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Effects of Drought the Lipid Content in Fundulus Catenatus

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EFFECTS OF DROUGHT THE LIPID CONTENT IN *FUNDULUS CATENATUS*

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ABSTRACT

During severe drought, small streams often dry to small isolated pools with subsequent high densities of fishes. Under such conditions, fish may be stressed by high temperatures, low food availability, and lack of refugia from predators. I was interested in whether fish restricted to isolated pools burn more calories than they take in, resulting in a loss of storage lipids (high energy molecules) in their bodies. During the 2007 drought, I collected *Fundulus catenatus* (northern studfish) from small stream communities in the Drake's Creek watershed in south central Kentucky. I examined storage lipid content by processing each fish through a series of petroleum ether washes and calculated the % storage lipid in each fish's body. The average % storage lipid of fish in isolated pools was decrease than that from free flowing streams. I found that fish from small isolated pools in the headwaters of the creek had higher % storage lipid content than those fish living in free-flowing sections of the creek downstream.

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Introduction

Evolution can and will take place in a population where genetic variation exists. The evolutionary processes may be sped up when the population is isolated and a stress is pressing the species to compete. Competition will intensify where resources are minimal, which could be an area created due to adverse environmental conditions or stress. An example of this is when drought forces stream fish into isolated pools, where they are forced to compete for the limited resources present. The lack of water means that a large number of fish of all types are trying to survive in isolated pool, where the resources present may not even fit the needs for the specific species present. Along with this the individual species that get pooled together may not even have the resources they need for maintenance and not just for food, or might depend on another species to exist. When there are two organisms that share a relationship that is required for their individual survival it is called a symbiotic relationship. An example of this relationship is seen in large bodied fish that have a suckerfish, which serves as their main means of cleaning their skin surface. In return, the suckerfish is receiving protection, due to being attached to a larger bodied fish opposed to swimming in the water column. During a drought, individuals involved in a symbiotic relationship can be geographically separated from their symbiont, under these conditions they will not thrive regardless of the resource levels.

All of the variables that go into determining the survival of individuals once they are separated into isolated pools are accounted for in the evolutionary mechanism of natural selection. Natural selection can be defined as the increase in favorable hereditary traits in successive generations and a decline of unfavorable traits due to the differential in reproduction of the genotype, which is the genetic make up of the cell and its specific alleles (Gillespie, 2004). Drought may make individuals that have larger body masses or mature reproductive organs have a better chance of surviving in stressed conditions due to the presence of storage lipids. In a low resource environment many individuals in the population will not be able to allow energy to go towards gonad development; therefore, individuals that are already sexually mature will not have to deal with the added stress of gonad development. Also the maturity of the fish means that all the energy can be allotted to the soma, or cells of the body not including the reproductive organs, which will help survival. Lastly the fish that have a high body mass are more likely to have storage lipids present and therefore can survive more readily when the environmental stresses onset.

The individuals in the population that are better able to allot energy to soma and gonad development will have a greater chance of reproducing, which will lead to their genes being passed on to the next generation. Another variable that may impact the survival rate of a certain species in comparison to another present in the same isolated pool is the type of resource available and if it can be utilized by the species present. For example a top minnow may eat a variety of microorganisms and insects depending on its size, but a stoneroller only eats a certain type of algae. Therefore if the pooling is too warm or cold, the algae may not develop, or if the larger fish that release feces pollute the pool or the pH is not ideal, resource development for stonerollers will not occur.

The evolutionary mechanism called genetic drift, which occurs when a random event affect the gene make up of the population and over time will lead to a change in gene frequencies causing microevolution to occur in the given population. In small populations, these random changes to the genes in a population have a larger impact on the gene frequencies and may lead to the loss of genetic diversity in the population. The few survivors that reproduce are the only genes represented for the given species in the total population. This could also carry through the entire population of the pool and not just one species. The smallmouth bass and creek chub adults may depend on the small fish like stonerollers as a food resource and a lack of their food source, like the elimination of stonerollers due to lack of algae, will lead to a change in the entire make up of the isolated population; therefore the lack of one variable can cause a dramatic change in a population's genetic make up and genetic drift will occur due to only the genes of surviving organisms being represented.

Not only the presence of resources in the population can affect the survival rate of the fish, but the actual physical make up of the pool can lead to population changes. For example, some fish may depend on rock to hide them from being prey for a larger species; therefore, in a rockless pool the larger fish will thrive due to completely consuming the small species of fish. Other fish may depend of the presence of physical structures to allow their food resource to thrive, like a flat rock for algae to grow on in the case of stonerollers. The stoneroller has a specialized mouth that is ideal for the scraping of algae off the surface of rocks. Mechanisms such as these will alter the chance of survival if their sources are eliminated.

