2011

The influence of predation on the feeding ecology of trinidadian guppies using chemical tracers

Caroline Dennis
University of Windsor

THE INFLUENCE OF PREDATION ON THE FEEDING ECOLOGY OF TRINIDADIAN GUPPIES USING CHEMICAL TRACERS

by

Caroline A. Dennis

A Thesis

Submitted to the Faculty of Graduate Studies
through the Great Lakes Institute for Environmental Research
in Partial Fulfillment of the Requirements for
the Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

2011

THE INFLUENCE OF PREDATION ON THE FEEDING ECOLOGY OF TRINIDADIAN GUPPIES USING CHEMICAL TRACERS

by

Caroline Dennis

APPROVED BY:

_____

Dr. I. Samson
Department of Earth and Environmental Sciences

_____

Dr. D. Haffner
Great Lakes Institute for Environmental Research

_____

Dr. T. Pitcher, Advisor
Department of Biological Sciences

_____

Dr. A.T. Fisk, Advisor
Great Lakes Institute for Environmental Research

_____

Dr. D. Heath, Chair of Defense
Great Lakes Institute for Environmental Research

August 26, 2011

DECLARATION OF CO-AUTHORSHIP/ PREVIOUS PUBLICATION

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research undertaken under the supervision of Drs. Aaron Fisk and Trevor Pitcher, as follows: Chapter 2 contains material from a manuscript entitled "Diet discrimination factors are inversely related to $\delta^{15}N$ and $\delta^{13}C$ values of food for fish under controlled conditions" that has been published in *Rapid Communications in Mass Spectrometry*. This manuscript is coauthored by Dennis CA, MacNeil MA, Rosati JY, Pitcher TE and Fisk AT. Chapter 3 contains material from a manuscript entitled "Feeding ecology and niche width differences among freshwater fish populations experiencing variable predation intensity" that has been submitted to the *Journal of Animal Ecology*. This manuscript is coauthored by Dennis CA, Pitcher TE, Ramnarine IW, Rush SA, McMeans BA and Fisk AT. Throughout the dissertation, the main ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author, and the contribution of co-authors was primarily through assistance with analytical and statistical procedures, as well as helping with revising early drafts.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-authors to include the above materials in my thesis. I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

II. Declaration of Previous Publication

This thesis includes 2 original papers that have been previously published/submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication title/full citation	Publication status
Chapter 1	Diet discrimination factors are inversely related to $\delta^{15}N$ and $\delta^{13}C$ values of food for fish under controlled conditions	Published
Chapter 2	Feeding ecology and niche width differences among freshwater fish populations experiencing variable predation intensity	Submitted

I certify that I have obtained a written permission from the copyright owner to include the above published material in my thesis. I certify that the above material describes work completed during my registration as a graduate student at the University of Windsor.

I declare that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary right and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner to include such material in my thesis.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

ABSTRACT

This dissertation demonstrates that diet discrimination factors (DDF) have a strong negative correlation with stable isotope values in food for $\delta^{15}N$ (slope=-0.59±0.02, r^2=0.95) and $\delta^{13}C$ (slope =-0.56±0.02, r^2=0.94). Based on these results, a reliable DDF was used to evaluate the influence of predation on the trophic ecology of wild guppy populations in Trinidad. The trophic position of the guppies was 2.7 and did not differ between high- and low-predation sites (p=0.77). Nevertheless, high-predation populations had significantly higher proportions of the fatty acids associated with algae than low-predation populations which had higher proportions of fatty acids associated with invertebrates (p= 0.005). Additionally, standard ellipse area, a measure of isotopic niche width, was greater at high-predation sites compared to low-predation sites within Quare (p<0.001), Turure (p=0.003) and Tacarigua/Tunapuna (p=0.016) but not Aripo (P=0.19). Therefore, low-predation guppy populations appear to have diets more specialized on invertebrates, while high-predation guppies consume resources more indiscriminately.

ACKNOWLEDGMENTS

I would like to start by thanking my supervisor, Dr. Aaron Fisk, for his patience and support throughout my studies at the University of Windsor. Not only has he provided invaluable guidance in my field of study, but he has also been an inspirational role model for me over the years. Special thanks to my co-supervisor, Dr. Trevor Pitcher for his advice and assistance in the field as well as my committee members Dr. Iain Samson and Dr. Doug Haffner for their involvement in this project.

I would like to acknowledge and thank Sandra Ellis, Anna Hussey, Eric Primeau and Mary-Lynn Mailloux for their assistance with data processing and analyses as well as Nigel Hussey and Jaclyn Brush for discussion and advice. I am also very grateful to the other students in both the Fisk and Pitcher labs at the University of Windsor for their encouragement, friendship, and input.

This research was funded by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grants to Trevor Pitcher and Aaron Fisk. Additionally support was provided in the form of NSERC-PGM and an Ontario Graduate Scholarship.

Most importantly I would like to thank my family for their continued support throughout my studies. In particular, I would like to thank my parents for their love and understanding, without which I would not have made it this far and my dear friend Erica who has always encouraged me to reach for the stars and climb every mountain higher. Finally, I would like to thanks my fiancé Gian who has always motivated me to be a

better person and who agreed to postpone our wedding so that I could get my degree. I can't wait to start our new life together in Florida!

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2

Chapter 3

LIST OF FIGURES

Chapter 2

Chapter 3

1.0 – GENERAL INTRODUCTION

1.1 THE INFLUENCE OF PREDATION ON PREY SPECIES

Predation is a major factor influencing ecosystem dynamics and functioning (Connell 1970; Paine 1974; Carpenter et al.1985). The idea of trophic interactions whereby the influence of a predator can be transmitted through the food web is exemplified by Paine's pioneering study on rocky intertidal shores that showed how the removal of a keystone predator can cause changes in community structure and the distribution of species (Paine 1974). Since then, numerous other studies have investigated manipulations of predation intensity and the propagation of its effects through multiple trophic links in the food web. For example Power et al. (1985) saw a decrease in algae grazing minnows (*Campostoma anomalum*) and an increase in algae in Oklahoma streams when bass species (*Micropterus salmoides and M. punctilatus*) were artificially introduced to a pool. Similarly, Schmitz et al. (1997) manipulated predatory spiders (*Pisurina mira*) to be harmless to their prey, grasshopper nymphs (*Melanoplus femurrubrum*), and witnessed a trophic cascade whereby the amount of plant biomass consumed by the grasshopper nymphs was reduced. The reduction in plant biomass consumed resulted from the grasshopper nymphs' decreased activity time and diet shifts and suggests that the feeding behaviour of an organism can vary due to the presence of a predator even when the predator does not cause a direct lethal effect to its prey. Therefore, changes that occur at one level in the food chain have the ability to influence other trophic levels either directly through reductions in biomass or indirectly through behavioural modifications of the prey species (Huang and Sih 1990; Spiller and Schooner 1990; Schmitz et al. 1997).

Behavioural changes under predation risks are well documented in the literature (review see Lima and Dill 1990). Often, an organism's vulnerability to predation will depend upon the activities it engages in and the decisions it makes. In particular, an animal must make important decision in regards to feeding and must constantly balance their need to intake energy against the risk of predation. Organisms have been shown to alter the timing of feeding, the location of foraging activity and diet choice depending on the predation pressure they are being exposed to (Werner et al. 1983; Dill and Fraser 1984; Brown et al. 1988; Lima and Dill 1990; Abramsky et al. 1996; Rothley et al. 1997; Schmitz et al. 1997). For example, desert rodents were more likely to forage in a safer bush microhabitat compared to a more risky open microhabitat when they perceived that a predator was present (Brown et al. 1988). Behavioural modifications due to predation are also well documented for fish. For example, juvenile coho salmon (*Oncorhynchus kisutch*) reduced their attack distance when they were presented with a photograph model of a predator (Dill and Fraser 1984). This reduction in attack distance likely decreased the probability of an individual being detected by a predator but also decreased the size of the prey captured (Dill and Fraser 1984). Furthermore, the attack distance was influenced by the level of the perceived predation risk, the presence of a competitor and the hunger level of the individual, demonstrating how interacting factors can affect the behaviour of fish under predation threat (Dill and Fraser 1984).

Understanding the role of predators and the consequences that the removal or addition of a predator can have on entire food webs is particularly important considering the large impact that human activities are having on ecosystems around the world. Significant declines in predatory fish have been reported in the ocean due to

industrialized fishing and numerous examples of introduced non-native predators have been observed (e.g. Witte et al. 1992; Myers and Worm 2003; Heithaus et al. 2008). For example, large sharks have declined in significant numbers releasing their prey from predation (Myers et al. 2007). Among the prey species released from predation is the cownose ray (*Rhinoptera bonasus*) which has increased by an order of magnitude since the 1970's in the northwest Atlantic Ocean (Myers et al. 2007). Cownose rays feed heavily on bivalves such as bay scallops (*Argopecten irradians*) and a potential cause of the collapsed bay scallop fishing industry in North Carolina in 2004. Clearly, the influence of human mediated predator changes has important consequences for the sustainability of ecosystems and human industry.

1.2 THE USE OF CHEMICAL TRACERS TO STUDY SPECIES INTERACTIONS

Chemical tracers, such as stable isotopes and fatty acids, provide a tool for testing the influence of predation on a system. Isotopes are atoms of an element that have the same number of protons and electrons in the nucleus, but differ in the number of neutrons. Since isotopes have the same number of protons and electrons, their chemical behaviour is nearly identical (Peterson and Fry 1987). However, the differing number of neutrons causes different isotopes of an element to have a different atomic mass. For example, ^{12}C has 6 protons and 6 neutrons while ^{13}C and ^{14}C have the same number of protons but 7 and 8 neutrons, respectively. Additionally, stable isotopes will persist in the same form in the environment and will not radioactively decay. Both ^{12}C and ^{13}C are stable while ^{14}C is radioactive and will decay to ^{14}N.

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are valuable ecological tools used to evaluate trophic relationships (Peterson and Fry, 1987), carbon and nutrient

sources (DeNiro and Epstein, 1978; Peterson and Fry, 1987), and the dietary breadth of an organism (Bearhop et al. 2004; Layman et al. 2007; Jackson et al. 2011). Stable isotopes are commonly expressed in δ notation ($\delta X=[(R_{sample}/R_{standard})-1] \times 10^3$) where X represents ^{13}C or ^{15}N and R represents the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987). The expression of stable isotopes using this notation enables comparisons to be made among labs. Increases in the heavy isotope relative to the light isotope will cause the δ value to increase while decreasing the amount of heavy isotope relative to the light isotope will cause the δ value to decrease (Peterson and Fry 1987).

Stable isotopes have valuable applications in ecology because of the way isotopes fractionate in nature. Although isotopes have similar chemical behaviour, the lighter isotope typically reacts faster during chemical reactions (Peterson and Fry 1987). For this reason, stable isotopes can be used to study diet and food web interactions among species. For example, stable isotopes of carbon are frequently used to determine the source of diets because the different sources have distinct stable isotope values and $\delta^{13}C$ values change little (about 1‰ per trophic link) as it is transferred from prey to predator (Peterson and Fry 1987; France 1995). Therefore, anything feeding on a particular source will have a $\delta^{13}C$ value reflective of what it is eating (Peterson and Fry 1987). For example, benthic algae have been found to have higher $\delta^{13}C$ values (by about 7‰) compared to planktonic algae due to limited CO_2 diffusion below a stagnant boundary layer (France 1995). Since CO_2 is limited in benthic environments, the algae will assimilate more ^{13}C than in planktonic environments where the ^{13}C would be discriminated against (France 1995). Organisms feeding on these two algae sources will then have a $\delta^{13}C$ value reflective of the environment from which the algae came from.

Conversely, stable isotopes of nitrogen increase, on average by about 3-5‰, with every increase in trophic level due to the preferential loss of ^{14}N in metabolism (Peterson and Fry 1987). Due to this progressive enrichment of ^{15}N at higher trohpic levels, $\delta^{15}N$ is often used to estimate the trophic position of an organism using the formula:

$$TL = \lambda + (\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}})/\Delta_n$$

where λ is the trophic position of the baseline organism and Δ_n is the enrichment of $\delta^{15}N$ per trophic level (Post 2002). Although a baseline organism is not required to study relative trophic position within a single system, a baseline correction is needed for studies that compare across multiple systems because stable isotope values at the base of the food chain can be variable (Rounick and Winterbourn 1986; Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999). Understanding trophic positions within a system is important because it provides information about ecological efficiencies and energy transfer within a system (Hairston and Hairston 1993). Hairston and Hairston (1993) suggest that the number of trophic levels present is an important predictor of the efficiency that energy is transferred to higher trophic levels.

Another important component of ecosystem health and stability that stable isotopes are able to assess is the niche width of an organism or group of organisms. Elton (1927) described the niche of an organism as the sum of its biological interactions with other species. Later, Hutchinson (1957) distinguished between the fundamental niche, the broad potential niche space set by taking all of the abiotic factors available to an organism into consideration, and the realized niche, the more specific niche of an organism which accounts for biological interactions. Traditionally, niche width has been analysed using measures of morphology, diet and habitat use (Blondel et al. 1988; Carrascal et al. 1994;

Gosler and Carruthers 1994; McDonald 2002). However, more recently, stable isotopes have been suggested as an additional measure of trophic niche width (Bearhop et al. 2004; Layman et al. 2007; Jackson et al. 2011). Bearhop et al. (2004) suggests that variability in stable isotope values reflect diet in terms of what is being consumed, how much of each diet item is being consumed, the trophic level of prey items, location of foraging, an individual`s physiology, and diet discrimination factors. For example, the variability in stable isotope values is expected to be greater for a generalist population than a specialist population (Bearhop et al. 2002). Furthermore, Layman et al. (2007) suggested the use of six metrics derived by plotting stable isotope values in $\delta^{13}C$-$\delta^{15}N$ bi-plot space to analyze the trophic niche width of a community or population. These metrics include the range of $\delta^{15}N$ values, range of $\delta^{13}C$ values, total area of the convex hull, mean distance to the centroid, mean nearest neighbour distance, and standard deviation of the nearest neighbour distance (Layman et al. 2007). By examining these six metrics, Layman et al. (2007) suggests that the extent of spacing and redundancy of a population or community can be assessed. Most recently, Jackson et al. (2011) expanded on Layman's idea and suggested the use of sample size corrected standard ellipses in a Bayesian framework to compare niche width among populations. However, there are several caveats to using stable isotopes to look at niche width, including the fact that the stable isotope variability of the prey species must be taken into consideration (Hoeinghaus and Zeug 2008)

Although chemical tracers like stable isotopes and fatty acids are commonly employed for a variety of purposes in ecological research, several assumptions about their use require further evaluation (Gannes et al. 1997; Jardine et al. 2006; Wolf et al. 2009).

One prominent example is the use of a constant diet discrimination factor based on published reviews (eg. Schaal et al. 2009; Syvaranta et al. 2010). Diet discrimination factors ($\Delta\delta X$) are defined as the difference in the $\delta^{13}C$ and $\delta^{15}N$ value between an organism and its diet ($\Delta\delta X = \delta X_{consumer} - \delta X_{food}$; $X = \delta^{13}C$ or $\delta^{15}N$). Diet discrimination factors are required for isotopic modelling (e.g. Ben-David et al. 1997; Szepanski et al. 1999; Phillips and Koch 2002) and to calculate trophic position (eg. Post 2002, Fisk et al. 2003). Nevertheless, studies have shown that diet discrimination factors are not constant and can vary across species (Post 2002; Caut et al. 2009) and taxonomic classes (Vanderklift and Ponsard 2003), within species across different temperature regimes (Frazer et al. 1997; Power et al. 2003), and among different tissues within the same organism (Hobson and Clark 1992). Most recently, diet discrimination factors have been shown to vary according to diet $\delta^{13}C$ and $\delta^{15}N$, with diet discrimination factors decreasing with increasing $\delta^{13}C$ or $\delta^{15}N$ values in the food (Overmyer et al. 2008; Caut et al. 2008, 2009). Evidentially, developing a species specific diet discrimination factor that considers the $\delta^{13}C$ or $\delta^{15}N$ values of the diet of the organism under study is of fundamental importance before conclusions can be drawn about stable isotope data and the effects of predation. Furthermore, the effect of variable $\delta^{13}C$ or $\delta^{15}N$ values in the food using a single food type has not been assessed.

Fatty acids are another tool that can be used to analyse trophic interactions. Fatty acids are a class of lipids consisting of carbons chains generally ranging in length between 12 and 24 carbon atoms (Napolitano 1999). Some fatty acids are considered essential meaning that they must be acquired in the diet in order to meet an organism's needs and cannot be produced at sufficient levels by the individual itself (Olsen 1999).

There are several well established fatty acid markers in freshwater and marine ecosystems (Napolitano 1999). One prominent example is the use of palmitoleic acid (16:1n-7) and eicosapentaenoic acid (20:5n-3) as markers for diatoms (Parrish et al. 1995; Napolitano et al. 1997; Torres-Ruiz et al. 2007).

In recent years, essential fatty acids have proven to be related to the health and stability of ecosystems (Müller-Navarra et al. 2000). For example, low levels of eicosapentaenoic acid (20:5n-3) in primary producers were associated with poor energy transfer efficiencies from primary producers to consumers in an aquatic ecosystem (Müller-Navarra et al. 2000). Fatty acids such as arachidonic acid (C20:4n-6) and docosahexaenoic acid (C22:6n-3) are also a strong indicator of an organism's physiological health and functioning. Diets manipulated to consist of low levels of docosahexaenoic acids caused impaired vision in larval sea bass (*Dicentrarchus labrax*) even under high light intensities and a diet high in arachidonic acid improved the survival of gilthead seabream larvae (*Sparus aurata*) following handling stress (Bell et al. 1996; Koven et al. 2001, respectively). Furthermore, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid were found to reduce triglyceride concentration which have been linked to heart disease in humans (Harris 1997).

1.3 TRINIDADIAN GUPPIES AS A STUDY SPECIES

An ideal way to study the effects of predation is within a system that is experiencing varying levels of predation intensity. Guppy populations from rivers in Northern Trinidad provide an excellent model system to study the impacts of predation on feeding behaviour. Guppies (*Poecilia reticulata*) are small freshwater fish that are members of the Poeciliidae family. They are live bearing fish and produce offspring

through internal fertilization using the male reproductive organ called the gonopodium (Magurran 2005). Adult males are typically smaller than females and vary greatly in their brightness and colouration patterns, which are heritable traits (Haskins et al. 1970; Endler 1980). Conversely, females are characterised by a dull grey-brown colour and do not display the bright colouration patterns of the males. Generation times are relative short for the guppy (approximately 210 days) and females chose mates based on secondary sexual characteristics, such as colour patterns (Reznick et al. 1997).

