

THE ROLE OF TERRESTRIAL SUBSIDIES IN FISH COMMUNITIES WITH A  
PARTICULAR FOCUS UPON CYPRINIDS

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THE ROLE OF TERRESTRIAL SUBSIDIES IN FISH COMMUNITIES WITH A  
PARTICULAR FOCUS UPON CYPRINIDS

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## **ABSTRACT**

### **THE ROLE OF TERRESTRIAL SUBSIDIES IN FISH COMMUNITIES WITH A PARTICULAR FOCUS UPON CYPRINIDS**

by

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The importance of the fluxes of energy and nutrients that cross the terrestrial-aquatic ecotone has been recognized for several decades. Allochthonous energy and nutrients, such as terrestrial leaf litter falling into a stream where it is utilized by aquatic macroinvertebrates, is known as an ecological subsidy. Research on ecological subsidies has extended into more food-web linkages that include terrestrial arthropods subsidizing fish communities, emergent aquatic insects subsidizing riparian spider communities, and marine derived nutrients from salmonids that migrate upstream to spawn and die and their decaying carcasses are utilized by many species of terrestrial

plants and animals. The primary objectives of this dissertation are to 1) assess the importance of terrestrial subsidies across a broader geographic and taxonomic level 2) determine the importance of terrestrial subsidies in a fish community inhabiting a semi-arid sub-tropical stream and examine the variation of terrestrial subsidy consumption among fish species in different habitat types, and 3) examine the variability in the carbon to nitrogen ratios of aquatic and terrestrial prey items that fishes commonly utilize and 4) determine the optimal growth temperature for Guadalupe bass (*Micropterus treculii*) to explore physiological similarities with co-occurring micropterids and assess how management of streamflow and the riparian area within the Llano River can help protect Guadalupe bass with respect to riparian and spring-flow influence. These results will help answer questions concerning the importance of terrestrial subsidies for fish communities outside of temperate salmonid streams, guide managers on the importance of terrestrial subsidies in systems that are markedly different from temperate salmonid streams, and assess how the nutritional quality of terrestrial and aquatic food items consumed by fishes might play a role in the consumption and nutrient dissemination between aquatic and terrestrial ecosystems.

## **CHAPTER 1**

### **TERRESTRIAL SUBSIDIES IN THE DIETS OF STREAM FISHES OF THE USA: COMPARISONS AMONG TAXA AND MORPHOLOGY**

#### **Abstract**

Terrestrial food subsidies are important energy sources to a variety of stream fishes across the globe but outside of salmonid communities, their importance is still not fully understood. Using published values of the terrestrial proportion of fish diets, we investigated the patterns of fish taxonomy and jaw morphology in the consumption of terrestrial food items by non-salmonid stream fishes in the USA. We made two predictions: in general, fishes would consume terrestrial food items to the extent of salmonids, and subsidy consumption would be associated with patterns in jaw morphology. Cyprinids and fundulids consumed terrestrial subsidies approaching levels observed in salmonids (20 – 44% terrestrial food) but others (e.g. catostomids and moronids) consumed little (< 2%) terrestrial food items. Fishes with terminal jaw positions tended to consume more terrestrial inputs than those with sub-terminal and inferior jaw positions. Within a species, there were highly variable propensities for consuming terrestrial subsidies. The results of this paper justify a focus on trophic linkages between fishes and riparian systems across a wide range of taxa and habitats. It

is necessary to assess the relative contribution of terrestrial subsidies in fish growth and population dynamics with a focus on nutritional and energetic benefits.

## **Introduction**

The transfer of energy and materials across aquatic and terrestrial ecotones has long been recognized (Minshall 1967; Likens and Bormann 1974). While terrestrial leaf litter provides an energy basis for many stream systems, terrestrial arthropods can also subsidize aquatic consumers, especially fishes. The flow of energy between ecosystems might be affected by the relative availability of *in situ* prey items versus the subsidy (Marczak *et al.* 2007), but can also be related to the relative productivity values of donor and recipient ecosystems (Polis *et al.* 1997). Such subsidy mediation of energy flow may be better understood by assessing the contribution of terrestrial subsidies to fish diets across taxa and geographic regions.

Studies on fish feeding behavior indicate that the consumption of terrestrial subsidies by fishes is a global phenomenon (Balcombe *et al.* 2005; Bosjen 2005) and there is also an increasing amount of evidence that fishes consuming terrestrial seeds may be important seed dispersers in both temperate and tropical systems (Horn 1997; Horn *et al.* 2011). One family of fishes, Salmonidae, appears to consume terrestrial food items with a greater consistency than other fishes. Terrestrial subsidies constituted 68% of the diet of coho salmon (*Oncorhynchus kisutch*) (Eberle and Stanford 2009) and contributed over 50% of a salmonid's annual energy budget (Nakano and Murakami 2001; Utz and Hartman 2007). In other cases, salmonids consume only moderate amounts of terrestrial subsidies, in the 10 – 20% range (Thomas 1962; Tippetts *et al.* 1978; Cada *et al.* 1987).

In order to assess general taxonomic and morphological patterns of the consumption of terrestrial foods by fishes, we used published values of percent terrestrial subsidy contribution to fish diets. Because of the great taxonomic and geographic variability of fish communities, we restrict our analysis to the USA. To our current knowledge, there is a general underrepresentation of work performed for fish communities outside of salmonid-dominated systems that explicitly focus on the importance of terrestrial food in the USA (but see Cloe and Garman 1996). We did not set out *a priori* to test if other fish species consume more or fewer terrestrial food items than salmonids, but we do predict that terrestrial subsidy inputs are important among many fish taxa outside of ecosystems where salmonids evolved.

We analyzed several fish families (Catostomidae, Centrarchidae, Cottidae, Cyprinidae, Fundulidae, Ictaluridae, Moronidae, and Percidae) to determine which, if any, tend to utilize more terrestrial subsidy inputs and to what extent. We predicted that if there were families that tended to consume terrestrial food items more than others, there would be an association with that family's jaw position (e.g. inferior, sub-terminal, and terminal jaw types). Since terrestrial subsidy inputs must enter the aquatic system from the water's surface, we predict that fishes with more upturned jaw positions (i.e. terminal) would consume more terrestrial inputs than those with more downward opening mouths. Jaw morphology has been shown to be an important factor in the sizes and types of prey fishes consume (Hugueny and Pouilly 1999; Keeley and Grant 2001). We also assess patterns in the types of terrestrial food items consumed by fishes. This is an important component because understanding the particular types of prey items fishes consume can guide more exacting riparian management strategies.

## Methods and Materials

We used 37 published diet references that represent 55 fish species and 74 total diet observations from the USA (see Accessory Publication) using the percentage of terrestrial food items in diet as a response variable. We restricted our analyses to this geographic boundary due to both taxonomic and environmental variability making comparisons of diet difficult. However, this study encompasses a large geographic range within the USA. The percent contribution of terrestrial food items was used as a response variable. The percent terrestrial contribution was averaged across seasons or size classes, depending on which way the data were presented. In cases where the authors reported both, we opted for a mean across seasons in order to capture temporal variability. We recorded only explicitly identified terrestrial food items. For example, if the original reference combined winged-adult aquatic insects with terrestrial food items, those observations were discarded. The category of “terrestrial food items” therefore includes both plant and animal remains but the majority of the terrestrial diet remains are of arthropods. The dataset we obtained was primarily represented by diets of fishes in lotic systems. Five of the 37 references analyzed represented diets of fishes collected in lentic systems, all of which were reservoirs.

For each reference, our database was populated using both environmental and biological variables. We used fish species (for original papers that include multiple species, each species was entered as an independent observation) and fish family. We categorized each species based on jaw morphology (inferior, sub-terminal, and terminal) from Barton (2006) and Goldstein and Simon (1999). Superior mouth positions were not included as none of the species analyzed possess this mouth configuration. This was

determined by species accounts in Pflieger (1997), Etnier and Starnes (2001) and Thomas *et al.* (2007). We accepted three quantitative diet methods for our analyses that were categorized and interpreted using Hyslop (1980):

(1) Percent by abundance of food items (PBA), calculated as the total number of a particular prey items over the grand total number of prey items ingested by fish.

(2) Percent by mass of food items (PBM), calculated as the mass of a particular prey category over the total mass of all prey items ingested by fish. This method is most often expressed as dry weight in grams or milligrams.

(3) Percent by volume of food items (PBV), similar to PBM but using volume displacement instead of weight. A prey category is placed into a graduated container with liquid and the net volume is recorded. The percent is the composition (%) of food items over the grand total of displacement in fish stomach contents for all food categories. Because of the high variability and sensitivity to food abundance, we did not use percent by occurrence or frequency of occurrence.

## Results

Across all diet methods, terrestrial subsidies occurred among representative taxa in five of the eight families (63%) and 37 of the 55 species (68%) surveyed in this study. Percent contribution of terrestrial inputs ranged from 0% among representative taxa of three families (i.e., Catostomidae, Cottidae, and Moronidae) to 41% among fundulids and 44% among cyprinids. Terrestrial inputs were rare in percids (<1%), centrarchids (5%), and ictalurids (up to 13%). Among species and reported diets ( $N = 74$ ), terrestrial food items were absent to rare (0 – 15% contribution) in 88% ( $N = 65$ ) of observations,



moderate (16 – 30%) in 8% ( $N = 6$ ) of observations, and high (30 – 44%) in 4% ( $N = 4$ ) of observations (Figure 1). Of the studies that explicitly identified terrestrial subsidy taxa ( $N = 20$  studies and  $N = 34$  diet observations), hymenopterans were the most frequent terrestrial subsidy across diet methods, occurring in 30 (88%) of observations. Coleopterans occurred in 59%, orthopterans in 32%, and plant materials occurred in 41%. Cyprinids consumed all terrestrial subsidy types identified, hymenopterans being the most frequent, occurring in 25 (93%) of the 27 cyprinid diet observations (Table 1).

The percentages of terrestrial subsidies in fish diets were greatest in small-bodied cyprinids and fundulids. Mean percent volume of terrestrial inputs was 15% (range: 1 – 44%) among three cyprinid genera and 14 species (Table 2). Mean percent volume was 33% (range: 22 – 41%;  $N = 2$ ) in *Luxilus* diets, 18% (range: 2 – 44%;  $N = 4$ ) in *Cyprinella* diets, and 9.5% (range: 1 – 23%;  $N = 8$ ) in *Notropis* diets (Table 3). All of the studies ( $N = 7$ ) where cyprinids consumed moderate to high (>20%) terrestrial subsidies were sampled from stream systems with dense forest cover in portions of the sampling reach suggesting a relationship between terrestrial subsidy availability and habitat. However, there were also instances where cyprinids within the same forested stream consumed little or no terrestrial subsidies. Mean percent volume was 15% (range: 1 – 41%) among three species of fundulids. No habitat mediated variability in terrestrial diet contribution for fundulids could be ascertained; all three species were sampled from lentic systems and only *Fundulus notatus* was observed to consume a high amount of terrestrial food (41%).

Terrestrial subsidy abundance was relatively low but consistently consumed among one of the most popular non-salmonid sport fish genera (*Micropterus*). Mean

percent terrestrial inputs were 1% (range: 1 – 2%) by volume for *M. dolomieu*, *M. punctulatus*, and *M. salmoides* and 3% (range 2 – 3%) by percent biomass for (*M. dolomieu* and *M. salmoides*). Within year, percent contributions were highest in spring for both *M. dolomieu* (4%) and in *M. salmoides* (7%). Among studies, micropterids were primarily sampled from streams, but the greatest average terrestrial inputs were observed (4% by abundance) from lentic habitats.

Terrestrial food items were reported in the diets of fish with inferior, sub-terminal, and terminal jaw positions. Across diet technique categories, mean percent terrestrial subsidies in the diets were lowest in fishes with inferior jaw positions and highest in fishes with terminal jaw positions (Table 4). Maximum percent terrestrial subsidies in fish diets were 7% for inferior, 13% for sub-terminal, and 44% for terminal jaw positions. Greatest familial diversity of jaw morphology was observed within cyprinids, which were represented by all three jaw positions. Among cyprinids, mean percent volume of terrestrial inputs were 2% (N = 3 observations) for inferior jaw positions, 2% (N = 7) for sub-terminal jaw positions, and 12% (N = 24) for terminal positions.

## Discussion

Terrestrial food subsidies frequently occurred within the diets of fishes (68%) reviewed in this study and had moderate to high (11 to 44%) contributions in 12% of the fishes. Greater terrestrial food subsidies were associated with smaller-bodied fishes and fishes with terminal jaw positions, and input of terrestrial invertebrates was greatest in stream segments with dense vegetation cover along the stream margin. In general, percent contribution of terrestrial food subsidies was notably lower (<45%) in non-

salmonid stream fishes, although some species of Cyprinidae and Fundulidae examined approached those levels observed in Salmonidae. Salmonids from stream habitats consume >50% terrestrial subsidies, (Wipfli 1997; Eberle and Stanford 2007; Rosenfeld and Raeburn 2009), attributed in part to competition and to low productivity of salmonid streams.

Large amounts of terrestrial subsidies in the diets of salmonids, especially larger conspecifics, are attributed in part to interspecific and intraspecific competition (Nakano 1995). Salmonids in stream habitats display hierarchical feeding behavior, where larger individuals are more likely to occupy focal feeding spaces that have more optimal water velocities and better access to drifting invertebrates (Fausch 1981; Sabo 1997), suggesting that larger salmonids will consume a greater amount of terrestrial food as well. We were unable to test effects of size and amount of terrestrial food consumed within species (e.g., intraspecific competition), but interspecific competition likely is not a contributing factor in influencing which non-salmonid family will consume large amounts of terrestrial subsidies, given that the smaller-bodied cyprinids and fundulids had the highest percent contribution of terrestrial subsidies. In fact, length of fish as a predictor of the amount of terrestrial subsidies is not consistent among salmonids. Coho salmon (40 – 180 mm fork length), consumed similar amounts of terrestrial food items (40 – 60%) across study sites (Wipfli 1997; Eberle and Stanford 2009; Rosefeld and Raeburn 2009). Native Dolly Varden charr (*Salvelinus malma*) were empirically shown to be inferior competitors for terrestrial and drifting food items when compared to similarly sized, introduced rainbow trout (Baxter *et al.* 2004). Intermediate sizes (70 – 120 mm) of brown trout (*Salmo trutta*) consumed greater amounts of terrestrial food

(23%) when compared to smaller (<70mm) and larger (>120 mm) individuals with 5 and 18% terrestrial food contribution to diet, respectively (Elliott 1967).

Large amounts of terrestrial subsidies in the diets of salmonids also are attributed in part to low instream productivity. The importance of a trophic subsidy can be mediated by the relative productivities of the adjacent systems is conceptualized by Polis and Hurd (1996) and further explored by Marczak *et al.* (2007) and Paetzold *et al.* (2008). In general, macroinvertebrates in tropical and subtropical streams generally have greater in-stream secondary productivity (Benke and Jacobi 1986; Huryn and Wallace 2000) and obtain greater body mass (Morin 1997) than macroinvertebrates in relatively cold streams (Allen 1951). This suggests that *in situ* energy limitation in cold-water systems may be driving the reliance upon terrestrial food subsidies observed among salmonids. Stream fish communities in warmer waters, especially in the South Eastern USA might rely less upon terrestrial food items because of greater in-stream productivity due to relatively warm annual temperatures.

Fishes in biographical regions outside of the nearctic region of North America consume high amounts of terrestrial inputs seasonally and year round. Terrestrial food subsidies range from 35% in a cyprinid within subtropical regions of Asia (Chan *et al.* 2008) to 70% among several characins in Ecuador (Bosjen 2005). The consumption of terrestrial food items from Bosjen (2005) was particularly great, ranging between 65 and 71%. Stream fishes in Australia have also been observed to consume considerable amounts of terrestrial food items (Balcombe *et al.* 2005; Davis *et al.* 2010), especially *Melanotaenia splendida tatei* (Melanotaeniidae) which was observed to consume an average of 66% terrestrial food items (Balcombe *et al.* 2005). Consumption of terrestrial

inputs can be higher during the monsoon season by a retropinnid in Australia (Balcombe *et al.* 2005) and year round by a galaxid at higher latitudes of New Zealand (West *et al.* 2005). Fruit and seed-eating fishes are most abundant within tropical regions of South American with some small-bodied characins and cichlids consuming fruits and seeds seasonally and opportunistically along with other aquatic and terrestrial invertebrates, whereas some larger bodied and herbivorous fishes can consume fruits and seeds either opportunistically or preferentially (Horn *et al.* 2011). Horn *et al.* (2011) suggested that specialization on terrestrial inputs by the larger bodied, herbivorous fishes might be adaptive based on the evolutionary history and diverse lineages of fruit-eating fishes within the long-persisting tropical rainforests of South America.

Collectively, salmonids and fishes in tropical regions consumed higher amounts of terrestrial inputs than the majority (88%) of the non-salmonid, stream fishes surveyed in this study that consumed <15% of terrestrial inputs. Lower consumption of terrestrial food subsidies outside of salmonid streams of the USA are attributed to two plausible mechanisms. First, antiquity of the land-water flora and fauna communities in the tropics likely provided longer and more persistent biotic and abiotic interactions, allowing fishes to specialize on available terrestrial nutrient sources (Horn *et al.* 2011). Plants and animal communities of higher latitudes (i.e., temperate and subtropical regions) are more variable because they have been more heavily influenced by cyclic warm/cold climates during the last 2 MA. Radiations of specialized terrestrial food consumers within fish communities are inhibited because the land-water communities changed substantially with each advancing and retreating glacial event. This mechanism, described by Horn *et al.* (2011) to explain the high occurrence of fruit- and seed-eating fishes in the tropics of

South American, offers an explanation on why terrestrial inputs comprised a greater proportion of the diets in tropical fishes versus temperate fishes, but the patterns were not consistent with high frequency and abundance of terrestrial inputs in the diets of salmonids at higher latitude of North America. A second, smaller-scale mechanism might be operating within areas of glacial influence and regulated by the relative productivities of stream and adjacent riparian communities. Within the USA, cooler more temperate streams in which salmonids inhabit are relatively less productive than the warmer streams in the subtropical regions. Therefore, our observation that fishes in warmer, more subtropical streams rely less upon terrestrial food subsidies than salmonids in higher latitudes might reflect a smaller differential between the productivity within the stream and the adjacent riparian area.

Regardless of the relatively low biomass, volume, and abundance of terrestrial inputs, non-salmonid, stream fishes of the USA frequently consumed terrestrial inputs and at times consumption comprised high proportions of the total diet. Terrestrial invertebrate inputs to forest stream ecosystems often represent both high quality and high quantity food source as an important trophic link between riparian zones and stream food webs (Mason and MacDonald 1982; Allan *et al.* 2003; Francis and Schindler 2009). This suggests that terrestrial inputs, independent of the amount, are important in the survival, growth, and productivity of the fish communities or simply opportunistic feeding by highly voracious, non-selective, drift-feeding invertivores (Goldstein and Simon 1999). Even among salmonids, linkages between terrestrial inputs and health of the fish community remain unclear with respect to the abundance and productivity of fishes (Baxter *et al.* 2005; Zhang and Richardson 2011). Nakano and Murakami (2001)

estimated the mean annual energetic contribution of terrestrial food items to a stream fish community in a forested Japanese stream to equal 44%, but this ranged from 12.0 to 57% depending on species. Among the five species examined, four were salmonids and the fifth was a sculpin (Cottidae) which consumed the lowest amount of terrestrial food. Growth rates of Dolly Varden charr decreased by 31%, when terrestrial inputs were experimentally excluded from their diets, but Kawaguchi *et al.* (2003) found no detectable change growth rates among salmonid species with similar experimental exclusions. Using a bioenergetics model, Sweka and Hartman (2008) predicted that a 25% annual reduction in the terrestrial inputs into a stream would reduce growth of brook trout (*Salvelinus fontinalis*) by approximately 25%. Additional experimental manipulations on fish growth are necessary, especially outside of salmonid communities, to determine if terrestrial inputs are necessary and selective among some members of the fish community or simply an opportunistic feeding event with little regulating role of the aquatic community. From a management perspective, understanding the role of terrestrial inputs in the nutrient uptake of stream fishes will identify additional benefits of intact watershed and riparian vegetation cover to the biotic community of streams.

