2016

A Comparative Analysis of the Metabolism and Energetics of Darters (Percidae)

Emma Kirsten Kist
levinnielsen@marshall.edu

A COMPARATIVE ANALYSIS OF THE METABOLISM AND ENERGETICS OF DARTERS (PERCIDAE)

A thesis submitted to
the Graduate College of
Marshall University
In partial fulfillment of
the requirements for the degree of
Master of Science
in
Biological Sciences
by
Emma Kirsten Kist
Approved by
Dr. Jeff Kovatch, Committee Chairperson
Dr. Brian Antonsen
Dr. Anne Axel

Marshall University
May 2016

APPROVAL OF THESIS

We, the faculty supervising the work of Emma Kirsten Kist, affirm that the thesis, *A Comparative Analysis of the Metabolism and Energetics of Darters (Percidae)*, meets the high academic standards for original scholarship and creative work established by the Department of Biological Sciences and the College of Science. This work also conforms to the editorial standards of our discipline and the Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

Dr. Jeff Kovatch, Department of Biological Sciences — Committee Chairperson — Date

4/29/16

Dr. Brian Antonsen, Department of Biological Sciences — Committee Member — Date

29/Apr/16

Dr. Anne Axel, Department of Biological Sciences — Committee Member — Date

Anne C. Axel — 29 April 2016

ACKNOWLEDGMENTS

I would like to express my gratitude to my advisor, Dr. Jeff Kovatch, for all of his support and patience. I would not have made it through this journey without his guidance. I am thankful for the advice and assistance provided by Dr. Brian Antonsen and Dr. Anne Axel. For funding assistance, I thank The Lemotto Smith – John Goodno Fellowship for Aquatic Biology Research and Marshall University Department of Biological Sciences Graduate Assistantship. I would also like to thank my fellow lab mates Thad, Mark, Steve, and Tuesday for equipment training, power tool lessons, project relocation assistance, comic relief, and so much more; without their help, this project would not have been possible. I would like to thank my family, especially my parents, for providing immense support and help throughout this process. Finally, I would like to thank my husband, Thomas, and son, Daniel, for their love and patience. This has been a crazy journey, and without their support, I would never have succeeded.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Ecological niche theory suggests two species cannot live in the same ecological niche and differences should exist among species that appear to cohabitate. Variations in physiology and behavior that potentially enable species coexistence can be reflected in metabolism. This study investigated coexisting darter fishes by measuring the metabolism of greenside (*Etheostoma blennioides*) and variegate (*E. variatum*) darters over 48 h using intermittent-flow respirometry. Activity was analyzed using time-lapse videos. *E. blennioides* mean metabolic rate (154.64 ± SE 52.54 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$; n=14; p=0.0006) was significantly greater than and varied more than *E. variatum's* mean rate (92.51 ± SE 32.70 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$; n=15). *E. blennioides* had consistently elevated activity compared to *E. variatum*, but was not significantly different over time ($F_{1,4}$=3.24, p=0.5173); while *E. variatum's* activity was ($F_{1,4}$=20.50, p=0.0004). The results may suggest that *E. blennioides* is a bolder, more active species than *E. variatum.* Physiological and behavioral differences revealed between *E. blennioides* and *E. variatum* could potentially explain their coexistence. Second, a comparison of published studies on darter metabolism was conducted to elucidate interspecific variation among a greater number of darter species. This search resulted in metabolic data on a total of 11 darter species, which were compared to the current study in three ways (using the 48 h mean rates, the first 4.5 h mean rates, and the middle 24 h mean rates). The results showed interspecific variation in metabolic rates among darters, but also illustrated the importance of developing methods that measure metabolism accurately. Finally, this study helped uncover differences among darters using a physiological approach and will hopefully lead to an increased interest in quantitative darter physiological ecology.

CHAPTER 1

GENERAL INTRODUCTION

The concept of ecological niche was first described in detailed and studied by Grinnell (1928), in which he focused on animal distribution. Over the years, the definitions and ideas involving ecological niche have evolved and been refined. Work by Hutchison (1957) began to shape the idea of ecological niche that is most often today and which focuses not only on the place of the organism in the environment, but also the role it has in the ecosystem. Hutchinson distinguished between fundamental and realized niches and provided a definition of niche that focuses more on the role of the species within its community. Additionally, the competitive exclusion principle developed from niche theory and states that two closely related species cannot coexist in the same ecological niche, and eventually one species will be excluded from the niche (Gause 1932; Hardin 1960). This principle incorporates the distribution of the species, as well as the role each plays in the community. Therefore, it is hypothesized that if two closely related species appear to coexist in the same ecological niche, there are subtle differences that exist, one outcompetes the other, and the species evolve to occupy specialized niches. Though there has been much discussion about defining ecological niche and the competitive exclusion principle (Vandermeer 1972; Pianka 1981; Leibold 1995; Cushing et al. 2004), it continues to be useful background for research focusing on differences among coexisting species (Kuno 1992; Dijkstra et al. 2008; Violle et al. 2011).

As previously mentioned, subtle differences are hypothesized to exist among species that may not initially appear ecologically distinct (Vandermeer 1972). Therefore,

researchers seek out these differences in several areas of their ecology, including niche and/or resource partitioning, animal behavior, and physiology. An excellent example of this is the diverse group of cichlids found in the Great Ancient Lakes of East Africa. Resource partitioning, behavior, and physiology of these fish that exhibit high levels of sympatry among closely related species have been studied by many researchers (Haesler and Seehausen 2005; Dijkstra et al. 2008; Martin and Genner 2009; Takahasi and Koblmuller 2011). These cichlids are of great interest due to the explosive speciation and adaptive radiation that has occurred among this group in Lake Malawi, Tanganyika, and Victoria in particular (Turner et al. 2001). To gain an understanding of how this rapid speciation and adaptive radiation has taken place, various aspects of the cichlid ecology and life history have been investigated. Morphology, phylogeny, and behavior have been the focus of many studies including those targeting female mating preference (Haesler and Seehausen 2005), resource partitioning (Martin and Genner 2009), and phenotypic plasticity of characteristics such as male nuptial coloration and jaw structure (Magalhaes et al. 2009). However, as is the case for other species of non-game fish, there is not an extensive amount of research devoted to differences in specific physiological functions among the cichlids. Dijkstra et al. (2013) and Dijkstra et al. (2016) were interested in determining physiological differences that could have influenced the rapid speciation among the cichlids, specifically targeting metabolism. Dijkstra et al. (2013) found a correlation between agonistic behavior and metabolic differentiation among males of two cichlid species known for aggressive territorial interactions. Dijkstra et al. (2016) studied three sympatric female color morphs of the cichlid *Neochromis omnicaeruleus* and found differences among their metabolic rates

and territorial behavior. This use of behavioral analysis with physiological data can create a clearer picture of differences among these sympatric species, but this technique has not been used for most of the research on smaller fishes. Though this provides some insight into the physiology of cichlids, there has been limited attention placed on determining how physiological differences impact niche overlap and the high levels of sympatry found among these highly studied species.

A physiological approach to understanding differences among closely related sympatric fish can offer information that cannot be as easily obtained by observing and examining morphological features. For a fish, the external anatomy can reveal important information (Moyle and Cech 2004), but studying and incorporating physiology and behavior can provide researchers with a more valuable and reliable understanding of a particular organism's life history and ecology (Dwyer et al. 2014; Eme et al. 2014). Additionally, being able to obtain physiological information becomes increasingly beneficial when an organism is a cryptic species and little information can be easily obtained from field observations.

Metabolic rate, a fundamental biological rate, can help explain and make predictions about variations in basic individual life history traits, as well as more complex ecological patterns (Brown et al. 2004). The Metabolic Theory of Ecology (MTE) (Brown et al. 2004) suggests that metabolic rate sets the pace of life by determining the demands an organism puts on its environment, and can be used to make predictions about survival and mortality, population density, and even interspecific interactions. For this reason, researchers interested in broad ecological or behavioral aspects of an organism sometimes study metabolic rates and bioenergetics models

(Small 1975; Fischer 2000; Garcia et al. 2012). Ecosystem energetics can even be studied, but for a more comprehensive understanding, the major components and organisms of the ecosystem need to be understood. Therefore, for species for which little is known physiologically, are understudied, or are simply difficult to observe naturally, measuring their metabolic rate is a valuable means to start answering more complex ecological questions.

Scientists often pursue the development of general rules or laws predicting metabolism for various groups of organisms (Bennett 1978; West et al. 1997; Clarke and Johnston 1999; White and Seymour 2003). When looking specifically at fish, it is often difficult to obtain a general law because metabolic rate is strongly influenced by many intrinsic and extrinsic factors. Metabolic rate is affected by body size (e.g. Kleiber 1932; Schmidt-Nielsen 1984; West et al. 1997; Urbina and Glover 2013), environmental temperature (Clarke and Johnston 1999), oxygen tension (Ultsch et al. 1978; Dwyer 2014), substrate availability (Fischer 2000), and turbulent flow (Enders et al. 2003). These factors influence metabolic rate differently and must be taken into consideration when comparing rates found in the literature. For example, the relationship of body size and metabolism has been extensively investigated for many organisms (Peters 1983; Schmidt-Nielsen 1984; West et al. 2002), however, as noted by Clarke and Johnston (1999), this relationship is relatively understudied in fish.

Of the studies published on fish metabolic scaling, relatively little is reported for smaller, non-game fish, which can make up large portions of the biomass. For example, in some large/medium rivers, the largest constituent of biomass in the fish community is often a sucker species (Catostomidae) (Cooke et al. 2005) and in some small streams,

89% of the biomass is made up of benthic fishes which is often dominated by darters (Percidae) (Small 1975). Metabolic studies that incorporate other factors such as temperature and oxygen tension also tend to focus on larger fish, such as salmonids (Brett 1972; Cho et al. 1982; Metcalfe et al. 1995; Huang et al. 2013). It has even been shown that scaling laws for fish do not work well in predicting metabolism of cyprinids and darters (Ultsch et al. 1978). Ultsch et al. discovered that reported equations used to predict freshwater fish metabolic rate greatly overestimated the actual metabolism of the darters he investigated. The predicted values were also well above the actual rates reported for many fish in Cyprinodontidae, the minnow family, which is also mostly small fishes. It was suggested by Ultsch et al. that the extreme small size of these fishes makes the use of generated curves inaccurate for predicting their metabolic rate. Clarke and Johnston (1999) reported a more recent scaling equation that could be used to predict metabolism of smaller fishes, but the model was constructed from a large set of studies on teleost fish. Due to the large scope of that model, it may be unlikely that this equation will more accurately predict small, non-game fish metabolism. Therefore, there is a clear need for more laboratory research to be conducted on many smaller species of fish that are overlooked and often have great ecological importance.

Darters (Percidae) are small, perch-like, freshwater fish common in streams throughout eastern United States, though their range extends through all of North America (Figure 1). Percidae is the second-largest family of North American freshwater fishes (Page and Burr 2011), comprising roughly 20% of North American fish diversity (Carlson and Wainwright, 2010) and darters can encompass up to 89% of fish biomass in some systems (Small 1975). There are four genera of darters (*Etheostoma*, *Percina*,

Ammocrypta, and *Crystallaria*) with a total of about 200 species (Table 1). *Etheostoma* is the largest genus of North American fishes (Page 1983).

Figure 1. Male greenside darter (*Etheostoma blennioides*) resting on substrate. This fish is about 7 cm in length. Photo by E. Kist.

Table 1. General information on the four genera of darters in Percidae.

Genus	Number of species*	Note
Etheostoma	151	Some of the most common and colorful freshwater fishes
Percina	46	Generally larger than other darters
Ammocrypta	6	Sand darters, spend time buried in sandy streams
Crystallaria	2	Slender, translucent species

*Estimated from Page and Burr (2011) and recent updates of species lists.

Many species of darters inhabit wide ranges, while others have very limited distributions. Darters often dominate fish assemblages in riffle communities and even make up a large portion of vertebrate biomass (Hlohowskyj and White 1983). Perhaps one of the most interesting facts about darters is that they often exist sympatrically. In some areas of the Kentucky River, coexistence of up to 14 darter species has been observed (Carlson et al. 2009). This high level of sympatry has been investigated focusing on resource partitioning (Wehnes 1973; Fisher and Pearson 1987),

microhabitat use (Chipps et al. 1994; Harding et al. 1998; Weston et al. 2010), temperature selection (Hill and Matthews 1980; Ingersoll and Claussen 1984), and even skull morphology (Carlson and Wainwright 2010); but no large focus has been placed on physiological differences. Physiological, as well as behavioral, differences between two species of darters, *Etheostoma blennioides* (greenside darter) and *E. variatum* (variegate darter), will be the focus of the second chapter of this thesis.

Additionally, despite their high diversity, darters remain relatively understudied in many areas when compared to larger fishes (i.e. bass, salmonids). A large portion of current literature tends to focus on the genetics and phylogeny, (Piller et al. 2008; Switzer et al. 2008; Camak 2012) and updated distributions, statuses, and general life history of darters (Hargrave and Johnson 2003; Driver and Adams 2013; Johnston et al. 2013) with little emphasis on physiology. Darters are of particular interest for genetic research due to the high diversity of this clade of fishes, and the high level of sympatry that often occurs among them (Keck and Near 2009). The polytypic greenside darter (*E. blennioides*) is one species that tends to be of focus for many geneticists (Miller 1968; Haponski and Stepien 2008), with controversial classification of four subspecies (Piller et al. 2008; Stepien and Haponski 2010). Few known studies have been published regarding darter metabolism, and the comparison of these will be the focus of the third chapter of this thesis.

A shift to physiological experiments can begin answering questions about these understudied fish. The high levels of sympatry have been of interest to researchers, but as of yet, no major physiological method has been taken to address this occurrence. Food and resource partitioning, microhabitat selection, and thermal preference have

been investigated for various darter species to address their common coexistence. Kessler and Thorp (1993) suggested that the microhabitat partitioning observed between the spotted (*E. maculatum*) and orange fin (*E. bellum*) darters is linked to their behavioral and morphological differences. By use of underwater snorkeling observations, Stauffer et al. (1996) found that substrate size and water depth and velocity were key factors in the segregation of eleven darter species in the Allegheny River. Behavioral isolation resulting from male visual signals has even been shown to aid sympatry of *E. zonale* and *E. barrenense* (Williams and Mendelson 2010). Temperature selection reveals information about darter physiology and sympatry, because fish tend to select temperatures based upon optimal temperature ranges for physiological processes (Brett 1971). Temperature selection differs between the commonly sympatric *E. flabellare* and *E. nigrum*, correlating with their preferred habitat types (Ingersoll and Claussen 1984). These and various other studies provide evidence that the common coexistence of darters appears to be influenced by many factors, but their physiology is not one that has been studied in great detail. Taking a more physiological approach can reveal how metabolic differences can facilitate sympatry among darters and enhance our knowledge of habitat selection and species coexistence.

Darters are also important to study because they are often used as indicators species and one of the seven metrics (DMS metric: Darter, madtom, sculpin richness) in the Kentucky Index of Biotic Integrity (KIBI) (KDOW 2003). The KIBI evaluates stream health based upon fish communities, and many darters are relatively intolerant and sensitive to pollution, making them useful indicators of water quality. Darters serve

important roles as prey items for larger fishes, such as bass, and host for many species of glochidia, the larval stage of most mussels in Unionidae. The latter point, the use of darters as hosts, illustrates the importance and influence that darters have on benthic biomass. Darters (Percidae), as well as sunfishes (Centrarchidae) and minnows (Cyprinidae), are used heavily as hosts (Strayer 2008); interestingly, closely related mussels tend to use closely related fish species as hosts. For example, mussels in the genus *Epioblasma* tend to use fish in Percidae as hosts. Strayer also emphasis that some mussels are very species specific in terms of host use, and any changes in these fish populations and distributions can greatly impact the mussel populations. Therefore, researching darter ecology and physiology could aide in a more developed understanding of benthic community dynamics.

Studying darter energetics will be even more beneficial with the rising issue of climate change. It is known that aquatic organisms will be impacted by rising temperatures (Throp et al. 1998; Ficke et al. 2007; Hester and Doyle 2011). Climate change is predicted to have greater impacts on smaller streams (the habitat of most darters) than larger rivers due to relationships between the water temperature and air temperature of these streams (Heino et al. 2009). Aquatic ecosystems are also anticipated to be affect by shifts in riparian vegetation, alterations of nutrient loading and cycling, and warmer temperatures affecting fish habitat availability (Meyer et al. 1999). Therefore, how darters respond to climate change will be better understood by gaining insight into their energetics by measuring their metabolism. Thus, this information could aid in the conservation of many rare or threatened species.

Finally, the physiological data gathered for darters could provide important information about how these fish respond to altered flow regimes created by dams. Darters, as well as many other aquatic organisms, are likely impacted by the effects that dams have on the natural flow regime of streams and rivers. The practice of pulsed-flows or “e-flows” (environmental flow) from dams is being investigated in an attempt to return lotic systems to a more natural flow regime (Bednarek and Hart 2005; Richter and Thomas 2007; Bunn et al. 2014). Biotic composition within a river or stream is largely influenced by how the flow shapes the physical habitat (Richter and Thomas 2007). Thus in streams or rivers in which the flow level is maintained without natural fluctuations, selection could occur for species that may have physiological advantages in more constant environments. Understanding energetic requirements for darters could reveal that constant flows created by dams may select for some species over others, impacting darter community composition.

The two species studied in the laboratory metabolic experiments were the greenside (*E. blennioides*) (Figure 1) and variegate (*E. variatum*) darters. Both of these species are commonly found in riffles with fast currents and can even be found in small to medium rivers (Page 1983) in North America. *E. blennioides* has a larger range extending from the Great Lakes basin in New York to parts of the Mississippi River basin in Kansas and Oklahoma (Page and Burr 2011). The range of *E. variatum* is limited to the Ohio River basin from southwest New York to eastern Kentucky and southern Indiana, cohabitating much of the same waters as *E. blennioides* (Page and Burr 2011). Both species feed mostly on insect larvae, which is the case for most darters (Wehnes 1973). The spawning season occurs between April and June for *E.*

blennioides and eggs are laid on green filamentous algae (Miller 1968). *E. variatum* lay their eggs in sand and gravel between April and May (May 1969). *E. blennioides* is the largest *Etheostoma* species, with a maximum total length of 17 cm; *E. variatum* can reach up to 11 cm long (total length) (Page and Burr 2011). Darters are among the most beautiful freshwater fish in North America, especially breeding males. *E. blennioides* breeding males are bright green with dark green bars on the sides and back, and a dark green head and breast. The females maintain a yellow-green appearance year-round (Page 1983). *E. variatum* is closely related to the candy darter (*E. osburni*) which has been considered the most vivid freshwater fish in North America (Page and Burr 2011). *E. variatum* is olive-colored with blue-green bars separated by yellow-orange spaces. They have a light green belly and a blue-tinted breast. The breeding males take on a vivid coloration pattern in which the anal, pelvic, and pectoral fins, and the head and breast become deep blue-black (Page 1983).