Within Trinidad, waterfalls restrict the movement of guppy predators thereby producing varying levels of predation between upstream and downstream populations. Previous research on these populations has demonstrated genetic divergence across populations under different environmental pressure that have been manifested in varying characteristics, such as life history traits (Reznick and Endler, 1982) and colouration patterns (Endler 1980), that differ depending on the level of predation that the guppies have experienced. The existence of different predation regimes within a river system is replicated a number of times in Northern Trinidad allowing for comparisons to be made among high and low predation sites. The pike cichlid, *Crenicichla alta*, is generally the most significant predator of guppies at the high predation locations but a variety of other predators including other fish species, invertebrates, and avian predators may also be present (Endler 1983; Magurran 2005). At low predation sites, the gape limited *Rivulus hartii* is the most significant predator and this species is only able to prey upon juvenile guppies (Magurran 2005). Although, difference between high predation and low predation sites is often attributed to differences in predation intensity, a number of other characteristics differ between these locations. For example, it has been suggested that the

guppies at high predation sites have a greater amount of resources available to them than the guppies at low predation populations due to higher levels of primary productivity and lower guppy densities (Reznick et al. 2001). Additionally, there is variation among high predation sites, with different predator and fish assemblages present at each high predation location (Endler 1983). However, other characteristics, such as water depth, flow regime, water clarify and substratum, are fairly similar between proximal sites within a river system (Magurran 2005). Furthermore, predation is a major driving force on the evolution of guppies in Trinidad as several studies have shown that guppies transplanted from a high-predation location to a low-predation location rapidly evolved characteristics typical of the low-predation populations in both natural and laboratory experiments (Endler, 1980; Reznick and Bryga 1987; O'Steen et al. 2002).

Guppies have been shown to behave differently under different predation regimes in Trinidad. For example, schooling behaviour is well developed in high predation environments but much less pronounced under low predation threat (Seghers 1974). Feeding behaviour of guppies has also been shown to differ under different predations regimes. Guppies experiencing more intense predations pressure have been observed to restrict their feeding activities to the periphery and to shallow areas of the river (Seghers, 1970) and spend less time foraging (Magurran and Seghers 1994). High predation guppies also were less likely to reduce their feeding rates when confronted with a predator stimulus (Fraser and Gilliam 1987).

Although the diet of wild guppy populations in Trinidad has been studied (Dussault and Krammer 1981; Bassar et al. 2010; Zandonà 2010; Zandonà et al. 2011), most of these studies used conventional gut analysis and did not employ chemical tracers,

such as fatty acids and stable isotopes. There are many caveats to the use of gut content analysis including unequal digestibility of food items, difficulty in content identification, empty stomach contents and temporal bias (Vander Zaden and Rasmussen 1996; Sheffield et al. 2001). Additionally, the studies found opposing results among different rivers, years, and seasons (Dussault and Krammer 1981; Bassar et al. 2010; Zandonà 2010; Zandonà et al. 2011). For example, low predation guppy populations had a greater proportion of invertebrates in their guts than high predation populations during the wet season but the pattern was reversed during the dry season (Zandonà 2010). Despite these difficulties, these studies suggest that the guppy's diet consisted mainly of algae, detritus, and benthic invertebrates (Dussault and Krammer 1981; Bassar et al. 2010; Zandonà 2010; Zandonà et al. 2011). Although guppies can live on a diet consisting exclusively of algae, guppies fed an algae diet displayed slower growth rates than diets that included animal material (Dussault and Krammer 1981). A proposed explanation for the difference in diet between high and low predation sites is increased interspecific competition for invertebrate prey at high predation locations where there are more diverse fish assemblages than at low predation locations (Dussault and Krammer 1981). Alternatively, Murdoch et al. (1975) demonstrated that guppies will consume a disproportionate amount of the type of food that is most available. Therefore differences in available resources at high and low predation sites could account for difference in the diet of fish populations from contrasting predation environments (Magurran 2005; Reznick et al. 2001).

Several differences in feeding behaviour between the sexes have been reported for the guppy. Dussault and Krammer (1981) found that males consume more food per peck than females and spend less time foraging. Furthermore, Magurran and Seghers (1994)

found that females allocate more of their time to foraging while males dedicate more time to mating strategies.

1.4 RATIONAL AND OBJECTIVES

This thesis investigates the stable isotope values and fatty acid signatures of a freshwater fish species experiencing variable predation intensity and evaluates some of the underlying assumptions regarding the use of stable isotopes in ecological studies. Using chemical tracers, this research will aid in the understanding of the influence that predation can have on the trophic position, niche width, and diet of a prey species. In addition, this thesis will generate information on the trophic ecology of an important model organism used in studies of evolution and ecology.

The main objectives of this study are:

1) To determine if diet discrimination factors are affected by the stable isotope composition and food type of an organism's diet so that more reliable diet discrimination factors can be employed in the field to develop a better understanding of trophic interactions.
2) To compare the niche width, trophic position, and diet of guppy populations from high and low predation regimes in order to better understand how predation affects the feeding behaviour of an organism.

1.5 REFERENCES

Abramsky Z, Strauss E, Subach A, Kotler BP, Riechman A (1996) The effect of barn owls (*Tyto alba*) on the activity and microhabitat selection of *Gerbillus allenbyi* and *G. pyramidum.* Oecologia 105: 313-319

Bassar RD, Marshall MC, López-Sepulcre A, Zandonà E, Auer SK, Travis J, Pringle CM, Flecker AS, Thomas SA, Fraser DF, Reznick DN (2010) Local adaptation in Trinidadian guppies alters ecosystem processes. Proc Natl Acad Sci USA 107: 3616-3621

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73: 1007-1012

Bell MV, McEvoy LA, Navarro JC (1996) Deficit of didocosahexaenoyl phospholipid in the eyes of larval sea bass fed an essential fatty acid deficient diet. J Fish Biol 49: 941-952

Ben-David M, Flynn RW, Schell DM (1997) Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia 111: 280–291

Blondel J, Chessel D, Frochot B (1988) Bird species impoverishment, niche expansion, and density inflation in mediterranean island habitats. Ecology 69: 1899-1917

Brown JS, Kotler BP, Smith RJ, Wirtz WO II (1988) The effects of owl predation on the foraging behavior of heteromyid rodents. Oecologia 76: 408-415

Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. Proc Natl Acad Sci USA 93:10844-10847

Carpenter SR, Kitchell JF, Hodgson JR (1985) Cascading trophic interactions and lake productivity. BioScience 35: 634-639

Carrascal LM, Moreno E, Valido A (1994) Morphological evolution and changes in foraging behaviour of island and mainland populations of Blue Tit (*Parus caeruleus*) – a test of convergence and ecomorphological hypotheses. Evol Ecol 8: 25-35

Caut S, Angulo E, Courchamp F (2008) Discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$) in an omnivorous consumer: effect of diet isotopic ratio. Funct Ecol 22: 255-263

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46: 443-453

Connell JH (1970) A predator-prey system in the marine intertidal region. I. *Balanus glandula* and several predatory species of *Thais*. Ecol Monogr 40**:** 49-78

DeNiro MJ, Epstein S (1978) Carbon isotopic evidence for different feeding patterns in two hydrax species occupying the same habitat. Science 201**:** 906–908

Dill LM, Fraser AHG (1984) Risk of predation and the feeding behavior of juvenile coho salmon (*Oncorhynchus kisutch*). Behav Ecol Sociobiol 16: 65-71

Dussault GV, Kramer DL (1981) Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). Can J Zool 59: 684-701

Elton CS (1927) Animal ecology. Sidgwick and Jackson, London, UK

Endler JA (1980) Natural selection on color patterns in *Poecilia reticulata*. Evolution 34: 76-91

Endler JA (1983) Natural and sexual selection on color patterns in poeciliid fishes. Enviro Biol Fishes 9: 173-190

Fisk AT, Hoekstra PF, Gagnon JM, Duffe J, Norstrom RJ, Hobson KA, Kwan M, Muir DCG (2003) Influence of habitat, trophic ecology and lipids on, and spatial trends of,

organochlorine contaminants in Arctic marine invertebrates. Mar Ecol Prog Ser 262: 201-214

France RL (1995) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol Oceanogr 40: 1310-1313

Fraser DF, Gilliam JF (1987) Feeding under predation hazard: response of the guppy and Hart's rivulus from sites with contrasting predation hazard. Behav Ecol Sociobiol 21: 203-209

Frazer TK, Ross RM, Quetin LB, Montoya JP (1997) Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: a stable isotope approach. J Exp Mar Biol Ecol 212: 259-275

Gannes LZ, O'Brien DM, Martinez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78: 1271-1276

Gosler AG, Carruthers TD (1994) Bill size and niche breadth in the Irish Coal Tit *Parus ater hibernicus*. J Avian Biol 25: 171-177

Hairston NG Jr., Hairston NG Sr. (1993) Cause-effect relationships in energy flow, trophic structure and interspecific interactions. Am Nat 142: 379-411

Harris WS (1997) n-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 65: 1645S-1654S

Haskins CP, Young P, Hewitt RE, Haskins EF (1970) Stabilised heterozygosis of supergenes mediating certain y-linked colour patterns in populations of *Lebistes reticulatus*. Heredity 25: 575-589

Heithaus MR, Frid A, Wirsing AJ, Worm B (2008) Predicting ecological consequences of marine top predator declines. Trends Ecol Evol 23: 202-210

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor 94: 189-197

Hoeinghaus DJ, Zeug SC (2008) Can stable isotope ratios provide for community-wide measures of trophic structure? Comment. Ecology 89: 2353-2357

Houde AE (1987) Mate choice based upon naturally occurring color-pattern variation in a guppy population. Evolution 41: 1-10

Huang C, Sih A (1990) Experimental studies on direct and indirect interactions in a three trophic-level stream system. Oecologia 85: 530-536

Hutchinson GE (1957). Concluding remarks. Cold Spring Harb Symp Quant Biol 22: 415-427

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80: 595-602

Jardine TD, Kidd KA, Fisk AT (2006) Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. Environ Sci Technol 40: 7501-7511

Koven W, Barr Y, Lutzky S, Ben-Atia I, Weiss R, Harel M, Behrens P, Tandler A (2001) The effect of dietary arachidonic acid (20:4n-6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. Aquaculture 193: 107-122

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88: 42-48

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68: 619-640

Magurran AE (2005) Evolutionary Ecology: The Trinidadian Guppy. Oxford University Press, Oxford.

Magurran AE, Seghers BH (1994) Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata,* populations in Trinidad. Proc R Soc Lond B Biol Sci 255: 31-36

McDonald RA (2002) Resource partitioning among British and Irish mustelids. J Anim Ecol 71: 185-200

Müller-Navarra DC, Brett MT, Liston AM, Goldman CR (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403: 74-77

Murdoch WW, Avery S, Smyth MEB (1975) Switching in predatory fish. Ecology 56: 1094-1105

Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science 315: 1846-1850

Myers RA,Worm B (2003) Rapid worldwide depletion of predatory fish communities. Nature 423: 280-283

Napolitano GE (1999) Fatty acids as trophic and chemical markers in freshwater ecosystems. In: Arts MT, Wainman BC (eds) Lipids in freshwater ecosystems. Springer-Verlag, New York, pp 21-44

Napolitano GE, Pollero RJ, Gayoso AM, MacDonald BA, Thompson RJ (1997) Fatty acids as trophic markers of phytoplankton blooms in the Bahia Blanca Estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). Biochem Syst Ecol 25: 739-755

Olsen Y (1999) Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture. In: Arts MT, Wainman BC (eds) Lipids in freshwater ecosystems. Springer-Verlag, New York, pp 161-202

O'Steen S, Cullum AJ, Bennett AF (2002) Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). Evolution 56: 776-784

Overmyer JP, MacNeil M, Fisk AT (2008) Fractionation and metabolic turnover of carbon and nitrogen stable isotopes in black fly larvae. Rapid Commun Mass Spectrom 22: 694-700

Paine RT (1974) Intertidal community structure. Oecologia (Berl.) 15: 93-120

Parrish CC, McKenzie CH, MacDonald BA, Hatfield EA (1995) Seasonal studies of seston lipids in relation to microplankton species composition and scallop growth in South Broad Cove, Newfoundland. Mar Ecol Prog Ser 129: 151-164

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18: 293-320

Phillips DL, Koch PL (2002) Incorporating concentration dependence in stable isotope mixing models. Oecologia 130: 114–125

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703-718

Power M, Guiguer KRRA, Barton DR (2003) Effects of temperature on isotopic enrichment in *Daphnia magna*: implications for aquatic food-web studies. Rapid Commun Mass Spectrom 17: 1619-1625

Power ME, Matthews WJ, Stewart AJ (1985) Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. Ecology 66: 1448-1456

Reznick DN, Bryga H (1987) Life-history evolution in guppies (*Poecilia reticulata*):1. Phenotypic and genetic changes in an introduction experiment. Evolution 41: 1370-1385

Reznick D, Butler MJ IV, Rodd H (2001) Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. Am Nat 157: 126-140

Reznick D, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36: 160-177

Reznick DN, Shaw FH, Rodd FH, Shaw RG (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275: 1934-1937

Rothley KD, Schmitz OJ, Cohon JL (1997) Foraging to balance conflicting demands: novel insights from grasshoppers under predation risk. Behav Ecol 8: 551-559

Rounick JS, Winterbourn MJ (1986) Stable carbon isotopes and carbon flow in ecosystems. BioScience 36: 171-177

Schaal G, Riera P, Leroux C (2009) Trophic significance of the kelp *Laminaria digitata* (Lamour.) for the associated food web: a between-sites comparison. Estuar Coast Shelf Sci 85: 565-572

Schmitz OJ, Beckerman AP, O'Brien KM (1997) Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. Ecology 78: 1388-1399

Seghers BH (1970) Behavioral adaptations of natural populations of the guppy, *Poecilia reticulata*, to predation. Am Zool 10: 489-490

Seghers BH (1974) Schooling behavior in the guppy (*Poecilia reticulata*): An evolutionary response to predation. Evolution 28: 486-489

Sheffield G, Fay FH, Feder H, Kelly BP (2001) Laboratory digestion of prey and interpretation of walrus stomach contents. Mar Mamm Sci 17: 310-330

Spiller DA, Schoener TW (1990) A terrestrial field experiment showing the impact of eliminating top predators on foliage damage. Nature 347: 469-472

Syväranta J, Cucherousset J, Kopp D, Crivelli A, Céréghino, R, Santoul F (2010) Dietary breadth and trophic position of introduced European catfish *Silurus glanis* in the River Tarn (Garonne River basin), southwest France. Aquat Biol 8: 137-144

Szepanski MM, Ben-David M, Van Ballenberghe V (1999) Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. Oecologia 120: 327–335

Torres-Ruiz M, Wehr JD, Perrone AA (2007) Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. J N Am Benthol Soc 26: 509-522

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia 136: 169-182

Vander Zanden MJ, Rasmussen JB (1996) A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. Ecol Monogr 66: 451-477

Vander Zanden MJ, Rasmussen, JB (1999) Primary Consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80: 1395-1404

Werner EE, Gilliam JF, Hall DJ, Mittelbach GG (1983) An experimental test of the effects of predation risk on habitat use in fish. Ecology 64: 1540-1548

Witte F, Goldschmidt T, Wanink J, van Oijen M, Goudswaard K, Witte-Maas E, Bouton N (1992) The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. Environ Biol Fishes 34: 1-28

Wolf N, Carleton SA, Martinez del Rio C (2009) Ten years of experimental animal isotopic ecology. Funct Ecol 23: 17-26

Zandonà E (2010) The trophic ecology of guppies (*Poecilia reticulata*) from the streams of Trinidad. PhD dissertation, Department of Biology, Drexel University, Philadelphia, Pennsylvania, USA

Zandonà E, Auer SK, Kilham SS, Howard JL, López-Sepulcre A, O'Connor MP, Bassar RD, Osorio A, Pringle CM, Reznick DN (2011) Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. Funct Ecol 25, doi: 10.1111/j.1365-2435.2011.01865.x

2.0 - DIET DISCRIMINATION FACTORS ARE INVERSELY RELATED TO $\delta^{15}N$ AND $\delta^{13}C$ VALUES OF FOOD FOR FISH UNDER CONTROLLED CONDITIONS

2.1 INTRODUCTION

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values in fish can be used to evaluate trophic relationships and assess carbon and nutrient sources (DeNiro and Epstein 1978; Minagawa and Wada 1984; Peterson and Fry 1987). Although the use of stable isotopes as chemical tracers has become a common and powerful tool in ecological research, literature relating to this subject has identified many caveats to their use and has called for controlled calibration of these tracers (Gannes et al. 1997; Jardine et al. 2006; Wolf et al. 2009). Specifically there is currently limited mechanistic understanding of diet discrimination factors ($\Delta\delta X = \delta X_{consumer} - \delta X_{food}$; X = $\delta^{13}C$ or $\delta^{15}N$). Diet discrimination factors (DDF) are required for a variety of stable isotope studies including those that employ isotopic modeling in order to estimate the proportional contribution of different diet items to the isotopic composition of the tissue under consideration and those that estimate trophic position following the logic that the isotopic value of an organism will increase by one DDF each trophic level as you move up the food chain (Peterson and Fry 1987; Hobson and Welch 1992; Ben-David et al. 1997; Szepanski et al. 1999; Phillips and

*modified from: Dennis CA, MacNeil MA, Rosati JY, Pitcher TE, Fisk AT (2010) Diet discrimination factors are inversely related to $\delta^{15}N$ and $\delta^{13}C$ values of food for fish under controlled conditions. Rapid Commun Mass Spectrom 24: 3515-3520

Koch 2002). For these studies, a single DDF is often selected based on published reviews despite the fact that these factors have been shown to vary across species and taxonomic classes, within species across different temperature regimes and among different tissues within the same organism (Hobson and Clark 1992; Post 2002; Power et al. 2003; Vanderklift and Ponsard 2003).

Diet isotopic composition has recently been shown to affect DDFs. An extensive review of 66 publications concluded that diet isotopic value had a significant impact on DDFs and recommended the use of diet-dependent discrimination factors (Caut et al. 2009). Additionally, DDFs were shown to decrease linearly with increasing $\delta^{15}N$ in the black fly, *Simulium vittatum IS*-7 (Overmyer et al. 2008). However, this study stated that the negative $\delta^{15}N$ DDFs could be due to elimination processes such as molting or elimination of feces, lower assimilation of food due to high metabolism and growth rates, and differential assimilation of diet components (Overmyer et al 2008). Therefore there is a need to perform controlled laboratory studies to determine the relationship between diet isotopic value and diet discriminations.