There are stages to water loss during a drought and in return there is variation in population isolation. In a phase one drought water levels drop below the consistent level but flow continues in the form of shallower pools that connect lager bodies, which could stay at a constant for the whole duration of the stress or may proceed on to phase two. In phase two drought, the flow of small shallow riffles is gone and isolation of pools begins to occur. If the drought persists surface flow along all riffles ceases and the pools become completely isolated, which is the beginning of phase three of a drought. In phase three there is complete isolation of pools and the community will behave like a shallow pond under intense predation. If a constant loss of water volume continues the drought will begin to enter the final phase. When a drought enters into phase four, all water has evaporated and all species are dead and the stream bed is now a terrestrial environment. (Matthews,1998)

Drought does not only omit water volume but also reduces the surface area of the environment, due to shallow areas being evaporated first. For example in a stream setting the dry and high temperatures that occur during a drought will cause the shallow, maybe rock or rapid areas, to dry up first, which would lessen the flow of the stream system. This is a slow and constant process that will occur at a higher rate at the upstream site most of the time. The water will flow to the lower pools due to them normally being deeper; therefore, there is a higher speciation in these pools. Also with more species being present the lipid percentages are higher in all individual located in the downstream pools in comparison to the upstream pools. There are more resources present in a body of water that is larger along with having a higher surface area for reaction to occur with the terrestrial environment. This becomes imperative when food resources become limited in the water and fish have to utilize other reservoirs of energy like insects. Fish can also feed off of plant material and other organisms, which is more prone to surface in a larger body of water due to terrestrial species using it as a water source.

To be able to compare the difference in evolutionary fitness you must find a solid variable to measure, and in this case this is the percentage of storage lipids present in individual organisms. There are many different types are lipid present in the body, but the only ones of concern to this study are storage lipids or ones being used directly for soma or gonad development along with metabolic energy needed for life functions. So the extraction method must leave all membrane bound lipids intact due to the fact that they are already been devoted to soma or gonad cells and are not free energy. To be able to extract all lipids from the body of the fish without interfering with membrane bound lipid is to use a lipid soluble solvent like petroleum ether. Petroleum ether can extract lipids from a sample, but is not strong enough to remove membrane bound lipids.

To perform an extraction, the fish from the survey must be dry, which means that all the water must be removed. This is accomplished by placing the organisms in a drying oven around sixty degrees Celsius, which will allow the removal of water but will not alter the chemical properties of the fish. The individual must be massed every 24 hours until it is at a constant weight and at this point the weight should be recorded. Then the fish are placed in volume of petroleum ether that is 100 times its dry mass. The fish will stay in the solvent for pproximately 48 hours, and then are placed in another volume of the same size of clean petroleum ether for another 72 hours. This rotation from one petroleum ether volume to another is due to the properties of saturation and equilibrium. The rate that the lipids leave the body of the fish for solvent will lessen to a point where there is an equal number of lipids leaving the fish's body for the solvent as there is lipids going to the fish's body from the solvent, which is the equilibrium state; therefore the entire amount of lipids present can not be extracted in one extraction. So a rotation of the fish to a fresh petroleum ether volume will allow more lipids to leave the fish's body and give a more accurate account of the actual amount of lipids present in the individual. After all of the petroleum ether extractions the fish will be placed back into the drying oven until a constant dry weight is reached. At this point final mass can be compared to the initial weight to calculate the percentage of the initial weight that was attributable to the storage lipids.

A percent is used to display the amount of lipids due to the difference in body size and mass of each fish in the sample. For example a fish with a body length 8.3 cm in a lower pool may have a dry weight of 1.158g before lipid extraction and 1.011g after extract, which yields a lipid percentage of 12.7%, but the mass of the lipids that were present is 0.147g. Another fish with a body length 8.15 cm in the same pool has a initial dry weight of 0.88g and a dry weight of 0.852 g after the lipids are extracted, which gives a lipid percentage of 3.86% and a lipid mass of 0.0342g. Two fish from the same pool may have close to the same length, but to be able to place those in comparison without dealing with the variable of body mass a percentage must be used. The percentage places the lipid amount in a direct relationship to the body mass of the entire organism; therefore fish with completely different sizes can be compared.

When the percentages are placed into categories based on location and a relationship can be distinctly seen between the ratios of lipids present in the lower pools to the upstream pools. The downstream pool had larger body masses and also had higher lipid percentages in comparison to upstream pools where the body masses are also smaller. The difference found between the two populations can be directly linked to the environmental stresses that drought causes, by lowering the resources available. Due to the lack of flow of the stream leaving less area of water with the same amount of organisms with the same demand that the full stream could provide. So the downstream populations are larger but still have a larger environment and do not have to compete as much for resources and have the ability to have more storage or free lipids to convent to soma or gonad development along with the management of body function.