Across stream ecosystems, the importance of intact riparian areas is well established for they provide shading for temperature mitigation, woody debris and undercut banks for refugia, sedimentation buffering, and also trap nutrients and contaminant pollution (Naiman and Décamps 1997; Pusey and Arthington 2003; Whitledge *et al.* 2006). Although the specific role of riparian zones providing a food resource for stream fishes outside of salmonid communities has not been assessed to the same extent (but see Garman 1991 and Cloe and Garman 1996), the results here support

that more research is needed to explore the importance of terrestrial food items for fish population dynamics across a wide variety of taxa and regions. Because the consumption of terrestrial food items by fishes, in general, is highly variable, it will be necessary to address the importance of terrestrial inputs in a particular system on a case by case basis with consideration of spatial and temporal variation in subsidy availability. A question of how important terrestrial food items are for non-salmonid fishes from an energetic standpoint still remains open. Terrestrial food items were estimated to be more energy rich on a per-gram basis than aquatic food items (Francis and Schindler 2009). If this is true as a generality, terrestrial food items might contribute greatly to a fish's energy budget, even at a low relative contribution, especially during a season with the low biomass availability of benthic macroinvertebrates (Cloe and Garman 1996; Nakano and Murakami 2001). Thus, the results of this study will justify further research into the estimation of the energetic contribution of terrestrial food items to stream fish community dynamics across a variety of ecoregions.



### Literature Cited

- Allan, J.D., Wipfli, M.S., J.P. Caouette, Prussian, A., and Rodgers, J. (2003). Influence of streamside vegetation on inputs of terrestrial invertebrates to salmonid food webs. *Canadian Journal of Fisheries and Aquatic Sciences* **60**, 309–320.
- Allen, K.R. (1951). The Horokiwi stream: A study of a trout population. New Zealand Marine Department of Fisheries Bulletin No. 10. (New Zealand Marine Department, Wellington, N.Z.) 238 p.
- Atmar, G.L. and Stewart, K.W. (1972). Food, feeding selectivity, and ecological efficiencies of *Fundulus notatus* (Cyprinodontidae). *American Midland Naturalist* **88**, 76–89.
- Balcombe, S.R., Bunn, S.E., F. McKenzie-Smith, and Davis, P.M. (2005). Variability of diets between dry and flood periods in an arid zone floodplain river. *Journal of Fish Ecology* **67**, 1552–1567.
- Benke, A.C. and Jacobi, D.I. (1986). Growth rates of mayflies in a subtropical river and their implications for secondary production. *Journal of the North American Benthological Society* **5**, 107–114.
- Barton, M. (2006). ‘Bond’s Biology of Fishes’ 3<sup>rd</sup> Edn.’ (Thomson Learning, Inc.: Australia.)
- Bosjen, B.H. (2005). Diet and condition of three species (Characidae) of the Andean foothills in relation to deforestation. *Environmental Biology of Fishes* **73**, 61–73.
- Cada, G.F., Loar, J.M., and D.K. Cox. 1987. Food and feeding preferences of rainbow and brown trout in Southern Appalachia streams. *American Midland Naturalist* **117**, 374–385.

- Chan, E.K.W., Zhang, Y., and Dudgeon, D. (2008). Arthropod 'rain' into tropical streams: the importance of intact riparian forest and influence on fish diets. *Marine and Freshwater Research* **59**, 653–660.
- Cloe, W., III and Garman, G. (1996). The energetic importance of terrestrial arthropod inputs into three warm-water streams. *Freshwater Biology* **36**, 104–114.
- Eberle, L.C., and Stanford, J. (2009). Importance and seasonal availability of terrestrial invertebrates as prey for juvenile salmonids in floodplain spring brooks of the Kol River (Kamchatka, Russian Federation). *River Research Applications* **26**, 682–694.
- Etnier, D.A. and W.C. Starnes. (1993). 'The Fishes of Tennessee.' (University of Tennessee: Knoxville). 668 p.
- Fausch, K.D. and White, R.J. (1981). Competition between brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for positions in a Michigan stream. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 1220–1227.
- Francis, T.B. and Schindler, D.E. (2009). Shoreline urbanization reduces terrestrial insect subsidies to fishes in North American lakes. *Oikos* **118**, 1872–1882.
- Garman, G.C. (1991). Use of terrestrial arthropod prey by a stream-dwelling cyprinid fish. *Environmental Biology of Fishes* **30**, 325–331.
- Goldstein, R.M. and Simon, T.P. (1999). Toward a united definition of guild structure for feeding ecology of North American fishes. In 'Assessing the Sustainability and Biological Integrity of Water Resources Using Fish Communities.' (Ed. Simon, T.P.) pp. 123–139 (CRC Press: Boca Raton, U.S.A.)

- Horn, M.H. (1997). Evidence for seed dispersal of fig seeds by the fruit-eating characid fish *Brycon guatamalensis* Regan in a Costa Rican tropical rain forest. *Oecologia* **109**, 259–264.
- Horn, M.H., S.B. Correa, P. Parolin, B.J.A. Pollux, J.T. Anderson, C. Lucas, P. Widmann, A. Tjiu, M. Galetti, and M. Goulding. (2011). Seed dispersal by fishes in tropical and temperate fresh waters: The growing evidence. *Acta Oecologia* **37**, 561–577.
- Hugueny, B. and Pouilly, M. (1999). Morphological correlates of diet in an assemblage of West African freshwater fishes. *Journal of Fish Biology* **54**, 1310–1325.
- Huryn, A.D. and Wallace, J.B. (2000). Life history and production of stream insects. *Annual Reviews of Entomology* **45**, 83–110.
- Hyslop, E.J. (1980). Stomach content analysis-a review of methods and their applications. *Journal of Fish Biology* **17**, 411–429.
- Kawaguchi, Y., Taniguchi, Y. and Nakano, S. (2003). Terrestrial invertebrate inputs determine local abundance of stream fishes in a forested stream. *Ecology* **84**, 701–708.
- Keeley, E.R. and Grant, J. (2001). Prey size of salmonid fishes in streams, lakes, and oceans. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1122–1132.
- Likens, G.E. and Bormann, F.H. (1974). Linkages between terrestrial and aquatic ecosystems. *BioScience* **24**, 447–456.
- Marczak, L.B., Thompson, R.M., and Richardson, J.S. (2007). Meta-analysis: trophic level, habitat, and productivity shape the food web effects of resource subsidies. *Ecology* **88**, 140–148.

- Mason, D. F., and MacDonald, S. M. (1982). The input of terrestrial invertebrates from tree canopies to a stream. *Freshwater Biology* **12**, 305–311.
- Minshall, G.W. (1967). Role of allochthonous detritus in the trophic structure of a woodland springbrook community. *Ecology* **48**, 139–149.
- Morin, A. (1997). Empirical models predicting population abundance and productivity in lotic systems. *Journal of the North American Benthological Society* **16**, 319–337.
- Naiman, R.J. and Décamps, H. (1997). The ecology of interfaces: riparian zones. *Annual Review of Ecology and Systematics* **28**, 621 – 658.
- Nakano, S. (1995). Individual differences in resource use, growth, and emigration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. *Journal of Animal Ecology* **64**, 75-84.
- Nakano, S. and Murakami, M. (2001). Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences* **98**, 166–170.
- Pflieger, W.L. (1997). ‘The Fishes of Missouri.’ (Missouri Department of Conservation, Missouri.
- Polis, G.A. and Hurd, S. (1996). Linking marine and terrestrial food webs: allochthonous input from the ocean supports high secondary productivity on small island communities and coastal land communities. *The American Naturalist* **147**, 396 – 423.
- Polis, G.A., Anderson, W.B., and Holt, R. (1997). Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology* **28**, 289–316.

- Pusey, B. J., and Arthington, A.H. (2003). Importance of the riparian zone to the conservation and management of freshwater fish: a review. *Marine and Freshwater Research* **54**, 1–16.
- Richardson, J.S., Zhang, Y.Z., and Marczak, L.B. (2010). Resource subsidies across the land-freshwater interface and responses in recipient communities. *River Research and Applications* **26**, 55–66.
- Rosenfeld, J.S. and Raeburn, E. (2009). Effects of habitat and internal prey subsidies on juvenile coho salmon growth: implications for stream productive capacity. *Ecology of Freshwater Fish* **18**, 572–584.
- Sabo, J.L. and Pauley, G.B. (1997). Competition between stream-dwelling cutthroat trout (*Oncorhynchus clarki*) and coho salmon (*Oncorhynchus kisutch*): effects of relative size and population origin. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 2609–2617.
- Starnes, L.B. and Starnes, W.C. (1985). Ecology and life history of the mountain madtom, *Noturus eleutherus* (Pisces: Ictaluridae). *American Midland Naturalist* **114**, 331–341.
- Sweka, J.A. and Hartman, K.J. (2008). Contribution of terrestrial invertebrates to yearly brook trout prey consumption and growth. *Transactions of the American Fisheries Society* **137**, 224–235.
- Thomas, C., Bonner, T.H. and Whiteside, G. (2007). 'Freshwater Fishes of Texas: A Field Guide.' (Texas A&M Press: College Station, TX.)
- Thomas, J.D. 1962. The food and growth of brown trout (*Salmo trutta* L.) and its feeding relationships with the salmon parr (*Salmo salar* L.) and the eel (*Anguilla anguilla* (L.)) in the River Teify, West Wales. *Journal of Animal Ecology* **31**, 175–205.

- Tippets, W.E. and Moyle, P.B. 1978. Epibenthic feeding by rainbow trout (*Salmo gairdneri*) in the McCloud River, California. *Journal of Animal Ecology* **47**, 549-559.
- Utz, R.M., and Hartman, K.J. (2007). Identification of critical prey items to Appalachian brook trout (*Salvelinus fontinalis*) with emphasis on terrestrial organisms. *Hydrobiologia* **575**, 259–270.
- VanderKooy, K.E., Rakocinski, C., and Heard, R.W. (2000). Trophic relationships of three sunfishes (*Lepomis spp.*) in an estuarine bayou. *Estuaries* **23**, 621–632.
- West, D.W., Jowett, I.G., and J. Richardson. (2005). Growth, diet, movement, and abundance of adult banded kokopu (*Galaxias fasciatus*) in five Coromandel, New Zealand streams. *New Zealand Journal of Marine and Freshwater Research* **39**, 915–929.
- Whitledge, G.W., Rabeni, C., G. Anne, and S. Sowa. (2006). Riparian shading and groundwater enhance growth potential for smallmouth bass in Ozark streams. *Ecological Applications* **16**, 1461–1473.
- Wipfli, M.S. (1997). Terrestrial invertebrates as salmonid prey and nitrogen sources in streams: contrasting old-growth and young-growth riparian forests in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1259–1269.
- Wipfli, M. S., and Baxter, C. V. (2010). Linking ecosystems, food webs, and fish production: Subsidies in salmonid watersheds. *Fisheries* **35**, 373–387.
- Zhang, Y. X., and Richardson, J. S. (2011). Contrasting effects of cross-ecosystem subsidies and predation on benthic invertebrates in two Pacific coastal streams. *Aquatic Sciences* **73**, 53–62.

## Tables and Figures

**Table 1.1** – Frequency of terrestrial subsidy categories within fish families and diet method (PBA = percent by abundance, PBM = percent by mass, and PBV = percent by volume) for the studies that specifically identified terrestrial remains ( $N = 20$  studies and  $N = 34$  diet observations). Note that species within families consume multiple terrestrial subsidy types.

	Terrestrial Subsidy Category			
	Hymenoptera	Coleoptera	Orthoptera	Seeds/Plant Material
<b>Centrarchidae</b>	<b>3</b>	<b>1</b>	<b>0</b>	<b>0</b>
PBA	1	0	0	0
PBM	1	1	0	0
PBV	1	0	0	0
<b>Cyprinidae</b>	<b>25</b>	<b>18</b>	<b>11</b>	<b>11</b>
PBA	5	2	1	1
PBM	5	3	2	0
PBV	15	13	8	10
<b>Fundulidae</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>2</b>
PBV	1	0	0	2
<b>Ictaluridae</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>1</b>
PBA	1	1	0	1
<b>Grand Total</b>	<b>30</b>	<b>20</b>	<b>11</b>	<b>14</b>

**Table 1.2** – Summary of the range of percent terrestrial contribution for each family analyzed. Cell values are ranges and the number of observations for each cell (parentheses) is given for each diet method (PBA = Percent by abundance of food items, PBM = Percent by mass of food items, and PBV = Percent by volume of food items).

	<b>Diet Technique</b>		
	<b>PBA</b>	<b>PBM</b>	<b>PBV</b>
<b>Catostomidae</b>	0.0-0.0 (1)	0.0-0.0 (2)	0.0-0.0 (1)
<b>Centrarchidae</b>	0.0-4.1 (6)	2.3-5.3 (3)	0.7-2.2 (3)
<b>Cottidae</b>	0.0-0.0 (2)	.	.
<b>Cyprinidae</b>	0.0-15.0 (8)	0.3-7.2 (6)	0.0-44.0 (25)
<b>Fundulidae</b>	.	.	1.0-40.8 (3)
<b>Ictaluridae</b>	0.0-3.8 (4)	0.0-13.0 (2)	0.0-0.0 (1)
<b>Moronidae</b>	.	0.0-0.0 (1)	0.0-0.0 (1)
<b>Percidae</b>	0.0-1.0 (5)	.	.

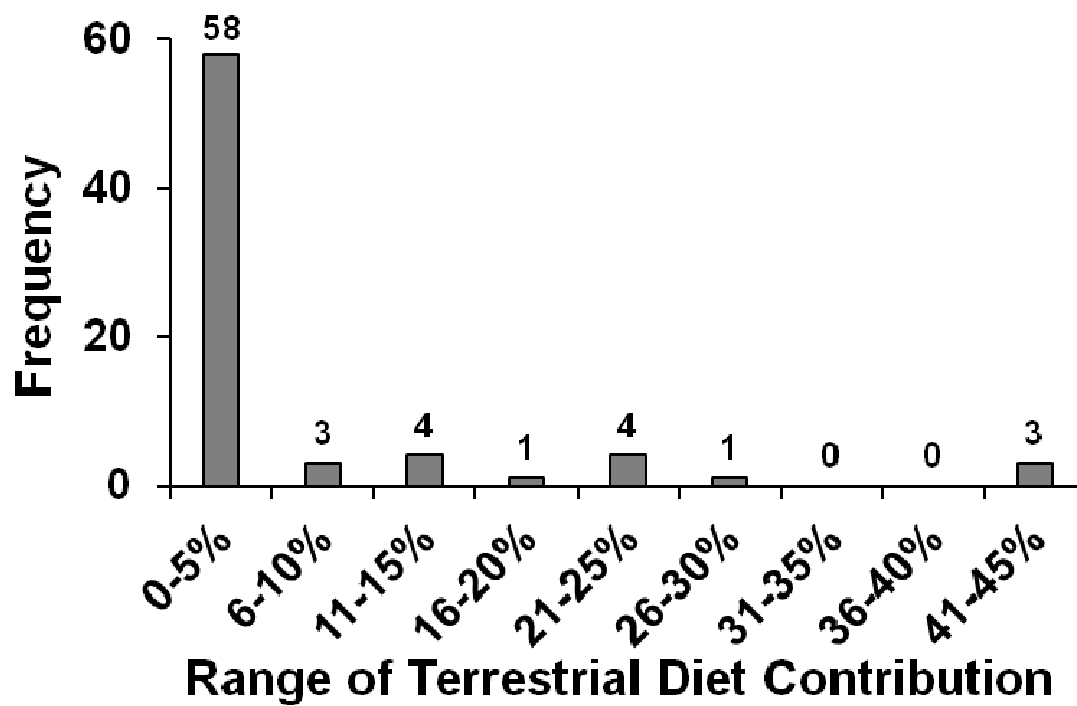


**Table 1.3** – Summary of the mean percent terrestrial contribution to diet for each family and diet method (PBA = Percent by abundance of food items, PBM = Percent by mass of food items, and PBV = Percent by volume of food items). Cell values represent mean terrestrial contribution to diet (%) and standard deviations are in parentheses.

<b>Fish Family</b>	<b>Diet Method</b>		
	<b>PBA (n = 26)</b>	<b>PBM (n = 14)</b>	<b>PBV (n = 34)</b>
<b>Catostomidae (n = 4 )</b>	0.0	0.0	0.0
<b>Centrarchidae (n = 7)</b>	1.1 (1.7)	3.6 (1.5)	1.4 (0.8)
<b>Cottidae (n =1)</b>	0.0	.	.
<b>Cyprinidae (n = 29)</b>	4.7 (6.3)	3.9 (2.5)	10.8 (13.3)
<b>Fundulidae (n = 3)</b>	.	.	14.7 (22.6)
<b>Ictaluridae (n = 5)</b>	1.3 (1.7)	6.5 (9.2)	0.0
<b>Moronidae (n = 1)</b>	.	0.0	0.0
<b>Percidae (n = 5)</b>	0.3 (0.5)	.	.

**Table 1.4** – Summary of data analyzed for jaw positions inferior, sub-terminal, and terminal. The mean of terrestrial contribution to diet is given along with sample sizes for each jaw position and diet technique category (samples sizes are in parentheses). **PBA** = Percent by abundance of food items, **PBM** = Percent by mass of food items, and **PBV** = Percent by volume of food items

<b>Jaw Orientation</b>	<b>PBA</b>	<b>PBM</b>	<b>PBV</b>	<b>Total N</b>	<b>Range %TI</b>
<b>Inferior</b>	0.0 (1)	0.2 (2)	2.3 (3)	6	0.0-6.8
<b>Sub-terminal</b>	0.9 (8)	4.2 (6)	2.0 (7)	21	0.0-13.0
<b>Terminal</b>	2.5 (17)	3.7 (6)	12.4 (24)	47	0.0-44.0



**Figure 1.1** – Frequency distribution of fish diet observations ( $N = 74$ ) across ranges of % terrestrial contribution to diet.

## CHAPTER 2

### INFLUENCE OF AQUATIC AND TERRESTRIAL FOOD AVAILABILITY AND AQUATIC HABITAT ON GENERALIST CYPRINID DIETS

#### **Abstract**

Understanding the relationship between habitat and food availability is important for a detailed understanding of trophic dynamics in ecology. The primary food resource for most insectivorous minnows (Cyprinidae) is aquatic macroinvertebrates and in streams, aquatic macroinvertebrate abundance and community structure is closely linked to physical in-stream habitat characteristics such as substrate and current velocity. However, work on individual foraging habits has revealed that species which appear to be generalist foragers might actually be aggregates of specialized individuals at a population level. This variability in foraging habits can be related to context and site dependent habitat factors. The primary objective of this study was to assess how two insectivore generalist minnow species, Blacktail Shiners (*Cyprinella venusta*) and Texas Shiners (*Notropis amabilis*) alter their foraging habits within different stream habitats with swift and slack current velocities, particularly as it relates to the availability of different prey items within the water columns. Both species studied feed upon more terrestrial and surface prey items in slack-water habitats than in swift-water but Texas Shiners doubled consumption of terrestrial and surface prey items in slack-water versus swift-water, although the difference was not significant in an analysis of similarity. In multivariate

space, Texas Shiner diets separated by both habitat gradients as well as amounts of terrestrial and surface items consumed while blacktail shiner diets separated primarily along gradients of the consumption of infrequent, relatively large prey items. When considering the importance of habitat availability and heterogeneity in regulated rivers, this study provides evidence that foraging habits of even generalists can become specialized under certain habitat conditions and that water conservation plans should not only consider habitat associations of certain species but also the trophic aspects of their life histories.