The objective of this study was to estimate DDFs across a range of $\delta^{13}C$ and $\delta^{15}N$ values in food for a fish under controlled conditions. To quantify this relationship, we developed a series of experimental foods of consistent composition over an exceptionally-wide range of $\delta^{13}C$ and $\delta^{15}N$ that would unambiguously determine whether DDFs are concentration-dependent. The $\delta^{13}C$ values of the food in this study were in the range of values (-22.9 to -6.6‰) seen naturally in the environment but the $\delta^{15}N$ of the food ranged from values seen in the environment (6.5‰) to those much higher (1586‰). This range

of stable isotope values permitted strong inferences to be made about the relationship between diet stable isotope value and DDF, and avoided the difficulty in selecting suitable diets with variable $\delta^{15}N$. Using enriched $\delta^{15}N$ has not biased results in other studies (MacNeil et al. 2006; Fisk et al. 2009), and the $\delta^{15}N$ values in this study are well within the range where the relationship of $\delta^{15}N$ with $\%^{15}N$ remains linear (Fry 2008). The guppy (*Poecilia reticulata*) is an ideal organism to use for this study due to its small size, which allows it to come into equilibrium with the stable isotope values of a new diet relatively quickly (see Figure 2.1).

2.2 MATERIALS AND METHODS

Fish and aquarium

A total of 7 treatments were used for this study. All guppies were held in quarantine for a minimum of four weeks prior to the start of the experiment and fed TetraMin Tropical Flakes in order to establish a common dietary baseline and to allow the fish to acclimate to their surroundings. The aquaria, 20 L, were exposed to a 12h light and 12h dark cycle, dechlorinated water held at a constant temperature of 25°C and thoroughly cleaned biweekly with approximately a third of the water being replaced. Approximately 10 guppies were held in each aquarium at the start of the experiment and multiple aquaria were assigned to each treatment.

Food preparation

Two types of basic food were used for this study, TetraMin, a standard commerical fish food, and pulverized maggots. Maggots, *Ophyra aenescens*, were

selected as a food source because their stable isotope values can be manipulated without creating biases in amino acid composition, which can influence stable isotope dynamics (Schmidt et al. 2004). An adult fly colony was established using wild caught flies from the Windsor-Essex area. Flies were maintained in the lab with a diel cycle, 16(on) eight (off), at a humidity of 50-60% and temperature of 21-22°C. The flies were fed water and sugar *ad libitum* and held until gravid, at which time they were given a small amount of No Name Meat Mix (Loblaws Companies Limited, Brampton, Canada) dog food with no chemicals added on which to lay eggs. The maggot eggs were transferred into 1L glass mason jars containing paper towels and one of three different rearing treatments.

Isotopically-distinct control and treatment maggots were created by raising maggots on dog food that either had no chemicals added (called *control* maggot treatment) or approximately 0.25g each of non-labeled sodium acetate (99.0%, Sigma-Aldrich Inc., St. Louis, MO, USA) and ammonium chloride (99.9%, J.T. Baker, Phillipsburg, NJ, USA) added to 624g of dog food (called *low* maggot treatment); or 0.25g ^{15}N-enriched (99%) ammonium chloride and ^{13}C-enriched (99%) sodium acetate (Cambridge Isotope Laboratories, Andover, MA, USA) (called *high* maggot treatment). The mixtures were left to stand for a minimum of six days prior to adding the maggots to allow bacteria present in the mixture to absorb the chemicals that had been added. The maggots were allowed to feed *ad libitum* on the dog food until the pre-pupal stage of development. During this time, the maggots were monitored to ensure an adequate moisture level, food supply, and maggot density. Once, they reached the pre-pupal stage, maggots were collected and allowed to wander without food until the contents of their gut had emptied.

After emptying their guts, maggots were frozen for 24 hours, washed and freeze-dried for 48 hours in a VLP200 ValuPump freeze drier (Thermo Savant Instruments Inc., Holbrook, NY, USA). Maggots were pulverized using a mortar and pestle and frozen until fed to the fish. Seven different foods were used that consisted of 6 maggot diets with varying stable isotope values and a single TetraMin diet (see Table 2.1). The $\delta^{13}C$ and $\delta^{15}N$ values of the different foods were monitored throughout the experiment to ensure that $\delta^{15}N$ and $\delta^{13}C$ values did not vary over time for any of the food types.

Experimental Protocol

Of the 7 treatments (see Table 2.1) used for this study six involved a diet consisting of only maggots (Treatment A-F) and one involved a diet consisting of only TetraMin Tropical Flakes (Treatment G). Treatments A, B, and F represent the control, low, and high maggot diets while treatments C, D, and E represent mixtures of the control and high maggots (proportions of each mixture are given in Table 2.1). All fish were fed a consistent amount of food six days a week and treatments were maintained until the stable isotope values in the fish came into equilibrium with their diet. Stable isotope values were monitored for the fish throughout the experiment to determine when they had reached an apparent steady state (isotope values remained constant across multiple sampling days) with the diet (see Figure 2.1 as an example). Six to 10 fish from each treatment were sampled to calculate DDFs once the fish had come into equilibrium with their diet.

The guppies were sacrificed using a lethal dose of MS-222 (Finquel, Redmond, WA, USA), and weight, standard and total length measurements were taken. The

gastrointestinal tract of each fish was removed under a dissecting microscope, in order to ensure that undigested food would not interfere with the stable isotope values recorded.

Stable Isotope Analysis

Prior to stable isotope analysis, samples were freeze dried for 48 hours and homogenized. Samples were weighed into 0.5mg tin capsules and analyzed with a Delta V Advantage isotope ratio mass spectrometer (Thermo Electron Corporation, Bremen, Germany) and 4010 Elemental Combustion System (Costech Instruments, Valencia, CA, USA). Every tenth sample was run in triplicate and lab and National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) reference standards were used for quantification. The analytical precision based on the standard deviation of an internal lab (fish muscle) and NIST standard 8414 (bovine liver) for $\delta^{15}N$ ranged from 0.14‰ to 0.21‰, respectively, and for $\delta^{13}C$ was 0.05‰ to 0.08‰, respectively, during the analysis of these samples. The analysis of NIST standards (sucrose and ammonia sulphate; n = 3 for each) during the analysis of samples generated values that were within 0.01‰ and 0.07‰ of certified values for $\delta^{15}N$ and $\delta^{13}C$, respectively. Stable isotope values are conveyed in δ notation using the following equation:

$$\delta X = [R_{sample}/R_{standard})-1] \times 1000$$

where X is ^{13}C or ^{15}N and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Lipid contents were normalized mathematically using the equation suggested by Post (2007):

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} -3.32 + 0.99 \times C:N$$

Statistical Analyses

Growth rate was calculated as $g = \ln(W_f / W_o) / t$, where W_f is the weight at the time of sampling (g), W_o is the initial weight at the start of the experiment (g) and t is time (days). All statistics were calculated using Sigmastat 3.5 (Systat Software Inc., Point Richmond, CA, USA). A simple least squares linear regression was applied to a plot of food isotopic composition against DDF in order to determine the relationship between these two variables. A t-test was used to compare DDFs of guppies fed different food types.

2.3 RESULTS

General Health and Growth

Fish grew throughout the experiment at a rate of 0.01 day^{-1} across all treatments. Although most of the fish appeared to be in good health throughout the experiment, approximately 20% of the fish died of natural causes, which is a normal rate for aquarium held guppies (unpublished data), and had to be removed. Differences in mortality among aquaria were very minor, differing only by approximately 1-2 fish deaths per treatment.

Diet Discrimination Factors

All seven treatments achieved an apparent steady state with the diet based on constant stable isotope values across multiple sampling days (see Figure 2.1). Diet discriminations factors ranged from -7.0±0.3 to 1.1±0.1‰ for $\delta^{13}C$ and from -849±43.6 to 7.1±2.2‰ for $\delta^{15}N$ depending on food type and stable isotope value of the food (see Table 2.1), and had a significant negative relationship with the $\delta^{13}C$ and $\delta^{15}N$ value of the food (see Figure 2.2).

Food $\delta^{15}N$ values were similar for treatment B (maggots) and treatment G (TetraMin) allowing for comparison among DDFs from differing food types. However, C:N differed for the two food types (See Table 2.2). DDFs were significantly higher (see Table 2.1) for treatment B compared to treatment G ($t=2.222$, $p=0.045$). Food $\delta^{13}C$ values were not similar among treatments therefore a comparison of DDFs could not be made.

Lipid Normalized $\delta^{13}C$ values

Caution must be used when applying stable isotopes to tissue with high lipid contents because the low $\delta^{13}C$ values in lipids compared to other tissue may bias interpretation (DeNiro and Epstein 1977). In general it is not necessary to correct for lipid content when the C:N ratio of the tissue being sampled is below 3.5 for aquatic animals (Post 2007). Since the C:N ratio of the guppies ranged from 4.2±0.1 to 6.5±0.5 in this study (see Table 2.2), $\delta^{13}C$ values were corrected using equations for fish and diet from Post (2007). A negative linear relationship between lipid corrected food $\delta^{13}C$ and lipid corrected DDF was also observed (see Figure 2.3). The slope of this relationship (slope=-0.57±0.04) was very similar to the slope reported for the non-lipid corrected data (slope=-0.56±0.2).

2.4 DISCUSSION

The results of this study clearly demonstrate that DDFs are dependent on diet, both in terms of isotopic composition of the food and food type. Although some DDFs were within the range of values typically reported in the literature (e.g., 2.9‰ for $\delta^{15}N$ and -0.4 to 1.1‰ for $\delta^{13}C$), not all treatments followed this trend. Guppies fed a diet that consisted of a highly enriched food displayed DDFs much more negative than previously

reported values demonstrating that organisms fed a diet with high $\delta^{15}N$ or $\delta^{13}C$ values will be more depleted in the heavy isotope than their diet (Peterson and Fry 1987; Post 2002; Caut et al. 2009). Negative DDFs have also been reported for other organisms including winter flounder, *Pseudopleuronectes americanus*, black fly larvae, *Simulium vittatum IS-7*, and the rat, *Rattus rattus* (Bosley et al. 2002; Caut et al. 2008; Overmyer et al. 2008). Therefore DDFs are dependent on the isotopic composition of the diet, varying more widely among organisms than is currently assumed.

Typically, a single DDF value, based on published reviews, is used in food web studies involving stable isotopes of carbon and nitrogen (Schaal 2009; Syväranta et al. 2010). However, the DDF used in these studies have failed to consider the influence of diet $\delta^{15}N$ and $\delta^{13}C$ values. In this study, DDFs decreased linearly with increasing $\delta^{13}C$ and $\delta^{15}N$ values of the food. This negative relationship between DDFs and the $\delta^{13}C$ and $\delta^{15}N$ values of the food has also been reported for black fly larvae and rats (Caut et al. 2008; Overmyer et al. 2008). A recent study by Caut et al. (2009) which consisted of an analysis of 66 reviewed publications, concluded that diet appears to have a strong influence on DDFs. Our study showed that the relationship between DDFs and $\delta^{13}C$ or $\delta^{15}N$ of the food is very strong ($r^2 > 0.94$) when the organism is fed a constant food source. The slope of the regression lines for DDF and $\delta^{13}C$ in the food (-0.56±0.02) and the slope of the regression line for DDF and $\delta^{15}N$ in the food (-0.59±0.02) were both similar but more negative than the slopes reported for organisms in the Caut et al. (2009) review, which ranged from -0.417 to -0.113 for $\delta^{13}C$ and -0.311 to -0.141 for $\delta^{15}N$. The C:N value of the maggot diet treatments was consistent among treatments (see Table 2.2)

demonstrating that diet quality was maintained across all treatments used for the regression analysis. For this reason, TetraMin was not used for the regression analysis.

Although some of the $\delta^{15}N$ values used in this study were outside the range usually seen in the published literature, high $\delta^{15}N$ values in the food were used to establish the relationship between the $\delta^{15}N$ value of the diet and DDF and to avoid potential problems with a smaller range and the difficulty of selecting similar diets with variable $\delta^{15}N$ values. Even within an environmentally relevant $\delta^{15}N$ range, the DDFs were found to vary by over 10‰ according to the regression obtained in this study. To our knowledge there are no studies suggesting that the use of enriched ^{15}N foods may influence stable isotope dynamics and the use of enriched values in previous studies has not been problematic (MacNeil et al. 2006; Fisk et al. 2009). Additionally, $\delta^{13}C$ values were well within the range of $\delta^{13}C$ values found in the environment and the similarity in the regression slopes between $\delta^{15}N$ and $\delta^{13}C$ suggests that similar processes are operating for both isotopes at high concentrations of ^{13}C and ^{15}N in the food. Finally, the similarities in DDF isotope value in food slopes between our study and those of Caut et al. (2009), which used DDFs from studies in the wild, provides evidence that our enriched $\delta^{15}N$ in food did not influence the behavior or DDFs calculated. We can think of no plausible mechanism by which highly-enriched foods would be unduly biased relative to natural foods, therefore our results clearly demonstrate that DDFs are concentration-dependent.

The mechanism underlying the negative relationship between diet $\delta^{15}N$ and $\delta^{13}C$ values and DDF is not well understood at this time. Fractionation of stable isotopes

occurs through a dynamic balance of absorption from the gut and excretion through the formation of excretory products (Ponsard and Averbuch 1999). Since the lighter isotope typically reacts faster chemically (Peterson and Fry 1987), the product absorbed from the gut can be isotopically lighter than the food. An organism feeding on a food source with a very high $\delta^{13}C$ or $\delta^{15}N$ value may absorb and break down a lower portion of the compounds containing the heavy isotopes present in an enriched food than a more depleted food. The product that is assimilated from the gut is then subject to metabolic processes typically responsible for the enrichment in stable isotope values between an organism and its diet but from a substrate pool that is isotopically-depleted relative to the ingested food. If this is the case then we would expect the feces to be enriched in ^{15}N; Checkley & Entzeroth (1985) showed that the feces of copepods was isotopically heavy compared to the diet and Overmyer *et al*. (2008) also observed very high $\delta^{15}N$ values for the feces of black fly larvae. This is analogous to parasitic organisms that have an abundance of food (the host organism); the parasite is not able to absorb all of the potential food sources and a disproportional amount of the compounds containing the light isotope is absorbed because it is more reactive than the heavy isotope (Olive et al. 2003). Alternatively, the differential distribution of heavy and light isotopes in the maggot tissue, due to isotopic routing, followed by the guppy's preferential assimilation of certain tissues could contribute to the difference in DDFs observed (Phillips and Koch 2002; Felicetti et al. 2003).

If DDFs are dependent on the food $\delta^{13}C$ and $\delta^{15}N$ values, then studies applying stable isotopes to natural systems must be sure the DDFs used reflect the diet $\delta^{13}C$ and $\delta^{15}N$ values of the food. If this is not done, the results of isotopic modeling and the

interpretation of diet reconstruction studies may lead to biased results, particularly where prey stable isotope values span a wide isotopic range. This study also has implications for research employing $^{15}NH_4$ as a tracer to study nitrogen cycling within an ecosystem (e.g. Hall et al. 1998; Wollheim et al. 1999; Dodds et al. 2000). In these studies, very high $\delta^{15}N$ values are often observed that could bias both reported results and comparisons among different experiments (Overmyer 2008).

As observed elsewhere, food type also influenced the DDFs observed for $\delta^{15}N$. When comparing the $\delta^{15}N$ DDF for treatments fed the maggot and the TetraMin diet with similar $\delta^{15}N$ (treatment B compared to treatment G), the treatment fed maggots had a higher DDF than those typically reported in the literature while the $\delta^{15}N$ DDF for the treatment fed TetraMin only, was very close to the range of 3 to 5‰ commonly used in the literature (Minagawa and Wada 1984; Peterson and Fry 1987). Using C:N as an estimate for protein content of the diet, it appears that the protein content of the diets could account for the discrepancy in DDFs observed for these two treatments since C:N differed among maggot and TetraMin diets. Previous studies have shown opposing results for the influence of protein content on DDFs. For example, Focken (2001) and Pearson et al. (2003) found that $\delta^{15}N$ DDFs increased with protein consumption for Nile tilapia, *Oreochromis niloticus* and wild yellow-rumped warblers, *Dendroica, coronate,* respectively. Conversely, Tsahar et al. (2008) found that Yellow-vented bulbuls, *Pycnonotus xanthopygos,* had higher $\delta^{15}N$ DDFs when fed a lower protein diet compared to a higher protein diet and Webb et al. (1998) found that Locusts, *Locusta migratoria* had higher $\delta^{15}N$ DDFs when fed a low quality maize diet than when fed a higher quality

wheat diet. Additionally, Robbins et al. (2005) found no significant relationship between protein content or C:N and DDF.

In the current study, TetraMin tropic flakes are specifically designed to provide fish with a nutritionally balanced diet and are likely more representative of the guppy's natural diet than maggots. Since the TetraMin diet displayed a lower C:N value than the maggot diet, this suggests that the TetraMin diet has a higher protein content. Webb et al. (1998) attributed the higher DDFs for the low quality maize diet to substrate recycling. If the maggot diet represents a suboptimal diet for the guppy then substrate recycling may also be responsible for the higher DDFs observed for fish fed the maggot diet. Therefore DDFs calculated from the regression analysis obtained in this study may be higher than the DDF for a guppy feeding in the wild Nevertheless, the negative relationship between DDF and food $\delta^{15}N$ values remains.

2.5 CONCLUSIONS

This study has experimentally evaluated DDFs of $\delta^{15}N$ and $\delta^{13}C$ in guppies and has profound implications for the application of stable isotopes in other organisms. DDFs were found to depend on both the $\delta^{15}N$ and $\delta^{13}C$ value of the diet and the food type. Although the negative linear relationships between DDF and diet $\delta^{15}N$ and $\delta^{13}C$ values provides an initial estimate of how DDFs may vary through a food web, additional research is warranted to determine this relationship in other species. Reliable DDFs that have been tested under controlled laboratory experiments should be acquired before stable isotopes are applied in the field to investigate diet and food web interaction. Furthermore, DDFs were found to differ depending on the food type demonstrating that

food protein content may influence DDFs. Overall, our findings highlight the considerable need for more controlled laboratory experiments to interpret stable isotopes dynamics in the field and understand the mechanism underlying the relationships observed.