When water levels are lowered and so is the surface area, the water temperature will tend to increase and may not be idea for the inhabitance. Along with the water temperature change there is also the increase of perturbation in a shallower pool. Some professionals would argue that the change in body mass from the upstream populations to the downstream populations could be due to predators eliminating larger fish from the shallower pools, which on average are the upstream pools. When reaching the high temperatures and perturbation, the fish are also living in a low oxygen environment. Surface dwelling fish are the most successful species during these types of conditions due to being acclimated for shallow/surface interactions. Fundulus do quiet well in this type of environment as long as they can cope with the sunfish, bluegill, or bass that might consume then for survival. The problem for these fish arises when they are competing for a long period of time for resources and that is why it is key to study the least effected individual like the Fundulus species (Matthews, 1998).

When the water levels drop due to the high temperature, that can be increased to five degrees Celsius during the day in comparison to the evening temperature which occurs at the surface and not in the lower water column in the downstream pooling can affect the dispersal of fish in the water column. When the flow is completely eliminated the oxygenation of the water is completely dependent on photosynthesis, which is only occurring in the higher water column where floating algae is present (Matthews, 1998). Now the fish are no longer dispersed due to natural patterns, they might inhabit the lower water column to avoid predators and higher water temperatures, but need to rise to the upper water column due to the lack of oxygen in the lower water column.

Over time if environmental stresses occur at a steady rate such as successive drought, there will be an change in the stream community. The larger species of fish that could not survive in the small pools due to predators and resources will be eliminated from the population make up over time and small species will succeed like *Fundulus*. This is due to the smaller resources needs and that they can exists and even thrive in the upper water column and are small enough to not be preyed on heavily. Looking into the survival mechanisms of this smaller species due to them begin able to adapt the best to a drought stricken environment is important because they will have the longest survival rate along as the drought does not reach phase four, where there is complete desiccation of the stream. So studying the *Fundulus* will allow the observer to see changes all the way to the final phases of a drought.

When analyzing an individual it is important to eliminate all variable except for the one the researcher is focusing on. In this case all of the attention is turned to the storage lipid levels. If you are looking at the individual organism and sampling at random then the specimens will be a good representation of the population. Therefore perturbation and water temperatures/water levels will affect all members of the population the same, which allows the storage ability of each fish to be analyzed with no other variable acting on the study. By focusing on the level of storage lipids and body mass in general conclusions on the values of each fishes energy budget can be attained, which will lead to understand the population in the pools. There will be fish on the lower and higher ends of the energy storage production spectrum leading to an average, which will be used in comparison between upstream and downstream pools.

The energetics of an organism can be described by the equation $C = P + R + E$ presented in the book entitled <u>Fish Energetics: New Perspectives</u>. (Callow and Tytler,

1985) Where (C) is ingested food energy that is equal to the sum of (P), the production of tissue, plus (R), energy used for metabolic processes, and (E), energy in the form of waste to yield a tangible ratio of energy allotment in an organism. This equation is a simple display of the conservation of energy that has to occur in all living systems including fish. So energy that is taken into the body will be dispersed in the areas of metabolism, physical activity, growth, and excretion. When a stress is added to the system the fish may shift the normal pattern of energy dispersal to a pattern that allows the maximum fitness level. To be considered evolutionarily fit an organism must develop the ability to reproduce and the offspring are able to survive along with the parental generation. (Ashton et al., 2004) In the stressed environment individual will go against the natural mechanism to reproduce and will shift energy toward growth and metabolism, which means the conventional pattern of life development is altered. So in this case the fish that survive in the stressed environment are by definition considered more evolutionarily fit then the individuals that do not survive. This is where the discrepancy between actual population mechanics and evolutionary intervals is present and important to note. Therefore in this type of study fitness must be looked at as a present generation trend or individuals that are affected by the drought, and not as a comparison between past and future generations.

When the stress causes competition for resources a fish will allot all energy to fuel their metabolism and will not develop reproductive capabilities. For example, the energy available is directly linked to the ability to produce eggs in female fish. Callow and Tytler (2004) develop a rationality of food consumption and egg production that is predicted by using experimental values of body mass, food intake levels, and eggs produced for each individual of the population and an average was the result. On average the larger the body mass, the higher the fecundity, the ability to reproduce, of the individual which means that reproduction does have a direct relationship to energy intake because body mass growth depends on available resources. During mating season, typically between four and seven days, females are capable of spawning several times. The amount of eggs produced per spawn is a direct function of body size, which can be seen in the equation $n = aL^b$, where (n) is the rate of fecundity per spawning, (L) is the body length, and (a and b) are constants. Therefore if the body length doses not reach a certain point the fish will not invest energy into the gonades; or if energy is invested the reproductive fitness of the female is directly linked to the body length to produce a high fecundity (Callow and Tytler, 2004).

