

processes within smaller sub-reservoirs that vary in the direction and magnitude of flux. However, to date progress in our ability to predict future atmospheric CO_2 concentrations has been limited by large knowledge gaps in our understanding of local C cycling within many of these sub-reservoirs and the physicochemical and biological processes that control them. As a result, this has prompted increased efforts to quantify and predict long-term trends in natural and anthropogenic sources and sinks of CO_2 in sub-reservoirs. This thesis specifically focuses on C cycling among sub-reservoirs within the continental biosphere and their C exchange with the atmosphere.

1.2 Inland waters and the carbon cycle

Inland waters (e.g. lakes, reservoirs, ponds, wetland, rivers, and streams) represent less than 4% of the earth's surface (Downing *et al.* 2006). However, these systems are energetically and dynamically linked to their much larger counterpart, terrestrial ecosystems. Until recently, inland waters have been incorporated into global C budgets only as passive transport routes of terrestrial C from land to sea. However, the input of terrestrial C to inland waters is considerably greater than the final C delivered by these systems to the sea (Cole *et al.* 2007, Battin *et al.* 2009). As a result, aquatic ecosystems likely play a disproportionate role in global and regional C dynamics relative to the area they occupy, as they store and process large quantities of terrestrial C (e.g. DOM, leaves, wood) (Cole *et al.* 2007).

Terrestrial ecosystems deliver about 1.9-2.7 PgC yr^{-1} or 3-5 % of their annual primary production to aquatic systems (Field *et al.* 1998, Cole *et al.* 2007, Battin *et al.* 2009). For small streams, ponds, and lakes terrestrial C is generally delivered directly from riparian zones (Rau 1976), conversely the major route of terrestrial C inputs to large rivers and lakes is through runoff from the surrounding watershed (Findlay *et al.* 1998). Of the terrestrial C annually delivered to freshwater ecosystems, it is estimated that about 0.8-1.2 PgC yr^{-1} is mineralized and returned to the atmosphere as CO_2, while another 0.2-0.6 PgC yr^{-1} is buried in aquatic sediments (Cole *et al.* 2007, Battin *et al.* 2009). These numbers suggest that inland waters not only transport less than 50% of the terrestrial C delivered to them from elsewhere, but store and process up to 1.8 PgC yr^{-1}, a

number that is similar to both land and ocean uptake of anthropogenic CO_2 emissions (1.9 PgC yr^{-1}) (IPCC 2007).

For many aquatic ecosystems, especially those in temperate regions, terrestrial C is often more significant than autochthonous C and is a large subsidy to aquatic food webs (Fisher and Likens 1973, Richardson 1991, Webster and Meyer 1997a and b, Wallace *et al.* 1999, Webster *et al.* 1999, Oquist *et al.* 2009, Richardson *et al.* 2010). When terrestrial C is processed and respired by aquatic organisms it has large effects on the CO_2 concentrations and fluxes. Many aquatic systems have greater respiration of organic C (OC) than its combined gross primary production and OC burial and export, and as a result are net heterotrophic in respects to their metabolism (Cole *et al.* 1994, Mulholland *et al.* 2001). These net heterotrophic aquatic ecosystems are generally natural sources of CO_2 to the atmosphere (Cole *et al.* 2007, Battin *et al.* 2009); however, studies investigating their influence on local, regional, and global CO_2 dynamics are scarce. Although a few studies have attempted to incorporate inland waters into global C budgets and the abiotic and biotic mechanisms which control carbon flux in these systems (Cole *et al.* 2007, Battin *et al.* 2008, Battin *et al.* 2009), many ecosystems (e.g., streams, phytotelmata, and small ponds) have been largely overlooked in all aspects of this research. As CO_2 has radiative forcing potential, whether ecosystems act as sources or sinks of CO_2 to the atmosphere, and the magnitude of those fluxes are crucial to the regulation of global temperatures (Lovelock 1972, Woodwell *et al.* 1998, Whiting and Chanton 2001).

1.3 Biotic controls on CO_2: from individuals to ecosystems

The net carbon balance of an ecosystem, and thus whether it acts as a source or sink for atmospheric CO_2, is determined by the difference between the gross fixation of CO_2 from the atmosphere through gross primary production (GPP), and the release of metabolized fixed carbon to the atmosphere as CO_2 or methane (CH_4). The rate of gross primary production of an ecosystem is the sum of the individual photosynthetic rates of all the autotrophic individuals of the ecosystem (Allen *et al.* 2005, Yvon-Durocher *et al.* 2010). Similarly, ecosystem respiration (ER), or the release of metabolized fixed carbon

back to the atmosphere as CO_2, is the sum of respiration rates of individual heterotrophs and autotrophs in the system (Woodwell and Whittaker 1968, Woodwell 1995, Enquist *et al.* 2003, and Lopez-Urrutia *et al.* 2006), plus CO_2 transported from upstream environments in lotic ecosystems and ground water intrusion. The metabolic rate of an individual, which determines its respiration rates, is determined by the amount of energy expended for growth, reproduction, foraging activity, predator avoidance, physiological regulation, and other maintenance (Brown *et al.* 2004). Because each individual within an ecosystem contributes to the net carbon balance of that ecosystem (Allen *et al.* 2005, Yvon-Durocher *et al.* 2010), the overall carbon storage of an ecosystem can ultimately be influenced by the average life span of organisms and their metabolic rates.

Fundamentally, the death of an individual will have the greatest effect on its contribution to either GPP if it is an autotroph or ER if it is a heterotroph, as mortality permanently removes the individual from the equation. Although the removal of a single individual is unlikely to cause large changes in the GPP or ER, population level changes in the abundance of species could have large influences on ecosystem carbon storage, especially in species poor systems. This line of reasoning suggests that any stressors that create large changes in populations of organisms within an ecological community have the potential to alter the magnitude of CO_2 fluxes of that ecosystem, and potentially whether they act as a source or a sink to the atmosphere.

1.4 Food webs, community structure, and predators

Predators, for the most part, are now recognized as important regulators of food web structure (Carpenter *et al.* 1985, Carpenter *et al.* 1987, Power 1990, Pace *et al.* 1999, Halaj and Wise 2001, Knight *et al.* 2005, Shurin and Seabloom 2005). Empirical and theoretical research demonstrating the influence of predators on food webs stretches back over 50 years. Hairston, Smith, and Slobodkin (1960) were the first to popularize the idea of a top-down controlled ecosystem with the "Green World Hypothesis" (HSS). In general this theory suggested that the abundance of each trophic level was regulated by predators at the top of the food chain through their direct or indirect effects on the intensity of consumptive pressure on that trophic level. Paine (1980) popularized the term

"trophic cascade" with his research on the predatory starfish *Pisaster* spp. Today the classical view of trophic cascades is that carnivores suppress herbivore populations through predation, which reduces herbivore grazing, allowing plant populations to flourish. However when top predators are removed, herbivore populations increase in the absence of predation, increasing grazing on plants, and ultimately reducing plant abundance. Thus a trophic cascade is an ecological phenomenon by which changes to the abundance of a predator creates reciprocal changes in the relative populations of lower trophic levels, ultimately influencing the assemblage's structural appearance and production (Paine 1980).

1.4.1 Interaction strengths

Interaction strength between predators and prey is a critical parameter in understanding what shapes food webs and influences ecosystem function in both experimental and theoretical approaches (Lawton 1989, Thebault and Loreau 2003, Ives *et al.* 2005, Goudard and Loreau 2007). Interaction strength is the estimated magnitude of the effects of one species on another. Although most studies measure direct effects between species, indirect effects (or non-trophic interactions) may also play an important role in ecosystem functioning (Mulder *et al.* 2001, Cardinale *et al.* 2002, Goudard and Loreau 2007) and can occur at frequencies and magnitudes equal to or greater than consumptive interactions (Preisser *et al.* 2005). Most food webs are characterized as having a few strong interactions (both direct and indirect) embedded in a majority of weak ones (McCann *et al.* 1998, Berlow 1999, Neutel *et al.* 2002, O'Gorman *et al.* 2010). Strong interactions are thought to have a greater influence on local distribution and abundance of organisms than weak interactions, and can have cascading effects on trophic levels if they are between predator and prey (Paine 1980, Petchy 2008). Conversely, weak interactions are thought to be important for maintaining biodiversity that may stabilize community dynamics (Berlow 1999, McCann 2000, O'Gorman and Emmerson 2009). However, the magnitude and duration of species interactions are not static; they can vary dramatically over space and time, and are influenced by both biotic and abiotic factors (Strong 1992, Mills *et al.* 1993, Menge *et al.* 1994, Sanford 1999,

Kordos and Dudgeon 2009). Shifting interaction strengths due to anthropogenic stressors such as climate change, eutrophication, and changes to predator abundance can have differing effects on many aspects of food web structure, such as shape and stability (Mitcheli 1999, Barton *et al.* 2009, Kratina *et al.* 2012, Shurin *et al.* 2012). As the shape of food webs and food web stability underpin ecosystem processes, changes to interactions strength via anthropogenic stressors may have dramatic consequences for the functioning of ecosystems and ecosystem services (Estes *et al.* 2011).

1.5 Key species and ecosystem function

Natural communities are a mosaic of complex relationships related to both community and food web structure. The topology of an ecological network produces unique ecosystem properties and is a function of how the trophic and non-trophic interactions within a community affect population dynamics and community stability. Over the past couple of decades a central goal in ecology has been to determine factors that shape food web structure and understand whether food webs are controlled by bottom-up or top-down processes (May 1986, Power 1992, Paine 1988, Hunter and Price 1992, Huryn 1998, Nystrom *et al.* 2003, MacIntosh *et al.* 2005). However, in the face of large-scale anthropogenic and natural environmental changes there has been a recent focal shift to identifying the relationships between food web structure, food web stability, and ecosystem functioning (Neutel 2002, Hooper *et al.* 2005, Lecerf and Richardson 2010, Romero and Srivastava 2010).

Although both biotic and abiotic factors influence food web structure, many theoretical food web models suggest a type of "topological keystone species" (Vasas and Jordan 2006) that give rise to community-trophic dynamics and control ecosystem function. In addition to these theoretical developments, experiments and observations support the idea that functional characteristics of organisms and the distribution and abundance of those organisms over space and time are some of the key regulators of ecosystem properties (sizes of energy compartments and fluxes of energy among compartments), and can have a greater effect than abiotic characteristics (Chapin *et al.* 2000, Hooper *et al.* 2005). This idea of "topological keystone species" has led to a

6

growing number of studies which attempt to identify and define key traits in species that determine food web structure and ecosystem function (Neubert *et al.* 2000, Jennings *et al.* 2002, Rossberg *et al.* 2010). The identification of these traits not only makes interpretation of empirical food webs easier, but also significantly helps in management efforts of that ecosystem.

1.6 Trophic cascades, food web structure, and CO_2 fluxes of freshwaters

Food web structure substantially influences ecological functions and processes (Schmitz 2008, Estes *et al.* 2011), and both theoretical and empirical evidence suggests that predator-induced trophic cascades can influence CO_2 fluxes of aquatic ecosystems (Schindler *et al.* 1997, Flanagan *et al.* 2006, Wilmers *et al.* 2012). In the section "Biotic controls on CO_2: From individuals to ecosystems" I discussed how the net carbon balance of an ecosystem is largely determined by the sum of individual population contributions to either ecosystem GPP or ER. Additionally, above I talked about the ability of predators to control populations of lower trophic levels through trophic cascades. Together, these concepts predict that predators could influence carbon dioxide fluxes of aquatic ecosystems by regulating populations of both heterotrophs and autotrophs. Empirically, predator influences on CO_2 fluxes have been documented in lentic and marine ecosystems (Schindler *et al.* 1997, Flanagan *et al.* 2006, Wilmers *et al.* 2012). Both ecosystems showed that changes to the abundance of predators, either through predator removal or additions, had cascading effects on heterotroph and autotroph populations that ultimately led to changes in the rates of CO_2 exchange with the atmosphere. In the most extreme cases, alterations to trophic structure shifted whether the ecosystem acted as a source or a sink for atmospheric CO_2 (Schindler *et al.* 1997, Carpenter *et al.* 2001). Although these studies provided pioneering research on how predator loss may be influencing regional and global greenhouse gas dynamics, an expansion on this theory is greatly needed.

1.7 Thesis objectives and overview

The main objectives of my thesis were to empirically determine how predator effects on CO_2 fluxes of freshwater ecosystems compared with other trophic groups (Chapter 2), and how ecosystem type (Chapter 3), interspecific competition between predators and predator identity (Chapter 4), and interactions with climate warming and eutrophication (Chapter 5) influenced predator effects on the CO_2 fluxes of freshwater ecosystems.

In Chapter 2, I experimentally determined which feeding guild (grazers/herbivores, detritivores, or predators) had the greatest effect on CO_2 flux from stream ecosystems. Using stream mesocosms, I manipulated the presence of a large-bodied grazer, detritivore or predator and measured their effects on community composition, leaf litter decomposition, primary production, and CO_2 flux from streams. I then compared the effects from all treatments to determine which feeding guild had the largest effect on CO_2 flux to the atmosphere.

In Chapter 3, I corroborated the work done by Schindler *et al.* (1997) and Flanagan *et al.* (2006) showing that predators and trophic cascades can influence CO_2 emissions in lentic systems. I then further developed the theory by determining whether the direction and magnitude of the effect of predators on CO_2 emissions was ecosystem dependent. Here, I manipulated the presence of top predators within three freshwater ecosystem types (ponds, streams, and the aquatic insect communities of bromeliads) that differed in predator type (invertebrate or vertebrate), flow regime (lentic or lotic), climatic region (temperate or tropical), ecological zone (pelagic or benthic), and level of *in situ* primary production (autochthonous, allochthonous, or mixed) to determine the overall pattern of the effect of predators on the CO_2 flux of freshwater ecosystems. I provided a mechanistic understanding behind predator effects on CO_2 flux for each ecosystem with measurements of total consumer biomass, plant biomass, and pCO_2 concentrations within all three ecosystem types.

In Chapter 4, I further explored the relationship between predators, trophic cascades and CO_2 fluxes of freshwater ecosystems by investigating how predator identity and interspecific competition between predators influenced CO_2 flux from freshwater ecosystems. In this study I compared the individual and combined effects of two predator species on CO_2 emissions from bromeliad phytotelmata mesocosms. Again, to provide a mechanistic understanding of the pathways by which these predators are able to alter CO_2 flux, I measured their effects on community composition and leaf litter decomposition.

In my final data chapter (Chapter 5), I investigated whether warming and eutrophication alter the importance of top-down control on CO_2 flux of aquatic ecosystems. In this study I manipulated warming, nutrient enrichment and predator presence in freshwater pond mesocosms to test their individual and combined effects on CO_2 flux. Similar to my previous chapters, I measured herbivore biomass, algal biomass, and pCO_2 concentrations to provide a mechanistic understanding of how direct and indirect predator effects on these factors influenced CO_2 emissions.

CHAPTER 2: THE INFLUENCE OF TROPHIC LEVEL ON CO$_2$ EMISSIONS OF EXPERIMENTAL STREAM ECOSYSTEMS

2.1 Summary

Due to accelerating rates of species extinctions, a few studies have investigated how the loss of predators mediates trophic cascades that influence CO$_2$ flux of aquatic ecosystems. However, to date, no studies have investigated how species extinctions at other consumer trophic levels are likely to influence CO$_2$ fluxes of aquatic ecosystems. In this study I added either predatory *Hesperoperla pacifica*, detritivorous *Pteronarcys californica*, or the grazer *Ascaphus truei* tadpoles to stream food webs and measured their effects on community composition, leaf litter decomposition, primary production, and CO$_2$ flux from experimental stream channels. The presence of the predator *H. pacifica* created trophic cascades that decreased CO$_2$ emissions from stream channels. The addition of a large functionally dominant detritivore (*P. californica*) or grazer (*A. truei* tadpoles) had no significant effect on total invertebrate biomass, leaf litter decomposition, chlorophyll-a concentrations, or CO$_2$ flux. However, there was a numerical increase in the densities of detritivores in treatments that lacked *P. californica* and an increase in the densities of grazers in treatments that lacked *A. truei* tadpoles. My results suggest that experimental stream communities were able to compensate for the loss of a functionally dominant detritivore or grazer, but not for the loss of a functionally dominant predator.

2.2 Introduction

Alterations to biotic communities as a result of species loss and the introduction of non-native species are occurring at alarming rates across all of Earth's ecosystems (Pimm and Raven 2000, Barnosky *et al.* 2011). As a result, ecologists are tasked with understanding how changes to biodiversity will influence ecosystem processes and functions, and ultimately ecosystem services. Although discrepancies exist over which measure of biodiversity best explains the biodiversity-ecosystem function relationship, there is an overwhelming consensus that biodiversity in one form or another is inherently

linked to the functioning of ecosystems (Loreau *et al.* 2001, Hooper *et al.* 2005, Balvanera *et al.* 2006, Cardinale *et al.* 2006, Worm *et al.* 2006, Cardinale *et al.* 2012). Results from both empirical and theoretical studies strongly suggest that the functional traits of organisms, and their abundance and distribution are important controllers of ecosystem processing rates, and thus the functioning of ecosystems (Tilman 1996, Hector 1998, vander Heijden *et al.* 1998., Tilman 1999, Loreau 2000).

As a result of their charismatic nature and their high susceptibility to extinction due to their slow population growth and high vulnerability to over-harvesting and habitat loss (Tracy and George 1992, McKinney 1997, Cardillo and Bromham 2001, Cardillo *et al.* 2005, Collette *et al.* 2011), predators have been the focus of research investigating how alterations to a consumer species' abundance is likely to influence ecosystems (Duffy 2003). Predators not only alter communities through trophic cascades, but their effects on food web structure also influence ecosystem processes (Finke and Denno 2005, Schmitz 2008, Estes *et al.* 2011). Trophic cascades created by the addition or loss of a predator have been shown to fundamentally alter the CO_2 fluxes of aquatic ecosystems, potentially influencing regional and global carbon cycles (Schindler *et al.* 1997, Flanagan *et al.* 2006, Wilmers *et al.* 2012, Atwood *et al.* 2013). Through trophic cascades, predators alter community respiration and carbon storage in plant biomass by having striking effects on both consumer and plant populations. The potential link between predator abundance, greenhouse gas dynamics, and global change has added to a growing concern that proper management of predator populations is necessary to conserve fundamental ecosystem processes.

Predators, however, are not the only animals being threatened with extinction (Owen-Smith 1989, Pinsky *et al.* 2011). Many species currently listed by the IUCN as extinct in the wild or critically endangered are members of lower trophic levels. Independent of trophic cascades, lower trophic level consumers have been shown to alter the structure and dynamics of both animal and plant communities through direct consumption of plant biomass or through the facilitative or competitive interactions with other organisms (Kohler 1992, Olff and Richie 1998, Lamberti *et al.* 1992, Parker *et al.* 2006, Lecerf and Richardson 2011). This suggests that the loss of functionally dominant

lower trophic level consumers could also impact carbon processing and storage. However, to date no studies have investigated how the loss of functionally dominant lower trophic level consumers could influence CO_2 fluxes of aquatic ecosystems.

For the first time, I investigated the individual effects of the addition of a functionally dominant grazer (tailed-frog tadpole, *Ascaphus truei*) and detritivore (stonefly, *Pteronarcys californica*) on community biomass, plant biomass, and CO_2 emissions of stream ecosystems. Additionally, I re-tested the effects of the addition of the stream predator (stonefly, *Hesperoperla pacifica*) on the same responses as above. As multiple studies have already shown the effects of predator abundance changes on community biomass, plant biomass, and CO_2 fluxes of aquatic ecosystems, I also compared the effects of the grazer and detritivore with those of the predator. These results can provide insight into which groups of animals deserve the greatest attention when managing communities for the protection of carbon cycling in ecosystems.

2.3 Methods

2.3.1 Focal species

This study experimentally manipulated the presence or absences of a large functionally dominant predator, grazer, or detritivore to compare their effects on community composition, primary production, leaf litter decomposition rates, and CO_2 flux to the atmosphere. I manipulated the presence or absence of *H. pacifica* (predator), *A. truei* (grazer), and *P. californica* (detritivore) from experimental stream ecosystems. These species were chosen because they all represent relatively large members, in terms of biomass, of their respective functional feeding groups within small coastal British Columbian streams, and because previous research has suggested that they have large influences on both community composition and primary producers. *Hesperoperla pacifica* is a large (21 ± 0.96 mg s.e. wet mass) predatory stonefly that primarily feeds on other benthic invertebrates and is capable of creating strong trophic cascades both within algal and detritivore food chains (Lecerf and Richardson 2011). *Pteronarcys californica* is a large (164 ± 5 mg) detritivore which feeds on decaying litter and is reported to both

increase leaf litter breakdown rates and alter both intra- and inter-guild species compositions (Andersen and Sedell 1979, Lecerf and Richardson 2011). Finally, *A. truei* (tailed frog) tadpoles are large grazers (567 ± 20 mg) that feed on periphyton from the surfaces of rocks and have been reported to reduce periphyton and chlorophyll-a concentrations by as much as 98% (Atwood and Richardson 2012). Additionally, it has been documented that *A. truei* tadpoles can alter community composition through interference and exploitative competition with other smaller grazers (Lamberti *et al.* 1992, Kiffney and Richardson 2001).

2.3.2 Stream mesocosms

I tested the influence of large functionally dominant species from the functional feeding groups; predators, grazers, and detritivores on stream ecosystem properties and functions using twelve experimental stream channels (15 m x 0.20 m). Channels were located in the Pacific coastal rain forest within the University of British Columbia's Malcolm Knapp Research Forest (MKRF) in Maple Ridge, Canada. Channels were lined with plastic and natural substrata consisting of sand, gravel, pebbles, and cobble were added to the stream beds, producing a series of small runs and riffles. Prior to the start of the experiment, channels were drained, cleaned, and sediments were homogenized. Experimental channels were then dried for 14 day to remove any previous communities. One month prior to the start of the experimental manipulations, channels were connected to a continuous flow of natural stream water from Mayfly Creek, an oligotrophic perennial stream within the research forest. Water from Mayfly Creek was mixed in two settling tanks before entering the stream channels. A flow velocity of ~1.0 L sec^{-1} was maintained in all channels for the duration of the experiment. The use of these experimental channels allowed us to maintain similar physical and chemical water properties (e.g., temperature, pH) to Mayfly creek, but also allowed us to control stream channel properties that can cause variation in aqueous gas dynamics (e.g. velocity, depth, and groundwater CO_2 intrusion).

2.3.3 Experimental communities and manipulations

After the restoration of water flow (day 0), stream communities were left to colonize for 30 days. Colonization occurred through drift from Mayfly Creek and from ovipositing by adult insects directly into the stream channels. Richardson (1991) found that colonization of experimental channels leads to similar levels of food web complexity to that of the natural donor stream. Large, functionally dominant focal species (*H. pacifica*, *A. truei*, *P. californica*) were prevented from colonizing the channels by passing water through a 4 mm-mesh filter before entering stream channels. Every third day, focal species were removed from the community caught in filters, and the remaining organisms were emptied into their respective stream channels.

Following the 30 d colonization of basal communities *H. pacifica* (predator), *A. truei* (grazer), or *P. californica* (detritivore) were randomly added to experimental channels. Each treatment was replicated three times, and all focal species were added at naturally occurring densities. In control treatments, *H. pacifica, A. truei*, and *P. californica* were absent. In predator treatments, I added 2.66 *H. pacifica* per m^2. To grazer treatments I added 0.66 *A. truei* tadpoles per m^2. Finally, to detritivore treatments I added 1 *P. californica* per m^2. Throughout the study, densities of focal species were maintained using the same methods as during colonization by passing water through a 4-mm-mesh filter before entering the channels. Stream channels were left for 70 days following treatment manipulations and then sampled for community composition, leaf litter decomposition, primary production, and CO_2 flux.

2.3.4 Community composition

On the final day of the study (70 d) benthic samples were collected using a Surber sampler with a sampling area of 0.023 m^2. Three samples were taken, one each from randomly assigned positions within each third of the stream (beginning, middle, and end sections). Substrata within the sampling area designated by the Surber were disturbed down to the plastic lining. Samples were placed in plastic containers, put on ice, and transported to the laboratory. Within 12 h of sampling, invertebrates were picked and

placed in 70% ethanol until identified. Invertebrates were identified to the lowest taxonomic group to determine functional feeding group. Following Merritt *et al.* 2008), invertebrates were assigned one of the following functional feeding groups: grazer, detritivore, predator or unknown. Total invertebrate dry biomass of each functional feeding group [detritivore, grazer and meso-predators (all predator species except *H. pacifica*)] was determined by weighing the combined taxa within a functional feeding group, and total invertebrate biomass was calculated as the sum of all functional feeding groups. Densities of the three functional feeding groups were calculated by taking the number of individuals found within the sampling area of the Surber sampler and extrapolated to 1 m^2.

2.3.5 Leaf litter decomposition

This study used *Alnus rubra* leaves, one of the largest components of terrestrial leaf litter in Mayfly Creek (Richardson 1992), to measure the effects of predator, grazer, and detritivore manipulations on percent remaining leaf litter. Freshly senesced *A. rubra* leaves were dried at 60° C, stacked into groups of 2.0 ± 0.01 g, then re-wetted and stapled together. Leaves were stapled together rather than using traditional mesh bags in order to prevent the use of the mesh as a refuge by smaller detritivores and to allow *P. californica* full access to the leaf litter. On day one of experimental manipulations, single leaf packs were randomly secured within the beginning, middle and end third of stream channel, for a total of three leaf packs per stream channel. An additional 0.5 g of loose leaf litter was added to each channel each week to ensure that stream organisms did not become food limited.

Leaf packs from each section of the stream were retrieved on the final day of the study (70 d), placed in a ziplock bag, and then placed on ice until processed. In the laboratory, invertebrates and sediments were carefully rinsed from each leaf surface. Leaf litter was dried to constant mass at 60° C, and weighed. Final leaf pack mass was subtracted from initial leaf pack mass to determine percent loss over 70 d.

2.3.6 Primary production

To determine standing periphyton biomass, a single 25 cm^{-2} unglazed ceramic tile was placed in each section of the stream (upper, middle, and bottom), for a total of three tiles per stream, on day one of the experimental manipulations. It has been previously shown that tiles support algal communities similar to that found on natural rock (e.g., Rosemond *et al.* 2000, Mallory and Richardson 2005). Tiles were collected on day 70, placed inside a ziplock bag, and then transported to the laboratory on ice. Tiles were kept in a dark freezer until analyzed. The surface of each tile was scraped with a razor blade, brushed using a toothbrush, and rinsed until all algae had been removed. Periphyton biomass was determined fluorometrically following acetone extraction of chlorophyll-*a* pigments from tile scrapings.