2.6 REFERENCES

Ben-David M, Flynn RW, Schell DM (1997) Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia 111: 280–291

Bosley KL, Witting DA, Chambers RC, Wainright SC (2002) Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder *Pseudopleuronectes americanus* with stable isotopes. Mar Ecol Prog Ser 236: 233-240

Caut S, Angulo E, Courchamp F (2008) Discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$) in an omnivorous consumer: effect of diet isotopic ratio. Funct Ecol 22: 255-263

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46: 443-453

Checkley DMJ, Entzeroth LC (1985) Elemental and isotopic fractionation of carbon and nitrogen by marine, planktonic copepods and implications to the marine nitrogen cycle. J Plankton Res 7: 553-568

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197: 261-263

DeNiro MJ, Epstein S (1978) Carbon isotopic evidence for different feeding patterns in two hydrax species occupying the same habitat. Science 201**:** 906–908

Dodds WK, Evans-White MA, Gerlanc NM, Gray L, Gudder DA, Kemp MJ, López AL, Stagliano D, Strauss EA, Tank JL, Whiles MR, Wollheim WM (2000) Quantification of the nitrogen cycle in a prairie stream. Ecosystems 3**:** 574-589

Focken U (2001) Stable isotopes in animal ecology: the effect of ration size on the trophic shift of C and N isotopes between feed and carcass. Isot Environ Healt S 37: 199-211

Felicetti LA, Schwartz CC, Rye RO, Haroldson MA, Gunther KA, Phillips DL, Robbins CT (2003) Use of sulfur and nitrogen and stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears. Can J Zool 81: 763-770

Fisk AT, Sash K, Maerz J, Palmer W, Carroll JP, MacNeil MA (2009) Metabolic turnover rates of carbon and nitrogen stable isotopes in captive juvenile snakes. Rapid Commun Mass Spectrom 23: 319-326

Fry B. *Stable Isotope Ecology*. Springer: New York, 2008.

Gannes LZ, O'Brien DM, Martinez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78: 1271-1276

Hall RO, Peterson BJ, Meyer JL (1998) Testing a nitrogen-cycling model of a forest stream by using a nitrogen-15 tracer addition. Ecosystems 1: 283-298

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor 94: 189-197

Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 84: 9

Jardine TD, Kidd KA, Fisk AT (2006) Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. Environ Sci Technol 40: 7501-7511

MacNeil MA, Drouillard KG, Fisk AT (2006) Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. Can. J. Fish. Aquat. Sci. 63: 345-353

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48: 1135-1140

Olive PJW, Pinnegar JK, Polunin NVC, Richards G, Welch R (2003) Isotope trophic-step fractionation: a dynamic equilibrium model. J Anim Ecol 72: 608-617

Overmyer JP, MacNeil M, Fisk AT (2008) Fractionation and metabolic turnover of carbon and nitrogen stable isotopes in black fly larvae. Rapid Commun Mass Spectrom 22: 694-700

Pearson S, Levey D, Greenberg C, del Rio CM (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia 135: 516-523

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18: 293-320

Phillips DL, Koch PL (2002) Incorporating concentration dependence in stable isotope mixing models. Oecologia 130: 114–125

Ponsard S, Averbuch P (1999) Should growing and adult animals fed on the same diet show different $\delta^{15}N$ values? Rapid Commun Mass Spectrom 13: 1305-1310

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703-718

Post D (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152: 179-189

Power M, Guiguer KRRA, Barton DR (2003) Effects of temperature on isotopic enrichment in *Daphnia magna*: implications for aquatic food-web studies. Rapid Commun Mass Spectrom 17: 1619-1625

Robbins CT, Felicetti LA, Sponheimer M (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144: 534-540

Schaal G, Riera P, Leroux C (2009) Trophic significance of the kelp *Laminaria digitata* (Lamour.) for the associated food web: a between-sites comparison. Estuar Coast Shelf Sci 85: 565-572

Schmidt K, McClelland JW, Mente E, Montoya JP, Atkinson A, Voss M. (2004) Trophic-level interpretation based on $\delta^{15}N$ values: implications of tissue-specific fractionation and amino acid composition. Mar Ecol Prog Ser 266: 43-58

Syväranta J, Cucherousset J, Kopp D, Crivelli A, Céréghino, R, Santoul F (2010) Dietary breadth and trophic position of introduced European catfish *Silurus glanis* in the River Tarn (Garonne River basin), southwest France. Aquat Biol 8: 137-144

Szepanski MM, Ben-David M, Van Ballenberghe V (1999) Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. Oecologia 120: 327–335

Tsahar E, Wolf N, Izhaki I, Arad Z, del Rio CM (2008) Dietary protein influences the rate of ^{15}N incorporation in blood cells and plasma of Yellow-vented bulbuls (*Pycnonptus xanthopygos*). J Exp Biol 211: 459-465

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia 136: 169-182

Webb SC, Hedges RE, Simpson SJ (1998) Diet quality influences the $\delta^{13}C$ and $\delta^{15}N$ of locusts and their biochemical components. J Exp Biol 201**:** 2903-2911

Wolf N, Carleton SA, Martinez del Rio C (2009) Ten years of experimental animal isotopic ecology. Funct Ecol 23: 17-26

Wollheim WM, Peterson BJ, Deegan LA, Bahr M, Hobbie JE (1999) A couples field and modeling approach for the analysis of nitrogen cycling in streams. J N Am Benthol Soc 18: 199-221

Table 2.1: DDFs and values of $\delta^{13}C$ and $\delta^{15}N$ of the food for Treatments A through G (mean ± SE). See methods for details.

Treatment	**Isotope**	**Food $\delta^{13}C$ or $\delta^{15}N$**	**Food Composition**	**N**	**DDF**
Maggots only					
A	$\delta^{15}N$	6.5 ± 0.2	Control	6	6.02 ± 0.6
	$\delta^{13}C$	-20.0 ± 0.1			0.1 ± 0.1
B	$\delta^{15}N$	8.2 ± 0.2	Low	7	7.1 ± 2.2
	$\delta^{13}C$	-19.5 ± 0.04			0.03 ± 0.1
C	$\delta^{15}N$	151.7 ± 68.7	10% High, 90% Control	9	-54.4 ± 7.4
	$\delta^{13}C$	-18.6 ± 0.6			-0.4 ± 0.1
D	$\delta^{15}N$	747.1 ± 151.8	50% High, 50% Control	10	-491.3 ± 20.4
	$\delta^{13}C$	-14.2 ± 1.0			-3.6 ± 0.3
E	$\delta^{15}N$	1154.8 ±65.9	90% High, 10% Control	8	-729.3 ± 26.2
	$\delta^{13}C$	-10.7 ± 0.5			-5.3 ± 0.2
F	$\delta^{15}N$	1586 ± 39.0	High	6	-849 ±43.6
	$\delta^{13}C$	-6.6 ± 0.4			-7.0 ± 0.3
TetraMin only					
G	$\delta^{15}N$	8.2 ± 0.8	TetraMin	9	2.9 ±0.4
	$\delta^{13}C$	-22.9 ± 0.05			1.1 ± 0.1

Table 2.2: Lipid normalized $\delta^{13}C$ DDFs and lipid corrected $\delta^{13}C$ of the food (mean ± SE) with C:N (mean ± SE) for Treatments A through G.

Treatment	**Food $\delta^{13}C$**	**Food C:N**	**DDF**	**Guppy C:N**
A	-15.8 ± 0.2	7.6 ± 0.5	-1.7 ± 0.3	5.6 ± 0.2
B	-15.5 ± 0.2	7.4 ± 0.6	-1.4 ± 0.4	5.4 ±0.2
C	-14.9 ± 0.4	7.2 ± 0.2	-1.2 ± 0.5	6.4 ± 0.4
D	-9.8 ± 0.8	7.7 ± 0.3	-4.9 ± 0.6	6.5 ± 0.5
E	-6.8 ± 0.5	7.3 ± 0.3	-6.6 ± 0.4	6.0 ± 0.4
F	-2.8 ± 0.3	7.2 ± 0.6	-8.3 ± 0.4	5.0 ± 0.2
G	-20.8 ± 0.05	5.5 ± 0.05	0.3 ± 0.1	4.2 ± 0.1

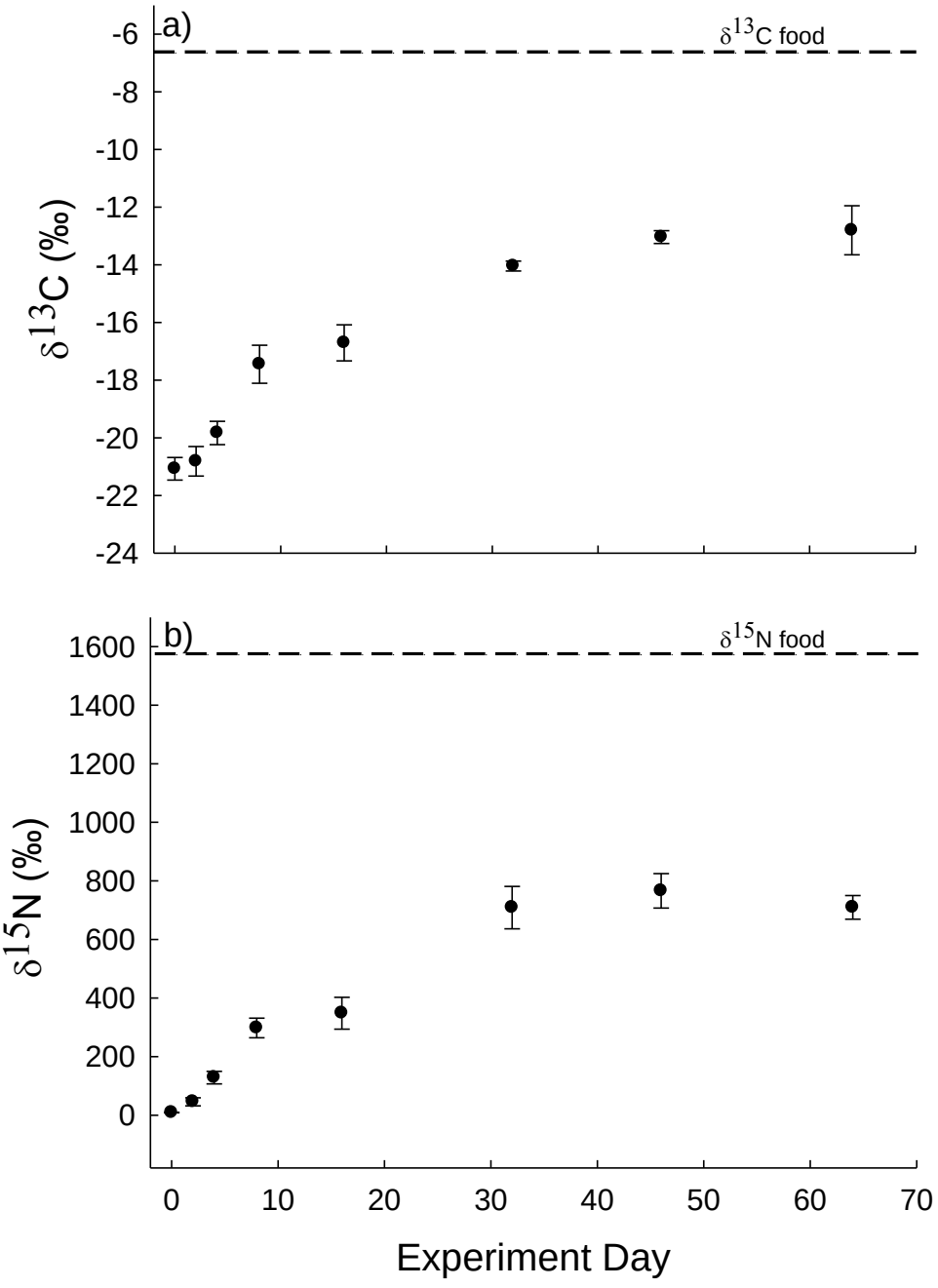

Figure 2.1: Treatment F stable isotope values for (a) $\delta^{13}C$ and (b) $\delta^{15}N$ in guppies (each point is the mean ±SE, n=3) fed a maggot diet ($\delta^{13}C$=-6.6 ± 0.4 and $\delta^{15}N$=1586 ± 39.0). Dashed lines indicate the (a) $\delta^{13}C$ and (b) $\delta^{15}N$ values of the food.

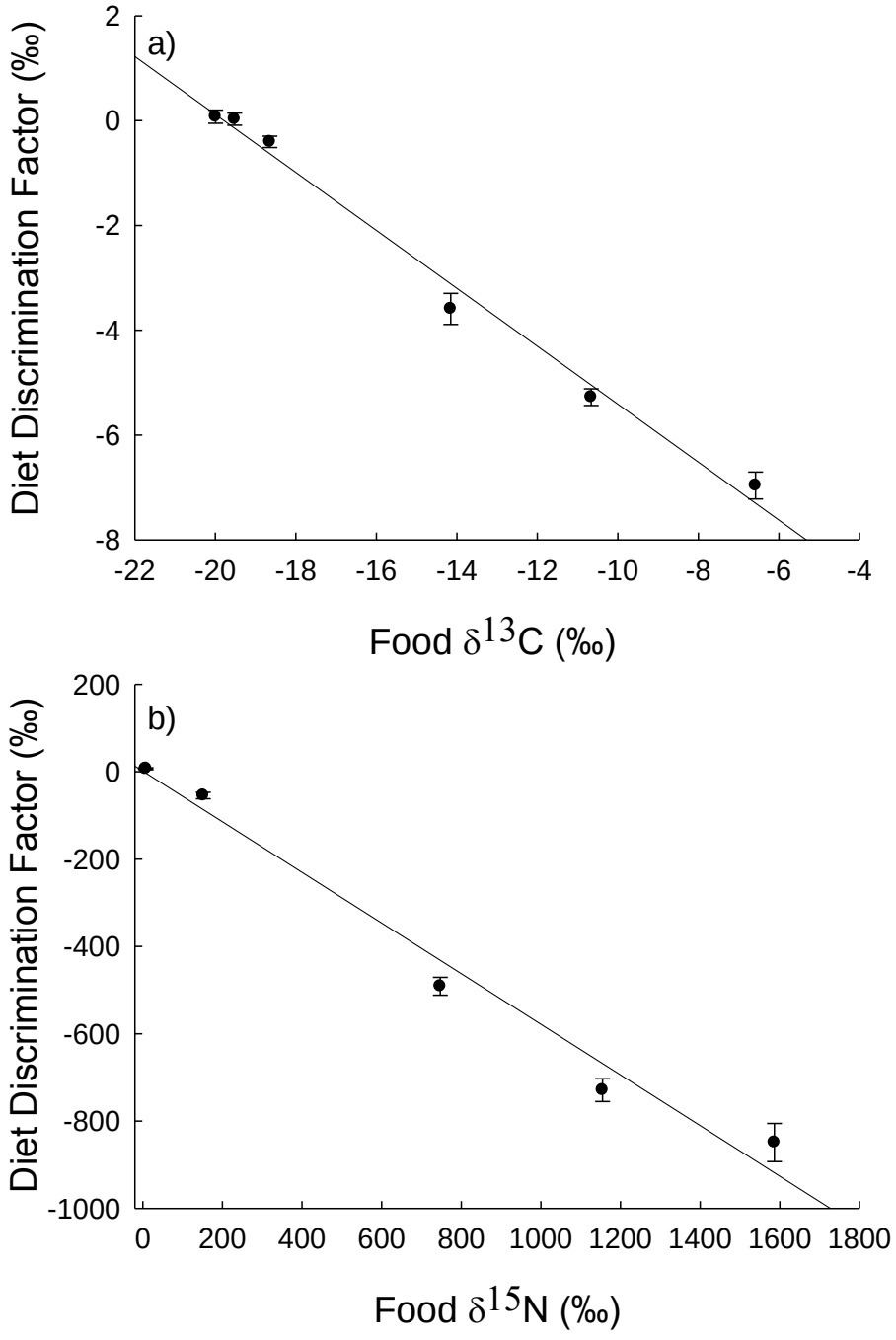

Figure 2.2: Relationship between DDFs (mean ±SE) and (a) $\delta^{13}C$ and (b) $\delta^{15}N$ values in the guppy food for treatments A through F (maggots only). Lines represent linear regression for (a) $\delta^{13}C$ (DDF = -11.07 - (0.56 * $\delta^{13}C_{diet}$); r^2=0.94, p<0.001) and (b) $\delta^{15}N$ (DDF = 1.44 - (0.59 * $\delta^{15}N_{diet}$); r^2=0.95, p<0.001).

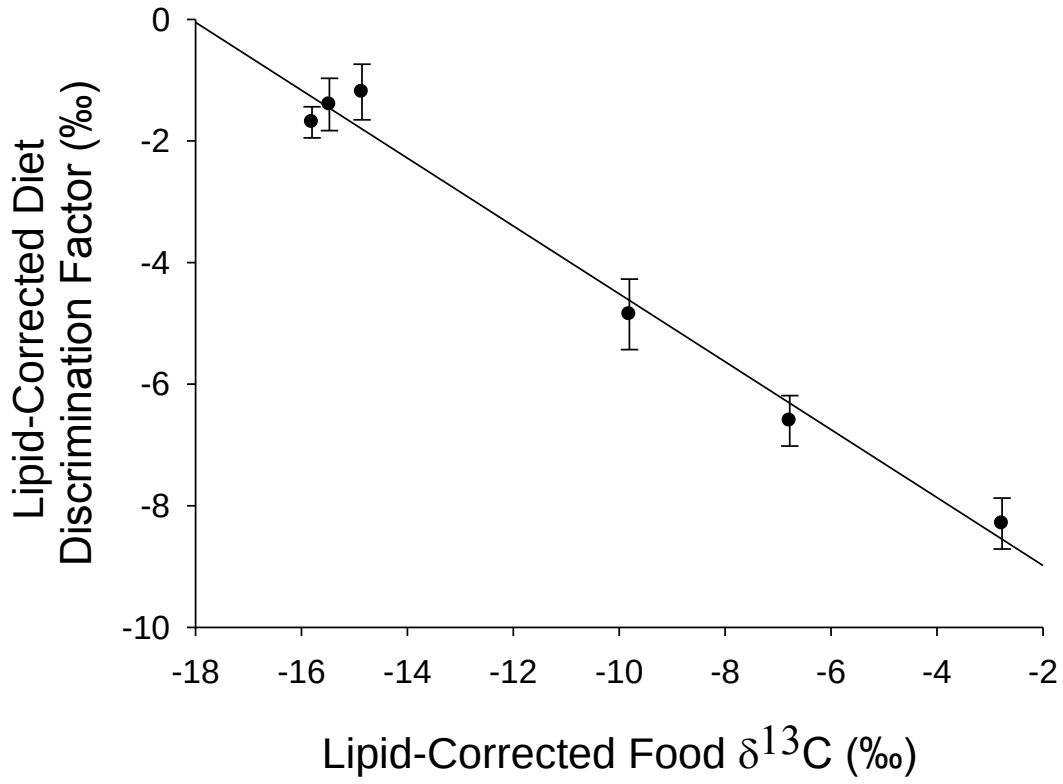

Figure 2.3: Relationship between lipid corrected DDFs (mean ±SE) and lipid corrected $\delta^{13}C$ values in the guppy food for treatments A through F (maggots only). Line represents linear regression (DDF = -10.21 - (0.57 * $\delta^{13}C_{\text{Lipid Corrected Food}}$); r^2=0.80, p<0.001).