In this study, the overall aim was to evaluate the common coexistence of darters by examining interspecific variance of their metabolic rates and energetics. First, the diel cycle of metabolic rate over forty-eight hours for *E. blennioides* and *E. variatum* were determined using an intermittent flow respirometer. The hypothesis that *Etheostoma blennioides* will have a significantly different mass-specific metabolic rate than *E. variatum* was tested. The use of forty-eight hour trials eliminated potential erroneous estimations, as suggested by Steffensen (1989), which exist when metabolic rate is measured only during portions of the day. In addition, activity levels were monitored throughout the metabolic trials with the expectation that change in activity will correlate with change in metabolic rate. Levels of activity were compared between

periods of light and dark, hypothesizing that there will be a difference in activity between the periods with lights on and off. Incorporating behavior analysis with the metabolic experiments is presumed to provide a clearer picture of differences between these species and how they coexist. Secondly, a comparison of published studies on darter metabolic rates and energetics was conducted to elucidate any interspecific variation for a greater number of darter species. The null hypothesis was that there will be no difference in mass-specific metabolic rate among the darter species.

CHAPTER 2

RESPIROMETRY AND BEHAVIORAL EXPERIMENTS

Introduction

The ecological niche theory stems back to the concept that no two ecologically similar species can occupy the same ecological niche (Grinnell 1928), which later came to be known as the competitive exclusion principle (Gause 1932; Hardin 1960). G.E. Hutchison (1957) contributed to this principle the notion of fundamental and realized niches. The fundamental niche can be described as the range of environmental conditions inhabitable by a particular species without competition or predation pressure. Interactions with other organisms (i.e. competition and predation) limits the species to a range of environmental conditions in which it actually lives, defining the realized niche (Moyle and Cech 2004). However, Hutchison (1961) later noted that there is a paradox found in nature that closely related species are often able to coexist and hypothesized that there must be differences among them. Therefore, researchers that study closely related, coexisting species often seek out the subtle ecological differences that are not initially evident (Vandermeer 1972).

Ecological differences among closely related species may be found in differing physiology that is not easy recognizable. Metabolism is an important physiological process that can be used to make some powerful predictions about the ecology of living organisms. Metabolic rate, the rate at which an organism takes up, converts, allocates, and expends energy and materials, governs the demands the organism places on its surroundings (Brown et al. 2004). Therefore, testing for differences in metabolic rate can lead to a better understanding of life histories, population dynamics, and species'

diversity patterns (West et al. 1997). However, estimating the metabolic rate of many organisms, especially aquatic species, can often be difficult. Metabolism has long been shown to be affected by many intrinsic and extrinsic factors, including body size (Kleiber 1932; Urbina and Glover 2013), environmental temperature (Clarke and Johnston 1999), oxygen tension (Ultsch et al. 1978; Dwyer 2014), substrate availability (Fischer 2000), and turbulent flow (Enders et al. 2003). Respiration rate (oxygen consumption) is often used as a measure of metabolic rate for aquatic organisms, including fish, and respirometry experiments need to control for these factors to measure a more accurate metabolic rate.

Aquatic Respiration. There are known problems that can be associated with aquatic respirometry experiments and accepted ways of dealing with experimental and methodological issues (Steffensen 1989; Lighton 2008). Mainly, Steffensen discusses the issues associated with flow-through and closed respirometry systems. Two issues with closed systems are that there can be unwanted accumulation of CO_2 and excretory products and oxygen tension decreases over time, which can cause a change in respiratory behaviors. In a flow-through system, there tends to be a washout effect caused by the sensitivity of oxygen to dilution by CO_2 production resulting in a lag time for the change in oxygen consumption that is measured (Lighton 2008). The solution to these problems is intermittent-flow respirometry, in which respiration rate is measured with a closed system, but periodic flushing of the system occurs to remove wastes and reoxygenate the water. This also allows for longer experiments because depleted oxygen is no longer an issue. It is most common in current literature to see the use of intermittent-flow respirometry.

Diel cycles in fishes are not uncommon for various activities and physiological processes. Sea bass exhibit diel swimming activity patterns (Anras et al. 1997); two Sonoran Desert stream fishes have presented two distinct diel feeding patterns (Fisher et al. 1981); diel oxygen consumption rates have been reported for bluegill (*Lepomis macrochirus*) (Pierce and Wissing 1974), sockeye salmon (*Oncorhynchus nerka*) (Brett and Zala 1975), and California halibut (*Paralichthys californicus*) (Merino et al. 2011). The studies focusing on oxygen consumption patterns incorporated feeding times into the experiments and thus provide different information then experiments with unfed fish. However, these studies on diel cycles can provide a basis for an understanding the diel cycles of physiological processes. These studies also help to illustrate that the duration and timing of the experiments can affect the estimates of metabolic rate. Steffensen (1989) provides an example of an experiment measuring the oxygen consumption of a rainbow trout, *Salmo gairdneri*, over 18 hours. For this experiment, intermittent-flow respirometry was used allowing for a longer experiment that tracked a pattern in the oxygen consumption of the trout. After sufficient acclimation, the rate reached a relatively constant lower rate for most of the night and increased rapidly at sunrise. Steffensen notes that an erroneously high metabolic rate would have been recorded for this fish if flow-through respirometry would have been used, during which acclimation would have occurred over night and measurements taken the following morning. Though the author is commenting on the use of different respirometry methods, this example illustrates an important issue that may be evident in more current studies. Research studies that measure the respiration of an organism during short periods of time or only during the day can miss important information about the diel cycle of

respiration and erroneous estimates can be made (Steffensen 1989). Evans (1984) even pointed out that when estimating annual energy budgets for fish, single-season experiments may provide inadequate information.

Therefore, it is important to be clear what information is being sought out and what measurements are being taken. When trying to determine the metabolic rate of an organism, it can be important to know what metabolic rate is actually being measured. Fry (1947) emphasizes that many researchers attempting to measure standard metabolic rate (SMR), the minimum oxygen consumption of an unfed, but not starving, fish at rest, are often more appropriately measuring the routine metabolic rate (RMR), the rate for an unfed, but not starving, fish with movement of spontaneous swimming with minimal influence from the environment. Active metabolic rate (AMR), the total cost of swimming (SMR plus the energy required for movement), may also be the rate of interest, and can be measured using a swim tunnel (Boisclair and Tang 1993). Roche et al. (2013) was interested in discovering the best method for estimating metabolic rate in coral reef fish. From their experiments, Roche et al. stressed that knowing the ecology and behavior of the organism is important in determining the appropriate methodology to measure accurate metabolic rate. The authors also warned that caution should be taken when comparing estimates of metabolic rate when different methods have been used. As a result, prior to conducting metabolic experiments, it can be essential to determine the time and length of trials, as well as establish which type of metabolic rate is desired and use appropriate methods. These preparations can ensure more valuable and accurate information is being obtained and used for comparisons (Fry 1947; Steffensen 1989).

Behavioral Analysis. In addition to the use of metabolic rate to extrapolate differences among sympatric species and their ecology, behavioral analysis can provide important ecological information. Combining physiological traits with behavioral traits, researchers can begin to better answer questions about organism's life history, ecology, and effects on community composition (Dwyer et al. 2014). Killen et al. (2011) found that routine metabolic rate can influence risk-taking behavior in juvenile sea bass, but the effect is context-dependent. This research suggested that fish with relatively high intrinsic energy demands have an increase in the likelihood of being predated when food is scarce. On the other hand, the way fish behaviorally respond to environmental conditions can invoke metabolic costs (Facey and Grossman 1990; Lankford et al. 2005; Blank et al. 2007). Consequently, though it is often not feasible or practical, understanding differences among species can be better refined with the use of physiological and behavior studies.

Darter Introduction. Darters are a large group of freshwater fish that have excellent potential to study as a group to understand how closely related species can often coexist with high levels of sympatry. Most darters are small, benthic species, living on the substrate, with reduced swim bladders (Page 1983). They inhabit much of the eastern United States where they are often found in riffle communities of small streams. As their name indicates, darter fish are mostly still but when they move it is a very quick, darting motion. The family comprising darters, Percidae, is the second to Cyprinidae (minnows) as the largest family of North American freshwater fishes (Page and Burr 2011). Percidae encompasses roughly 20% of the fish diversity in North America (Carlson and Wainwright, 2010) and in some systems, darters dominate the

composition of benthic fishes which can make up to 89% of the fish biomass (Small 1975). What makes this group of fish fascinating to study is the high levels of sympatry that can often be observed among these fish in many systems. For example, the coexistence of up to 14 darter species has been observed in some areas of the Kentucky River (Carlson et al. 2009). Unfortunately, much of the literature published on darters focuses on general life history and conservation, with no major interest in physiology. Studies aimed at discovering how these species coexist in the similar niches tend to focus on resource partitioning (Wehnes 1973; Fisher and Pearson 1987), microhabitat use (Chipps et al. 1994; Harding et al. 1998; Weston et al. 2010), temperature selection (Hill and Matthews 1980; Ingersol and Claussen 1984), and interests in skull morphology (Carlson and Wainwright 2010), with little effort focused on physiology. Studies of microhabitat use and resource partitioning can have important ties to physiological differences; specifically, what facilitates darters living in different microhabitats or behaviorally partitioning resource items may have important connections to their physiology. Additionally, improved understandings of habitat requirements for declining populations and how they interact with sympatric species can ensure thorough management actions for imperiled darters (Weston et al. 2010). Therefore, there is a great need for a more physiological approach to understanding the high levels of sympatry among darters which can aid in a better understanding of overall darter ecology and improve conservation efforts.

Fish physiological studies tend to focus on large game-species, such as largemouth bass (*Micropterus salmoides*), that are economically important. Small stream fishes, like darters, are, however, ecologically important and necessary to study.

Darters have routinely been used as indicator species of water quality due to their relative intolerance and sensitivity to pollution (KDOW 2003). Many darters are prey items to the economically important species, such as trout and bass. Darters also play an important role in the aquatic ecosystem as many species are hosts for numerous species of freshwater mussel larvae. O'Dee and Watters (1998) studied twelve species of darters (among other fish) and found they were all hosts for at least one species of mussel larvae, with some even being hosts for federally endangered species, such as *Epioblasma torulosa rangiana*. For these reasons, darters have important ecological functions and more efforts should be focused towards these. In addition, with the current plight of climate change, it is increasingly important to study fish energetics to determine how these aquatic organisms will respond to many unavoidable changes to their habitats and ecosystems. It is projected that small streams will be more greatly impacted than larger rivers (Heino et al. 2009), with most aquatic systems to experience shifts in riparian vegetation, changes in nutrient loading, and warmer temperatures impacting the availability of fish habitat (Meyer et al. 1999). Therefore, it is evident that there is a lack of knowledge on the physiology of these fish and a need to for more laboratory research to examine the physiological differences and energetics of these ecologically important, sympatric species.

Etheostoma blennioides (greenside darter) and *E. variatum* (variegate darter) are the two species study for the current experiments. *E. blennioides* is common in the Ohio River basin and inhabits a wide range throughout eastern North America (Page and Burr 2011). It is one of the largest darters and can reach up to 17cm in total length. *E. blennioides* feed mostly on invertebrate larvae, such as mayfly larvae, and inhabit rock

riffles of small streams, some medium sized rivers, and even shores of large lakes (Page and Burr 2011). *E. variatum* have a smaller, but similar, range as *E. blennioides* inhabiting a large portion of the Ohio River Basin. Consequently, it is not uncommon for these two species to coexist in the same waters. *E. variatum* is a smaller species, reaching a maximum total length of about 11 cm (Page and Burr 2011). They also prey on various species of aquatic invertebrates, while inhabiting fast gravel and rubble riffles in small to medium rivers.

Study Objectives. For the current study, the main objective is to measure the mass-specific metabolic rate for *E. blennioides* and *E. variatum* to discover any physiological differences that may enable these species to coexist. *E. blennioides* and *E. variatum* are both common and dominate species in Twelvepole Creek, the collection point for the fish used in this experiment. Therefore, they are good representatives of dominating and coexisting species to study how differences in physiology may permit sympatry. These experiments were conducted over a 48-hour period to gain information about the metabolic cycle, physiology, and behavior of the darters. The goal was to measure the most representative and accurate estimate for metabolic rate, and as previously discussed and illustrated by Steffensen (1989), the longer trial was expected to capture any diel fluctuations to achieve this goal. The hypothesis tested was that the mean mass-specific metabolic rate of *E. blennioides* will be significantly different than the mean mass-specific metabolic rate of *E. variatum*. Additionally, with interest in differences in metabolic rate over time and during light and dark periods, the hypothesis tested was that there will be a difference among the mean metabolic rates for each period of light and dark for each species individually. To incorporate behavioral analysis

to supplement the physiological data measured, time-lapse videos over the 48 h experiments were used. It was hypothesized that there will be a significant difference in the activity of the fish between period of light and dark and over time.

Materials and Methods

Specimen Collection and Maintenance. Fishes were collected from Twelvepole Creek, a fourth-order stream in Wayne County, WV (38°17'54.71"N, 82°26'47.07"W) by electrofishing in October 2014. A Halltech-2000 Battery Backpack Electrofisher (Halltech Aquatic Research Inc., Ontario, Canada) was used to stun the fish in a swift riffle, while two large hand nets caught the fish as they moved downstream. The fish were transported back to an aquatics laboratory at Marshall University in buckets filled with stream water. The mean total length for *E. variatum* (*n*=15) was 8.3 cm (0.7 SD) and the mean wet weight was 6.8 g (1.2 SD). The mean total length was 7.7 cm (0.8 SD) and the mean wet weight was 4.8 g (1.6 SD) for *E. blennioides* (*n*=17). Upon completion of respirometry experiments, the fish were returned to point of collection. The procedure used for fish collection, maintenance and respirometry experiments was approved by the Institutional Animal Care and Use Committee (protocol numbers 586876 and 587294) at Marshall University (Appendix A). A scientific collecting permit for *E. blennioides* and *E. variatum* was obtained from the West Virginia Division of Natural Resources.

The fish were maintained in an aquatics laboratory at Marshall University for 6 months prior to experimentation. A simulated stream was used to house the fish for maintenance and the duration of the experiments. The stream consisted of an agricultural feeding trough with the bottom covered with gravel and small rocks, and a

filtration system at the base of the stream into which water would drain. A pump was in the reservoir of the filtration system and pumped water through a chiller back to the top of the stream. The total volume of water in the system was ~215 L. The water in the stream was maintained at 18°C (range 17.28 – 18.48°C) using an Oceanic 1 HP Aquarium Chiller (Oceanic Systems, Franklin, WI). The filtration system used three filter methods (physical, biological, and chemical) to maintain high water quality. The physical filter was a flat sheet of plastic filled with holes that the water initially flowed over. This allowed for capture of any large debris as well as appropriate dispersal of the water over the biological filters. The biological filters were Amiracle Bio Balls which provided large surface area for bacterial growth for the removal of ammonia and nitrites. After filtering over the biological filter, the water flowed through activated carbon to remove any other impurities before being pumped back into the stream. Water quality parameters (DO%, temperature, pH, and conductivity) were monitored daily. Ammonia, nitrate, and nitrite levels were measured weekly. The fish in the stream were kept in similar conditions (14L:10D light cycle and 18°C) as later used in the respirometry experiments. Fish were fed commercial bloodworms daily.

Respirometry Equipment. Intermittent-flow respirometry experiments utilized Loligo Systems respirometry equipment (Loligo Systems, ApS, Tjele, Denmark) to measure the oxygen-consumption rate for the fish over a 48 h period. Respirometry trials began in May 2015 and ended in August 2015. The respirometry chamber was custom built (772 mL) for use with the small darters. Gravel covered portion of the bottom of the chamber to reduce increases in metabolic rate resulting from lack of substrate availability for benthic fishes (Fischer 2000). Tubing used for the recirculating

circuit of the system was 56 mL in volume. The entire intermittent-flow respirometry system consisted of the respirometry chamber to which a recirculating and a flush pump were connected by tubing at the front end and tubing connecting an oxygen probe holder to the back end of the chamber and then to the recirculating pump (Figure 2). The respirometry equipment was placed in a water bath (34.5 L) for maintaining the water temperature for each trial. A separate reservoir of water was cooled with an Oceanic 1 HP Aquarium Chiller in which a pump carried water through a cooling coil that was placed in the water bath. This enabled a more precise temperature control (17.74°C, 0.09 SD) for the trials. Given the equipment that was used, it was not possible to hold the temperature at exactly 18° C.

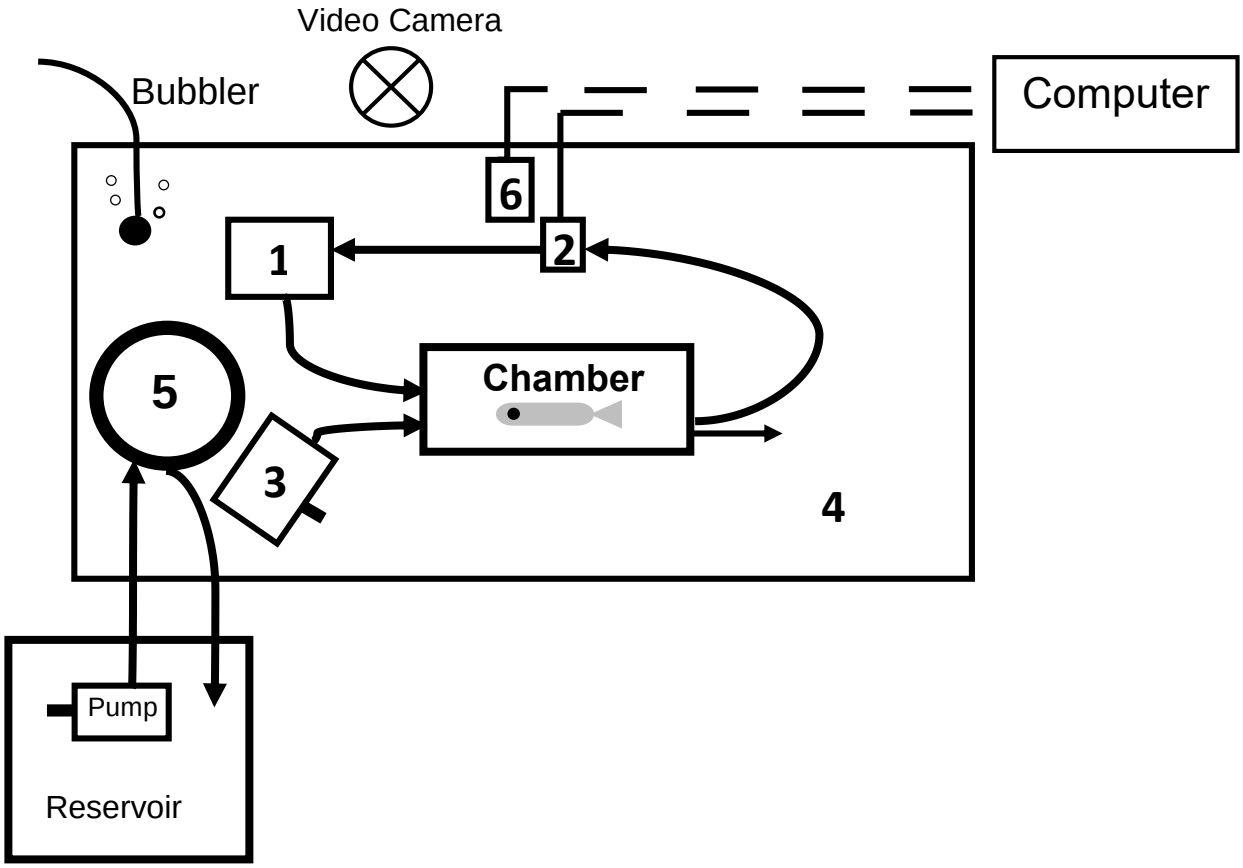

Figure 2. Setup of respirometry equipment used to measure the metabolic rate of the fish: For measurement cycles, the recirculation pump (1) pumps water through the chamber containing the fish, across the oxygen probe (2), then back to the pump. For flush cycles, the flush pump (3) turns on and flush water out of the chamber into the water bath (4). Water is pumped from a cold water reservoir through a cooling coil (5) to maintain water temperature. A bubbler maintains the oxygen concentration in water bath, which was monitored by the ambient oxygen probe (6). Arrows indicate tubing and direction of water flow.