To be able to even develp mature ovarian capabilities needed for spawning the ovary must increase from 8% to 20-30% of the total body weight. (Callow and Tytler, 2004) This proves that the reproductive capabilities are fully depended on energy intake, which is limited do to the present stress and lowering of resource levels. As a rule of existence the fish that do not reach their levels needed for reproduction, or in this specific example energy needed to produce eggs, the gonads will begin to atrophy in a developed adult or will not produce in a maturing fish. The fragility of this mechanism of the living body is key to understanding the mechanics of environmental stresses. Being able to monitor and measure the body length and reproductive rates along with offspring numbers will allow conclusions to be draw between a population experiencing drought, food shortage, and one in normal state conditions.

The ability to develop secondary sexual characteristics like coloration and physical morphologies such as external sex organs are also directly linked to the energy budget. Forming of secondary structure is not as energy costing as mandatory reproductive features, but it is still important in the large game of reproduction. For example, male fish may have a bright coloration to attract a female, but if the energy is not present to develop this coloration then the male may struggle in receiving attention. This energy into secondary characteristics can also be seen in males that morph their kidneys into a mucus producing organ to glue the nest together. (Callow and Tytler, 2004) It was experimentally determined that males with a low mucus production and less viable sperm had smaller kidneys, which were most likely due to the lack of energy to convert and enlarge the kidney during mating season.

Energy in reproduction budget can not be calculated in a direct sense, but can be predicted due to life history studies. The principle of tradeoff between the ability to reproduce now versus the ability to reproduce in the future is a notion that many debate upon (Duggan et al., 1983). This is a concept based in life history studies about certain species of fish and how they can adapt to the environmental changes by choosing to reproduce, which is evolutionarily sound in most stressed situations. This choice is key in the success of progeny compared to success of the community of species. For example, if a stream population is under the stress of drought and the resources are depleting the female may wait till the next mating season to spawn. This occurs because the population has a better chance of surviving with individual that have already achieved mature body mass, due to the fact that fecundity in females is based on body size. Also, if there are not enough resources the offspring will not be able to reach mature body size and will be competing with the mature members of the population. Therefore a mature female may choose to wait to spawn because of environmental stresses, because her fecundity is higher than that of an immature offspring and will be able to further the population at next spawn more efficiently when resources are plentiful.

This regulatory ability between gonads and soma, which are reproductive cells and body cells respectively, is the main way to see the environmental stress impact. There can be a shift from one type of development to the next and there is a tradeoff between the two types of cells and how energy is budgeted. There are energy expenditures for soma that have to be met before there is any investment into the gonads. This type of budgeting is the same as the females waiting to spawn due to the fact that their offspring will have to accrue a great deal of energy to become mature in a soma and gonad sense; therefore it us more ideal for the mature female to wait and spawn compared to immature offspring struggling to get this mature level to reproduce. The energy need for soma development is extremely more taxing than that of the gonad production, but there are metabolic requirement that also take precedence over reproductive maturity. Some metabolic requirements, like the energy dependent break down of carbohydrates, lipids, and protein create an energy reservoir for locomotion, respiration, and growth. Therefore metabolic processes require a small investment of energy to produce a larger pool of energy that could further the growth and life of the organism.

The energy budget of an affected individual is compared to the stardard energy storage potential of an individual that is not experiences any environmental stress and the deviation is measured. At this point an average is settled on for the given pool and the location of the pools, upstream, middle, or downstream, is placed in a ranking of most to least efected. Thoretically the population under the most stress should have the lowest energy budget. (Eckmann, 2004) This could be seen in their lack of reproduction, body mass, and body length along with the already measured low lipid levels. Therefore the upstream populations are experences more stress and will have shifts in there budgets and this is proven by the lower lipid levels in comparison to the downstream populations. (Guether and Spacie, 2006) The populations that continue to be completely isolated will inturn experience a constant pull away from evolutionary fitness. If the condition is waved and then reinstated like an annual drought you will begin to see genetic dift in the population. Larger species will be present in the downstream populations only, which will allow for variation in life systems. The concept of evolution in these small populations is likely or unlikely depending on what type of ariable you are focusing on. For example evolution cannot apply due to the fact that all individuals are subject to environental stress and the ones that achieve reproductive abilities my not reproduce. If reproduction is not occuring niether is evolution. On the other hand some might reproduce and the generation that carries their genes on allows a shift in the population gene pool, but the gene pool is very small due to the overall lack of reproduction. So if there is only a few individual in new generations that genes of the previous generation are still more prevelent and gene flow or genetic drift dose not occur. In conclusion there are may mechanism involved in the budgeting of energy in fishes that can be adapted to ensure a higher fitness level in a time of stress. Along with this populations can be subject to evolution through gene flow or genetic dift if some individuals in the population are more readily able to endure the stresses that are put in place.