3.0 – FEEDING ECOLOGY AND NICHE WIDTH DIFFERENCES AMONG FRESHWATER FISH POPULATIONS EXPERIENCING VARIABLE PREDATION INTENSITY

3.1 INTRODUCTION

Understanding interspecific interactions and the consequences that these interactions can have within an ecosystem is of profound importance to ecologists; of particular interest are predator-prey relationships. In order to survive, organisms must constantly balance their need to forage against predator avoidance (Sih 1980; Dill 1987; Lima and Dill 1990). It is well understood predation pressure can influence diet and foraging in terms of the food items consumed, the feeding location, and the timing of foraging activity (Werner et al. 1983; Dill and Fraser 1984; Brown et al. 1988; Lima and Dill 1990; Abramsky et al. 1996; Rothley et al. 1997; Schmitz et al. 1997; Houtman and Dill 1998; Zandona 2010). However, the actual diet of an organism in the presence of a predator may differ from the predicted response under natural conditions when other factors, such as hunger or competition, are considered (Skutelsky 1996; Dill and Fraser 1984). For example, scorpions (*Buthus occitanus Israelis)* were more likely to forage on risky moonlit nights if they had low energy reserves (Skutelsky 1996) and juvenile Coho Salmon (*Oncorhynchus kisutch*) were less responsive to a predator when they perceived that a competitor was present (Dill and Fraser 1984). This emphasizes the need to conduct field experiments to better understand the influence of a predator on the diet of an

*modified from: Dennis CA, Pitcher TE, Ramnarine IW, Rush SA, McMeans BC and Fisk AT. Feeding ecology and niche width differences among freshwater fish populations experiencing variable predation intensity. Submitted to the *Journal of Animal Ecology*

organism under natural conditions rather than laboratory conditions where interacting factors may not be present.

Guppy (*Poecilia reticulata*) populations in Trinidad provide a unique opportunity to study the influence of predation in a natural system. Within streams of Trinidad's northern mountainous range, guppies are exposed to natural variations in predation intensity (Magurran 2005). This variation can occur over a relatively short distance due to the presence of rapids and waterfalls which restrict the upstream colonization of most major adult guppy predators. Therefore, upstream locations tend to have low levels of predation for the guppy, where the only fish predator is the gape limited *Rivulus hartii,* which only preys on juvenile guppies (Magurran and Seghers 1994; Endler and Houde 1995). Conversely, downstream guppies are typically exposed to a variety of predators, most notably *Crenicichla alta* and *Hoplias malabaricus,* which frequently prey on adult guppies (Magurran and Seghers 1994; Endler and Houde 1995). Numerous examples of evolutionary divergence between upstream and downstream guppy populations are documented including differences in diet and foraging behaviour (Dussault and Kramer 1981; Bassar et al. 2010, Zandonà et al.2011).

Based on previous studies, the guppy's diet in Trinidad consists mainly of algae, detritus and benthic invertebrates, with invertebrates thought to represent a high quality food item (Dussault and Kramer 1981; Bassar et al. 2010; Zandonà et al. 2011). The importance of animal material in the diet of guppies was highlighted by Dussault and Kramer (1981) who found that guppies had slower growth rates when fed a diet consisting exclusively of algae compared to diets consisting of Tetra-Min or *Daphnia pulex*. Predation has been shown to restrict the feeding activities of guppies to the

periphery and to shallow areas of the stream and may result in high-predation guppies having more limited access to high quality food items (Seghers 1970; Fraser and Gilliam 1992). Furthermore, guppies are more vulnerable to predation when they are feeding on more rewarding food patches and invertebrate prey may require greater handling times than algae which could compromise predator vigilance (Godin and Smith 1988; Houtman and Dill 1998).

Although the diet of wild guppy populations in Trinidad has been studied (Dussault and Kramer 1981; Bassar et al. 2010; Zandonà 2010; Zandonà et al. 2011), these studies found conflicting results in regards to the relative amount of invertebrates in the guts of high-predation guppies compared to low-predation guppies. Furthermore, three of the four studies examined only two rivers and employed conventional gut analysis. Because there are a number of caveats with the use of gut content analyses including misidentified food items, partially digested foods, etc. (Vander Zanden and Rasmussen 1996; Sheffield et al. 2001), stable isotopes and fatty acids offer alternative solutions to examine the diet of guppies under differing predation regimes.

The fatty acid composition of an organism is often related to the fatty acid composition of its diet and can provide insights into trophic links in aquatic ecosystems (reviewed by Napolitano 1999). For example, the fatty acids palmitoleic acid (16:1n7) and eicosapentaenoic acid (20:5n3) are commonly used as markers for diatoms (Parrish et al. 1995; Napolitano et al. 1997; Torres-Ruiz et al. 2007). Among other things, stable isotopes can be used to infer trophic relationships including diet, trophic position and niche width (Peterson and Fry 1987; Bearhop et al. 2004; Layman et al. 2007; Jackson et al. 2011). Stable isotopes of nitrogen ($\delta^{15}N$) are used to estimate the trophic position of an

organism because $\delta^{15}N$ values show a progressive enrichment in the heavy isotope at higher trophic levels in the food web (Peterson and Fry 1987). On the other hand, carbon stable isotopes are used to trace dietary sources due to differences in baseline carbon stable isotope values among different diet items and environments (Peterson and Fry 1987). Owing to these relationships, stable isotopes have recently been suggested as a measure of niche width at the community and population level (Bearhop et al. 2004; Layman et al. 2007; Jackson et al. 2011).

The objective of this study was to compare the diet and trophic niche width of wild guppy populations in Trinidadian streams. We took advantage of natural variations in predation intensity and collected guppies from four high- and four low-predation populations in order to determine if differences were widespread and consistent among rivers. Samples were analysed for fatty acid and stable isotopes to estimate diet, trophic position and niche width. Optimal foraging strategy predicts that niche width will increase when food becomes more limited (Emlen 1966; MacArthur and Pianka 1966; Tinker et al. 2008). Therefore, we predicted that predation would limit the resources available to the guppy and high-predation guppy populations would be less selective and have a greater niche width than low-predation guppy populations which will specialize on high quality food items like invertebrates. Consequently, we also expected the low-predation guppy populations to have a higher trophic position than the high-predation guppy populations.

3.2 MATERIALS AND METHODS

Guppy collection

In September 2009, guppies (*Poecilia reticulata*) were collected from 8 populations in the Northern Mountain Range of Trinidad. The populations consisted of 4 paired high- and low-predation sites, with 3 pairs located within the same river systems (Airipo, Quare and Turure) and one pair with a high-predation site in one river system (Tacarigua) and a low-predation site in another river system (Tunapuna). Since the low-predation site at Tunapuna and the high-predation site at Tacarigua are part of the same watershed system, these sites have been paired in other studies (e.g. Neff et al. 2008; Elgee et al. 2010). Low-predation sites are located in the upstream regions of the river and high-predation sites were located at downstream regions. All sites were selected based locations where predation intensity has been surveyed and monitored previously (Magurran and Seghers 1994; Endler and Houde 1995; Evans et al. 2003; Neff et al. 2008).

Both male and female guppies were collected from each site (see Table 3.1) using seine nets and butterfly nets and transported back to the lab alive in aerated buckets. Additionally, epilithon (algae and detritus) and invertebrates (Family: Chironomidae) were collected as potential food items and snails (Family: Lymnaeidae) were collected to establish an isotopic baseline, where available. Only epilithon and fish were analyzed for fatty acid methyl esters (FAME).

Sample preparation

Guppies were euthanized using a lethal dose of MS-222 (Finquel, Redmond, Washington). Once sacrificed, weight and total length measurements were taken and the gastrointestinal tract of each fish was removed to insure that undigested food items would not interfere with stable isotope analysis. Samples were stored in liquid nitrogen until

transferred to a -80°C freezer. Prior to analysis, samples were freeze-dried for 48 hours and homogenized.

Fatty Acid Analysis

Analysis of FAME followed established methods similar to Kainz et al. 2010. Briefly, this procedure involved extraction using (2:1 vol:vol) chloroform:methanol (Bligh and Dyer 1959), derivatization, and quantification on a gas chromatograph. An internal standard (cholestane) was added to all samples to provide an estimate of extraction efficiency.

FAME were analysed using a 6890 Series Gas Chromatography System (Agilent, Mississauga, Ontario) which was configured as follows: splitless injection; column = Supelco (SP-2560 column) 100 m X 0.25 mm ID X 0.20 µm film thickness; oven = 140°C (hold for 5 min) to 240°C at 4°C min^{-1}, hold for 15 min; carrier gas = helium, 1.2 mL min^{-1}; detector = FID at 260°C; injector = 260°C; total run time = 45 min per sample. A 37-component fatty acid standard (Supelco 47885-U) was used to identify FAME in the samples by comparing their retention times to those of the fatty acid standard. Quantification of individual fatty acid components was calculated on the basis of known amounts of injected standard dilutions of the 37-component fatty acid mix. FAME are expressed as concentrations (µg FAME /mg dry weight of tissue extracted) and proportions (percent). Six fatty acids were analysed for this study including palmitoleic acid (C16:1n-7) and the essential fatty acids linoleic acid (C18:2n-6c), α-linolenic acid (C18:3n-3), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3), and docosahexaenoic acid (C22:6n-3). The ratio ∑n-3/∑n-6 fatty acids was also examined as

a marker for terrestrial compared to aquatic sources (Pollero et al. 1981; Desvilettes et al. 1994).

Stable Isotope Analysis

All samples were lipid extracted with (2:1 vol:vol) chloroform:methanol prior to stable isotope analysis (Post et al. 2007). A small sub sample of approximately 500µg (for fish and snails) or 4000 µg (for epilithon) was weighed out into 0.5mg tin capsules which were analyzed for $\delta^{13}C$ and $\delta^{15}N$ using a Delta V Advantage isotope ratio mass spectrometer (Thermo Electron Corporation, Bremen, Germany) and 4010 Elemental Combustion System (Costech Instruments, Valencia, CA, USA). All stable isotope values were converted into δ notation using the formula: $\delta X = [R_{sample}/R_{standard})-1] \cdot 1000$ where X is ^{13}C or ^{15}N and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. To assess precision, every 12^{th} samples was run as a triplicate and the standard deviation of these samples was generally less than 0.2‰. Additionally, lab and National Institute of Standards and Technology (NIST) standards were analyzed after every 12 samples for quantification of samples. The analytical precision (standard deviation) for NIST standard 8414 (bovine muscle, n=65) and an internal lab standard (tilapia muscle, n = 65) for $\delta^{13}C$ was 0.1 and 0.1, respectively, and for $\delta^{15}N$ was 0.2 and 0.3, respectively. The NIST standards (sucrose and ammonia sulphate, n=3) were within 0.01‰ and 0.07‰ of certified values for $\delta^{15}N$ and $\delta^{13}C$, respectively.

Data Analysis

For each fish, Fulton's condition factor (K) was estimated as $K = 100{,}000 * W/L^3$ where W is the whole body wet weight (g) and L is the total length (mm; Ricker 1975 as

cited in Nash et al. 2006). Ratios of ∑n-3/∑n-6 fatty acids were compared among high- and low-predation sites within each river using t-tests.

Principal component analysis (PCA) was performed on a correlation matrix to examine patterns of variation in fatty acid proportions (i.e. fatty acid concentration divided by the sum of all fatty acid concentrations, expressed as a %) using SYSTAT Version No. 11.00.01 (Systat Software Inc.). Prior to the PCA, fatty acid proportions were tested for univariate normality through visual inspection of normal probability plots and Shapiro-Wilk's tests. Since some of the fatty acids were not normally distributed, fatty acid proportions were transformed using the formula: $x_{trans}= \ln (x_i/c_r)$, where x_i is a given fatty acid proportion, c_r is the proportion of C18:0, and x_{trans} is the transformed fatty acid proportion (Aitchison 1986 as cited in Budge et al. 2006). In order to reduce the number of variables included in the PCA, only fatty acids thought to differentiate the potential diet items of the guppy were included in the PCA and were as follows: C16:1n-7, C18:2n-6, C18:3n-3, C20:4n-6, C20:5n3 and C22:6n3 (Hanson et al. 1985; Ackman and Takeuchi 1986; Napolitano et al. 1996; Napolitano 1999; Kainz et al. 2004; Torres-Ruiz et al. 2007). In addition to fatty acid variables, weight (g) was also included in the PCA. All data was standardized to a mean of zero and unit variance prior to the PCA. All principal components with eigenvalues >1 were retained.

Linear regression was used to assess the relationship between stable isotope values and fish length. Estimates of trophic position were calculated as follows using the formula: trophic position = $\lambda + (\delta^{15}N_{secondary\ consumer} - \delta^{15}N_{base})/\Delta_n$, where λ is the trophic position of a baseline organism and Δ_n is the enrichment of $\delta^{15}N$ per trophic level. Snails (λ=2) were used as baseline organisms for this study. An enrichment factor of 2.9 for

$\delta^{15}N$ was selected based on a previous laboratory study with the guppy (Dennis et al. 2010) which provided an estimate of the enrichment factor based on the stable isotope value of the guppy's diet items. An estimate of the isotopic niche width of the guppy was calculated using the standard ellipse-based metrics suggested by Jackson et al. (2011) which are unbiased with respect to sample size. Standard ellipse areas (SEA) were calculated using the package Stable Isotope Analysis in R (siar: Parnell and Jackson 2011; R Development Core Team 2011). Additionally, SEA_b, which is an estimate of sample size corrected version of SEA using a Bayesian framework, was analyzed by determining the proportion of ellipses that are smaller for one population compared to the other within the high-low-predation pairs (Jackson et al. 2011). Since stable isotope values can vary substantially among primary producers, the Levene median test was used to examine differences in the variance of snails and epilithon collected from high compared to low-predation sites within a river.

Differences in trophic positions among predation levels were analyzed using linear mixed-effects models fit using restricted maximum likelihood in the lmer function of the lme4 package of R Version 2.13 (Bates et al. 2011, R Development Core Team 2011). Because predation levels varied between upstream and downstream locations in most rivers, we accounted for the effect of the different river systems by treating river as a random effect in each model (random intercepts). The trophic position was treated as the response variable and a dichotomous variable representing predation level (i.e, '0' reflecting low-predation and '1' denoting high-predation) entered as a fixed effect. We tested whether mean trophic positions differed between high- and low-predation sites using single degree-of-freedom multiple comparison tests via the glht function in the

multcomp package (Hothorn et al. 2008). The linear mixed-effects model was repeated using ratios of ∑n-3/∑n-6 fatty acids, principal component scores (from the PCA performed on fatty acid proportions), Fulton's condition factor or % lipid as the response variable. A similar analysis was performed to assess variations in $\delta^{13}C$ and $\delta^{15}N$ among sexes. In this analysis, sex was treated as the fixed effect and river was treated as a random effect.

3.3 RESULTS

Ratios of ∑n-3/∑n-6 fatty acids were higher at the high-predation site compared to the low-predation site (Figure 3.1) within three of the four rivers but this difference was only significant within the Quare (t_{33}=6.30, $p<0.001$) and the Tacarigua/Tunapina (t_{38}=-8.58, $p<0.001$) river systems. Conversely, the ratio of ∑n-3/∑n-6 fatty acids was significantly higher within the low-predation site compared to the high-predation site at Aripo (t_{38}=8.05, $p<0.001$).

Fatty acid proportions were highest for C22:6n-3 within all populations except Tunapuna, where C18:2n-6 was highest, and Upper Quare, where C20:4n-6 was highest (Table 3.2). Relative differences in fatty acid concentrations among high- and low-predation sites generally agreed with the proportional data (Table 3.3). The first two components extracted by the PCA of fatty acid proportions had eigenvalues >1 and the variances explained by these components were 36.9% for component 1 (PC1) and 29.6% for component 2 (PC2). PC1 displayed high positive loadings for C16:1n-7 (0.89), C18:3n-3 (0.77), and C20:5n-3 (0.69) and a high negative loading for C20:4n-6 (-0.70). PC2 displayed a high positive loading for C18:2n-6 (0.86) and a high negative loading for

C22:6n-3 (-0.91, Figure 3.2). Guppy weight did not load highly for either component (<0.35).

Although both $\delta^{15}N$ and $\delta^{13}C$ values differed among populations (Table 3.4), there were no relationships between either $\delta^{13}C$ and fish length ($F_{1,140}$= 2.78, p = 0.10) or $\delta^{15}N$ and fish length ($F_{1,140}$= 0.81, p = 0.37) across all samples or within populations, with the exception of a negative relationship between $\delta^{13}C$ and fish length within Upper Turure ($F_{1,17}$=7.32, p=0.02) and a positive relationship between $\delta^{15}N$ and fish length within Upper Aripo ($F_{1,17}$=4.94, p=0.04).

SEA_b, a measure of trophic niche width, was greater at high-predation sites compared to low-predation sites within 3 of the 4 rivers but did not differ among high- and low-predation populations at Aripo (Table 3.5, figure 3.3). The variance in $\delta^{15}N$ values did not differ among high- and low-predation sites for snails within Aripo ($F_{1,7}$=2.18, p=0.18) or Turure ($F_{1,5}$=0.82, p=0.41) nor did $\delta^{15}N$ values differ for epilithon among predation regimes within Aripo ($F_{1,10}$=0.60, p=0.46) or Quare ($F_{1,7}$=0.52, p=0.49). The variance in $\delta^{13}C$ values did not differ among high- and low-predation sites for snails within Aripo ($F_{1,7}$=1.73, p=0.23) or Turure ($F_{1,5}$=0.001, p=0.99) nor did $\delta^{13}C$ values differ for epilithon among predation regimes within Aripo ($F_{1,10}$=0.56, p=0.47) or Quare ($F_{1,7}$=3.16, p=0.12).

Mixed effects model results indicated that trophic position had a mean value of 2.7 and did not differ between high- and low-predation sites (z=0.29, df=1, p=0.77). Nevertheless, trophic position could only be compared within two river systems (Turure and Airipo) due to a lack of available baseline organisms (snails) at the other sampling locations. Mixed effects model results indicate that the ratio of ∑n-3/∑n-6 fatty acids did

not differ among high- and low-predation populations (z=0.46, df=1, p=0.65). Low-predation guppy populations had significantly more negative scores on PC1 (PC1 mean ± SE: -0.24 ± 0.14) than high-predation guppy populations (PC1 mean ± SE: 0.15 ± 0.33; z= -2.84, df=1, p= 0.005). Although component scores on PC2 were more negative at high-predation populations (PC2 mean ± SE: -0.13 ± 0.12) compared to low-predation populations (PC2 mean ± SE: 0.13 ± 0.16), this difference was not significant (z= 0.26, df=1, p=0.12). Both Fulton's condition factor (z=-0.59, df=1, p = 0.56) and % lipid (z=-0.19, df=1, p= 0.85) did not differ significantly among predation regimes. Additionally, there was no difference in the $\delta^{13}C$ (z = 1.08, df = 1, p = 0.28) and $\delta^{15}N$ (z = 1.05, df = 1, p = 0.29) values of males or females.