2.3.7 CO$_2$ collections

Direct measurements of dissolved CO_2 (pCO_2) concentrations within each stream were measured at dusk on day 70 using methods from Teodoru *et al.* (2009). Water samples for headspace analysis were collected 2 cm above the substrate from the beginning, middle, and end sections of the stream using a 50-ml Pressure-Lok® syringe (VICI Precision Sampling Corp., Baton Rouge, LA). Syringes were filled with 25-ml of sample water and 25-mL of air that had been scrubbed with soda lime to create a CO_2-free headspace. The valve on the syringe was then closed and the syringe was shaken for 5 minutes to equilibrate the gases in the water and headspace. Headspace gases were then sampled and transferred into 5 ml gas tight vacutainers (Labco Limited High, Wycombe, UK). Sample CO_2 concentrations were analyzed on a 5890 Series II gas chromatograph within 24 h, and total CO_2-C concentrations (mg C l^{-1}) in the water were calculated using equations from Appendix A.

2.3.8 Carbon dioxide flux

CO_2 fluxes (mg C m^{-2} d^{-1}) from stream channels to the atmosphere were calculated using the following equation.

$$CO_{2flux} = k \ * \ (pCO_{2water} - pCO_{2air})$$

where, k is the CO_2 exchange velocity coefficient, pCO_2 is the partial pressure of CO_2 measured in the water, and pCO_2 is the global average of the partial pressure of CO_2 in the overlying atmosphere (390 ppm). Stream k values (4 m d^{-1}) were calculated using the equation from Butman and Raymond (2011).

2.3.9 Data Analysis

The individual effects of the addition of a functionally dominant predator, detritivore, or grazer on total invertebrate biomass, total predator biomass, total detritivore biomass, total grazer biomass, total predator density, total detritivore density, total grazer density, percent remaining leaf litter, chlorophyll-a concentrations, and CO_2 flux were tested using analysis of variance (ANOVA). Subsequent post-hoc Tukey's analyses were used to determine differences among treatments (predator, detritivore, and grazer) on the individual responses. To meet assumptions for normality and equal variance, detritivore density was log transformed. All statistical analyses were performed in R 2.12.1 (R Development Core Team, 2010).

2.4 Results

The magnitude of trophic cascades and their influence on CO_2 flux from stream ecosystems was dependent on the functional feeding group manipulated. All stream mesocosms, regardless of treatment, were net sources of CO_2 to the atmosphere. Invertebrate detritivorous and predaceous invertebrates made up the bulk of the total invertebrate biomass within all stream treatments (Fig. 2.1). Members of the family Chironomidae were the most abundant taxa found in the stream channels followed by stoneflies, mayflies, and oligochaetes. Orthocladiinae (Chironomidae) and several mayflies numerically dominated the grazer taxa, Tanytarsini (Chironomidae) and oligochaetes dominated the detritivore taxa, and Tanypodinae (Chironomidae) dominated predatory taxa. The presence of large detritivores (*P. californica*) or grazers (*A. truei*) had no effect on total invertebrate biomass (Table 2.1, Fig. 2.1). However, *P. californica* significantly influenced the density of smaller detritivores (Table 2.1, Fig. 2.1b) and *A.*

truei tadpoles influenced the density of smaller grazers (Table 2.1, Fig. 2.1c.). Post-hoc Tukey's analysis showed that the detritivore treatment containing *P. californica* had lower densities of smaller detritivores than control, and grazer treatments (p = 0.004 and p = 0.003, respectively; Table 2.2). Post-hoc Tukey's analysis also showed that treatments containing the large grazer *A. truei* had significantly lower densities of smaller grazers than control treatments (p = 0.01; Table 2.2). Neither *P. californica* nor *A. truei* significantly influenced the density of meso-predators.   Furthermore, the presence of *P. californica* or *A. truei* had no effect on percent remaining leaf litter, chlorophyll-a concentrations, or CO_2 emissions from streams (Table 2.1, Fig. 2.2 and 2.3).

The presence of the large invertebrate predator *H. pacifica* had a significant effect on invertebrate biomass, density of detritivores and grazers, percent remaining leaf litter, chlorophyll-a concentrations and CO_2 emissions from stream channels (Table 2.1, Fig. 2.1-2.3). Post-hoc Tukey's analysis showed that predator treatments had significantly lower total invertebrate biomass by 48 ± 4 % (± s.e.) compared to the control treatments, but were similar in magnitude to detritivore and grazer treatments (Table 2.1 and 2.2, Fig. 2.1a). The greatest reduction in consumer biomass in predator treatments compared to control treatments occurred in the detritivore functional feeding group (Table 2.1). Predator treatments had no significant influence on the density of meso-predators (Table 2.1). Post-hoc Tukey's also showed that predator treatments had significantly lower detritivore densities than both control and grazer treatments (p = 0.047 and p = 0.027, respectively; Table 2.2) and significantly lower grazer densities than control and detritivore treatments (p = 0.002 and p = 0.011; Table 2.2). Furthermore, predator treatments had significantly greater percent remaining leaf litter and chlorophyll-a concentrations, and significantly lower CO_2 emissions compared to control, detritivore, and grazer treatments (Tables 2.1 and 2.2). Predators had 10 ± 8 % more leaf litter than controls treatments, 11 ± 9 % more leaf litter than detritivore treatments, and 8 ± 7 % more leaf litter than grazer treatments (Fig. 2.2a). For chlorophyll-a concentrations, predator treatments had 272 ± 21 %, 358 ± 50 %, and 970 ± 57 % more algal standing biomass compared to control, detritivore, and grazer treatments, respectively (Fig. 2.2b). Finally, predator treatments emitted 94 ± 7 %, 95 ± 6%, 94 ± 4% less CO_2 to the

atmosphere compared to control, detritivore, and grazer treatments, respectively (Fig. 2.3).

2.5 Discussion

The loss of consumer species from Earth's ecosystems may have far-reaching effects on regional and global biogeochemical cycles (Estes *et al.* 2011). However, investigations of the effects of predator loss on CO_2 fluxes of aquatic ecosystems has dominated the literature (Schindler *et al.* 1997, Wilmers *et al.* 2012, Atwood *et al.* 2013), despite that habitat loss and fragmentation, over harvesting, climate change, and invasive species create extinction risks for species across a wide range of trophic groups. This study is the first to my knowledge to investigate how changes to the abundance of functionally dominant grazers and detritivores influences the CO_2 emissions of an ecosystem and how the effects of grazers, detritivores and predators on CO_2 emissions compare with one another. This study showed that within experimental stream channels in the Pacific Northwest, the functionally dominant grazer *A. truei* and detritivore *P. californica* did not significantly alter the consumer biomass, algal biomass, leaf litter decomposition, or CO_2 emissions of the stream ecosystem. However, the addition of *A. truei* or *P. californica* did influence the densities and the size structure of the different function groups (grazers, detritivores, and meso-predators) within the community. Conversely, predatory *H. pacifica* created strong trophic cascades, which reduced CO_2 emissions. Results from my study suggest that stream ecosystems may be able to compensate for the loss of a functionally dominant grazer or detritivore, but not the loss of a functionally dominant predator.

The presence of the functionally dominant grazer *A. truei* did not significantly reduce standing algal biomass relative to control channels. This result conflicts with those from Lamberti *et al.* (1992) who found that *A. truei* tadpoles reduced chlorophyll-a concentrations by 98% in streams in Washington, USA. However, there has been some debate on the ability of *A. truei* tadpoles to significantly reduce algal biomass compared to streams where they are absent (Atwood and Richardson 2012). Studies done by Rosenfeld (1997) and Kiffney and Richardson (2001) in British Columbia both found that

A. truei tadpoles did not significantly reduce algal biomass. Difference in the strength of the effect of *A. truei* on algal biomass seen in this study could have been due to the fact that either the effects of *A. truei* tadpoles on standing algal growth is variable across time or habitats (Atwood and Richardson 2012), or stream channels contained functionally redundant species (i.e. the biological insurance hypothesis).

Presence of *A. truei* tadpoles also did not influence total consumer biomass or the individual biomasses of grazer, detritivore, or predator taxa groups. However, streams lacking *A. truei* tadpoles had 67% more grazers per m^2 than streams containing *A. truei* tadpoles. My results showing that the number of grazer individuals was higher in streams lacking *A. truei* tadpoles is supported by other studies, suggesting that *A. truei* tadpoles may be competing with other grazer taxa (Lamberti *et al.* 1992, Rosenfeld 1997, Kiffney and Richardson 2001). My results suggest that although *A. truei* tadpoles did not influence the biomass of consumers within streams, they influenced their densities and the size structure of other grazers. Additionally, the increase in the number of grazers present when *A. truei* tadpoles were excluded may help explain why algal biomass was not significantly different between control treatments and grazer treatments. In ecosystems where multiple species play a functionally similar role, ecosystem processes may be less susceptible to species loss (Walker 1995, Duffy *et al.* 2001). If the abundance of smaller grazer taxa within the stream channels are controlled by exploitative or interference competition by *A. truei* tadpoles, then the removal of *A. truei* tadpoles should release the smaller taxa from competition. Additionally, if these smaller grazers are functionally similar to *A. truei* tadpoles, then an increase in their abundance could lead to similar levels of algal grazing.

Based on the allometric equation; metabolic rate α (body size)$^{0.69}$ for aquatic insects (Ruesink and Srivastava 2001), the presence of *P. californica* should have increased leaf biomass loss within stream channels by 85%. However, in my study *P. californica* did not significantly reduce percent leaf litter loss compared to control treatments. Although I did not see a response in total invertebrate biomass or detritivore biomass with the loss of *P. californica* from stream mesocosms, I did detect a numerical response in detritivore density. Control streams had nearly 3x as many detritivores per m^2

compared to detritivore treatments. This result suggests that the higher densities of remaining detritivore species in control channels were effective at maintaining leaf litter breakdown when *P. californica* was absent. Furthermore, this suggests that stream systems in this study were capable of maintaining the ecosystem process of leaf litter decomposition through functional redundancy, despite the loss of a large detritivore. However, in order for a numerical response (increase in the number of other detritivores) in the remaining detritivore assemblage to increase leaf litter breakdown without an increase in total biomass, a per capita response must also have occurred (increase in feeding rates). Although investigating the per capita responses of other detritivores in the absence of *P. californica* was outside the scope of this study, my hypothesis is consistent with results from Ruesink and Srivastava (2001). In their study, the authors found that both numerical and per capita responses in the remaining detritivore assemblage restored leaf litter breakdown rates when *P. californica* was removed from stream systems.

Alterations to the abundance of the top predator *H. pacifica* caused trophic cascades that influenced both consumer biomass and plant biomass. Here, I saw that *H. pacifica* reduced total invertebrate biomass ~48% and leaf litter loss by ~10%, and increased chlorophyll-a concentrations by ~272% compared to controls. At first these results seem contradictory to what I would expect from an even-numbered food chain (Schindler *et al.* 1997, Halaj and Wise 2001). Theoretically, in this food chain the presence of *H. pacifica* should have reduced meso-predator abundance leading to an increase in grazers and detritivores and a decrease in both leaf litter and chlorophyll-a. However, our results showed that the predator treatments did not affect meso-predator biomass or densities compared to control channels, but did reduce detritivore biomass and the densities of both detritivores and grazers. These results suggest that meso-predators were not the target diet for *H. pacifica* in this system. The apparent diet of *H. pacifica* provides support for why I saw trophic cascade results similar to that of an odd-numbered food chain.

The lack of an effect by the large detritivore *P. californica* or grazer *A. truei* on total invertebrate biomass, leaf litter loss, or chlorophyll-a suggests that, biologically, they could have only influenced CO_2 flux of stream channels relative to controls through

their own contributions to community respiration (Allen *et al.* 2005). This however, did not occur and the presence of *P. californica* or *A. truei* had no effect on CO_2 emissions relative to controls. Conversely, the presence of predators decreased CO_2 emissions of streams relative to controls by ~94% through multiple mechanisms. Predator presence decreased community respiration through decreases in the total invertebrate biomass of streams, increased primary production through relaxation of grazing pressure, and decreased remineralization of leaf litter to CO_2 through a reduction in leaf litter decomposition by detritivores. My results are consistent with other studies which found that changes to predator abundance substantially altered CO_2 fluxes of freshwater and marine systems (Schindler *et al.* 1997, Flanagan *et al.* 2006, Wilmers *et al.* 2012, Atwood *et al.* 2013). Both the community data and the ecosystem processes data from this study suggest that my experimental stream communities were able to compensate for the loss of a functionally dominant detritivore or grazer, but not for the loss of a functionally dominant predator.

This study compared the consequences of changes to the abundance of functionally dominant species in different trophic levels on the CO_2 emissions of experimental aquatic ecosystems. My study found that effects of consumers on CO_2 emissions of streams differed between predators, grazers, and detritivores. In my study only the alterations to the functionally dominant predator influenced CO_2 emissions of streams. Results from this study suggest that the influence of biodiversity loss on CO_2 fluxes of aquatic ecosystems is idiosyncratic, and largely dependent on the ability of the remaining community members to compensate for the functional loss of that species. Although some studies have suggested that the quantitative effects of species loss on ecosystem function are similar among trophic groups (Cardinale *et al.* 2006, Cardinale *et al.* 2012), others suggest that predator loss may have larger effects on ecosystem functions than other trophic groups as a result of their high susceptibility to anthropogenic stressors, low diversity and functional redundancy, and their high interaction strengths (Duffy 2003). In order to determine whether species loss influences on CO_2 fluxes of ecosystems are trophically skewed, much more research is needed in both aquatic and other ecosystems.

Table 2.1 Summary statistics for the effect of treatment (grazer, detritivore, or predator additions) on total consumer biomass, biomass and densities of the different functional feeding groups (detritivores, grazers, and meso-predators), decomposition, primary producer biomass and CO_2 emissions of experimental streams. P-values in bold text represent statistically significant factors.

Response	$F_{(3,8)}$	p-value
Consumer biomass (mg m^{-2})	6.52	**0.015**
Detritivore biomass (mg m^{-2})	3.91	0.054
Grazer biomass (mg m^{-2})	0.07	0.974
Meso-predator biomass (mg m^{-2})	4.20	**0.046**
Detritivore density (ind. m^{-2})	13.32	**0.002**
Grazer density (ind. m^{-2})	13.63	**0.002**
Meso-predator density (ind. m^{-2})	2.59	0.125
% remaining leaf litter	22.61	**< 0.001**
Chlorophyll-a (µg cm^{-2})	24.73	**< 0.001**
CO_2 emissions (g C m^{-2} d^{-1})	32.67	**< 0.001**

Table 2.2 P-values for post-hoc Tukey's statistics between control, predator, detritivore, and grazer treatments for total consumer biomass, biomass and densities of the different functional feeding groups (detritivores, grazers, and meso-predators), decomposition, primary producer biomass and CO_2 emissions of experimental streams. ANOVA results for grazer biomass and meso-predator density were not significant. P-values in bold text represent statistically significant factors.

Response	Predator vs Control	Predator vs Detritivore	Predator vs Grazer	Detritivore vs Control	Grazer vs Control	Detritivore vs Grazer
			Treatment comparisons			
Consumer biomass (mg m^{-2})	**0.013**	0.365	0.059	0.144	0.694	0.565
Detritivore biomass (mg m^{-2})	**0.044**	0.800	0.388	0.157	0.425	0.862
Meso-predator biomass (mg m^{-2})	0.135	0.099	**0.036**	0.996	0.787	0.887
Detritivore density (ind. m^{-2})	**0.047**	0.379	**0.027**	**0.004**	0.976	**0.003**
Grazer density (ind. m^{-2})	**0.002**	**0.011**	0.511	0.511	**0.011**	0.083
% remaining leaf litter	**< 0.001**	**< 0.001**	**0.002**	0.954	0.639	0.374
Chlorophyll-a (µg cm^{-2})	**0.001**	**< 0.001**	**< 0.001**	0.971	0.481	0.719
CO_2 emissions (g C m^{-2} d^{-1})	**< 0.001**	**< 0.001**	**< 0.001**	0.975	0.286	0.466
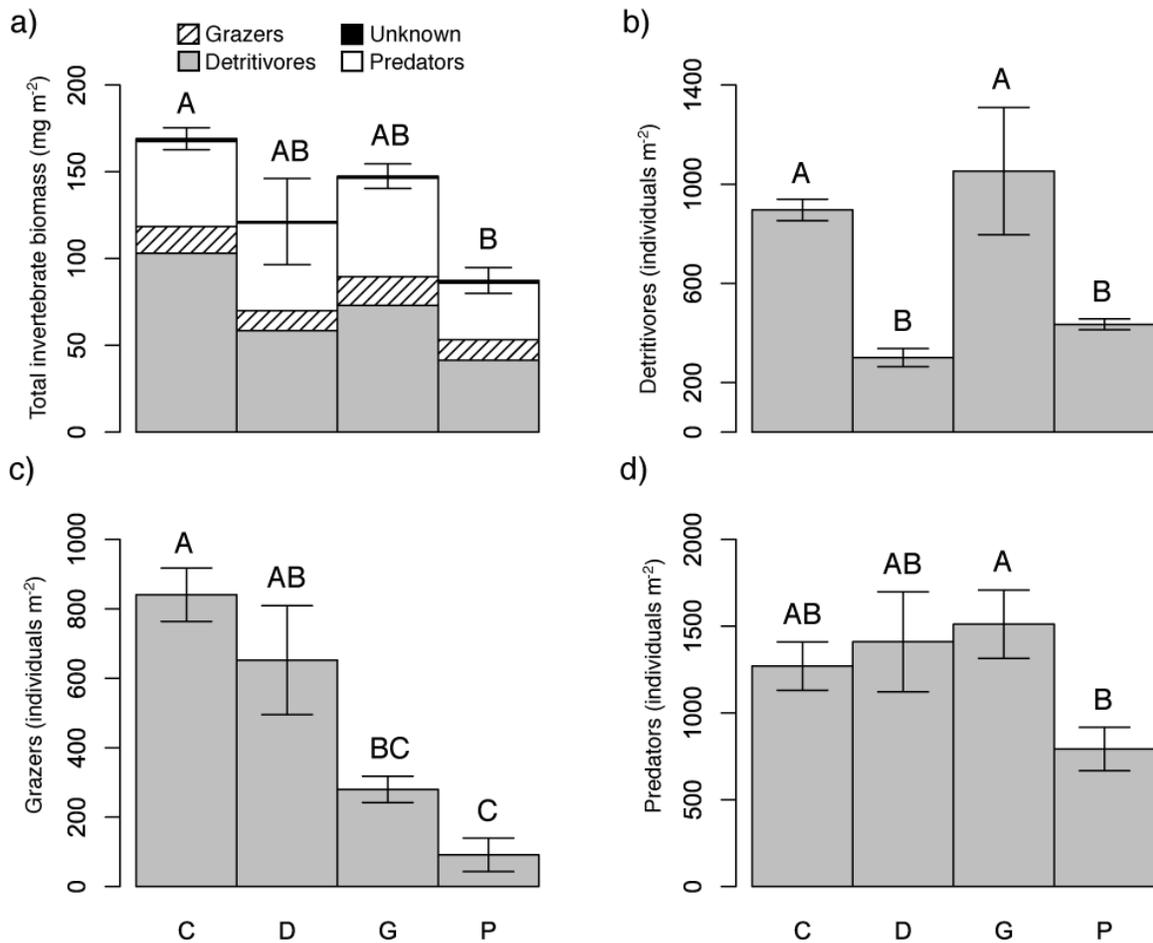
Figure 2.1 Community composition of control stream channels (C) and streams with the addition of a large functionally dominant detritivore *Pteronarcys californica* (D), grazer *Ascaphus truei* (G), or predator *Hesperoperla pacifica* (P). (a) Effects of treatment on total invertebrate biomass and the biomass of different functional feeding groups (meso-predators, detritivores, and grazers). Effects of treatment on the density of detritivores (b), the density of grazers (c), and density of meso-predators (d). Data are means (± s.e.). Letters above bars represent results from Tukey's analyses, where differing letters represent a significant difference between treatments.
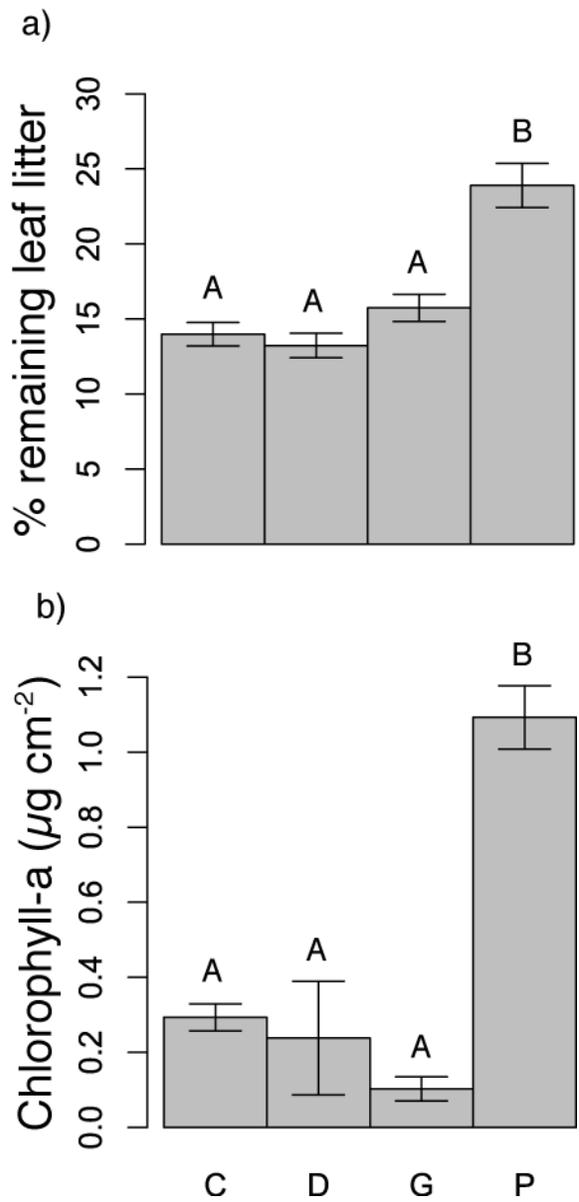
Figure 2.2 Effects of control experimental stream food webs (C) and stream food webs with the addition of a large functionally dominant detritivore *Pteronarcys californica* (D), grazer *Ascaphus truei* (G), or predator *Hesperoperla pacifica* (P) on the percent remaining leaf litter (a) and chlorophyll-a concentrations (b). Data are means (± s.e.). Letters above bars represent results from Tukey's analyses, where differing letters represent a significant difference between treatments.
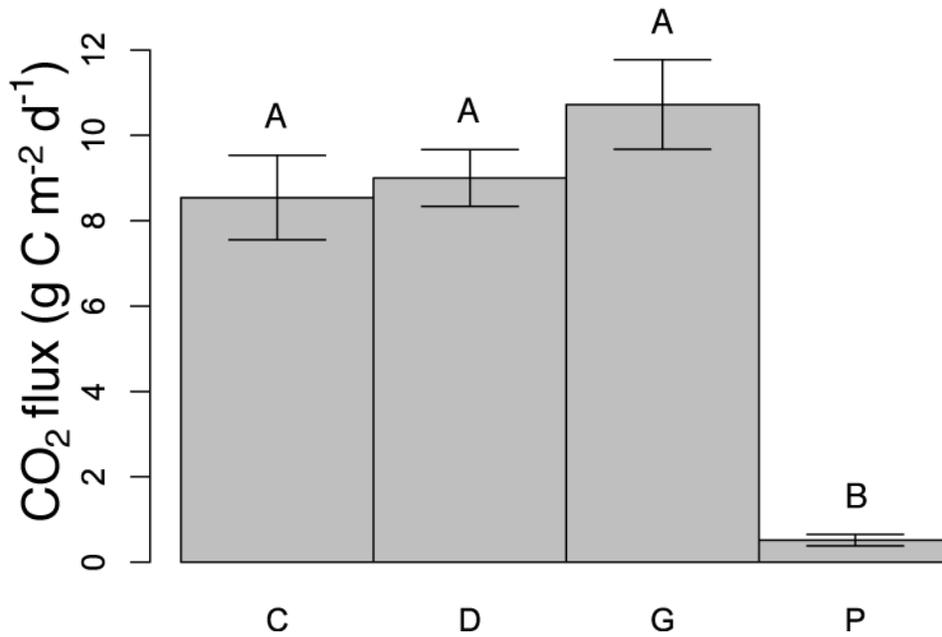
Figure 2.3 Effects of control experimental stream food webs (C) and stream food webs with the addition of a large functionally dominant detritivore *Pteronarcys californica* (D), grazer *Ascaphus truei* (G), or predator *Hesperoperla pacifica* (P) on CO_2 emissions from stream channels. Data are means (± s.e.). Letters above bars represent results from Tukey's analyses, where differing letters represent a significant difference between treatments.

CHAPTER 3: PREDATOR-INDUCED REDUCTION OF FRESHWATER CARBON DIOXIDE EMISSIONS OF THREE DISTINCT FRESHWATER ECOSYSTEMS

3.1 Summary

The top-down effects of predators on their prey have been shown to have cascading effects on lower trophic levels across a wide variety of ecosystems. Additionally there is some empirical evidence that changes to the abundance of predators can influence the chemical nature of ecosystems. Here, I report on experiments in three-tier food chains in experimental ponds, streams and bromeliads in Canada and Costa Rica in the presence or absence of fish (*Gasterosteus aculeatus*) and invertebrate (*Hesperoperla pacifica* and *Mecistogaster modesta*) predators. I monitored carbon dioxide fluxes along with prey and primary producer biomass. I found substantially reduced carbon dioxide emissions in the presence of predators in all systems, despite differences in predator type, hydrology, climatic region, ecological zone and level of *in situ* primary production. I also observed lower amounts of prey biomass and higher amounts of algal and detrital biomass in the presence of predators. I conclude that predators have the potential to markedly influence carbon dioxide fluxes in freshwater systems.

3.2 Introduction

The Earth is currently experiencing its sixth mass species extinction, which like those before it, is drastically altering the abundance and diversity of predator species (Barnosky *et al.* 2011, Estes *et al.* 2011). The loss and global homogenization of predators due to extinctions and introductions is expected to have far-reaching effects on biogeochemical cycling and the functioning of ecosystems (Estes *et al.* 2011, Duffy 2003). Predators play a potentially important, but currently unclear role in local and global carbon cycling. The removal or introduction of predators can trigger alternating changes in the relative populations of lower trophic levels, a phenomenon called a trophic cascade. Trophic cascades can have striking effects on the abundance or biomass of both

heterotrophs and autotrophs within virtually every type of ecosystem (Brooks and Dodson 1965, Ripple and Beschta 2004). Changes in the abundance or biomass of heterotrophs and autotrophs can alter the rates of photosynthesis and community respiration, two biologically driven processes that underpin global carbon cycling (Allen *et al.* 2005) (Fig. 3.1).

