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Experimental analysis of metabolic adaptation of *Cottus carolinae* in
response to photoperiod and food availability

Linda L. Roman
Southern Illinois University, Carbondale

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Undergraduate Honors Thesis

Thesis Advisor: Dr. Brooks M. Burr

Abstract

Epigeal (surface) and hypogean (cave) habitats differ significantly, thereby influencing organisms that inhabit these environments in varying ways. As organisms move from surface to cave environments, they adapt to cave conditions: constant darkness, relatively constant temperatures year-round, low food availability, and high humidity. Fish adjusted to cave life often experience reductions in pigmentation, eye size, and metabolic rate. Metabolism is, in general, influenced by temperature, seasonal changes, photoperiod, and food availability. The objective of this research was to understand the alteration of metabolic rate in laboratory acclimated *Cottus carolinae* (banded sculpin) in response to photoperiod and food availability.

Metabolic rates of *C. carolinae* were measured after acclimation to laboratory aquaria. After initial metabolic measurement, *C. carolinae* were placed into one of four treatments: 1) 24-hours dark, low food availability, 2) 24-hours dark, high food availability, 3) 12-hours light: 12-hours dark, low food availability, and 4) 12-hours light: 12-hours dark, high food availability. After eight weeks of acclimation to experimental treatments, metabolic rates of fish were measured following the same protocol used in initial measurements. Results indicated no statistically significant differences existed in *C. carolinae* as a result of photoperiod, food availability, or the interaction of the two. Also, no significant differences existed between laboratory and field measurements from cave and surface environments. However, many variables were identified that may have influenced fish metabolism in the laboratory. Further study is needed to determine influences of photoperiod and food availability on metabolism of *C. carolinae*.

Introduction

Cave and Epigeal Environments

Cave environments are unique habitats, which often harbor organisms which exhibit unusual characteristics (Poulson, 1963; Huppopp, 1986; Langecker, 2000; Huppopp, 2000; Burr, et al., 2001). Most caves are relatively stable in relation to epigeal (surface) habitats. Poulson (1963) characterizes caves as being better “buffered” than epigeal systems against sudden changes in temperature, water chemistry, and turbidity. Caves exhibit relatively constant temperature and water quality year-round (Huppopp, 1986) rather than abrupt changes due to season, natural disaster, etc. as commonly exists in epigeal habitats. As opposed to changing photoperiods and light availability, constant, complete darkness is one characteristic of cave surroundings, which has been shown to have an influence on such fish characteristics as pigmentation, eye size, pelvic fin length, length of appendages, and metabolism (Langecker, 2000; Burr et al., 2001; Adams, personal communication). Also, food availability is reduced in many cave systems with the primary exception of those that experience flooding or presence of bat guano (Huppopp, 1986; Poulson, 1963). Huppopp (2000) states, “The basic food resource in most caves is organic matter from external sources: wind, percolating rainwater, floodings and streams provide input of dissolved organic matter, micro-organisms, detritus, feces and accidental or dead animals” (p. 159). Also, caves typically have a rather high humidity (approximately 100% in most systems).

Metabolism

Although the individual parameters that affect metabolism are not well understood in caves, we can examine what is known to influence temperate surface fish.

Rather than constant environmental conditions often characteristic of caves (Huppopp, 1986), epigeal (surface) environments exhibit changes in food, temperature, photoperiod, season, and reproductive activity, all of which may influence metabolic rate.

Food, typically, is more readily available in surface habitats than cave systems. Variation in light and daylength are typical characteristics of surface freshwater systems. Photoperiod influences growth and reproduction of primary producers that utilize photosynthesis, thereby affecting other organisms which exploit these beings for growth and consumption. On the other hand, primary production is almost completely absent in caves. Cave organisms, instead, rely primarily on food from external sources (Huppopp, 1986; Huppopp, 2000). Food can exist in caves in different ways, including: 1) low, but continuously entering levels, 2) food washed into the cave during flooding or rains, and 3) food existing in an relatively unlimited amount, but difficult to attain (Huppopp, 2000). Therefore, food intake opportunities experienced by cave organisms may be considerably less than that of surface organisms.

Fish adapt to limited food availability in many ways. Langecker (2000) discusses research showing a trend to pedomorphosis, reduced growth rate, increased lipid storage, and a reduction in metabolic rate in response to low food availability and a stable environment. Also, behavioral changes, including effectiveness in food-finding ability based on somatosensory adaptation, have been observed in amblyopsid fishes in response to food scarcity (Poulson, 1963). Literature review by Huppopp (1986) implies that when insufficient amounts of foods are available, organisms may resist starvation by reducing metabolic rate. Reduction in metabolism may be due to low energy demand (Huppopp, 2000). However, even though routine activity may become low, a reduction in metabolic

rate of pike in response to short term food scarcity has not been observed in research conducted by Diana (1982) or Ince and Thorpe (1976).