3.4 DISCUSSION

This study found that guppy populations experiencing contrasting predation intensity display different diets and isotopic niche widths in Trinidad. Although all guppies most likely consume a combination of invertebrates, algae and detritus, fatty acid and stable isotope data suggest that guppies from low-predation sites are more specialized and consume a greater amount of invertebrates, while guppies from high-predation sites feed more indiscriminately, consuming a greater amount of green algae and diatoms. In general, guppies sampled from sites with high-predation intensity had higher proportions of the fatty acids C16:1n7, C18:3n3, and C20:5n3 whereas guppies from low-predation sites had higher proportions of C20:4n6. Previous studies have used C16:1n7 and C20:5n3 as markers for diatoms and C18:3n3 as a marker for green algae in freshwater streams (Napolitano et al. 1996; Napolitano 1999; Torres-Ruiz et al. 2007). For example periphyton consisting of a large volume of diatoms had higher levels of C20:5n3 than

other periphyton samples and high concentrations of C18:3n3 and C20:5n3 in minnows (*Campostoma anomalum*) were attributed to green algae and diatoms, respectively (Napolitano et al. 1996). Additionally Torres-Ruiz et al. (2007) found that diatoms generally had higher levels of C16:1n7 and C20:5n3 than the other primary producers and invertebrates studied. On the other hand, C20:4n6 is generally low in most types of algae but consistently occurs in aquatic invertebrates comprising up to 7.2% of total fatty acids (Hanson et al. 1985; Napolitano 1999; Torres-Ruiz et al. 2007). Indeed, Torres-Ruiz et al. (2007) found invertebrates to generally have higher levels of C20:4n6 compared to primary producers with the exception of the moss *Hygrohypnum luridum*. Similarly, Ackman and Takeuchi (1986) attributed higher C20:4n6 in wild salmon (*Salmo salar*) compared to hatchery salmon to the consumption of aquatic insects and Kainz et al. (2004) found that macrozooplankton had 13 times more ARA than seston. The general trend of higher proportions of C20:4n6 and lower proportions of C16:1n7, C18:3n3, and C20:5n3 among low-predation guppies could reflect a greater amount of invertebrates and less green algae and diatoms in their diet.

The relative amount of omega-3 fatty acids compared to omega-6 fatty acids was lower at the low-predation site within Quare, Turure and Tacarigua/Tunapuna, although the difference was not significant at Turure. Lower relative amounts of omega-3 fatty acids compared to omega-6 fatty acids suggest a more allochthonous food source for guppies from the low-predation sites (Pollero et al. 1981; Desvilettes et al. 1994; Torres-Ruiz et al. 2007). Most of the guppy's major invertebrate prey items can be classified as collectors or predators (Merritt et al. 2007 as cited in Zandonà 2010; Bassar et al. 2010). Isopods collector-gatherers had lower omega-3 fatty acids relative to omega-6 fatty acids

than periphyton and other invertebrates that were thought to be consuming algae (Torres-Ruiz et al. 2007). Therefore we would also expect a more terrestrial signature for guppies consuming a higher proportion of collector-gatherer invertebrates in their diet. Indeed, invertebrates have been shown to reflect a terrestrial carbon source in streams using stable isotopes (e.g. Doucett et al. 1996). However, carbon sources generally become more autochthonous downstream and the relative amount of omega-3 fatty acids compared to omega-6 fatty acids could reflect difference in the relative position of populations within the rivers rather than differences in diet (Vannote et al. 1980; Doucett et al. 1996).

Variation in fatty acid signatures of the guppies among predation regimes could be affected by differences in physiological state (condition) of the guppies or differences in the fatty acid signatures of prey species among high- and low-predation regimes. However, our results provide little evidence to suggest that this is the case in the current study. Both Fulton's condition factor and % lipid did not differ among predation regimes suggesting similar condition of the fish. Additionally, epilithon fatty acid signatures generally did not differ among high- and low-predation populations and when they did differ, the fatty acid signatures of the epilithon were opposite to those of the guppies. For example, C18:3n3 was higher in epilithon from the low-predation site at Aripo but fish from this site had higher C18:3n3 than their low-predation counterparts. However, this was not always the case and we were not able to collect epilithon at all locations.

Guppy populations experiencing high-predation intensity had greater isotopic niche widths than guppies under low-predation intensity within three of the four high-low-predation pairings. A greater niche width within high-predation populations compliments the fatty acid data and suggests that high-predation guppies have a more

varied diet while low-predation guppies are more specialized on invertebrates. Invertebrates are generally considered a more high quality diet item than algae and detritus (Dussault and Kramer 1981; Zandona et al. 2011). Predators may reduce the amount of time guppies spend foraging and limit the safe foraging areas available to them thereby reducing resource availability and causing the guppies to feed more indiscriminately (Seghers 1970; Fraser and Gilliam 1992; Magurran and Seghers 1994). Additionally, higher handling times associated with invertebrate prey compared to other diet items may compromise vigilance and favour the inclusion of foods that require less handling time when predation is intense (Houtman and Dill 1998). A recent study found that guppy morphology changed to one that enhanced foraging ability rather than predator avoidance when predation pressure was reduced (Palkovacs et al. 2011). Other studies have found that low-predation guppy populations have a higher trophic position and a higher proportion of invertebrates in their guts compared to high-predation guppy populations during the wet season (Dussault and Kramer 1981; Zandonà 2010). Nevertheless, Zandonà et al. (2011) and Bassar et al. (2010) found more invertebrates in the guts of guppies from high-predation sites during the dry season. These differences in diet between seasons were attributed to alterations in guppy densities and resource availability due to flooding and washouts during the wet season (Zandonà 2010). Although it is possible that seasonality affects the guppy`s diet, these previous studies employed gut content analysis which may not accurately represent assimilated food items and may instead reflect accidentally ingested items that were consumed while foraging for more desirable prey (Zandona 2010). Furthermore, the studies conducted during the dry season only examined guppies collected from the Aripo and Guanapo drainages

(Zandonà et al. 2011; Bassar et al. 2010). In a study that compared stream characteristics among rivers in Trinidad, invertebrate biomass was much greater (up to 62 times greater) at the high predation sites at both Aripo and Guanapo compared to high predation sites at Turure and Quare (Zandonà, 2010). We did not examine the Guanapo drainage in the current study, but both fatty acid and stable isotope data were anomalous at Aripo compared with our other rivers. This anomaly could be related to the very high invertebrate availability within this drainage. With such a high abundance of invertebrates, high-predation guppy populations within the Aripo drainage may be able to specialize on invertebrates and have a similar isotopic niche width to low-predation guppies, despite the risk of predation. Therefore both predation and resource availability may interact to influence the diet of guppies in Trinidad, with predation having a greater influence when resources are more limited.

When comparing the isotopic niche width of a species among sites, it is important to consider the intrinsic variability of stable isotope values for basal resources (Hoeinghaus and Zeug 2008). For example, if the intrinsic variability of basal resources is higher in one population compared to another, a larger isotopic niche width may be evident even if that population does not have a greater niche width. In the current study, we found little evidence that intrinsic variability of basal resources could account for the differences in isotopic niche width between high- and low-predation sites. Indeed, the variance of $\delta^{15}N$ and $\delta^{13}C$ values for a baseline organism and epilithon did not differ among high- and low-predation sites.

Although niche width differed among predation regimes, it is difficult to determine if the greater isotopic niche width at high-predation intensity sites reflected a

population composed of individual guppies consuming a variety of diet items or a population of individual guppies each specializing on different diet items (Van Valen 1965; Bearhop et al. 2004; Tinker et al. 2008). In particular, guppies from the high-predation site at Tacarigua displayed $\delta^{13}C$ and $\delta^{15}N$ values that clustered into two groups (Figure 3.3). Rather than all fish being generalists, these fish could be specializing on two different diet items. The individuals with higher $\delta^{15}N$ also tended to have higher C20:4n6 but lower C16:1n7 and C18:3n3, suggesting the consumption of more invertebrates and less green algae and diatoms compared to other guppies in the same population. On the other hand, standard ellipses at Turure and Quare overlapped among predation regimes and did not appear to cluster into separate groups within a population. Bearhop et al. (2004) suggest two methods for distinguishing between the two types of generalist populations; a comparison of stable isotope variance among tissues with long and short turnover rates or a comparison of stable isotope variance of the same tissue over time to that of the entire population at a particular time. Unfortunately, neither of these comparisons were possible in the current study due to the small size of the guppy and time constraints.

Guppy trophic position ranged between approximately 2.6 to 2.8, which we would expected for fish feeding on a diet consisting mainly of algae, detritus and invertebrates. Based on the isotopic niche width results, we anticipated that trophic position would be greater within the low-predation populations. Indeed, a previous study found that trophic position was slightly higher within low-predation populations at Aripo, Turure, and Quare (Zandonà 2010). However, we found no evidence that predation intensity influence guppy trophic position. Alternatively, Zandonà (2010) found that trophic position, calculated

using $\delta^{15}N$ values, was not related to the proportion of invertebrates in the guts. In our study, $\delta^{15}N$ values were not distinguishable between epilithon and invertebrates (Table 3.4). Therefore, the lack of differences in trophic positions between high- and low-predation sites may not reflect differences in guppy diets that exist between high and low intensity predation regimes.

Our results support the prediction that predation influences the feeding ecology and isotopic niche width of guppies. Predation has been shown to be a major driving factor for the evolution of guppies. Studies have shown that guppies transplanted from a high-predation location to a low-predation location rapidly evolved characteristics typically of the low-predation populations in both natural and laboratory experiments (Endler, 1980; Reznick and Bryga 1987). However, in addition to predation, several factors differ between high- and low-predation locations. For example, high-predation locations tend to be larger streams with lower guppy densities (Reznick et al. 2001). In particular, higher resource availability at high-predation sites could be responsible for the greater niche width at the high-predation locations. Nevertheless, more resources within a site does not necessarily equate to more resources available to guppies within that site. Predation restricts guppy foraging to the periphery and to shallow areas of the stream which could limit the amount of resources accessible to guppies (Seghers 1970; Fraser and Gilliam 1992). Furthermore, Zandonà et al. (2011) found that the proportion of invertebrates in the guts of wild guppies was not related to the invertebrate biomass available in the environment and Bassar et al. (2010) showed that guppies placed in mesocosms stocked with equal resources displayed diets that differed according to the predation regime from which the guppies were collected.

It should be noted that guppy size could influence some of the observed differences between predation regimes. In general, guppies from high-predation regimes are smaller than those from low-predation sites (e.g. Rodd and Reznick 1997). In the current study, this was only true at Aripo and Tacarigua and there were no differences in size among predation regimes at Quare and Turure (data not shown). Although adult female guppies are often larger than males, we found no differences in stable isotope values among sexes nor were stable isotope values related to guppy size. It might be expected that larger guppies can consumer larger prey. However, Dussault and Kramer (1981) found that larger female guppies did not consume larger food items than smaller females and found no differences between male and female guppies in regards to the diet items consumed at Upper Aripo and Tacarigua with the exception of diatoms and algal remains at Tacarigua (Dussault and Kramer 1981). Similarly, Zandonà (2010) found that guppy trophic position was not related to guppy size and there was little evidence of a relationship between diet and size. Therefore, there are probably few diet items that larger guppies can consume that smaller guppies cannot. Nevertheless, differences in feeding behaviour among sexes that did not result in different diet items being consumed, have been observed and these differences are likely due to differences in mating effort. For example, female guppies have lower ingestion rates and devote a greater percentage of their time to foraging than male guppies (Dussault and Kramer 1981; Magurran and Seghers 1994).

3.5 CONCLUSIONS

It is well established that predation can influence an organism's feeding ecology and that these alterations can have profound impacts on ecosystem structure (Lima and Dill 1990; Bassar et al. 2010). Understanding the role of predators is of particular interest considering the large impact that humans are having on natural systems around the world, from the decline of top predators in the oceans to the introduction of non-native predators (e.g. Witte et al. 1992; Myers et al. 2007). This study examined the diet and isotopic niche widths of a fish species in a natural environment and found differences among populations experiencing contrasting predation intensity. With the exception of one river, the results suggest that low-predation populations were more specialized on invertebrates while high-predation guppies consumed resources more indiscriminately. Trade-offs between quality of food and predation risk is not unique to guppies. Threespined sticklebacks (*Gasterosteus aculeatus*) consumed smaller and less profitable prey in the presence of a predator and Juvenile coho salmon (*Oncorhynchus kisutch*) reduced their attack distance in the presence of a predator, especially for the highly profitable larger size classes of prey (Dill and Fraser 1984; Ibrahim and Huntingford 1989). In conclusion, predation appears to play a major role in shaping the diet and niche width of guppy populations in Trinidad, however, future studies should consider the influence of other variables that differ among predation regimes, including intra- and interspecifi competition and resource availability.

3.6 REFERENCES

Abramsky Z, Strauss E, Subach A, Kotler BP, Riechman A (1996) The effect of barn owls (*Tyto alba*) on the activity and microhabitat selection of *Gerbillus allenbyi* and *G. pyramidum.* Oecologia 105: 313-319

Ackman RG, Takeuchi T (1986) Comparison of fatty acids and lipids of smolting hatchery-fed and wild Atlantic salmon *Salmo salar*. Lipids 21:117-120

Bassar RD, Marshall MC, López-Sepulcre A, Zandonà E, Auer SK, Travis J, Pringle CM, Flecker AS, Thomas SA, Fraser DF, Reznick DN (2010) Local adaptation in Trinidadian guppies alters ecosystem processes. Proc Natl Acad Sci USA 107: 3616-3621

Bates D, Maechler M, Bolker B (2011) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-39

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73: 1007-1012

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917

Brown JS, Kotler BP, Smith RJ, Wirtz WO II (1988) The effects of owl predation on the foraging behavior of heteromyid rodents. Oecologia 76: 408-415

Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar Mamm Sci 22: 759-801

Dennis CA, MacNeil MA, Rosati JY, Pitcher TE, Fisk AT (2010) Diet discrimination factors are inversely related to $\delta^{15}N$ and $\delta^{13}C$ values of food for fish under controlled conditions. Rapid Commun Mass Spectrom 24: 3515-3520

Desvilettes C, Bourdier G, Breton JC, Combrouze P (1994) Fatty acids as organic markers for the study of trophic relationships in littoral cladoceran communities of a pond. J Plankton Res 16: 643-659

Dill LM (1987) Animal decision making and its ecological consequences: the future of aquatic ecology and behaviour. Can J Zool 65: 803-811

Dill LM, Fraser AHG (1984) Risk of predation and the feeding behavior of juvenile coho salmon (*Oncorhynchus kisutch*). Behav Ecol Sociobiol 16: 65-71

Doucett RR, Power G, Barton DR, Drimmie RJ, Cunjak RA (1996) Stable isotope analysis of nutrient pathways leading to Atlantic salmon. Can J Fish Aquat Sci 53: 2058-2066

Dussault GV, Kramer DL (1981) Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). Can J Zool 59: 684-701

Elgee KE, Evans JP, Ramnarine IW, Rush SA, Pitcher TE (2010) Geographic variation in sperm traits reflects predation risk and natural rates of multiple paternity in the guppy. J Evol Biol 23: 1331-1338

Emlen JM (1966) The role of time and energy in food preference. Am Nat 100: 611-617

Endler JA (1980) Natural selection on color patterns in *Poecilia reticulata*. Evolution 34: 76-91

Endler JA, Houde AE (1995) Geographic variation in female preferences for male traits in *Poecilia reticulata*. Evolution 49: 456-468

Evans JP, Pilastro A, Ramnarine IW (2003) Sperm transfer through forced matings and its evolutionary implications in natural guppy (*Poecilia reticulata*) populations. Biol J Linn Soc 78: 605-612

Fraser DF, Gilliam JF (1992) Nonlethal impacts of predator invasion: facultative suppression of growth and reproduction. Ecology 73: 959-970

Godin J-GJ, Smith SA (1988) A fitness cost of foraging in the guppy. Nature 333: 69-71

Hanson BJ, Cummins KW, Cargill AS, Lowry RR (1985) Lipid content, fatty acid composition, and the effect of diet on fats of aquatic insects. Comp Biochem Physiol 80B: 257-276

Hoeinghaus DJ, Zeug SC (2008) Can stable isotope ratios provide for community-wide measures of trophic structure? Comment. Ecology 89: 2353-2357

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50: 346-363

Houtman R, Dill LM (1998) The influence of predation risk on diet selectivity: a theoretical analysis. Evol Ecol 12: 251-262

Ibrahim AA, Huntingford FA (1989) Laboratory and field studies of the effect of predation risk on foraging in three-spined sticklebacks (*Gasterosteus aculeatus*). Behaviour 109: 46-57

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80: 595-602

Kainz MJ, Arts MT, Mazumder A (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnol Oceanogr 49:1784-1793

Kainz MJ, Johannsson OE, Arts MT (2010) Diet effects on lipid composition, somatic growth potential, and survival of the benthic amphipod *Diporeia* spp. J Great Lakes Res 36: 351-356

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88: 42-48

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68: 619-640

MacArthur RH, Pianka ER (1966) On optimal use of a patchy environment. Am Nat 100: 603-609

Magurran AE (2005) Evolutionary Ecology: The Trinidadian Guppy. Oxford University Press, Oxford

Magurran AE, Seghers BH (1994) Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata,* populations in Trinidad. Proc R Soc Lond B Biol Sci 255: 31-36

Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science 315: 1846-1850

Napolitano GE (1999) Fatty acids as trophic and chemical markers in freshwater ecosystems. In: Arts MT, Wainman BC (eds) Lipids in freshwater ecosystems. Springer-Verlag, New York, pp 21-44

Napolitano GE, Pollero RJ, Gayoso AM, MacDonald BA, Thompson RJ (1997) Fatty acids as trophic markers of phytoplankton blooms in the Bahia Blanca Estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). Biochem Syst Ecol 25: 739-755

Napolitano GE, Shantha NC, Hill WR, Luttrell AE (1996) Lipid and fatty acid compositions of stream periphyton and stoneroller minnows (*Campostoma anomalum*): trophic and environmental implications. Arch Hydrobiol 137: 211-225