Studies investigating the impact of changes in predator abundance on carbon cycling have largely been conducted in terrestrial ecosystems (Wardle *et al.* 2005, Staddon *et al.* 2010, Hawlena *et al.* 2012), despite the fact that freshwater ecosystems often experience stronger top-down control than terrestrial ones (Shurin *et al.* 2012) and are estimated to emit as much CO_2 gas (up to 1.65 Pg C y^{-1}) as emissions due to land-use change (Cole *et al.* 2007, Le Quere *et al.* 2009). However, evidence for top-down effects on CO_2 fluxes of freshwater ecosystems comes from only two studies conducted in experimental lentic ecosystems (Schindler *et al.* 1997, Flanagan *et al.* 2006), one of which was unreplicated (Schindler *et al.* 1997). Although the results of those studies suggest that predators can indirectly influence CO_2 fluxes of more complex ecosystems, they provide only an inductive generalization from a single ecosystem type. To predict how changes to predator abundance may influence carbon cycling more generally, broader experimental testing is needed.

I manipulated the presence of predators within small-scale experimental ponds, streams, and bromeliad phytotelmata to determine the effects of predators on prey biomass, decomposition rates, algal biomass, *in situ* CO_2 concentrations, and CO_2 flux to the atmosphere. Experimental food chains used contained three trophic levels and predator types consisted of both vertebrate (*G. aculeatus* in ponds) and invertebrate (*H. pacifica* in streams and *M. modesta* in bromeliads) primary predators that largely feed on invertebrate herbivores (mainly zooplankton), grazers, and detritivores. I focused our study on pond, stream and bromeliad freshwater ecosystems for three reasons. First, despite their small global surface area, ponds, streams, and bromeliads have been shown to be large sources of CO_2 and methane, and thus, represent an integral part of regional carbon cycles (Cole *et al.* 2007, Martinson *et al.* 2010, Butman and Raymond 2011, Abnizova *et al.* 2012). Second, these ecosystems allowed us to test our hypothesis that

predators influence the CO_2 fluxes of freshwater ecosystems, regardless of differences with respect to predator type (invertebrate or vertebrate), hydrology (lentic or lotic), climatic region (temperate or tropical), ecological zone (pelagic or benthic), and level of *in situ* primary production (autochthonous, allochthonous, or mixed). Finally, these systems can be easily replicated using mesocosms that support naturally complex food webs, but control for physical characteristics within ecosystem types that may influence CO_2 flux (e.g., flow rate, depth, surface area, wind speed). Thus, indirect predator effects on CO_2 flux generated through trophic cascades can be more easily isolated, providing a mechanistic understanding of how predators influence CO_2 fluxes of freshwater ecosystems.

3.3 Methods

3.3.1 Stream experiments

I tested the influence of the presence or absence of the predatory invertebrate *Hesperoperla pacifica*, on total consumer biomass, leaf litter decomposition, primary production, CO_2 concentrations and CO_2 flux from six experimental stream channels (surface area = 7.52 m^2). Channels were located in the Pacific coastal rain forest within the University of British Columbia's Malcolm Knapp Research Forest (MKRF) in Maple Ridge, Canada. Channels were lined with plastic and natural substrata consisting of sand, gravel, pebbles, and cobble were added to the stream beds, producing a series of small runs and riffles. Prior to the start of the experiment, channels were drained, cleaned, and sediments were homogenized. Experimental channels were then dried for 14 day to remove any previous communities. One month prior to the start of the experimental manipulations, channels were connected to a continuous flow of natural stream water from Mayfly Creek, an oligotrophic perennial stream within the research forest. Water from Mayfly Creek was mixed in two settling tanks before entering the stream channels. A flow velocity of ~1.0 L sec^{-1} was maintained in all channels for the duration of the experiment. The use of these experimental channels allowed us to maintain similar physical and chemical water properties (e.g., temperature, pH) to Mayfly creek, but also

allowed us to control stream channel properties that can cause variation in aqueous gas dynamics (e.g. velocity, depth, and groundwater CO_2 intrusion).

3.3.1.1 Experimental communities and manipulations

After the restoration of water flow (day 0), stream communities were left to colonize for 30 days. Colonization occurred through drift from Mayfly Creek and from ovipositing by adult insects directly into the stream channels. Richardson (1991) found that colonization through the discussed methods lead to similar levels of food web complexity to that of the natural donor stream. *Hesperoperla pacifica* were prevented from colonizing the channels by passing water through a 4 mm-mesh filter before entering stream channels. Every third day, *H. pacifica* were removed from the invertebrate community caught in filters, and the remaining organisms were emptied into their respective stream channels.

Following the 30 d colonization of basal communities, I experimentally manipulated the presence *H. pacifica* . Each treatment was replicated three times. In predator treatments, I added *H. pacifica* at naturally occurring densities (2.66 ind. per m^2). Throughout the study, densities of *H. pacifica* were maintained by passing water through a 4-mm-mesh filter before entering the channels. Every third day, *H. pacifica* were removed from the invertebrate community caught in filters, and the remaining organisms were emptied into their respective stream channels. Stream channels were left for 70 days following treatment manipulations and then sampled for total consumer biomass, leaf litter decomposition, primary production, and CO_2 concentrations.

3.3.1.2 Total consumer biomass

On the final day of the study (70 d) benthic samples were collected using a Surber Sampler with a sampling area of 0.023 m^2. Samples were taken randomly from three assigned positions (beginning third of the stream, middle third, and bottom third) within the stream channel. Substrata within the sampling area designated by the Surber were disturbed down to the plastic lining. Samples were placed in plastic containers, put on

ice, and transported to the laboratory. Within 12 h of sampling, invertebrates were picked and placed in 70% ethanol. Total invertebrate wet biomass was determined by averaging the three samples taken within each stream.

3.3.1.3 Total plant biomass

This study used *Alnus rubra* leaves, one of the largest components of terrestrial leaf litter in Mayfly Creek (Richardson 1989), to measure the effects of predators on percent remaining leaf litter. Freshly senesced *A. rubra* leaves were dried at 60° C, stacked into groups of 2.02 ± 0.01g, then re-wetted and stapled together. Leaves were stapled together rather than using traditional mesh bags in order to prevent the use of the mesh as a refuge by smaller detritivores. On day one of experimental manipulations, three leaf packs were secured to random locations within a stream channel.

Leaf packs from each stream were retrieved on the final day of the study (70d), placed in a ziplock bag, and then placed on ice until processed. In the laboratory, invertebrates and sediments were carefully rinsed from each leaf surface. Leaf litter was dried to a constant mass at 60° C, and weighed. Final leaf pack mass was subtracted from initial leaf pack mass then multiplied by 100 to determine percent loss over 70 d. Percent remaining leaf litter was averaged across the three pseudo-replicates within a stream.

To determine standing periphyton biomass, three unglazed ceramic tiles were placed randomly in each stream channel on day one of the experimental manipulations. It has been previously shown that tiles support algal communities similar to that found on natural rock (Rosemond *et al.* 2000, and Mallory and Richardson 2005). Tiles were collected on day 70, placed inside a ziplock bag, and then transported to the laboratory on ice. Tiles were kept in a dark freezer until analyzed. The surface of each tile was scraped with a razor blade, brushed using a toothbrush, and rinsed until all algae had been removed. Periphyton biomass was determined fluorometrically following acetone extraction of chlorophyll-*a* pigments from tile scrapings. Pseudo-replicates of chlorophyll-a within each channel were pooled.

3.3.2 Pond experiments

I tested the influence of the presence or absence of the predatory fish *Gasterosteus aculeatus* on total consumer biomass, primary production, CO_2 concentrations and CO_2 flux from ten experimental open-air, experimental ponds (surface area = 2.16 m^2) located at the University of British Columbia, Vancouver, Canada. Experimental ponds were filled with 1,136 L of municipal water and left to de-chlorinate for one week prior to the assembly of food webs.

3.3.2.1 Experimental communities

Experimental food webs were assembled by inoculating ponds with ~20 zooplankton taxa, ~ 50 phytoplankton taxa, and microbes from nearby ponds (Greig *et al.* 2012, Kratina *et al.* 2012). Zooplankton and phytoplankton were collected from nearby ponds using 64 μm-mesh conical tow nets. Additionally, 1 L of sediment containing eggs, spores, and microbes was added from nearby ponds to aid in community assembly. Aquatic insects were colonized in ponds using natural dispersal. Food webs were left for three weeks before the addition of experimental treatments. Five *G. aculeatus* (three-spined stickleback) of 52.4 ± 0.05 mm in standard body length were added per tank as predator treatments. In the event of death, stickleback were removed from the tank and replaced with a similar-sized fish.

3.3.2.2 Total consumer biomass and plant biomass

Consumers, primary producers, and *in situ* CO_2 concentrations were measured on a single date starting 336 d after the implementation of experimental treatments. Zooplankton were collected using 10 L, depth-integrated zooplankton samples, filtered through a 64 μm-mesh sieve. Spatial variation of zooplankton biomass within the ponds was minimized by pooling samples from multiple depths and locations within the tank (Kratina *et al.* 2012). Zooplankton were identified (usually to genus), counted, and measured under a 10x magnification. Biomass of zooplankton was estimated using length-mass regressions (Kratina *et al.* 2012). Benthic macro-invertebrates were sampled

by collecting organisms from the bottom and walls of the tank and then summed together. Macro-invertebrates on the bottom of the ponds were sampled with standard sweeps within a 0.02 m^2 cylinder, in two areas of the pond. Macro-invertebrates inhabiting the walls of the ponds were collected by sweeping two locations of the wall from the bottom of the pond to the water's surface using a 12 cm, 0.5 mm-mesh net. Macro-invertebrates were identified and their biomasses calculated using length-mass regressions (Greig *et al.* 2012). Biomass of both benthic and pelagic consumers (not including *G. aculeatus*) were combined to estimate total consumer biomass. Phytoplankton biomass was measured with *in vivo* fluorometry (Trilogy, Turner Designs, Sunnyvale, California, USA) using the concentration of chlorophyll *a* in the water column. Periphyton biomass was determined fluorometrically from algal growth collected from 25 cm^2 unglazed tiles (Thompson and Shurin 2009). Phytoplankton biomass and periphyton biomass were added together to get total primary producer biomass.

Biomass was converted to g C m^2 for all organism groups by dividing grams of carbon per tank by the tank surface area. Grams of carbon were estimated by assuming a carbon biomass to chlorophyll biomass ratio of 1:40 for phytoplankton (Cole *et al.* 2002, Kritzberg *et al.* 2004), 1:50 for periphyton (Azim 2010), and an average carbon content of 48% of dry mass for zooplankton (Andersen and Hessen 1991) and 51.8% of ash-free dry mass for benthic invertebrates (Salonen *et al.* 1976).

3.3.3 Bromeliads

I tested the influence of the presence or absence of the predatory invertebrate *Mecistogaster modesta* on total consumer biomass, primary production, CO_2 concentrations and CO_2 flux from in 20 bromeliad phytotelmata mesocosms (surface area = 0.02 m^2) located in the Estación Biológica Pitilla, Área de Conservación Guanacaste, Costa Rica . Mesocosms were constructed from 100 ml plastic cups filled with 85 ml of purified bottled water and 5 ml of filtered bromeliad water to inoculate microbial communities. Three artificial leaves made from fine rigid mesh were fashioned into a rosette pattern and placed inside each mesocosm to mimic the natural structure of a

bromeliad plant. All mesocosms were assembled using naturally occurring levels of habitat complexity (Srivastava 2006) and water surface area (Zotz and Thomas 1999).

3.3.3.1 Experimental communities

Experimental food webs were assembled using invertebrates collected from *Vriesea gladioliflora*, *Guzmania scherzeriana*, and *Vriesea sanguinolenta* bromeliads located in both primary and secondary forest surrounding the station. Three of the most common species of detritivores were added to all mesocosms, at naturally occurring abundances (Srivastava 2006). Ten chironomids (*Polypedilum* sp.), two scirtids (*Scirtes* spp. Coleoptera), and one tipulid (*Trentepholia* sp.) were randomly distributed within the mesocosms. Twenty-four hours after detritivores were placed in mesocosms, a single damselfly larva was added to five mesocosms. All predators were starved for 24 h prior to their placement inside the mesocosms. Three chironomids and two scirtids were added mid-way through (20 d) the experiment to simulate re-colonization. Additional predators were not added mid-experiment because ovipositing by damselfly larvae is less frequent in the rainy season and predator larval stages tend to last much longer than the detritivore species used in this experiment (Srivastava 2006). After mesocosm food webs were assembled, mesocosms were placed inside a large black cup and then the larger black cup was covered with mesh to prevent insects from ovipositing. Mesocosms were then placed on a bench outdoors under a rain shelter.

3.3.3.2 Total consumer biomass

On day 40, remaining leaf litter and detritivores were removed from all mesocosms. Leaves were carefully rinsed and inspected to ensure that all detritivore had been removed. Detritivores lengths were measured using a dissecting microscope. Total remaining detritivore biomass after 40 d for each mesocosm was calculated by summing the dry biomass of the remaining detritivores. Dry biomass was calculated using length-mass regressions (Srivastava unpublished data).

3.3.3.3 Total plant biomass

This study used *Conostegia xalapensis* Bonpl. leaf litter to measure the effects of predator, on percent remaining leaf litter. Freshly senesced *C. xalapensis* leaves collected from around the station were dried at 60° C, stacked into groups of 1.05 ± 0.01 g, then placed inside mesocosms prior to the release of detritivores or predators. On day 40, remaining leaf litter was removed from all mesocosms. Leaf litter was rinsed to remove all detritivores and then dried to a constant mass at 60°C, and weighed. Percent leaf litter remaining was subtracted from initial leaf pack mass then multiplied by 100 to determine percent loss over 40 d.

3.3.4 CO_2 collection and flux calculations

Water samples for dissolved CO_2 concentrations were extracted at dusk using 50-mL Pressure-Lok® syringes (VICI Precision Sampling Corp., Baton Rouge, LA, USA) and stored in vacutainers (Labco Limited High, Wycombe, UK). Sample CO_2 concentrations were analyzed on a 5890 Series II gas chromatograph within 24 h for ponds and streams or 72 h for bromeliads using headspace equilibrium analysis (Teoduro *et al.* 2009). Total CO_2-C concentrations (mg C l^{-1}) in the water were calculated using equations from Appendix A.

CO_2 flux (g C m^{-2} d^{-1}) to the atmosphere was calculated as follows:

$$CO_{2flux} = (pCO_{2water} - pCO_{2air})*k$$

Here, pCO_{2water} is the temperature corrected partial pressure of CO_2 measured in the water, and pCO_{2air} is the partial pressure of CO_2 in the overlying atmosphere (390 ppm), k is the CO_2 exchange velocity coefficient (m d^{-1}). Stream k values (4 m d^{-1}) were calculated using the equation from Butman and Raymond (2011). Bromeliad and pond k values were estimated using literature values for no wind (bromeliads; $k = 0.48$ m d^{-1}) and low wind (ponds; $k = 0.63$ m d^{-1}) conditions (Cole and Caraco 1998).

3.2.5 Statistical analyses

I contrasted predator versus non-predator treatments for all response variables using a multivariate analysis of variance (MANOVA; test = "Pillai trace"). To compare the predator effects across different ecosystems using a single MANOVA analysis, ecosystem response variables (prey biomass, percent leaf litter remaining, algal biomass, and CO_2 flux) for each ecosystem type (pond, streams, and bromeliads) were converted into z-scores. Because *in situ* CO_2 concentrations and CO_2 flux were co-linear factors, only CO_2 flux was added to the MANOVA model. I found no significant differences among ecosystem type ($F_{2,32} = 0.00$, p = 1.00) and so removed this factor from subsequent analyses. To determine where significant difference occurred in our model, subsequent univariate analyses were performed on the individual response variables.

3.4 Results

I found strong effects of predators on prey biomass, plant biomass, *in situ* CO_2 concentrations, and CO_2 flux across all three ecosystems (MANOVA, $F_{1,34} = 32.97$, p < 0.001; Fig. 3.2, Table 3.1). Predators in each system significantly reduced prey biomass by ~75 ± 67% (mean ± SD) ($F_{1,34} = 50.96$, p < 0.001; Fig. 3.2), and cascading indirect effects led to ~47 ± 10% lower detrital loss ($F_{1,34} = 38.49$, p < 0.001; Fig. 3.2) and 65 ± 15% higher algal biomass ($F_{1,34} = 14.19$, p < 0.001; Fig. 3.2). Furthermore, predators significantly decreased *in situ* CO_2 concentrations by ~ 42 ± 23% (Fig. 3.2). These effects were also manifested in the CO_2 flux, where predators negatively influenced CO_2 emissions ($F_{1,34} = 27.25$, p < 0.001; Fig. 3.2). Here, predator treatments emitted ~ 93 ± 44% less CO_2 gas to the atmosphere per day compared to non-predator treatments (Fig. 3.3).

3.5 Discussion

Recent studies have shown the potential effects loss of predators can play on the CO_2 fluxes of ecosystems (Shindler *et al.* 1997, Flanagan *et al.* 2006, Wilmers *et al.* 2012). Consistent with these studies, my results provide experimental evidence that

predators can alter CO_2 emissions to the atmosphere in freshwater ecosystems, and suggest that predators have the potential to play a key role in local and global C cycles. However, this study is the first to show that predators had a consistent negative indirect effect on CO_2 emissions from different freshwater ecosystems despite differences in predator type, hydrology, climatic region, ecological zone, and level of *in situ* primary production.

The magnitude of the indirect effect of predators on CO_2 emissions is dependent on the strength of the trophic cascade. The use of experimental ecosystems with low diversity and simplified physical structure can result in stronger top-down effects of predators on communities and ecosystem processes. However, a graphical comparison of the trophic cascade strengths for our three experimental ecosystems to averages of natural partner ecosystems calculated in a meta-analysis by Shurin *et al.* (2002) showed that top-down control of plant biomass in natural ecosystems was, if anything, greater compared to our experimental ones (Fig. 3.4). This shows that predators in complex ecosystems are capable of generating trophic cascades at equal or greater magnitudes to those demonstrated in this study, and suggests that trophic cascades could have a greater influence on CO_2 fluxes in natural ecosystems. Additionally, the effects of predators on communities and CO_2 fluxes in natural ecosystems may be further exaggerated by other anthropogenic influences, such as climate warming (Kratina *et al.* 2012) and increased nutrient loading to freshwater ecosystems (Schindler *et al.* 1997, Flanagan *et al.* 2006). Despite broad differences in predator type, all predators in our study were capable of creating trophic cascades that influenced CO_2 fluxes of their ecosystems. However, the magnitude of trophic cascades can be influenced by the biological characteristics of the predator, and thus effects on CO_2 fluxes may also be influenced by predator identity (Borer *et al.* 2005, Schmitz 2008).

I showed that predators decreased CO_2 emissions to the atmosphere in predominantly three-tier food chains consisting of predators, primary consumers and primary producers. However, the direction of the indirect effect of predators on CO_2 emissions is dependent on food chain length (Schindler *et al.* 1997). In odd number trophic-level systems, such as the systems presented in our study, predators are

hypothesized to decrease CO_2 emissions. Conversely, the indirect effect of predators in even number trophic-level systems is predicted to cause an increase in CO_2 emissions.

The consistency in the effect of predators on CO_2 emissions in our study was remarkable, given the substantial differences among our experimental systems. Perhaps most surprisingly, predators had similar indirect effects on CO_2 flux for both detrital-based (bromeliads and streams) and algal-based (ponds and streams) food webs. This suggests that although predators may affect different underlying processes (photosynthesis or community respiration) behind the changes in CO_2 concentrations of the ecosystem, their effects on carbon storage generate a similar ecosystem response. The consistency of our results combined with the comparison of our trophic cascade strengths to those of natural ecosystems, provides evidence that predators have the potential to markedly influence CO_2 fluxes of freshwater ecosystems, and further supports evidence that predators can have strong effects on biogeochemical processes (Schmitz 2008, Vanni 2002). The dramatic influence of predators on CO_2 emissions from our freshwater ecosystems also indicates that human-induced removal of predators, or introduction of non-native predators, may have complex consequences for regional and global C cycles. Although predators are well known to shape ecological communities, our multisystem approach provides evidence that changes to predator abundance can extend beyond the biotic realm of an ecosystem and may fundamentally alter biogeochemical cycling and greenhouse gas dynamics.

Table 3.1 Mean (± 95% confidence intervals) effects of predator manipulations on prey biomass, algal biomass, leaf litter decomposition, in situ CO2 concentrations ([pCO2]), and CO2 flux with the atmosphere for pond, stream, and bromeliad experimental ecosystems.

	Predator absent	**Predator present**
Ponds		
Prey biomass (g C m^{-2})	0.171 ± 0.098	0.034 ± 0.034
Algal biomass (g C m^{-2})	0.406 ± 0.315	0.640 ± 0.071
[pCO$_2$] (mg C l^{-1})	5.4 ± 0.1	4.9 ± 0.3
CO$_2$ flux (g C m^{-2} d^{-1})	-0.024 ± 0.040	-0.841 ± 0.160
Streams		
Prey wet biomass (g m^{-2})	0.769 ± 0.255	0.263 ± 0.128
Algal biomass (μg m^{-2})	0.206 ± 0.154	1.224 ± 0.341
Detritus (% remaining)	14.703 ± 4.274	25.607 ± 4.588
[pCO$_2$] (mg C l^{-1})	87.8 ± 0.5	5.9 ± 0.1
CO$_2$ flux (g C m^{-2} d^{-1})	8.330 ± 1.882	0.509 ± 0.221
Bromeliads		
Total remaining prey biomass (g)	0.0018 ± 0.0003	0.0004 ± 0.0004
Detritus (% remaining)	75.58 ± 2.91	90.75 ± 3.06
[pCO$_2$] (mg C l^{-1})	81.5 ± 16.8	48.1 ± 9.0
CO$_2$ flux (g C m^{-2} d^{-1})	37.313 ± 8.083	21.255 ± 4.318
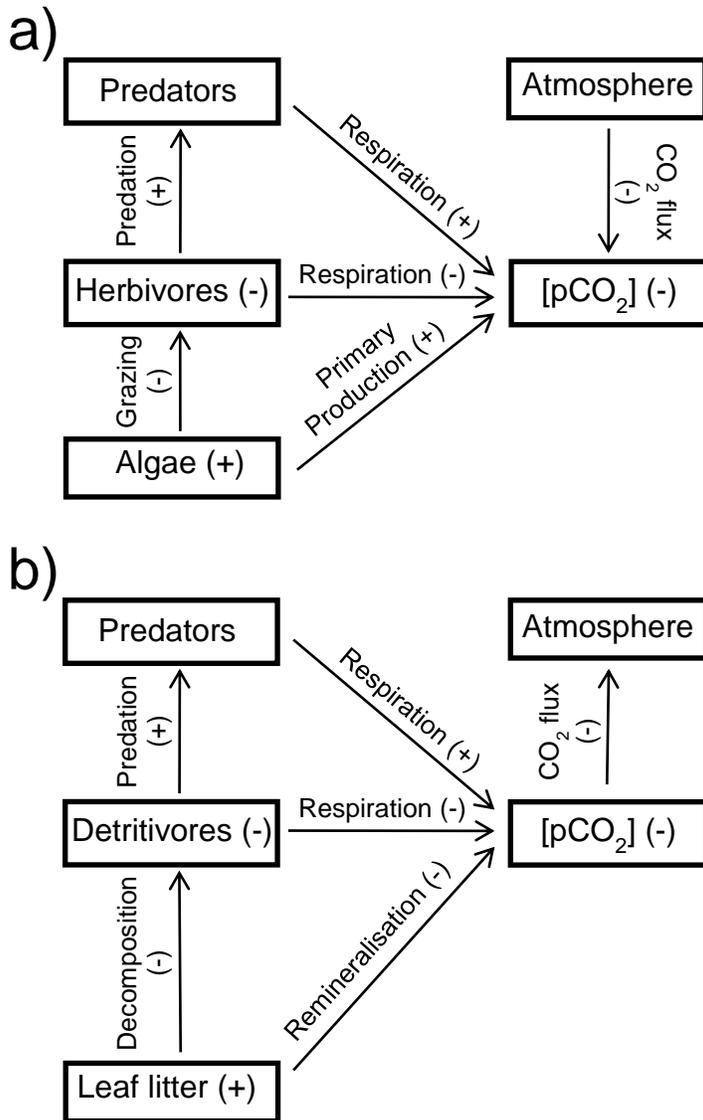
Figure 3.1 Hypothesized effects (depicted by + or -) of predators on community composition, ecosystem processes, and carbon flux to the atmosphere. a) Predators in algal-based freshwater ecosystems can negatively influence in situ CO_2 concentrations ([pCO_2]) and CO_2 flux with the atmosphere by creating trophic cascades that increases primary production and alters community respiration. b) Predators in detrital-based freshwater ecosystems can negatively influence [pCO_2] and CO_2 flux with the atmosphere by creating trophic cascades that reduce remineralization of leaf litter and alter community respiration. Predator effects depicted are representative of odd-numbered food chains; opposite effects are predicted for even-numbered food chains.
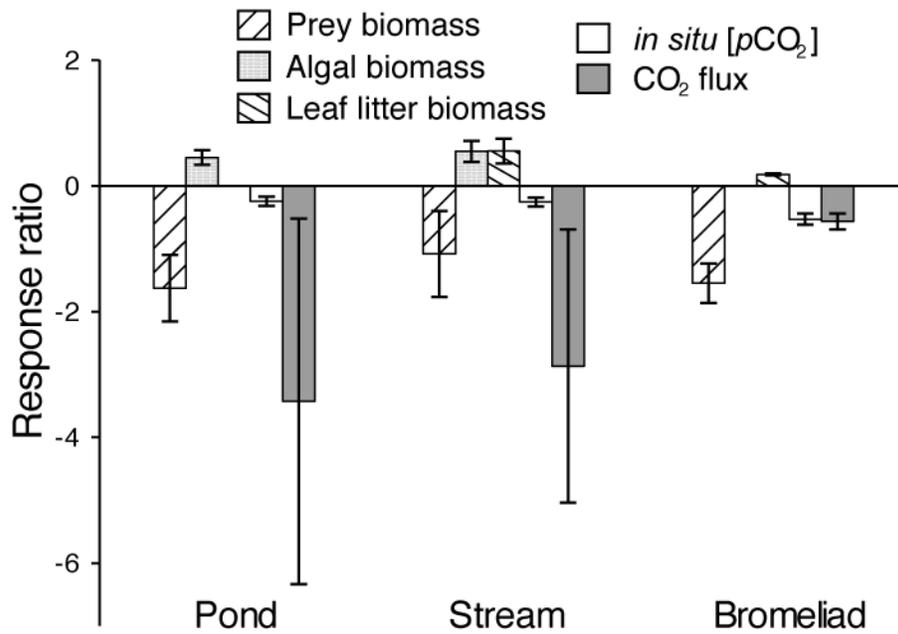
Figure 3.2 Demonstrated effect sizes of predators on prey, primary producers, and CO_2 flux of ponds, streams, and bromeliads. Results are shown as log-ratios ± 95% C. I.
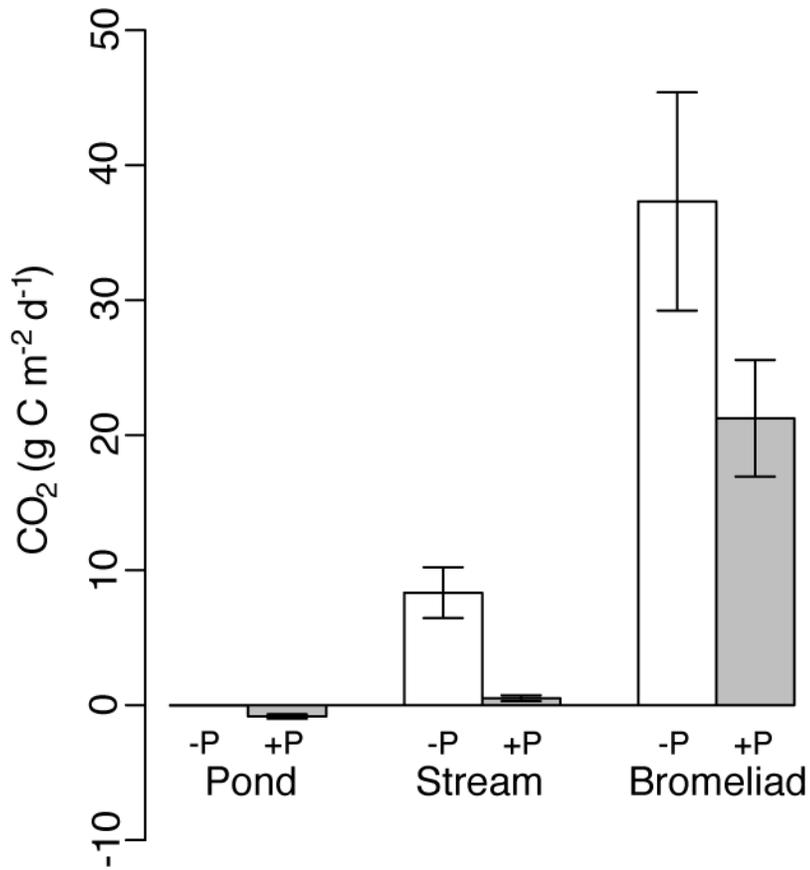
Figure 3.3 Effects of predator manipulations on mean (± 95% confidence intervals) CO_2 flux of ponds, streams, and bromeliads. Ponds exposed to no predator treatments were at equilibrium with the atmosphere.
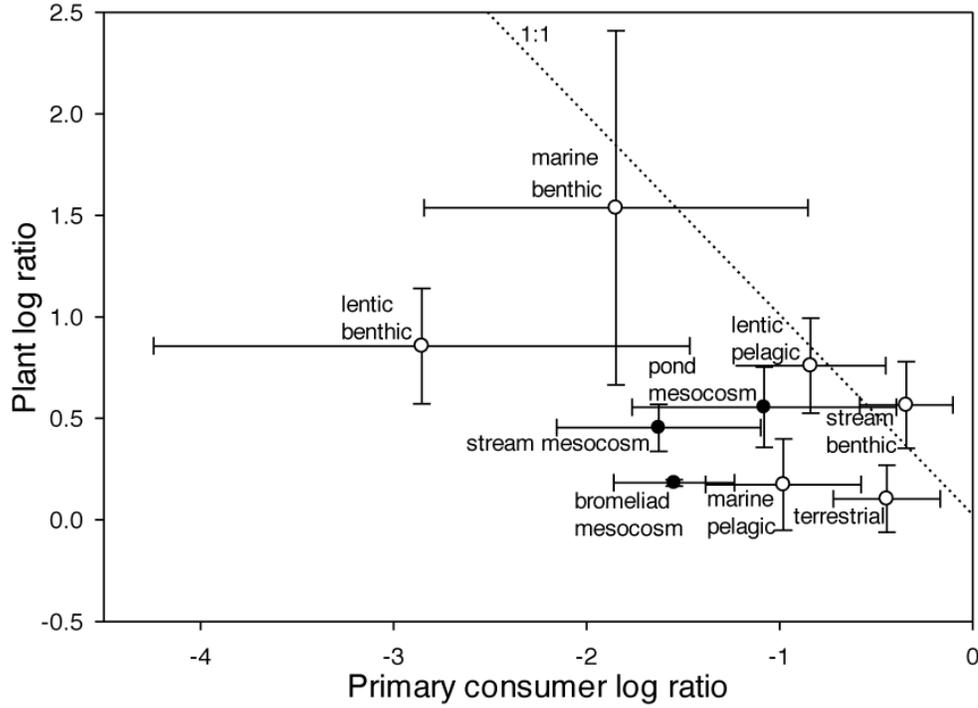
Figure 3.4 Comparison of trophic cascade strength from the current study to natural ecosystems. Effect sizes (log-ratio ± 95% C.I.) of predators on primary producers vs. primary consumers from our experimental ponds, streams, and bromeliads (●), and those calculated from natural ecosystems (O) (Shurin *et al.* 2002). Primary producer data for stream mesocosms is representative of the effect size of predators on leaf litter biomass, however, predator effect size on algal biomass was similar in magnitude. The dotted line shows the 1: -1 relationship. Data for lentic benthic, lentic pelagic, stream benthic, marine pelagic, marine benthic, and terrestrial ecosystems were replotted from Shurin *et al.* (2002).