Environmental Background

Samples were collected at three separate sites, two of which were in Buck Creek and the third in Trammel Fork. Trammel Fork is the larger stream that had a substantially larger size, flow, and depth in comparison to Buck Creek. The sample site at Trammel Fork is downstream from the confluence of Buck Creek and Trammel fork. In the Trammel Fork site there was two samples taken, one in the deeper portion and another in a backwater, where there was substantially more *Fundulus*. Buck Creek is a smaller tributary of Trammel Fork Creek. The Claudis Harris Rd. survey site is upstream from the intersection of Buck Creek and Trammel Fork, and is marked by a bridge at the turn of the road where a hog farm is located. The New Buck Creek Rd. site of Buck Creek is further upstream from the Claudis Harris Rd. site and was marked by a bridge crossing.

Field Sampling Procedure

While sampling we used an electrofishing backpack and combed through the sample site. While one individual was wearing the backpack the other was netting the fish. During the sampling we focused on collecting *Fundulus* primarily and concentrated more time sampling in areas where there might be a larger population of *Fundulus*. For example, at the Trammel Fork site 1 there was a wide, deep pool where there was flow and very few Fundulus to be found. At Trammel Fork site 2 we spent time in a backwater and shallow grass area herding the *Fundulus* to be collected.

At each of the sampling locations there where two to three different sitesmeasured. At each of the independent site I took measurements of stream length, width, and depth. The length was measured at the point where it was the longest, and the width was taken along three transects; one in the middle and then one at each end of the pool. Along each of the three transects I measured depth at every meter going across the complete width of the pool. The depths were averaged at a given recorded in the table below, along with the maximum and minimum depth.

Environmental Data

Trammel Fork					
Survey Site	Length (m)	Average Width (m)	Average Depth (cm)	Maximum Depth (cm)	Minimum Depth (cm)
Site 1	84.05	7.67	20.5	43	2

Site 2	102.07	18.03	21.05	44	1
Claudis Harris Rd. (Buck Creek)					
Survey Site	Length (m)	Average Width (m)	Average Depth (cm)	Maximum Depth (cm)	Minimum Depth (cm)
Site 1	14.23	2.45	7.30	12	4
Site 2	22.44	6.06	12.06	27	2
Site 3	41	7.69	10.55	30	1
New Buck Creek Rd. (Buck Creek)					
Survey Site	Length (m)	Average Width (m)	Average Depth (cm)	Maximum Depth (cm)	Minimum Depth (cm)
Site 1	30	2.60	6.33	25	6
Site 2	34.34	6.33	4.56	11	0
Site 3	23.40	6.26	12.82	35	1

The Trammel Fork site 1 had an area of backwater on one extremity of the pool and then on the other after a small riffle there was a grassy area where a great deal more *Fundulus* were present. We started on the side with the grassy area and combed down to the backwater on the other end of the pool. At the Trammel Fork site 2 the water was really deep next to the bedrock riffle on one side and the other side of the pool lead to backwater, where their were some *Fundulus*. Claudis Harris Rd. site 1 had a large fallen tree trunk between the second and third width measurement points. There was a great deal of Fundulus that were captured next to this fallen tree trunk. At the Claudis Harris Rd. site 2 there was a large tree that was rooted in the water where we electrofished for a little bit longer due to the large population of *Fundulus* present. In site 3 of Claudis Harris Rd. there was a separation of pools by a bed of rock between the second and third width points. New Buck Creek Rd. site 1 was very shallow on one side and site 2 was very shallow as you move from one side to the other. The site 3 of New Buck Creek Rd. was around a bridge where the water was very deep, but away from the bridge was the sampling area, where there was a bedrock.

After collection, we prepared the specimens for storage. The fish were placed in plastic bags according to survey site and then frozen. As the lipid extraction was occurring they were defrosted and analyzed.