### **Introduction**

Consumer populations are linked to the food resources upon which they rely via multiple pathways. Reduction in resource abundance or quality can result in physiological stress (Kitaysky et al. 1999) and lower consumer population densities (Bianchi et al. 1989). However, consumers adjust for this by behavioral changes that allow them to use a different resource (Malmquist 1992; Rowe and Richardson 2001) which might result in related changes in morphological characteristics (Mittelbach et al. 1992; Matthews et al. 2010). For fishes, arthropods, especially aquatic and terrestrial insects, are a staple food item but their availability is not homogenously distributed in space and time. Stream insects distribute primarily along abiotic gradients such as substrate and current velocity (Rabeni and Minshall 1977; Sheldon and Haick 1981; Schlosser 1982) and terrestrial invertebrate availability primarily varies along seasonal and vegetative gradients (Edwards and Huryn 1996; Cloe and Garman 1996). Despite large taxonomic dissimilarities among streams within the Western Gulf Slope, fishes largely capture and process nutrients similarly within mesohabitats (e.g. riffles and pools)

across a sub-basin spatial scale. This suggests that across relatively large spatial scales, fishes associated with pools feed upon similar food items and fishes associated with riffles feed upon similar food items (Hoeinghaus et al. 2007). However, if we peer directly into these particular mesohabitats, we find that stream fishes segregate within the water column and this is reflected both in where they are collected and in their diets (Mendelson 1975).

The apparent generalities behind fish feeding habits might be the manifestation of conspecifics specializing on food items that represent a sub-set of the entire trophic niche. This is the niche variation hypothesis (Bolnick et al. 2007) and challenges oversimplifying trophic relationships. Habitat specialization (benthic vs. pelagic) among lentic sticklebacks (*Gasterosteus aculeatus*) led to divergence in trophic position, which is related to changes in morphological factors such as gill raker length and body size (Matthews et al. 2010). An important factor in adaptive radiation is ecological opportunity, or the availability of a new resource due to an environmental change (Losos 2010) and the ability of an individual organism to utilize this resource will be related to dispersal ability and capacity to alter behavior (Losos 2010). The implication for stream fishes, particularly those groups that consume a wide array of prey items (e.g. Cyprinidae) is habitat heterogeneity will be important for trophic relationships because it might afford the presence of new resources and the connectivity necessary for organisms to utilize those resources, whether the change in foraging is related to morphology, physiology, or behavior. Globally, the family Cyprinidae naturally occurs on every continent except South America and within North America, a majority of the ichthyofaunal diversity is within this family (Lévêque et al. 2008). *Notropis* and

*Cyprinella* are categorized as insectivores (Goldstein and Simon 1999), but the particulars of what they feed upon can be very different from diet reference to diet reference, which are likely due to differences in large-scale environmental factors such as climate and geology that influence insect life cycles. For example, the terrestrial component of *Cypinella spiloptera* diets might be as low as 2% to as great as 25% by volume (Starrett 1950; Whitaker 1977). Given the habitat heterogeneity across river basins and differential availability of certain food resources, we propose that the trophic role of cyprinids is under appreciated for two main reasons; first, cyprinids being a large group of consumers are likely important for nutrient processing, especially since they feed upon both terrestrial (Sullivan et al. 2012) and aquatic food resources, and second, their appearance of a group of “generalists” may be the result of a heterogeneous aggregation of individually specialized components (Bolnick (2007). In this case of this paper, when cyprinid diets are pooled, they are classified as general insectivores because their diets are largely insects (Simon and Goldstein 1999) but certain individuals might specialize on particular component of the suite of potential insect prey.

The purpose of this study was to assess the consumption of terrestrial and aquatic food items among cyprinids collected in different mesohabitats to determine how habitat influences cyprinid diets, testing the hypothesis that fishes would focus upon particular prey items with these different habitats. Additionally, I used morphological features to determine and if any differences in diet could be attributed to morphological variation and individual niche separation. Because terrestrial arthropod abundance is closely related to season and habitat characteristics, we predict differences in the feeding ecology among cyprinids will largely be explained by invertebrate availability and abundance but

nuanced variation in foraging patterns, especially if related to morphological characteristics, would support the individual niche hypothesis. We collected data on prey availability and cyprinid diets from the Llano River basin in Central Texas. We used two regionally ubiquitous cyprinid species in Central Texas; the blacktail shiner (*Cyprinella venusta*) and the Texas shiner (*Notropis amabilis*). We simultaneously collected benthic aquatic invertebrates, drifting invertebrates, and invertebrates falling into the stream and all diet and prey availability samples were constricted by mesohabitats designated as swift and slack flow velocities. Because aquatic macroinvertebrate communities tend to segregate along mesohabitat gradients, we predicted that most of the variability in cyprinid diets among these mesohabitats would be due to availability of different macroinvertebrates among these different habitats. On the other hand, some cyprinid diets could be the result of some level of specialization, for example feeding solely from the benthos or the surface. Therefore, our study objective was to determine the relative role of habitat heterogeneity and individual feeding specialization on the diets of two generalist-insectivore cyprinids. Particularly as they relate to terrestrial and aquatic arthropod availability. Additionally, morphological features were measured to determine if morphological differences could partially account for variability in diets among different mesohabitats with special focus on the consumption of terrestrial and winged aquatic adult food items.

## **Methods and Materials**

### *Study Area*

The Llano River is a semi-arid spring-fed stream located on the Edwards Plateau of Central Texas with an encompassing watershed of approximately 11,568 square



kilometers (Heitmuller and Hudson 2009). The Llano River watershed is composed of a mesquite-juniper savannah and receives ca. 532 mm of precipitation per year. Primary source of flow is through springs emerging from the Edwards-Trinity Aquifer to create the North Llano River in Sutton County and the South Llano River in Edwards County. The two meet in Junction, Texas and flow approximately 161 river kilometers to Lake Lyndon B. Johnson on the Colorado River (Perkin et al. 2010). Upper reaches and tributaries are characterized by limestone bedrock with substantial amounts of alluvial deposits, moderately flowing habitats and relatively dense riparian zones transitioning to braided channels with finer substrates, granitic outcroppings, and a grassland-savannah type riparian zone.

### *Field Sampling*

Sampling occurred at several sites throughout the Llano River system from December 2010 – June 2011 (Figure 1). Sites were sampled three times throughout the year to best represent seasonality: winter (December), spring (March) and summer (June). Site 1, the most upstream site is characterized by a relatively narrow stream width (18 m) and overhanging riparian canopy ( $25\% \pm 7$ ) that consists primarily of young sycamore trees and oak species. Substrates at Site 1 ranged from cobble-boulder on bedrock to bare clay. Site 2, the middle site, is characterized by a wider stream channel (22 m) with riffle-island complexes within the sample area. Riparian vegetation at Site 2 was sparse ( $9\% \pm 23$ ) and primarily consisted of grasses and some oak. The substrates at Site 2 primarily consisted of cobble-gravel but also had patches of granitic bedrock. Site 3, the most downstream site, had a similar stream width (25 m) to Site 2 and had similar riparian vegetation but overall, had less riparian coverage ( $<10\%$  at all sample points).

The substrate at Site 3 is generally characterized by cobble-sand and granitic bedrock outcroppings.

Average depth in meters and current velocity (m/s) were measured in each mesohabitat and current velocity was measured using a Marsh-McBirney Flo-mate 2000 flow meter. Fish were collected with the use of straight seines (3.0 x 1.8 m with 3.2 mm mesh) and bag seines (4.3 x 1.8 x 1.8 m with 3.2 mm mesh). Fish were administered a lethal dose of tricainemethanesulfonate (MS-222), fixed in a 10% formalin solution. Fish abundances and habitat associations were quantified in representative geomorphic units consisting of slack-water (0.00-0.04 m/s) and swift-water (0.05-1.0 m/s) habitats.

Terrestrial arthropods were collected using 81.6 x 48.6 x 17.8 cm pan traps anchored in the stream with rebar stakes and survey string either against the stream bank or on the stream bed. All pan traps were set within one meter of the stream edge. Pan traps were filled with ca. 10 L of water and one drop of scentless, clear soap was added as a surfactant and set for 24 hrs (N = 8 per site but one was lost at site one in March and two were lost in June, one at Site 1 and one at Site 3). Aquatic arthropods were collected using Surber samplers (0.091 m<sup>2</sup> frame), D-frame kick nets, and drift nets (45.7 x 25.4 x 91.4 cm set for 4-5 hrs). Because the sampling efficiency of Surber samplers is influenced by stream-flow and stream-flow was not homogenous among seasons, it was necessary to supplement Surber samples with D-frame kick net samples and since the overall Renkonen similarity between these two gear types was 68%, they were combined.

#### *Laboratory Measurement*

Fish were measured for total length (mm), mass (g), gape width (mm), and body depth (mm). Gape width was defined as the widest part of the mandible when jaw is closed (Perkin et al. 2009). Aquatic and terrestrial arthropod samples were identified to family and counted. Fish diets were identified down to the lowest taxonomic resolution practical, at least to order. Diet items were primarily identified based upon recalcitrant structures such as head capsules. Whenever practical, the head capsule width (mm) and body length (mm) of prey items was measured for converting to estimated dry mass from Benke et al. (1999). Diet analyses are presented here based upon relative abundance and relative mass estimated from head-capsule to dry mass regressions. Fish diets are assumed to accurately reflect feeding habits at site of capture because other cyprinids have been reported to evacuate their gut tract within 12 hours in 16-24 °C water temperature (Persson 1982) and tend to remain in habitats where they forage (Garner et al. 1998).

### *Statistical Analyses*

Diet differences among and within fish species between swift and slack-water habitats were first assessed using Renkonen similarity which sums the minimum proportional contribution of diet items between the two habitats. This was then tested using analysis of similarity (ANOSIM). In order to visualize differences in feeding habits among Texas and blacktail shiners between the two habitats, we used Multi-dimensional scaling (MDS) because it does not assume linear relationships between samples and variables as does PCA. We used Ivlev's selectivity index to assess selectivity of prey items relative to their abundance.

## Results

### *Aquatic and Terrestrial Arthropod Density*

Arthropod density ( $\text{no}/\text{m}^2 \pm 1 \text{ SD}$ ) in pan traps was low ( $173 \pm 151$ ) relative to benthic arthropod density ( $979 \pm 307$ ) in Surber samples although terrestrial arthropods were collected in all sample months. The terrestrial component sampled in pan-traps was significantly lower than the adult aquatic component across all sample months ( $F_{5,42} = 13.5$ ,  $P < 0.01$ ). Among mesohabitats, overall mean benthic invertebrate density ( $\text{no}/\text{m}^2 \pm 1 \text{ SD}$ ) was greater in swift ( $1,101 \pm 376$ ) than slack ( $809 \pm 91$ ) mesohabitats but this difference was only significant within March ( $F_{2,18} = 4.0$ ,  $P = 0.036$ ).

### *Benthic Macroinvertebrate Community*

The most dominant members of the benthic community by abundance were elmids (Coleoptera) adults and larvae, which contributed to 70% of the total relative abundance (Table 1). While other coleopteran families Gyrinidae, Haliplidae, Hydrophilidae, Leutrochidae, and Psphenidae were present, they contributed collectively <2% by relative abundance. Coleopteran adults were collected primarily in swift-water (33%) compared to 8% in slack-water. Conversely, Coleopteran larvae were more frequent in slack-water (17%) compared to 13% in swift-water. Ephemeroptera larvae were 9% of the community and were primarily collected in swift-water (7% in swift-water, 2 % in slack water). Within Ephemeroptera, the dominant families were Leptophlebiidae (32%) and Baetidae (29%). Within swift water-habitats, the ephemeropteran families of Baetidae, Heptageniidae, and Leptohyphidae accounted for >50% of the community and within

slack-water habitats, the most dominate ephemeropterans were Baetidae (26%) and Leptophlebiidae (16%).

### *Invertebrate Drift Community*

In drift nets, mean benthic aquatic arthropod density ( $\text{no}/\text{m}^2 \pm 1 \text{ SD}$ ) was  $559 \pm 202$  and was greater ( $F_{8,33} = 4.0$ ,  $P < 0.01$ ) than terrestrial arthropod ( $56 \pm 9$ ) and aquatic adult density ( $53 \pm 32$ ) for all sample months. Terrestrial arthropods were observed in drift net samples during all sample months. Drift samples were dominated by elmids larvae (22%), diptera larvae (15%), elmids adults (10%), and diptera adults (7%), Table 2. Seasonally, the greatest overall contributions in the drift were observed in March and June (together contributing 88% of the total abundance) and the remaining 12% in December. Terrestrial arthropods were observed in drift nets in all seasons suggesting that these prey items are available throughout the year. Overall, the terrestrial component of the drift was decreased during December, contributing about 12% by abundance but 40% by mass.

### *Fish Diet Results*

A total of 276 cyprinids were taken (blacktail shiners,  $N = 144$  and Texas shiners,  $N = 132$ ) across sites and seasons. Both species primarily consumed aquatic invertebrate larvae although post-larval fish remains were found in two blacktail shiners. When diets for both species are pooled across sites, seasons, and mesohabitats, their feeding spaces largely overlap in multivariate space when excluding prey items that were not shared among the two species (Figure 2). The gradient along the first axis separates the foraging habits of individuals collected in swift water habitats between feeding upon

ephemeropteran larvae (loading negatively) from individuals found in slack water habitats consuming more terrestrial and winged aquatic adults (loading positively).

Among the 179 blacktail shiners captured, 51 (35%) were empty and the frequency of unidentifiable insect remains was 4% out of 575 total prey remains counted. General debris such as silt or filamentous algae was observed in 6 (4%) of the blacktail shiner diets. By percent abundance, the overall diet of blacktail shiners consisted primarily of Diptera larvae (54%). Of the dipterans consumed, Simuliidae was the most common (31%) and Chironomidae was second (21%). By percent mass, the dominant prey items were Simuliidae larvae (38%) and Ephemeroptera larvae (24%). Seasonal shifts in diet were detected. The strongest seasonal pattern (was among the dipteran adults which were nearly absent from the diets in October (3% by abundance but not measurable by % mass) and June (0.6% by mass and 2% by abundance) and then contributed appreciable proportions in March (20% by mass and 33% by abundance) and December (17% by mass and 24% by abundance). Although Ephemeroptera larvae did not contribute a large amount to the overall diet, this group appears to be a seasonally consistent food item for blacktail shiners. By percent abundance, this group contributed 8% in March, 10% in June, and 18% in December. Terrestrial formicids were observed in diets from December (5% by abundance and 1% by mass) and October (17% by abundance).

Among the 132 Texas shiners captured, 32 (24%) had empty stomachs and the frequency of unidentifiable remains was 35 (13%) of the 278 total prey remains counted. The stomachs of only two individuals contained miscellaneous debris such as silt or filamentous algae but in each case, the entire gut tract was noted to be completely packed

with this material. By percent abundance, Texas shiners primarily consumed Ephemeroptera larvae (47%). While most ephemeropteran remains were not identifiable to family, of those that were, Isonychidae was the most abundant (3%) followed by Baetidae and Ephemeridae. Together, these latter families contributed <2% of the total. Diptera larvae were the next most common prey item (12% by abundance and 8% by mass). The most common families were Chironomidae and Simuliidae. Chironomidae contributed 8% by abundance and 3% by mass while Simuliidae contributed 3% by abundance and 4% by mass. Trichoptera larvae contributed 10% by abundance and of the individuals that we were able to identify to family, 4% were Hydropsychidae and <1% were Philopotamidae. There were not sufficient quantifiable Trichoptera larvae remains to analyze their contribution by % mass. Ephemeropteran larvae provided a bulk of the overall diet for Texas shiners but by season this contribution was highly variable, the greatest contribution was in March (65% by abundance and 55% by mass) and then in October (26% by abundance and 75% mass). Ephemeropteran larvae were largely absent in diet samples from December (4% by abundance and <0.5% by mass). The only aquatic adults detected in Texas shiner diets were dipterans and June was the only month in which they were consumed to a great extent (21% by abundance and 52% by mass). Terrestrial food items were consumed by Texas shiners during parts of the winter. By percent abundance, a semi-aquatic group known as the shore bugs (Saldidae; Hemiptera) were consumed by 11 (38%) of the 29 individual fish analyzed and contributed 58% of the December diet by abundance and 26% by mass. Hymenopterans (Formicidae) were consumed at low levels (0-2% by abundance and mass) throughout the year except in December where they contributed 12% by abundance.

### *Diets by Mesohabitat*

The diets of both fish species shifted dependent upon mesohabitat because both species tended to utilize more terrestrial and adult aquatic prey items in slack water habitats than in swift. This shift was not as apparent for blacktail shiners (Figure 3) and the Renkonen similarity was 83% between diets of blacktail shiners from swift and slack habitats (ANOSIM;  $R = 0.015$ ,  $P = 0.013$ ). The points that appear to separate from the rest of the cloud in Figure 6 are the result of four individual blacktail shiners utilizing larger prey items absent from Texas shiner diets (e.g. megalopteran larvae and post-larval fishes). Texas shiner diets were 49% similar between swift and slack habitats but this difference was not significant (ANOSIM;  $R = 0.15$ ,  $P = 0.15$ ). Texas shiner diets from swift water habitats can be viewed as a subset of the suite of available prey (Figure 4). Texas shiners from swift habitats largely consumed Ephemeroptera larvae, diptera larvae, and Trichoptera larvae. Texas shiners from slack water habitats utilized these same taxa but additionally consumed winged aquatic and terrestrial forms that are almost entirely missing from the diets of individuals from swift water habitats. A gradient of individuals from slack-habitats consuming relatively more winged aquatic adults and terrestrial forms (loading positively) to individuals from swift-water habitats largely consuming Ephemeroptera larvae (loading negatively), Figure 4. The multivariate patterns are supported by Ivelev's electivity values for Texas shiner diets (Figure 5). Texas shiners appear to prefer benthic Ephemeroptera, Diptera and Trichoptera larvae across mesohabitats but in slack water habitats the sum (1.2) and mean (0.2) of electivity values were greater than those in swift water mesohabitats (sum = -2.1, mean = -0.3).



We detected some morphological variation among individuals collected in swift versus slack-water. Differences were between blacktail shiners among swift and slack habitats for total length ( $t = 7.6$ ,  $Df = 105.5$ ,  $P < 0.01$ ) and body depth ( $t = 8.0$ ,  $110.9$ ,  $P < 0.01$ ) where the longer, deeper individuals were collected from swift habitats (Figure 6). No significant morphological differences were detected among Texas shiners between mesohabitats. The mean standard length ( $\pm 1$  SD) for blacktail and Texas shiners was 55.7 ( $\pm 13.5$ ) and 43.4 ( $\pm 4.7$ ), respectively and there were no significant differences in either species in gape width between the two habitats. The mean gape width to standard length ratio ( $\pm 1$  SD) for blacktail shiners was  $0.07 \pm 0.008$  and for Texas shiners was  $0.07 \pm 0.013$ .