For each 48 h trial, there were 15 min cycles which consisted of a 10 min measurement period, followed by a 3 min flush period, then a 2 min wait period. During the measurement period, the system was closed and the flush pump was off to measure the oxygen consumption of the fish as the decrease in oxygen concentration in the chamber. The flush period was an open-system period in which the flush pump was on and flushed fresh water into the chamber from the water bath. The wait period, after the flush pump was turned off, was used to account for the lag in the response time of the system. This 15 min cycle continued for the duration of the 48 h trial resulting in 4

measurements of oxygen consumption every hour. Respiration rates for each fish were measured from the slope of the regression of oxygen concentration over time for each measurement period.

Video Analysis. To correlate metabolic rate with activity level, activity of the fish was recorded for 10 s during every measurement period (every 15 min) using a time-lapse video camera. Video analysis started after the 3 h acclimation period. Preliminary analysis of fish behavior in the chamber revealed that, for the most part, the fish would not move, but if they did, it was short bursts (typically less than 5 s) of rapid darting. Therefore, scoring of the videos was based upon a change in position from the previous video, resulting in 4 possible activity scores: 0 = no change in position; 1 = small position change (<2.5 cm); 2 = large position change (>2.5 cm); 3 = actively moving. For example, a score of 0 was given to the video if the fish had not moved from the position it held in the previous video (15 min prior). A score of 1 was given if the fish had moved less than 2.5 cm. If the fish was actively moving in the video clip, a score of 3 was given. A score corresponded to each measurement period of oxygen consumption rate. Statistical analysis of correlation between activity and metabolic rate will be described in the data analysis section.

Experimental Setup. Prior to each trial, the fish was removed from the simulated stream and placed in a holding tank to ensure it was unfed for 24 h before the initiation of the trial. This provided that the metabolic rate measured was postabsorptive. The holding tank had the same water quality and temperature as the simulated stream, which was used in the respirometry experiments. Water was taken from the simulated stream to fill the water bath (34L) that held the respirometry chamber and equipment.

The simulated stream water was used to limit stress experienced by the fish and ensure the same quality of water was used for each trial. Deionized water treated with commercial aquarium salt (Jungle Aquarium Salt) was then added to the simulated stream to maintain a consistent volume and conductivity. All the respirometry equipment (tubing, chamber, and pumps) were assembled submerged in the water bath to eliminate the presence of air bubbles in the system. The chamber was closed and the equipment ran for two hours, measuring the respiration of the bacteria in the water, prior to the addition of the fish to the chamber. Therefore, there were eight measurement periods determining the oxygen consumption of the bacteria in the water before the fish was in the chamber. Subsequently, bacterial respiration was measured for two hours (eight measurement periods) after the fish trial was complete. The calculated bacterial respiration rates determined from the before and after trials were used to correct the fish respiration rates.

Prior to placing the fish in the chamber, the wet mass, total length, and volume of the fish was measured. The fish was then placed in the respirometry chamber and the system was closed. The video camera was turned on and set to record 10 s videos during each measurement period. The first 3 h were used as an acclimation period, and these data were not used in oxygen consumption calculations. Each fish was transferred to the chamber around noon, and measurements began around 3:00 pm. The average temperature across all trials was 17.74°C (0.09 SD). A 14L:10D light cycle was maintained during the duration of each trial. A bubbler was placed in the water bath to maintain a high level of oxygen in the water for the flush periods. The fish remained in the chamber for at least 48 h after the 3 h acclimation (at least 204 measurement

periods), at which point the fish was removed from the chamber and placed into a separate large aquarium, guaranteeing each fish was used only once. The two-hour bacterial trial began immediately after the removal of the fish from the chamber. At the very end of each trial, the water was drained from the water bath and all respirometry equipment was disassembled. The tubing, chamber, and pumps were cleaned with 0.5M HCl to remove any bacterial growth.

The oxygen concentration of the water was measured every second using a fiber-optic oxygen meter (Loligo Systems, ApS, Tjele, Denmark). The oxygen consumption rate was calculated from the slope of the decline in dissolved oxygen in the chamber, corrected for the chamber volume, over time (mg/s) for each measurement period. The oxygen consumption rate was later converted to a mass-specific rate (mg O2 kg^{-1} hr^{-1}) for each fish. Thirty respirometry trials were completed, but due to technical malfunctions such as power outages and leaks in the system, only twenty-nine trials were used for metabolic rate data analysis (15 *E. variatum* and 14 *E. blennioides*). Due to some instances of excess glare on videos or camera malfunctions, only 23 of the 29 trials were used for activity data analysis (12 *E. variatum* and 11 *E. blennioides*).

Data Analysis. Data analyses were conducted using Microsoft *Excel* 2013 and *SAS* 9.4. Linear regressions were run for each measurement period to obtain a slope that corresponded to the oxygen consumption rate for each 15 min measurement period. Due to how the program incorporates water temperature into the algorithm for oxygen concentration, the slight change in temperature (total range 17.5-18.1°C) during each trial caused some fluctuations in the reading for oxygen concentration. Therefore,

a temperature correction had to be conducted on the data to correct the oxygen concentration and produce a more accurate calculation of oxygen consumption rate. The modal temperature was calculated for each measurement period. Then the data points that were not recorded at the modal temperature were dropped. The oxygen consumption rate for each measurement period was calculated from the slope of the decrease in oxygen concentration measured only at the modal temperature. Once the corrections were performed, the oxygen consumption rate (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) for each measurement period was calculated for each fish. The average metabolic rate at each measurement period was then calculated and graphed for each species to examine their diel metabolic cycle. For visual representation of rates over time, time was presented in 00:00 time scale beginning with start time of the experiment (~1500) and increased by hour, not starting over at 0000 for the second day, but rather became 2400, 2500, 2600, etc.

Correlation analyses were performed on the metabolic rate, activity scores, and time for both species. Due to the use of ranked data for activity analysis, the Spearman Correlation Coefficient (*R*; Proc CORR) was utilized. After correlation analysis, the effects of light and time on metabolic rate were investigated. In order to statistically analyze these effects, the measured rates were averaged for the different periods of light and dark throughout the 48-hour trials. Specifically, there were five distinct periods for each trial: Light1, Dark1, Light2, Dark2, and Light3 (Table 2).

Table 2. Time periods used for statistical analyses of metabolic rate and activity of *E. blennioides* and *E. variatum.*

Time period	Label	Hour Time Scale
3:30 pm - 8:00 pm	Light 1	15.50 – 20.00
8:00 pm - 6:00 am	Dark 1	20.00 – 30.00
6:00 am - 8:00 pm	Light 2	30.00 – 44.00
8:00 pm - 6:00 am	Dark 2	44.00 - 54.00
6:00 am - 2:00 pm	Light 3	54.00 - 61.75

The start and end of each trial was approximately around 1500, but because each trial did not start at exactly the same time (between 11:30am and 12:30pm), the data sets had to be truncated to include only rates between 1530 on day one and 1400 (6200 on new time scale) on day 3. This ensured that the same sample size would be used for calculations of mean metabolic rate for each measurement period. Therefore, data analyzed were for 46.5 h instead of 48 h.

Test for normality revealed the mean metabolic rates at some of the time periods for each fish were not normally distributed. Therefore, a natural log transformation was conducted, improved normality, and further analysis was performed on the mean natural log of metabolic rate for each species. A repeated measures ANOVA (Proc GLM) was conducted to examine the effects of light, time, and species on mean natural log of metabolic rate. The Tukey HSD test (Proc GLM / Tukey) was used to test the hypothesis that there is a difference among the mean metabolic rates for each light period. These comparisons were conducted for each species, but not between species.

Finally, the activity data were analyzed by averaging the activity scores for each of the different light periods (Table 2), and performing a Friedman's Test (Proc FREQ) as a non-parametric alternative to one-way repeated measures ANOVA. The non-

parametric test was used because the activity data were ordinal. This tests for a difference in the distribution of the activity scores among the five different periods. Then, a Wilcoxon Signed-Rank test (Proc UNIVARIATE) was used to make multiple comparisons following the results of the Friedman's Test to determine for which periods the distributions for activity scores were different. For the Wilcoxon Signed-Rank test, the alpha level had to be adjusted for the number of comparisons performed; the adjusted alpha was 0.005.

Results

Metabolic Rates. Both species had weak (R<0.5) but significant positive correlations between activity and metabolic rate and negative correlations between time and metabolic rate (Table 3). Activity and time had a positive, but very weak correlations for *E. blennioides* and was not significant (R= 0.02244; p=0.3009), but the relationship was negative, very weak, and significant for *E. variatum* (R= -0.0932; p<0.0001). The diel metabolic cycle for the *E. blennioides* and *E. variatum* have produced different patterns over the 48 h experiments (Figure 3). Both species show a pattern of decrease in metabolic rate over the length of the trial, but the *E. variatum* decreases more rapidly and levels off at a more constant rate than *E. blennioides*. The error bars (±SE) provided in Figure 3 offer a distinct comparison between the two species. *E. variatum* have much less variation in recorded values of metabolic rate, and for both species, when the rate is low, there is also less variation than when their rates are higher. *E. blennioides'* rates were consistently higher than *E. variatum* throughout the duration of the 48 h trials (Figure 4). It is only at night (indicated by the black bars) that the rates of the two species are consistently close to the same values. The complete set of rates

presented in Figure 3 and Figure 4 are used to compare patterns of change and variation between the two species.

Table 3. Results of Spearman Correlation (*R*) analysis among activity, time, and metabolic rate for *E. blennioides* and *E. variatum.*

Species	*n*	Variables	*R*	*p*
E. blennioides	2127	Activity vs Metabolic Rate	0.3347	<0.0001
		Time vs Metabolic Rate	-0.3009	<0.0001
		Activity vs Time	0.02244	0.3009
E. variatum	2312	Activity vs Metabolic Rate	0.3528	<0.0001
		Time vs Metabolic Rate	-0.4192	<0.0001
		Activity vs Time	-0.09320	<0.0001

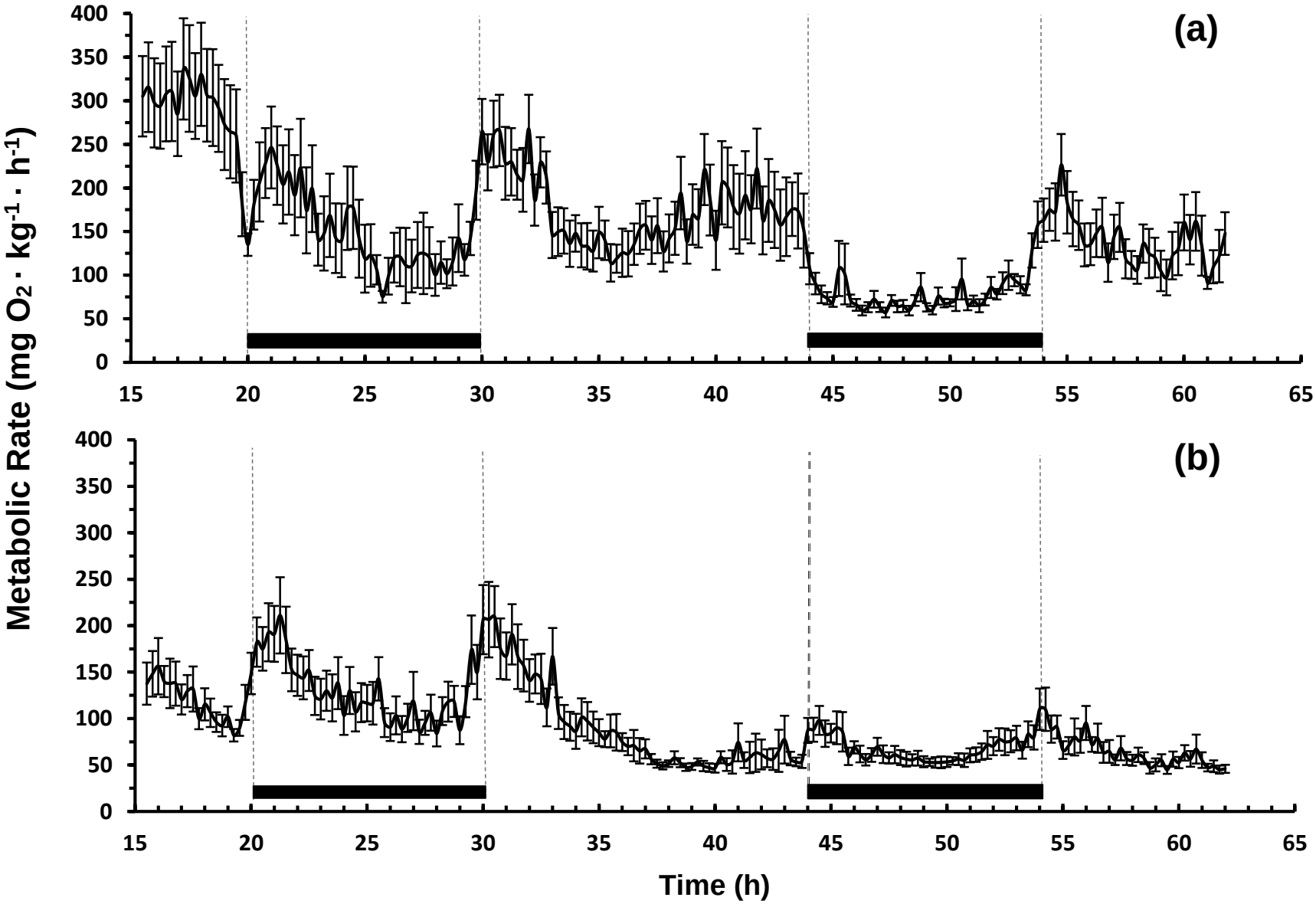

Figure 3. Diel cycle of metabolic rate of (a) *E. blennioides* (*n*=14) and (b) *E. variatum* (*n*=15). Metabolic rate is plotted as mean oxygen consumption rate (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) (±SE) for 15 min intervals connected by straight lines. The start of the time scale,15, represents 15:00, the start of each experiment. Black bars indicate dark.

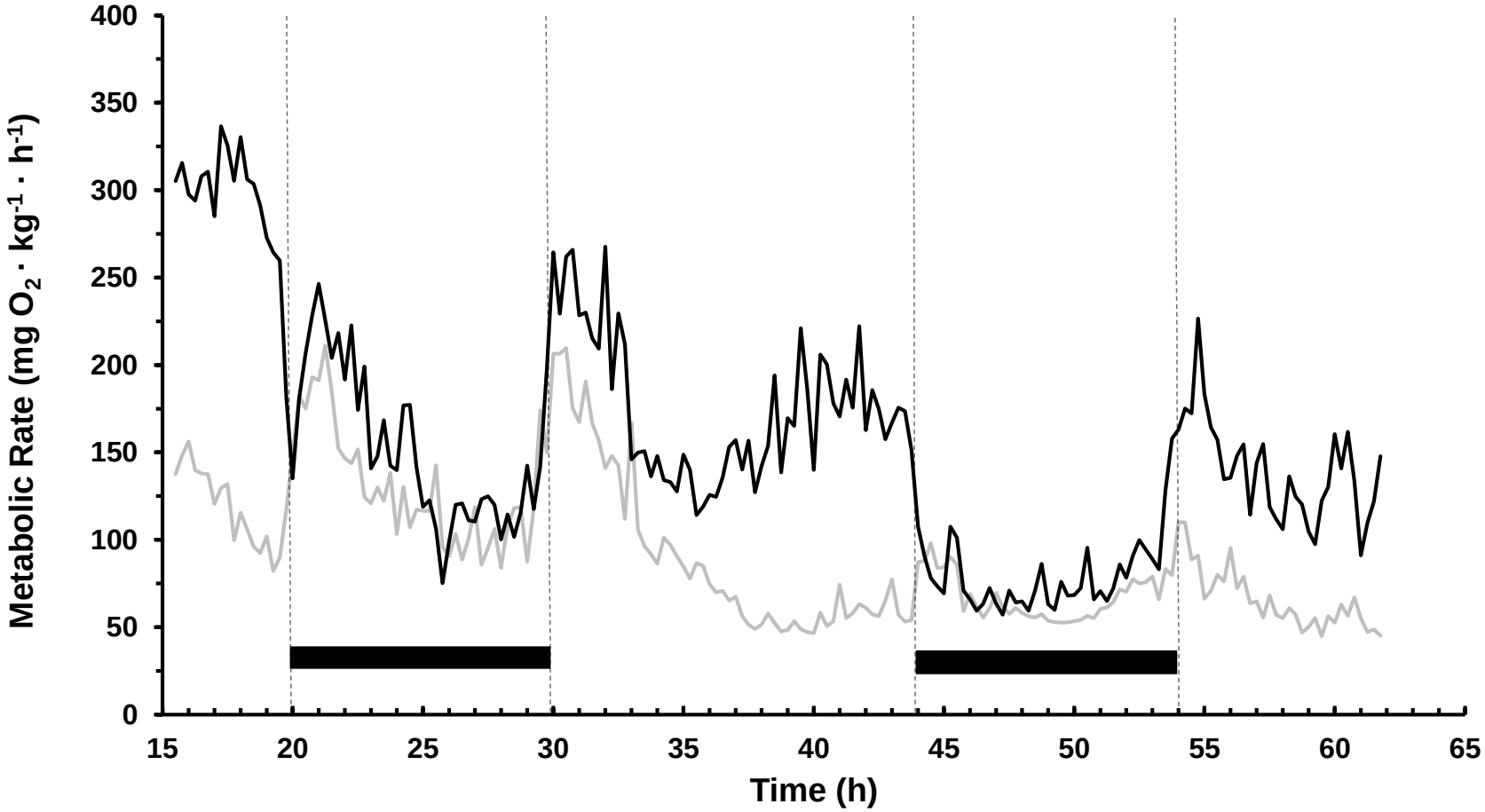

Figure 4. A comparison of diel metabolic rate between *E. blennioides* (*n*=14; black line) and *E. variatum* (*n*=15; grey line). Metabolic rate is plotted as mean oxygen consumption rate for 15 min intervals connected by straight lines. The start of the time scale,15, represents 15:00, the start of each experiment. Black bars indicate dark.