Temperature varies in most temperate surface environments usually in response to season and location. Water quality, vegetation growth, metabolism, and reproductive time of fish are just a few factors influenced by variation in temperature on an aquatic ecosystem. On the other hand, in the dark zone of caves, temperature remains relatively stable, varying no more than about 1°C in any one geographic location, and is correlated to the yearly mean temperature (Hackney, Adams, & Martin, 1992). Temperature has been shown to affect the critical swimming speed of certain fish. Increasing temperatures led to increasing swimming speed of white crappie to a maximum temperature before declining (Smiley & Parsons, 1997). In his study on northern pike, Diana (1982) considered effects of temperature on metabolism since "The metabolism of poikilotherms is directly dependent on temperature" (p. 395). He explained the importance of equivalence of laboratory temperatures and field temperatures. In a laboratory examination of metabolism of lake charr and walleye, oxygen consumption as well as swimming speed of juvenile lake charr increased significantly in response to increasing temperature (Beamish, 1990).

Photoperiod may play a significant part in influencing physiology and behavior of some organisms. In their research on white crappie, Smiley and Parsons (1997) found photoperiod significantly affected swimming speed, with highest mean swimming performance occurring at an 8L: 16D photoperiod, as opposed to other experimental photoperiods (24L: 0D, 16L: 8D, 12L: 12D, and 0L: 24D). An 8L:16D photoperiod is congruent with winter photoperiods and Smiley and Parsons (1997) suggest fish may be

compensating for lower temperatures that tend to occur during this season. Kolok (1991) agrees that photoperiod alters critical swimming speed of fish, suggesting that effects of photoperiod may not be constant over varying water temperatures and that performance is dependent upon season. However, continuous darkness has been shown to have significant effects on fish without influence of temperature change (Poulson, 1963; Huppopp, 1987; and Langecker, 2000) . Langecker (2000) noted that circadian rhythms and levels of hormones have been shown to change as a direct result of light. He also indicates that in the absence of photoperiod, fish may experience a reduced metabolic rate as the result of a stable environment. Crayfish acclimated to a 24-hour dark photoperiod exhibited a significantly lower metabolic rate than those who experienced a 12L: 12D photoperiod (Boyd, 1997). Significant metabolic differences were found after only a six week acclimation period, indicating that response to photoperiod occurs in a relatively short time period in some organisms.

Metabolism also seems to be influenced by season. Roberts (1964) concluded metabolic rate of sunfish was dependent on seasonal daylengths and seasonal reproduction. He found *Lepomis gibbosus* exhibited higher metabolic rates at a 9-hr. photoperiod as opposed to a 15-hr. photoperiod, at temperatures above 10°C. Research conducted by Burns (1975) attempted to explain factors influencing changes in respiration of *Lepomis gibbosus*, including temperature, natural day length, and reproductive cycle by comparing results to a previous study of sunfish respiration (Roberts, 1964). He found that, in general, respiration increased with increasing temperatures and that day length had an influence on respiration as well. Metabolic changes may also occur in stream fish based on many seasonally changing factors

including temperature, photoperiod, and other stimuli (Facey & Grossman, 1990). In fact, seasonal effects on respiration have been shown to take place even without temperature change (Facey & Grossman, 1990). Boyd (1997) compared metabolic rate of *C. carolinae* among seasons, finding significant influence of season on metabolism in a cave resurgence system in Arkansas. Temperature during measurements at each season varied by no more than 2°C seasonally, yet oxygen consumption in winter was significantly lower than oxygen consumption during all other seasons. (Figure I). Little variation in oxygen consumption occurred among other seasons.

Gonad development and reproductive cycles also seem to have a strong influence on metabolic rate (Facey & Grossman, 1990; Diana, 1982; Beamish, 1990; Roberts, 1964; Burns, 1975). Some seasonal variation in respiration of stream fishes has been attributed to spawning preparation in that spring spawners tend to show higher metabolic rates in early spring and fall spawners tend to show higher metabolic rates in early fall (Facey and Grossman, 1990). It has also been suggested that the rise in metabolism during reproduction may be attributed to gonadogenesis (Beamish, 1990). In studying the influence of photoperiod on fresh-water sunfish, Roberts (1964) concluded that physiological sensitivity to photoperiod is lost once gonadal development begins and until spawning is complete. Due to the active reproductive season, high metabolic rates have been observed regardless of photoperiod, perhaps due to increased hormones (Burns, 1975).

Objectives

The purpose of this study was to compare cave and epigeal-adapted populations using laboratory acclimation. I wanted to understand the influence of particular cave conditions on metabolism, specifically photoperiod and food availability. Laboratory acclimation was used to isolate effects of photoperiod and food availability. Also, I wanted to understand trends in metabolism associated with cave adaptation. Much is understood about such trends as reductions of pigmentation and eye size, but I wanted to understand changes in metabolism using *C. carolinae* as study organisms. Four objectives were arranged in this study: 1) compare cave and surface populations, 2) determine effect of photoperiod on metabolic rate, 3) determine effect of food availability on metabolism, and 4) examine the interaction of photoperiod and food availability on metabolism.