### Discussion

These results support the hypothesis that in-stream habitats will partially mediate the arthropod prey items would focus on, specifically terrestrial and surface prey. Although the terrestrial component of cyprinid diets was relatively small in this study, terrestrial food items were consumed and available during all sampling month which was also reported in Cloe and Garmen (1996) but runs counter to the general prediction where terrestrial arthropods will be unavailable in more temperate regions (Wipfli 1997; Nakano and Murakmi 2000). While some of these differences are most likely explained by abundance, both cyprinid species consumed more terrestrial and winged aquatic adult prey items in slack water habitats than swift and this pattern was consistent among the three sampling locations with the exception of one site where blacktail shiners consumed 23% surface-drifting prey compared to 11% benthic prey in swift water habitats. There were no morphological features that aid in explaining these feeding differences. Both

cyprinid species consumed more prey items in swift water than in slack water habitats and there was substantially more benthic prey available in the swift habitats. Overall, Texas shiners consumed more surface-drifting prey than blacktail shiners. Variation of foraging behavior as it relates to terrestrial food consumption has been observed in other stream-fishes where native charr (*Salvelinus malma*) had significantly decreased terrestrial food consumption when introduced rainbow (*Oncorhynchus mykiss*) trout were present (Baxter et al. 2004) and the nuances between the different propensities of the cyprinids in this study to exploit prey items from the surface as it relates to the habitats they select could be a result of variation in their individual foraging behaviors. Environmental changes will put different selective pressures on different foraging strategies and because all of this occurs on a temporally dynamic scale and fishes are probably often energy limited (Schindler and Eby 1997), the availability of terrestrial foods are important in both short, seasonal time scales but also longer time scales that allow natural selection to act.

If physical stream characteristics such as stream-flow regulate an aquatic organisms' ability to capture prey on the surface of the water (Marczak et al. 2010), these results suggest, particularly for the Texas shiner, habitat heterogeneity can be important in maintaining their diet breadth. For both species, our results support coexistence of stream fishes through plasticity in their foraging behaviors (Nakano et al. 1999) by being able to shift where they feed within the water column, whether this results from the presence of competitors (Nakano et al. 1999) or, in the present paper, differences in habitat characteristics and food availability. The ability of Texas shiners to utilize surface prey more regularly might also partially explain their association with run

habitats (Bean et al. 2007), where they have access to drifting prey as well as surface prey. Blacktail shiners appear to be more opportunistic; while they consumed fewer surface prey than Texas shiners, they consume prey items that Texas shiners did not (e.g.  $N = 3$  Megaloptera larvae and  $N = 2$  post-larval fishes). This is most likely explained by the disparity in the maximum body sizes between blacktail and Texas shiners. Despite this size disparity, Texas shiners successfully coexist with blacktail shiners and are often in high relative abundance (Cantu and Winemiller 1997; Bonner et al. 2005; Bean et al. 2007) and have increased in abundance in a West Texas stream over the past few decades (Bonner et al. 2005). Blacktail shiners tend to increase in relative abundance in altered stream habitats and are potential native invaders (Brad Littrell, Master's Thesis). Both of these species demonstrate plastic feeding behaviors but blacktail shiners appear to be more opportunistic, feeding upon more similar proportions of drifting and benthic prey regardless of habitat. Blacktail shiners fed upon similar food items more or less independent of habitat conditions which suggests blacktail shiners will be successful over a large gradient of habitats, especially when altered, and other native species may no longer be able to tolerate and this is supported by the broader, geographical range of the Blacktail shiner versus the Texas shiner (Thomas et al. 2007). Coarse scale categorization of functional feeding groups of fishes can be important for coarse scale studies but understanding the importance of habitat mediation and fish diets for individual watershed management is going to require a finer scale. Goldstein and Simon (1999) attempted to categorize the functional role of fishes by taxa in a standardized fashion and we feel their scheme was largely beneficial but the finer details on where they feed in the water column are largely missing for Cyprinids.

Morphological features have been correlated to feeding ecology of several fish taxa (De Silva et al. 1979) but we did not detect morphological variability in relation to diet. The differences in blacktail shiner total length and body depth between swift and slack water habitats are probably more related to swimming ability than feeding ecology (McLaughlin and Grant 1994; Ojanguren, and Braña 2003). Divergence in cichlids has occurred in African rift lakes over relatively short periods (within 300,000 years) of geologic time (Seehausen 2006) which suggests cyprinid feeding behaviors could also result in speciation relatively rapidly given enough isolation. The Edwards Plateau experienced Miocene uplift ca. 15 mya (Oetting et al. 1996) but a more recent reshuffling of habitats and species occurred within the last 10,000 years via glaciation and this event likely forged the extant watercourses and species distributions we observe in streams flowing through the Edwards plateau. The cyprinids in the current study do demonstrate differences in their foraging habits and given enough time without large-scale and substantial change in the environment, could eventually equate to morphological variation. Therefore, our results are relevant to the individual niche variation hypothesis as a viable mechanism for species divergence where individuals of a population might be forced into specializing on a food resource which can then lead to morphological changes observed in (Matthews et al. 2010). The mechanism behind this could then be the differential rates of morphological change in different structures that will be based upon performance trade-offs (Holzman et al. 2012). In this case, the performance trade-off would be the ability to capture terrestrial or surface drifting food resources in slack-water habitats that might be associated with higher predator densities in other similarly sized Texas streams (Cantu and Winemiller 1997).

Terrestrial inputs were a relatively small component of the fish diets we analyzed but were utilized. When we included terrestrial and winged-aquatic adult forms of potential prey, we found that both Texas shiners and blacktail shiners tended to consume these surface oriented prey more often than those in swifter water but this shift was more pronounced among Texas shiners. Because Central Texas is prone to drought and flooding, it will be important to understand how the amount of water in the river will influence habitat availability as we have confirmed that, at least to some extent, the available habitats for these fishes will alter their foraging patterns. Since cyprinids are ubiquitous in North American streams, this should be investigated in other streams to begin to further understand the relationships between fish foraging modes, food consumption, and habitat availability.

### Literature Cited

- Bean, P.T., T.H. Bonner, and B.M. Littrell. 2007. Spatial and temporal patterns in the fish assemblage of the Blanco River, *Texas. Texas J. Sci.*, **59**: 179-200.
- Benke, A.C., A.D. Huryn, L.A. Smock, and J.B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *J. N. Benthol. Soc.*, **18**: 308-343.
- Bianchi, T.S., C.G. Jones, and M. Shachak. 1989. Positive feedback of consumer population density on resource supply. *Trends in Ecol. and Evol.*, **4**: 234-238.
- Bolnick, D.I., R. Svanbäck, J.A. Fordyce, L.H. Yang, J.M. Davis, C.D. Hulsey, and M.L. Forister. 2003. The ecology of individuals: incidence and implications of individual specialization. 161: *Am. Nat.*, **161**: 1-28.
- Bolnick, D.I., R. Svanbäck, M.S. Araújo, and L. Persson. 2007. Comparative support for the niche variation hypothesis that more generalized populations also are more heterogenous. *Proc. Natl. Aca. Sci.*, **104**: 10075-10079.
- Bonner, T.H., C. Thomas, C.S. Williams, and J.P. Karges. 2005. Temporal assessment of a west Texas stream fish assemblage. *Southwestern Assoc. Nat.*, **50**: 74-78.
- Cantu, N.E.V. and K.O. Winemiller. 1997. Structure and habitat associations of Devils River fish assemblages. *Southwestern Assoc. Nat.*, **42**: 265-278.
- Cloe, W.W. III and G.C. Garman. 1996. The energetic importance of terrestrial arthropod inputs to three warm-water streams. *Freshwater Biol.*, **36**: 105-114.

- De Silva, S., P.T. Cummaranatunga, and C.D. De Silva. 1979. Food, feeding ecology and morphological features associated with feeding of four co-occurring cyprinids (Pisces: Cyprinidae). *Netherlands J. Zool.*, **30**: 54-73.
- Edwards, E.D. and A.D. Huryn. 1996. Effect of riparian land use on contributions of terrestrial invertebrates to streams. *Hydrobiologia*, **337**: 151-159.
- Garner, P., S. Clough, S.W. Griffiths, D. Deans, and A. Ibbotson. 1998. Use of shallow marginal habitat by *Phoxinus phoxinus*: a trade-off between temperature and food? *J. of Fish Biol.* **52**: 600-609.
- Goldstein, R.M. and T.P. Simon. 1999. Toward a united definition of guild structure for feeding ecology of North American fishes. In 'Assessing the Sustainability and Biological Integrity of Water Resources Using Fish Communities.' (Ed. Simon, T.P.) pp. 123-139 (CRC Press; Boca Raton, U.S.A.).
- Heitmuller, F.T. and P.F. Hudson. 2009. Downstream trends in sediment size and composition of channel-bed, bar, and bank deposits related to hydrologic and lithologic controls in the Llano River watershed, central Texas, USA. *Geomorphology*, **112**: 246-260.
- Hoeinghaus, D.J., K.O. Winemiller, and J.S. Birnbaum. 2007. Local and regional determinants of stream fish assemblage structure: inferences based on taxonomic vs. functional groups. *J. Biogeogr.*, **34**: 324-338.

- Holzman, R.D., D.C. Collar, S.A. Price, C.D. Hulsey, R.C. Thomas, and P.C. Wainwright. Biochemical trade-offs bias rates of evolution in the feeding apparatus of fishes. *Proc. Biol. Sci.* **279**:1287-1292.
- Kitayski, A.S., J.C. Wingfield, and J.F. Piatt. 1999. Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. *Functional Ecol.*, **13**: 577-584.
- Lévêque, C., T. Oberdorff, D. Paugy, M.L.J. Stiassny, and P.A. Tadesco. 2008. Global diversity of fish (Pisces) in freshwater. *Hydrobiologia*, **595**: 545-567.
- Losos, J.B. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. *Am. Nat.*, **175**: 623-639.
- Malmquist, H.J. 1992. Phenotype-specific feeding behavior of two arctic charr *Salvelinus alpinus* morphs. *Oecologia*, **92**: 354-361.
- Marczak, L.B., T.M. Hoover, and J.S. Richardson. 2007. Trophic interception: how a boundary-foraging organism influences cross-ecosystem fluxes *Oikos*, **116**: 1651-1662.
- Matthews, B., K.B. Marchinko, D.I. Bolnick, and A. Mazumder. 2010. Specialization of trophic position and habitat use by sticklebacks in an adaptive radiation. *Ecology*, **91**: 1025-1034.
- McLaughlin, R.L. and J. Grant. 1994. Morphological and behavioral differences among recently-emerged brook charr, *Salvelinus fontinalis*, foraging in slow-vs. fast-running water. *Env. Biol. Fishes*, **39**: 289-300.



- Mendelson, J. 1975. Feeding relationships among species of *Notropis* (Pisces: Cyprinidae) in a Wisconsin stream. *Ecol. Monographs*, **45**: 199-232.
- Mittelbach, G.G., C.W. Osenberg, P.C. Wainwright. 1992. Variation in resource abundance affects diet and feeding morphology in the pumpkinseed sunfish (*Lepomis gibbosus*). *Oecologia*, **90**: 8-13.
- Nakano, S., K.D. Fausch, and S. Kitano. Flexible niche partitioning via a foraging mode shift: a proposed mechanism for co-existence in stream-dwelling charrs. *J. Anim. Ecol.*, **68**: 1079-1092.
- Oetting, G.C., J.L. Banner, and J.M. Sharp. 1996. Regional controls on the geochemical evolution of saline groundwaters in the Edwards aquifer, central Texas. *J. Hydrol.*, **181**: 251-283.
- Ojanguren, A.F. and F. Braña. 2003. Effects of size and morphology on swimming performance in juvenile brown trout (*Salmo trutta* L.). *Ecol. of Freshwater Fishes*, **12**: 241-246.
- Perkin, J.S, C. Williams, and T.H. Bonner. 2009. Aspects of chub shiner *Notropis potteri* life history with comments on native distribution and conservation status. *Am. Midl. Nat.*, **162**: 276-288.
- Perkin, J.S., Z. Shattuck, P.T. Bean, and T.H. Bonner. 2010. Movement and microhabitat associations of Guadalupe bass in two Texas Rivers. *N. Am. J. Fisheries Mgmt.*, **30**: 33-46.

- Persson, L. 1982. Rate of food evacuation in roach (*Rutilus rutilus*) in relation to temperature, and the application of evacuation rate estimates for studies on the rate of food consumption. *Freshwater Biol.*, **12**: 203-210.
- Rabeni, C.F. and G.W. Minshall. 1977. Factors affecting microdistribution of stream benthic insects. *Oikos*, **29**: 33-43.
- Rowe, L. and J.S. Richardson. 2001. Community responses to experimental food depletion: resource tracking by stream invertebrates. *Oecologia*, **129**: 473-480.
- Schlosser, I.J. 1982. Fish community structure and function along two habitat gradients in a headwater stream. *Ecol. Monographs*, **52**: 395-414.
- Sheldon, A.L. and R.A. Haick. 1981. Habitat selection and association of stream insects: a multivariate analysis. *Freshwater Biol.*, **11**: 395-403.
- Starrett, W.C. 1950. Food relationships of the minnows of the Des Moines River, Iowa. *Ecology*, **31**: 216-233.
- Seehausen, O. 2006. African cichlid fish: a model system in adaptive radiation research. *Proc. Biol. Sci.*, **273**: 1987-1998.
- Sullivan, M.L., Y. Zhang, and T.H. Bonner. 2012. Terrestrial subsidies in the diets of stream fishes of the USA: comparisons among taxa and morphology. *Marine and Freshwater Research*, **63**: 409-414.
- Thomas, C., T.H. Bonner, and B.G. Whiteside. 2007. Freshwater fishes of Texas, Texas A&M Press. College Station, Texas, U.S.A.

Whitaker, J.O. Jr. 1977. Seasonal changes in food habits of some cyprinid fishes from the White River at Petersburg, Indiana. *Am. Midl. Nat.*, **97**: 411-418.

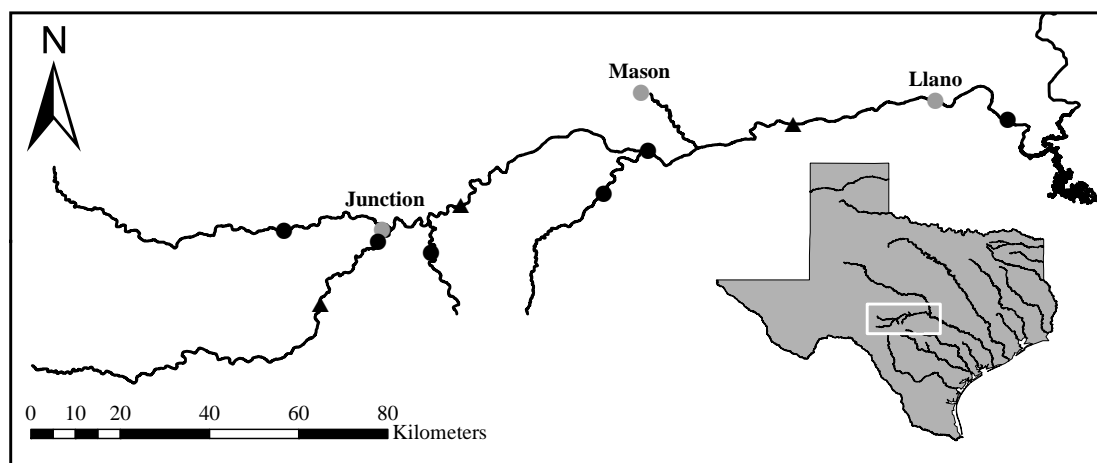
## Tables and Figures

**Table 2.1** – Benthic community sampled by Surber samples (SS) and kick nets (KN) expressed as percent relative abundance. Table is separated by those samples collected in swifter habitats (riffles and fast runs) versus those that were collected in slack water habitats (pools, backwaters, and slow runs).

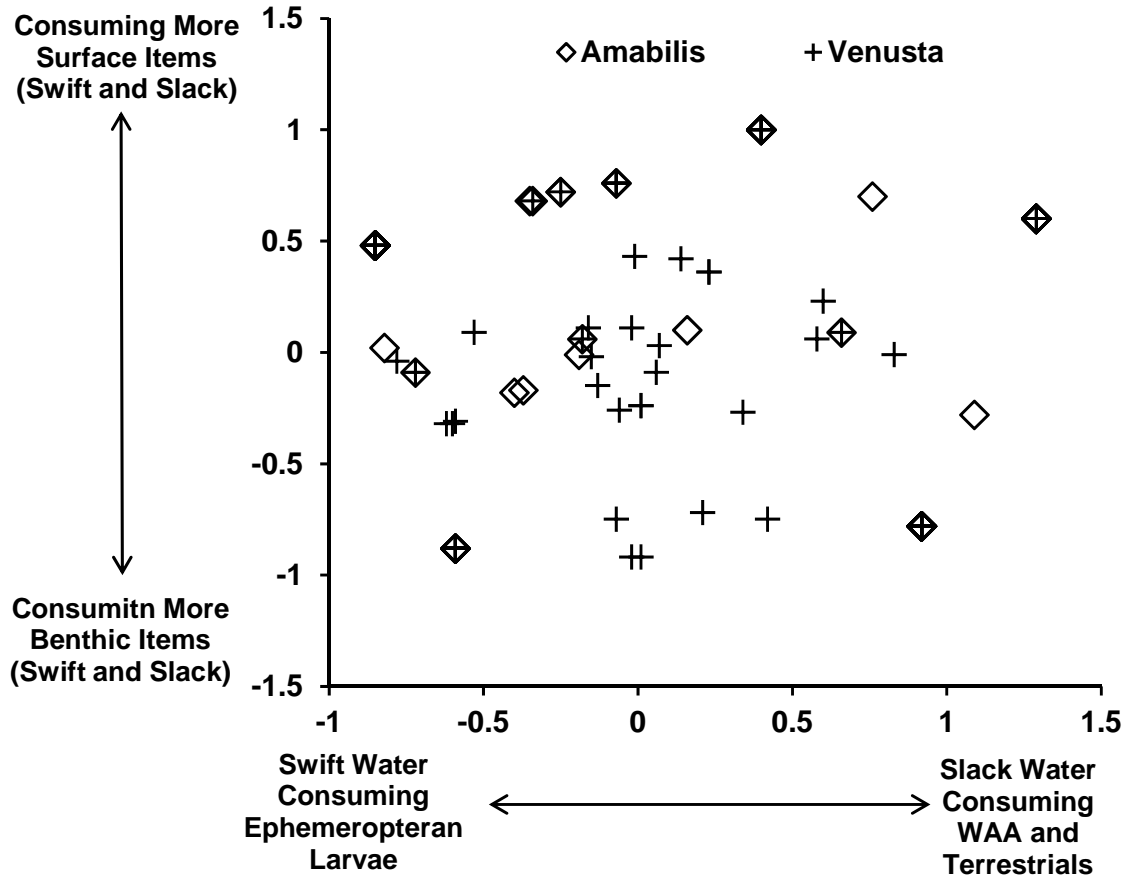
	March	June	December	
<b>Riffle/Swift Run Community</b>	<b>(n = 24 SS, 12 KN)</b>	<b>(n = 12 SS, 22 KN)</b>	<b>(n = 18 SS, 24 KN)</b>	<b>Totals</b>
Coleoptera Adults	12.9	7.7	12.1	<b>32.7</b>
Coleoptera Larvae	8.4	2.8	1.4	<b>12.5</b>
Diptera Larvae	3.3	0.4	0.6	<b>4.3</b>
Ephemeroptera Larvae	2.3	2.9	1.7	<b>6.8</b>
Hemiptera	0.6	0.4	0.8	<b>1.7</b>
Lepidoptera Larvae	0.0	0.0	0.0	<b>0.1</b>
Megaloptera Larvae	0.2	0.1	0.5	<b>0.7</b>
Odonata Larvae	0.6	0.3	0.3	<b>1.2</b>
Trichoptera Larvae	1.8	1.2	1.7	<b>4.7</b>
<b>Pool/Slow Run Community</b>				
Coleoptera Adults	0.7	4.1	2.9	<b>7.8</b>
Coleoptera Larvae	10.2	2.8	3.6	<b>16.7</b>
Diptera Larvae	3.0	0.9	1.9	<b>5.7</b>
Ephemeroptera Larvae	0.7	0.8	0.4	<b>1.9</b>
Hemiptera	0.0	0.1	0.0	<b>0.1</b>
Lepidoptera Larvae	0.1	0.0	0.0	<b>0.1</b>
Megaloptera Larvae	0.0	0.1	0.0	<b>0.1</b>
Odonata Larvae	0.7	0.3	0.7	<b>1.7</b>
Trichoptera Larvae	0.0	0.5	0.6	<b>1.1</b>
<b>Totals</b>	<b>45.5</b>	<b>25.2</b>	<b>29.3</b>	<b>100.0</b>

**Table 2.2** – Drift community sampled by month expressed as percent by relative abundance. Totals are calculated out of total number of organisms sampled.