However, the rates were averaged for the light and dark periods of the trial to analyze significant differences in metabolic rate for each species among periods of light and dark over the 48 h. A two-way ANOVA with repeated measures was used to analyze these data (Table 4). The mean metabolic rate for *E. blennioides* (154.64 ± 52.54 mg $O2 \cdot kg^{-1} \cdot hr^{-1}$ SD) is significantly greater than the mean metabolic rate for *E. variatum* (92.51 ± 32.70 mg $O2 \cdot kg^{-1} \cdot hr^{-1}$ SD) ($F_{1,27}$=15.33, p=0.0006). There was also a significant effect of time on metabolic rate ($F_{4,108}$=21.54, p<0.0001), as well as a significant time*species interaction ($F_{4,108}$=5.14, p=0.0013).

Table 4. Repeated-measures ANOVA results on effects of time and species on the mean metabolic rate for *E. blennioides* and *E. variatum* for 48 h respirometry experiments.

Source	*df*	*F*-value	*p*
Species	1	15.33	0.0006
Time	4	21.54	< 0.0001
Time*Species	4	5.14	0.0013

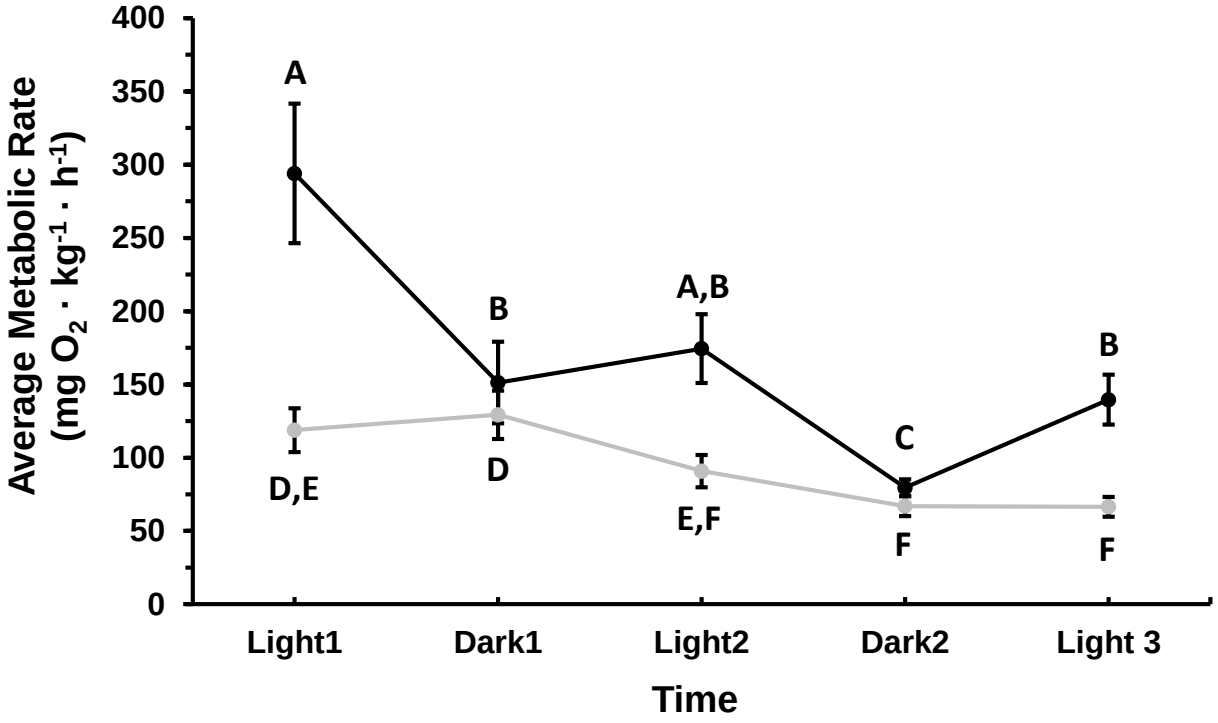

Figure 5. Average metabolic rate (±SE) for *E. blennioides* (*n*=14; black line) and *E. variatum* (*n*=15; grey line) for different light treatments during 48 h long respirometry experiments. “Light” indicates day time; “Dark” indicates night time. The number following each treatment refers to the day of the trial; for example, Light1 is day time of day one. Means with the same letter are not significantly different. Comparisons were not made between species.

The average rates during the light and dark periods were calculated for both species and presented in Figure 5. Comparisons of the average metabolic rates for the five different light periods were performed for each species, but not between species. For *E. blennioides*, the mean metabolic rate during the light of day one (Light1) was significantly greater than all other periods (Dark1, Dark2, and Light3) except the light of day 2 (Light2) of which it was not significantly different. Interestingly, the second night period (Dark2) had a significantly smaller mean metabolic rate than all the other periods; tests of differences were performed on the ln-transformed data. There was no significant difference among the mean metabolic rate for night of day 1 (Dark1) and light of days 2 and 3 (Light2 and Light3 respectively).

For *E. variatum*, the light of day 1 (Light1) was only significantly greater than night of day 2 (Dark2) and light of day 3 (Light3). Therefore, Light1 was not significantly different from night of day 1 (Dark1), but Dark1 was significantly greater than all other periods (Light2, Dark2, and Light3). There was no significant difference among the mean metabolic rate for Light2, Dark2, and Light3; tests of differences were performed on ln-transformed data. The change in metabolic rate over the 48-hour trials presents two different patterns between the two species. *E. variatum* metabolic rate starts to level off after an increase during night of day 1 (Dark1), whereas the metabolic rates for *E. blennioides* fluctuated more throughout the five periods during the 48-hour trials.

Activity Results. Of the 29 trials for which metabolic rate was analyzed, only 23 trials were analyzed for activity levels due to technical malfunctions. Due to glare on some video clips, the fish were not visible to score activity levels. The activity of the fish over the 48 h trials produced different patterns between the two species (Figure 6). Over all, *E. blennioides* appeared to have consistently more elevated activity levels, which dropped down during the middle of the dark periods. *E. variatum* had lower activity levels during the course of the trials, but their activity increased more during the dark periods. Statistically, there was no evidence that the distributions of the activity scores where different among the five time periods for *E. blennioides* ($F_{1,4}$=3.24, p=0.5173); however, the distributions of the activity scores were different for *E. variatum* ($F_{1,4}$=20.50, p=0.0004) (Table 5). When determining which periods were different in activity for *E. variatum*, it was found that Dark1 had a different distribution of activity scores than Light1 (S=-39, p=0.0005, Light2 (S=38, p=0.0010, and Light3 (S=38,

p=0.0010), but was not significantly different than Dark2 (*S*=20.5, *p*=0.0371) (Figure 7). All other pairwise comparisons were not found to be significantly different (α=0.005).

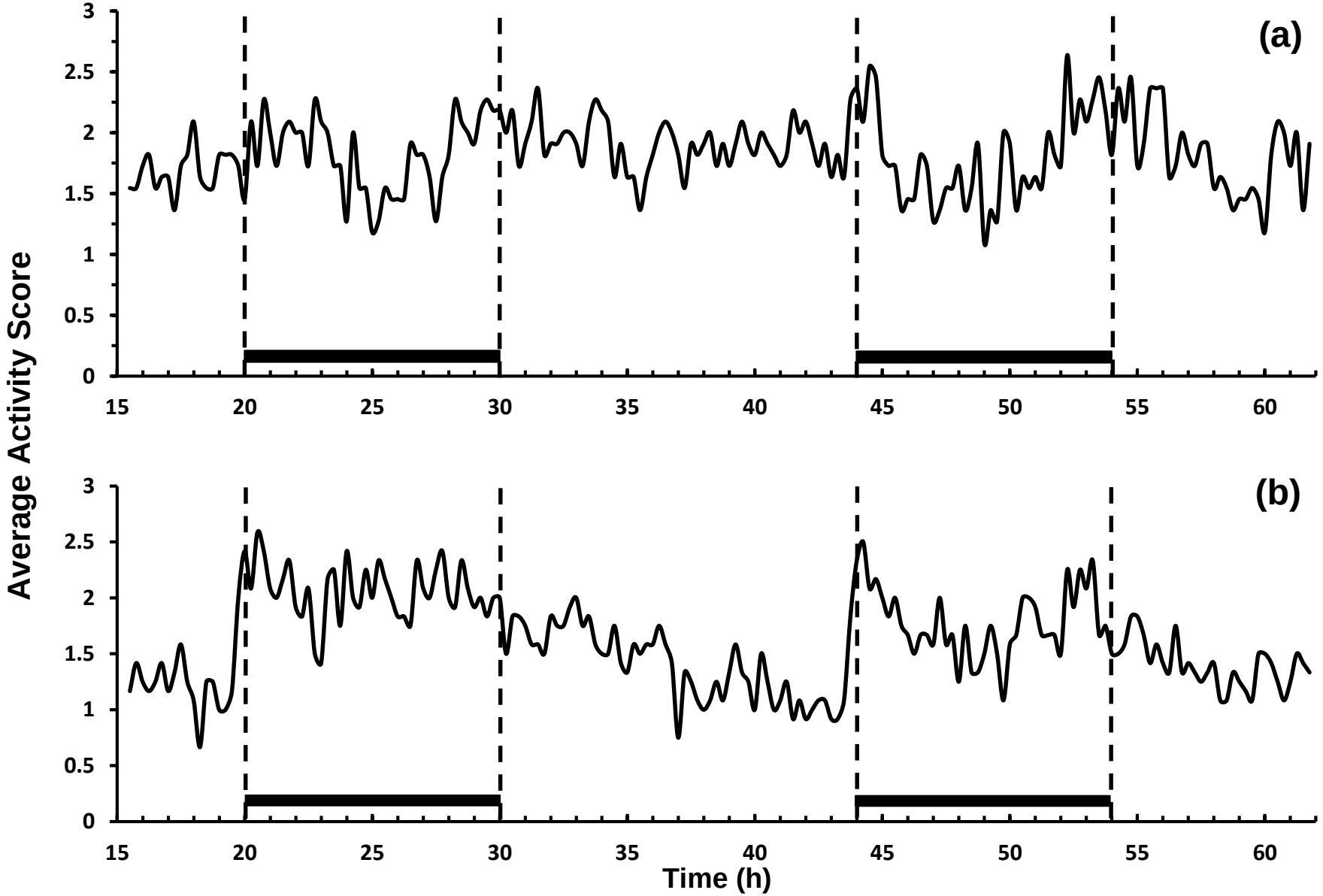

Figure 6. Diel cycle of activity of (a) *E. blennioides* (*n*=11) and (b) *E. variatum* (*n*=12). Activity is plotted as average activity score for 15 min intervals. The start of the time scale, 15.5, represents 15:30, the start of each experiment. Black bars indicate dark.

Table 5. Friedman's test (*F*) results on effects of time on the distributions of activity scores for *E. blennioides* and *E. variatum* for 48 h respirometry experiments.

Species	*df*	*F*	*p*
E. blennioides	4	3.2477	0.5173
E. variatum	4	20.5042	0.0004

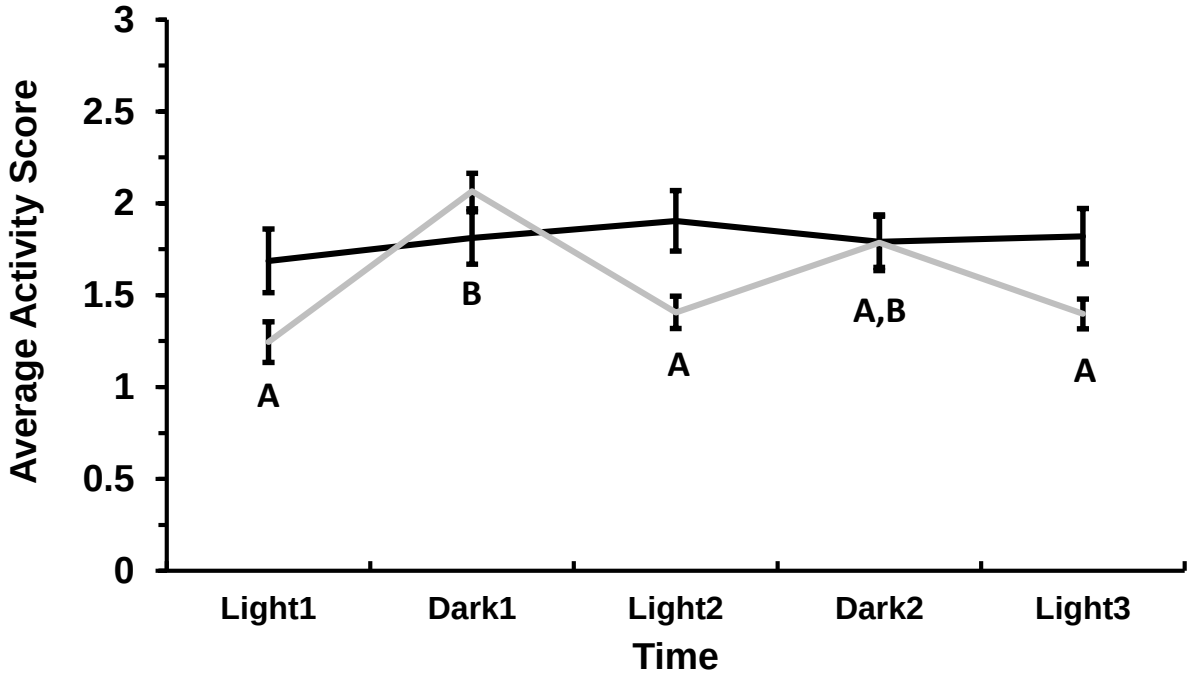

Figure 7. Average activity score (±SE) for *E. blennioides* (*n*=11; black line) and *E. variatum* (*n*=12; grey line) for different light treatments during 48 h long respirometry experiments. "Light" indicates day time; "Dark" indicates night time. The number following each treatment refers to the day of the trial; for example, Light1 is day time of day one. Means with the same letters are not significantly different. Comparisons were not made between species. There was no significant difference among the activity scores for *E. blennioides* over time.

Discussion

This study compared the diel metabolic rates of *E. blennioides* and *E. variatum* and illustrated many differences between the two species. Both species had weak (*R*,0.5) positive correlations between activity and metabolic rate, but negative

correlations between time and metabolic rate (see Table 3). Thus, as activity increased, metabolic rate increased; this is not suggested to be a causal relationship and will be discussed in further detail with interpretation of the activity results. Additionally, there could be several reasons as to why there was a negative relationship between metabolic rate and time. The fish could have become less active, began downregulating their metabolism, or increased acclimation to the chamber. The relationship between time and activity was very weak for both species, and only significant for *E. variatum*, for which it was negatively correlated. Therefore, activity decreased as the experiments progressed for *E. variatum.*

The mean metabolic rate over the 48 h trials for *E. blennioides* was significantly greater than the mean rate recorded for *E. variatum*. Therefore, at the most basic level, this study has shown that there are some physiological differences between these two coexisting species resulting in a higher metabolic rate for *E. blennioides* than *E. variatum*, supporting the hypothesis. This is also important, because it provides basic physiological information about these darters which has not been the focus of most darter research. Studies that attempt to estimate total stream/system metabolism are likely to benefit more from empirical data such as this, rather than estimating fish metabolism from reported equations which tend to overestimate the rates for small fish (Ultsch et al. 1978).

However, by reporting a simple number for metabolic rate as many studies do, important information can be missed. It could be stated that *E. blennioides* have a greater demand for oxygen than *E. variatum*, but potentially important physiological and behavioral causes for these observed differences are ignored or overlooked. Therefore,

what is more meaningful with these results is what happened metabolically and behaviorally over the 48 h. The repeated-measures ANOVA reported that there were time and time*species effects in addition to the species effect on mean metabolic rate for these two species. For both species then, metabolic rate changed over time, but the time*species effect means that metabolic rate changed differently over time for the two species. Thus, not only were the overall mean rates significantly different, the pattern by which the metabolic rate changed over time differed between the two species. This difference is well illustrated in Figure 4, in which the metabolic rate for *E. variatum* begins a steady decline after the end of the first night period (Dark1). Although the metabolic rate for *E. blennioides* does decrease over time, the metabolic rates fluctuate more than the metabolic rates of *E. variatum*. Specifically, *E. blennioides'* metabolic rate increases during the day and decreases at night. Even with the fluctuations and decrease over time, the rates for *E. blennioides* remained higher than the rates for *E. variatum* for the duration of the 48-hour experiments. Therefore, *E. blennioides* maintained a higher energetic cost throughout the experiments. Dwyer et al. (2014) report a strong correlation between routine metabolic rate and lifestyle among three species of freshwater fish, of which one was a small, benthic fish similar to darters in lifestyle. Though the Dwyer et al. study investigated a much larger range of lifestyles among fish, it is possible that the differences in the metabolic rates for *E. blennioides* and *E. variatum* are linked to lifestyle differences. For example, metabolic rate for *E. blennioides* shows a pattern of increase before the onset of dark and light, the times of dusk and dawn. A pattern such as this in metabolic rate for *E. blennioides* could suggest a dusk/dawn activity or feeding habits (Svendsen et al. 2014). This pattern is not as

prevalent for *E. variatum,* which could suggest different feeding times or that *E. variatum* is more of an ambush predator, while *E. blennioides* may engage in more risk-taking or endurance foraging behavior (Dwyer et al. 2014).

Another interesting pattern that can be missed by simply reporting a single estimate of metabolic rate is the change in variation that can be seen in Figure 3. For both species, the standard error decreases when the mean metabolic rate is low, whereas during times of higher metabolic rate, there is more variability. Also, when comparing the two species, *E. blennioides* generally has more variability for the mean metabolic rates recorded than *E. variatum.* Some of this variation is likely due to activity (Killen et al. 2011) and even individual plasticity of activity patterns which is common among fishes (Reebs 2002). Therefore, less variation for *E. variatum* could suggest these fish to be more consistently calm than *E. blennioides,* which could be a bolder, more active species (Killen et al. 2012), which has not been previously suggested for these species. The decrease in variability at times for which metabolic rates are low also suggests that the fish are less active and, on average, calmer.

When looking at each species individually, there are interesting differences in the mean metabolic rate for light versus dark periods and day one versus days two and three. Statistically and graphically (Figure 5), the rates for *E. variatum* produce a more distinct pattern of decrease in metabolism over time. The first night is significantly different then the light periods of days two and three, but it is also significantly greater than the second night. Therefore, there is no clear distinction between the metabolic rate during the day versus the night for *E. variatum*, but day one (first 24 hours) is different than day two (the second 24 hours). This is mainly due to the fact that the

metabolic rate for *E. variatum* simply decreased over time resulting in lower rates for the second compared to the first day. This species, consequently, appears to have downregulated its metabolism as the trials progressed and reached a constant, perhaps basal, rate. In comparison, the metabolic rate for *E. blennioides* is significantly lower at Dark2 then all other periods and increases during Light3. Therefore, unlike the metabolic rate of *E. variatum* which simply declined over time, the metabolic rate for *E. blennioides* continued to exhibit cyclic differences during night and day throughout the experiment. Additionally, the metabolic rate of *E. blennioides* for Light1 is higher and more variable than at other times. This is likely due to stress from experimental introduction and this species may require a longer acclimation period (Steffensen 1989). The observation of more variation in rates and fluctuations of mean rates over time for *E. blennioides* versus the less variable, more consistently decreasing mean rates of *E. variatum,* is an important beginning to understanding the differences between these two species, but additional information is gained by combining this with their activity patterns.