Study Species

Cottus carolinae (banded sculpin) are benthic dwellers that reside primarily in upland streams of the Mississippi River Basin. Some morphological characteristics of *C. carolinae* include a broad, flattened head with conspicuous spines; large dorsal eyes; lack of a swim bladder; large pectoral fins; and small, closely spaced pelvic fins (Pflieger, 1997). *Cottus carolinae* are primarily nocturnal, “sit-and-wait” predators and opportunistic feeders, commonly feeding on crayfish, insect larvae, and small fish, and even exhibit cannibalism on occasion. Adams (personal communication) suggests that *C. carolinae* commonly enter caves and may move upstream into caves to spawn, perhaps to avoid predation.

Cottus carolinae from caves in Perry County, Missouri exhibit unique morphologies. Burr et al. (2001) reported *C. carolinae* in caves of Perry County often exhibited troglomorphic characteristics including reduced pigmentation, eye size, and pelvic fin rays in response to cave environments. *Cottus carolinae* often enter caves, but the only populations to show cave adaptation exist in Perry County (Burr et al., 2001). Therefore, morphological or physiological attributes exhibited by populations outside Perry County can be compared to those populations within Perry County. *Cottus carolinae* from this area provide a unique opportunity to compare populations from surface and cave habitats. Also, comparisons can be made between *in-situ* metabolic measurements in the cave and acclimation measurements of the laboratory. Observed brain morphology of *C. carolinae* in response to the cave environment may provide information on optic adaptation due to continuous darkness as well (Adams, personal communication).

Methods

Collection

Seventy adult *C. carolinae* were collected from Big Creek, a surface creek located in Union County, Illinois (approximately N37°26', W89°12'). Collection primarily occurred just downstream of a freshwater spring. *Cottus carolinae* were collected in early evening by dip-netting and seining techniques, then placed into 5-gallon buckets and treated with "Stress Coat" (Aquarium Pharmaceuticals) to prevent loss of their slime coat due to stress of handling. A small bubbler was placed into each bucket during transport to the SIUC cold laboratory. Due to difficulty in finding *C. carolinae* of appropriate age and size, more than one collection date was needed. Collections took place on 5 January 2000, 5 July 2000, and 10 July 2000.

Forty adult sculpin were also collected from Tom Moore Cave in Perry County, Missouri. Collections took place on 23 June 2000 and 14 July 2000.

Acclimation

After collection, buckets were placed in a 15°C room for a period of at least 24 hours to acclimate *C. carolinae* to the constant temperature of the room. After approximately 24 hours, fish were placed into a 3-tier system of twenty 10-gallon aquaria (Figure II). Water moved through the system using a submersible pump and all tanks shared the same water. Temperature remained constant throughout the room (15°C) using a cooling unit and fan system attached to the room. Fish from Big Creek were placed into the upper tier of the system, approximately 39.8 cm from the fluorescent lighting and initially acclimated in a 12 hour-dark: 12 hour-light photoperiod. Fish from Tom Moore Cave were placed 24-hour dark treatments in the system. Sculpin were

trained, beginning with diets of shrimp and bloodworms, to feed on a diet of krill and fed a small piece of krill everyday during the acclimation period. Clay or plastic covers were placed within tanks to provide refuge for fish. The acclimation period was a minimum of four weeks before initial metabolic rates were recorded.

During acclimation, many specimens experienced mortality due to common aquaria illnesses and cannibalism. Although only occasional in specimens from Big Creek, occurrence of such illnesses was quite common in *C. carolinae* from Tom Moore Cave. When these fish were placed in experimental treatments in the laboratory, they quickly developed illnesses such as fungus and ich and many mortalities occurred. Currently, Kelly Smith is conducting a research study at Southern Illinois University exploring gut content of sculpin from various locations. She has found that *C. carolinae* from Tom Moore Cave have high loads of parasites in their stomachs. This may have influenced response of *C. carolinae* to experimental treatments. It was decided that experimentation on cave sculpin would no longer take place so that no more fish from this cave would be collected at this time.

Metabolism Methods

Initial metabolic measurements were taken following methods of Boyd (1997), beginning on 24 September 2000. Fish were dip-netted from their tanks and placed directly into 950 ml glass jars, which were used as habituation chambers. Chambers were covered with 1 mm² mesh material, then placed directly under a stream of water within the treatment tank of the fish for a minimum of twelve hours before measurements were taken. Habituation chambers were also used as testing chambers. Chambers were

removed from treatment tanks and placed within a clay enclosure to minimize effects of data recorded.

To measure metabolism, a rubber stopper was placed into the top of the chamber, creating an air-tight seal. A stirrer and a dissolved oxygen probe were inserted through two holes in the rubber stopper. Another hole was cut into the stopper to allow a small rubber piece to be inserted into the stopper, forming a tight seal and preventing air bubbles from being trapped within the chamber. The dissolved oxygen probe was connected to an Orion 810 DO meter. Measurements were recorded by hand from the meter at approximately 5-minute intervals and temperature was taken at the end of each trial. Also, fish movement was recorded at 5-minute intervals using a 0-3 scale (Refer to Table I). Weights and lengths were recorded immediately following metabolic testing.