Lipid Analysis Procedure

The fish were frozen until ready to be analyzed for lipid content in plastic bags separated by sampling site; therefore they were only thawed right before drying. A random group of *Fundulus*, 8 to 17 specimens per site, were dried in a drying oven at 60 degrees Celsius for 48 hours. At this point the fish were individually massed and then massed again at 72 hours to make sure a constant dry weight was recorded. Along with the dry weight being recorded the length in centimeters was also recorded. The *Fundulus* were then placed in glass jars with enough petroleum ether to submerge the complete surface of the fish. The specimens were left in this wash for 48 hours and then placed into a second wash of fresh petroleum ether for an additional 72 hours. After the second wash most storage lipids should be extracted from the fish, while leaving the membranous lipids intact. The fish were removed from the petroleum ether and placed back in the drying oven for 48 hours and a final dry weight is recorded. The difference of the initial dry mass and the final dry mass is equivalent to the mass of the storage lipids extracted. The differences of body mass of individuals are taken into consideration as a variable, so the calculation of storage lipid percentages of total body mass was used for comparison. So the difference between the initial dry weight and the final dry weight is divided by the initial dry weight and multiplied by 100 to equal body mass percent of storage lipid present.

The use of petroleum ether for the extract is key to the mission of the experiment due to the fact that the storage lipids can be removed with a non-polar solvent or just slightly polar solvent. Membrane-associated lipids take a more polar solvent to be extracted due to the electrostatic forces and hydrogen bonding of lipids in the phospholipids bilayer.

Analyses

All analyses were performed using Statistica (version 7.0). To determine if there was any influence of body size on storage lipids, I used a regression of percent lipid content on total fish length. Based on the results, I restricted further analyses to fish greater than 6 cm.

To determine if there were differences between the sites (pools) within each sampling location, I used an analysis of variance (ANOVA) or a t-test. Based on the results of those tests, I pooled the fishes from all the sites within each sampling location. I used a one-way ANOVA to determine if there was a difference in the percent lipid content among the fish from the three sampling locations.

Results

A total of 81 *Fundulus catenatus* were collected and processed for lipid content (table 1). A regression analysis on all of the fish collected indicated that small fish had a significantly higher percentage of storage lipids ($F=22.2$, $P<0.0001$). To eliminate any effects of size on the amount of lipid in the body, I eliminated all individuals under 6 cm from the dataset. After eliminating small fish, there was no relationship between total length and percent lipid content ($F= 1.38$, $P = 0.243$).

Table 1. Total length, initial dry weight, final dry weight, and % lipid content for 81 *Fundulus catenatus* collected from sites in Trammel Creek, and its tributary, Buck Creek, in south-central Kentucky. Sites were sampled during August and September, 2007, when the region was experiencing a drought.

	Trammel Fork		
length (cm)	initial dry weight	final dry weight	%lipid content
8.51	1.0413	1.01992	2.0532
10.3	2.32716	2.26931	2.48586
3.7	0.15912	0.15052	5.40472
11.5	2.39042	2.3454	1.88
9.7	1.14813	1.12317	2.173969
9.4	1.335	1.30753	2.057677
12.35	3.87285	3.73083	3.667066
8.2	1.05047	1.01959	2.939636
3.8	0.09519	0.08994	6.10711

Buck Creek Claudis Harris Rd. Site 2

length (cm)	initial dry weight	final dry weight	%lipid content
7.75	1.37122	1.33856	2.3818
3.85	0.11202	0.10493	6.3292
8	1.05365	1.01295	3.8627
7.75	0.86324	0.82882	3.9873
6.9	0.55302	0.5341	3.4212
7.8	0.76875	0.74957	2.4949
7.4	0.62818	0.61638	1.8784
4.2	0.17685	0.16246	8.1368
8.3	0.85184	0.83479	2.0015
6.9	0.55995	0.54721	2.2752
7.1	0.70906	0.66809	5.7781

Buck Creek Claudis Harris Rd. Site 3

length (cm)	initial dry weight	final dry weight	%lipid content
8.5	1.25753	1.22	2.9844
5.8	0.36947	0.365	1.2098
6.8	0.54699	0.531	3.10608
6.6	0.49851	0.48	3.7131
5.3	0.28	0.271	3.5714
6.4	0.54345	0.53	2.4749
10.9	3.05654	2.941	3.8128
8.9	1.49816	1.45	3.2146
6.9	0.63504	0.61	3.94305
9.2	1.07895	1.0601	1.7563
8.4	1.07453	1.05	2.28285
8.7	1.22117	1.1911	2.45901
6.7	0.51564	0.5002	3.03312
6.1	0.422	0.4201	0.4739
3.5	0.10397	0.0901	13.4365
6	0.43282	0.42	2.96197
6.4	0.48443	0.4702	2.97875