Though patterns in metabolic rate can often be explained by behavioral patterns, this is not necessarily the case for these data. With more fluctuations in metabolic rate over time for *E. blennioides,* it may be assumed that these fluctuations would correlate with similarly fluctuating activity levels. However, Figure 6 shows that the activity of *E. blennioides* was consistently higher, only showing somewhat of a dip in the middle of the night. This decrease in activity does correspond well with the decreased pattern of metabolic rate during the night (Figure 5), but there is not an overall decrease in activity for *E. blennioides* that may have been expected from the overall decrease in metabolic

rate. Comparing mean activity levels among light and dark periods and over the two days (Figure 7) further illustrates this point. There was no effect of time on activity for *E. blennioides* and, therefore, no significant differences in the mean activity levels among the different periods (Table 5). Interestingly, *E. variatum* shows more of a repeatable pattern in activity levels over the 48 h. This species becomes more active during the night and decreases in activity over the course of the day, repeating this pattern for day two. Again the repeatability is well illustrated in Figure 7, where there is no significant difference in the mean rates for the light periods and no significant difference during the dark periods, which were higher than the light periods. The increase in activity for *E. variatum* was not associated with an equivalent increase in metabolic rate. Pierce and Wissing (1974) reported opposite findings for bluegill (*Lepomis macrochirus*), for which higher metabolic rates recorded during the dark were not associated with increased swimming behavior. Consequently, although increased activity often leads to increased metabolic rate, this type of a causal relationship should not be assumed for all data (Careau et al. 2008; Biro and Stamps 2010). Some changes in metabolic rate could simply be due to normal cyclic changes in metabolism (Pierce and Wissing 1974; Svendsen et al. 2014) or even excitement from changes in light condition (Pierce and Wissing 1974; Steffensen 1989).

When combining the metabolic and behavioral data, clear and interesting distinctions can be made between *E. blennioides* and *E. variatum* that could facilitate their coexistence. *E. blennioides* has a greater metabolic rate than *E. variatum* which means that this species has greater energetic requirements. A higher energetic cost could lead to different behaviors exhibited by *E. blennioides* that enable the two species

to inhabit similar areas. For example, some fish are more likely to engage in risk-taking behaviors after food deprivation, leading to increases in metabolic rate, thus reinforcing the problem (Killen et al. 2011). The higher metabolic rate for *E. blennioides* could influence these fish to exhibit more risk-taking foraging behavior, while *E. variatum* may energetically be able to afford to sit and wait to ambush prey. Also, fish with higher metabolic rates are often bold and exhibit dominant, aggressive behavior (Metcalfe et al. 1995; Cutts et al. 1998). The consistently higher activity and metabolism of *E. blennioides* relative to *E. variatum* could suggest that this species is more aggressive and bold, while *E. variatum* tends to be more reserved.

These results have important ecological implications regarding the survivability and habits of these two species. If *E. blennioides* is bolder and aggressive in its natural habitat, they are likely to be more susceptible to predation than *E. variatum.* Also, during stressful conditions, such as inadequate habitat or low food availability, the high metabolic rate of *E. blennioides* may produce risky behaviors or vice versa, and greatly impact the health of this species. Another possible disadvantage of a higher metabolic rate for *E. blennioides* could be that this species is more susceptible to starvation when there are limited resources. Whereas, in similar situations, *E. variatum* may simply downregulate its metabolism and exhibit more reserved behavior, thus conserving energy and persisting longer in an unsuitable environment. This has important implications about how these species may respond to climate change. Under changing thermal conditions, *E. blennioides* may be more adversely affected than *E. variatum.* *E. blennioides* has been shown to have a lower tolerance to fluctuating and extreme environmental conditions, including higher temperatures (Hlohowskyj and Wissing

1987), and many of their populations could be greatly impacted by climate change. Unintentionally, to an extent, these experiments mimicked an environment with low substrate and food availability. Darters are often found beside or under larger rocks which provide flow refugia and shelter from predators. Though there was some substrate available on the bottom of the chamber for the fish to rest upon, there were not large rocks present. Also, the length of the experiments may have caused the fish to enter a stage of starvation. Therefore, these results could possibly mimic how these species respond to suboptimal conditions. Though it is beyond the scope of this study, it would be interesting for future research to investigate changes in the metabolic rate and activity of these species with different substrate availability and feeding conditions.

From a management and conservation stand point, these results could shed some light on how dam operations potentially favor a particular species over another, altering community composition. If there are extreme thermal changes downstream of a dam as a result of cold-water releases, this could perhaps favor species like *E. variatum* that have a lower metabolic rate and appear to be more reserved in behavior, waiting out the periods of extreme change (Bednarek and Hart 2005). As previously mentioned, *E. blennioides* has been shown to have a low tolerance to fluctuating environmental conditions (Hlohowskyj and Wissing 1987). However, community structure is also largely influenced by the change in flow regime as a result of dam operations (Richter and Thomas 2007). From the results of the current study, it could be suggested that maintained flow could possibly favor *E. blennioides* over *E. variatum.* As the more aggressive species, *E. blennioides* could have advantage in a more constant environment; if there were more natural flows with seasonal flooding and low flow

events, *E. variatum* may do better in the summer pools with high temperatures, where this could be more energetically costly for *E. blennioides.* Therefore, as suggested (Bednarek and Hart 2005; Richter and Thomas 2007), dam mitigation and pulsed flows could help return natural conditions to streams and rivers and increase biodiversity by providing variation that allows for physiologically different species, such as *E. blennioides* and *E. variatum*, to coexist.

In addition to providing physiological data about these fish, this study also highlighted ways in which metabolic experiments could be improved to produce more accurate estimates of metabolism for fish. Measuring the metabolic rate over a longer period of time and including periods of dark can capture important periods of the metabolic cycle. For instance, *E. variatum* had the highest mean metabolic rate during the first dark period, which would have been missed if a short experiment during the day was performed. However, experiments that are too long could cause the fish to enter a state of starvation and downregulate their metabolism. Therefore, reported rates in the literature could be inaccurate and affect estimates of energetics for these fish and the whole ecosystem. This will be discussed in more detail in the next chapter of this thesis.

This study provided important information about the physiology and behavior of the understudied *E. blennioides* and *E. variatum* that can help to develop a better understanding of their ecology and coexistence. It appears that *E. blennioides* is a bolder species which may exhibit riskier behavior increasing energetic demands, while *E. variatum* had a subtler and repeatable behavior with overall lower metabolic rate. These recorded differences could play important roles in their life histories that enable these species to coexist. In conclusion, this study began a necessary and key step

towards the discovery of physiological differences among closely related fish species that facilitate coexistence, and provided novel information on the diel metabolic and behavioral cycles of *E. blennioides* and *E. variatum.*

CHAPTER 3

DARTER SPECIES METABOLIC COMPARISONS

Introduction

Darters (Percidae), a species-rich group of mostly benthic fish, are very common freshwater fish inhabiting many small streams and rivers throughout the eastern United States. Considered perch-like fish, they are characterized as small fish (most less than 10 cm in length) with two dorsal fins, the first spiny and the second rayed (Page and Burr 2011). Most species lack a swim bladder and occupy the benthos of streams, rivers, and lakes. Darters are considered to be some of the most beautiful freshwater fish in North America (Page 1983) with vibrant nuptial colors. This group of fish is also the second-most diverse family of North American freshwater fishes (Page and Burr 2011), over 200 darter species currently classified. It has been known for some time that darters can make up a large portion of the biomass in a stream and in some locations in which 89% of the biomass is benthic fish, darters are often the dominating species (Small 1975).

Despite the large abundance and diversity of these fish within North America, darters are relatively understudied when compared to large, more recreationally and economically important game-fish found in often the same waters as darters. However, it is important and of particular interest to study darters for several reasons. First, darters play several important ecological roles. Many species of darters are prey items for large fish that are often economically important, such as bass and trout. Second, darters are usefully indicator species and are often used by many agencies as metric of water quality. For example, the Kentucky Index of Biotic Integrity (KIBI) considers the

presence of darters as an indication of good water quality and include their presence or absence in determinations of overall water health (KDOW 2003). Thirdly, darters have a major impact on the benthic biomass in streams they occupy because many are important hosts for freshwater mussel larvae (glochidia). As obligate ectoparasites, many species of glochidia attach to the gills of fish and thus rely on these host fish for dispersal. O'Dee and Watters (1998) identified twelve species of darters that were host for at least one of the ten mussel species that were investigated and emphasize the importance of host species for maintaining and conserving threatened and endangered mussels. Most species of mussels have a range of known host species, often from 2-20, but there are a few mussels that are highly specialized and have a single known host species (Strayer 2008). Finally, the most frequent reason that darters are a particular research interest is that many species often coexist at high levels of sympatry; that is, many closely related darters coexist within the same area of a stream, river, or lake. For example, in some areas of the Kentucky River, 14 species of darters have been observed coexisting (Carlson et al. 2009). And the reason for research interest and intrigue in this phenomenon has its roots in theories of ecological niches. Among attempts to define what an ecological niche is (Grinnell 1928; Hutchison 1957; Vandermeer 1972; Pianka 1981; Leibold 1995; Cushing et al. 2004), the competitive exclusion principle (Gause 1932; Hardin 1960) was theorized, which presents the idea that no two ecologically similar species can coexist. This principle hypothesizes that two species of similar ecology cannot exist in the same area without one outcompeting the other and forcing it from the area (Hardin 1960). Therefore, when observing this

phenomenon of species coexistence, it is suggested that there must be subtle differences among the species that facilitate such coexistence.

Seeking out these subtle differences among various species of darters is precisely what many researchers have attempted and continue to attempt to do. However, research that has focused on explaining this phenomenon tends to focus on resource and niche partitioning. Gaining a better understanding of the microhabitat use and selection of many species of darters has received much attention (McCormick and Aspinwall 1983; Chipps et al. 1994; Kessler et al. 1993; Harding et al. 1998; Welsh and Perry 1998; Skyfield and Grossman 2008; Ashton and Layzer 2010; Weston et al. 2010). For many of these studies, key factors within the habitat such vegetation and substrate type and availability and water depth and velocity correlated with the distribution of the species of interest. Hlohowskyj and White (1983) noted for three species of darters there was overlap in prey items selected, but seasonal shifts led to times of food resource partitioning and reduced competition. Researchers have even investigated the thermal preferences (Ingersoll and Claussen 1984; Hlohowskyj and Wissing 1987; Strange et al. 2002), behavioral isolation based on visual signals (Williams and Mendelson 2010; Williams and Mendelson 2013), and jaw morphology (Carlson and Wainwright 2009) to better understand the sympatry and diversification of darters.

Unfortunately, despite the numerous studies pursuing the differences among darters that enable their coexistence, there is a lack of attention placed on darter physiology. Physiology can provide details of how, for example, darter behavior or morphology is limited, facilitating their coexistence and ultimately leading to the high

levels of diversification of this group of fish. Metabolic rate is a fundamental biological rate that researchers can explore to help explain variations in the ecology and life history of organisms. Brown et al. (2004) present the Metabolic Theory of Ecology (MTE) and suggest that because metabolism sets the pace of life by limiting rates of resource uptake and allocation, metabolic rate regulates life history traits and ecological processes. This theory is grounded on the three-quarter-power scaling of metabolism with mass that occurs at all levels of an ecosystem, and explanation of this scaling law is based on the branching patterns of distribution networks of organisms (i.e. blood vessels of mammals) (West et al. 1997). Therefore, Brown et al. (2004) propose that how metabolism is affected by body size, chemical kinematics, and resource supply can explain many broad and complex ecological patterns.

For these reasons, researchers study metabolic rate and bioenergetics models to understand on organism's life history and ecology. Unfortunately, the use of various methods for measuring metabolic rate provide different information and it is cautioned to be fully aware of these differences where comparing metabolic rates (Roche et al. 2013). Fish respirometry experiments may differ in the length of the acclimation period, if or how long the fish is unfed prior to the experiments, duration of the trials, and measurement frequency. Steffensen (1989) illustrates that metabolic rates may fluctuate throughout the course of a day and measuring for only during a portion of the day may lead to erroneously high or low values for the organism. Also, longer experiments may provide information about the daily fluctuations in metabolic rate, providing more information about the ecology of the organism that may have been otherwise overlooked. For example, Pierce and Wissing (1974) found that nocturnal

metabolic rates were 26% higher than during the day for bluegill (*Lepomis macrochirus*). The authors noted that this increase is likely due to some other process than prey capture, and thus reveals important information about the physiology of this fish. Consequently, experiments that are performed over longer periods of time, can potentially provide more information about the ecology of the organism than studies that simply report a single number as the metabolic rate.

The current study seeks to gather information about darter metabolism and energetics to make comparisons and elucidate any physiological differences among these related species. Thus, this study is a comparative analysis, connecting the experiments performed on *E. blennioides* and *E. variatum* of the current paper to previously published literature on darter metabolism and energetics. It is hypothesized that there will be a significant difference in the mean mass-specific metabolic rates among the different darter species. Therefore, one main goal is to compile all known darter energetic information for easy access and comparison. Additionally, this analysis aims to inform researchers of the large lack of knowledge on the physiology of these fish and illustrate that more research needs to be conducted to gain a better understanding of their physiological ecology.

Methods

Data Compilation. To obtain a complete list of studies that provided metabolic information on darters, searches of electronic databases (Google Scholar and Academic Search Premier) were conducted from February to August 2015. The search was performed for all known species of darters as of February 2015. This included 205 species from four genera, *Etheostoma* (151), *Percina* (46), *Ammocrypta* (6), and

Crystallaria (2). Searches were conducted using the scientific name and then common name for each species with the following search terms: metabolism, metabolic rate, oxygen consumption, hypoxia, energetics, and energy budgets. The original publication for each study was examined to ensure it met criteria necessary to perform the comparative analyses. The criteria used were:

1. The fish were post-larval.
2. All fish were in a post-absorptive state.
3. Measurements taken were of resting (standard) or routine metabolic rate.
4. Experimental temperature was controlled and reported.

Statistical Analysis. Few studies met the search criteria and were analyzed. The data acquired from the literature were reported in various units for oxygen consumption rate and measured at different temperatures. Prior to converting all oxygen consumption data to the same units, reported rates were standardized to 18°C. A temperature coefficient, Q_{10}, was used to make these calculations. For one of the studies (Ultsch et al. 1978), species-specific Q_{10} values were calculated and reported. The data produced from that study were corrected using the Q_{10} values reported. For oxygen consumption data for the other studies, a Q_{10} of 1.83 was used, which was estimated by Clarke and Johnston (1999) to be the between-species Q_{10} for 69 species of teleost fish. After temperature standardizations, oxygen consumption units were converted to mg $O_2 \cdot kg^{-1} \cdot h^{-1}$. Conversion of units was performed assuming STP (one mol of oxygen gas per 22.391 L).

Measure of variance or error for metabolic rates and sample size was not reported for all the studies. Therefore, in order to statistically compare results among

species, 95% confidence intervals were calculated from each species that had a measure of variance provided. The rates were considered to be significantly different if the mean for one species, in which measures of variance were not provided, did not fall within the 95% confidence interval that was calculated for another species; these tests for mean differences were equivalent to one-sample *t*-tests. For the species that had a measure of variance provided, an *F*-test was first performed to determine if the variances were equal to proceed with a two-sample *t*-test. However, the results of the *F*-tests were that variances were not equal (data not shown). Therefore, the mean rates for these species were considered to be significantly different if the 95% confidence intervals of two species did not overlap.

The rates calculated for *E. variatum* and *E. blennioides* from the 48 h experiments were compared in three different ways to the rates recorded in the literature for other species. First, the average metabolic rate with the 95% confidence interval for the entire 48 h trials for each species was compared to metabolic rates reported for other darter species. Second, the average metabolic rate with 95% confidence interval for the first light period (Light1) for *E. variatum* and *E. blennioides* was compared to the other reported darter rates. Finally, the average metabolic rate with the 95% confidence interval consisting of the first dark and second light period (Dark1 and Light2) for *E. variatum* and *E. blennioides* was compared to the rates reported for the other darter species.

Results

A total of three published papers met the criteria to perform the comparative analysis of metabolic rate among darters (Table 6). Ultsch et al. (1978) reported rates

for 6 species of darters in the genus *Etheostoma*: *E. rufilineatum, E. flabellare, E. duryi, E. squamiceps, E. boschungi,* and *E. fusiforme*. Hartline (2013) studied, among other small freshwater fish, three species of darters: *E. jordani, Percina nigrofasciata,* and *P. palmaris*. Clausen (1936) also studied several species of freshwater fish, including one darter, *E. blennioides*. The metabolic rates for these species in addition to the rates calculated from the current project for *E. variatum* and *E. blennioides* resulted in a total of 11 darter species for which quantitative metabolic rate information was available for comparison (*E. blennioides* had metabolic rate recorded by Clausen (1936) and by the current project).

Table 6. Details of metabolic experiments found in the literature performed on different darter species.

Source	Darter species studied	Metabolic rate measurement methods	Duration of trials and measurement frequency	Acclimation time
Clausen 1936	*Etheostoma blennioides*	Winkler titration; Flow-through system	Sampled every h for 24 h	12 h
Ultsch et al. 1978	*E. rufilineatum, E. flabellare, E. duryi, E. squamiceps, E. boschungi, E. fusiforme.*	Oxygen partial pressure with Radiometer PHM 71 Mk2 Acid-base Analyser with 0_2 electrode; Closed system	1-4 h sampling intervals; trial durations varied	At least 2 h
Hartline 2013	*E. jordani, Percina. palmaris P. nigrofasciata*	Intermittent-flow respirometry; Strathkelvin Instruments, model 1302 oxygen electrode	2 measurements taken	1 h

Due to reports of metabolic rate at different temperatures and units, 95% confidence intervals could only be calculated for means of metabolic rate for *E. blennioides* (Clausen 1936), and *E. variatum* and *E. blennioides* (current project). As previously mentioned, the *F*-test results indicated that the variances were not equal for the mean metabolic rates of *E. blennioides* from Clausen 1936, *E. blennioides* from the

current study, or *E. variatum* from the current study (data not shown). Therefore, statistical significance was determined if 95% confidence intervals did not overlap. The average rate for *E. variatum* and *E. blennioides* over the full 48 h trials was first compared to the 10 reported rates for darters found in the literature (Figure 8). The results show that *E. blennioides* has the significantly greatest mean metabolic rate of the darter species presented. Interestingly, the rate for *E. blennioides* reported by Clausen (1936) is significantly greater than the average rate reported for the current project over 48 h. Both rates for *E. blennioides* are significantly greater than the metabolic rate of *E. variatum*. *E. variatum* has a mean metabolic rate that falls within the middle of the rates reported for the other 9 species of darters. It is greater than the rate for *E. jordani*, which has the lowest rate, but is less than rates reported for several of the other species (*E. boschungi, E. fusiforme,* and *P. palmaris*). Therefore, there is some variation among the mean metabolic rates for the different species of darters, but *E. blennioides* and *E. jordani* are most distinct with their high and low rates respectively.