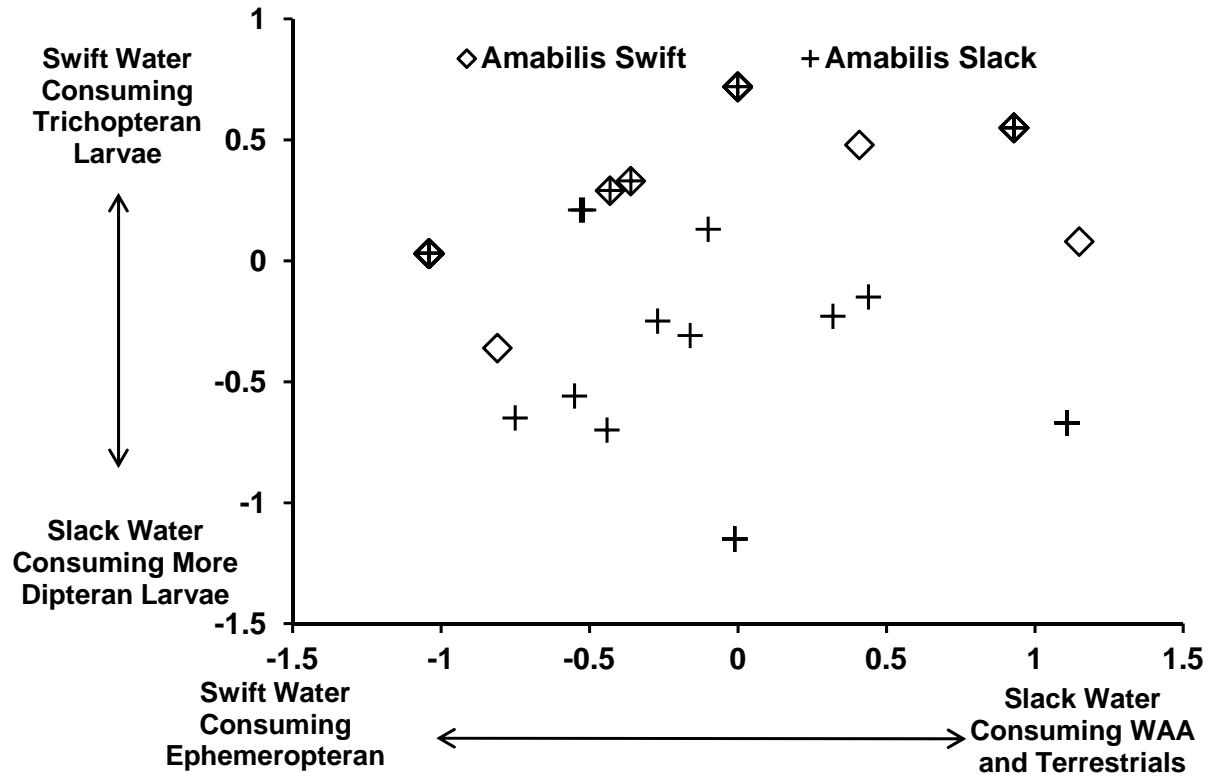
	<b>March</b>	<b>June</b>	<b>December</b>	
	<b>(N = 6)</b>	<b>(N = 7)</b>	<b>(N = 7)</b>	<b>Totals</b>
<b><u>Aquatic Larvae</u></b>				
Coleoptera, Elmidae Larvae	16.1	5.5	0.0	21.6
Diptera Larvae	7.4	3.5	3.9	14.8
Ephemeroptera Larvae	2.3	5.5	1.3	9.0
Lepidoptera Larvae	1.0	0.6	0.6	2.3
Odonata Larvae	0.0	1.9	0.3	2.3
Trichoptera Larvae	3.2	3.9	1.0	8.1
<b><u>Aquatic Adults and Pupae</u></b>				
Coleoptera, Elmidae Adults	4.5	5.8	0.0	10.3
Diptera Adults	3.9	3.2	0.3	7.4
Diptera Pupae	0.3	0.3	0.0	0.6
Ephemeroptera Adults	0.3	0.0	0.0	0.3
Hemiptera, Naucoridae	1.0	11.9	0.0	12.9
Trichoptera Adults	0.6	0.0	1.3	1.9
<b><u>Terrestrial</u></b>				
Araneae	0.3	0.0	0.0	0.3
Coleoptera	0.0	0.0	1.0	1.0
Hemiptera (Saldidae) and Homoptera	0.3	0.3	0.6	1.3
Hymenoptera, Formicidae	2.3	1.6	1.0	4.8
<b>Totals</b>	<b>44.2</b>	<b>44.2</b>	<b>11.6</b>	<b>100.0</b>



**Figure 2.1** – Map of study area including fish collection sites (black circles), arthropod collection sites (black triangles) and town localities (grey circles).

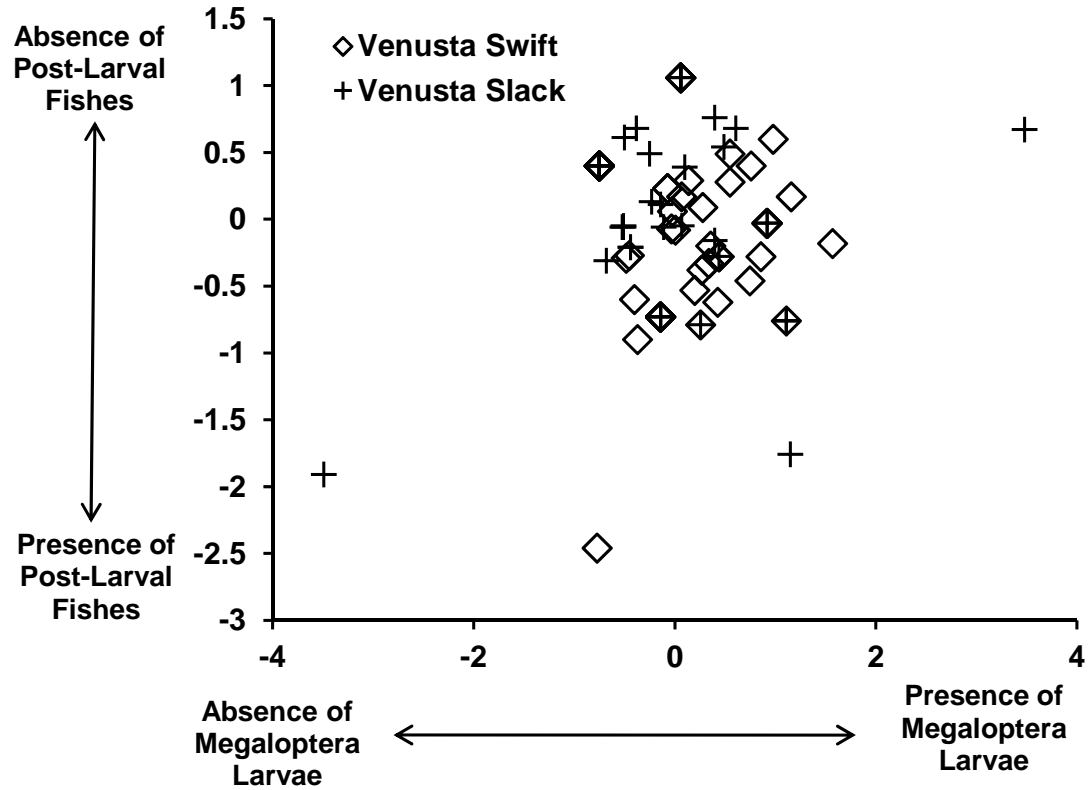


**Figure 2.2** – Scatterplots of multi-dimensional scaling (MDS) of black tail shiner (*Cyprinella venusta*) and Texas shiner (*Notropis amabilis*) loadings for their diets across sites, seasons, and mesohabitats. Qualitative gradients are added to aid in interpreting the gradients for each axis. WAA = winged aquatic adults.

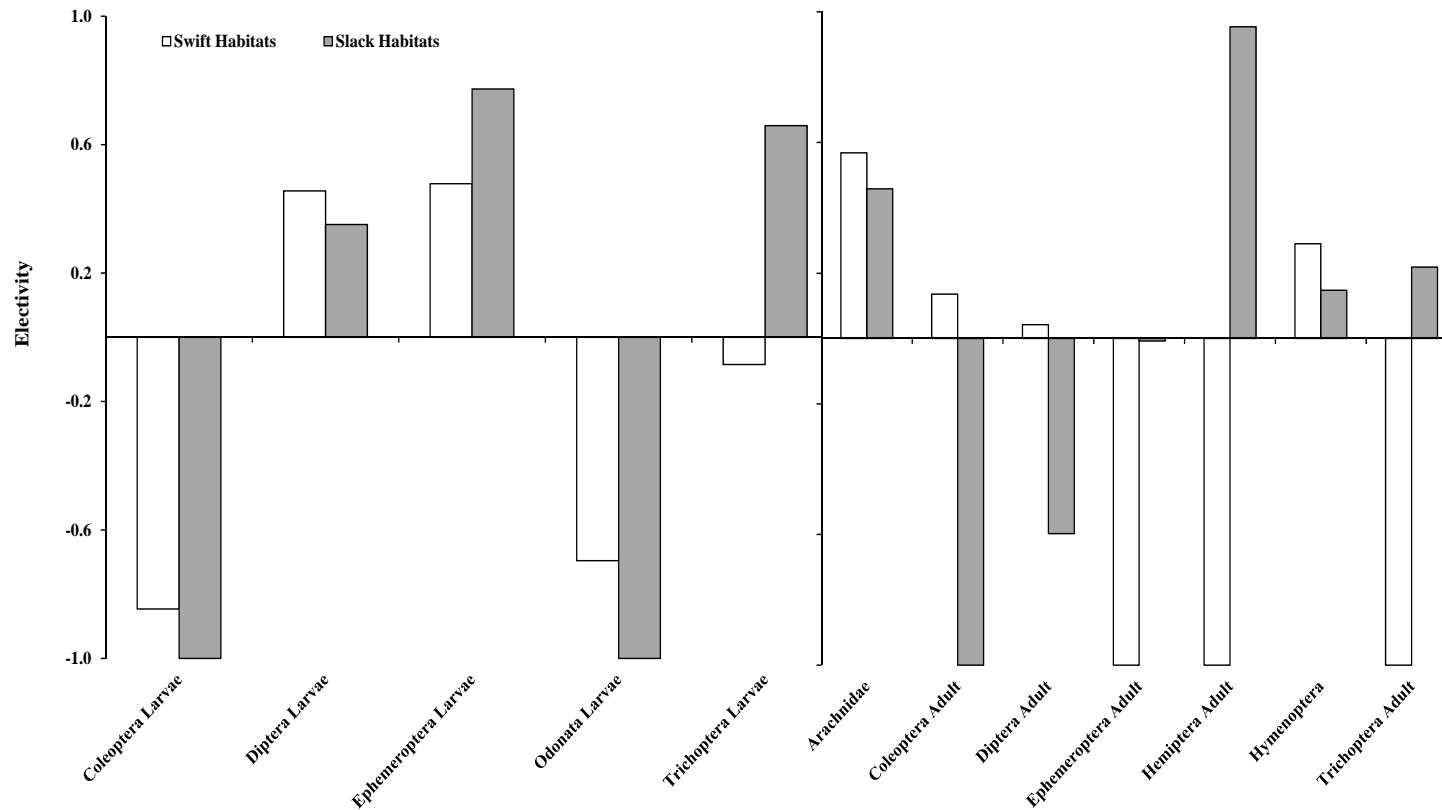


**Figure 2.3** – Scatterplots of multi-dimensional scaling (MDS) for Texas shiner loadings for diets from both swift water habitats and slack water habitats. Along axis one, individuals are largely separated by consumption of winged aquatic adults (WAA) and terrestrials that were mostly consumed by individuals in slack water habitats. Along axis two, another habitat mediated gradient occurs due to consumption of trichopteran larvae (swift water habitats) versus dipteran larvae (slack water habitats).

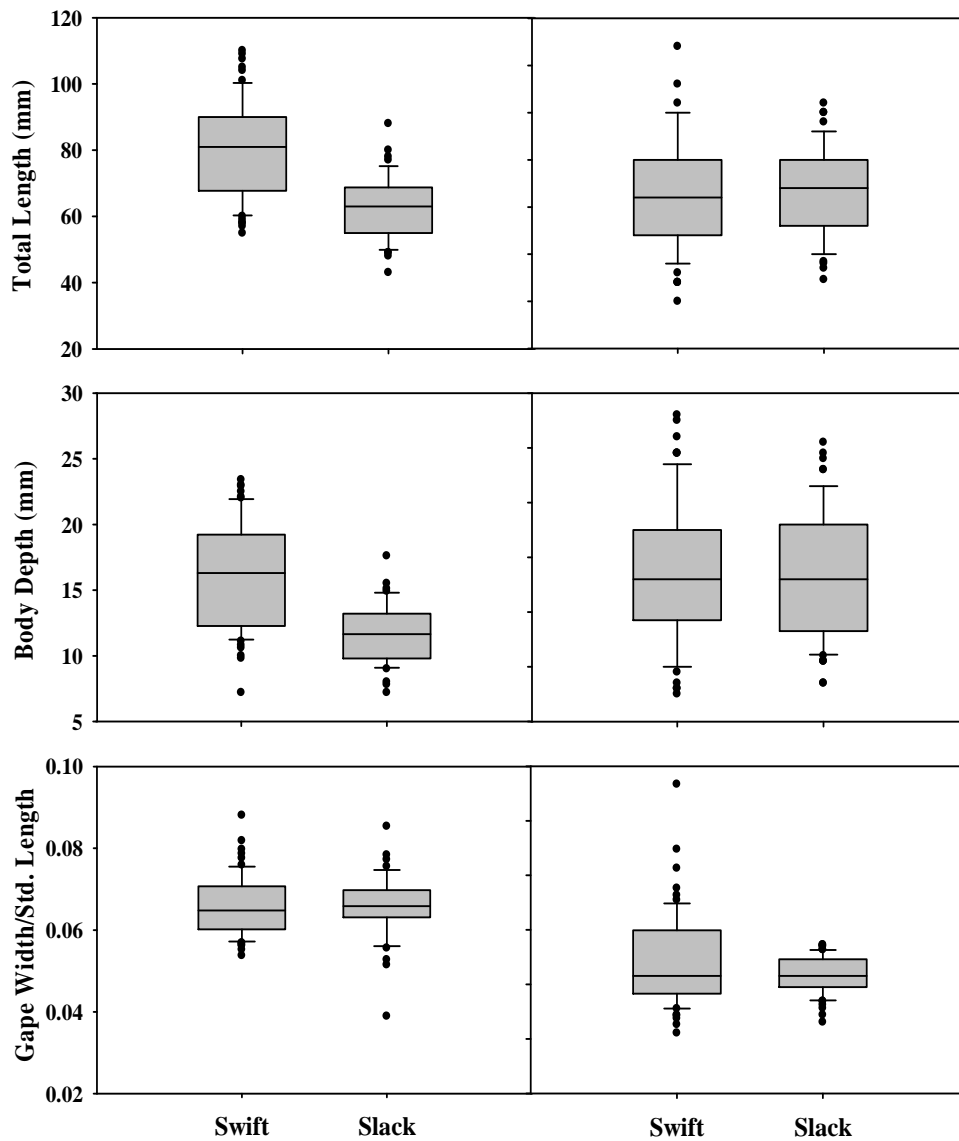




**Figure 2.4** – Scatterplots of multi-dimensional scaling (MDS) for blacktail shiner loadings for diets from both swift and slack water habitats. Blacktail shiner diets do not appear to separate based on a habitat gradient; the first axis largely separates individuals that ate rare, relatively large prey items such as fish and Megaloptera larvae.



**Figure 2.5** – Electivity values for Texas shiners between swift (hollow bars) and slack habitats (grey bars). Along the x-axis are prey categories; on the left half of the panel are benthic and drifting prey items and the right half of the panel are surface prey items (terrestrial and winged-aquatic adults). Values that are near zero indicate fish are feeding commensurate with availability, values close to negative one indicate avoidance or inaccessibility while values close to one indicate selectivity.



**Figure 2.6** – Morphological measurements (gape width/standard length, body depth, and total length) for blacktail shiners (left) and Texas shiners (right) sampled from swift and slack water habitats. Significant differences were detected for blacktail shiners among swift and slack habitats for total length and body depth. No significant differences were detected among Texas shiners collected from swift and slack habitats.

## **CHAPTER 3**

### **INFLUENCE OF TIMING ON THE NUTRITIONAL VALUE OF TERRESTRIAL AND AQUATIC ARTHROPODS FOR FISH COMMUNITIES**

#### **Abstract**

Ecological subsidies are materials and energy that cross a boundary between two ecosystems such as leaf litter that falls into a stream to become the basis of the stream food web. Terrestrial arthropods that fall into streams can be an important resource for fishes however differences in the nutritional quality of terrestrial and aquatic arthropods have not been fully assessed. The ratio of carbon to nitrogen in a food resource can be an indicator of food quality and in order to better understand potential differences in the food quality of terrestrial versus aquatic arthropods, we sampled terrestrial and aquatic arthropods during winter, spring, and summer and measured their molar C:N values. We tested terrestrial and aquatic C:N values for origin (terrestrial versus aquatic), taxonomy (order level), and time of year (winter, spring, and summer). We did not detect significant differences in any of these comparisons and terrestrial and aquatic arthropods had very similar mean molar C:N values and variability around these means. Any trends in molar C:N variability in arthropods, whether they be terrestrial or aquatic, is probably due to the amount of skeletal chitin versus their relative amounts of carbohydrates and

proteins but since chitin is largely undigested in fish diets, molar C:N might still be an indicator of food quality and a potential predictor of food assimilation in fishes.

### **Introduction**

The nutritional quality of a food item is related to the ratios of carbon, nitrogen, and phosphorous and all organisms must balance their nutritional requirements with what is available in the environment, evolutionary constraints on life-history, and the need to maintain support structures and life systems (Fagan et al. 2002; Sterner and Elser 2002). Invertebrate fecundity and higher growth rates are increased when fed diets that are balanced in carbon, nitrogen, and phosphorous (Twombly and Burns 1996; Edwards et al. 1999; Frost and Elser 2002). This suggests that for all consumers, the nutritional value of a food item will have implications for their foraging behavior and fitness but the focus of this relationship has largely been upon herbivores and primary producers (Elser et al. 2000; Frost and Elser 2002). Community level models based upon nutritional interactions between larger sets of primary producers, herbivores, and predators have been proposed (Simpson et al. 2009).