Nash RDM, Valencia AH, Geffen AJ (2006) The origin of Fulton's condition factor – setting the record straight. Fisheries 31: 236-238

Neff BD, Pitcher TE, Ramnarine IW (2008) Inter-population variation in multiple paternity and reproductive skew in the guppy. Mol Ecol 17: 2975-2984

Palkovacs EP, Wasserman BA, Kinnison MT (2011) Eco-evolutionary trophic dynamics: loss of top predators drives trophic evolution and ecology of prey. PLoS ONE 6: e18879

Parnell A, Jackson A (2011) siar: Stable Isotope Analysis in R. R package version 4.1

Parrish CC, McKenzie CH, MacDonald BA, Hatfield EA (1995) Seasonal studies of seston lipids in relation to microplankton species composition and scallop growth in South Broad Cove, Newfoundland. Mar Ecol Prog Ser 129: 151-164

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18: 293-320

Pollero RJ, Brenner RR, Gros EG (1981) Seasonal changes in lipid and fatty acid composition of the freshwater mollusk, *Diplodom patagonicus*. Lipids 16: 109-113

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152: 179-189

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reznick DN, Bryga H (1987) Life-history evolution in guppies (*Poecilia reticulata*):1. Phenotypic and genetic changes in an introduction experiment. Evolution 41: 1370-1385

Reznick D, Butler MJ IV, Rodd H (2001) Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. Am Nat 157: 126-140

Rodd FH, Reznick DN (1997) Variation in the demography of guppy populations: the importance of predation and life histories. Ecology 78: 405-418

Rothley KD, Schmitz OJ, Cohon JL (1997) Foraging to balance conflicting demands: novel insights from grasshoppers under predation risk. Behav Ecol 8: 551-559

Schmitz OJ, Beckerman AP, O'Brien KM (1997) Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. Ecology 78: 1388-1399

Seghers BH (1970) Behavioral adaptations of natural populations of the guppy, *Poecilia reticulata*, to predation. Am Zool 10: 489-490

Sheffield G, Fay FH, Feder H, Kelly BP (2001) Laboratory digestion of prey and interpretation of walrus stomach contents. Mar Mamm Sci 17: 310-330

Sih A (1980) Optimal behavior: can foragers balance two conflicting demands? Science 210: 1041-1043

Skutelsky O (1996) Predation risk and state-dependent foraging in scorpions: effects of moonlight on foraging in the scorpion *Buthus occitanus*. Anim Behav 52: 49-57

Tinker MT, Bentall G, Estes JA (2008) Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proc Natl Acad Sci USA 105: 560-565

Torres-Ruiz M, Wehr JD, Perrone AA (2007) Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. J N Am Benthol Soc 26: 509-522

Vander Zanden MJ, Rasmussen JB (1996) A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. Ecol Monogr 66: 451-477

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. Can J Fish Aquat Sci 37: 130-137

Van Valen L (1965) Morphological Variation and Width of Ecological Niche. Am Nat 99: 377-390

Werner EE, Gilliam JF, Hall DJ, Mittelbach GG (1983) An experimental test of the effects of predation risk on habitat use in fish. Ecology 64: 1540-1548

Witte F, Goldschmidt T, Wanink J, van Oijen M, Goudswaard K, Witte-Maas E, Bouton N (1992) The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. Environ Biol Fishes 34: 1-28

Zandonà E (2010) The trophic ecology of guppies (*Poecilia reticulata*) from the streams of Trinidad. PhD dissertation, Department of Biology, Drexel University, Philadelphia, Pennsylvania, USA

Zandonà E, Auer SK, Kilham SS, Howard JL, López-Sepulcre A, O'Connor MP, Bassar RD, Osorio A, Pringle CM, Reznick DN (2011) Diet quality and prey selectivity

correlate with life histories and predation regime in Trinidadian guppies. Funct Ecol, 25, doi: 10.1111/j.1365-2435.2011.01865.x

Table 3.1: Fulton's K ($g \cdot mm^{-3}$), % lipid and the ratio of ∑n-3 /∑n-6 fatty acids for guppies and epilithon collected at 8 sites within 5 river systems in Trinidad (mean ± SE).

River	Predation Regime	N	Fulton's K ($g \cdot mm^{-3}$)	% Lipid	∑n-3 /∑n-6
Guppies					
Upper Airipo	Low	10 male, 10 female	0.91 ± 0.03	11.30 ± 0.79	1.85 ± 0.07
Lower Aripo	High	10 male, 10 female	0.99 ± 0.03	15.97 ± 0.56	1.00 ± 0.08
Tunapuna	Low	10 male, 10 female	1.01 ± 0.03	17.58 ± 0.70	0.97 ± 0.10
Tacarigua	High	10 male, 10 female	1.04 ± 0.07	12.99 ± 0.51	2.16 ± 0.09
Upper Quare	Low	5 male, 10 female	1.12 ± 0.04	11.86 ± 0.93	0.76 ± 0.04
Lower Quare	High	10 male, 10 female	0.98 ± 0.02	11.65 ± 0.67	1.47 ± 0.14
Upper Turure	Low	9 male, 10 female	0.95 ± 0.07	11.69 ± 0.85	1.01 ± 0.05
Lower Turure	High	1 male, 11 female	1.06 ± 0.10	12.97 ± 0.74	1.03 ± 0.10
Epilithon					
Upper Aripo	Low	6		1.77 ± 0.25	1.17 ± 0.31
Lower Aripo	High	5		7.06 ± 1.64	1.74 ± 0.14
Tacarigua	High	2		6.96 ± 0.16	1.66 ± 0.09
Upper Quare	Low	4		2.51 ± 0.35	0.54 ± 0.28
Lower Quare	High	4		1.44 ± 0.24	0.44 ± 0.13

Table 3.2: Fatty acid proportions (% total fatty acid concentration**)** for guppies and epilithon collected at 8 sites within 5 river systems in Trinidad (mean ± SE). Sample sizes are equal to table 3.1.

River	C16:1n-7	C18:2n-6	C18:3n-3	C20:4n-6	C20:5n-3	C22:6n3
Guppies						
Upper Airipo	5.41 ± 0.74	5.11 ± 0.24	1.85 ± 0.27	8.75 ± 0.73	3.45 ± 0.19	16.44 ± 1.12
Lower Aripo	3.40 ± 0.28	9.61 ± 0.44	1.14 ± 0.03	6.94 ± 0.44	2.25 ± 0.24	11.64 ± 0.67
Tunapuna	6.43 ± 0.49	9.62 ± 0.48	1.40 ± 0.09	5.97 ± 0.33	2.61 ± 0.45	8.17 ± 0.70
Tacarigua	5.36 ± 0.34	4.62 ± 0.29	2.80 ± 0.16	6.32 ± 0.30	2.95 ± 0.08	16.00 ± 0.40
Upper Quare	2.20 ± 0.14	10.46 ± 0.37	1.21 ± 0.07	11.02 ± 0.56	1.40 ± 0.10	10.41 ± 0.71
Lower Quare	4.24 ± 0.55	7.24 ± 0.55	1.81 ± 0.23	8.61 ± 0.51	2.15 ± 0.24	15.43 ± 1.03
Upper Turure	2.10 ± 0.18	8.39 ± 0.32	1.29 ± 0.10	10.59 ± 0.54	1.50 ± 0.14	15.05 ± 0.90
Lower Turure	5.15 ± 0.39	8.39 ± 0.68	1.48 ± 0.20	8.28 ± 0.62	2.28 ± 0.41	11.32 ± 1.23
Epilithon						
Upper Aripo	7.30 ± 1.14	5.96 ± 0.50	8.79 ± 1.64	12.79 ± 4.74	7.79 ± 0.80	2.13 ± 0.53
Lower Aripo	3.40 ± 0.53	12.23 ± 0.49	20.76 ± 0.75	2.88 ± 0.07	3.56 ± 0.17	0.43 ± 0.18
Tacarigua	3.11 ± 0.11	14.71 ± 0.32	21.81 ± 0.93	2.83 ± 0.08	4.23 ± 0.30	0.50 ± 0.10
Upper Quare	5.55 ± 1.40	20.89 ± 6.52	9.64 ± 4.92	3.09 ± 1.06	1.14 ± 0.67	0 ± 0
Lower Quare	9.74 ± 0.46	17.19 ± 1.93	6.39 ± 0.54	1.91 ± 0.30	1.86 ± 0.68	0.60 ± 0.60

Table 3.3: Fatty acid concentrations (μg FAME mg · dry weight^{-1}) for guppies and epilithon collected at 8 sites within 5 river systems in Trinidad (mean ± SE). Sample sizes are equal to table 3.1.

River	C16:1n-7	C18:2n-6	C18:3n-3	C20:4n-6	C20:5n-3	C22:6n3
Guppies						
Upper Airipo	4.43 ± 1.02	3.47 ± 0.49	1.55 ± 0.42	4.65 ± 0.18	2.17 ± 0.27	9.10 ± 0.45
Lower Aripo	3.51 ± 0.45	9.41 ± 0.67	1.12 ± 0.08	6.36 ± 0.23	2.23 ± 0.29	10.77 ± 0.35
Tunapuna	8.77 ± 0.94	12.51 ± 0.81	1.83 ± 0.16	7.40 ± 0.23	3.57 ± 0.71	10.21 ± 0.65
Tacarigua	3.68 ± 0.33	3.07 ± 0.22	1.89 ± 0.15	4.12 ± 0.17	1.97 ± 0.10	10.62 ± 0.42
Upper Quare	1.46 ± 0.19	6.84 ± 0.66	0.79 ± 0.08	6.80 ± 0.38	0.90 ± 0.11	6.60 ± 0.67
Lower Quare	2.80 ± 0.49	4.97 ± 0.80	1.30 ± 0.28	5.01 ± 0.25	1.24 ± 0.13	8.99 ± 0.50
Upper Turure	1.27 ± 0.25	5.01 ± 0.65	0.79 ± 0.12	5.52 ± 0.25	0.76 ± 0.07	7.86 ± 0.44
Lower Turure	3.64 ± 0.37	6.06 ± 0.67	1.02 ± 0.14	5.48 ± 0.23	1.66 ± 0.34	7.49 ± 0.60
Epilithon						
Upper Aripo	0.48 ± 0.16	0.38 ± 0.11	0.61 ± 0.21	0.45 ± 0.13	0.42 ± 0.08	0.09 ± 0.01
Lower Aripo	0.78 ± 0.15	3.26 ± 0.87	5.71 ± 1.64	0.76 ± 0.20	0.94 ± 0.25	0.07 ± 0.02
Tacarigua	0.86 ± 0.06	4.10 ± 0.33	6.11 ± 0.88	0.79 ± 0.06	1.17 ± 0.04	0.14 ± 0.01
Upper Quare	0.23 ± 0.05	0.87 ± 0.26	0.42 ± 0.21	0.13 ± 0.04	0.05 ± 0.03	0 ± 0
Lower Quare	0.29 ± 0.04	0.52 ± 0.10	0.19 ± 0.01	0.06 ± 0.00	0.05 ± 0.02	0.02 ± 0.02

Table 3.4: Stable isotope data (‰) for guppy, epilithon, chironomidae and snail samples collected at 8 sites within in 5 river systems in Trinidad (mean ± SE).

River	Predation Regime	N	δ13C (‰)	δ15N (‰)	C:N
Guppies					
Upper Airipo	Low	10 male, 9 female	-27.0 ± 0.2	8.9 ± 0.2	3.2 ± 0.0
Lower Aripo	High	10 male, 10 female	-24.0 ± 0.2	8.8 ± 0.1	3.2 ± 0.0
Tunapuna	Low	9 male, 10 female	-24.7 ± 0.3	8.0 ± 0.2	3.2 ± 0.0
Tacarigua	High	9 male, 10 female	-31.9 ± 0.8	8.0 ± 0.2	3.2 ± 0.0
Upper Quare	Low	5 male, 10 female	-26.2 ± 0.2	7.1 ± 0.1	3.3 ± 0.0
Lower Quare	High	10 male, 10 female	-28.5 ± 0.7	7.5 ± 0.1	3.3 ± 0.0
Upper Turure	Low	9 male, 10 female	-26.8 ± 0.2	6.7 ± 0.1	3.2 ± 0.0
Lower Turure	High	1 male, 11 female	-26.7 ± 0.5	7.2 ± 0.3	3.2 ± 0.0
Epilithon					
Upper Airipo	Low	6	-23.2 ± 1.3	5.0 ± 0.3	24.6 ± 2.2
Lower Aripo	High	6	-30.2 ± 1.9	5.1 ± .5	8.2 ± 1.1
Tacarigua	High	3	-32.2 ± 0.3	4.2 ± 0.1	7.1 ± 0.1
Upper Quare	Low	5	-26.0 ± 2.1	-0.8 ± 0.4	15.1 ± 3.3
Lower Quare	High	4	-23.2 ± 0.2	-0.5 ± 0.2	9.1 ± 0.2
Chironomidae					
Upper Airipo	Low	3	-26.0 ± 0.1	5.0 ± 0.1	4.1 ± 0.0
Lower Aripo	High	2	-25.4 ± 0.3	5.1 ± 0.1	4.0 ± 0.0
Upper Turure	Low	2	-27.6 ± 0.2	2.7 ± 0.0	4.0 ± 0.0
Lower Turure	High	1	-27.1	2.5	4.1
Snails (Lymnaeidae)					
Upper Airipo	Low	5	-27.9 ± 0.3	7.2 ± 0.3	4.1 ± 0.2
Lower Aripo	High	4	-26.1 ± 0.1	7.2 ± 0.1	3.8 ± 0.2
Upper Turure	Low	4	-27.5 ± 0.5	5.1 ± 0.8	3.8 ± 0.2
Lower Turure	High	3	-27.9 ± 0.4	4.2 ± 0.3	4.1 ± 0.1

Table 3.5: Average standard ellipse area (SEA_b) values for 8 guppy populations in Trinidad. SEA_b is the size-corrected standard ellipse area, calculated using $\delta^{13}C$ and $\delta^{15}N$ values for population members within each sampling area (see methods for details). P-values reflect comparisons between sites of differing predation intensity within each river.

River	Predation Regime	SEA_b	p-value
Aripo	Low	2.3	0.19
	High	1.8	
Tunapuna[a] Tacarigua	Low	3.5	0.016
	High	7.0	
Quare	Low	1.7	<0.001
	High	6.6	
Turure	Low	1.7	0.003
	High	6.0	

[a]Because of their similar location within the watershed, Tunapuna and Tacarigua were treated as the same river system.

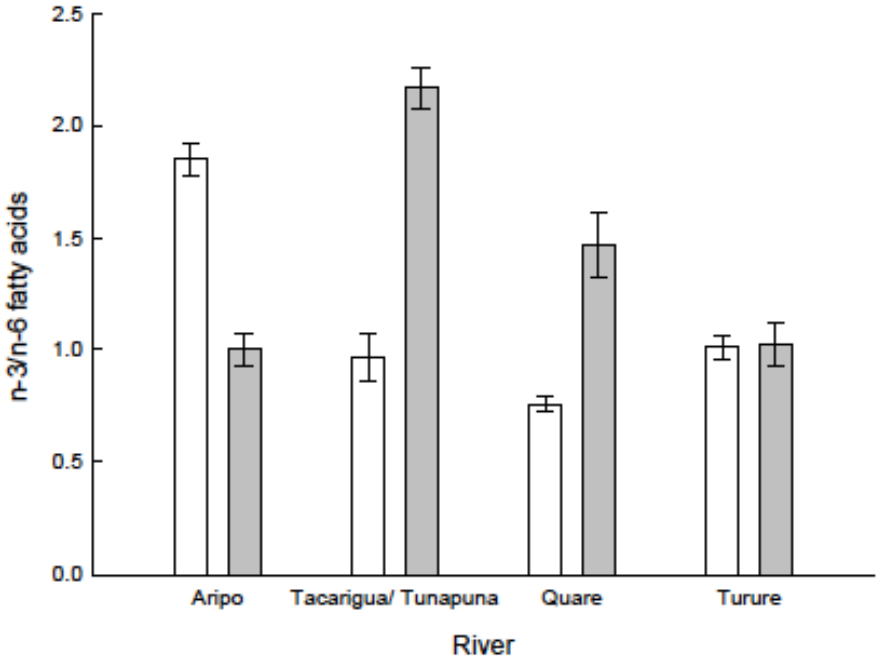

Figure 3.1 Comparison of the relative amount of omega-3 and omega-6 fatty acids (∑n-3/∑n-6 fatty acids, mean ± SE) among high- (filled bars) and low-predation (open bars) guppy (*Poecilia reticulata*) populations

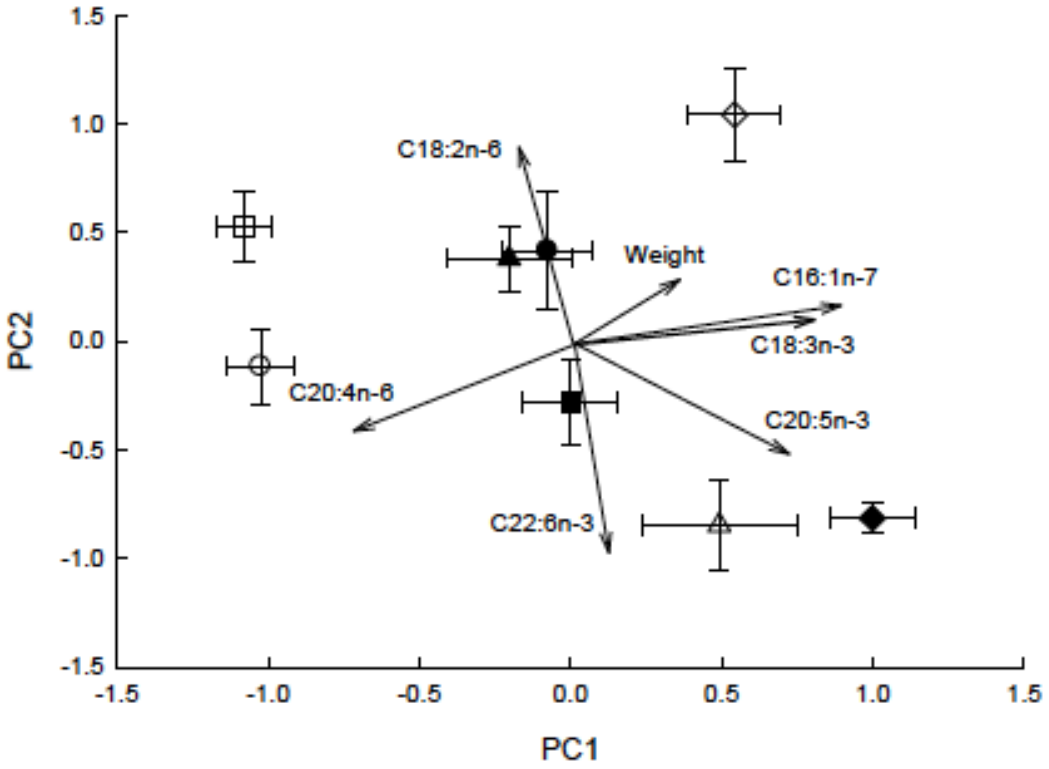

Figure 3.2 Biplot of the component scores (mean ± SE) and fatty acid variable loadings on the first two principal components from a PCA performed on transformed and standardized fatty acid proportions of guppies from high- (filled symbols) and low-predation (open symbols) populations within 4 rivers including Aripo (triangles), Quare (squares), Tacarigua/Tunapuna (diamonds) and Turure (circles)