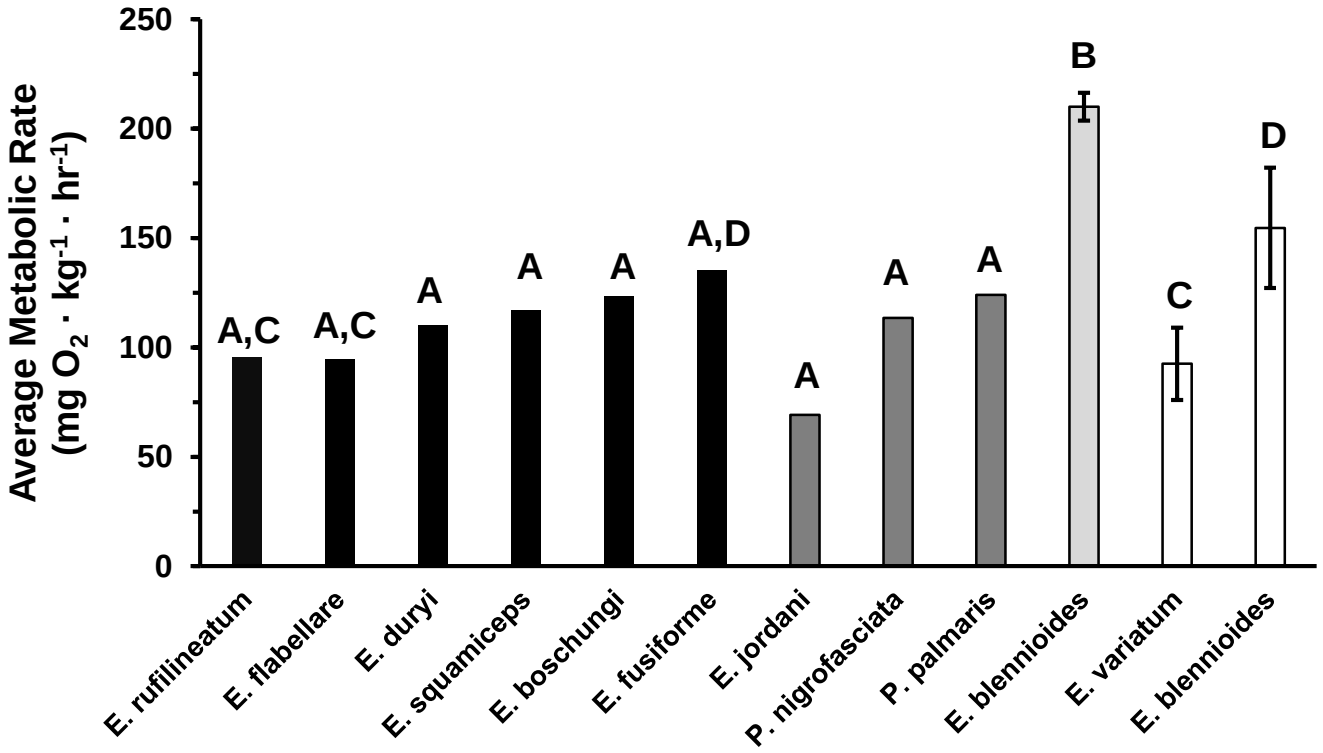

Figure 8. The average mass-specific metabolic rate for 11 species of darters, temperature standardized to 18°C and converted to mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$ (95% CI). Rates were obtained from published studies, with the color of the bar indicating different studies (▬, Ultsch et al. 1978; ▬, Hartline 2013; ▬, Clausen 1936). The white bars are the average rates calculated from the current project. Therefore, two mean rates are reported for *E. blennioides*, one published by Clausen 1936 and one from the current project. Means with the same letters are not significantly different.

The measurements of metabolic rate from the three published papers, were taken over a shorter period of time than the current study and during light hours. Therefore, the rates measured for *E. variatum* and *E. blennioides* over 48 h were truncated to only calculate the mean rate for the 4.5 h light period of the first day (Light1). The comparison of the mean metabolic rate for this period to the other published rates is presented in Figure 9. For these comparisons, the mean rate for *E. blennioides* during the first light period of the 48 h trials is greater than the rates reported for the other species. It is also important to note that there is much more variation reported for the mean rate for *E. blennioides*, as indicated by the wide range of

the 95% confidence interval. The rate for *E. variatum* is slightly greater when only looking at the first light period. Therefore, in relation to the other species, it is within the same range of metabolic rates and does not appear to be much different, other than being greater than the rate for *E. jordani*.

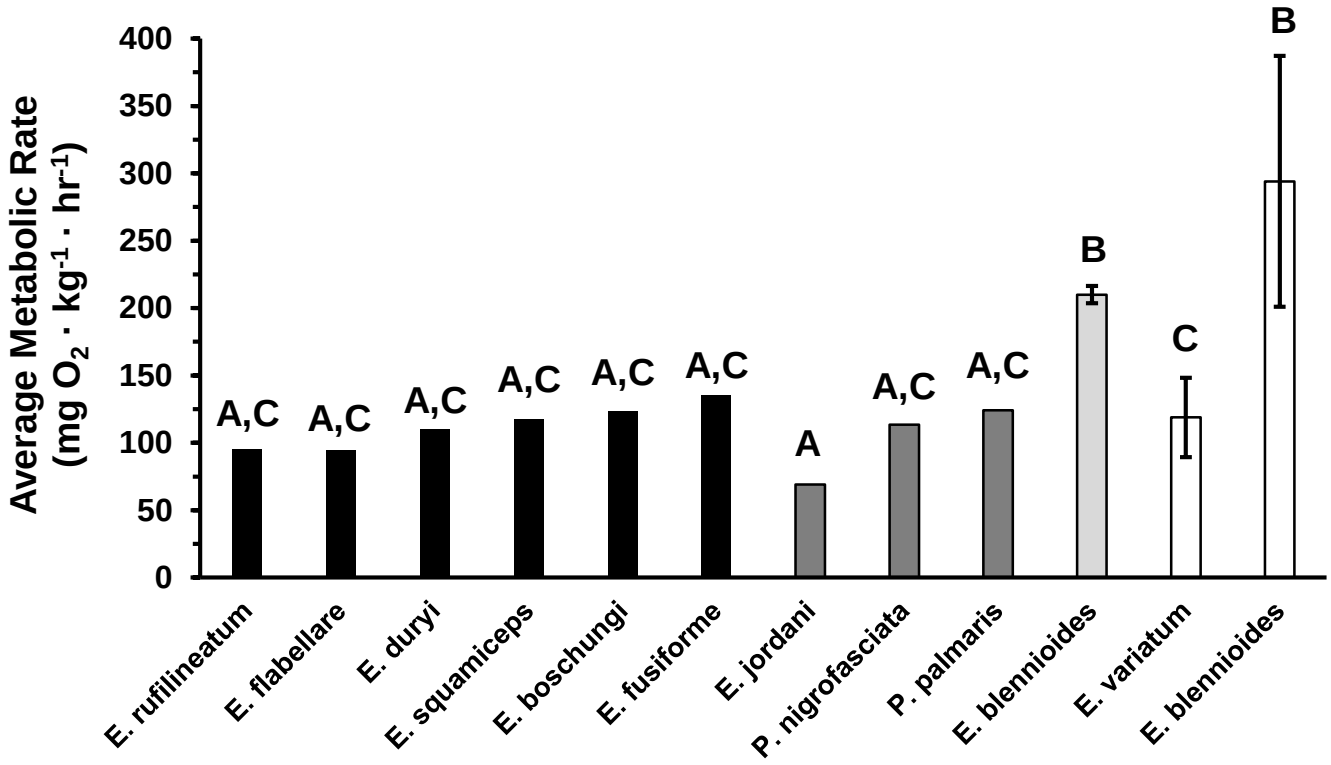

Figure 9. The average mass-specific metabolic rate for 11 species of darters, temperature standardized to 18°C and converted to mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$ (95% CI). Rates were obtained from published studies, with the color of the bar indicating different studies (▬, Ultsch et al. 1978; ▬, Hartline 2013; ▬, Clausen 1936). The white bars are the average rates calculated from the first light period (4.5 hours) from the current project. Therefore, two mean rates are reported for *E. blennioides*, one published by Clausen 1936 and one from the current project. Means with the same letters are not significantly different.

Finally, the metabolic rates recorded from the 48 h trials were truncated to a 24 h period from the onset of dark on day one (Dark1) to the end of light on day two (Light2). This was chosen as potentially a better representation of metabolic rate measured for these two species as it agreed more with the measurement durations from the other studies; this section excludes the likely unrealistically high rates from the first light

period, as well as the low rates observed as the trial extended into the third day. The comparisons for these data are presented in Figure 10, in which the mean rate for *E. blennioides* is much lower than when looking only at the first light period (Figure 9). The mean rate for *E. blennioides* from these trials is lower than the mean rate recorded by Clausen (1936). However, the 95% confidence intervals do overlap and these rates cannot be considered significantly different. Due to the wide range of the 95% confidence interval for the mean rate of *E. blennioides* from the 48 h trials, the confidence interval also overlaps with those for *E. variatum*, meaning that these mean rates cannot be considered significantly different. However, the mean rate recorded by Clausen for *E. blennioides* is significantly greater than the mean rate of *E. variatum*. Again, the mean rate for *E. variatum* is similar to that of the other 9 species, except it appears to still be greater than *E. jordani* when only including the 24-hour truncated data set for *E. variatum*.

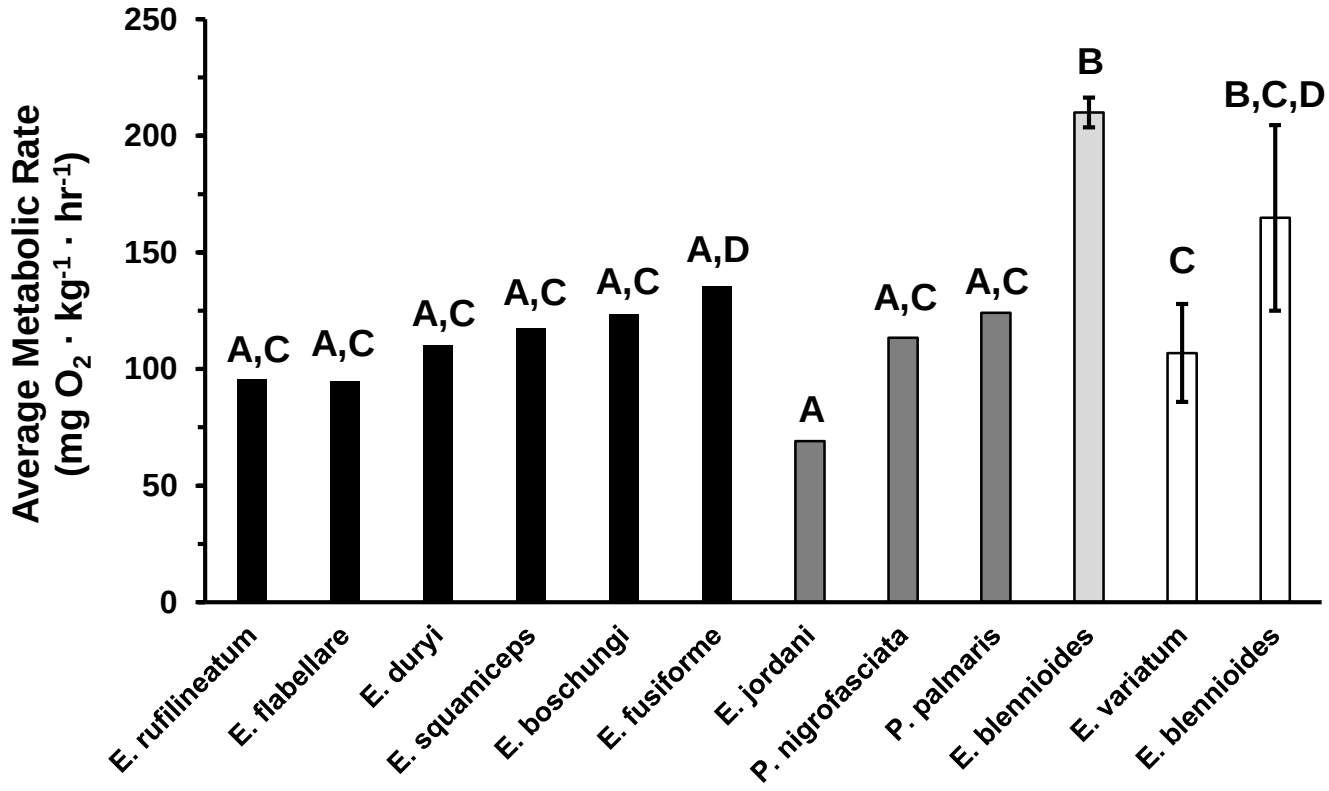

Figure 10. The average mass-specific metabolic rate for 11 species of darters, temperature standardized to 18°C and converted to mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$ (95% CI). Rates were obtained from published studies, with the color of the bar indicating different studies (▬, Ultsch et al. 1978; ▬, Hartline 2013; ▬, Clausen 1936). The white bars are the average rates calculated from the first dark and second light period (24 hours) from the current project. Therefore, two mean rates are reported for *E. blennioides*, one published by Clausen 1936 and one from the current project. Means with the same letters are not significantly different.

Discussion

Due to the lack of comparable data, there is limited, but important information that can be taken from these results. When initially comparing the results derived from the current study with the ten species rates found in the literature, there is interesting variation present. For instance, the highest rate recorded is for *E. blennioides* from Clausen 1936, with the lowest rate recorded for *E. jordani* being nearly a third of the rate for *E. blennioides.* Therefore, there is some variation among these species, but most of the other species produced rates around 100 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$. The mean 48 h

rate recorded from the current study for *E. variatum* fell within the range of most of the other species, with perhaps not much significant variation among these species. However, the mean rate recorded for *E. blennioides* was greater than all the other species rates, significantly greater than *E. variatum* and likely to be significantly greater than *E. jordani*. Therefore, *E. blennioides* does appear to be on the upper end of the metabolic spectrum for darter species and may impact their behavior and life history, enabling it to coexist with some of the other species. Perhaps what is most interesting from the initial comparisons of darter metabolic rates to the 48 h mean rates from the current study is the significant difference between the two different rates for *E. blennioides.* This perfectly illustrates the warnings of researchers (Steffensen 1989; Roche et al. 2013) to cautiously examine and compare metabolic data from studies using different methods. The difference between the two rates for *E. blennioides* is likely due to differences in equipment, technology, and methods. Clausen (1936) used Winkler titrations and a very different setup to house the fish and collect samples. Additionally, there was a longer acclimation period (12h) than the current study and sampling occurred every hour, whereas rates were estimated every 15 min in the current study. There was also no mention of the light cycle used during the experiments. However, it was consistent that both studies resulted in higher rates for *E. blennioides* than the other darter species in this comparison.

In an attempt to more reliably compare the results of the current study to the other reported darter rates, the rates for *E. blennioides* and *E. variatum* were truncated to the mean rate for the first 4.5 h of the 48 h trials. This comparison also helped to illustrate the impacts of methodology on the accuracy of the metabolic rates reported.

The reasoning behind the truncation of data to the first 4.5 h is that often metabolic studies will only measure over a short period of time during the day. The first 4.5 h of the current study's trials were the best representation of this methodology. When this was conducted, the rates for *E. variatum* and *E. blennioides* increased from 92.51 and 154.64 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ to 118.83 and 294.04 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$, respectively. It also changed the results of the comparisons. The two rates for *E. blennioides* were no longer significantly different, and the rate for the current study was much higher than all the other rate with a large amount of variation (LCI=200.9, UCI=387.2). The rate for *E. variatum* also increased, but was still within the range of rates recorded for the other species. The greater variation and higher mean rates when only the first 4.5 h of data were used is likely due to the fish still being stressed from transfer to experimental chamber (Steffensen 1989; Reebs 2002). By measuring the metabolic rate for these fish during these short periods, estimates for energetic models and whole ecosystem metabolism could become less accurate. For example, the metabolic rate per day for *E. blennioides* estimated from the 48 h average data is 3,711.36 mg $O_2 \cdot kg^{-1} \cdot d^{-1}$, and the yearly metabolic budget is estimated to be 1,354,646.40 mg $O_2 \cdot kg^{-1} \cdot y^{-1}$. If the daily and yearly budgets were to be extrapolated from the average calculated for only the first 4.5 h during the day, the estimates would be 7,056.96 mg $O_2 \cdot kg^{-1} \cdot d^{-1}$ and 2,575,790.40 mg $O_2 \cdot kg^{-1} \cdot y^{-1}$. That results in a 190% increase in the yearly energy budget. For *E. variatum*, estimates from the 48 h average data would be 2,220.24 mg $O_2 \cdot kg^{-1} \cdot d^{-1}$ and 810,387.60 mg $O_2 \cdot kg^{-1} \cdot y^{-1}$. If the average from the first 4.5 h is used, the estimates would be 2851.92 mg $O_2 \cdot kg^{-1} \cdot d^{-1}$ and 1,040,950.80 mg $O_2 \cdot kg^{-1} \cdot y^{-1}$. This is a 128% increase in the annual energy budget for *E. variatum.*

The final comparison made was made among the published rates and the data from the current study truncated to include only a 24 h period starting with the first dark period (Dark1) and continuing through the second light period (Light2) of the 48 h trials. It was apparent from the experimental section of this study that the 48 h trials were likely too long in duration and the fish appeared to have begun downregulating their metabolism towards the end of the experiments. Therefore, this 24 h section of data excluded the lower rates of the later stages of the experiments, as well as the high and variable rates of the early hours of the experiment. Thus, this was an effort to evaluate data that is believed to represent the most accurate levels of metabolism for these species. As a result, the rates for *E. blennioides* and *E. variatum* fell between the mean rates reported for these two species for the full 48 h mean and the first 4.5 h mean. Consequently, the 24 h mean rates for *E. blennioides* and *E. variatum* were not significantly different from each other and appear to not be different from most of the other species, with the exception of *E. jordani*, which continued to have the lowest rate. The two mean metabolic rates for *E. blennioides* were not significantly different and the range between them could be an accurate range for the standard metabolic rate for *E. blennioides.*

By performing these comparisons, it is now possible to see the effects of different experimental design and technique on the precision of metabolic data. As previously mentioned, studies have illustrated for quite some time that important information can be lost if only short trials are used to measure metabolism (Pierce and Wissing 1974; Evans 1984; Steffensen 1989). It is equally important to have a good understand of the ecology and habits of the fish prior to metabolic experiments if possible (Roche et al.

2013). Having this prior knowledge can assist in developing techniques that will most accurately measure the metabolism of the fish. For example, the species in this study could potentially be more active at times when these studies did not conduct their experiments and could have missed significant changes in their metabolism or their normal cyclic pattern of metabolic rate. Therefore, from the current comparison results, it is suggested that accurate measurements of metabolism of darter species would involve trials longer than a few hours, but not too long that it results in the beginning phase of starvation and downregulation of metabolism. For example, the fish could be fed during a longer acclimation period in the chamber to allow it to adapt to the chamber better, but not go without being fed for too long. An elimination of the fasting period prior to introduction into the chamber and a long enough acclimation period that would double as the fasting period would be another alternative to avoid some of these issues.