Invertebrate arthropods contribute a substantial portion of fish diets, across taxa and ecosystems (Goldstein and Simon 1999) and terrestrial arthropods can provide a substantial portion of stream fish diets (Baxter et al. 2005). Despite the importance of terrestrial arthropods to fishes, knowledge on how their nutritional quality directly compares to aquatic arthropods is limited. Elser et al. (2000) reviewed the nutritional values of aquatic and terrestrial herbivorous arthropods and Cross et al. (2003) assessed the C:N of aquatic and terrestrial invertebrates which ranged from 5.2-6.4 in molar ratios. In temperate zones, terrestrial arthropod flux into fish diets peaks in summer, coinciding

with peak terrestrial vegetative production. Conversely, aquatic insect availability is at an annual low because the aquatic larvae are hatching and being exported to the terrestrial system (Wipfli 1997; Nakano and Murakami 2001; Sweka and Hartman 2007). The asynchronous pattern in the abundance and use of aquatic and terrestrial food items is predicted to stabilize stream-fish biomass (Takimoto et al. 2002) and the nutritional quality of these food items may also vary seasonally due to changes in the nutritional quality of primary producers during the growing season, perhaps enhancing the benefits to fishes.

Texas shiners (*Notropis amabilis*, Cyprinidae) and blacktail shiners (*Cyprinella venusta*, Cyprinidae) in the Llano River, as well as other cyprinid species consume terrestrial arthropods but this consumption is not heterogeneous throughout the year (Sullivan 2013, Ph.D. Dissertation). Fishes are selective foragers (Gardner 1981) but diet selectivity interacts with ontogeny, morphology, habitat selection, prey size, and prey abundance (Mittelbach 1981; Newman 1987; Osenburg and Mittelbach 1989; Mittelbach et al. 1992) therefore the consumption of terrestrial arthropods by cyprinid fishes can be attributed to multiple factors and perhaps variation in nutritional quality between aquatic and terrestrial arthropods is yet another factor. If terrestrial arthropods are found to be of lesser nutritional quality than aquatic arthropods, then the true benefits of terrestrial subsidies to fish communities are unresolved. Terrestrial arthropod prey items appear to be more energy dense than aquatic prey (Utz et al. 2007; Francis and Schindler 2010) but this energy may not be fully assimilated in prey items that have a relatively high amount of indigestible carbohydrates (especially chitin) relative to protein.

My primary objective was to determine whether the nutritional quality, in terms of mean molar C:N ratios, between aquatic and terrestrial arthropods frequently utilized by fishes varies. The ratio of carbon to nitrogen is indicative of the relative amounts of carbohydrates to protein and is commonly used as a measure of nutritional quality in studies on grazing herbivores and other herbivore producer interactions (Van der Wal 2000; Alldredge 2002). Because arthropods can be largely made of chitin, this will likely be an important factor in the variation between taxa. Chitin is made of *N*-Acetylglucosamine chains  $(C_8H_{13}O_5)_N$  (C:N = 8:1) embedded in sclerotin. Chitin contributes about 23% per 10 mg of insect dry mass (Lease and Wolf 2010) and contains 5-8% nitrogen (Kumar 2000). Aquatic arthropod taxa tend to be less robustly chitinized because gravity does not affect their physiology as much as those in the terrestrial landscape (Cauchie 2002). If aquatic and terrestrial arthropods vary greatly in their amounts of chitin production relative to body mass (Cauchie 2002; Lease and Wolf 2010) and the increase in nitrogen content of terrestrial plants during growing seasons (Mattson 1980), I hypothesize that aquatic and terrestrial arthropods will have different molar C:N ratio values. Specifically, I hypothesize that terrestrial arthropods should have a lower molar C:N than aquatic arthropods during the summer due to an increase in nitrogen concentrations of terrestrial primary producers during the growing season. This might partially explain their consumption by fishes. Understanding the nutritional composition of potential arthropod prey for fishes will increase knowledge of how the foraging ecology of fishes might be related to food quality. Additionally, these results will provide more detail on how terrestrial subsidies truly benefit fish communities.

## **Methods and Materials**

All samples were taken from three sites within the Llano River in Central Texas during December 2010, and March and June 2011. The Llano River is a semi-arid spring-fed stream located on the Edwards Plateau of Central Texas with an encompassing watershed of approximately 11,568 square kilometers (Heitmuller and Hudson 2009). The Llano River watershed is composed of a mesquite-juniper savannah and receives ca. 532 mm of precipitation per year. Primary source of flow is through springs emerging from the Edwards-Trinity Aquifer to create the North Llano River in Sutton County and the South Llano River in Edwards County. The two meet in Junction, Texas and flow approximately 161 river kilometers to Lake Lyndon B. Johnson on the Colorado River (Perkin et al. 2010). Upper reaches and tributaries are characterized by limestone bedrock with substantial amounts of alluvial deposits, moderately flowing habitats and relatively dense riparian zones transitioning to braided channels with finer substrates, granitic outcroppings, and a grassland-savannah type riparian zone.

The three sites were chosen because they offered longitudinal gradients of substrates and riparian vegetation cover. I collected aquatic arthropods using D-frame kick-nets (KN) and Surber samplers (SS). Terrestrial arthropods were sampled using a combination of sweep nets (SN) and pan-traps (PT). Because of variability in stream-flow during the sample period, Surber samplers were not always efficient and sampling effort was made up by D-frame kick-nets and all types of mesohabitats (i.e. riffles, runs, and pools) were sampled. During December, we performed a total of 18 SS and 24 KN, March 24 SS and 12 KN, and June 12 SS and 22 KN. The number of aquatic samples at each site varied with stream-flow and available mesohabitats. Because the relative



abundances of major orders agreed between the KN and SS, they were combined to obtain a more accurate estimate of community composition. Samples using KN were standardized by time (one minute in each mesohabitat) and SS samples were also standardized by time (one min for each sample). Each month at each site, 8 SN were performed and 8 PT were deployed. Sweep-net samples were standardized by time and area (two minutes within a two square meter area) and PT samples were standardized by time (24 hours).

Terrestrial samples were kept frozen until they could be processed, while aquatic samples were processed 12 hours upon entering the lab. In both cases, arthropod samples were assumed to evacuate most of their gut contents. Due to the water content in the aquatic samples, freezing was not practical. Each sample was sorted to the lowest taxonomic resolution practical but because of frequently low sample sizes within the families, analyses were performed at the order level. Origin indicates whether the individual began its life-cycle in the aquatic or terrestrial environment. For example, emergent-adult aquatic insects captured in pan-traps or sweep nets were still recorded as aquatic. Because our sampling occurred within the edge of the stream, we assumed all adult dipterans aquatic unless the origin is well established at the family level (i.e. Muscidae and Calliphoridae which lay eggs in carcasses and other decaying organic matter). Once separated by taxa, samples were desiccated for 48 hours at 60°C and weighed (0.001, mg).

A Thermo Flash EA 1112 series NC soil analyzer was used to measure %C and %N by dry mass (mg) calibrated on aspartic acid and used marine sediments as a certified reference material. Because our C:N measurements were based on dry mass, it was

difficult to collect enough biomass of each taxa in all seasons to reach the desired dry mass of 2.000-4.000 mg, especially adult aquatic coleopterans (primarily of the family Elmidae), it required up to 20 dried individuals to equal an adequate sample for nutrient analyses. I used t-tests to determine significant differences of molar C:N between terrestrial and aquatic taxa across seasons and used a one-way ANOVA to test for variation in mean molar C:N values between arthropod orders that were most frequently consumed in Goldstein and Simon (1999), Sullivan et al. (2012) and Chapter 2 of this dissertation. A two-way ANOVA was used to assess variation in molar C:N values between aquatic and terrestrial arthropods across sample month. All response variables were  $\log x + 1$  transformed to alleviate issues with normality. We used  $\alpha = 0.05$  to judge significance and tested homogeneity of variances for all hypothesis tests and used an LSD *post hoc* test on ANOVAs to assess pair-wise differences.

## Results

The mean molar C:N values ( $\pm 1$  SD), pooled across sample month, life stage, and taxa, were  $5.0 \pm 0.6$  for aquatic ( $N = 81$  samples) arthropods and  $5.1 \pm 0.7$  for terrestrial) arthropods ( $N = 42$  samples). The maximal molar C:N values were the same for both aquatic (range = 4.7-5.6) and terrestrial taxa (range = 4.4-5.6). The greatest molar C:N ratios were observed in Trichoptera larvae among the aquatic arthropods and Coleoptera among the terrestrial arthropods (Table 1). Molar C:N values were not different ( $Df = 74.3$ ,  $T = -0.995$ ,  $P = 0.418$ ) among aquatic and terrestrial arthropods, nor were they different across arthropod taxa ( $F_{7,50} = 1.9$ ,  $P = 0.087$ ), Figure 1.

Seasonal asynchronies in terrestrial arthropod abundance were observed (Table 1) where they were most abundant in June but aquatic arthropod abundance remained

relatively constant throughout the sampling months. The terrestrial arthropod taxa most frequently consumed by fishes were the greatest in relative abundance (Arachnida = 28.4%, Coleoptera = 21.4%, and Hymenoptera = 19.1%). A significant interaction was detected for mean molar C:N values between sample month and origin ( $F_{4,117} = 3.5$ ,  $P = 0.007$ ), Figure 2. When mean molar C:N values for aquatic and terrestrial arthropods were separately analyzed across month, mean molar C:N values for aquatic arthropods was significantly lower in December than in March ( $F_{2,78} = 8.0$ ,  $P = 0.009$ ) and June ( $F_{2,78} = 8.0$ ,  $P < 0.01$ ) but no monthly differences were found for terrestrial arthropods ( $F_{2,39} = 0.88$ ,  $P = 0.47$ ).

### Discussion

Molar C:N values did not differ between aquatic and terrestrial pooled across seasons and taxa and the arthropods most frequently consumed by fishes did not differ in their mean molar C:N values. Our molar C:N values for both aquatic and terrestrial arthropods are within the range of those reported in Cross et al. (2003) and do not support the hypothesis that terrestrial arthropods are of a greater nutritional quality than aquatic arthropods. Differences were detected in the mean molar C:N values across sample month within aquatic arthropods only where their mean molar C:N values were significantly lower in December than in March or June but this difference runs counter to the prediction that terrestrial an increase in terrestrial arthropod availability will coincide with increased nutritional quality during the summer. Aquatic and terrestrial arthropods might not vary in their overall molar C:N values because of compensation in their foraging habits. Herbivorous insects can alter their foraging patterns in order to balance their carbohydrate and protein intake (Raubenheimer and Simpson 1999; Raubenheimer

and Simpson 2004; Behmer 2009) and the result would be similar nutrient concentrations of C and N, despite the varied quality of their food resources. For this paper, the variation in carbon resources available to aquatic and terrestrial arthropod consumers is reflected in the aquatic versus terrestrial origin.

These results suggest that terrestrial arthropod availability will be more important than their nutrient concentrations for fishes. Fishes are more often energy limited than nutrient limited (Schindler and Eby 1997) which suggests any caloric benefit fishes receive from terrestrial arthropods is more important than their nutrient content but a fish's ability to assimilate these calories will be related to the nutritional quality and digestibility of prey. Consumption of terrestrial arthropods by fishes is primarily due to availability and not nutritional quality. If this is generally true across freshwater stream ecosystems and their adjacent riparian subsidy contributors, conceptual models for the importance of terrestrial arthropod prey for fishes such as that proposed by Wipfli and Baxter (2011) are valid because on per unit mass basis, an aquatic prey item is more or less equal to that of a terrestrial prey item.

When we consider the relative importance of food items, such as aquatic versus terrestrial food items, nutritional balance is an important factor because in other cases various foods can offer different nutritional benefits or physiological hindrances such as in the case of herbivores that must overcome potentially harmful secondary plant compounds (Freeland and Janzen 1974). Among predaceous lady beetle larvae (Coleoptera: Coccinellidae) of different species, lady beetle larvae fed diets supplemented with different aphid species produced more eggs than those that were fed only one aphid species (Evans et al. 1999) but on the contrary, herbivorous copepods that

were fed a diet mixed with a lower quality species of phytoplankton experienced depressed development (Twombly and Burns 1996). In the fish *Tilapia aurea*, experimentally manipulated diet combinations of macrophytes, detritus, and invertebrates had a significant effect on their feeding preferences. Food consumption was related to the protein and energy densities of the various food items. However, increased food consumption could only overcome energy limitation but not protein limitation (Bowen et al. 1995). Therefore, even if terrestrial arthropods are not of a higher quality with respect to molar C:N ratios, they are still a source of both energy and protein. Fishes also experience compensatory growth (Skalski et al. 2005) which means individuals that experience a period of starvation or low food abundance will make up for the loss by modulating their food intake during periods of high food abundance which leads to enhanced growth rates. Terrestrial subsidies might then provide a very proximate benefit but could aid fish in compensating for low aquatic food abundance and in some cases, might mean the difference between starvation and survival.

While terrestrial food items can be very important to fishes in some cases, a broader review of fish diets across taxa and morphology indicates that terrestrial foods are consistently fed upon by fishes but generally in low abundances (Sullivan et al. 2012). Taken with the current results, terrestrial food items can be important to fishes but only as a complement to the energy and nutrient concentrations of aquatic foods. However, it might be that terrestrial arthropods provide different elements than those measured here, such as phosphorous (P). Cyprinid gut contents varied across lakes in their C:N and C:P but whole body tissue mass of cyprinids remained similar, indicating that cyprinids will adjust food ingested to remain in stoichiometric balance (Sterner and George 2000) and

terrestrial foods might offer another option for fishes as a P resource to maintain this balance. Given the temporally dynamic nature of terrestrial subsidies in fish communities, whatever energetic and nutritional benefits fishes receive from terrestrial subsidies could be extremely important when put in the context of starvation or the inability to maintain nutritional balance but this probably occurs at acute time scales (within hours or days) that are difficult to attribute to increases in fitness and growth in an empirical fashion. More quantitative analyses on the variation in nutritional quality and energy densities of terrestrial and aquatic arthropods is needed, especially on a temporal scale similar to the work that has been done on the energetic contribution of terrestrial arthropods to salmonid communities (Utz et al. 2007). However, when considering insectivorous fishes, the digestibility of various food items must be considered simultaneously since the primary constituent of arthropod exoskeleton is chitin, which is largely undigested in fish feces (Lindsay et al. 1984) and additional chitin in *Tilapia aurea* diets as a protein supplement significantly depressed growth rates (Shiau and Yu 1999). A bioenergetics framework would be useful to continue this work, especially for insectivorous minnows, as it would provide a mass balance that would estimate growth and more directly relate aspects of biological fitness.

Terrestrial and aquatic arthropods did not differ in their nutritional quality with respect to molar C:N ratios. If fishes are more energy limited than nutrient limited, then fishes that feed upon both resources are adding to their energy and nutrient intake, not supplementing it. While chitin might explain some of the minor differences among taxa (for example aquatic chironomic larvae versus terrestrial adult beetles), this variability was not significant in our ANOVA. However, it may still be the case where terrestrial

invertebrates provide other elemental resources not measured here such as phosphorous (P) and other trace elements such as calcium or magnesium. We did not detect any order level (taxonomic) differences, even within an order that shares aquatic and terrestrial representatives (e.g. Hemiptera and Coleoptera). To fishes, the differences, or in this case the similarities between terrestrial vs. aquatic taxa, is probably the varying amounts of structural chitin between taxa and origin.

### Literature Cited

- Aldredge, M.W., J.M. Peek, and W.A. Wall. 2002. Nutritional quality of forages used by elk in Northern Idaho. *Journal of Range Management* **55**, 253-259.
- Behmer, S.T. 2009. Insect herbivore nutrient regulation. *Annual Reviews of Entomology* **54**, 165-187.
- Bowen, S.H., E.V. Lutz, and M.O. Ahlgren. 1995. Dietary protein and energy as determinants of food quality: trophic strategies compared. *Ecology* **76**, 899-907.
- Cauchie, H. 2002. Chitin production by arthropods in the hydrosphere. *Hydrobiologia* **470**, 63-69.
- Cross, W.F., J.P. Benstead, A.D. Rosemond, and J.B. Wallace. 2003. Consumer-resource stoichiometry in detritus-based streams. *Ecology Letters* **6**, 721-732.
- Edwards, W.E., Stevenson, A.T., and D. Richards. 1999. Essential versus alternative foods of insect predators: benefits of a mixed diet. *Oecologia* **121**: 107 – 112.
- Elser, J.J., Fagan, W.F., R.F. Denno, D. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S.S. Kilham, E. McCauley, K. Schulz, E. Siesmann, and R.W. Sterner. 2000. Nutritional constraints and freshwater food webs. *Nature* **408**, 578 – 580.
- Fagan, W.F., Siemann, E., C. Mitter, R.F. Denno, A. F. Huberty, H.A. Woods, and J.J. Elser. 2002. Nitrogen in insects: implications for trophic complexity and species diversification. *The American Naturalist* **160**, 784 – 802.
- Fancis, T.B. and D.E. Schindler. 2009. Shoreline urbanization reduces terrestrial insect subsidies to fishes in North America. *Oikos* **118**, 1872-1882.



- Frost, P.C. and Elser, J.J. 2002. Growth responses of littoral mayflies to the phosphorous content of their food. *Ecology Letters* **5**, 232 – 240.
- Gardener, M.B. 1981. Mechanisms of size selectivity by planktivorous fish: a test of hypotheses. *Ecology* **62**, 571-578.
- Goldstein, R.M. and Simon, T.P. (1999). Toward a united definition of guild structure for feeding ecology of North American fishes. In ‘Assessing the Sustainability and Biological Integrity of Water Resources Using Fish Communities.’ (Ed. Simon, T.P.) pp. 123 – 139. (CRC Press: Boca Raton, U.S.A.) .
- Lease, H.M. and B.O. Wolf. 2010. Exoskeletal chitin scales isometrically with body size in terrestrial insects. *Journal of Morphology* **271**, 759-768.
- Lyndsay, G., M.J. Walton, J.W. Adron, T.C. Fletcher, C.Y. Cho, and C.B. Cowey. 1984. The growth of rainbow trout (*Salmo gairdneri*) give diets containing chitin and its relationship to chitinolytic enzymes and chitin digestibility. *Aquaculture* **37**, 315-334.
- McIntyre, P.B., A.S. Flecker, M.J. Vanni, J.M. Hood, B.W. Taylor, and S. A. Thomas. 2008. Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots? *Ecology* **89**, 2335-2346.
- Mittelbach, G.G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. *Ecology* **62**, 1370-1386.
- Mittelbach, G.G., C.W. Osenberg, and P.C. Wainwright. 1992. Variation in resource abundance affects diet and feeding morphology in pumpkinseed sunfish (*Lepomis gibbosus*). *Oecologia* **90**, 8-13.

- Nakano, S. and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Science* **98**, 166-170.
- Newman, R.M. 1987. Comparison of encounter model predictions with observed size-selectivity in trout. *Journal of the North American Benthological Society* **6**, 56-64.
- Osenberg, C.W. and G.G. Mittelbach. 1989. Effects of body size on the predator-prey interaction between pumpkinseed sunfish and gastropod. *Ecological Monographs* **4**, 405-432.
- Pilati, A. and M. Vanni. 2007. Ontogeny, diets, and nutrient stoichiometry in fish. *Oikos* **116**, 1663-1674.
- Raubenheimer, D., and Simpson, S.J. 1999. Integrating nutrition: a geometrical approach. *Entomologia Experimentalis et Applicata* **91**, 67 – 82.
- Raubenheimer, D. and Simpson, S.J. 2004. Organismal stoichiometry: quantifying non-independence among food components. *Ecology* **85**, 1203 – 1216.
- Schindler, D.E. and Eby, L.A. 1997. Stoichiometry of fishes and their prey: implications for nutrient recycling. *Ecology* **78**, 1816 – 1831.
- Shiau, S. and Y. Yu. 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus* X *O. aureus*. *Aquaculture* **179**, 439-446.
- Simpson, S.J., D. Raubenheimer, M.A. Charlston, F.J. Cissold, and the ARC-NZ Vegetation Function Network Herbivory Group. *Trends in Ecology and Evolution* **1152**: 1-8.
- Sterner, R.W. and N.B. George. 2000. Carbon, nitrogen, and phosphorous stoichiometry of Cyprind fishes. *Ecology* **81**, 127-140.