Buck Creek New Buck Rd. Site 1

length	initial dry	final dry	%lipid content

(cm)	weight	weight	
10.35	1.86137	1.78	4.371511306
8.8	1.2311	1.19	3.338477784
7.3	0.77774	0.71	8.709851621
8.1	0.96972	0.93	4.096027719
6.8	0.68314	0.65	4.851128612
7.6	0.98118	0.91	7.254530259
9.7	1.65155	1.55	6.148769337
7.4	0.87395	0.81	7.317352251
7.7	0.84665	0.81	4.328825371
9.2	1.07656	1.03	4.324886676
8.7	1.36037	1.26	7.378139771
8.0	0.9156	0.88	3.888160769
3.9	0.10955	0.095	13.28160657

Buck Creek New Buck Rd. Site 2

length (cm)	initial dry weight	final dry weight	%lipid content
8.9	1.2053	1.15	4.58806936
8.2	1.01167	0.97	4.118932063
9.9	1.89666	1.8	5.096327228
7.8	0.62598	0.6	4.150292342
7.2	0.68691	0.66	3.917543783
9.4	1.50972	1.45	3.955700395
9.1	1.30019	1.25	3.860205047
8.5	1.3227	1.23	7.008391926
8.4	1.2928	1.24	4.084158416
3.2	0.07214	0.0701	2.827834766
7.6	0.7878	0.76	3.52881442
7.0	0.64115	0.61	4.858457459
7.2	0.66865	0.63	5.780303597
4.0	0.15086	0.14	7.198727297
3.5	0.08914	0.083	6.888041283
3.0	0.07888	0.0698	11.51115619

Buck Creek New Buck Rd. Site 3

length	initial dry	final dry	%lipid content

(cm)	weight	weight	
8.9	1.07068	1.03	3.799454552
8.2	1.01879	0.98	3.807457867
8.4	1.16935	1.13	3.365117373
7.4	0.93332	0.84	9.998714267
3.8	0.09718	0.08	17.67853468
10.5	2.19831	2.08	5.381861521
5.2	0.2534	0.24	5.288082084
6.6	0.54611	0.52	4.781088059
8.1	0.95485	0.92	3.649787925
7.2	0.79309	0.72	9.215851921
6.9	0.7936	0.74	6.754032258
6.4	0.51501	0.47	8.739636124
7.5	0.92809	0.86	7.336572962
6.7	0.45892	0.44	4.122722915
6.3	0.61006	0.59	3.288201161

There was no significant difference among the fish in sites within three sampling locations. I used a t-test to compare the lipid content of the fishes in Buck Creek at the two sites within the Claudis Harris location ($t = 0.709$, $df = 21$, $P = 0.48$). There was also no difference in the lipid content of the fish in the three sites at the New Buck Creek Road location along Buck Creek ($F = 1.314$, $df = 2,34$, $P = 0.28$). Only 7 fish were collected from the two sites within Trammel Creek. Based on these results, we pooled fish from all sites within each sampling location for further analyses. There was significantly higher lipid content in the fish from the New Buck Creek Road location than in the other two locations (Figure 1; $F = 21.53$, $df = 2, 64$, $P < 0.0001$).