There are other limitations on the extent by which comparisons can be made for these eleven darter species. For example, Hartline (2013) was the only other study besides the current study to utilize intermittent-flow respirometry which has been long been the recommended method (Steffensen 1989). The closed system used by Ultsch et al. (1978) could have resulted in accumulation of CO_2 and waste products impacting the results. Clausen (1936) used a flow-through system which has problems associated with wash-out and lag in a change in oxygen consumption (Steffensen 1989). Therefore, it should be cautioned not to make large assumptions or conclusions regarding the variation in metabolic rate among these eleven darter species due to the different methods used.

However, it can be useful to note the similarity among the rates for these species and that there is some variation among them in regards to the mean metabolic rate. These results can begin to provide some insight into the physiological differences or similarities found among these closely related species. This study builds on a story about darter physiology that has not previously been a subject of much interest. The metabolic variation could be a result of many factors but determining what the factors are is beyond the scope of this study. This research served to initiate the path towards understand the physiological differences among darters and illustrate the need for additional research.

Finally, the main goal of this comparative analysis was to search the literature to find studies on the metabolism and/or energetics of darters to compare with the current study and gain a broader understanding of the variation among darters. However, of the roughly 200 species of darters, there were three published papers on their metabolism for a total of ten species studied (not including the current study). Though it was known going into this study that darters were understudied, it was surprising to find how truly underrepresented these species are in the metabolic literature. A great deal of novel information can be learned about these ecologically important species from simple metabolic experiments. Therefore, it is a hope that this study will enlighten the scientific community of the great need for darter research and spark an interest in these ecologically important and fascinating fish species.

CHAPTER 4

GENERAL DISCUSSION

Darter Physiology and Behavior

Darters, a large group of understudied but ecologically important fish species, were the subject of this research to determine physiological differences that could facilitate their common coexistence. Through respirometry experimentation, physiological and behavioral differences between *E. blennioides* and *E. variatum* were examined. The results of the experiments supported the hypothesis that the metabolic rate of *E. blennioides* is significantly different than the metabolic rate of *E. variatum; E. blennioides* had a significantly greater mean metabolic rate (154.64 ± SD 52.54 mg $O2 \cdot kg^{-1} \cdot hr^{-1}$) over the 48 h experimental period than *E. variatum* (92.51 ± SD 32.70 mg $O2 \cdot kg^{-1} \cdot hr^{-1}$). However, unlike other studies on fish metabolism that only report a single estimate for metabolic rate, providing limited information, this study was able to investigate more areas of the physiological ecology of *E. blennioides* and *E. variatum.* The patterns of the metabolic and behavioral cycles and the differences among light and dark periods, proxies for diel light cycles, for these two species provide novel information. *E. blennioides* had more variation associated with the mean rates recorded over the 48 h and the rates fluctuated more than the rates recorded for *E. variatum* which decreased and eventually leveled out after the first night. The variation for *E. blennioides* suggests that this species is more active than *E. variatum,* which was supported by the activity analysis. *E. blennioides* had consistently high activity levels that only appeared to decrease slightly during the two night periods. The activity levels of *E. variatum* fluctuated more; activity was highest at night and decreased over the

course of the daylight periods. Therefore, *E. variatum* may be more active at night during times when *E. blennioides* appears to have the least amount of activity.

These findings suggest differences in the behavior and ecology of these two species that could facilitate and perhaps help explain their coexistence. The higher metabolic rate of *E. blennioides* and increased activity may suggest that it is a bolder, more aggressive species than *E. variatum* (Metcalfe et al. 1995; Cutts et al. 1998; Killen et al. 2012). *E. blennioides* may perform more risk-taking behaviors during the day while *E. variatum* may be more conservative and only active at night. There has been some evidence of behavioral differences among other darter species that allow them to cohabitate (Winn 1958; Kessler and Thorp 1993; Williams and Mendelson 2010; Williams and Mendelson 2011), and could also be the case for *E. blennioides* and *E. variatum*. Dijkstra et al. (2013) studied the behavior of cichlids (*Pundamilia*) and found a correlation between agonistic behavior and metabolic differentiation of two sister species. The differences that Dijkstra et al. found provided evidence of behavioral isolation and metabolic differences that enable these cichlids to coexist. These results are applicable to the current study due to the similar adaptive radiation and high levels of sympatry that occur for cichlids and darters.

The studies on cichlid metabolic and behavioral differences can provide a useful template and direction for future research on darters. For example, though the activity analysis of the current study provided novel information about the behavior of *E. blennioides* and *E. variatum,* the way in which activity was measured missed large portions of activity and potentially important behavioral information. Specifically, the video recorded a ten s clip for each 15 min measurement period; thus a large portion of

the cycle was not monitored. Studies that focus on behavior that facilitates coexistence of cichlids, as well as other fish, can be important resources for darter research. Therefore, further investigation of the behavior of *E. variatum* and *E. blennioides,* such as continuous behavior monitoring, might further elucidate interspecific behavioral differences.

Metabolic Scaling. It has been known for quite some time that the metabolism of animals scales with body mass; Kleiber (1932) showed whole-organism metabolism to scale with body mass to ¾-power. The allometric scaling equations for the relationship between body mass (*M*) in grams and oxygen consumption ($\hat{V}_{O_2}$) in mmol O_2 h^{-1} produced for *E. blennioides* and *E. variatum* was compared to a more recent equation reported by Clarke and Johnston (1999) for all teleost fish,

$$\ln \hat{V}_{O_2} = 0.80 \ln M - 5.43.$$

Scaling functions for *E. blennioides* (*n*=14; R^2 =0.4283; *p*=0.0111) and *E. variatum,* (*n*=15; R^2 =0.1202; *p*=0.2056) respectively had a mass scaling equation of

$$\ln \hat{V}_{O_2} = 0.8753 \ln M - 5.196.$$

and

$$\ln \hat{V}_{O_2} = 0.8052 \ln M - 5.543.$$

The interspecific scaling equation (*n*=29; R^2 = 0.1037; *p*=0.0884) was:

$$\ln \hat{V}_{O_2} = 0.4302 \ln M - 4.6759.$$

Therefore, only the scaling equation for *E. blennioides* was significant (*p*=0.0111) However, the scaling exponents produced for the intraspecific equations are similar to the that in the reported equation from Clarke and Johnston as well as the Metabolic Theory of Ecology (¾-power scaling exponent), which attempts to describe the broad

patterns of ecology by means of the universal scaling of metabolism (Brown et al. 2004). The confidence intervals for the scaling exponent of *E. blennioides* (Table 7) show that the exponent estimated from the current study is not significantly different from the scaling exponents reported by Clarke and Johnston (1999) and Brown et al. (2004).

Table 7. Scaling exponent for allometric scaling equation for the relationship between body mass and metabolic rate for *E. blennioides* as measured during 48-hour respirometry experiments.

Source	*n*	Scaling Exponent	*p*	95% Confidence Interval	R^2
E. blennioides	14	0.8753	0.0111	0.2392 - 1.5113	0.4283

Killen et al. (2008) attribute some variation in scaling equations to lifestyle and locomotory ability. In the current study, no attempt was made to control for sex, age, activity, or personalities, which could contribute to the variation. The intent of mass-based scaling equations is to show the major factors (i.e. body mass and temperature) that contribute to the variation of metabolic rate and that these relationships can help explain ecological dynamics (Brown et al. 2004). Therefore, a more controlled study focused on understanding the scaling laws for darters could better regulate for these factors and further develop an understanding of the relationship between body size and metabolism for darters. Fortunately, the trade-off is that with the less controlled metabolic study presented, perhaps more universally applicable equations were obtained, providing a realistic representation of darter metabolism in nature.

Interspecies Comparisons

The comparative analysis of all published and comparable darter metabolic data was intended to compile energetic information for as many darter species as possible to

make a broader comparison among these closely related species. To an extent, this comparison was conducted. However, it successfully illustrated the significant lack of physiological data available for this diverse and ecologically important group of fish. A mere eleven species out of about ~200 known darter species had metabolic information available. Therefore, it is clear that there needs to be more effort focused on the physiological ecology of these species as information from ~5% of the species may not be representative of the group and likely misses ecological important interspecific variations among conspecific fishes.

The comparisons of the species that were available resulted in some variation in metabolism among the eleven species (Figures 8, 9, 10). *E. blennioides* had the highest metabolic rate while *E. jordani* had a much lower rate than all the other species. Most of the other species had a mean metabolic rate around 100 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$, with the average rate for the darters species presented being 146.27 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$. Therefore, without a further detailed investigation, it can be concluded that darters do exhibit some interspecific variation in regards to metabolic rate, with rates on the ends of the spectrum being different enough that their physiological ecology could facilitate cohabitation of these species.

Utilizing the data from the current study of 48-hour trials enabled interesting comparisons to be made that were not the initial intention of this analysis. The length of the current trials made it possible to compare the mean rates for *E. blennioides* and *E. variatum* to the species of the other studies that used slightly different results. For the other studies that were shorter in length or did not even include measurements during the night, this type of a comparison revealed that differences in diel cycles may be

missed. At least for the case of *E. variatum*, there was increased activity at night and changes in metabolism that would not have been reflected in a mean rate for the species if a trial was only performed during light hours. As cautioned by several researchers (Pierce and Wissing 1974; Evans 1984; Steffensen 1989; Roche et al. 2013), data from studies using different methods should be examined with the knowledge that limited conclusions can be made. The current comparative analysis then illustrated the importance of this caution by manipulating the results of the current study's respirometry experiments. The data were truncated to only include parts of the trial that were more comparable to methods of the other studies. The mean rates for *E. blennioides* and *E. variatum* changed as did the significance of the differences between them and the other species. Therefore, the longer trials allowed for more comparisons to be made revealing variation in the interspecific rates of the darters.

Conclusion

This study did seek to find out more information about the physiological ecology of *E. blennioides* and *E. variatum* rather than simply determining a single rate to represent these species. The study was successful in achieving this goal, but it also helped to guide future metabolic research. It was evident that 48 h trials with a 24 h fasting period prior to the start may have caused the fish to downregulate their metabolism and produce lower than normal metabolic rates. However, in the case of *E. blennioides,* this species did not appear to have a long enough acclimation period and the rates in the beginning of the trial were likely greater than normal. As Roche et al. 2013 suggested, it is important to have a good understanding of the ecology and habits of the fish prior to experimenting to produce more accurate results.

Finally, this research provided novel information in several areas of darter physiology and behavior that will assist in the understanding of differences among these highly sympatric species. The variation observed in the results support the ideas of niche theories that suggest that ecologically similar species must have subtle differences to coexist (Grinnell 1928; Gause 1932; Hutchison 1957; Hardin 1960). Whether it was trial length, activity, metabolic cycling, or some other source, the main cause of the variation recorded was not determined and is beyond the scope of this study. Therefore, metabolic and behavioral differences found in this research provide a starting point for future studies and hopefully increase interest in the physiological ecology of darters.

REFERENCES

Anras, M.-L.B., J.P. Lagardere, and J.-Y. Lafaye. 1997. Diel activity rhythm of seabass tracked in a natural environment: group effects on swimming patterns and amplitudes. Canadian Journal of Fisheries and Aquatic Sciences. 54: 162-168.

Ashton, M.J. and J.B. Layzer. 2010. Summer microhabitat use by adult and young-of-year snail darters (*Percina tanasi*) in two rivers. Ecology of Freshwater Fish. 19: 609-617.

Bednarek, A.T. and D.D. Hart. 2005. Modifying dam operations to restore rivers: ecological responses to Tennessee river dam mitigation. Ecological Applications. 15: 997-1008.

Bennett, A.F. 1978. Activity metabolism of the lower vertebrates. Annual Review of Physiology. 40: 447-469.

Biro, P.A. and J.A. Stamps. 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends in Ecology and Evolution. 25: 653-659.

Blank, J.M., C.J. Farwell, J.M. Morrissette, R.J. Schaller, and B.A. Block. 2007. Influence of swimming speed on metabolic rates in juvenile pacific bluefin tuna and yellowfin tuna. Physiological and Biochemical Zoology. 80: 167-177.

Boisclair, D. and M. Tang. 1993. Empirical analysis of the influence of swimming pattern on the net energetic cost of swimming in fishes. Journal of Fish Biology. 42: 169–183.

Brett, J.R. 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). American Zoologist. 11:99–113.

Brett, J.R. 1972. The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. Respiration Physiology. 14: 151-170.

Brett, J.R. and A. Zala. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. Journal of the Fisheries Research Board of Canada. 32: 2479-2486.

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

Bunn, S.E., N.R. Bond, J.A. Davis, B. Gawne, M.J. Kennard, A.J. King, R.T. Kingsford, J.D. Koehn, S. Linke, J.M. Olley, E.E. Peterson, C.A. Pollino, F. Sheldon, N.C. Sims, R.M. Thompson, D. Ward, and R.J. Watts. 2014. Ecological responses to altered flow regimes: Synthesis report. CSIRO Water for a Healthy Country Flagship, Australia.

Camak, D.T. 2012. Assessing the impact of low-head dams and life history on fine scale genetic structure of three Etheostomatine darters (Percidae). M.S. Southeastern Louisiana University, Ann Arbor.

Careau, V., D. Thomas, M.M. Humphries, and D. Reale. 2008. Energy metabolism and animal personality. Oikos. 117: 641-653.

Carlson, R.L., P.C. Wainwright, and T.J. Near. 2009. Relationship between species co-occurrence and rate of morphological change in Percina darters (Percidae: Etheostomatinae). Evolution. 63: 767–778.

Carlson, R.L. and P.C. Wainwright. 2010. The ecological morphology of darter fishes (Percidae: Estheostomatinae). Biological Journal of the Linnean Society. 100: 30-45.

Chipps, S.R., W.B. Perry, and S.A. Perry. 1994. Patterns of microhabitat use among four species of darters in three Appalachian streams. American Midland Naturalist. 131:175-180.

Cho, C.Y., S.J. Slinger, and H.S. Bayler. 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry and Physiology. 73: 25-41.

Clarke, A. and N.M. Johnston. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. Journal of Animal Ecology. 68: 893-905.

Clausen, R.G. 1936. Oxygen consumption in fresh water fishes. Ecology. 17: 216-226.

Cooke, S.J., C.M. Bunt, S.J. Hamilton, C.A. Jennings, M.P. Pearson, M.S. Cooperman, D.F. Markle. 2005. Threats, conservation strategies, and prognosis for suckers (Catostomidae) in North America: insights from regional case studies of a diverse family of non-game fishes. Biological Conservation. 121: 317-331.

Cushing, J.M., S. Levarge, N. Chitnis, and S.M. Henson. 2004. Some discrete competition models and the competitive exclusion principle. Journal of Difference Equations and Applications. 10: 1139-1151.

Cutts, C.J., N.B. Metcalfe, and A.C. Taylor. 1998. Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. Journal of Fish Biology. 52: 1026-1037.

Dijkstra, P.D., O. Seehausen, T.G.G. Groothuis. 2008. Intrasexual competition among females and the stabilization of a conspicuous colour polymorphism in a Lake Victoria cichlid fish. Proceedings of the Royal Society B: Biological Sciences. 275: 519-526.

Dijkstra. P.D., O. Seehausen, and N.B. Metcalfe. 2013. Metabolic divergence between sibling species of cichlids *Pundamilia nyereri* and *Pundamilia pundamilia*. Journal of Fish Biology. 82: 1975-1989.

Dijkstra. P.D., M.E.R. Pierotti, O. Seehausen, and N.B. Metcalfe. 2016. Metabolism, oxidative stress, and territorial behaviour in a female colour polymorphic cichlid fish. Behavioral Ecology and Sociobiology. 70: 99-109.

Driver, L.J., and G.L. Adams. 2013. Life history and spawning behavior of the western sand darter (*Ammocrypta clara*) in Northeast Arkansas. American Midland Naturalist. 170: 199-212.

Dwyer, G.K., R.J. Stoffels, and P.A. Pridmore. 2014. Morphology, metabolism, and behavior: responses of three fishes with different lifestyles to acute hypoxia. Freshwater Biology. 59: 819-831.

Eme, D., F. Malard, C. Colson-Proch, P. Jean, S. Calvignac, L. Konecny-Dupre, F. Hervant, and C.J. Douady. 2014. Integrating phylogeography, physiology and habitat modelling to explore species range determinants. Journal of Biogeography. 41: 687-699.

Enders, E.C., D. Boisclair, A.G. Roy. 2003. The effect of turbulence on the cost of swimming for juvenile Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatics Sciences. 60: 1149-1160.

Evans, D.O. 1984. Temperature independence of the annual cycle of standard metabolism in the pumpkinseed. Transactions of the American Fisheries Society. 113: 494-512.

Facey, D.E. and G.D. Grossman. 1990. The metabolic cost of maintaining position for four North American stream fishes: effects of season and velocity. Physiological Zoology. 63: 757-776.

Ficke, A.D., C.A. Myrick, and L.J. Hansen. 2007. Potential impacts of global climate change on freshwater fisheries. Reviews in Fish Biology and Fisheries. 17: 581-613.

Fischer, P. 2000. An experimental test of metabolic and behavioural responses of benthic fish species to different types of substrate. Canadian Journal of Fisheries and Aquatic Sciences. 57: 2336-2344.

Fisher, S.G., D.E. Busch, and N.B. Grimm. 1981. Diel feeding chronologies in two Sonoran Desert stream fishes, *Agosia chrysogaster* (Cyprinidae) and *Pantosteum clakri* (Catostomidae). The Southwestern Naturalist. 26: 31-36.

Fisher, W.L. and W.D. Pearson. 1987. Patterns of resource utilization among four species of darters in three central Kentucky streams. Pg.69-76. In: Matthews, W.J. and D.C. Heins (eds.). Community and Evolutionary Ecology of North American Stream Fishes. University of Oklahoma Press, Norman, Oklahoma.

Fry, F.E.J. 1947. Effects of the environment on animal activity. University of Toronto Studies, Biological Series No. 55. Publications of the Ontario Fisheries Research Laboratory No. 68.

Garcı´a, A., C. Sobenesa, O. Link, and E. Habit. 2012. Bioenergetic models of the threatened darter *Percilia irwini*. Marine and Freshwater Behaviour and Physiology. 45: 17-28.

Gause, G.F. 1932. Experimental studies on the struggle for existence. Journal of Experimental Biology. 9: 389-402.

Grinnell, J. 1928. Presence and absence of animals. University of California Chronicle. 30: 429-450.

Haesler, M.P. and O. Seehausen. 2005. Inheritance of female mating preference in a sympatric sibling species pair of Lake Victoria cichlids: implications for speciation. Proceedings of the Royal Society B: Biological Sciences. 272: 237-245.

Haponski, A. E., and C. A. Stepien. 2008. Molecular, morphological, and biogeographic resolution of cryptic taxa in the greenside darter *Etheostoma blennioides* complex. Molecular Phylogenetics and Evolution. 49: 69-83.

Hardin, G. 1960. The competitive exclusion principle. Science. 131: 1292-1297.

Harding, J.M., A.J. Burky, and C.M. Way. 1998. Habitat preferences of the rainbow darter, *Etheostoma caeruleum*, with regard to microhabitat velocity shelters. Copeia 1998: 988-997.

Hargrave, C.W., and J.E. Johnson. 2003. Status of Arkansas darter, *Etheostoma cragini*, and least darter, *E. microperca*, in Arkansas. The Southwestern Naturalist. 48: 89-92.