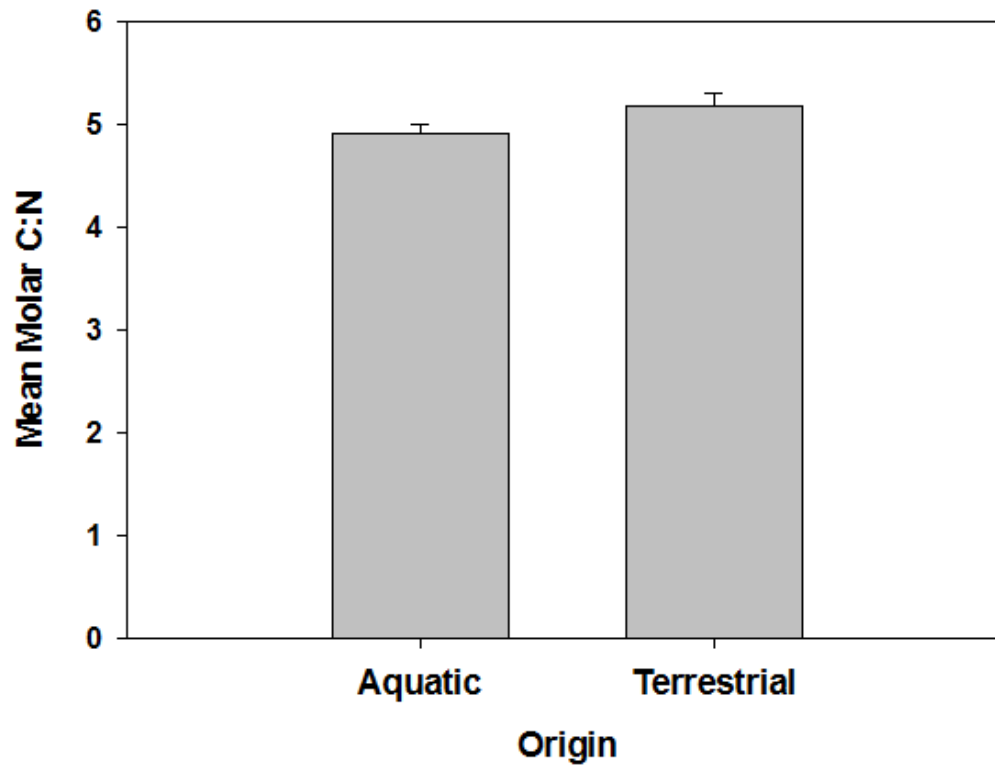
- Sterner , R.S. and Elser, J.J. 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton, New Jersey, U.S.A.
- Sullivan, M.L., Y. Zhang, and T.H. Bonner. 2012. Terrestrial subsidies in the diets of stream fishes of the USA: comparisons among taxa and morphology. *Marine and Freshwater Research*, **63**: 409-414.
- Sweka, J.A. and Hartman, K.J. 2008. Contribution of terrestrial invertebrates to yearly brook trout prey consumption and growth. *Transactions of the American Fisheries Society* **137**, 224 – 235.
- Takimoto, G., Iwata, T., and M. Murakami. 2002. Seasonal subsidy stabilizes food web dynamics: balance in heterogeneous landscapes. *Ecological Research* **17**, 433 – 439.
- Twombly, S. and Burns, C.W. 1996. Effects of food quality on individual growth and development in the freshwater copepod (*Boeckella triarticulata*). *Journal of Plankton Research* **18**, 2179 – 2196.
- Utz, R.M. and K.J. Hartman. 2007. Identification of critical prey items to Appalachian brook trout (*Salvelinus fontinalis*) with emphasis on terrestrial organisms. *Hydrobiologia* **575**, 259-270.
- Van der Wal, R., N. Madan, S. van Lieshout, C. Dormann, R. Langvatn, and S.D. Albon. 2000. Trading forage quality for quantity? Plant phenology and patch choice by Svalbard reindeer. *Oecologia* **123**; 108-115.
- Wipfli, M.S. 1997. Terrestrial invertebrates as salmonid prey and nitrogen sources in streams: contrasting old-growth and young-growth riparian forests in southeastern Alaska, U.S.A. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1259–1269.

Wipfli, M.S. and C.V. Baxter. 2011. Linking ecosystems, food webs, and fish production: subsidies in salmonid watersheds. *Fisheries* **35**, 373-387.

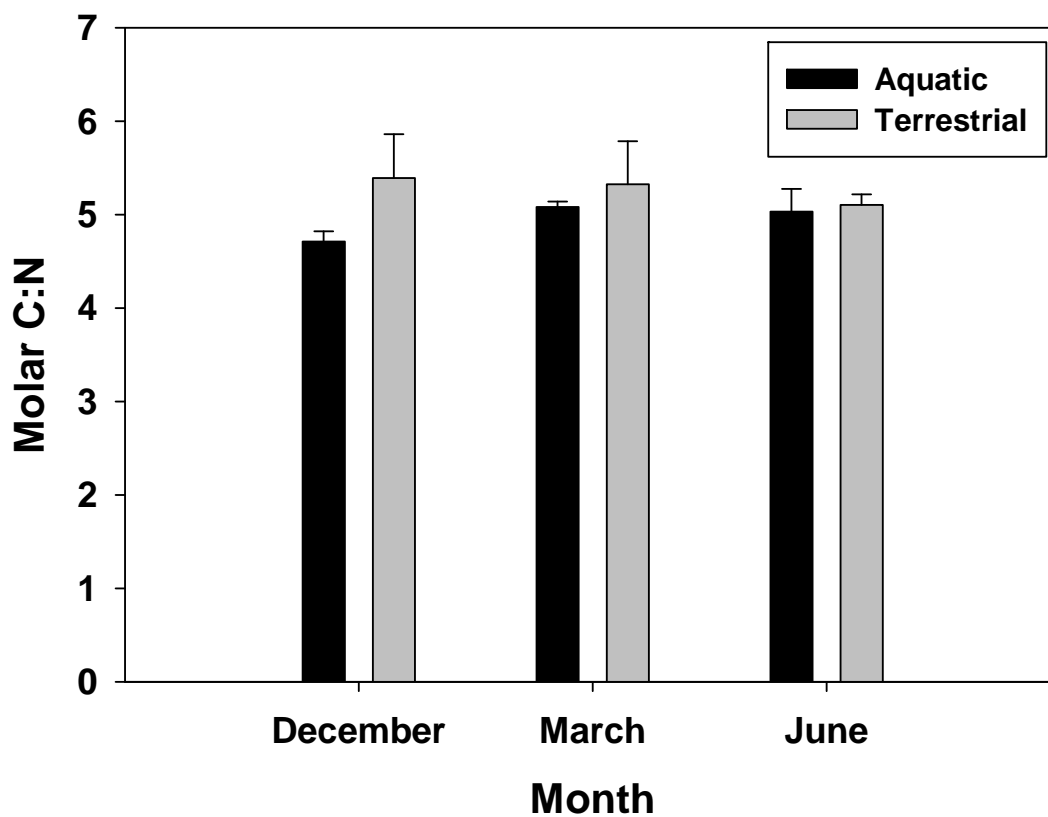
## Tables and Figures

**Table 3.1** – Summary table of mean molar C:N values and the %N for each terrestrial and aquatic taxa collected (standard deviations in parentheses). Letters in parentheses next to order names represent life stage (A = adult and L = larvae). Monthly totals represent total number of individuals sampled and %RA = percent relative abundance within each origin (aquatic or terrestrial).

Order	March	June	December	Total	% RA	Molar C:N	%N
<i>Aquatic Taxa</i>							
Coleoptera (A)	434	456	456	1346	44.3	5.2 (0.6)	10.0 (1.2)
Coleoptera (L)	23	233	144	400	13.2	5.1 (0.4)	10.3 (1.2)
Diptera (A)	27	37	36	100	3.3	5.2 (0.6)	11.1 (1.9)
Diptera (L)	241	71	74	386	12.7	4.7 (0.9)	10.2 (0.8)
Ephemeroptera (A)	13	0	8	21	0.7	5.0 (.)	11.9 (.)
Ephemeroptera (L)	58	92	128	278	9.1	4.9 (0.1)	9.3 (1.7)
Hemiptera (A)	35	11	29	75	2.5	5.1 (0.2)	11.1 (0.5)
			14				
Megaloptera (L)	13	4		31	1.0	4.8 (0.6)	10.5 (0.8)
Odonata (A)	2	7	0	9	0.3	4.8 (0.2)	11.7 (0.3)
Odonata (L)	48	30	30	108	3.6	4.7 (0.4)	11.3 (0.5)
Plecoptera (L)	35	0	15	50	1.6	5.3 (0.1)	10.7 (0.4)
Trichoptera (A)	16	4	12	32	1.1	4.8 (.)	11.7 (.)
Trichoptera (L)	110	17	69	196	6.4	5.6 (0.2)	9.8 (0.8)
<b>Total</b>	<b>1055</b>	<b>962</b>	<b>1015</b>	<b>3032</b>	<b>100.0</b>		
<i>Terrestrial Taxa</i>							
Arachnida	12	27	22	61	28.4	4.8 (0.4)	12.0
Coleoptera (A)	16	21	9	46	21.4	5.6 (0.8)	10.4
Hemiptera (A)	6	13	13	32	14.9	5.6 (.)	10.9
Hymenoptera	6	26	9	41	19.1	5.3 (0.7)	13.1
Lepidoptera (A)	5	9	2	16	7.4	4.4 (0.2)	11.3
Orthoptera	3	15	1	19	8.8	5.3 (0.3)	10.7
<b>Total</b>	<b>48</b>	<b>111</b>	<b>56</b>	<b>215</b>	<b>100.0</b>		



**Figure 3.1** – Mean molar C:N between aquatic and terrestrial arthropods pooled across sample month and taxa (Df = 74.3,  $T = -0.995$ ,  $P = 0.418$ ).



**Figure 3.2** – Mean molar C:N values for aquatic (black bars) and terrestrial arthropods (grey bars) by season. The interaction between origin (aquatic vs. terrestrial) and season was significant ( $F_{4,117} = 3.5$ ,  $P = 0.007$ ). Aquatic and terrestrial molar C:N values were analyzed separately across seasons.

**CHAPTER 4**  
**TEMPERATURE MODULATION OF GROWTH AND**  
**PHYSIOLOGY OF JUVENILE GUADALUPE**  
**BASS *MICROPTERUS TRECULII***

**Abstract**

Guadalupe bass *Micropterus treculii* fingerlings were cultured in 18, 21, 24, 27, or 30°C for six weeks. Temperature affected the growth of the Guadalupe bass when it was measured by increases in total length ( $P = 0.042$ ), but not when measured by increase in mass ( $P = 0.100$ ). The optimal temperature for growth of Guadalupe bass was 27-28°C. Condition factor increased linearly ( $P = 0.049$ ) with temperature, indicating that the fish were plumper at warmer temperatures. The mean hematocrit was 37 and not significantly affected by temperature, although a trend of increasing hematocrit with increasing temperature was apparent. The liver index varied significantly ( $P = 0.041$ ) with temperature, and the maximum index was at 24°C. The results of this study provide basic thermal information and some base line hematological values for hatchery production and management of Guadalupe bass.

**Introduction**

The Guadalupe bass *Micropterus treculii* is a stream dwelling micropteryd native to the Edwards Plateau region of South-Central Texas. It is a species of state special



concern due to limited natural distribution and genetic introgression with introduced micropterids, particularly the smallmouth bass *Micropterus dolomieu* (Whitmore 1983; Warren et al. 2000; Littrell et al. 2007; Bonner and Bean 2008). The stocking of hatchery-produced fingerlings is a part of recovery effort for this species. Beyond some information on spawning and hatching conditions (Carmichael and Williamson 1986) and toxicity of nitrogenous wastes (Tomasso and Carmichael 1986), little is known of the environmental requirements of Guadalupe bass. To develop a broader base of environmental information for hatchery managers, this study determined the effects of temperature on growth and body condition of Guadalupe bass. We also observed the effect of rearing temperature on hematocrit, liver index, and body condition which are indicators of general fish health (Barton et al. 2002).

### **Methods and Materials**

Approximately 400 Guadalupe bass fingerlings (59 -108 mm total length) were obtained from the A.E. Wood Fish Hatchery (Texas Parks and Wildlife Department) in May 2011. The fish were trained to consume prepared feed by generally following Carmichael and Williamson (1986). Briefly, fish were held in indoor fiberglass tanks receiving constantly flowing spring water (22 C). Initially, fish were offered a mixture of dried, crushed krill and silverside and a prepared pellet (BioDiet Grower<sup>®</sup>; Bio-Oregon, Longview, Washington: 43% protein, 14% oil, 1% fiber). During training, feed was offered at least 10 times per day, and the proportion of prepared pellet was increased as fish began to feed consistently. During training, approximately 5% of the fish died. Fish were transferred to experimental systems after they had been readily taking prepared pellets for at least two weeks.

Thirty individuals were stocked into each of six 300-L recirculating systems (Living Streams, Frigid Units, Toledo, Ohio), and then water temperatures were adjusted at a rate of approximately  $\pm 0.5^{\circ}\text{C}$  per day (starting water temperature was approximately  $22^{\circ}\text{C}$ ) until temperatures in Living Streams were nominal 18, 21, 24 (two Living Streams), 27, or  $30^{\circ}\text{C}$  with a thermostat resolution of  $\pm 1.0^{\circ}\text{C}$ . After all Living Streams reached their nominal temperatures, fish in each were anesthetized (2 g/L tricaine methanesulfonate), weighed individually and returned to the Living Stream from which they were collected. Fish averaged  $4.7 \pm 1.47$  g. Mean fish weight varied significantly (ANOVA;  $P \leq 0.001$ ) among Living Streams (Living Stream means ranged from 3.9 to 5.3 g) with higher mean weights in the warmer tanks due to thermally controlled differential growth during the acclimation period. After weighing, fish were exposed to experimental temperatures for six weeks and were fed to satiation once per day. During the six-week study, 6% of the fish died (7 fish in the  $18^{\circ}\text{C}$  treatment and 3 fish in the  $21^{\circ}\text{C}$  treatment).

Temperature and dissolved oxygen were monitored daily using a YSI 85-10 meter (YSI, Inc., Yellow Springs, Ohio). The mean temperature for each Living Stream was the same as the nominal temperature with a maximum coefficient of variation of 2.1%. Mean oxygen concentrations ranged from 87% of saturation in the highest temperature treatment to 96% of saturation in the lowest temperature treatment, with a maximum coefficient of variation of 3.8%. Total ammonia nitrogen (TAN) was measured by direct Nesslerization (APHA 1989) three times during the experimental period and fell below detectable limits (0.01 mg/L) each time. The pH was measured using an Accumet 15 pH meter (Fisher Scientific, Pittsburgh, Pennsylvania) three times during the experimental

period and ranged from 8.5 to 8.6. Artificial sea salt was added to the culture water to maintain adequate ions and buffering capacity. It was measured daily using the YSI 85-10 and was  $1.2 (\pm 0.2)$  during the course of the study.

After a six week exposure to experimental temperatures, fish were anesthetized as described previously, weighed, measured (total length), and liver and blood were collected. For both length and mass, growth was calculated as percent gain. Blood was collected into a heparinized capillary tube from the hemal arch after severing the caudal peduncle. Capillary tubes were immediately centrifuged and hematocrit determined. After fish were weighed, livers were removed and weighed separately. Liver index was expressed as a percent of body mass represented by the liver. Fulton's condition factor ( $K$ ) was calculated according to Anderson and Neumann (1996).

Regression analysis was applied to all data sets and an  $\alpha$  of 0.05 was considered significant. Based on previous experience defining thermal-growth relationships in fishes (e.g., (Atwood et al. 2003; Sullivan and Tomasso 2010), we fitted a second-degree polynomial to the growth data. Mean responses of individuals in each Living Stream were entered into analyses, resulting in an  $N$  of six in each analysis.

## **Results and Discussion**

Temperature affected the growth of the Guadalupe bass when it was measured by increases in total length (Figure 1a). However, a significant treatment effect was not observed when growth was measured by mass (Figure 1b). The polynomials used to describe the temperature and growth relationships for both lengths and masses described 88 and 78% of the variability in the observations, respectively. The optimal temperature for growth of Guadalupe bass was estimated to be 28°C for length and 27°C for mass.

Decreases in growth rates were observed at 30°C, indicating that Guadalupe bass were in a thermal environment that was beyond their maximum metabolic scope (Neill and Bryan 1991). Estimated optimal growth temperature for Guadalupe bass was similar to that reported for largemouth bass *Micropterus salmoides* (26 – 30°C; Jobling 1981) but differed from that of smallmouth bass (22°C; Whitledge et al. 2006). The low  $N$  (6) may be responsible for the failure to observe a significant treatment effect when measuring mass and also creates some uncertainty with regard to our estimate of optimal temperature.

Condition factor increased linearly with temperature (Figure 2), indicating that the fish were plumper at warmer temperatures. This is similar to results of largemouth bass where significantly greater condition factors were observed at 26 and 32 °C versus 20°C (Tidwell et al. 2003). To try and understand why  $K$  was different across treatments we plotted mass versus length for all fish at the end of the study. The relationship was continuous ( $Mass = 0.000009Length^{3.004}$ ) and consistent ( $r^2 = 0.95$ ), indicating that warmer (faster-growing) fish and cooler (slower-growing) fish will have the same  $K$  at a given mass. Hence, the differences we detected in  $K$  may be attributed to normal allometric growth rather than a thermal effect.

The mean hematocrit was 37 and not significantly affected by temperature, although a trend of increasing hematocrit with increasing temperature was apparent. As temperature increases, oxygen demand by fish will increase while the solubility of oxygen in water and plasma decreases. Any increase in hematocrit under these conditions is probably directed toward increasing the ability of the animal to deliver

oxygen to the tissues (reviewed in Nikinmaa 2006). However, diet and activity may also play a role in variability in hematocrit (Denton and Yousef 1975).

The liver index varied significantly with temperature with a maximum index at 24°C (Figure 3b). During periods of surplus energy intake, fish store energy in muscle and liver leading to changes in liver size relative to fish body size (Busacker et al. 1990). As stored energy is used, liver size decreases. In this study, relative liver size increased in fish reared at lower temperatures, peaked, and declined in fish reared at the higher temperatures. Relative liver size peaked at temperatures 3-4°C below the temperature for maximum growth. Perhaps the earlier decline in relative liver size represents a period when energy stored as liver fat and glycogen supplemented daily rations to support thermally controlled increases of metabolic rate and growth. The temperature at which fish growth decreases represents the point where metabolic scope is decreasing due to the rapid increase of standard metabolism (Neill and Bryan 1991). Similar peaks in relative liver size have been reported for largemouth bass (Heidinger and Crawford 1977; Brown and Murphy 2004).

### **Ecological Implications**

Current recovery efforts are focused on the South Llano River (Texas) Guadalupe bass population which is <3% hybridized with smallmouth bass (T. Bonner, unpublished data). Recovery efforts include capturing of broodstock from the South Llano River and subsequent back-stocking of hatchery-produced fingerlings. The results of this study provide basic thermal information for use during hatchery production of Guadalupe bass and management of populations in the field. Our estimated optimal growth temperature

for Guadalupe bass (27 – 28 °C) is similar to that reported for largemouth bass (26 – 30 °C) of various sizes (Jobling 1981) but differs from that reported for smallmouth bass *Micropterus dolomieu* (22 °C, Whitledge et al. 2003). We observed decreased growth for weight and total length at 30 °C, indicating that Guadalupe bass were in a thermal environment that was beyond their maximum metabolic scope (Neill and Bryan 1991). Water temperatures correspond with distributions of micropterids and other fishes. For example, the historical range of smallmouth bass is generally restricted to higher latitudes than largemouth bass although there is considerable overlap (Lee et al. 1981), and smallmouth bass have a lower optimal growth temperature.