CHAPTER 4: INFLUENCE OF PREDATOR IDENTITY AND INTERSPECIFIC INTERACTIONS ON CO$_2$ EMISSIONS FROM A BROMELIAD ECOSYSTEM

4.1 Summary

Competition and facilitation between predator species has the potential to either enhance trophic cascades or dampen them, ultimately influencing the total predator effects on CO$_2$ fluxes of ecosystems. I tested how interspecific interactions between a predatory damselfly and a predator diving beetle influenced the magnitude of trophic cascades and their effects on emissions of CO$_2$ gas in experimental, bromeliad ecosystems. Here I show that, relative to multiplicative null models, multiple predators in a detrital-based system resulted in antagonistic relations that eliminated trophic cascades. The elimination of trophic cascades in treatments containing both predators lead to greater leaf litter decomposition, reducing carbon storage and increasing CO$_2$ emissions from bromeliads. Furthermore, I showed that the antagonistic effects between predators occurred due to a tactile response that culminated in the emigration of the damselfly larvae from the ecosystem. My results suggest that predator-predator interactions are an important mechanism regulating top-down effects on carbon flux of ecosystems.

4.2 Introduction

Predators influence carbon cycling of their ecosystems through their direct interactions with their prey and indirect interactions with lower trophic levels (Greig *et al.* 2012, Leroux *et al.* 2012). Trophic cascades that substantially alter the biomass of lower trophic levels influence ecosystem processes that underpin carbon storage (i.e., decomposition and secondary and primary production) by altering carbon turnover times or the size of biologically active carbon pools (Schmitz *et al.* 2000, Estes *et al.* 2011, Greig *et al.* 2012). Top-down effects of predators on carbon storage processes can extend to alter the exchange of the greenhouse gas CO$_2$, between ecosystems and the atmosphere by influencing the amount of CO$_2$ that is sequestered or respired by the ecosystem community (Schindler *et al.* 1997, Wilmers *et al.* 2012, Atwood *et al.* 2013). Studies showing the importance of top-down processes on CO$_2$ fluxes of ecosystems are

beginning to emerge as a result of concern over the widespread removal and invasion of predators from Earth's ecosystems. However, to date these studies have been single-predator experiments, which ignore the influence of multi-predator species interactions (Schindler *et al.* 1997, Flanagan *et al.* 2006, Atwood *et al.* 2013). As such, our current knowledge of top-down effects on CO_2 emissions from ecosystems is an over-simplified abstraction of the much more prevalent multi-predator ecosystems seen in nature.

Higher predator diversity increases the potential for both positive and negative interactions among predator species, and can influence trophic cascade strength (Soluk and Richardson 1997, Hart 2002, Finke and Denno 2004, Casula *et al.* 2006). Predator-predator interactions can alter prey populations differently depending on whether their interactions result in additive mortality of prey (equivalent to the expected value of prey consumed), synergism (greater than the expected value of prey consumed), or antagonism (less than the expected value of prey consumed). Synergistic relationships between predators can create stronger trophic cascades as a result of greater consumptive or behavioral effects on prey populations (Sih *et al.* 1998, Duffy 2002). Conversely, antagonistic relationships between predators can dampen trophic cascades by reducing predation pressure on prey populations (Finke and Denno 2005). Both synergistic and antagonistic interactions among predators can arise from several different mechanism including direct and indirect interactions, and consumptive and behavioral interactions (Polis *et al.* 1989, Siddon and Witman 2004, Hart 2008, Steffan and Snyder 2010)

Territorial behavior, an antagonistic relationship, is common amongst predators and is expressed by representatives from nearly every group in the animal kingdom (Keen and Reed 1985, Stamps 1995, Hargrave *et al.* 2011). Predators will defend resources by attacking or manifesting other threatening behaviors towards heterospecific competitors. By defending feeding sites, territorial predators can often monopolize large amounts of resources and reduce the consumption rates of other predators purely through behavioral interactions. The reduction in consumption rates of one predator can have large impacts on how the predator guild as a whole influences community functioning (Ives *et al.* 2005, Steffan and Snyder 2010), especially if the predatory species that becomes dominant is unable to exert strong top-down effects on the community (negative

selection effect) (Jiang *et al.* 2008). Thus, cascading diversity effects transmitted by non-lethal behavioral interactions among predators can alter the rates of ecosystem processes that underpin carbon storage (e.g., primary production or decomposition) (Steffan and Snyder 2010). To date, no studies have investigated how antagonistic behaviors among predators (e.g., territorial behavior) influences top-down effects on biogeochemical cycling.

The aim of this study was to examine how the propagation of predator diversity effects, specifically antagonistic effects, influences the magnitude of trophic cascades and the CO_2 exchange between an ecosystem and the atmosphere. Our study examined the individual and combined effects of two predator species, a sit-and-wait predatory damselfly larva (*Mecistogaster modesta,* Selys 1980) and an active foraging adult diving beetle (Dytiscidae: *Copelatus* sp.), on detritivore biomass, leaf litter decomposition and CO_2 flux to the atmosphere using bromeliad mesocosms. In order to better understand the predator-predator interactions in our experiment, a series of behavioral studies were also conducted to determine the mechanism (visual, chemical, tactile, or combinations of the three) behind the observed antagonistic behaviors of our predator found in our study.

I used the bromeliad ecosystem to examine the effects of inter-species antagonism between predators on community processes and emissions of CO_2 because: i) bromeliads are hypothesized to be large sources of CO_2 and methane within neotropical forests (Martinson *et al.* 2010) ii) territorial behavior in odonate larvae, is well documented (Cobert 1980, Shaffer and Robinson 1996) iii) the strength of cascading effects on ecosystem functions have been shown to be dependent on predator hunting-mode (Schmitz 2008, Woodcock and Heard 2011), and iv) it has been documented that predators have strong effects on intra- and inter-ecosystem nutrient cycling, leaf litter decomposition rates, and insect emergence within bromeliad ecosystems (Ngai and Srivastava 2006, Starzomski *et al.* 2010). Additionally, bromeliad mesocosms offer an ideal ecosystem to test the effects of predators and their interactions on community processes and CO_2 as they provide an easily replicated ecosystem that supports naturally complex food webs and are similar in spatial area and habitat complexity, but also

controls for physical characteristics of bromeliads that may influence CO_2 flux (e.g., water depth, surface area, wind speed).

4.3 Methods

4.3.1 Experimental design

This study was conducted during the wet-season (September-November), a time when bromeliad aquatic communities are at their peak, at the Estación Biológica Pitilla, Área de Conservación Guanacaste, Costa Rica. Invertebrates used in all experiments were collected from *Vriesea gladioliflora*, *Guzmania scherzeriana*, and *Vriesea sanguinolenta* bromeliads located in both primary and secondary forest surrounding the station. Mesocosms were constructed from 100 ml plastic cups filled with 85 ml of purified bottled water and 5 ml of filtered bromeliad water to inoculate microbial communities. Three artificial leaves made from a fine rigid mesh were fashioned into a rosette pattern and placed inside each mesocosm to mimic the natural structure of a bromeliad plant and to provide a perch for predators and for insect emergence. Detrital inputs into each mesocosm consisted of ~ 2 g of dried, *Conostegia xalapensis* Bonpl. leaf litter. After mesocosm food webs were assembled, mesocosms were placed inside a large black cup and then covered with mesh to prevent insects from ovipositing. Mesocosms were then placed on a bench outside under a rain shelter. Black cups and mesh tents were checked daily for emigration of damselfly larvae and emerged detritivores.

Experimental food webs consisting of leaf litter and detritivores were exposed to one of four predator treatments (each n = 10); 1) no predator, 2) predatory diving beetle present, 3) predatory damselfly larva present, and 4) both predators present. Three of the most common species of detritivores were added to all mesocosms at naturally occurring abundances (Srivastava 2006). Ten chironomids (*Polypedilum* sp.), two scirtids (*Scirtes* sp. Coleoptera), and one tipulid (*Trentepholia* sp.) were randomly distributed within the mesocosms. Twenty-four hours after detritivores were placed in mesocosms, a single damselfly larva, adult diving beetle, or one of each predator in the combined treatment

were added to predator treatments. All predators were starved for 24 h prior to their placement inside the mesocosms. Three chironomids and two scirtids were added mid-way (20 d) through the experiment to simulate re-colonization. Predators were not added mid-experiment because ovipositing by damselfly larvae is less frequent in the rainy season and predator larval stages tend to last much longer than the detritivore species used in this experiment (Srivastava 2006).

4.3.2 Sample collection

A single water sample from each mesocosm was collected for dissolved CO_2 concentrations at dusk on day 39 of the experiment using 50-mL Pressure-Lok® syringes (VICI Precision Sampling Corp., Baton Rouge, LA, USA). Water samples were then stored in gas tight vacutainers (Labco Limited High, Wycombe, UK.) and transported to the Department of Civil Engineering, Environmental Laboratory at the University of British Columbia for analysis of dissolved CO_2 gas using headspace equilibrium within 72 h. While in transport, two water samples from the combined predator treatment and three samples from the diving beetle treatment were compromised and removed from future analyses. Collections for sample water were done following procedures described in Hope *et al.* (1995), and total CO_2-C concentrations (mg C l^{-1}) in the water were calculated using equations from Appendix A.

On day 40, remaining leaf litter and detritivores were removed from all mesocosms. The diving beetle and damselfly larva within one of the combined predator treatments were both found dead, thus this replicate was removed from future analyses. Decomposition was measured as detritus loss of 40 days. Leaf litter was rinsed to remove all detritivores and then dried to a constant mass at 60°C, and weighed. Total remaining detritivore biomass after 40 days for each mesocosm was calculated by summing the dry biomass of the remaining detritivores. Dry biomass of detritivores was estimated by measuring the lengths of remaining detritivores using a dissecting microscope and then calculating dry biomass using length-mass regressions (Srivastava unpublished data).

4.3.3 CO_2 flux calculations

CO_2 flux (g C d^{-1}) to the atmosphere was calculated using the following equation:

$$CO_{2flux} = (pCO_{2water} - pCO_{2air})*k*SA$$

Here, pCO_{2water} is the partial pressure of CO_2 measured in the water, and pCO_{2air} is the partial pressure of CO_2 in the overlying atmosphere (390 ppm), k is the CO_2 exchange velocity coefficient, and SA is the surface area of the mesocosm (m^2). The k value corresponding to no wind conditions ($k = 0.48$ m d^{-1}, Cole and Caraco (1998)) was used to compute CO_2 flux.

4.3.4 Statistical analysis

I used multiplicative risk models to test whether the two predators acted independently of one another (Soluk and Collins 1988, Soluk 1993, Sih *et al.* 1998). The multiplicative risk model states,

$$C_{fs} = (P_1 + P_2) - (P_1 * P_2)$$

where, C_{fs} was the combined predicted effect of the two predators on the response, P_1 was the effect of damselflies on the response, and P_2 was the effect of diving beetles on the response. Unlike the additive model, the multiplicative risk model ensured that the combined predicted effect of the predators on the response was never greater than 100%. Additionally, this model took into account the change in predator densities between the single predator treatment and the combined predator treatments. In order to test the effects of the multiplicative risk model all data must be log-transformed or linked to a log function.

I examined multiple predator effects of a predatory diving beetles and damselfly larvae on detritus loss over 40 d and CO_2 flux to the atmosphere using a 2 x 2 factorial design crossing the presence or absence of diving beetles with the presence or absence of damselfly larvae. As a result of total prey consumption in some predator treatments, multiple predator effects on total detritivore biomass were tested using a fully crossed generalized linear model with a quasi-poisson error structure and a loglink function. A

significant interaction between diving beetles and damselfly larvae suggest either a synergistic (response variable > multiplicative risk model prediction) or antagonistic (response variable < multiplicative risk model prediction) relationship between the two predators. In order to ensure that the absence of an interaction represented multiplicative effects of predators, all data were log transformed or linked to a log function.

To disentangle multiple predator effects from those of changes in predator densities, I compared my measured response variable to those predicted from the multiplicative risk model (Otto *et al.* 2003, Vonesh and Osenberg 2003, Romero and Srivastava 2010). A predicted value inside the s.e. of the measured value indicates that the effects of the two predators were additive; a predicted value outside the s.e. of the measure value indicates that the effect of the two predators were non-additive.

In addition to testing for non-additive effects of predators, the effects of predator identity on detrital loss and CO_2 flux was also analyzed using analysis of variance and total detritivore biomass was tested using a fully crossed generalized linear model with a quasi-poisson error structure. Subsequent post-hoc Tukey's analyses on the individual response variables were used to determine where significant differences occurred. Carbon dioxide flux data were log transformed in order to meet test assumptions of normality and homogeneity of variance. Differences among treatments in detritivore emergence were also tested using a One-way ANOVA to determine whether differences in total remaining detritivore biomass among treatments could be explained by detritivore emergence as opposed to predation. All statistical analyses were performed in R 2.12.1 (R Development Core Team, 2010).

4.3.5 Behavioral experiments

To evaluate possible mechanisms behind the emigration observed by damselfly larvae in the biodiversity study, I conducted stimuli behavioral experiments. Mesocosms containing a single damselfly larva were exposed to one of five treatments; visual, chemical, visual + chemical, or tactile stimuli or a control treatment (n = 5 per treatment). Visual treatments contained a single diving beetle within a clear 50 ml tube placed inside

the mesocosm. Chemical treatments consisted of a single diving beetle within an opaque 50 ml tube placed inside the mesocosm. To allow chemical cues to circulate through the mesocosm, the tubes had two large holes covered with 80 μm Nitex mesh on either side of it near the bottom. Visual + chemical treatments had a single diving beetle within a clear 50 ml tube with mesh covered holes drilled near the bottom that was placed inside the mesocosm. Tactile treatments contained a free roaming diving beetle and an empty 50 ml tube within the mesocosm. Because tactile response could not be separated from chemical and visual cues, these treatments actually represent a combination of tactile, chemical and visual cues. Beetles added to tactile treatments were placed directly on top of the damselfly to ensure tactile interactions. Control treatments contained a single damselfly larva and an empty 50 ml tube. All predators within centrifuge tubes were fed three chironomids daily and six chironomids were placed within mesocosms. Mesocosms were placed inside a larger black cup and checked every 6 h for emigration over 3 days. I used one-way ANOVA and a subsequent Dunnett's test to test statistical differences between stimuli treatments and the control treatment.

4.4 Results

The magnitude of trophic cascades and their influence on CO_2 emission from bromeliad mesocosms were dependent on interactions between predators and predator identity. Significant interactions between damselfly larvae and diving beetles showed that mesocosms containing both predators had non-additive, antagonistic effects on detritivore biomass, leaf litter decomposition and CO_2 flux to the atmosphere (Table 4.1; Fig. 4.1). In predator treatments containing only damselfly larvae, strong top down effects significantly reduced detritivore biomass by 79% (95% CI; 55-96%) ($F_{1,35} = 16.19$, p < 0.001; Fig. 4.1a), leaf litter decomposition by 63% (95% CI; 50-75%) ($F_{1,35} = 22.49$, p < 0.001, Fig. 4.1b) and CO_2 flux to the atmosphere by 46% (95% CI; 28-59%) ($F_{1,31} = 11.34$, p = 0.002; Fig. 4.1c). Conversely, diving beetles had only a minimal effect on detritivore biomass ($F_{1,35} = 0.73$, p = 0.397; Fig. 4.1a), leaf litter decomposition ($F_{1,35} = 2.13$, p = 0.153; Fig. 4.1b) and CO_2 flux to the atmosphere ($F_{1,31} = 2.70$, p = 0.109; Fig. 4.1c).

Tukey's analysis showed that treatments containing only damselfly larvae as predators had significantly lower detritivore biomass ($p < 0.001$), leaf litter decomposition ($p < 0.001$), and CO_2 emissions ($p = 0.001$) compared to treatments containing no predators (Fig. 4.1). Additionally, treatments containing only damselfly larvae as predators had significantly lower detritivore biomass ($p = 0.001$), leaf litter decomposition ($p < 0.001$), and CO_2 emissions ($p = 0.017$) compared to treatments containing only diving beetles (Fig 4.1). Tukey's analysis also showed that treatments containing only diving beetles were not significantly different in detritivore biomass, leaf litter decomposition, or CO_2 emissions compared to treatments containing no predators ($p = 0.337$, $p = 0.060$, $p = 0869$, respectively; Fig. 4.1) or combined predator treatments ($p = 0.742$, $p = 0.354$, $p = 0.999$), respectively; Fig. 4.1).

ANOVA results revealed that there was a significant difference among treatments in detritivore emergence ($F_{3,35} = 8.55$, $p < 0.001$). Subsequent Tukey's analysis showed that the control treatment was significantly different than all three predator treatments in detritivore emergence, but predator treatments did not statistically differ from one another.

4.4.1 Behavioral experiments

Two to seven days from the start of the biodiversity experiment, I observed that damselflies in the combined predator treatment were emigrating out of the bromeliads and into the surrounding black cups. This behavior occurred in 9 of the 10 replicates. Behavioral experiments between damselfly larvae and diving beetles showed that neither chemical, visual, nor a combination of the two stimuli from diving beetles had any effect on emigration of damselfly larvae from the mesocosms (all $t = 2.04$, $p > 0.05$). However, tactile cues from diving beetles increased emigration of damselfly larvae from the mesocosms by 100% ($t = 1.84e^{16}$, $p < 0.001$).

4.5 Discussion

Although still understudied, a few studies have demonstrated that alterations to predator abundance can influence CO_2 fluxes of ecosystems through indirect effects on primary producers at the base of food webs (Schindler *et al.* 1997, Flanagan *et al.* 2006, Atwood *et al.* 2013). This study demonstrated that interactions between predators and predator functional identity can cause quantitative changes in the strength of trophic cascades and the transmission of indirect predator effects on CO_2 fluxes. Furthermore, our study reveals that behavioral adaptations that allow multiple predators to co-exist within an ecosystem can create non-additive predator effects on community properties and ecosystem function.

Results from our study demonstrated that the two top predators in the bromeliad ecosystem, diving beetles and damselfly larvae, were not functionally similar and elicited different responses in the bromeliad community and the ecosystem functioning of the bromeliad with respect to leaf litter decomposition and CO_2 emissions. These results suggest that the lack of redundancy in the top predator role places bromeliad ecosystems at high risk of loss or degradation to critical processes. The pronounced differences between the strength of trophic cascades created by damselfly larvae or diving beetles, and ultimately their effects on CO_2 emissions from bromeliad mesocosms, likely arose from striking differences in their overall consumption of detritivores. Diving beetles consumed ~72% less detritivore biomass than damselfly larvae did. This difference in consumption rates was in part likely due to greater food requirements of larval damselflies for somatic growth, maintenance and storage compared to the adult diving beetles, which only need to allocate food resources to maintenance and reproduction (Honek 1993, Sokolovska *et al.* 2000).

In addition to consumption rates, differing behavioral responses of the prey to the two different predator hunting modes displayed by damselfly larvae (sit-and-wait) and diving beetles (active hunter) in our experiment may have also attributed to the effect of predator identity on the strength of trophic cascades and ecosystem function (Otto *et al.* 2008, Schmitz 2008). Sit-and-wait predators have a continuous presence in a fixed

location that creates point source cues, which signals that the area is high risk to prey. In response to these predator cues, prey respond with antipredator behavioral changes that often reduce foraging efficiency (Werner and Anholt 1993, Peacor and Werner 2001). Thus, the cascading indirect effects of a sit-and-wait predator on ecosystem processes may be amplified by trait-mediated effects on prey (Schmitz 2008). In contrast to sit-and-wait predators, active hunters provided diffused predator cues throughout the entire habitat. As a result of diffused predator cues, prey can become less responsive to such predators, and trait-mediated indirect effects on prey resources and ecosystem processes can become dampened. Thus in our study, the indirect effects of sit-and-wait damselfly larvae on leaf litter decomposition and CO_2 emissions may have been amplified by eliciting greater trait-mediated effects on prey foraging behavior compared to active hunting diving beetles. Our results support previous findings that predator identity has important consequences for predator effects on ecosystems and that the ecosystem level effects of predator loss or replacement is species dependent (Chalcraft and Resetaritis 2003, Schmitz 2008).

Weakened trophic cascades in mesocosms containing both predators were likely caused by predator-predator interactions that reduced the effects of damselfly larvae on detritivore abundance. The dampening of damselfly larvae effects was due to predator-predator intimidation, culminating in the emigration of the damselfly from the mesocosm. This behavior by damselfly larvae was observed in nine of ten replicates. Retreat by damselfly larvae is a common strategy used by damselflies to avoid aggression and competition from both hetero-and conspecific species (Cobert 1980, Baker 1983, McPeek and Crowley 1987). However, no studies to date have shown that this behavior results in the complete emigration of a damselfly from the ecosystem. In our study, when predators co-occurred within a mesocosm, territorial behavior expressed by one or both predators diminished top-down effects of damselfly larvae on detritivores. Additionally, because diving beetles had only a minimal effect on the bromeliad community, indirect predator effects on levels of CO_2 flux from mesocosms containing both predators were completely eliminated. The low consumptive effect of adult diving beetles on the bromeliad community was likely due to the fact that many female dytiscids primarily use

bromeliads as nursery habitat for their larvae (Greenly 2001, Frank and Lounibos 2009). Although our system shows an example of an extreme case of antagonism between predator species, lethal and non-lethal interactions resulting in the reduction of prey consumption by one or more predators is common in nature (Hart 2002, Finke and Denno 2004, Finke and Denno 2005, Ives *et al.* 2005, Steffan and Snyder 2010). Our results provide experimental evidence that multiple predator effects can diminish the magnitude of top-down effects on CO_2 flux to the atmosphere from freshwater ecosystems.

Ecologists have made great strides in understanding how species interactions and species diversity influence ecosystem function (Cardinale *et al.* 2006, Cardinale *et al.* 2012). However, despite its importance for both conservation and global change biology, previous studies have only explored how the presence or absence of predators influences CO_2 fluxes of ecosystems (Schindler *et al.* 1997, Atwood *et al.* 2013). Our study demonstrates the need to investigate how species interactions at higher trophic levels influence top-down effects on carbon cycling. I showed that multiple predator effects on CO_2 flux from bromeliad mesocosms were driven purely by non-lethal antagonistic interactions between the two predators. Additionally, our results indicate that predator identity may have functional consequences on CO_2 flux from ecosystems. These findings have important implications for how humans respond to changes in predator abundance and diversity, as predator effects on carbon cycling may depend on the particular predator species removed or added to the ecosystem and their interactions with other predators. Although our study focused on a small-scale experimental ecosystem, it is nevertheless the first study to our knowledge to show that interactions between predators determined the overall effect of predators on CO_2 flux to the atmosphere. Without a more complete understanding of how predator effects influence carbon storage, we cannot predict how the introduction and loss of predators will influence the exchange of CO_2 and other greenhouse gases between ecosystems and the atmosphere.