Cottus carolinae were randomly placed into one of four treatments upon completion of initial metabolic testing, on 6 February 2001 (Figure III). Treatments included the following: 1) 24-hours dark, low food availability, 2) 24-hours dark, high food availability, 3) 12-hours dark: 12-hours light, low food availability, and 4) 12-hours dark: 12-hours light, high food availability. Dark treatments were completely enclosed using black plastic. Light provided for 12:12 photoperiods, came from three long fluorescent lights suspended from the ceiling of the room. Emission of light was measured using a Light meter. Light intensity of 2.16 lumens/m² was emitted in all dark treatments, while light intensity of 1922.4 lumens/m² was emitted in the light treatments of the top tier and light intensity of 355.32 lumens/m² was emitted in the light treatments of the middle tier. High food treatments included feeding a small piece of krill everyday, while low food treatments were fed a small piece of krill two days per week. Fish in dark

food treatments were fed with the aid of a dim red light so as they were not exposed to fluorescent lighting. Temperature remained constant (ca. 15°C) throughout the entire acclimation. Water quality was measured regularly to assure fish health.

Final metabolic measurements were taken between 9 April and 17 April 2001, eight weeks from initial measurements. This time length was based on research conducted by Boyd (1997), who found significant metabolic differences in ringed crayfish based on photoperiod after an acclimation period of six weeks. Fish were taken from tanks in a random order and final testing was performed in the exact same fashion as the aforementioned initial testing. Weights and lengths were taken to compare fish conditions. Each fish received a caudal fin clip before it was returned to its treatment tank.

Data analysis

Effects of treatments on metabolism were analyzed using analysis of covariance (ANCOVA) with mass as the covariate. Oxygen consumption and mass were log-adjusted. Tests of homogeneity of variance, homogeneity of slopes, and normality were made to assure that assumptions of ANCOVA were met with each of these variables. To prevent pseudoreplication, average metabolic rates of separate tanks were used as data points rather than individual fish since each tank should exhibit identical environmental conditions, including water velocity, temperature, and light intensity.

Fish condition was analyzed using ANCOVA, with length as the covariate. Condition comparisons provided analysis of plumpness and helped in ascertaining effect of food treatments.

Results

Water temperature remained constant throughout experimental testing. Temperature was recorded during metabolic measurement, but differed by no more than 2°C. Also, fish movement and activity was minimal during measurement. Oxygen consumption of 26 *C. caroliniae* was measured initially. After approximately eight weeks of acclimation to laboratory conditions, oxygen consumption for each of 51 specimens was measured: 10 fish from 24 hour dark/ high food treatment; 15 fish from 24 hour dark/ low food treatment; 14 fish from 12 hour light: 12 hour dark/ high food treatment; and 12 fish from 12 hour light: 12 hour dark/ low food treatment. Analysis of covariance was used to analyze metabolic rate with mass as the covariate. Mass was found to significantly affect metabolic rate (Analysis of Variance: $F_{1,38} = 9.4145$, $\underline{P} = 0.0040$). Assumptions of ANCOVA were tested for metabolic rate (Test of Parallelism: $F_{4,34} = 0.0877$, $\underline{P} = 0.9856$; Levene's Test for Homogeneity of Variances: $F_{4,39} = 1.1514$, $\underline{P} = 0.3470$), and mass (Levene's Test for Homogeneity of Variances: $F_{4,39} = 0.6384$, $\underline{P} = 0.6382$). No significant differences occurred among metabolic rates of initially measured fish and fish in experimental treatments or among metabolic rates of fish in each treatment ($F_{4,38} = 1.6226$, $\underline{P} = 0.1885$) (Figure IV). However, a trend for higher metabolism in high food treatments compared to low food treatments is noticeable in Figure IV.

Fish condition was also analyzed using Analysis of Variance (ANOVA). Assumptions of ANCOVA were tested for mass (Test of Parallelism: $F_{4,33} = 0.6632$, $\underline{P} = 0.6220$; Levene's Test for Homogeneity of Variances: $F_{4,38} = 0.7181$, $\underline{P} = 0.5848$), and length (Levene's Test for Homogeneity of Variances: $F_{4,38} = 0.1310$, $\underline{P} = 0.9701$). There

was a significant effect of length on mass (Analysis of Variance: $F_{1,37} = 318.333$, $P < 0.001$), therefore, ANOVA was used. No significant differences were found to exist between initial and final measurements or among treatments ($F_{4,37} = 1.674$, $P = 0.1768$) (Figure V). However, a trend in higher fish condition can be observed among the high food treatments indicated in Figure V.

Table II gives a detailed description of the results acquired from each treatment. Samples size is the number of individuals within each treatment and varies little among treatments. Mean mass refers to the average weight of fish in each treatment and mean length refers to the average length of fish within each treatment. Condition is the relative “fatness” of the fish expressed as log mass (g) adjusted. It is often used as an indicator of fish health. A measurement of two standard errors is also provided. Finally, log adjusted mean metabolic rate is indicated on the table, measured in mg O₂/hr. Two standard errors are provided for metabolic rate as well.

ANCOVA was used to compare metabolic measurements begun in the morning (9:00am-2:00pm) to those begun in the afternoon (2:01pm-8:30pm), as well. No significant differences existed between measurement time ($F_{1,46} = 0.1290$, $P = 0.7211$).