Warmer or cooler optimal growth temperatures could be a physiological response to residing in and being restricted to certain climates (i.e. phenotypic plasticity) because within some species of Centrarchidae, metabolic rate and preferred temperature are positively associated with acclimation temperature (Schnell and Pigg 1975; Díaz et al. 2007). Alternatively, warmer or cooler optimal growth temperatures could be a derived condition, enabling a higher fitness in a given climate. Between sympatrically-occurring micropterids, largemouth bass are positively associated with warmer water temperatures, and smallmouth bass are positively associated with cooler water temperatures (Sowa and Rabeni 1995), suggesting optimal growth temperatures are derived and influencing distributions. Additionally, growth rate and latitude have been shown to be positively correlated in striped bass (Conover et al. 1997) where cooler temperatures and shorter growing seasons may have selected for a shorter life history but more rapid growth rates. Furthermore, the availability of oxygen in water decreases with increases in water temperature. Thus, thermal optima in fishes might be closely coupled to a species'

ability to acquire oxygen (Beitinger and Fitzpatrick 1979) which depends on the configuration of hemoglobin which is integrally linked to genotype. However, the addition of oxygen to warmer water has not been shown to increase the preferred temperature in microterids (Beamish 1970) as it has in some salmonids (Brett 1964).

Introduced smallmouth bass have genetically swamped out or ecologically-displaced Guadalupe bass in several drainages of Central Texas. Naturally occurring Guadalupe bass populations are now restricted to a few tributaries of the Colorado and San Antonio River drainages in central Texas (Koppelman and Garrett 2002). One of these tributaries is the South Llano River (Perkin et al. 2010) and each year the Texas Parks and Wildlife Department stocks several thousand Guadalupe bass fingerlings in this river for population augmentation. Success of the smallmouth bass introgression and displacement is attributed to repeated historical stockings and likely other variables, but cooler optimum growth temperatures of smallmouth bass obviously were not a limiting factor in the successful establishment of an introduced population 500 km south of its native range.

The annual variability in temperature within the Llano River can range from 8 °C in the winter up to 32 °C in the warmest parts of the summer (USGS Gaging Station No. 08151500) and therefore approaches temperatures that would cause thermal stress in Guadalupe bass. Adult Guadalupe bass were strongly associated with in-stream cover, especially woody debris, boulders and ledges (Perkin et al. 2010) and within the Llano River drainage, tend to be associated with medium-sized, spring-fed streams that are relatively thermally stable although they are not directly associated with spring-outflow (Curtis, S. Master's Thesis). These results suggest that, similar to smallmouth bass in

spring fed streams in the Ozark region (Whitledge et al. 2006), maintenance of in-stream habitat will be important for Guadalupe bass from a thermal standpoint as the structural features they are associated with can provide shade and spring-flows up-stream of their primary habitats will help mitigate the potentially adverse effects of high temperature during the warmest periods of the summer.

*All procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC No. 1036\_1102\_32)*



### Literature Cited

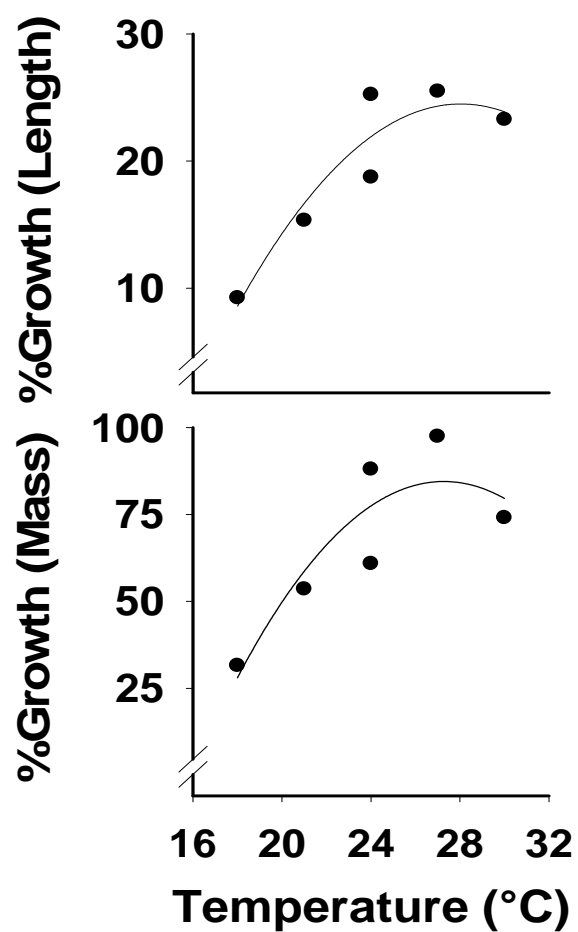
- APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater, 17<sup>th</sup> Ed. APHA, Washington, D.C., U.S.A.
- Anderson, R.O. and R.M. Neumann. 1996. Length, weight, and structural indices. Pages 447 – 482 *in* Fisheries Techniques, B.R. Murphy and D.W. Willis, editors. American Fisheries Society, Bethesda, Maryland, U.S.A.
- Atwood, H.L., S.P. Young, J.R. Tomasso and T.I.J. Smith. 2003. Effect of temperature and salinity on survival, growth, and condition of juvenile black sea bass *Centropristis striata*. Journal of the World Aquaculture Society 34:398-402.
- Barton, B.A., J.D. Morgan, and M.M. Vijayan. 2002. Physiological and condition-related indicators of environmental stress in fish. Pages 111 – 148 *in* Biological Indicators of Aquatic Ecosystem Stress, S.M. Adams, editor. American Fisheries Society, Bethesda, Maryland, U.S.A.
- Beamish, F.W. 1970. Oxygen consumption of largemouth bass, *Micropterus salmoides*, in relation to swimming speed and temperature. Canadian Journal of Zoology 48:1221 – 1228.
- Beitinger, T.L., and L.C. Fitzpatrick. 1979. Physiological and ecological correlates of preferred temperature in fish. American Zoologist 19:319 – 329.

- Bonner, T.H. and P.T. Bean. 2008. Threatened fishes of the world: *Micropterus treculii* (Vaillant and Bocourt 1874) (Centrarchidae). Environmental Biology of Fishes 83:367 – 368.
- Brett, J.R. 1964. The respiratory and swimming performance of young sockeye salmon. Journal of the Fisheries Research Board of Canada 21:1183 – 1226.
- Brown, M.L. and B.R. Murphy. 2004. Seasonal dynamics of direct and indirect condition indices in relation to energy allocation in largemouth bass *Micropterus salmoides* (Lacèpde). Ecology of Freshwater Fish 13:23 – 36.
- Busacker, G.P., I.R. Adelman, and E.M. Goolish. 1990. Growth. Pages 363-387 in Methods for Fish Biology, C.B. Schreck and P.B. Moyle, editors. American Fisheries Society, Bethesda, Maryland, U.S.A.
- Carmichael, G.J. and J.H. Williamson. 1986. Intensive production of Guadalupe bass. The Progressive Fish Culturist 48:133 – 136.
- Denton, J.E. and M.K. Yousef. 1975. Seasonal changes in hematology of rainbow trout, *Salmo gairdneri*. Comparative Biochemistry and Physiology Part A: Physiology 51: 151 – 153.
- Heidinger, R.C. and S.D. Crawford. 1977. Effect of temperature and feeding rate on the liver-somatic index of the largemouth bass, *Micropterus salmoides*. Journal of the Fisheries Research Board of Canada 34:633 – 638.

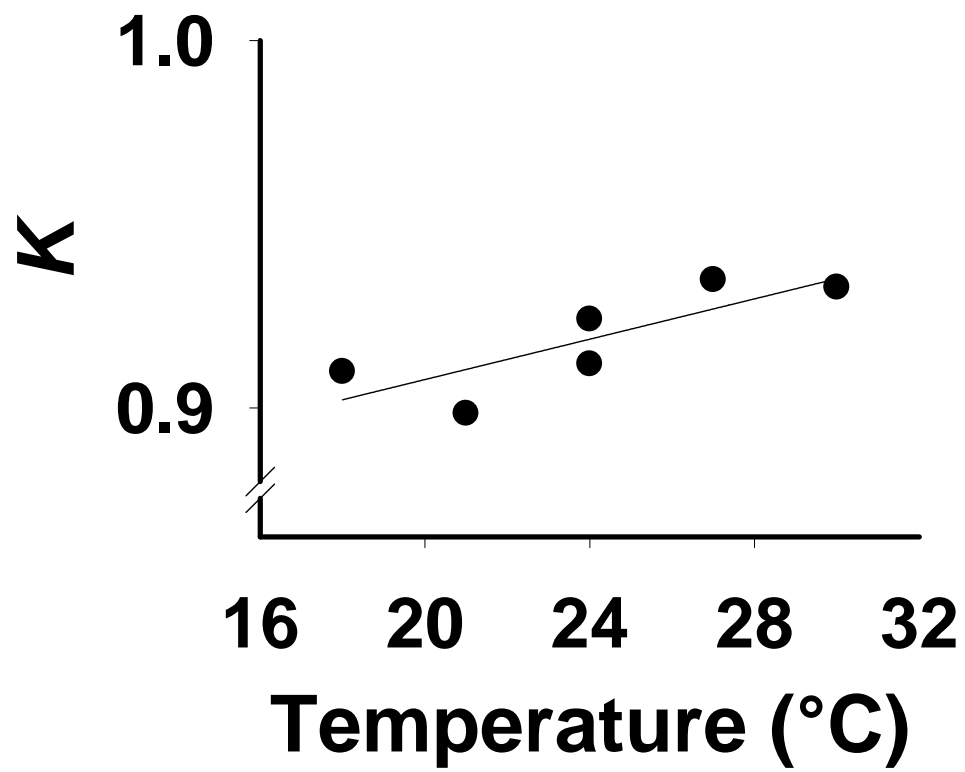
- Jobling, M. 1981. Temperature tolerance and the final preferendum – rapid methods for the assessment of optimum growth temperatures. *Journal of Fish Biology* 19:439 – 455.
- Lee, D.S., C.R. Gilbert, C.H. Holcutt, R.E. Jenkins, D.E. McAllister, J.R. Stauffer, Jr. 1980. Atlas of North American Freshwater Fishes. North Carolina Biological Survey, North Carolina State Museum of Natural History.
- Littrell, B.M., D.J. Lutz-Carillo, T.H. Bonner, and L.T. Fries. 2007. Status of an introgressed Guadalupe bass population in a central Texas stream. *North American Journal of Fisheries Management* 27:785 – 791.
- Neill, W.H. and J.D. Bryan. 1991. Response of fish to temperature and oxygen, and response integration through metabolic scope. Pages 30 – 57 *in* Aquaculture and Water Quality, D.E. Brune and J.R. Tomasso, editors. World Aquaculture Society, Baton Rouge, Louisiana.
- Nikinmaa, M. 2006. Gas Transport. Pages 153-174 *in* The Physiology of Fishes, 3<sup>rd</sup> Edition, D.H. Evans and J.B. Claiborne, editors. CRC Press, Boca Raton, Florida.
- Sullivan, M.L. and J.R. Tomasso. 2010. Limiting and optimal temperatures for the northern Atlantic population of black sea bass. *North American Journal of Aquaculture* 72:258-260.
- Sowa, S.P. and C.F. Rabeni. 1995. Regional evaluation of the relation of habitat to distribution and abundance of smallmouth bass and largemouth bass in Missouri streams. *Transactions of the American Fisheries Society* 124:240 – 251.

- Tidwell, J.H., S.D. Coyle, L. Bright, A. VanArnum, and D. Yasharian. 2003. Effect of water temperature on growth, survival, and biochemical composition of largemouth bass *Micropterus salmoides*. *Journal of the World Aquaculture Society* 34:175 – 183.
- Tomasso, J.R., and G.J. Carmichael. 1986. Acute toxicity of ammonia, nitrite and nitrate to Guadalupe Bass. *Bulletin of Environmental Contamination & Toxicology* 36:866-870.
- Warren, M.L., Jr., B.M. Burr, S.J. Walsh, H.L. Bart, Jr., R.C. Cashner, D.A. Etnier, B.J. Freeman, B.R. Kuhajda, R.L. Mayden, H.W. Robison, S.T. Ross, and W.C. Starnes. 2000. Diversity, distribution, and conservation status of the native freshwater fishes of the southeastern united states. *Fisheries* 25:7 – 31.
- Whitledge, G.W., C. Rabeni, G. Anne, and S. Sowa. 2006. Riparian shading and groundwater enhance growth potential for smallmouth bass in Ozark streams. *Ecological Applications* 16: 1461 –1473.
- Whitmore, D.H. 1983. Introgressive hybridization of smallmouth bass (*Micropterus dolomieu*) and Guadalupe bass (*Micropterus treculii*). *Copeia* 1983:672 – 679.

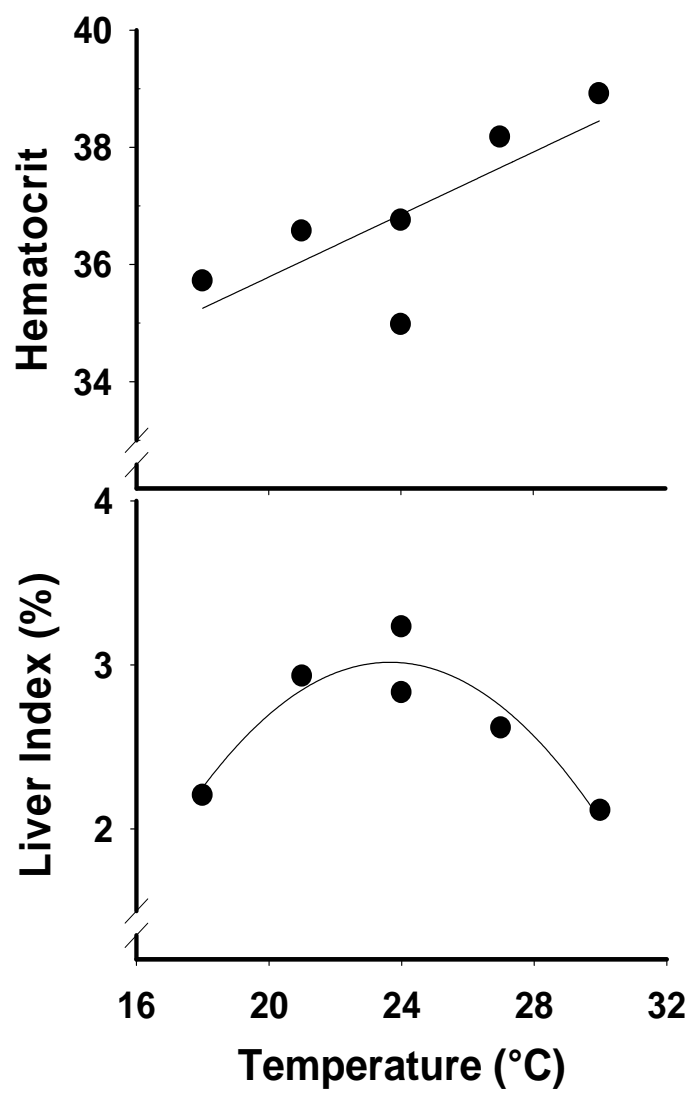
## Figures



**Figure 4.1**-The effect of temperature on growth (total length in panel a; wet weight in panel b) of Guadalupe bass (*Micropterus treculli*) after six weeks of exposure to experimental temperatures.



**Figure 4.2-**The effect of temperature on body condition ( $K$ ) of Guadalupe bass (*Micropterus treculii*) after six weeks of exposure to experimental temperatures.



**Figure 4.3-**The effect of temperature on hematocrit (panel a) and liver index (panel b) in Guadalupe bass (*Micropterus treculli*) after six weeks of exposure to experimental temperatures.

## CHAPTER 5

### SUMMARY AND FUTURE DIRECTIONS

The consumption of terrestrial arthropods by fishes is frequent across taxa but generally at low levels. Groups of fishes that are going to be most likely to utilize terrestrial food items are stream-dwelling trout (Salmonidae), minnows (Cyprinidae, especially the genera *Cyprinella* and *Notropis*), and topminnows (Fundulidae). Additionally, I discovered fish jaw morphology is a factor as well. Fishes with more terminal jaws are more likely to consume terrestrial food items but fishes with inferior jaw configurations still consumed some terrestrial food items. While there are patterns, the consumption of terrestrial food items by fishes can be highly variable. For example, one diet account of a certain minnow species will indicate a low level of terrestrial food consumption while another will indicate terrestrial food consumption is quite high, up to 40% by abundance. Therefore, much of this foraging upon terrestrial arthropods might be context dependent habitat factors such as riparian condition and in-stream habitat conditions that might regulate how well fishes can access terrestrial prey items. In regulated streams, this might be very important when considering water conservation efforts as in-stream habitat heterogeneity is not only related to associations with species and communities but also helps keep trophic interactions in-tact. While I discovered terrestrial and aquatic arthropods do not appear to differ in their nutritional quality with respect to molar C:N values, terrestrial food availability is still very likely important for



generalist stream-dwelling minnows in terms of complimenting their energy intake.

Terrestrial habitat components can be important for stream fishes beyond providing a food resource. Riparian canopy shading can mitigate extremely hot summer temperatures and since the Llano River where the Texas state fish, the Guadalupe Bass inhabits are sparsely shaded by riparian vegetation; the work on their physiological responses to temperature will help guide managers in management actions on Llano River. The water temperatures during the summer in the Llano River can approach or exceed the point at which Guadalupe Bass are outside of their metabolic scope ( $>28^{\circ}\text{C}$ ) so riparian management might be an important factor in recovery efforts for this species.

Future directions based on the results of this dissertation should be an assessment of terrestrial consumption by fishes on a global scale; testing whether or not there are other groups of fishes that frequently consume terrestrial food items that do not inhabit the political boundaries of the United States. Additionally, I would like to continue to investigate whether or not terrestrial food consumption by fishes is related to availability along latitudinal gradients as well as patterns related to different ecoregions that can reflect vegetation community differences and variation in annual primary productivity. For the question of in-stream habitat and consumption of terrestrial food items by fishes, an experimental approach would be useful where mesohabitats could be blocked off with cages, fishes would be stocked into the cages, and they would be presented with different treatments of terrestrial and aquatic food availability. Observations and counts would be made on how many of each prey item are consumed. A more thorough examination of potential differences between the food quality between terrestrial and aquatic food items would also be useful. This study would at a minimum incorporate phosphorous

concentration and potentially also include analyses of trace minerals important to fishes such as magnesium and calcium. Along with this study, a more detailed understanding for the digestibility and energy content of different food items for fishes is needed but with a focus on insects and the bioenergetics and nutritional requirements of small-bodied, insectivorous fishes such as those in the family Cyprinidae. For Guadalupe Bass conservation efforts within the Llano River, more detailed analyses of riparian vegetation and the influence of canopy shading are needed. I provide some baseline physiological responses of temperature for this species but a stronger link to ecology is required truly understand the potential importance of temperature mitigation for this species.

## **VITA**

Mario L. Sullivan earned his B.S. in fisheries biology from Colorado State University in 2005 and M.S. in biology in 2008 from Western Kentucky University. Mr. Sullivan was admitted into the aquatic resources doctoral program at Texas State University-San Marcos in 2008 and received funding from Texas EcoLab as well as a fellowship from the National Science Foundation's GK-12 Flowing Waters Program.

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