Table 4.1 Summary statistics for the effects of a predatory damselfly or diving beetle (beetle) and the interaction term (damselfly:beetle) for total detritivore biomass, leaf litter decomposition, and CO2 emissions of experimental bromeliads. A significant interaction term suggests predators had non-additive effects on the response variable measured. P-values in bold text represent statistically significant factors

	$F_{(df)}$	p-value
Total detritivore biomass (mg)		
Damselfly	$F_{1,35} = 16.19$	**p < 0.001**
Beetle	$F_{1,35} = 0.74$	p = 0.397
Damselfly:Beetle	$F_{1,35} = 10.84$	**p = 0.002**
Detritus loss (g)		
Damselfly	$F_{1,35} = 22.5$	**p < 0.001**
Beetle	$F_{1,35} = 2.13$	p = 0.153
Damselfly:Beetle	$F_{1,35} = 11.62$	**p = 0.002**
CO_2 flux (g C d^{-1})		
Damselfly	$F_{1,31} = 11.34$	**p = 0.002**
Beetle	$F_{1,31} = 2.72$	p = 0.109
Damselfly:Beetle	$F_{1,31} = 7.6$	**p = 0.009**
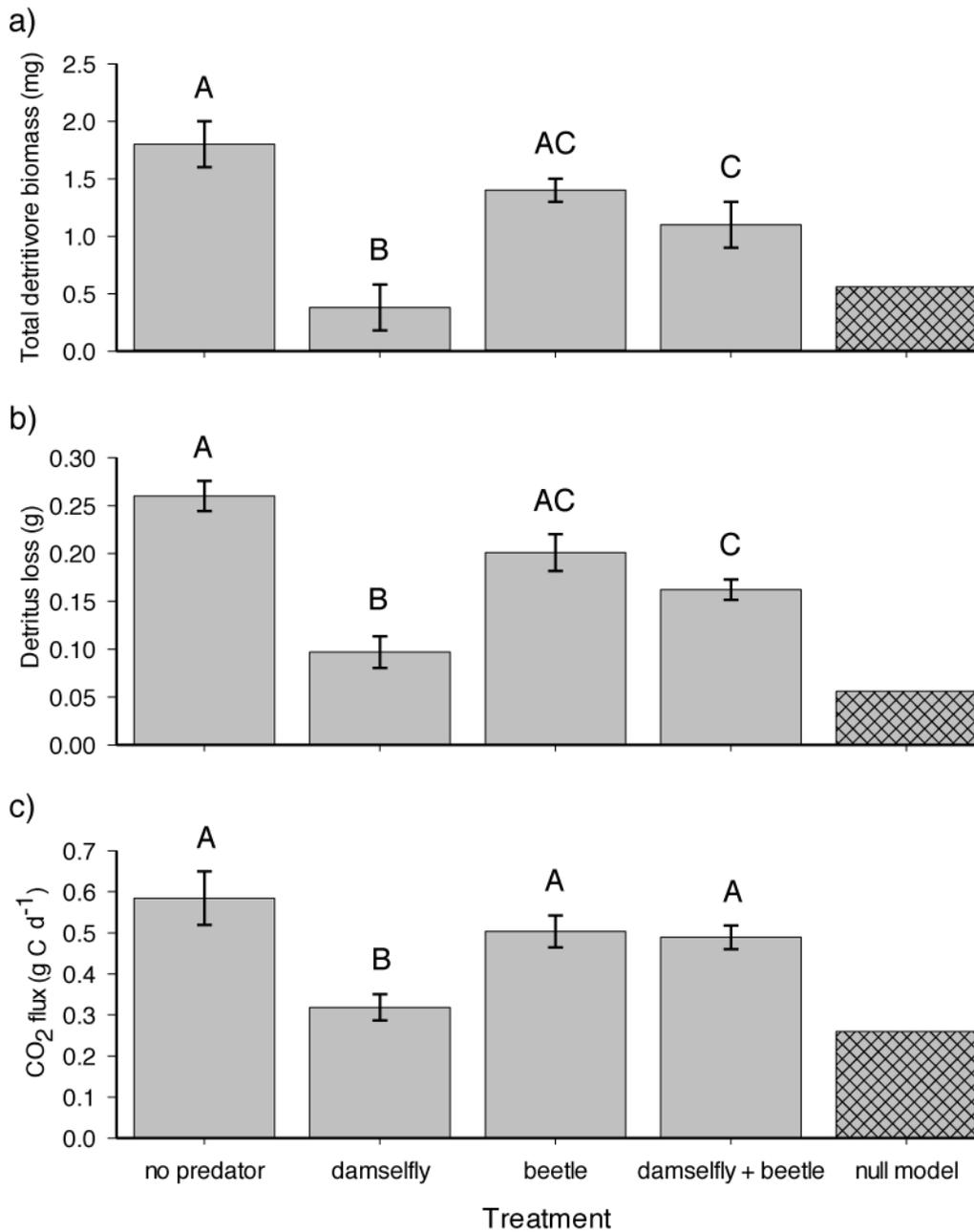
Figure 4.1 Individual and joint effects of predatory diving beetles and damselfly larvae on the magnitude of trophic cascades and CO_2 flux to the atmosphere from bromeliad mesocosms. Data are means (± s.e.). a) Predator effects on total detritivore biomass. b) Predator effects on detrital loss over 40 days. c) Predator effects on carbon dioxide flux to the atmosphere. Cross-hatched null model bars represent the predicted expectations for additivity under the multiplicative risk model. Differing letters above bars represent significant differences ($p > 0.05$).

CHAPTER 5: INDIVIDUAL AND INTERACTIVE EFFECTS OF WARMING, PREDATORS, AND EUTROPHICATION ON CO$_2$ FLUXES IN EXPERIMENTAL PONDS

5.1 Summary

Interactions among climate change, eutrophication and changes to predator abundance have the potential to alter the strength of trophic cascades and the influence of predator effects on carbon cycling within freshwaters. In this study I used a 2 x 2 x 2 factorial design, manipulating predator presence, water temperature, and nutrients to test their individual and interactive effects on consumer and primary producer biomass and CO$_2$ flux of experimental pond ecosystems. I show that warming altered top-down versus bottom-up control of consumers and primary producer biomass leading to changes in the direction and magnitude of CO$_2$ fluxes with the atmosphere. Warmer conditions generally increased top-down control of predators on the community and weakened nutrient effects on primary producers. In all cases, increased water temperatures relative to ambient temperatures reduced CO$_2$ sequestration in ponds, and in many cases switched ponds from being net sinks for atmospheric CO$_2$ to net sources. My results provide empirical evidence that increased water temperatures and nutrient additions alters top-down regulation of CO$_2$ fluxes of freshwater ecosystems, and has the potential to alter the role freshwater ecosystems will play in the future global carbon cycle.

5.2 Introduction

Until relatively recently, fluxes and storage of carbon were thought to occur between only three main pools, the atmosphere and the terrestrial and oceanic biomes. Freshwater ecosystems were seen as only passive conduits of terrestrial carbon to the ocean (IPCC 2007), despite the fact that they store or mineralize over half (~ 1.8 Pg C yr^{-1}) of their annual carbon inputs, emitting similar levels of CO$_2$ gas (1.65 Pg C yr^{-1}) as anthropogenic land-use change (Cole *et al.* 2007, Battin *et al.* 2009). As a result of this omission, predictions on how global change (e.g., warming, eutrophication, and changes to predator abundance) will influence freshwater CO$_2$ fluxes and its ramifications on the

global carbon cycle are lacking and far behind those for terrestrial and marine ecosystems.

The rate at which carbon is cycled and the direction of CO_2 fluxes between freshwater ecosystems and the atmosphere are largely dependent on food web structure (Schindler *et al.* 1997, Atwood *et al.* 2013). Primary producers and consumer efficiency in processing carbon can influence internal carbon cycling and fluxes of CO_2 between ecosystems by altering the ratio of respiration (R) to production (P) (Schindler *et al.* 1997, Lopez-Urrutia 2006). Freshwater ecosystems that generate more CO_2 than can be fixed by primary production (R > P) act as net sources of CO_2 to the atmosphere. Conversely, freshwater ecosystems that fix CO_2 through primary production at greater rates than it can be generated from respiration (R < P) are net sinks for atmospheric CO_2. Interactions among organisms that make up the food web structure, however, can be modified by global change (Greig *et al.* 2012, Kratina *et al.* 2012, Shurin *et al.* 2012), suggesting that feedback loops between freshwater food web structure, greenhouse gas dynamics, and global change are likely occurring.

Freshwater ecosystems are highly susceptible to anthropogenic stressors, such as alterations to predator abundance, eutrophication, and climate warming. The removal or addition of predators from freshwater food chains can create trophic cascades that can have striking effects on plants at the base of the food web (Brooks and Dodson 1965, Ripple and Beschta 2004). Recent studies have shown that alterations to predator abundance can create drastic changes to CO_2 fluxes of aquatic and marine ecosystems (Wilmers *et al.* 2012, Atwood *et al.* 2013). Additionally, predator removal in concert with eutrophication has been shown to intensify trophic cascades leading to even greater changes in the CO_2 fluxes of freshwater ecosystems (Schindler *et al.* 1997, Flanagan *et al.* 2006). In some cases, the combined effects of eutrophication and alterations to predator abundance caused lakes to switch from net sources of CO_2 to the atmosphere to net sinks for CO_2 (Schindler *et al.* 1997). Warming of aquatic ecosystems has been shown to both enhance trophic cascades (Kratina *et al.* 2012, Shurin *et al.* 2012) and increase respiration at both the individual and ecosystem levels (Allen *et al.* 2005, Yvon-Durocher *et al.* 2010). However, the cumulative effects of warming, eutrophication, and

alterations to predator abundance on CO_2 fluxes of ecosystems has yet to be explored. An understanding of their combined effects is important for making predictions about the future role freshwater ecosystems will play in the global carbon cycle as ecosystems often experience multiple stressors, and we are currently committed to a 3-5˚ C increase in global temperatures over the century (Houghton 2001).

Predicting the joint effects of multiple stressors on CO_2 fluxes of freshwater ecosystems in future climate scenarios can be challenging. Predictions can either come from long-term studies or manipulations of natural ecosystems, modeling, or manipulations to experimental ecosystems. Although long-term studies or manipulations of natural ecosystems provide more real world answers, they are expensive, often under-replicated, and in terms of understanding the influence of future climate change scenarios are often not possible. Models can provide relatively quick and inexpensive predictions, but currently we lack enough information from independent studies to adequately parameterize models. Finally, manipulations to freshwater mesocosms often over-simplify the complexity of natural ecosystems. However, mesocosms enable controlled manipulations of multiple stressors in identically replicated environments that support naturally complex food webs. Moreover, identical physical structure of ecosystems is imperative for isolating the effects of stressors on CO_2 flux, as physical characteristics such as wind speed, depth, surface area, and pressure can drastically influence the gas exchange velocities of ecosystems. Here, I investigated the individual and interactive effects of warming, nutrients, and predator loss on trophic cascades and CO_2 fluxes of experimental, freshwater ponds. Our study provides the first experimental evidence of how bottom-up and top-down influences on CO_2 fluxes of freshwater ecosystems will be altered by climate change.

5.3 Methods

5.3.1 Experimental design

Using a 2 x 2 x 2 factorial design I manipulated temperature ($+ 3 ˚C$), nutrients (nitrogen and phosphorus), and presence of predators (*Gasterosteus aculeatus*) to test

their interactive and individual effects on consumer biomass, primary producer biomass, and CO_2 flux with the atmosphere.   Experiments were performed in 40, open-air, experimental ponds (surface area = 2.16 m^2) located at the University of British Columbia, Vancouver, Canada. Experimental ponds were filled with 1,136 L of municipal water and left to de-chlorinate for one week prior to the assembly of food webs.

Experimental food webs were assembled by inoculating ponds with ~20 zooplankton taxa, ~ 50 phytoplankton taxa, and microbes from nearby ponds (Greig *et al.* 2012, Kratina *et al.* 2012). Zooplankton and phytoplankton were collected from nearby ponds using 64 µm-mesh conical tow nets. Additionally, 1 L of sediment containing eggs, spores, and microbes was added from nearby ponds to aid in community assembly. Aquatic insects were colonized in ponds using natural dispersal. Food webs were left for three weeks before the addition of experimental treatments.

Experimental ponds were randomly assigned one of eight treatments and replicated five times. Temperature manipulations, hereafter referred to as warming treatments, were achieved by warming ponds to 3.04˚ ± 0.05˚ C (mean ± s.e.) above ambient pond temperatures using 300 W submersible water heaters (Hagen, Montréal, Québec, Canada). A 3˚ C increase in ambient temperature falls within the range of predicted temperature projections for Northern Hemisphere temperate zones over the next 100 years (IPCC 2007). Temperatures of all ponds were monitored using HOBO Pendant data loggers (Onset Computer Corp. Bourne, Massachusetts, USA) in 30 min intervals. I manipulated nutrients through monthly additions of 264 µg of nitrogen/L (as NaNO$_3$) and 27 µg of phosphorus/L (as (KH$_2$PO$_4$), resulting in a N:P molar ratio of 22 (Kratina *et al.* 2012). Five *G. aculeatus* (three-spined stickleback) of 52.4 ± 0.05 mm in standard body length were added per tank as predator treatments. In the event of death, stickleback were removed from the tank and replaced with a similar-sized fish.

5.3.2 Sampling

Consumers, primary producers, and *in situ* CO_2 concentrations were measured on two dates during the 2010 growing season (May and October), starting 336 d after the implementation of experimental treatments. Phytoplankton biomass was measured with *in vivo* fluorometry (Trilogy, Turner Designs, Sunnyvale, California, USA) using the concentration of chlorophyll *a* in the water column. Periphyton biomass was determined fluorometrically from algal growth collected from 25 cm^2 unglazed tiles (Thompson and Shurin 2009). Zooplankton were collected using 10 L, depth-integrated zooplankton samples, filtered through a 64 µm-mesh sieve. Phytoplankton biomass and periphyton biomass were added together to get total primary producer biomass. Spatial variation of zooplankton biomass within the ponds was minimized by pooling samples from multiple depths and locations within the tank (Kratina *et al.* 2012). Zooplankton were identified (usually to genus), counted, and measured under a 10x magnification. Biomass of zooplankton was estimated using length-mass regressions (Kratina *et al.* 2012). Benthic macro-invertebrates were sampled by collecting organisms from the bottom and walls of the tank and then summed together. Macro-invertebrates on the bottom of the ponds were sampled with standard sweeps within a 0.02 m^2 cylinder, in two areas of the pond. Macro-invertebrates inhabiting the walls of the ponds were collected by sweeping two locations of the wall from the bottom of the pond to the water's surface using a 12 cm, 0.5 mm-mesh net. Macro-invertebrates were identified and their biomasses calculated using length-mass regressions (Greig *et al.* 2012). Biomass of both benthic and pelagic consumers (not including *G. aculeatus*) were combined to estimate total consumer biomass, hereafter referred to as consumer biomass.

Biomass was converted to g C m^{-2} for all organism groups by dividing grams of carbon per tank by the tank surface area. Grams of carbon were estimated by assuming a carbon biomass to chlorophyll biomass ratio of 1:40 for phytoplankton (Cole *et al.* 2002, Kritzberg *et al.* 2004), 1:50 for periphyton (Azim 2010), and an average carbon content of 48 % of dry mass for zooplankton (Andersen and Hessen 1991) and 51.8 % of ash-free dry mass for benthic invertebrates (Salonen *et al.* 1976).

5.3.3 CO_2 collection and flux calculations

Water samples for dissolved CO_2 concentrations were collected at dawn using 50-ml Pressure-Lok® syringes (VICI Precision Sampling Corp., Baton Rouge, LA, USA). Dissolved CO_2 gases were extracted from water samples using headspace equilibrium analysis, stored in gas tight vacutainers (Labco Limited High, Wycombe, UK.), and transported to the Department of Forest and Conservation Sciences, at the University of British Columbia for analysis. Gas samples were analyzed within 24 h using a 5890 Series II gas chromatograph. Collections for CO_2 from sample water were done following similar procedures to those described in Hope *et al.* (1995) and total CO_2-C concentrations (mg C l^{-1}) in the water were calculated using equations from Appendix A.

CO_2 flux (g C m^{-2} d^{-1}) to the atmosphere was calculated using the following equation:

$$CO_{2flux} = (pCO_{2water} - pCO_{2air})*k$$

Here, pCO_{2water} is the partial pressure of CO_2 measured in the water, and pCO_{2air} is the partial pressure of CO_2 in the overlying atmosphere (390 ppm), k is the CO_2 exchange velocity coefficient ($k = 0.63$ m d^{-1}). Experimental pond k values were estimated using literature values for lentic ecosystems experiencing low wind speeds (Cole and Caraco 1998).

5.3.4 Statistical analyses

Interactive and individual effects of warming, nutrients, and predators on total primary producer biomass, total consumer biomass (not including *G. aculeatus*), and CO_2 flux were tested using linear mixed-effect models (LME) in R 2.12.1 (R Development Core Team, 2010). A significant interaction between treatments suggests non-additive effects between treatments on the response variable. Individual ponds and date were treated as random factors. Date was not included as a fixed factor because sampling only occurred during a single growing season. Total primary producer biomass and total consumer biomass were log_e transformed to meet test assumptions for normality and equal variance. Standard errors represented in the graphs for each of the fixed effects

within the model were approximated using the "predictSE.lme()" function in the "AICcmodavg" package. This function calculates predicted values based on fixed effects, and standard errors are approximated from the linear mixed effects model using the delta method (Oehlert 1992). Calculating standard errors from the LME model has the advantage that error assigned to random effects has been accounted for in the estimates. A linear regression was used to determine how much of the variance in CO_2 flux of ponds could be explained by total primary producer biomass. For this analysis each sample represents an individual data point.

5.4 Results

Treatment effects of nutrients, predators, and warming on consumer biomass had only independent effects (Fig. 5.1a and b). The addition of only predators to ponds increased predation leading to a 70% ± 2% (mean ± s.e.) reduction in consumer biomass ($F_{1,32} = 28.24$, $p < 0.001$, Fig. 5.1a). Conversely, the addition of only nutrients to ponds increased consumer biomass by 50% ± 5% ($F_{1,32} = 47.21$, $p < 0.001$, Fig. 5.1b). There were no significant interactive effects between predators and nutrients, predators and warming, nutrients and warming, or a three-way interaction between warming, nutrients, and predation (Table 5.1, Fig. 5.1a and b). Additionally, warming had no significant effects on consumer biomass (Table 5.1).

Treatment effects on primary producer biomass had both interactive and independent effects (Fig. 5.1c and d). Primary producer biomass in ponds was significantly influenced by a three-way interaction between warming, nutrients, and predators ($F_{1,32} = 10.58$, $p < 0.001$, Fig. 5.1b). The combined effects of warming, nutrients, and predators increased primary producer biomass by 163% ± 10% compared to control treatments (Table 5.1). Treatments containing a combination of warming and nutrients also had a slight increase in primary producer biomass by ~97% ± 7% compared to control treatments (Table 5.1, Fig. 5.1d). However, primary producer biomass was reduced by ~45% ± 13% with the addition of both warming and nutrients compared to treatments containing only nutrients. Individually, the addition of fish or nutrients resulted in a significant increase in primary producer biomass by ~260% ± 11% and

~32% $\pm$ 5%, respectively (Table 5.1c). Ponds receiving only warming, however, had a significant reduction in primary producer biomass by ~37% $\pm$ 3% (Table 5.1, Fig. 5.1d).

On average control, warming, and warming + predator treatments were net sources of CO_2 (Fig. 1e and f). Warming + nutrient treatments were at equilibrium with the atmosphere (Fig. 5.1f). Finally, nutrient only, predator only, nutrients + predators, and nutrients + predators + warming were all on average net sinks of atmospheric CO_2 (Fig. 5.1e and f). Interactive and independent effects of warming, nutrients, and predators influenced both the direction and magnitude of CO_2 fluxes of ponds (Table 5.1, Fig. 5.1e and f). A significant three-way interaction was shown between warming, nutrients, and predators. ($F_{1,32}$ = 6.57, p = 0.015) (Fig. 5.1f). In general, the addition of warming to any other treatment (nutrients or predators) reduced the amount of carbon sequestered by the ponds, and in many cases it switched ponds from being net sinks of CO_2 to net sources of CO_2 (Fig. 5.1f). However, when warming was added to treatments containing both predators and nutrients, CO_2 sequestration was increased relative to control treatments (Fig. 5.1e and 5.1f). Independently, predators switched ponds from a source of CO_2, as seen in control treatments, to a sink for CO_2, sequestering ~1.51 $\pm$ 0.66 g C m^{-2} d^{-1} (Table 5.1, Fig. 5.1e). However, when warming was added to predator treatments a significant interaction occurred. The effect of predators on CO_2 flux was greatly reduced and ponds switched to potential net sources of CO_2, emitting ~ 0.12 $\pm$ 0.36 g C m^{-2} d^{-1} (Fig. 5.1f). The addition of only nutrients shifted ponds from net sources of CO_2 to sinks for CO_2, sequestering ~ 1.30 $\pm$ 0.87 g C m^{-2} d^{-1} (Table 5.1, Fig. 5.1e). However, when warming was added to nutrient treatments a significant interaction occurred and sequestration was greatly reduced and ponds largely came to equilibrium with the atmosphere (Table 5.1, Fig. 5.1e). Overall, warming had a significant effect on CO_2 flux of ponds and substantially altered the relationships between nutrients, predators and CO_2 (Table 5.1). In general however, the individual effect of warming on CO_2 did not appear to influence the magnitude or direction of the flux compared to control ponds (Fig. 5.1e and f).

Linear regression analyses showed that differences in the relationship between carbon dioxide flux and $\log_e$ primary production depend on pond temperature. In ambient temperature ponds I found a significant negative relationship between CO_2 flux

and $\log_e$ primary production ($F_{1,38} = 20.31$, $p < 0.001$, $R^2_{adj} = 0.33$; Fig. 5.2a). In general, ponds with low primary producer biomass were either net sources of CO_2 to the atmosphere or relatively weak sinks for atmospheric CO_2. Regression analysis of warmed ponds showed only a marginally significant relationship between carbon dioxide flux and $\log_e$ primary production ($F_{1,38} = 3.92$, $p = 0.055$, $R^2_{adj} = 0.069$; Fig. 5.2b).

5.5 Discussion

Recent studies have shown the potential effects of future climate warming on the top-down and bottom-up forces structuring food webs (Barton *et al.* 2009, Binzer *et al.* 2012, Shurin *et al.* 2012). Consistent with these studies, I showed that warming altered the strength of predator and nutrient effects on experimental pond food webs. However, our study is the first to show that the effects of warming on top-down and bottom-up forces structuring food webs extends past the food web and influences CO_2 fluxes of the ecosystem. Here, interactive effects between warming, nutrient additions, and predator abundance not only influenced the magnitude of CO_2 fluxes, but also the direction. In some cases the interactive effects were so strong they altered whether the pond acted as a sink or a source for atmospheric CO_2 or a source of CO_2.

In ambient temperature, three-tier pond food chains, predators did not create trophic cascades, but did have an indirect influence on CO_2 fluxes of the ponds. The presence of predatory fish reduced consumer biomass in ponds; however, this did not lead to increased primary producer biomass. Primary producer biomass in predator only treatments was similar to that of control ponds. This suggests that primary producer biomass in both control treatments and predator-only treatments were not controlled by top predators, and were determined instead by bottom-up forces (nutrient limitation). Overall, the negative effect of predators on community respiration and no net change in primary production reduced *in situ* CO_2 concentrations in the ponds. In order to compensate for reduced CO_2 concentrations in the water, ponds increased sequestration of atmospheric CO_2. Here, the presence of predators shifted ponds from being primarily net sources of CO_2 to the atmosphere to net sinks of CO_2. The reduction of CO_2 emissions caused by effects of predators on community structure seen in our study is

consistent with results from other odd-numbered food chain studies (Schindler *et al.* 1997, Atwood *et al.* 2013).

The addition of warming to predator treatments enhanced trophic cascades, but had counter-active effects on CO_2 flux. Predators in warmed treatments negatively influenced consumer biomass at similar magnitudes to predators in non-warmed treatments. However, predator effects under warming also relaxed grazing on primary producers, increasing primary producer biomass. This suggests that under warmer conditions ponds with no nutrient additions switch from being controlled by both bottom-up and top-down processes, to largely being controlled by top-down forces. Our results support current theory and empirical evidence which showed that warming enhances trophic cascades (Barton *et al.* 2009, O'Connor *et al.* 2009, Shurin *et al.* 2012).

An increase in trophic cascades strength under warmer conditions should have resulted in an increase in CO_2 sequestration (Schindler *et al.* 1997, Atwood *et al.* 2013). However, our results showed that there was no net change in CO_2 flux when predatory fish were added to warmed ponds. This was likely due to physiological changes in both the predators and zooplankton under warmer conditions. Higher temperatures would have led to higher respiration rates in both predatory fish and consumers (Gillooly *et al.* 2001, Brown *et al.* 2004). Empirical evidence of the Metabolic Theory of Ecology shows a stronger response in respiration than photosynthetic rates to increased temperatures, resulting in a reduction in the production to respiration ratio with increased temperatures (Harris *et al.* 2006, Yvon-Durocher *et al.* 2010). Yvon-Durocher *et al.* (2010) found that an increase to ambient temperatures similar to this study lead to a faster increase in ecosystem respiration compared to primary production, resulting in a 13% reduction in CO_2 sequestration. In combination with higher respiration rates from higher temperature, respiration rates in zooplankton could have been further increased due to the fear of predation (Beckerman *et al.* 2007, Hawlena and Schmitz 2010). Because the direction of the effects of trophic cascades on CO_2 is dependent on whether food chains are even- or odd-numbered (Schindler *et al.* 1997), these results suggest that the effect of interactions between predators and warming on CO_2 will vary with food chain length. The strength of

trophic cascades in warmer conditions would have to be greater than physiological changes to respiration in predators and consumers in order to reduce CO_2 emissions or enhance CO_2 sequestration in odd-numbered food chains. Conversely, in even-numbered food chains warming effects on respiration would augment effects of trophic cascades on CO_2, further increasing CO_2 emissions in source ecosystems or reducing CO_2 sequestration in sink ecosystems.