Laboratory metabolic rates were compared to *in situ* metabolic rates of *C. carolinae* currently being researched by Adams (personal communication). No statistically significant differences were noted between field and laboratory measurements. Also, no significant differences were found between measurements from the surface environment and laboratory treatments or the cave environment and laboratory treatments.

Also important to this research is the observation of gravid females. A sample of *C. carolinae* were moved from acclimation aquaria, which were on the top tier of the water system, where a 12-hr light: 12-hr dark photoperiod was present, to a treatment aquaria where a 24-hr dark photoperiod was present. Some females in the dark treatments gained excess weight and began to develop eggs within the first 2 or 3 weeks after movement. Soon afterward, some fish in dark treatment tanks with gravid females began developing enlarged, darkened heads, a potential sign of reproductively-ready males (Adams, personal communication).

Discussion

There were no significant differences in metabolic rate of *C. caroliniae* between 12-hr light:12-hr dark and 24-hr dark photoperiods. This may imply that factors other than photoperiod play important roles in alteration of metabolic rate and, therefore, physiological adaptation of *C. caroliniae* to cave life. However, it is also possible that physiological alteration may not occur in *C. caroliniae* after a period of only eight weeks, as opposed to metabolic change that occurred in ringed crayfish after only six weeks of exposure to altered photoperiod (Boyd 1997).

No significant differences existed in metabolic rate of *C. caroliniae* as a result of high- or low-food diets, indicating that food consumption alone is not enough to alter respiration of *C. caroliniae* used in this study. In fact, no significant differences occurred in fish condition after treatment. It was expected that fish fed a high-food diet would experience better condition, essentially be fatter, than those fed a low-food diet, but results indicated no such difference. One possibility for similar fish conditions is low-food diets were not low enough or high-food diets not high enough. Previous research resulting in decreased fish condition as a result of low food availability often involved starvation of fish (Diana, 1982; Ince & Thorpe, 1976; Huppopp, 2000). However, when amount of food in low-food treatments was decreased in this study, cannibalism took place within aquaria and when amount of food fed to fish in high-food treatments was attempted to be increased, fish refused to take more food. Krill was used as fish diet due to its ease of feeding and high nutrient content, making it a good source of weight gain and health. Therefore, any other diet used would most likely have yielded similar results.

However, without significant changes in muscle protein or water, weight loss occurred in northern pike after only 1 month of starvation, which has been attributed to depletion of lipid and glycogen (Ince & Thorpe, 1974). Although weight loss occurred, the rate of weight loss during 1 or 3 months starvation did not change, indicating that northern pike were unable to influence their metabolic rate over time in response to starvation. Diana (1982) attributes reduction in metabolic rates of fish to such variables as fish activity, diurnal changes in oxygen consumption, and hormone levels rather than food scarcity, as he did not observe significant reductions in metabolic rate of northern pike as a result of starvation.

Although statistically significant differences were not observed in metabolic rate or condition, two important trends existed within the data. A trend toward lower metabolic rate occurred in the treatments with low food availability, indicating that food availability may be a factor in altering metabolic rates, as commonly supported in the literature (Huppopp, 1986; Huppopp, 2000). This trend indicates that perhaps the sample size used in this research was too small. Previous research indicates that interpretations of metabolic rates could not be made when sample sizes were too small (Huppopp, 1980). A trend toward better condition of fish subjected to high food available diets also existed, demonstrating that the two food treatments may have been different enough to have dissimilar effects on metabolism, if, in fact, food availability influences metabolism in these particular fish.

Metabolism of *C. caroliniae* used in this research was not significantly effected by the interaction of photoperiod and food availability, indicating that other variables or combinations thereof may have effected oxygen consumption, but that the combination of

these two did not in this study. *Cottus carolinae* entering caves of Perry County, Missouri may experience a reduction in metabolic rate (Adams, personal communication), but this reduction may rely on other factors or combinations other than leniency toward a 24-hr dark photoperiod and low food availability, two factors often exhibited by caves. Metabolic adaptation is also influenced by such environmental conditions as temperature, season, and reproductive activity as well. In fact, metabolic rate has been shown to be reliant on temperature (Diana, 1982; Beamish, 1990). Also, seasonal variables, including daylengths, temperature, photoperiod, food availability, and reproduction, have been shown to strongly affect fish metabolism (Roberts, 1964; Burns, 1975; Facey & Grossman, 1990; Boyd, 1997).

However, *C. carolinae* used in this study were from Big Creek, a creek in Union County, Illinois. Many reported adaptations of *C. carolinae* due to cave life occurred in Perry County, Missouri, from streams associated with cave resurgence and caves, themselves. As unique populations of *C. carolinae* exist in this area, they may possess a specific gene pool, allowing them to more readily adapt to cave conditions. Perhaps there is something unique in the plasticity of metabolic adaptation in populations in Perry County.