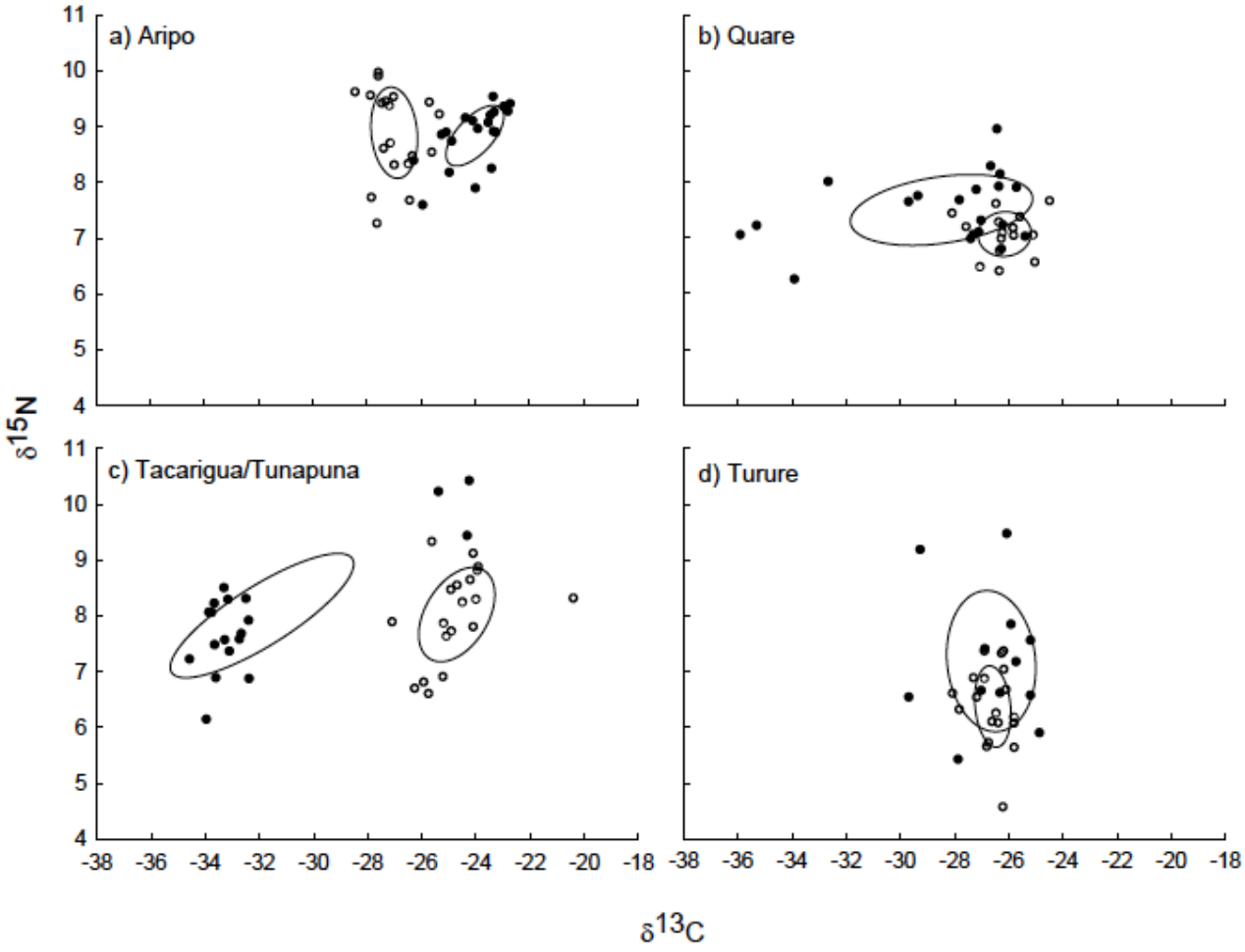

Figure 3.3 Stable Isotope Ellipses (SEA_c, see Jackson et al. 2011 for details) calculated for high- (closed circles) and low-predation (open circles) populations within a) Aripo (triangles), b) Quare (squares) c) Tacarigua/Tunapuna (diamonds) and d) Turure (circles)

4.0 – GENERAL DISCUSSION

4.1 SUMMARY

The main objective of this thesis was to evaluate the trophic ecology of a freshwater fish species in a natural habitat where predation intensity varies over a relatively short distance. In order to accomplish this goal, we used chemical tracers called fatty acids and stable isotopes to estimate diet, trophic position and niche width. However, some assumptions about the use of these tracers needed to be addressed and it was important to establish a reliable diet discrimination factor (DDF) for the species of interest prior to applying stable isotopes in the field.

Chapter two of this thesis addressed the assumption of a constant DDF in stable isotope studies. Most studies do not account for DDF variability even though there is strong evidence to suggest that DDFs are dependent on food $\delta^{13}C$ and $\delta^{15}N$ values (Caut et al. 2008; Overmyer et al. 2008; Caut et al. 2009). We created artificial diets using maggots with a wide range of $\delta^{13}C$ and $\delta^{15}N$ values which were fed to guppies until they came into equilibrium with the new diet. By creating variable $\delta^{13}C$ and $\delta^{15}N$ values within a single food source we avoided confounding factors, such a diet quality, that could arise if different food types had been used (MacNeil et al. 2006). As control treatments we also fed one group of guppies TetraMin Tropic Flakes, a commercial fish food, and a separate group of guppies was fed a maggot diet with no chemicals added. The results of this study showed that both food type and food $\delta^{13}C$ and $\delta^{15}N$ values influenced the DDF of the guppies. First, the fish fed the control diet consisting of TetraMin Tropic Flakes, had a lower DDF than fish fed a diet consisting of maggots with similar stable isotope values. Furthermore the DDF of fish fed the TetraMin diet had a DDF more consistent with

previously published values. Differences in DDF values among the two diet treatments likely arose due to differences in nutritional quality, with the TetraMin thought to represent a higher quality diet than the maggots. A second major finding of this study was a negative linear relationship between the food stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and DDF meaning that as the food stable isotope value increase, the difference between the food and the fish tissue decrease. This relationship resulted in some of the DDF values becoming negative at high $\delta^{13}C$ and $\delta^{15}N$ values of the food.

Chapter three of this thesis used the results of chapter two to apply stable isotopes in the field in conjunction with fatty acids. Guppy populations in Trindad are an important model system in ecology yet trophic relationships in this system have drawn attention only recently (Bassar et al. 2010; Zandona 2010; Zandona et al. 2011). Guppies from eight wild populations were collected in Trinidadian streams where predation pressure varies. The populations studied included 4 high-low predation pairs selected based on past studies (Magurran and Seghers 1994; Endler and Houde 1995; Evans et al. 2003; Neff et al. 2008) and were distributed among 5 rivers. Both fatty acid and stable isotope data were analysed and inferences were made about how predation affects the diet and niche width of guppies. First, guppies collected from high predation populations had fatty acid signatures reflecting a greater proportion of algae in the diet while guppies from low predation population had fatty acid signatures reflecting a diet that includes more invertebrates. The fatty acid results were in agreement with a previous study of gut content analysis that took place in the wet season but disagreed with studies from the dry season (Bassar et al. 2010; Zandona 2010; Zandona et al. 2011).

Stable isotope data suggested a smaller niche width among low predation guppies compared to high predation populations. The smaller niche width among low predation populations could indicate that guppies specialize on invertebrates when predation pressure is not intense but eat a more varied diet under high predation threat. Nevertheless, estimates of trophic position based on stable isotope values using a DDF selected from chapter 2 did not differ among predation regimes even though we would have expected a higher trophic position among low predation populated based on fatty acid and isotopic niche width data. Although predation appears to be a likely explanation for differences in diet among guppy populations, other factors could contribute to these differences, including differences in resource availability and baseline stable isotope values (Reznick et al. 2001; Hoeinghaus and Zeug 2008). However, Zandona et al. 2011 found that invertebrate availability did not affect the proportion of invertebrates in guppy guts and the intrinsic variability of the rivers did not differ among high and low predation sites in our study. In addition, both stable isotope and fatty acid data showed the opposite trend within one river system where high invertebrate biomass has been observed (Zandona 2010). High invertebrate availability could confound the influence of predation and enable guppies to specialize on invertebrates even when predation intensity is high.

4.2 IMPLICATIONS AND FUTURE DIRECTIONS

Chemical tracers such as stable isotope and fatty acids have the potential to provide a powerful means of evaluating food web structure and trophic interactions. However, these tracers are not always applied appropriately and their continued use in ecological studies demands a solid understanding of the assumptions underlying their

application (Gannes et al. 1997; Jardine et al. 2006; Caut et al. 2009; Wolf et al. 2009). The results of chapter two of this thesis highlighted the importance of selecting a suitable DDF that accounts for variability in food $\delta^{13}C$ and $\delta^{15}N$ values. Inappropriate DDFs can confound the interpretation of stable isotope studies that estimate trophic position, use $^{15}NH_4$ as a tracer for nutrient cycling or use isotopic mixing models for diet reconstruction. However, establishing an appropriate DDF may be difficult and time consuming, often requiring an independent laboratory experiment if insufficient data exists for the species and prey items of interest. Indeed, it took the guppy approximately 60 days to come into equilibrium with a new diet. Although establishing a reliable DDF may be problematic in some studies with strict time constraints, dismissing DDF variability among food sources could result in misleading conclusions and interpretations.

Currently the mechanism underlying the negative relationship between food stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and DDF requires further explanation. We proposed that the relationship could arise if food compounds containing the light isotope are preferentially absorbed from the gut while compounds containing the heavy isotope are preferentially excreted as waste. There is some evidence to suggest this is the case since black fly larvae and copepods both excreted products rich in the heavy isotope (Checkley and Entzeroth 1985; Overmyer et al. 2008). However a study that specifically targets the underlying cause of the relationships is warranted to establish a method for estimating DDFs without performing a controlled laboratory experiment.

Our study of wild guppy populations in Trinidad produced valuable information about the diet of this important model organism which may enhance our understanding of

how changes in predation intensity can affect an ecosystem. Numerous studies have used guppy populations in Trinidad to study the influence of predation on the evolution of guppy characteristic, including life-history traits, behaviour, and morphology (e.g. Reznick and Endler 1982; Fraser et al 2004; Palkovacs et al. 2011). However, differences in diet among guppy populations have only become a topic of interest in the past few years, despite the potential for diet to influence these other traits. In addition, concern over the decline of marine top predators and the introduction of invasive predators has received much interest in recent years (e.g. Witte et al. 1992; Myers et al. 2007). Wild guppy populations in Trinidad provide an opportunity to better understand how changes in predation intensity influence diet and niche width. Our study provides evidence that release from predation may enable animals to become more specialized on desirable food items. Similarly, Palkovacs et al. (2011) also found that morphology traits favouring foraging ability were enhanced when guppies were released from predation.

Recent studies concluded that high-predation guppy populations had a more specialized diet consisting of a higher proportion of invertebrates than low-predation populations (Bassar et al. 2010; Zandona 2010; Zandona et al. 2011). However, these conclusions were based on gut content analysis and only considered two rivers, both of which had a very high invertebrate biomass at the high-predation site compared to other rivers studied in this thesis (Bassar et al. 2010; Zandona 2010; Zandona et al. 2011). Furthermore, one of these studies also found higher trophic positions and more invertebrates in the guts of guppies from low-predation sites compared to high-predation sites within two other rivers (Zandona 2010). This discrepancy could be related to seasonal flooding and washouts which would affect population densities and resources

during the wet season (Zandona 2010). Indeed, a study targeting seasonal differences in diet among guppy populations replicated in multiple river systems would be beneficial. However, differences in guppy diets among seasons could easily have resulted from differences between the rivers being studied, especially considering the very high invertebrate biomass at the rivers studied by Zandona et al. (2011) during the dry season.

Another topic that deserves further investigation is the influence of competition. Competition levels may differ among predation regimes which could influence guppy diet and niche width. For example, increased competition could result from more diverse fish assemblages at high-predation locations or higher guppy densities at low-predation sites (Dussault and Kramer 1981; Reznick et al. 2001). To date, competition for food resources has not been assessed in relation to the guppy diet or foraging behaviour but this subject warrants consideration (Magurran 2005). Nevertheless, competition would likely have the opposite effect on niche width than what was found in this dissertation. High intraspecific competition at low-predation sites would likely favour niche expansion while high interspecific competition at high-predation sites would likely favour a narrow niche width (Gladfelter and Johnson 1983; Alatalo et al. 1985; Bolnick 2001; Tinker et al. 2008). On the other hand, predation may increase intraspecific competition by restricting habitat use and thereby increasing niche width (Fraser and Gilliam 1992). The broader niche width found at high-predation site compared to low-predation sites, therefore, provides evidence for the predation hypothesis rather than the competition hypothesis.

In conclusion this study has drawn attention to one of the major caveats regarding the broad application of stable isotopes in field studies and recommends the use of diet

dependent diet discrimination factors in future studies. Furthermore the trophic ecology of an important model organism has been evaluated with key findings that suggest predation causes populations of wild guppies to become less discriminate foragers. These conclusions should improve our understanding of how predators influence ecosystems and enhance the use of stable isotopes in field studies.

4.3 REFERENCES

Alatalo RV, Gustafsson L, Linden M, Lundberg A (1985) Interspecific competition and niche shifts in tits and the goldcrest: an experiment. J Anim Ecol 54: 977-984.

Bassar RD, Marshall MC, López-Sepulcre A, Zandonà E, Auer SK, Travis J, Pringle CM, Flecker AS, Thomas SA, Fraser DF, Reznick DN (2010) Local adaptation in Trinidadian guppies alters ecosystem processes. Proc Natl Acad Sci USA 107: 3616-3621

Bolnick DI (2001) Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. Nature 410: 463-466

Caut S, Angulo E, Courchamp F (2008) Discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$) in an omnivorous consumer: effect of diet isotopic ratio. Funct Ecol 22: 255-263

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46: 443-453

Checkley DM, Entzeroth LC (1985). Elemental and isotopic fractionation of carbon and nitrogen by marine , planktonic copepods and implications to the marine nitrogen cycle. J Plankton Res 7: 553-568.

Dussault GV, Kramer DL (1981) Food and feeding behavior of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). Can J Zool 59: 684-701

Endler JA, Houde AE (1995) Geographic variation in female preferences for male traits in *Poecilia reticulata*. Evolution 49: 456-468

Evans JP, Pilastro A, Ramnarine IW (2003) Sperm transfer through forced matings and its evolutionary implications in natural guppy (*Poecilia reticulata*) populations. Biol J Linn Soc 78: 605-612

Fraser, D.F. & Gilliam, J.F. (1992) Nonlethal impacts of predator invasion: facultative suppression of growth and reproduction. Ecology, 73, 959-970.

Fraser DF, Gilliam JF, Akkara JT, Albanese BW, Snider SB (2004). Night feeding by guppies under predator release: effects on growth and daytime courtship. Ecology 85: 312-319

Gannes LZ, O'Brien DM, Martinez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78: 1271-1276

Gladfelter WB, Johnson WS (1983) Feeding niche separation in a guild of tropical reef fishes (Holocentridae). Ecology 64: 552-563

Hoeinghaus DJ, Zeug SC (2008) Can stable isotope ratios provide for community-wide measures of trophic structure? Comment. Ecology 89: 2353-2357

Jardine TD, Kidd KA, Fisk AT (2006) Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. Environ Sci Technol 40: 7501-7511

MacNeil MA, Drouillard KG, Fisk AT (2006). Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. Can J Fish Aquat Sci 63: 345-353.

Magurran AE (2005) Evolutionary Ecology: The Trinidadian Guppy. Oxford University Press, Oxford

Magurran AE, Seghers BH (1994) Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. Proc R Soc Lond B Biol Sci 255: 31-36

Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science 315: 1846-1850

Neff BD, Pitcher TE, Ramnarine IW (2008) Inter-population variation in multiple paternity and reproductive skew in the guppy. Mol Ecol 17: 2975-2984

Overmyer JP, MacNeil M, Fisk AT (2008) Fractionation and metabolic turnover of carbon and nitrogen stable isotopes in black fly larvae. Rapid Commun Mass Spectrom 22: 694-700

Palkovacs EP, Wasserman BA, Kinnison MT (2011) Eco-evolutionary trophic dynamics: loss of top predators drives trophic evolution and ecology of prey. PLoS ONE 6: e18879

Reznick D, Butler MJ IV, Rodd H (2001) Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. Am Nat 157: 126-140

Reznick D, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36: 160-177

Tinker, M.T., Bentall, G. & Estes, J.A. (2008) Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences of the United States of America, 105, 560-565.

Witte F, Goldschmidt T, Wanink J, van Oijen M, Goudswaard K, Witte-Maas E, Bouton N (1992) The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. Environ Biol Fishes 34: 1-28

Wolf N, Carleton SA, Martinez del Rio C (2009) Ten years of experimental animal isotopic ecology. Funct Ecol 23: 17-26

Zandonà E (2010) The trophic ecology of guppies (*Poecilia reticulata*) from the streams of Trinidad. PhD dissertation, Department of Biology, Drexel University, Philadelphia, Pennsylvania, USA

Zandonà E, Auer SK, Kilham SS, Howard JL, López-Sepulcre A, O'Connor MP, Bassar RD, Osorio A, Pringle CM, Reznick DN (2011) Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. Funct Ecol 25: doi: 10.1111/j.1365-2435.2011.01865.x

VITA AUCTORIS

Caroline Dennis was born in 1986 in Windsor, Ontario, Canada. She graduated from Walkerville Collegiate Institute and the Windsor Centre for the Creative Arts in 2004. Following high school, she went on to obtain a B.Sc. in Environmental Geoscience at the University of Windsor graduating with Great Distinction in 2009. She is currently a candidate for the Master's degree in Environmental Science at the University of Windsor and hopes to graduate in October 2011.