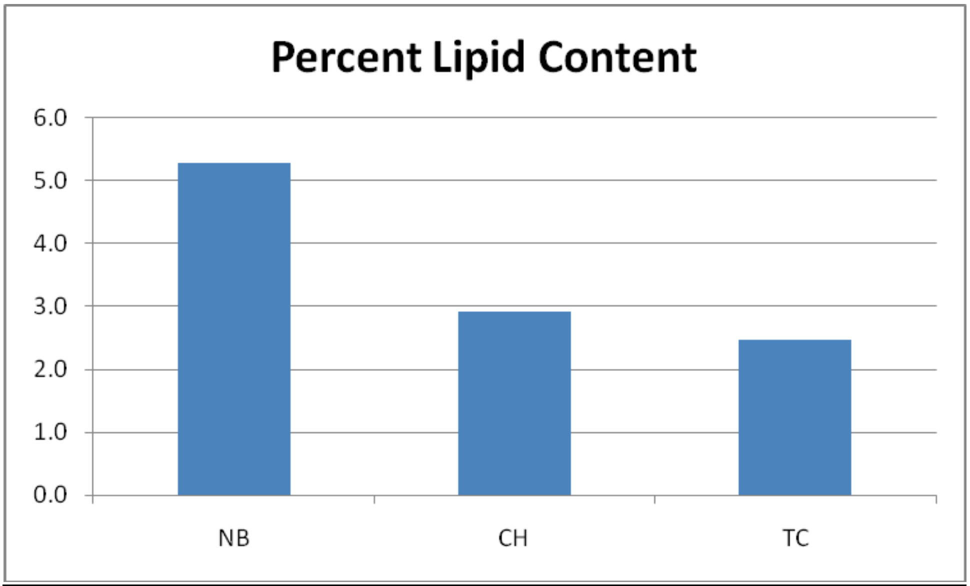

Figure 1. Mean percent lipid content for *Fundulus catenatus* from three sites in the Trammel Creek drainage, southcentral Kentucky. Location NB was at Buck Creek at the New Buck Creek Road bridge, CH was at Buck Creek at the Claudis Harris Road Bridge; and Trammel Creek was located just downstream from the confluence of Buck Creek and Trammel Creek. Sites were sampled during August and September, 2007, when the region was experiencing a drought.

Discussion

The upper most region of Buck Creek contained the highest lipid content which was the opposite of the expected outcome. We expected the upper most region of Buck Creek (New Buck Creek Rd.) to have the lowest lipid content due to the fact that there was less depth to the stream and there was no flow between pools. At the onset of the study it was hypothesized that upstream communities are under greater stress during droughts when environmental conditions become harsh compared to downstream communities. Some of the variables that would lead to the upstream community having a lower lipid content are: higher predation due to low water levels leading to less protection; competition for limited resources; with a shallower depth the temperature of the pool will rise and lead to higher metabolism and possibly additional stresses such as a change in pH or a change in food supply.

Since the results differed from what we expected we can now speculate on potential reasons why we got these results. For example, fish at downstream sites may have spawned so they lost more energy, whereas the upstream fish put off spawning because of the bad conditions. This could occur because when resources are limited the individual may not reach the lipid level to actually meet reproductive maturity or metabolically afford to allot energy to reproduce. *Fundulus catenatus* typically spawn between May and August (Pflieger, 1997) so fish collected for this study would have been post-spawn. If the upstream community was under enough stress to cause the resources to be highly limited, many fish may not be able to reach the lipid content to reproduce or choose not to reproduce because they would have to compete with their own offspring for the resources to survive. Therefore, choosing not to spawn and leaving them with a higher storage lipid concentration in comparison to the downstream community that would have just spawned and have a lower storage lipid concentration.

Another speculation of why the upstream community has a higher storage lipid content in comparison to the downstream site is that the upstream sites may have lost predators so fish could forage better. The elimination of a predator would allow the population to flourish at a higher level than one that still has a stress due to possible predation. Even though fish in the upstream environment are more vulnerable because of lower water depths and segregation of habitat, they are not at risk without predators. In this situation, the upstream community could obtain resources without the stress of a predator, leaving them with an opportunity to have a higher lipid content than those of the downstream sites where predatory fishes could still exist.

Another speculation to why the upstream community had higher lipid content is maybe due to the lack of competitors in their environment. In there is reduced competition then the chance of survival and having a higher lipid content is great than in those environments where competition exist. The competitors in the upstream community could have been under greater stress due to the drought or were eliminated. *Fundulus catenatus* in the downstream community will have a more difficult time foraging for resources in comparison to the upstream population. In conclusion, we found higher lipid content in *Fundulus catenatus* living in small shallow isolated pools compared to those in free-flowing downstream habitats. Futher studies on the effects of drought on stream fishes should incorporate analyses of community composition to gage possible effects of competition and predation.

Works Cited

Ashton, P., S. Harris, and A. Lowe. 2004. Ecological Genetics: Design, Analysis, and Application. Oxford: Blackwell Publishing.

Callow, P. and P. Tytler. 1985. Fish Energetic: New Perspectives. London: Croom Helm.

Duggan, R.T., T.J. Pitcher, and J.C. Rankin. 1983. Control Processes in Fish Physiology. New York: John Wiley & Sons.

Eckmann, R. 2004. "Overwinter Changes in Mass and Lipid Content of *Perca fluviatilis* and Gymnocephalus cernuus." Journal of Fish Biology 65(6):1498-1511.

Gillespie, J. H. 2004. Population Genetics: A Concise Guide. Baltimore: Johns Hopkins University Press.

Guenther, C.B. and A. Spacie. 2006. "Changes in Fish Assemblage Structure Upstream of Impoundments within the Upper Wabash River Basin, Indiana." Transactions of the American Fisheries Society 135(3): 570-583.

Matthews, W. J. 1998. Patterns in Freshwater Fish Ecology. London: Chapman & Hall.

Pflieger, W. J. 1997. The Fishes of Missouri. Missouri Department of Conservation, Jefferson City, Missouri.