The presence of gravid females may have strongly influenced results of this research. Roberts (1964) notes reduction in physiological sensitivity of fish to photoperiod regimes once gonadal development begins and until spawning has completed. Gravid females may have stopped responding to experimental photoperiods soon after being placed into treatments. Also, during spawning, fish tend to show higher metabolic rates (Facey & Grossman, 1990). However, Boyd (1997) attributes

food availability have been attributed to such physiological changes without the influence of reproductive season (Boyd, 1997). Little is actually known about spawning of *C. carolinae*. Seasons of reproduction have been deduced for specific areas (Jenkins & Burkhead, 1994) and clutches of eggs have been found, but gravid females have rarely been observed and reports of spawning do not appear to be reliable. Although egg clutches have seldom been observed in epigeal waters, *C. carolinae* larvae and clutches have been found within some caves (Herbert, 1994; Adams, personal communication), indicating that adults may move into caves to spawn. Thereby, the sudden gravidity of females in this study upon movement to a treatment lacking photoperiod is very interesting and may imply that constant darkness is a cue for reproductive activity in *C. carolinae*. As *C. carolinae* are common cave inhabitants, observed gravidity may also indicate that, when possible, *C. carolinae* will move into caves to spawn.

Stress may have also influenced metabolic rate of *C. carolinae* in the laboratory. Transfer of fish from the wild into the laboratory often is a source of stress. Stress Coat was placed into the water while transferring fish during this research, but that may not have been enough. Also, throughout the experiment, common aquaria illness, including that caused by *Ichthyophthirius multifiliis* occurred, possibly increasing level of stress experienced by fish. Fish were treated with fungicides and fish medications. However, it was common for fish to spread their illnesses as all tanks shared the same water. In addition, moving fish from treatment aquaria to habituation/testing chambers may have increased excitement level.

Future Direction

Further study is needed to understand plasticity of metabolism of *C. carolinae* and analyze variables existing in this research. Currently, fish used in this project remain in treatment aquaria and have been tested again to assure that length of acclimation to treatments is not an issue in this study. Also, examinations of oxygen consumption during various times of day are being conducted to understand whether metabolism changes diurnally.

In the future, it may be beneficial to conduct the aforementioned research procedure using *C. carolinae* juveniles. Although it may not be possible to accurately measure fish condition with juveniles, as they experience growth regardless of food availability, these fish would not be reproductively viable. Therefore, reproductive activity would not affect metabolic rate as it may have affected gravid females in this research. Metabolic rate may also be a more plastic in juveniles as opposed to adults. Furthermore, influence of environmental conditions may be enhanced in juveniles as they are quickly growing and changing.

Cave and epigeal *C. carolinae* could better be compared by conducting this study using *C. carolinae* collected from cave habitats and appearing to possess adaptive traits commonly possessed by cave organisms. One would first need to study causes of illnesses exhibited by cave *C. carolinae* such as those observed in this research, then find means of treating those illnesses. Using fish collected from caves with constant darkness and low food availability, one could make inferences based on how those fish respond to

a 12-hour light: 12-hour dark photoperiod and/or the sudden high availability of food. Plasticity of metabolic rate of cave-adapted *C. carolinae* could also be better analyzed in this way.

It may also be interesting to observe metabolic change exhibited by *C. carolinae* from surface and cave habitats if this research involved the presence of both types of fish in each treatment aquarium. Cave and epigeal organisms often demonstrate different behavioral patterns (Poulson, 1963; Huppopp, 1987). It may be interesting to note whether the two types of fish influence one another, especially with concern to food-finding ability under certain photoperiods.

Conclusion

Caves are unique ecosystems and typically more constant environments than surface habitats. Most caves exhibit constant darkness, relatively constant temperature year-round, low food availability, and relatively high humidity. Changes in metabolism seem to rely on such variables as food availability, temperature, photoperiod, season, and reproductive effort, some of which are very static in the cave environment, possibly resulting in reduced metabolic rate within cave organisms. Although reductions in metabolism often take place in *Cottus carolinae* as a result of cave adaptation, *C. carolinae* did not experience changes in metabolic rate due to photoperiod, food availability, or the interaction of the two during this study. However, many possible variants could have altered results of this experimentation, including gravid females, genetics, and stress. Further study is needed and is ongoing at Southern Illinois University, Carbondale to analyze plasticity of metabolic adaptation of *C. carolinae*.

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Table 1. Behavior ethogram of movement during metabolic testing of *Cottus carolinae* in the laboratory.

Ethogram numeric assignment	Behavioral indication
0	fish is non-moving, breathing normally
1	fish is non-moving, breathing is heavy
2	fish is moving or turning slightly
3	fish is exhibiting rapid movement or swimming frantically

Table II. *Cottus carolinae* adjusted metabolic rate and fish conditions among treatments in the laboratory.

Treatment	Sample Size	Mean Mass (g)	Mean Length (mm)	Condition (± 2 SE)	Log Adjusted Mean Metabolic Rate (mg O ₂ /hr) (± 2 SE)
Initial measurement	26	14.95	89.062	-1.822 (0.016)	0.214 (0.437)
24 hour dark/high food	10	17.086	91.386	-1.813 (0.056)	0.717 (0.372)
24 hour dark/ low food	15	15.010	91.045	-1.841 (0.042)	0.1667 (0.261)
12 hour light: 12 hour dark/high food	14	17.977	94.531	-1.777 (0.052)	0.182 (0.378)
12 hour light:12 hour dark/low food	12	14.557	88.514	-1.839 (0.038)	0.097 (0.294)

Figure 1. Seasonal metabolic rates (log mg O₂ hr⁻¹) for *Cottus carolinae* in Logan Cave in Benton County, Arkansas. Adapted from Boyd (1997). Error bars represent 2SE and numbers over error bars represent sample size.