The addition of nutrients to ambient temperature ponds caused bottom-up forces to dominate in ponds that lacked predators and top-down forces to dominate in ponds with predators. In both cases, nutrients increased CO_2 sequestration of ponds regardless of the presence of predators. Nutrient additions in predator-free ponds increased both primary producer and consumer biomasses, suggesting that consumer biomass was controlled by primary producers, and primary producer biomass was limited by nutrients. Despite that an increase in the consumer biomass would have led to greater community respiration, the five times higher increase in primary production due to nutrient additions switched ponds from being net sources of CO_2 to net sinks for atmospheric CO_2. When nutrients were added to ponds containing predators, trophic cascades were enhanced and the ecosystem switched from being bottom-up controlled to top-down controlled. The reduction in consumer biomass and the increase in primary producer biomass from trophic cascades increased CO_2 sequestration of the ponds compared to controls. The enhancement of trophic cascades and the overall increase in CO_2 sequestration in response to nutrient additions was consistent with results from other studies (Schindler *et al.* 1997, Flanagan *et al.* 2006).

The addition of warming to nutrient-enriched ponds weakened bottom-up control in ponds without predators. The overall effect of nutrients in warmed ponds was an increase in both consumer and primary producer biomass, despite that nutrient effects on primary producer biomass were somewhat reduced in warmed ponds compared to ambient temperature ones. Compared to ambient temperature ponds, the increase in consumers in warmed ponds was far greater than the increase in primary producers. This suggests that in the absence of predatory fish, nutrients and warming may have increased top-down control of primary producers by consumers (Kratina *et al.* 2012). This increase

in consumer biomass and community respiration relative to primary producer biomass in nutrient + warming treatments was likely responsible for no net change in CO_2 emissions, despite an increase in primary production.

Warming weakened top-down control in nutrient-enriched ponds with predators, which lead to weaker than expected changes in CO_2 sequestration of ponds. In ambient temperature ponds, nutrients enhanced trophic cascades from predatory fish. However, in warmed ponds nutrients had no effect on trophic cascade strength. Although predatory fish still decreased consumer biomass, this did not reflect an increase in primary producer biomass. Here, primary producer biomass remained at similar levels to nutrient + warming treatments, suggesting that any increase in primary producer biomass compared to warming only treatments was the result of nutrients and not relaxation of grazing pressure from top predators. The lack of response in primary producer biomass, despite a reduction in consumer biomass, may have been due to the adverse effects of warming on primary producers. Yvon-Durocher *et al.* (2010) found warming negatively affected phytoplankton biomass despite no net change in zooplankton biomass. Indeed I saw that overall, primary producer biomass was always lower in warmed ponds compared to non-warmed ponds. Finally, the significant decrease in consumer biomass and no change in primary producer biomass resulted in ponds switching from net source of CO_2 to net sinks for CO_2. However, because of the adverse effects of warming on primary producers the level at which ponds sequestered CO_2 in warmed, nutrient enriched ponds containing predators was far lower than their un-warmed counterparts.

Alterations to predator abundance and eutrophication represent two of the most pervasive effects humans have had on aquatic ecosystems. Recent studies have already shown the potential adverse effects these anthropogenic forces can have on CO_2 fluxes of freshwater ecosystems (Schindler *et al.* 1997, Atwood *et al.* 2013). However, these studies have not examined how the individual and joint effects of these perturbations on CO_2 fluxes are likely to change in response to climate warming. Currently, future climate projections show a 3-5˚ C increase in global temperatures. Our study represents the first empirical evidence showing that a 3˚ C increase in temperature will alter the relationships between predators, nutrients, and CO_2 fluxes of freshwater ecosystems. Overall, our

study shows that warming generally reduced CO_2 sequestration of ponds, often switching them from sinks to sources of atmospheric CO_2. However, the effects of warming on CO_2 fluxes in our study were dependent on both nutrients and the presence of predators, and their subsequent effects on primary producers and consumers. Warming enhanced predator effects on prey in pond communities that were exposed to warming + predators or warming + predators + nutrients. However, warming reduced the effects of nutrients on primary producers. In ponds simultaneously exposed to warming, nutrients and predators, the reduced effects of nutrients on primary producers and the enhanced effects of predatory fish on consumers caused by warming resulted in a decoupling of the food chain. Here, consumer biomass was controlled by predation and primary producer biomass was controlled by nutrients. Moreover, results from our regression analysis suggest that under current temperature conditions primary producer biomass is a relatively good ecological predictor of CO_2 flux. However, under future temperature conditions there was no relationship between primary producer biomass and CO_2 flux. These results in conjunction with previous studies suggest that other processes, such as the metabolic rates of individuals, may be more influential than primary production on CO_2 fluxes of ecosystems in a warmer world (Yvon-Durocher *et al.* 2010).

Decades of ecological research has attempted to demystify the effects of predators and nutrients on communities and the functioning of ecosystems (Shurin *et al.* 2006, Frank *et al.* 2007, Gruner *et al.* 2008). Climate change, however, stands to alter the nature and strength of nutrient and predator effects on ecological communities (Harrington *et al.* 1999, Emmerson *et al.* 2005, Tylianakis *et al.* 2008), threatening to make our current knowledge obsolete. Our study provides the first experimental evidence that global warming, in conjunction with eutrophication and alterations to predator abundance, has the potential to influence community dynamics, altering the role of freshwater ecosystems in future regional and global carbon cycles. Moreover, our results suggest that the effects of warming on freshwater communities may lead to an overall reduction in CO_2 sequestration or an increase in CO_2 emissions of freshwater ecosystems. These changes to carbon cycling in freshwater ecosystems have the potential to create positive

feedbacks between changes in freshwater food web structure, greenhouse gas dynamics and global change.

Table 5.1 Summary of linear models for the individual and interactive effects of warming (W), nutrient additions (N), and predator additions (P) on consumer biomass, primary producer biomass and CO_2 flux of experimental ponds. P-values in bold text represent statistically significant factors.

	Consumer biomass		Primary producer biomass		CO_2 flux	
	$F_{1, 32}$	p	$F_{1, 32}$	p	$F_{1, 32}$	p
N	47.206	**< 0.001**	132.154	**< 0.001**	33.947	**< 0.001**
P	28.238	**< 0.001**	25.318	**< 0.001**	39.119	**< 0.001**
W	3.108	0.875	28.996	**< 0.001**	67.133	**< 0.001**
W:N	0.090	0.766	16.746	**< 0.001**	5.138	**0.030**
N:P	1.621	0.212	3.391	0.075	0.236	0.630
W:P	0.034	0.855	2.114	0.156	11.267	**0.002**
W:P:N	0.064	0.802	10.579	**0.003**	6.573	**0.015**
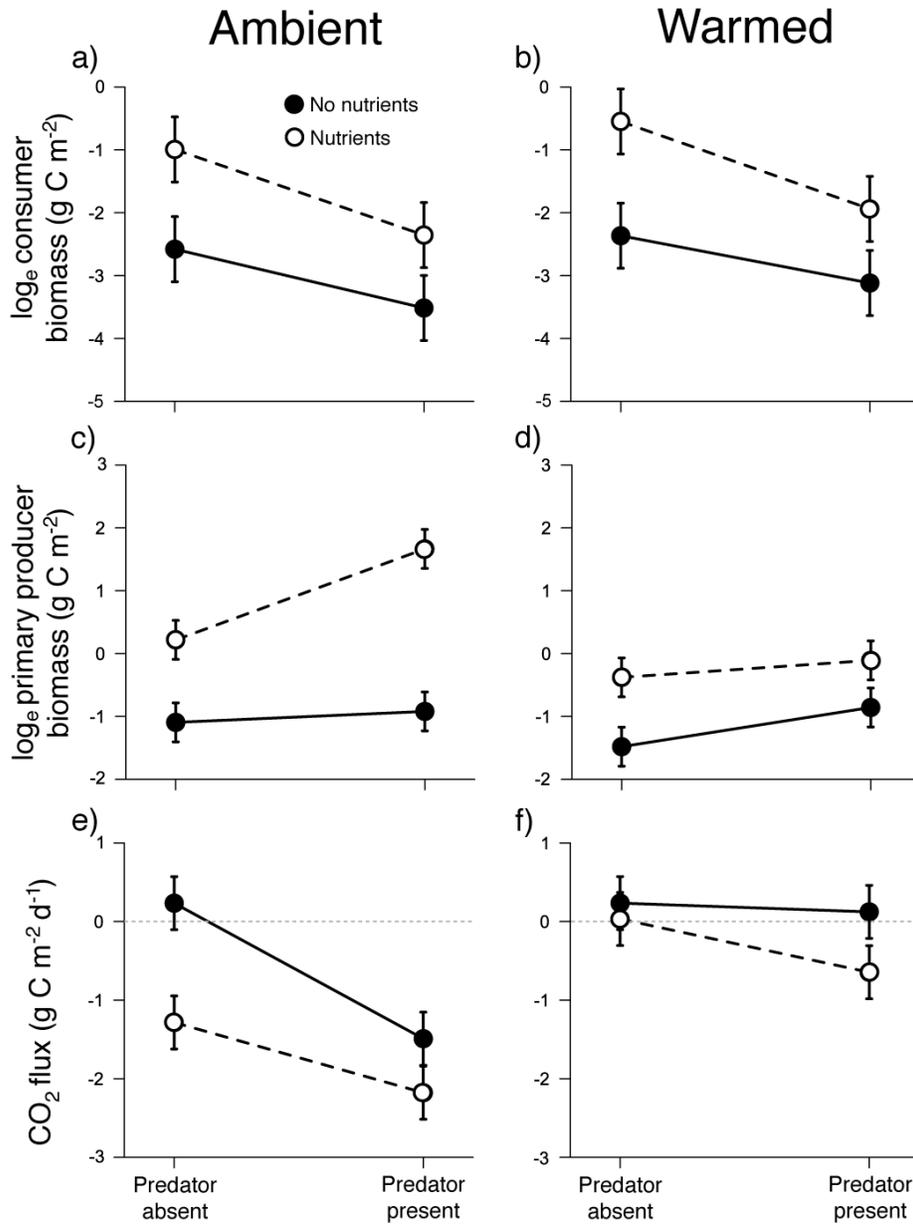
Figure 5.1 Interaction plots of the effects of warming, nutrient additions, and predator additions on the mean ± 95% C. I. of the fixed effects for community dynamics and CO_2 flux of experimental ponds. Individual and interactive effects of nutrients and predators on consumer biomass (a), primary producer biomass (c), and CO_2 flux (e) of ponds under ambient water temperature. Individual and interactive effects of nutrients and predators on consumer biomass (b), primary producer biomass (d), and CO_2 flux (f) of ponds under a 3˚ C increase in ambient water temperatures. The dotted line on graphs e and f represent where ponds are at equilibrium with the atmosphere, means below the line are net sinks for atmospheric CO_2 and data points above the line are net source.
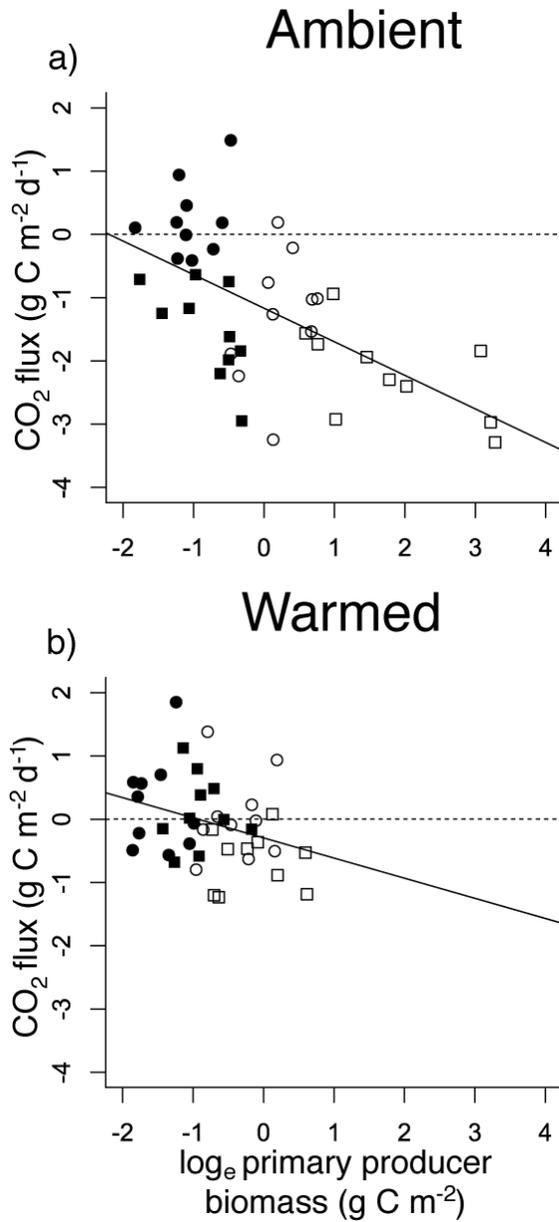
Figure 5.2 Relationship between primary producer biomass and CO_2 flux of experimental ponds under ambient temperature and warming. a) Primary producer biomass explained ~33 % of the variability in CO_2 flux of ambient temperature. b) No significant relationship was found between primary producer biomass and CO_2 flux of ponds exposed to a 3° C increase in ambient water temperatures. Symbols and colors show how different treatment effects clustered. Open symbols represent treatments exposed to nutrients and square symbols represent treatments where predators were present. The dotted line represents where ponds are at equilibrium with the atmosphere, ponds below the line are net sinks for atmospheric CO_2 and ponds above the line are net source.

CHAPTER 6: CONCLUSIONS: SYNTHESIS, LIMITATIONS, AND IMPLICATIONS FOR CONSERVATION

6.1 Overview

Due to accelerating rates of the species loss from Earth's ecosystems, this thesis sought to understand how predators and other trophic levels influenced the CO_2 fluxes of freshwater ecosystems. In Chapter 2 I tested which trophic level (predator, grazer, or detritivore) had the greatest effect on the CO_2 fluxes of experimental stream ecosystems. Chapter 2 showed that in my experimental stream channels, predators had the greatest effect on CO_2 emissions; I therefore focused the remainder of my thesis on understanding the generality of predator effects. The remaining data chapters were designed to further the understanding of the ubiquitous nature of predator effects in other freshwater systems, and specifically addressed how biotic and abiotic factors influenced predator effects on CO_2 emissions. In Chapter 3 I investigated predator effects on CO_2 fluxes across three different experimental freshwater ecosystems (streams, ponds, and bromeliads). In Chapter 4 I investigated how predator identity and interspecific competition between predators influences CO_2 emissions of experimental bromeliad ecosystems. Finally, in Chapter 5 I investigated how the individual and interactive effects of eutrophication, climate warming, and predator loss influenced CO_2 fluxes of experimental pond ecosystems. For all data chapters I provided a mechanistic understanding of how predators influenced CO_2 fluxes by testing their effects on total consumer biomass and plant biomass.

6.2 Prevalence of predator effects on CO_2 fluxes in freshwater ecosystems

One of the major goals of this thesis was to determine in which type of aquatic ecosystems changes to predator abundance is likely to influence the carbon exchange between that ecosystem and the atmosphere. The net carbon balance of an ecosystem is influenced by both GPP and ER (Woodwell and Whittaker 1968, Woodwell 1995, Enquist *et al.* 2003, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al.* 2010); this suggests that there are two pathways by which predators can influence the net carbon balance. However, aquatic ecosystems vary in their reliance on *in situ* primary

production (Fisher and Likens 1973, Richardson 1991, Webster and Meyer 1997a and b, Wallace *et al.* 1999, Webster *et al.* 1999, Thorp and Delong 2002, Oquist *et al.* 2009, Richardson *et al.* 2010), from ecosystems for which food webs are largely fueled by *in situ* primary production (e.g. some lakes and ponds) to ecosystems that solely rely on allochthonous organic matter from neighboring ecosystems (e.g., bromeliads). This suggests that the influence of GPP and ER on the net carbon balance of aquatic ecosystems varies, and predators must exert top-down effects on multiple ecosystem processes in order to affect the CO_2 fluxes of an ecosystem. In Chapter 3, I demonstrated that the absence of predators in odd-numbered food chains increased CO_2 emission, and that this effect was consistent across different aquatic ecosystem types. However, because my study lacked replication in ecosystem type, I could not determine statistically whether the magnitude of predator effects on CO_2 fluxes varied across the different aquatic ecosystems. I would hypothesize that because trophic cascade strength differs among aquatic ecosystems types (Brett and Goldman 1996, Shurin *et al.* at 2002, Chase 2003), and trophic cascades are an integral part of the ability of predators to influence CO_2 fluxes, we should see that the magnitude of predator effects on CO_2 fluxes would also differ across aquatic ecosystems. Thus, future studies should look at the effects of predator loss on carbon exchange between a plethora of aquatic ecosystems and the atmosphere, and eventually how the magnitude of these effects vary across ecosystem type.

6.3 The relationship between predator loss and CO_2 fluxes of ecosystems

Studies investigating the effects of species loss on ecosystem processes and function are not new in ecology. Several experimental studies, meta-analyses, and reviews have documented the relationships between biodiversity and ecosystem function (B-EF). Understanding the shape of B-EF relationships provides the opportunity to make predictions about how the loss of species will influence a specific ecosystem process, function, or service. Many studies suggest that biodiversity in some way scales positively with ecosystem function (Hooper *et al.* 2005, Cardinale *et al.* 2006, Cardinale *et al.* 2012). For aquatic ecosystems, most studies suggest either a redundancy or idiosyncratic B-EF relationship (Cardinale *et al.* 2006, Woodward 2009). A redundancy

relationship (a.k.a. Rivet Hypothesis) predicts that several species in an ecosystem are functionally redundant, and only when a guild of functionally redundant species is lost will there be implications for ecosystem function (Schlapfer and Schmid 1999). Conversely, an idiosyncratic relationship predicts that the B-EF relationship is context dependent and is influenced by both extrinsic and intrinsic factors (Schlapfer and Schmid 1999). There is less evidence supporting linear B-EF relationships in aquatic ecosystems (Cardinale *et al.* 2006, Woodward 2009) where process rates decline linearly with species loss. However, the shape of the relationship is likely to be dependent on the ecosystem functions being examined (Bengtsson 1998, Schwartz *et al.* 2000). Although this thesis generally focused on changes to the abundance of a single predator species (but see Chapter 4) results from these studies can still help form predictions about the shape of the relationship between predator species loss and CO_2 fluxes of ecosystems. Thus, results from this thesis enhance our ability to predict how predator loss will influence carbon exchange between ecosystems and the atmosphere, and helps identify future research directions.

Fundamental to the ability of predators to significantly influence CO_2 fluxes of their ecosystems is their ability to create trophic cascades. Among other requirements, the lack of functionally similar species is required in order for a predator to create a trophic cascade. There are several lines of evidence that suggest that many ecosystems may lack functionally redundant top predators. Evolutionarily, predators generally have lower speciation rates than other trophic levels because of longer generation times (Duffy 2002, Duffy 2003). Observations of well-studied ecosystems generally support the prediction that ecosystems tend to have fewer species in higher trophic levels (especially top predator levels) than lower trophic levels. Thus, species richness should be lowest at higher trophic levels, resulting in greater diversity effects on ecosystem function (Tilman *et al.* 1997, Schwartz *et al.* 2000). In Chapter 2, I demonstrated that my experimental stream communities were able to compensate for the loss of a functionally dominant detritivore or grazer, but not for the loss of a functionally dominant predator. Here, alterations to predator abundance created trophic cascades that propagated down to influence the magnitude of CO_2 emissions from the ecosystem. Conversely, alterations to

the functionally dominant grazer or detritivore abundances had no effect on food web structure, GPP or ER due to the presence of functionally similar species. Additionally, in Chapter 4 I showed that although both predators consumed the same prey species, their effects on food web structure and ecosystem processes underpinning CO_2 fluxes were not interchangeable. Results from both Chapters 2 and 4, along with the idea that predators often lack functionally similar species, suggest that the relationship between predator species loss and CO_2 fluxes is less likely to be represented by a redundancy relationship.

Several studies have suggested that a single species dominates the effects of its guild on a particular ecosystem function (Vanni *et al.* 2002, Smith and Knapp 2003, Otto *et al.* 2008). Thus, results from these studies suggest that species identity drives the overall effects of species diversity on a particular ecosystem function, producing an idiosyncratic relationship or a keystone relationship. A keystone relationship predicts that a singular species has a disproportionately positive or negative effect on an ecosystem function relative to its abundance (Schlapfer and Schmid 1999). Documented effects of species identity and keystone species on ecosystem functions have become especially prevalent in the predator literature (Chalcraft and Resetarits 2003, Otto *et al. 200*8*,* Schmitz 2008, Whilmers *et al.* 2012). Results from my Chapter 4 suggest that predator identity may play an important part in the ability of a predator to create trophic cascades that extend to influence the CO_2 fluxes of their ecosystem. As stated in the previous paragraph, the effects of a predatory diving beetle and a predatory damselfly larva on food web structure and CO_2 emissions of bromeliads were not interchangeable. I showed that although predatory diving beetles were able to out-compete predatory damselfly larvae for space in bromeliads, only damselfly larvae were capable of creating trophic cascades and altering CO_2 emissions. Additionally, results from Chapter 5 suggest that top-down control of CO_2 fluxes in aquatic ecosystems interacts with climate warming and eutrophication. This suggests that anthropogenic alterations to physical or chemical characteristics of a habitat may complicate our ability to make predictions about the effects predator loss will play in CO_2 fluxes of ecosystems. Together, results from Chapters 4 and 5 suggest that an idiosyncratic relationship may characterize the relationship between predator species loss and CO_2 fluxes of aquatic ecosystems. This is

worrying because an idiosyncratic relationship suggests that the effect of species loss or gain on the CO_2 fluxes is dependent on the biotic and abiotic conditions under which the extinction or addition occurs, making predictions difficult.

6.4 Benefits and limitations of mesocosm studies

Ecologists, conservation and natural resource managers, and policy makers are currently in a race against time to understand and plan for the implications our pervasive effects on Earth will have on the future of the natural world. However, ecological communities, ecosystem functions, fundamental biogeochemical cycles, and ecosystem services are controlled and regulated by complex networks of interconnected species interactions and abiotic factors that often have feedbacks with one another. Thus, the task at hand is unbelievably difficult and will require the combined consensus of theoretical and empirical studies that span from simplistic to holistic views of ecosystems. By understanding the unique limitations each type of study has, and combining their results will build towards a more holist understanding of the question at hand. My thesis sought to understand how predators influenced the CO_2 fluxes of freshwater ecosystems using artificial mesocosms. The use of mesocosms in this research allowed me to isolate the predator effects on CO_2 fluxes caused by trophic cascades with high reproducibility, but also limited my ability to extrapolate my findings to real ecosystems.

Experiments or observations from natural ecosystems have their obvious benefits for the applicability of their results to real systems. However, because of cost and logistical constraints, experiments in natural ecosystems often lack sufficient replication to analyze statistically. The use of comparative evaluations before and after an impact using a Before-After Control-Impact design (BACI) can help with the limitations of replication in natural experiments; however these studies are limited by the generalization of their results to other systems. As a result it is difficult to determine whether the results of those studies occurred due to the factor of interest or through happenstance. Additionally, because of the complexity of natural ecosystems it is also difficult to isolate a single factor responsible for the findings or to provide a mechanistic understanding for those results.

In terms of complexity, mesocosms represent an intermediate step between simplified laboratory microcosms and naturally complex ecosystems. If designed carefully, mesocosms can contain natural levels of habitat and food web complexity and can experimentally test questions from the individual to the ecosystem scale (Richardson 1991, Brown *et al.* 2011, Kratina *et al.* 2012). However, by their very nature mesocosms do not contain all the biotic and abiotic factors that make up a natural ecosystem, and thus, their findings have limitations. Because the size of the mesocosm relative to the natural system and the duration of the study are often small, mesocosms can distort or exclude important interactions in communities or the ecosystem (Carpenter 1996). Despite their limitations, mesocosms are commonly used in aquatic sciences because they allow for highly replicated, controlled environments that can be manipulate in complex ways to examine interactions between processes.

Control over the biological characteristics of my communities was essential in isolating predator effects on their CO_2 fluxes, as the biological structure of the community influences GPP and ER. In Chapter 1 I described that the net carbon balance of an ecosystem is the sum of the individual photosynthetic rates of all the autotrophic individuals of the ecosystem minus the sum of respiration rates of all the individual heterotrophs and autotrophs in the system (Woodwell and Whittaker 1968, Woodwell 1995, Enquist *et al.*2003, and Lopez-Urrutia *et al.* 2006). Because the carbon balance of an ecosystem works in terms of total biomass and not densities of organisms we must ensure that any differences in the biomass of organisms between predator and non-predator ecosystems is not confounded by some other habitat characteristic. For example, small streams often lack predatory fish and large vertebrates and macroinvertebrates due to space limitation, with the opposite being generally true for larger streams. However because there is a significant interaction between habitat size and the presence of predatory fish, we would be unable to isolate predator effects on community biomass and CO_2 fluxes as they are confounded in the differences in habitat size between streams with predators and without. Natural bromeliad ecosystems also provide an example of where habitat complexity would confound predator effects on community biomass and CO_2 fluxes. The top predator of bromeliads, *M. modesta*, occurs in nearly every Costa Rican

bromeliad larger than 100 ml, but does not occur in bromeliads smaller than 100 ml (Srivastava 2006). Additionally, as bromeliad size increases so does habitat complexity (Srivastava 2006). As habitat complexity is known to influence both interaction strengths among organisms and community composition (Beukers *et al.* 1998, Grabowski 2004), I again would have been unable to separate out predator effects on community biomass and CO_2 fluxes from habitat complexity effects. The use of mesocosms in this thesis allowed me to assemble identical food webs prior to the manipulation of predators. Thus, any differences seen between the two communities following manipulations could be identified as predator effects.

As previously stated, the physical characteristics of a habitat (e.g., complexity) can have implications for community structure and dynamics. However control over the physical characteristics of my study systems was also essential in isolating predator effects on CO_2 fluxes, as the physical structure of an ecosystem can influence the CO_2exchange velocities across their surface area. Carbon dioxide exchange velocities are an important component to calculating CO_2 flux [CO_2 flux = (pCO$_2$water - pCO$_2$air) * k; where k is the CO_2exchange velocity]. For lotic ecosystems CO_2 exchange velocities are heavily influenced by the slope and velocity of the individual stream (Butman and Raymond 2011), while lentic ecosystems are greatly influenced by wind speed across the surface area of the ecosystem (Cole and Caraco 1998). Because CO_2 exchange velocities are influenced by the physical characteristics of their ecosystems, and natural ecosystems greatly vary in their physical characteristic, this suggests that either caution should be taken when choosing previously published literature values for CO_2 exchange velocities or CO_2 exchange velocities should be calculated for the individual ecosystems at the same time as the CO_2 concentrations of the water are collected. By engineering my mesocosms, I could ensure that the physical structure of my mesocosms were identical and placed in the same location within each study. Thus, any differences in CO_2 fluxes of my ecosystems could be attributed to my manipulations as opposed to unaccounted for differences in CO_2 exchange velocities across mesocosms.

Although my thesis provides some of first broad evidence of the effects of predators on CO_2 flux of freshwater ecosystems, its results are not without limitation due

to the use of mesocosm as the study system. For all four of my data chapters I used modified food webs compared to their natural counterparts. Although I attempted to include all common species, rare species were likely excluded. This could have influenced results from my studies as there is a growing body of literature that now supports the idea that rare species are important for maintaining ecosystem function (Schwartz *et al.* 2000, Lyons and Schwartz 2001, Smith and Knapp 2003). Additionally, natural communities are dynamic and their populations change over time (Sousa 1984, McArdle and Gaston 1993, Stewart-Oaten 1995, Houlahan *et al.* 2000). Perhaps most importantly to the implications of this thesis are the findings that trophic cascade strength varies temporally (Pinnegar *et al.* 2000, Frank *et al.* 2007, Kratina *et al.* 2012). Chapters 2, 3, and 4 contained only a single measurement in time and Chapter 5 only contained a sample at the beginning and end of the growing season. This sampling regime cannot tell us anything about the potential for temporal variability in the effects of predators on the influence of CO_2 fluxes of the ecosystem. However, an understanding of the limitations of my thesis provides multiple opportunities for future research.

6.5 Future research directions for understanding the relationship between predator loss and CO_2 fluxes of ecosystems

Research investigating biodiversity effects on carbon exchange between ecosystems and the atmosphere provides a unique research opportunity to investigate an ecosystem function that may greatly influence future climate change, and is composed of multiple ecosystem processes. Because CO_2 fluxes are influenced by a multitude of ecosystem processes occurring at different levels of the food web (e.g., primary and secondary production, decomposition) they allow us to consider how biodiversity affects a variety of processes performed by different components of a food web that leads to a single measure of ecosystem function. So far, only this thesis and a few other studies (Schindler *et al.* 1997, Flanagan *et al.* 2006, Wilmers *et al.* 2012) have investigated the effects of biodiversity loss on the CO_2 fluxes of aquatic ecosystems, leaving the field virtually wide open for future research. Below I have provided just a few potential future directions that may help us better understand the relationship between predator species loss and carbon exchange between aquatic ecosystems and the atmosphere. However,

research investigating the influence of other trophic levels on CO_2 fluxes is also likely to be fruitful, and many of the concepts discussed below are applicable avenues for research involving other trophic levels.

Horizontal diversity, or the functional or taxonomic evenness and diversity of a trophic group, can influence predator effects on prey (Denoth *et al.* 2002, Balvanera *et al.* 2006, Cardinale *et al.* 2006). Thus, horizontal diversity is likely to influence the propagation of predator effects on GPP, ER, and ultimately CO_2 fluxes. Some studies have suggested that more diverse predator assemblages have greater effects on prey (Fox 2004), while others have shown that competition between predators can dampen trophic cascades (Skalski and Gilliam 2001). Perhaps a first avenue for future research is to determine whether more diverse predator assemblages influence CO_2 fluxes of aquatic ecosystems to a greater extent than species poor predator assemblages. Results from these types of studies may provide insights into whether competition or facilitation is a more important mechanism driving predator effects on CO_2 fluxes, or how prevalent functional redundancy is in the predator guild.

A second potential avenue for future studies would be to determine whether and what predator traits may be valid predictors of predator effects on carbon exchange between aquatic ecosystems and the atmosphere, and which traits are more likely to go extinct. Studies have shown that resource specialization, body size, and predator hunting mode may be good predictors of the effects a predator species has on ecosystem function (Thebault and Loreau 2003, Ives *et al.* 2005, Duffy *et al.* 2007, Schmitz 2008, Shurin and Seabloom 2005). Additionally, many of these traits are also predicted to make species more vulnerable to local extinctions (Pauly *et al.* 1998, Dobson *et al.* 2006). Identifying specific predator traits that are likely to influence CO_2 fluxes of ecosystems may make it easier to design experiments that mimic realistic scenarios of extinctions. Additionally, an understanding of which traits have the greatest effect on carbon exchange between ecosystems and the atmosphere and which traits are particularly vulnerable to extinction will make predictions about predator species loss on CO_2 fluxes less difficult.

Omnivory, intraguild predation, ontogenetic shifts in diet, and cannibalism are relatively common among aquatic predators (Thompson *et al.* 2007). These aspects of vertical niche breadth describe how discretely an organism fits into a single trophic level, and can have large influences on their effects on ecosystem function (Finke and Denno 2004, Finke and Denno 2005). Plasticity in the diet of predator species may influence CO_2 fluxes by altering the strength of trophic cascades and thus their ability to influence GPP and ER. Additionally, plasticity in the diet of predators may alter the distribution of biomass and productivity of different trophic levels over space and time. As a result, wide vertical niche breadth in predators may make predictions about their effects on CO_2 fluxes of ecosystems more difficult.

Food chain length, a measure of vertical diversity, has been shown to influence predator effects on ecosystem function (Borer *et al.* 2005, Duffy *et al.* 2005, Borer *et al.* 2006). Thus, food chain length is likely to influence predator effects on carbon exchange among aquatic ecosystems and the atmosphere. Food chain length is predicted to influence the direction of effects of predators on CO_2 fluxes of aquatic ecosystems (Schindler *et al.* 1997, Atwood *et al.* 2013). Theory suggests that the removal of a predator from an even-numbered food chain will negatively influence CO_2 concentrations in the water, while the removal of a predator from an odd-numbered food chain will positively influence CO_2 concentrations. Although the results from Schindler *et al.* (1997) support these predictions, their study design was unreplicated. Additionally, as this thesis focused on the effects of predators on CO_2 fluxes of three-tier food chains, empirical studies are needed to validate how food chain length will influence the effects of predators on carbon exchange between aquatic ecosystems and the atmosphere.

6.6 Implications for conservation

Past and continuing removal of predators has been characterized as one of humankind's most pervasive influences on the natural world (Estes *et al.* 2011). Over the last couple of decades more and more research has documented the potential for predator loss to create irreversible changes in ecological communities, the functioning of ecosystems and the services they provide (Pauly *et al.* 1998, Vanni 2002, Estes *et al.*

2011). This thesis provides compelling evidence that predators, via trophic cascades, can markedly influence the CO_2 fluxes of multiple types of freshwater ecosystems. My results also suggest that freshwater ecosystems may lack functionally redundant species at the top of the food chain, indicating that human-induced removal of aquatic predators may have irreversible consequences on the CO_2 fluxes of ecosystem. Additionally, results from my thesis showed that predator effects on food web structure and CO_2 are dependent on both biotic and abiotic interactions. Competition and facilitation of other predators can influence the total predator effects on populations and communities (Soluk and Richardson 1997, Hart 2002, Finke and Denno 2004, Casula *et al.* 2006). Thus, the invasion of non-native predators into ecosystems has the potential to alter the magnitude of CO_2 exchange between freshwater ecosystems and the atmosphere through biotic interactions with native predators. Finally, other anthropogenic stressors such as climate warming and nutrient loading threaten to alter the shape of the relationship between predators, trophic cascades, and CO_2 fluxes (Schindler *et al.* 1997, Flanagan *et al.* 1997, Yvon-Durocher *et al.* 2010, Greig *et al.* 2012, Shurin *et al.* 2012). Individually, nutrients and warming were shown to increase top-down forcing on CO_2 fluxes, while their combined effects decoupled food web effects on CO_2. The dramatic influence of predators on CO_2 emissions from aquatic ecosystems indicates that human-induced removal or introduction of aquatic predators, combined with nutrient loading and climate warming, may have far reaching consequences for regional and global C cycles and greenhouse gas dynamics.

REFERENCES

Abnizova, A., J. Siemens, M. Langer, and J. Boike. 2012. Small ponds with impact: The relevance of ponds and lakes in permafrost landscapes. Global Biogeochemical Cycles **26**: GB2041, DOI: 10.1029/2011GB004237.

Allen, A. P., J. F. Gillooly, and J. H. Brown. 2005. Linking the global carbon cycle to individual metabolism. Functional Ecology **19**:202-213.

Anderson, N. H., and J. R. Sedell. 1979. Detritus processing by macroinvertebrates in stream ecosystems. Annual Review of Entomology **24**:351-377.

Andersen, T., and D. O. Hessen. 1991. Carbon, nitrogen, and phosphorus-content of fresh-water zooplankton. Limnology and Oceanography **36**:807-814.

Anthony, R. I., J. C. Bradley, and E. S. William. 2005. A synthesis of subdisciplines: predator-prey interactions, and biodiversity and ecosystem functioning. Ecology Letters **8**:102.

Atwood, T. B., and J. S. Richardson. 2012. Trophic interactions between insects and stream-associated amphibians in steep, cobble-bottom streams of the pacific coast of North America. Insects **3**:432-441.

Atwood, T. B., E. Hammill, H. S. Greig, P. Kratina, J. B. Shurin, D. S. Srivastava, and J. S. Richardson. 2013. Predator-induced reduction of freshwater carbon dioxide emissions. Nature Geoscience **6**:191-194.

Azim, M. E. 2010. Photonsynthetic periphyton and surfaces. In plankton of inland waters (ed. G. E. Likens), pp. 175–182. San Diego, CA: Academic Press.

Baker, R. L. 1983. Spacing behaviour by larval Ischnura cervula Selys: effects of hunger, previous interactions, and familiarity with an area (Zygoptera: Coenagrionidae). Odonatologica **12**:201-207

Balvanera, P., A. B. Pfisterer, N. Buchmann, J.-S. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters **9**:1146-1156.

Barnosky, A. D., K. C. Maguire, B. Mersey, E. A. Ferrer, N. Matzke, S. Tomiya, G. O. U. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, and E. L. Lindsey. 2011. Has the Earth's sixth mass extinction already arrived? Nature **471**:51-57.

Barton, B. T., A. P. Beckerman, and O. J. Schmitz. 2009. Climate warming strengthens indirect interactions in an old-field food web. Ecology **90**:2346-2351.

Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold, and F. Sabater. 2008. Biophysical controls on organic carbon fluxes in fluvial networks.

Nature Geoscience 1: 95-100.

Battin, T. J., S. Luyssaert, L. A. Kaplan, A. K. Aufdenkampe, A. Richter, and L. J. Tranvik. 2009. The boundless carbon cycle. Nature Geoscience **2**:598-600.

Beckerman, A. P., K. Wieski, and D. J. Baird. 2007. Behavioural versus physiological mediation of life history under predation risk. Oecologia **152**:335-343.

Bengtsson, J. 1998. Which species? What kind of diversity? Which ecosystem function? Some problems in studies of relations between biodiversity and ecosystem function. Applied Soil Ecology **10**:191-199.

Berlow, E. L., 1999. Strong effects of weak interactions in ecological communities. Nature **398**: 330-334.

Beukers, J. S., and G. P. Jones. 1998. Habitat complexity modifies the impact of piscivores on a coral reef fish population. Oecologia **114**:50-59.

Binzer, A., C. Guill, U. Brose, and B. C. Rall. 2012. The dynamics of food chains under climate change and nutrient enrichment. Philosophical Transactions of the Royal Society B-Biological Sciences **367**:2935-2944.

Borer, E. T, E. W. Seabloom, J. B. Shurin, K. E. Anderson, C. A. Blanchette, B. Broitman, S. D. Cooper, and B. S. Halpern. 2005. What determines the strength of a trophic cascade? Ecology **86**:528-537.

Borer, E.T., B. S. Halpern, and E. W. Seabloom. 2006. Asymmetry in community regulation: effects of predators and productivity. Ecology **87**:2813–2820.

Brett, M. T. and C. R. Goldman. 1996. A meta-analysis of the fresh-water trophic cascade. Proceedings of the National Academy of Sciences of the United States of America **93**:7723–7726

Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science **150**:28-35.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology **85**:1771-1789.

Brown, L. E., F. K. Edwards, A. M. Milner, G. Woodward, and M. E. Ledger. 2011. Food web complexity and allometric scaling relationships in stream mesocosms: implications for experimentation. Journal of Animal Ecology **80**:884-895.

Bruno, J. F., and M. I. O'Connor. 2005. Cascading effects of predator diversity and omnivory in a marine food web. Ecology Letters **8**:1048-1056.

Butman, D. and P. A. Raymond. 2011. Significant efflux of carbon dioxide from streams and rivers in the United States. Nature Geoscience **4:**839-842.

Cardillo, M. and Bromham, L. 2001. Body size and risk of extinction in Australian mammals. Conservation Biology **15**: 1435–40

Cardillo, M., G. M. Mace, K. E. Jones, J. Bielby, W. Sechrest, and A. Purvis. 2005. Multiple causes of high extinction risk in large mammal species. Science **309**:1239-1241.

Cardinale, B. J., M. A. Palmer, and S. L. Collins. 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature **415**:426–429

Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature **443**:989-992.

Cardinale, B. J., D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, S. Naeem, J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, and D. Tilman. 2012. Biodiversity loss and its impact on humanity. Nature **486**:59-67.

Carpenter S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience **35**:634-639

Carpenter S. R., J. F. Kitchell, J. R. Hodgson, P. A. Cochran, J. J. Elser, M. M. Elser, D. M. Lodge, D. Kretchmer, X. He, and C. N. von Ende. 1987. Regulation of lake primary productivity by food web structure. Ecology **68**:1863-1876.

Carpenter S. R., J. J. Cole, J. R. Hodgson, J. F. Kitchell, M. L. Pace, D Bade, K. L. Cottingham, T. E. Essington, J. N. Houser, and D. E. Schindler. 2001. Trophic cascades, nutrients, and lake productivity: Whole-lake experiments. Ecological Monographs **71**:163-186

Casula, P., A. Wilby, and M. B. Thomas. 2006. Understanding biodiversity effects on prey in multi-enemy systems. Ecology Letters **9**:995-1004.

Chalcraft, D. R., and W. J. Resetarits. 2003. Predator identity and ecological impacts: functional redundancy or functional diversity? Ecology **84**:2407-2418.

Chapin III F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature **405**:234-242.

Chase, J. M. 2003. Strong and weak trophic cascades along a productivity gradient. Oikos **101**:187-195.

Cole J. J., N. F. Caraco, G. W. Kling, and T. K. Kratz. 1994. Carbon dioxide supersaturation in the surface waters of lakes. Science **265**:1568-1570.

Cole, J. J., and N. F. Caraco. 1998. Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF6. Limnology and Oceanography **43**:647-656.

Cole, J. J., S. R. Carpenter, J. F. Kitchell, and M. L. Pace. 2002. Pathways of organic carbon utilization in small lakes: Results from a whole-lake C-13 addition and coupled model. Limnology and Oceanography **47**:1664-1675.

Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg, and J. Melack. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems **10**:171-184.

Collette, B. B., K. E. Carpenter, B. A. Polidoro, M. J. Juan-Jorda, A. Boustany, D. J. Die, C. Elfes, W. Fox, J. Graves, L. R. Harrison, R. McManus, C. V. Minte-Vera, R. Nelson, V. Restrepo, J. Schratwieser, C. L. Sun, A. Amorim, M. B. Peres, C. Canales, G. Cardenas, S. K. Chang, W. C. Chiang, N. D. Leite, H. Harwell, R. Lessa, F. L. Fredou, H. A. Oxenford, R. Serra, K. T. Shao, R. Sumaila, S. P. Wang, R. Watson, and E. Yanez. 2012. High value and long life-double jeopardy for tunas and billfishes. Science **333**:291-292.

Corbet, P. S. 1980. Biology of odonata. Annual Review of Entomology **25**:189-217.

Crowley, T. J. 2000. Causes of climate change over the past 1000 years. Science **289**:270 – 277.

Enquist, B.J., E. P. Economo, T. E. Huxman, A. P. Allen, D. D. Ignace, and J. F. Gillooly. 2003. Scaling metabolism from organisms to ecosystems. Nature **423**: 639–642.

Dobson, A., D. Lodge, J. Alder, G. S. Cumming, J. Keymer, J. McGlade. H. Mooney, J. A. Rusak, O. Sala, V. Wolters, D. Wall, R. Winfree, and M. A. Xenopoulos. 2006. Habitat loss, trophic collapse, and the decline of ecosystem services. Ecology **87**:1915–1924.

Downing, J. A., Y. T. Prairie, J. Cole, C. M. Duarte, L. J. Tranvik, , R. G. Striegl, W. H. McDowell , P. Kortelainen, N. Caraco, and J. Melack, J. J. Middelburg. 2006. The global abundance and size distribution of lakes, ponds, and impoundments. Limnology and Oceanography **51**:2388-2397.

Denoth, M., L. Frid, and J. H. Myers. 2002. Multiple agents in biological control: improving the odds? Biological Control **24**:20–30.

Duffy, J. E. 2002. Biodiversity and ecosystem function: the consumer connection. Oikos **99**:201-219.

Duffy, J. E. 2003. Biodiversity loss, trophic skew and ecosystem functioning. Ecology Letters **6**:680-687.

Duffy, J. E., K. S. MacDonald, J. M. Rhode, and J. D. Parker. 2001. Grazer diversity, functional redundancy, and productivity in seagrass beds: an experimental test. Ecology **82**:2417-2434.

Duffy, J. E., B. J. Cardinale, K. E. France, P. B. McIntyre, E. Thébault, and M. Loreau. 2007. The functional role of biodiversity in ecosystems: incorporating trophic complexity. Ecology Letters **10**:522-538.

Emmerson, M., M. Bezemer, M. D. Hunter, and T. H. Jones. 2005. Global change alters the stability of food webs. Global Change Biology **11**:490-501.

Enquist, B.J., E. P. Economo, T. E. Huxman, A. P. Allen, D. D. Ignace, and J. F. Gillooly. 2003. Scaling metabolism from organisms to ecosystems. Nature **423**:639–642

Estes, J. A., J. B. C. Jackson, R. J. Marquis, L. Oksanen, T. Oksanen, R. T. Paine, E. K. Pikitch, W. J. Ripple, S. A. Sandin, M. Scheffer, T. W. Schoener, J. Terborgh, J. B. Shurin, A. R. E. Sinclair, M. E. Soule, R. Virtanen, D. A. Wardle, J. S. Brashares, M. E. Power, J. Berger, W. J. Bond, S. R. Carpenter, T. E. Essington, and R. D. Holt. 2011. Trophic downgrading of planet Earth. Science **333**:301-306.

Falkowski, P., R. J. Scholes, E. Boyle, J. Canadell, D. Canfield, J. Elser, N. Gruder, K. Hibbard, P. Horgberg, S. Linder, F. T. Mackenzie, B. Moore III, T. Pedersen, Y. Rosenthal, S. Seitzinger, V. Smetacek, and W. Steffen. 2000. The global carbon cycle: A test of our knowledge. Science **290**:291-296.

Field, C.B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. Science **281**:237–240.

Findlay S., R. L. Sinsabough, D. T. Fischer, and P. Franchini. 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. Ecosystems **1**:227-239.

Finke, D. L., and R. F. Denno. 2004. Predator diversity dampens trophic cascades. Nature **429**:407-410.

Finke, D. L., and R. F. Denno. 2005. Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. Ecology Letters **8**:1299-1306.

Fisher S. G., and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecological Monographs **43**:421-439

Flanagan, K. M., E. McCauley, and F. Wrona. 2006. Freshwater food webs control carbon dioxide saturation through sedimentation. Global Change Biology **12**:644-651.

Frank, J. H. and L. P. Lounibos. 2009. Insects and allies associated with bromeliads: a review. Terrestrial Arthropod Review 1:125-153.

Frank, K. T., B. Petrie, and N. L. Shackell. 2007. The ups and downs of trophic control in continental shelf ecosystems. Trends in Ecology & Evolution **22**:236-242.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science **293**:2248-2251.

Goudard A., and M. Loreau. 2007. Nontrophic interactions, biodiversity, and ecosystem functioning: an interaction web model. The American Naturalist **171**:91-106.

Grabowski, J. H. 2004. Habitat complexity disrupts predator-prey interactions but not the trophic cascade on oyster reefs. Ecology **85**:995-1004.

Greeney, H. F. 2001. The insects of plant-held waters: a review and bibliography. Journal of Tropical Ecology **17**:241-260.

Greig, H. S., P. Kratina, P. L. Thompson, W. J. Palen, J. S. Richardson, and J. B. Shurin. 2012. Warming, eutrophication, and predator loss amplify subsidies between aquatic and terrestrial ecosystems. Global Change Biology **18**:504-514.

Gruner, D. S., J. E. Smith, E. W. Seabloom, S. A. Sandin, J. T. Ngai, H. Hillebrand, W. S. Harpole, J. J. Elser, E. E. Cleland, M. E. S. Bracken, E. T. Borer, and B. M. Bolker. 2008. A cross-system synthesis of consumer and nutrient resource control on producer biomass. Ecology Letters **11**:740-755.

Hairston N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control and competition. The American Naturalist **92**:421-425.

Halaj, J., and D. H. Wise. 2001. Terrestrial trophic cascades: how much do they trickle? The American Naturalist **157**:262-281.

Hargrave, C. W., K. D. Hambright, and L. J. Weider. 2011. Variation in resource consumption across a gradient of increasing intra- and interspecific richness. Ecology **92**:1226-1235.

Harrington, R., I. Woiwod, and T. Sparks. 1999. Climate change and trophic interactions. Trends in Ecology & Evolution **14**:146-150.

Harris, L. A., C. M. Duarte, and S. W. Nixon. 2006. Allometric laws and prediction in estuarine and coastal ecology. Estuaries and Coasts **29**:340-344.

Hart, D. 2002. Intraguild predation, invertebrate predators, and trophic cascades in lake food

webs. Journal of Theoretical Biology **218**:111-128

Hawkins, C. P., L. J. Gottschalk, and S. S. Brown. 1988. Densities and habitat of tailed frog tadpoles in small streams near mt. st. helens following the 1980 eruption. Journal of the North American Benthological Society 7:246-252.

Hawlena, D., and O. J. Schmitz. 2010. Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. Proceedings of the National Academy of Sciences of the United States of America **107**:15503-15507.

Hawlena, D., M. S. Strickland, and O. J. Schmitz. 2012. Fear of predation slows plant-litter decomposition. Science **336**:1434-1438.

Hector, A. 1998. The effect of diversity on productivity: detecting the role of species complementarity. Oikos **82**:597-599.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos **66**:483-492.

Hooper, D. U., S. Naeem, B. Schmid, H. Setala, J. Vandermeer, D. A. Wardle, F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, and M. Loreau. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs **75**:3-35.

Hope, D., J. J. C. Dawson, M. S. Cresser, and M. F. Billett. 1995. A method for measuring free CO2 in upland streamwater using headspace analysis. Journal of Hydrology **166**:1-14.

Houghton, J. 2001. The science of global warming. Interdisciplinary Science Reviews **26**:247-257.

Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. Nature **404**:752-755.

Hudson, F. 2004. Sample preparation and calculations for dissolved gas analysis in water samples using a GC headspace equilibration technique. Method RSKSOP-175, U.S. Environmental Protection Agency (EPA) Region 1: Ground Water and Ecosystems Restoration Division. http://www.epa.gov/region1/info/testmethods/pdfs/RSKsop175v2.pdf

Hunter, M. D., and P. W. Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology **73**:724-732.

Huryn A. D. 1998. Ecosystem-level evidence for top-down and bottom-up control of production in a grassland stream system. Oecologia **115**:173-183.

IPCC. 2001. Climate change 2001: The scientific basis: contribution of working group I to the

third assessment report of the Intergovernmental Panel on Climate Change, edited by J. T. Houghton *et al.*, 881 pp., Cambridge Univ. Press, New York.

IPCC. 2007: Climate change 2007: The physical science basis. contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Ives, A. R., B. J. Cardinale, and W. E. Snyder. 2005. A synthesis of subdisciplines: predator-prey interactions, and biodiversity and ecosystem functioning. Ecology Letters **8**:102–116.

Jennings S., J. K. Pinnegar, N. V. C. Polunin, and K. J. Warr. 2002. Linking size-based and trophic analyses of benthic community structure. Marine Ecology Progress Series **266**: 77-85.

Jiang, L., Z. Pu, and D. R. Nemergut. 2008. On the importance of the negative selection effect for the relationship between biodiversity and ecosystem functioning. Oikos **117**:488-493.

John, T., D. L. Thomas, B. Luis, L. Lawrence, N. e. Percy, R. Madhu, S. Ghazala, O. Gabriela, R. Mailen, A. Rafael, and H. A. Greg. 2001. Ecological meltdown in predator-free forest fragments. Science **294**:1923-1926.

Keen, W. H., and R. W. Reed. 1985. Territorial defence of space and feeding sites by a plethodontid salamander. Animal Behaviour **33**:1119-1123.

Kiffney, P. M., and J. S. Richardson. 2001. Interactions among nutrients, periphyton, and invertebrate and vertebrate (ascaphus truei) grazers in experimental channels. Copeia **2001**:422-429.

Knight T. M., M. W. McCoy, J. M. Chase, K. A. McCoy, and R. D. Holt. 2005. Trophic cascades across ecosystems. Nature **437**:880-883

Kohler, S. L. 1992. Competition and the structure of a benthic stream community. Ecological Monographs **62**:165-188.

Kordas R. L. and S. Dudgeon. 2009. Modeling variation in interaction strength between barnacles
and fucoids. Oecologia **158**:717-731

Kratina, P., H. S. Greig, P. L. Thompson, T. S. A. Carvalho-Pereira, and J. B. Shurin. 2012. Warming modifies trophic cascades and eutrophication in experimental freshwater communities. Ecology **93**:1421-1430.

Kritzberg, E. S., J. J. Cole, M. L. Pace, W. Graneli, and D. L. Bade. 2004. Autochthonous versus

allochthonous carbon sources of bacteria: Results from whole-lake C-13 addition experiments. Limnology and Oceanography **49**:588-596.

Lamberti, G. A., S. V. Gregory, C. P. Hawkins, R. C. Wildman, L. R. Ashkenas, and D. M. Denicola. 1992. Plant-herbivore interactions in streams near Mount St Helens. Freshwater Biology **27**:237-247.

Lawton, J. H. 1989. Food webs. Pages 43–78 in J. M. Cherritt, editor. Ecological concepts. Blackwell Scientific, Oxford, UK.

Lecerf, A., and J. S. Richardson. 2011. Assessing the functional importance of large-bodied invertebrates in experimental headwater streams. Oikos **120**:950-960.

Le Quere, C. M. R. Raupach, J. G. Canadell, G. Marland, L. Bopp, P.Ciais, T. J. Conway, S.C. Doney, R. A. Feely, P. Foster, P. Fiedlingstein, K. Gurney, R. A. Houghton, J. I. House, C. Huntingford, P. E. Levy, M. R. Lomas, J. Majkut, N. Metzl, J. P. Ometto, G. P. Peters, I. Sarmiento, U. Schuster, S. Sitch, T. Takahashi, N. Viovy. G. R. van der Werf, and F. I. Woodward. 2009. Trends in the source and sinks in carbon dioxide. Nature Geoscience. **2**:831-836.

Leroux, S. J., D. Hawlena, and O. J. Schmitz. 2012. Predation risk, stoichiometric plasticity and ecosystem elemental cycling. Proceedings of the Royal Society of London B **279**: doi: 10.1098/rspb.2012.1315.