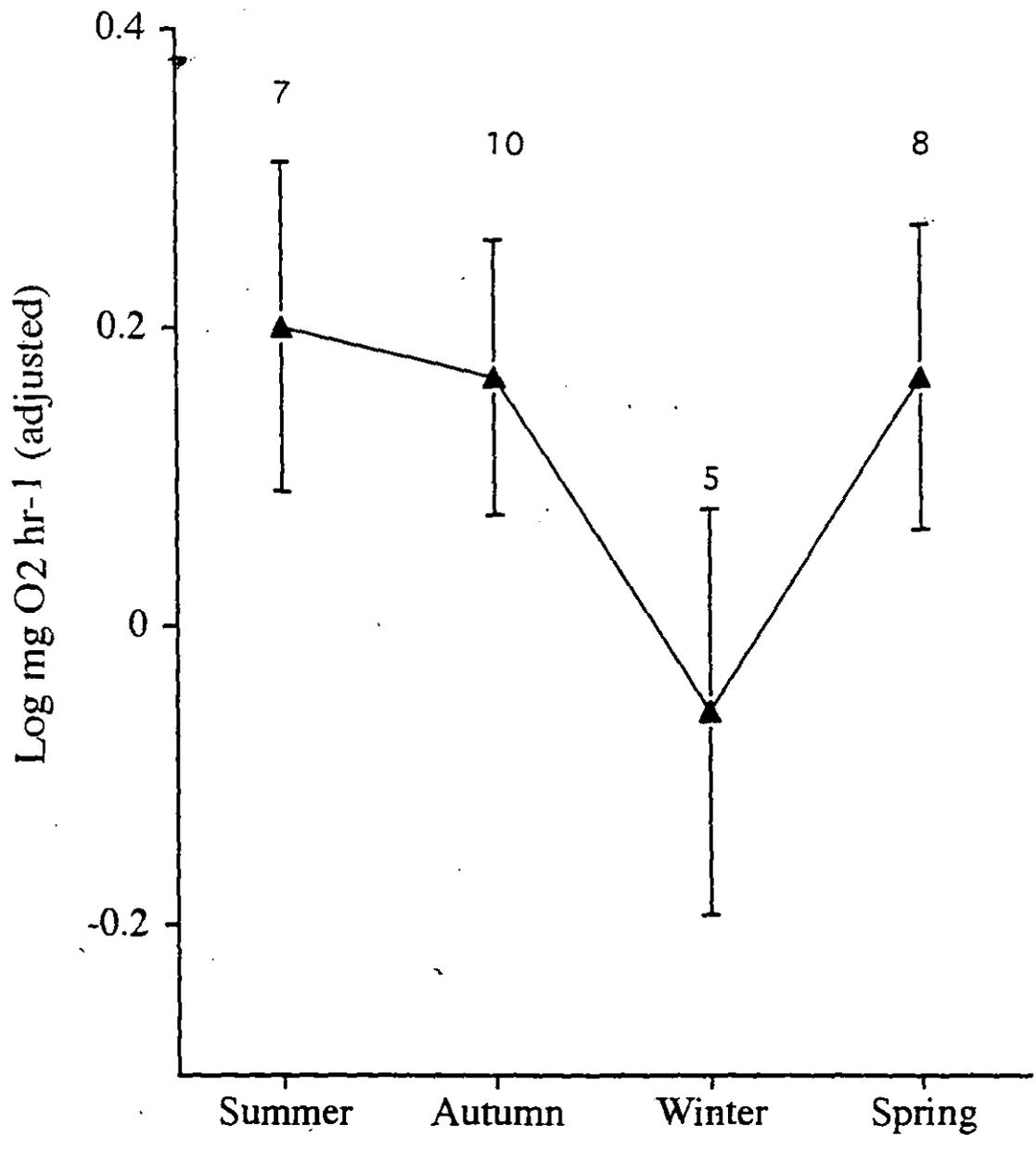


Figure II. Cold-room laboratory in Life Science II Building at Southern Illinois University, Carbondale. This room was kept at 15 ° C. Light treatments consisted of 12-hour light: 12-hour dark photoperiod, controlled by overhead fluorescent lighting.

Figure III. Detailed layout of treatment aquaria in cold-room laboratory. Four treatments are indicated: 1) 24-hours dark; low food availability, 2) 24-hours dark; high food availability, 3) 12-hours light: 12-hours dark; low food availability, and 4) 12-hours light: 12-hours dark; high food availability.

	Tank 1L 12-hr. light: 12-hr. dark High Food Availability	Tank 2L 12-hr. light: 12-hr. dark High Food Availability	Tank 3L 12-hr. light: 12-hr. dark High Food Availability	Tank 4L 12-hr. light: 12-hr. dark Low Food Availability	Tank 5L 12-hr. light: 12-hr. dark Low Food Availability	Tank 6L 12-hr. light: 12-hr. dark Low Food Availability
Tank 1D 24-hr. dark High Food Availability	Tank 2D 24-hr. dark High Food Availability	Tank 3D 24-hr. dark Low Food Availability	Tank 4D 24-hr. dark Low Food Availability	Tank 7L 12-hr. light: 12-hr. dark High Food Availability	Tank 8L 12-hr. light: 12-hr. dark Low Food Availability	Tank 9L 12-hr. light: 12-hr. dark Low Food Availability
Tank 5D 24-hr. dark High Food Availability	Tank 6D 24-hr. dark High Food Availability	Tank 7D 24-hr. dark Low Food Availability	Tank 8D 24-hr. dark Low Food Availability	Tank 9D 24-hr. dark Low Food Availability	Tank 10D 24-hr. dark Low Food Availability	Tank 11D 24-hr. dark High Food Availability

Figure IV. Metabolic rates (Log mg O₂/hr) of *Cottus carolinae* among treatments and compared to initial testing. Error bars represent 2 SE and numbers above bars represent sample size.

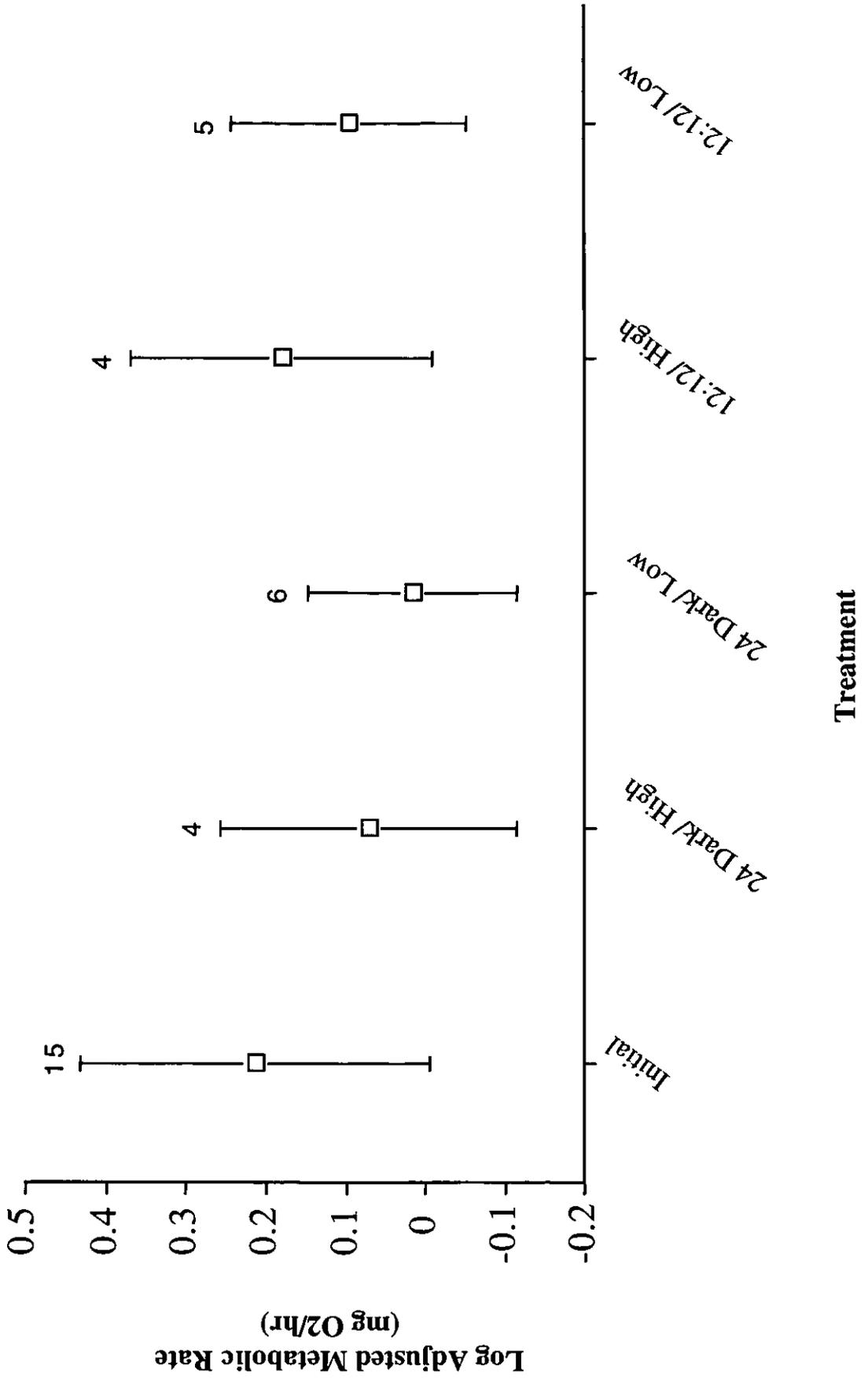


Figure V. Condition (Log Mass (g) Adjusted) for *Cottus carolinae* among laboratory treatments and compared to initial measurement. Error bars represent 2 SE and numbers above bars represent sample size.