Lopez-Urrutia, A., E. San Martin, R. P. Harris, and X. Irigoien. 2006. Scaling the metabolic balance of the oceans. Proceedings of the National Academy of Sciences of the United States of America **103**:8739-8744.

Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. Oikos **91**:3-17.

Loreau, M., B. Schmid, D. Tilman, D. A. Wardle, S. Naeem, P. Inchausti, J. Bengtsson, J. P. Grime, A. Hector, D. U. Hooper, M. A. Huston, and D. Raffaelli. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science **294**:804-808.

Lovelock, J. E. 1967. Gaia as seen through the atmosphere. Atmospheric Environment **6**:579-580.

Luyssaert, S., L. A. Kaplan, A. K. Aufdenkampe, A. Richter, L. J. Tranvik. 2009. The boundless carbon cycle. Nature Geoscience **2**:598-600.

Lyons, K. G., and M. W. Schwartz. 2001. Rare species loss alters ecosystem function - invasion resistance. Ecology Letters **4**:358-365.

Mallory, M. A., and J. S. Richardson. 2005. Complex interations of light, nutrients, and consumer density in a stream periphyton-grazer (tailed frog tadpoles) system. Journal of

Animal Ecology **74**:1020-1028.

Martinson, G. O., F. A. Werner, C. Scherber, R. Conrad, M. D. Corre, H. Flessa, K. Wolf, M. Klose, and S. R. Gradstein. 2010. Methane emissions from tank bromeliads in neotropical forests. Nature Geoscience **3**:766-769.

May, R. M. 1986. When two and two do not make four: nonlinear phenomena in ecology. Proceedings of the Royal Society of London B **264**:1249-1254

McArdle, B., and K. Gaston. 1993. The temporal variability of populations. Oikos **67**:187-191.

McCann, K., S. Hastings, and G. R. Huxel. 1998. Weak trophic interactions and the balance of nature. Nature **395**:794-798

McIntosh A. R., H. S. Greig, S. A. McMurtrie, P. Nystrom, and M. J. Winterbourn. 2005. Top-down and bottom-up influences on populations of a stream detritivores. Freshwater Biology **50**:1206-1218

McKinney, M. L. 1997. Extinction vulnerability and selectivity: combining ecological and paleontological views. Annual Review of Ecology and Systematics **28**:495-516.

McPeek, M. A., and P. H. Crowley. 1987. The effects of density and relative size on the aggressive behaviour, movement and feeding of damselfly larvae (Odonata: Coenagrionidae). Animal Behaviour **35**:1051-1061.

Meehl, G. A., W. M. Washington, W. D. Collins, J. M. Arblaster, A. Hu, L. E. Buja, W. G. Strand, and H. Teng. 2005. How much more global warming and sea level rise? Science **307**:1769-1772.

Menge, B. A., E. L. Berlow, C. A. Blanchette, S. A. Navarrete, and S. B. Yamada. 1994 The keystone species concept: Variation in interaction strength in a rocky intertidal habitat. Ecological Monographs **64**:249-286.

Merritt, R. W., K. W. Cummins, and M. A. Berg. (editors). 2008. An introduction to the aquatic insects of North America. 4th edition. Kendall/Hunt Publishing. Dubuque, Iowa.

Micheli, F. 1999. Eutrophication, fisheries, and consumer-resource dynamics in marine pelagic ecosystems. Science **285**:1396-1398.

Mills, L. S., M. E. Soule, and D. F. Doak. 1993. The keystone-species concept in ecology and conservation. BioScience **43**: 219-224.

Micheli, F. 1999. Eutrophication, fisheries, and consumer-resource dynamics in marine pelagic ecosystems. Science **285**: 1396-1398.

Montgomery, W. L. 1980. The impact of non-selective grazing by the giant blue damselfish,

microspathodon dorsalis, on algal communities in the gulf of california, mexico. Bulletin of Marine Science **30**:290-290.

Moore, M. V., C. L. Folt, and R. S. Stemberger. 1996. Consequences of elevated temperatures for zooplankton assemblages in temperate lakes. Archiv Fur Hydrobiologie **135**:289-319.

Mulder, C. P. H., D. D. Uliassi, and D. F. Doak. 2001. Physical stress and diversity-productivity relationships: the role of positive interactions. Proceedings of the National Academy of Sciences of the USA **98**:6704–6708.

Mulholland P. J., C. S. Fellows, J. L. Tank, N. B. Grimm, J. R. Webster, S. K. Hamilton, E. Martia, L. Ashkenas, W. B. Bowden, W. K. Dodds, W. H. McDowell, M. J. Paul, and B. J. Peterson. 2001. Inter-biome comparison of factors controlling stream metabolism. Freshwater Biology **46**:1503-1517

Neubert, M., S. Blumenshine, D. Duplisea, T. Jonsson, and B. Rashlei. 2000. Body size and food web structure: testing the equiprobability assumption of the cascade model. Oecologia **123**:241-251.

Neutel A., J. A. P. Heesterbeek, and P. C. de Ruiter. 2002. Stability in real food webs: weak links in long loops. Science **296**:1120-1123.

Ngai, J. T., and D. S. Srivastava. 2006. Predators accelerate nutrient cycling in a bromeliad ecosystem. Science **314**:963-963.

Niven, J. E., and P. W. Scharlemann Jr. 2005. Do insect metabolic rates at rest and during flight scale with body mass? Biology letters **1**:346-349.

Nystrom P., A. R. McIntosh, and M. J. Winterbourn. 2003. Top-down and bottom-up processes in grassland and forested streams. Oecologia **136**:596-608

O'Connor, M. I., M. F. Piehler, D. M. Leech, A. Anton, and J. F. Bruno. 2009. Warming and resource availability shift food web structure and metabolism. Plos Biology 7:e1000178-e1000178.

Oehlert, G. W. 1992. A note on the delta method. American Statistician **46**: 27-29.

O'Gorman, E. J., and M. C. Emmerson. 2009. Perturbations to trophic interactions and the stability of complex food webs. Proceedings of the National Academy of Science of the USA **106**:13393-13398.

O'Gorman, E. J., U. Jacob, T. Jonsson, and M. C. Emmerson. 2010. Interaction strength, food web topology and the relative importance of species in food webs. Hournal of Animal of Ecology **79**:682-692.

Olff, H., and M. E. Ritchie. 1998. Effects of herbivores on grassland plant diversity. Trends in

Ecology & Evolution **13**:261-265.

Oquist M. G., M. Wallin, J. Seibert, K. Bishop, and H. Laudon. 2009. Dissolved inorganic carbon export across the soil/stream interface and its fate in a boreal headwater stream. Environmental Science Technology **43**:7364-7369

Otto, S. B., E. L. Berlow, N. E. Rank, J. Smiley, and U. Brose. 2008. Predator diversity and identity drive interaction strength and trophic cascades in a food web. Ecology **89**:134-144.

Owen-Smith, N. 1989. Megafaunal extinctions: the conservation message from 11,000 years B.P. Conservation Biology **3**:405-412.

Paine, R. T. 1980. Food webs: linkage, interaction strength, and community infrastructure. Journal of Animal Ecology. **49**:667-685.

Paine, R. T. 1988. Food webs: road maps of interactions or grist for theoretical development? Ecology **69**:1648-1654.

Palmer, M. A., and S. L. Collins. 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature **415**:426-429.

Parker, J. D., D. E. Burkepile, and M. E. Hay. 2006. Opposing effects of native and exotic herbivores on plant invasions. Science **311**:1459-1461.

Pauly, D., V. Christensen, J. Dalsgaard, R. Froese, and F. Torres. 1998. Fishing down marine food webs. Science **279**:860-863.

Peacor, S. D., and E. E. Werner. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. Proceedings of the National Academy of Sciences of the United States of America **98**:3904-3908.

Petchey, O. L., A. Eklof, C. Borrvall, and B. Ebenman. 2008. Trophically unique species are vulnerable to cascading extinction. The American Naturalist **171**:568-579.

Pimm, S. L., and Raven, P. 2000. Biodiversity extinction by numbers. Nature **403**:843-845.

Pinnegar, J. K., G. D'Anna, C. Pipitone, N. V. C. Polunin, P. Francour, F. Badalamenti, R. Chemello, M. L. Harmelin-Vivien, B. Hereu, M. Milazzo, and M. Zabala. 2000. Trophic cascades in benthic marine ecosystems: lessons for fisheries and protected-area management. Environmental Conservation **27**:179-200.

Pinsky, M. L., O. P. Jensen, D. Ricard, and S. R. Palumbi. 2011. Unexpected patterns of fisheries collapse in the world's oceans. Proceedings of the National Academy of Sciences of the United States of America **108**: doi: 10.1073/pnas.1015313108

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. the ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics **20**:297-330.

Power M. E. 1990. Effects of fish in river food webs. Science **250**:411-415

Power M. E. 1992. Top-down and bottom-up forces in food webs: do plants have primacy. Ecology **73**:733-746

Preisser, E. L., D. I. Bolnick, and M. F. Benard. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology **86**:501-509.

Rau, G.H. 1976. Dispersal of terrestrial plant litter into a subalpine lake. Oikos **1**:153–160.

Richardson, J. S. 1991. Seasonal food limitation of detritivores in a montane stream: an experimental test. Ecology **72**:873-887.

Richardson, J. S. 1992. Coarse particulate detritus dynamics in small, montane streams of southwestern British-Columbia. Canadian Journal of Fisheries and Aquatic Sciences **49**:337-346.

Richardson J. S., C. R. Shaughnessy, and P. G. Harrison. 2004. Litter breakdown and invertebrate association with three types of leaves in a temperate rainforest stream. Archiv fur Hydrobiologie **159**:309-325.

Richardson J. S., Y. Zhang, and L. B. Marczak. 2010. Resource subsidies across the land-freshwater interface and responses in recipient communities. River Research and Applications **26**:55-66.

Ripple, W. J., and R. L. Beschta. 2003. Wolf reintroduction, predation risk, and cottonwood recovery in Yellowstone National Park. Forest Ecology and Management **184**:299-313.

Romero G. Q., and D. S. Srivastava. 2010. Food-web composition affects cross-ecosystem interactions and subsidies. Journal of Animal Ecology **79**:1122-1131.

Rosemond, A.D., P. J. Mulholland, and S. H. Brawley. 2000. Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and herbivores. Canadian Journal of Fisheries and Aquatic Sciences **57**:66–75.

Rosenfeld, J. S. 1997. The effect of large macroinvertebrate herbivores on sessile epibenthos in a mountain stream. Hydrobiologia **344**:75-79.

Rossberg, A. G., A. Brannstrom, and U. Dieckmann. 2010. How trophic interaction strength depend on traits. Theoretical Ecology **3**:13-24.

Ruesink, J. L., and D. S. Srivastava. 2001. Numerical and per capita responses to species loss: mechanisms maintaining ecosystem function in a community of stream insect detritivores. Oikos **93**:221-234.

Salonen, K., J. Sarvala, I. Hakala, and M. L. Viljanen. 1976. Relation of energy and organic-carbon in aquatic invertebrates. Limnology and Oceanography **21**:724-730.

Sanford E. 1999. Regulation of keystone predation by small changes in ocean temperature. Science **283**:2095–2097

Schindler, D. E., S. R. Carpenter, J. J. Cole, J. F. Kitchell, and M. L. Pace. 1997. Influence of food web structure on carbon exchange between lakes and the atmosphere. Science **277**:248-251.

Schlipfer, F. and B. Schmid. 1999. Ecosystem effects of biodiversity: a classification of hypotheses and exploration of empirical results. Ecological Applications **9**:893-912.

Schmitz, O. J. 2008. Effects of predator hunting mode on grassland ecosystem function. Science **319**:952-954.

Schmitz, O. J., P. A. Hamback, A. L. Mathew, and A. P. Beckerman. 2000. Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. The American Naturalist **155**:141-153.

Schmitz, O. J., V. Krivan, and O. Ovadia. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. Ecology Letters **7**:153-163.

Schwartz, M. W., C. A. Brigham, J. D. Hoeksema, K. G. Lyons, M. H. Mills, and P. J. van Mantgem. 2000. Linking biodiversity to ecosystem function: implications for conservation ecology. Oecologia **122**:297-305.

Shaffer, L. R., and J. V. Robinson. 1996. Do damselfly larvae recognize and differentially respond to distinct categories of macroinvertebrates? Journal of Insect Behavior **9**:407-419.

Shurin J. B., E. T. Borer, E. W. Seabloom, K. E. Anderson, C. A. Blanchette, B. Broitman, S. D. Cooper, and B. S. Halpern. 2002. Across-ecosystem comparison of the strength of trophic cascades. Ecology Letters **5**:785-791.

Shurin, J. B., and E. W. Seabloom. 2005. The strength of trophic cascades across ecosystems: predictions from allometry and energetic. Journal of Animal Ecology **74**:1029-1038

Shurin, J. B., D. S. Gruner, and H. Hillebrand. 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. Proceedings of the Royal Society B-Biological Sciences **273**:1-9.

Shurin, J. B., J. L. Clasen, H. S. Greig, P. Kratina, and P. L. Thompson. 2012. Warming shifts top-down and bottom-up control of pond food web structure and function. Philosophical Transactions of the Royal Society B-Biological Sciences **367**:3008-3017.

Siddon, C. E., and J. D. Witman. 2004. Behavioral indirect interactions: multiple predator effects and prey switching in the rocky subtidal. Ecology **85**:2938-2945.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends in Ecology & Evolution **13**:350-355.

Skalski G. T., and J. F. Gilliam. 2001. Functional responses with predator interference: viable alternatives to the Holling type II model. Ecology **82**:3083-3092

Smith, M. D., and A. K. Knapp. 2003. Dominant species maintain ecosystem function with non-random species loss. Ecology Letters **6**:509-517.

Sokolovska, N., L. Rowe, and W. J. Resetaritis. 2000. Fitness and body size of mature odonates. Ecological Entomology 25:239-248.

Soluk, D. A. 1993. Muliple predator effects: predicting combined functional responses of stream fish and invertebrate predators. Ecology **74**:219-225.

Soluk, D. A., and N. C. Collins. 1988. Synergistic inter- actions between fish and invertebrate predators: facilitation and interference among stream predators. Oikos **52**:94-100.

Soluk, D. A., and J. S. Richardson. 1997. The role of stoneflies in enhancing growth of trout: a test of the importance of predator-predator facilitation within a stream community. Oikos **80**:214-219.

Sousa, W. P. 1984. The role of disturbance in natural communities. Annual Review of Ecology and Systematics **15**:353-391.

Srivastava, D. S. 2006. Habitat structure, trophic structure and ecosystem function: interactive effects in a bromeliad-insect community. Oecologia **149**:493-504.

Srivastava, D. S., J. Emmett Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature **443**:989-992.

Staddon P., Z. Lindo, P. Crittenden, F. Gilbert, and A. Gonzalez. 2010. Connectivity, non-random extinction and ecosystem function in experimental metacommunities. Ecology Letters **13**:543-552.

Stamps, J. 1995. Territory acquisition in lizards: III. Competing for space. Animal Behaviour **49**:679-693.

Starzomski, B. M., D. Suen, and D. S. Srivastava. 2010. Predation and facilitation determine chironomid emergence in a bromeliad-insect food web. Ecological Entomology **35**:53-60.

Steffan, S. A., and W. E. Snyder. 2010. Cascading diversity effects transmitted exclusively by behavioral interactions. Ecology **91**:2242-2252.

Stewart-Oaten, A., W. W. Murdoch, and S. J. Walde. 1995. Estimation of temporal variability in populations. American Naturalist **146**:519-535.

Strong D. R. 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. Ecology **73**:747-754.

Teoduro, C. R., P.A. del Giorgio, Y. T. Prairie, and M. Camire. 2009. Patterns in pCO$_2$ in boreal streams and rivers of northern Quebec, Canada. Global Biogeochemical Cycles **23**: GB2012, DOI: 10.1029/2008GB003404.

Thebault, E., and M. Loreau. 2003. Food-web constraints on biodiversity–ecosystem functioning relationships. Proceedings of the National Academy of Sciences of the USA **25**:14949–14954.

Thompson, P. L., and J. B. Shurin. 2012. Regional zooplankton biodiversity provides limited buffering of pond ecosystems against climate change. Journal of Animal Ecology **81**:251-259.

Thompson R. M., M. Hemberg, B. M. Starzomski, and J. B. Shurin. 2007. Trophic levels and trophic tangles: the prevalence of omnivory in real food webs. Ecology **88**:612-617.

Tilman, D. 1999. The ecological consequences of changes in biodiversity: a search for general principles. Ecology **80**:1455-1474.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature **379**:718-720.

Tilman, D., C. L. Lehman, and K. T. Thomson. 1997. Plant diversity and ecosystem productivity: theoretical considerations. Proceedings of the National Academy of Sciences of the United States of America **94**:1857-1861.

Tracy, C. R., and T. L. George. 1992. On the determinants of extinction. The American Naturalist **139**:102-122.

Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters **11**:1351-1363.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines

plant biodiversity, ecosystem variability and productivity. Nature **396**:69-72.

Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. Annual Reviews in Ecology and Systematics **33**:341-370.

Vanni, M., A. Flecker, J. Hood, and J. Headworth. 2002. Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. Ecology Letters **5**:285-293.

Vasas V., and F. Jordan. 2006. Topological keystone species in ecological interaction networks: considering link quality and non-trophic effects. Ecological Modeling **196**:365-378.

Vonesh, J. R., and C. W. Osenberg. 2003. Multi-predator effects across life-history stages: non-additivity of egg- and larval-stage predation in an African treefrog. Ecology Letters **6**:503-508.

Walker, B. 1995. Conserving biological diversity through ecosystem resilience. Conservation Biology **9**:747-752.

Wallace J. B., S. L. Eggert, Judy L. Meyer, and J. R. Webster. 1999. Effects of Resource Limitation on a Detrital-Based Ecosystem. Ecological Monographs **69**:409-442.

Wardle, D. A., D. J. Bellingham, C. P. H. Mulder, and T. Fukami. 2005. Promotion of ecosystem carbon sequestration by invasive predators. Biology Letters **3**:479-482.

Webster J. R., and J. L. Meyer. 1997a. Stream organic matter budgets: An introduction. Journal of the North American Benthological Society **16**:3-13.

Webster J. R., and J. L. Meyer. 1997b. Stream organic matter budgets: A synthesis. Journal of the North American Benthological Society **16**:141-161.

Webster J. R, E. F. Benfield, T. P. Ehrman, M. A. Schaeffer, J. L. Tanks, J. J. Hutchens, and D. J. D'angelo. 1999. What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. Freshwater Biology **41**:687-705.

Werner, E.E., and B. R. Anholt, 1993. Ecological consequences of the tradeoff between growth and mortality rates mediated by foraging activity. American Naturalist **142**:242-272

Whiting, G. J., and J. P. Chanton. 2001. Greenhouse carbon balance of wetlands: methane emission versus carbon sequestration. Tellus B **53**:521-528.

Wilhelm, E., R. Battino, R. J. Wilcock. 1977. Low-pressure solubility of gases in liquid water. Chemical Reviews **77:**219-262.

Wilmers, C. C., J. A. Estes, M. Edwards, K. L. Laidre, and B. Konar. 2012. Do trophic cascades

affect the storage and flux of atmospheric carbon? An analysis of sea otters and kelp forests. Frontiers in Ecology and the Environment **10**:409-415.

Woodcock, B. A., and M. S. Heard. 2011. Disentangling the effects of predator hunting mode and habitat domain on the top-down control of insect herbivores. Journal of Animal Ecology **80**:495-503.

Woodward, G. 2009. Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. Freshwater Biology **54**:2171.

Woodwell, G. M. 1995. Biotic feedbacks from the warming of the Earth', in Woodwell, G. M. and Mackenzie, F. T. (eds.), Biotic feedbacks in the global climatic System. Will the warming feed the warming? Oxford University Press, New York, pp. 3–21.

Woodwell, G. M., and R. H. Whittaker. 1968. Primary production in terrestrial ecosystems. American Zoologist **8**:19–30.

Woodwell, G. M., F. T. Mackenzie, R. A. Houghton, M. Apps, E. Gorham, and E. Davidson. 1998. Biotic feedbacks in the warming of the earth. Climatic Change **40**:495-518.

Worm, B., S. R. Palumbi, E. Sala, K. A. Selkoe, J. J. Stachowicz, R. Watson, E. B. Barbier, N. Beaumont, J. E. Duffy, C. Folke, B. S. Halpern, J. B. C. Jackson, H. K. Lotze, and F. Micheli. 2006. Impacts of biodiversity loss on ocean ecosystem services. Science **314**:787-790.

Yvon-Durocher, G., J. I. Jones, M. Trimmer, G. Woodward, and J. M. Montoya. 2010. Warming alters the metabolic balance of ecosystems. Philosophical transactions of the Royal Society of London.Series B, Biological sciences **365**:2117-2126.

Zotz, G., and V. Thomas. 1999. How much water is in the tank? model calculations for two epiphytic bromeliads. Annals of Botany **83**:183-192.

APPENDIX A: CALCULATIONS FOR DISSOLVED CO_2 CONCENTRATIONS IN WATER SAMPLES USING HEADSPACE EQUALIBRIUM

According to Henry's law, the equilibrium value of the mole fraction of gas dissolved in a liquid is directly proportional to the partial pressure of the gas above the liquid surface. Here I used the US EPA Region 1 standard operating procedures (Hudson 2004) to calculate the total CO_2-C concentration (TC) in the original water sample. The total CO_2-C concentration (mg C l^{-1}) in the aqueous phase is calculated using the following equation:

$$TC = C_A + C_{AH}$$

where, C_A is the CO_2-C gas concentration in water after equilibrium (mg C l^{-1}), and C_{AH} is the CO_2-C gas concentration in headspace after equilibrium (mg C l^{-1}). The following steps provide the equations to calculate C_A and C_{AH}.

Step 1: Converting gaseous concentrations to partial pressure

The CO_2 values obtained for each sample using a calibration curve from CO_2 standards is expressed in units of ppm based on the volume of CO_2 in the total volume of sample. This concentration is converted to a partial pressure of CO_2 at atmospheric pressure (p_g) using the following equation:

$$p_g \text{ (atm } CO_2) = C_g * p_t$$

where, p_t is the total pressure (assumed to be 1 atm for this thesis), and C_g is the partial pressure of CO_2 expressed as a decimal fraction, calculated using the following equation:

$$C_g = CO_2 \text{ ppm} * 10^{-6}$$

where, 10^{-6} converts ppm to the decimal fraction.

Step 2: Calculating Henry's law constant for CO_2

Henry's law constant (atm per mol fraction) for CO_2 is calculated using the following equation:

$$K_h = 1 / [\exp \{(-317.65 + 17371.2 / (T_w + 273) + 43.06 \ln (T_w + 273) + -0.0022 * (T_w + 273)) / R\}]$$

where, T_w is the temperature of the sample (°C) and R is the universal gas constant 1.98719 cal K^{-1} mol^{-1}. Additional constants for CO_2 were obtained from Wilhelm *et al.* (1977).

Step 3: Calculating C_A (CO_2-C gas concentration in water after equilibrium)

The CO_2-C gas concentration in water after equilibrium (C_A) in units of mg C l^{-1} is calculated using the following equation:

$$C_A = C_{(H2O)} * ((p_g / K_h)*12* 10^3$$

where, $C_{(H2O)}$ is the molar concentration of water (55.5 mol l^{-1}), p_g is the partial pressure of CO_2 at atmospheric pressure (atm CO_2 mol fraction^{-1}) calculated in Step 1, K_h is the Henry Law constant (atm per mol fraction), 12 represents the carbon content of a mole of CO_2 (g C mol^{-1}), and 10^3 converts g C l^{-1} to mg C l^{-1}.

Step 4: Calculating the density of CO_2

For any CO_2, its density (ρ) in g C l^{-1} can be calculated at standard temperature by using the following equation:

$$\rho = ((12 / 44) / 22.4)) * (273 / (T_w + 273))$$

where 22.4 is the volume of one mole of an ideal gas at STP and 12/44 represents the carbon content of CO_2 (g C mol^{-1}).

Step 5: Calculating C_{AH} (CO_2-C gas concentration in headspace after equilibrium)

The CO_2 gas concentration in the headspace after equilibrium (C_{AH}) in units of mg C l^{-1} is calculated using the following equation:

$$C_{AH} = ((V_h / (V_b - V_h)) * C_g * \rho* 10^3$$

where, V_h is the volume in the headspace (ml), V_b is the volume of the bottle from which the gas was extracted (ml), C_g is the decimal fraction of the volumetric concentration of CO_2 calculated in Step 1, ρ is the density of CO_2 (g C l^{-1}) calculated in Step 4, and 10^3 converts g C l^{-1} to mg C l^{-1}.

Example calculation

Here, I provide a sample calculation for sample with an initial measured CO_2 value of 1000 ppm, a sample temperature of $25°$ C, an atmospheric pressure of 1 atm, a bottle volume of 20 ml, and a headspace volume of 15 ml.

Step 1: Converting gaseous concentrations to partial pressure

$$C_g = CO_2 \text{ ppm} * 10^{-6} = 1000 \text{ ppm} * 10^{-6} = 10^{-3} \text{ vol } CO_2 \text{ / vol sample}$$
$$p_g = C_g * p_t = 10^{-3} * 1 \text{ atm} = 10^{-3} \text{ atm}$$

Step 2: Calculating Henry's law constant for CO_2

$$K_h = 1 / [\exp\{(-317.65 + 17371.2 / (T_w + 273) + 43.06 \ln (T_w + 273) + -0.0022 * (T_w + 273)) / R\}]$$
$$K_h = 1 / [\exp\{(-317.65 + 17371.2 / (25 + 273) + 43.06 \ln (25 + 273) + -0.0022 * (25 + 273))/ 1.98719 \}] = 1629 \text{ atm/mol fraction}$$

Step 3: Calculating C_A (CO_2-C gas concentration in water after equilibrium)

$$C_A = C_{(H2O)} * ((p_g / K_h)*12) * 10^3 = 55.5 * ((10^{-3} / 1629) * 12) * 10^3 =$$
$$C_A = 0.408 \text{ mg C l}^{-1}$$

Step 4: Calculating the density of CO_2

$$\rho = ((12 / 44) / 22.4)) * (273 / (T_w + 273)) = ((12 / 44) / 22.4)) * (273 / (25 + 273)) = 1.12$$
$$\text{x } 10^{-2} \ \rho = 1.12 \text{ x } 10^{-2} \text{ g C l}^{-1}$$

Step 5: Calculating C_{AH} (CO_2-C gas concentration in headspace after equilibrium)

$$C_{AH} = ((V_h / (V_b - V_h)) * C_g * \rho * 10^3 = ((15 / (20 - 15)) * 10^{-3} * 1.12 \text{ x } 10^{-2} * 10^3$$
$$C_{AH} = 3.34 \text{ x } 10^{-2} \text{ mg C l}^{-1}$$

Step 6: Calculating total CO$_2$-C concentration (mg C l^{-1})

$$TC = C_A + C_{AH} = 0.408 + 3.34 \text{ x } 10^{-2} = 0.44 \text{ mg C l}^{-1}$$