Hartline, N.R. 2013. Differences in oxygen consumption and critical oxygen levels of five stream fishes. Thesis. Auburn University. Auburn, Alabama.

Heino, J., R. Virkkala, and H. Toivonen. 2009. Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. Biological Reviews. 84: 39-54.

Hester, E.T. and M.W. Doyle. 2011. Human impacts to river temperature and their effects on biological processes: a quantitative synthesis. Journal of the American Water and Resources Association. 47: 571-587.

Hill, L.G. and W.J. Matthews. 1980. Temperature selection by the darters *Etheostoma spectabile* and *Etheostoma radiosum* (Pisces: Percidae). American Midland Naturalist. 104: 412-415.

Hlohowskyj, I. and A.M. White. 1983. Food resource partitioning and selectivity by the greenside, rainbow, and fantail darters (Pisces: Percidae). The Ohio Journal of Science. 83: 201-208.

Hlohowskyj, I., and T.E. Wissing. 1987. Seasonal changes in the thermal preferences of fantail (*Etheostoma flabellare*) rainbow (*E. caeruleum*), and greenside (*E. blennioides*) darters. Community and Evolutionary Ecology of North American Stream Fishes. University of Oklahoma Press, Norman, OK. pp. 105-110.

Huang Q, Zhang Y, Liu S, Wang W, Luo Y. 2013. Intraspecific scaling of the resting and maximum metabolic rates of the crucian carp (*Carassius auratus*). PLoS ONE 8: e82837. doi: 10.1371/journal.pone.0082837.

Hutchison, G.E. 1957. Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology. 22: 415-427.

Hutchison, G.E. 1961. The paradox of plankton. The American Naturalist. 95: 137-145.

Ingersoll, C.G. and D.L. Claussen. 1984. Temperature selection and critical thermal maxima of the fantail darter, *Etheostoma flabellare*, and the johnny darter, *E. nigrum*, related to habitat and season. Environmental Biology of Fishes. 11: 131–138.

Johnston, C., A. Henderson, and W. Hartup. 2013. Precipitous decline and conservation of slackwater darter (*Etheostoma boschungi*) in tributaries of the Tennessee River, Tennessee and Alabama. Biodiversity and Conservation. 22: 3247-3259.

Keck, B.P., and T.J. Near. 2009. Patterns of natural hybridization in darters (Percidae: Etheostomatinae). Copeia. 4: 758.

Kentucky Division of Water (KDOW). 2003. Development and application of the Kentucky Index of Biotic Integrity (KIBI). Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Kessler, R.K., and J.H. Thorp. 1993. Microhabitat segregation of the threatened spotted darter (*Etheostoma maculatum*) and closely related orangefin darter (*E. bellum*). Canadian Journal of Fisheries and Aquatic Sciences. 50: 1084-1091.

Killen, S.S., D. Atkinson, D. Glazier. 2008. Ecological factors contributing to variation in the scaling of metabolic rate with body mass in fishes. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology. 150: Supplement. p. S111.

Killen, S.S., S. Marras, and D.J. McKenzie. 2011. Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. Journal of Animal Ecology. 80: 1024-1033.

Killen, S.S., S. Marras, M.R. Ryan, P. Domenici, and D.J. McKenzie. 2012. A relationship between metabolic rate and risk-taking behavior is revealed during hypoxia in juvenile European sea bass. Functional Ecology. 26: 134-143.

Kleiber, M. 1932. Body size and metabolism. Hilgardia. 6: 315-353

Kuno, E. 1992. Competitive exclusion through reproductive interference. Population Ecology. 34: 275-284.

Lankford, S.E., T.E. Adams, R.A. Miller, and J.J. Cech Jr. 2005. The cost of chronic stress: impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. Physiological and Biochemical Zoology. 78: 599-609.

Leibold, M. 1995. The niche concept revisited: mechanistic models and community context. Ecology. 76: 1371-1382.

Lighton, J.R.B. 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, Inc. New York, NY.

Magalhaes, I.S., S. Mwaiko, M.V. Schneider, and O. Seehausen. 2009. Divergent selection and phenotypic plasticity during incipient speciation in Lake Victoria cichlid fish. Journal of Evolutionary Biology. 22: 260-274.

Martin, C.H. and M.J. Genner. 2009. High niche overlap between two successfully coexisting pairs of Lake Malawi cichlid fishes. Canadian Journal of Fisheries and Aquatic Sciences. 66: 579-588.

May, B. 1969. Observations on the biology of the variegated darter, *Etheostoma variatum* (Kirtland). The Ohio Journal of Science. 69: 85-92.

McCormick, F.H. and N. Aspinwall. 1983. Habitat selection in three species of darters. Environmental Biology of Fishes. 8: 279-282.

Merino, G.E., D.E. Conklin, and R.H. Piedrahita. 2011. Diel rhythms of oxygen consumption rate of California halibut (*Paralichthys californicus*) under culture in a recirculating system. Aquacultural Engineering. 45: 28-34.

Metcalfe, N.B., A.C. Taylor, and J.E. Thorpe. 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. Animal Behavior. 49: 431-436.

Meyer, J.L., M.J. Sale, P.J. Mulholland, and N.L. Poff. 1999. Impacts of climate change on aquatic ecosystem functioning ad health. Journal of the American Water Resources Association. 35: 1373-1386.

Miller, R.V. 1968. A Systematic Study of the Greenside Darter, *Etheostoma blennioides* Rafinesque (Pisces: Percidae). Copeia. 1968: 1-40.

Moyle, P.B. and J.J. Cech Jr. 2004. Fishes, an introduction to ichthyology. Pearson Education Inc., Upper Saddle River, NJ.

O'Dee, S.H. and G.T. Watters. 1998. New or confirmed host identifications for ten freshwater mussels. Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium. Ohio Biological Survey. pp. 77-82.

Page, L.M. 1983. Handbook of darters. TFH Publications, Inc., Neptune City, NJ.

Page, L.M. and B.M. Burr. 2011. Peterson field guide to freshwater fishes of North America north of Mexico. 2nd ed. Houghton Mifflin Marcourt Publishing Company, New York, NY.

Peters, R.H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge, UK.

Pianka, E.R. 1981. Competition and niche theory. In: May, R.M, editor. Theoretical ecology: principles and applications. 2nd ed. Sunderland, MA. Sinauer Associates, Inc. pp. 167-196.

Pierce, R.J. and T.E. Wissing. 1974. Energy cost of food utilization in the bluegill (*Lepomis macrochirus*). Transactions of the American Fisheries Society. 103: 38-45.

Piller, K.R., H.L. Bart, Jr., and D.L. Hurley. 2008 Phylogeography of the greenside darter complex, *Etheostoma blennioides* (Teleostomi: Percidae): A wide-ranging polytypic taxon. Molecular Phylogenetics and Evolution. 46: 974-985.

Reebs, S.G. 2002. Plasticity of diel and circadian activity rhythms in fishes. Reviews in Fish Biology and Fisheries. 12: 349-371.

Richter, B.D. and G.A. Thomas. 2007. Restoring environmental flows by modifying dam operations. Ecology and Society. 12: 12. [online] URL: http://www.ecologyandsociety.org/vol12/iss1/art12/.

Roche, D.G., S.A. Binning, Y. Bosiger, J.L. Johansen, and J.L. Rummer. 2013. Finding the best estimates of metabolic rates in a coral reef fish. The Journal of Experimental Biology. 216: 2103-2110.

Small, J.W., Jr. 1975. Energy dynamics of benthic fishes in a small Kentucky stream. Ecology. 56: 827-840.

Schmidt-Nielsen, K. 1984. Scaling: why is animal size so important? Cambridge University Press, Cambridge, UK.

Skyfield, J.P. and G.D. Grossman. 2008. Microhabitat use, movements and abundance of gilt darters (*Percina evides*) in southern Appalachian (USA) streams. Ecology of Freshwater Fish. 17: 219-230.

Stauffer, J.R., Jr., J.M Boltz, K.A Kellogg, and E.S van Snik. 1996. Microhabitat partitioning of darters in the Allegheny River system. Environmental Biology of Fishes. 46: 37-44.

Steffensen, J. 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish Physiology and Biochemistry. 6: 49-59.

Stepien, C.A., and A.E. Haponski. 2010. Systematics of the greenside darter *Etheostoma blennioides* complex: Consensus from nuclear and mitochondrial DNA sequences. Molecular Phylogenetics and Evolution. 57: 434-447.

Strange, K.T., J.C. Vokoun, and D.B. Noltie. 2002. Thermal tolerance and growth differences in orangethroat darter (*Etheostoma spectabile*) from thermally contrasting adjoining streams. The American Midland Naturalist. 144: 120-128.

Strayer, D.L. 2008. Freshwater mussel ecology: a multifactor approach to distribution and abundance. 1st ed. University of California Press. Los Angeles, California.

Svendsen, J.C., J. Genz, W.G. Anderson, J.A. Stol, D.A. Watkinson, E.C. Enders. 2014. Evidence of circadian rhythm, oxygen regulation capacity, metabolic repeatability and positive correlations between forced and spontaneous maximal metabolic rates in lake sturgeon *Acipenser fulvescens*. PLoS ONE 9: e94693. doi:10.1371/journal.pone.0094693.

Switzer, J.F., S.A. Welsh, and T.L. King. 2008. Permanent genetic resources: microsatellite DNA primers for the candy darter, *Etheostoma osburni* and variegate darter, *Etheostoma variatum*, and cross-species amplification in other darters (Percidae). Molecular Ecology Resources. 8: 335-338.

Takahashi, T. and S. Koblmuller. 2011. The adaptive radiation of cichlid fish in Lake Tanganyika: a morphological perspective. International Journal of Evolutionary Biology. 2011. Article ID 620754.

Throp, J.H., J.E. Alexander, Jr., B.L. Bukaveckas. G.A. Cobbs, and K.L. Bresko. 1998. Response of Ohio River and Lake Erie dreissenid molluscs to changes in temperature and turbidity. Canadian Journal of Fisheries and Aquatic Sciences. 55: 220-229.

Turner, G.F., O. Seehausen, M.E. Knight, C.J. Allender, and R.L. Robinson. 2001. How many species of cichlid fishes are there in African lakes? Molecular Ecology. 10: 793-806.

Ultsch, G.R., H. Boschung, and M.J. Ross. 1978. Metabolism, critical oxygen tension, and habitat selection in darter (Etheostoma). Ecology. 59: 99-107.

Urbina, M.A. and C.N. Glover. 2013. Relationship between fish size and metabolic rate in the oxyconforming Inanga *Galaxias maculatus* reveals size-dependent strategies to withstand hypoxia. Physiological and Biochemical Zoology. 86: 740-749.

Vandermeer, J.H. 1972. Niche theory. Annual Review of Ecology and Systematics. 3: 107-132.

Violle, C., D.R. Nemergut, Z. Pu, and L. Jiang. 2011. Phylogenetic limiting similarity and competitive exclusion. Ecology Letters. 14: 782-787.

Wehnes, R.A. 1973. The food and feeding interrelationships of five sympatric darter species (Pisces: Percidae) in Salt Creek, Hocking County, Ohio. M. S. Thesis, Ohio State University, Columbus, Ohio.

Welsh, S.A and S.A. Perry. 1998. Habitat partitioning in a community of darters in the Elk River, West Virginia. Environmental Biology of Fishes. 51: 411-419.

West, G.B., J.H. Brown, and B.J. Enquist. 1997. A general model for the origin of allometric scaling laws in biology. Science. 276: 122-126.

West, G.B., W.H. Woodruff, and J.H. Brown. 2002. Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. Proceedings of the National Academy of Sciences. 99: 2473-2478.

Weston, M.R., R.L. Johnson, and A.D. Christian. 2010. Niche partitioning of the sympatric yellowcheek darter *Etheostoma moorei* and rainbow darter *Etheostoma caeruleum* in the Little Red River, Arkansas. American Midland Naturalist. 164: 187-200.

White, C.R. and R.S. Seymour. 2003. Mammalian basal metabolic rate is proportional to body mass$^{2/3}$. Proceedings of the National Academy of Sciences of the United States. 100: 4046-4049.

Williams, T.H. and T.C. Mendelson. 2010. Behavioral isolation based on visual signals in a sympatric pair of darter species. Ethology. 116: 1038-1049.

Williams, T.H. and T.C. Mendelson. 2011. Female preference for male coloration may explain behavioural isolation in sympatric darters. Animal Behavior. 82: 683-689.

Williams, T.H. and T.C. Mendelson. 2013. Male and female responses to species-specific coloration in darters (Percidae: Etheostoma). Animal Behavior. 85: 1251-1259.

Winn, H.E. 1958. Comparative reproductive behavior and ecology of fourteen species of darters (Pisces-Percidae). Ecological Monographs. 28: 155-191.

APPENDIX A

Letters from Institutional Animal Care and Use Committee

MARSHALL UNIVERSITY
www.marshall.edu

Animal Resource Facility

DATE:	April 22, 2014
TO:	Jeff Kovatch, Dr.
FROM:	Marshall University IACUC
IACUC #:	577
PROJECT TITLE:	[586876-2] Impacts of cold and hypoxic water conditions from dam releases on variegate (Etheostoma variatum) darters.
SUBMISSION TYPE:	New Project, Revised application
ACTION:	APPROVED
APPROVAL DATE:	April 22, 2014
EXPIRATION DATE:	April 21, 2017
REVIEW TYPE:	Full Committee followed by Designated Member Review

Thank you for your submission of Revision materials for this research project. The Marshall University IACUC has APPROVED your submission. All research must be conducted in accordance with this approved submission.

This submission has received Designated Member Review.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the appropriate revision forms for this procedure.

Please report all NON-COMPLIANCE issues regarding this project to this committee.

This project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Monica Valentovic at (304) 696-7332 or valentov@marshall.edu. Please include your project title and reference number in all correspondence with this committee.

Monica A. Valentovic, Ph.D.
Chairperson, IACUC

MARSHALL UNIVERSITY
www.marshall.edu

Animal Resource Facility

DATE:	April 22, 2014
TO:	Jeff Kovatch, Ph.D.
FROM:	Marshall University IACUC
IACUC #:	578
PROJECT TITLE:	[587294-2] Impacts of cold and hypoxic water conditions from dam releases on candy (Etheostoma osburni) darters.
SUBMISSION TYPE:	New Project Revsed application
ACTION:	APPROVED
APPROVAL DATE:	April 22, 2014
EXPIRATION DATE:	April 21, 2017
REVIEW TYPE:	Full Committee followed by Designated Member Review

Thank you for your submission of Revision materials for this research project. The Marshall University IACUC has APPROVED your submission. All research must be conducted in accordance with this approved submission.

This submission has received Designated Member Review.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the appropriate revision forms for this procedure.

Please report all NON-COMPLIANCE issues regarding this project to this committee.

This project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Monica Valentovic at (304) 696-7332 or valentov@marshall.edu. Please include your project title and reference number in all correspondence with this committee.

Monica A. Valentovic, Ph.D.
Chairperson, IACUC

MARSHALL UNIVERSITY
www.marshall.edu

Animal Resource Facility

DATE:	June 4, 2014
TO:	Jeff Kovatch, Ph.D.
FROM:	Marshall University IACUC
IACUC #:	577
PROJECT TITLE:	[586876-3] Impacts of altered flow from dam releases on variegate (Etheostoma variatum) darters.
SUBMISSION TYPE:	Amendment/Modification
ACTION:	APPROVED
APPROVAL DATE:	June 4, 2014
EXPIRATION DATE:	April 21, 2017
REVIEW TYPE:	Full Committee Review

Thank you for your submission of Amendment/Modification materials for this research project. The Marshall University IACUC has APPROVED your submission. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the appropriate revision forms for this procedure.

Please report all NON-COMPLIANCE issues regarding this project to this committee.

This project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Monica Valentovic at (304) 696-7332 or valentov@marshall.edu. Please include your project title and reference number in all correspondence with this committee.

Monica A. Valentovic, Ph.D.
Chairperson, IACUC

MARSHALL UNIVERSITY
www.marshall.edu

Animal Resource Facility

DATE:	June 4, 2014
TO:	Jeff Kovatch, Ph.D.
FROM:	Marshall University IACUC
IACUC #:	578
PROJECT TITLE:	[587294-3] Impacts of altered flow from dam releases on candy (Etheostoma osburni) darters.
SUBMISSION TYPE:	Amendment/Modification
ACTION:	APPROVED
APPROVAL DATE:	June 4, 2014
EXPIRATION DATE:	April 21, 2017
REVIEW TYPE:	Full Committee Review

Thank you for your submission of Amendment/Modification materials for this research project. The Marshall University IACUC has APPROVED your submission. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the appropriate revision forms for this procedure.

Please report all NON-COMPLIANCE issues regarding this project to this committee.

This project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Monica Valentovic at (304) 696-7332 or valentov@marshall.edu. Please include your project title and reference number in all correspondence with this committee.

Monica A. Valentovic, Ph.D.
Chairperson, IACUC

APPENDIX B

Vita

Emma Kist

Education

2013 B.S. Biology	West Virginia Wesleyan College Buckhannon, WV 26201 Cumulative GPA 4.0

Work Experience

January 2016-Present	Regulatory Specialist United States Army Corps of Engineers Huntington District Huntington, WV 25701
2014-2016	Graduate Teaching Assistant Marshall University Department of Biological Sciences
Summer 2012	Animal Care Intern Oglebay Good Zoo Wheeling, WV
Summer 2011	WV IDeA Network of Biomedical Research Excellence Intern Marshall University Department of Pharmacology, Physiology, and Toxicology

Field and Laboratory Experience

Fish Collection: Halltech-2000 Battery Backpack Electrofisher, seine, and kick-nets used to sample and collect specimens for thesis research. Electric seining, kick seine, Smith-Root GPP 5.0 boat electroshocking, Smith Root LR-20B backpack shocker used for Ichthyology field work.

Simulated-Stream Maintenance: Water quality and chemistry analyses of simulated stream for housing of fish using YSI 556 handheld Water Quality Meter and Barometer, pH probes, and nitrate and ammonia testing using YSI 9300 Photometer.

Fish Respirometry: Loligo Systems Respirometry equipment (Tjele, Denmark) and custom built respirometry chamber used to measure the metabolic rate of E. blennioides and E. variatum.

Computer Skills: Proficient with Microsoft Office Suite, SPSS, GIS, Outlook, and SAS

Presentations:

Kist, Emma and J.J. Kovatch. A comparative analysis of the metabolism and energetics of darters (Percidae). The West Virginia Academy of Science 91st Annual Meeting. Marshall University. Huntington, WV. April 9, 2016. Honorable Mention Graduate Student Presentation.

Kist, Emma and J.J. Kovatch. A comparative analysis of the metabolism and energetics of darters (Percidae). 31st Annual Ohio River Basin Consortium for Research and Education (ORBCRE) Scientific Symposium. Northern Kentucky University. Highland Heights, KY. October 11-13, 2015. First Place Student Oral Presentation.

Kist, Emma and J.J. Kovatch. A comparative analysis of the metabolism and energetics of darters (Percidae). 2015 Water Resources Conference of the Virginias. Stonewall Resort. Roanoke, WV. October 5-6, 2015.

Kist, Emma and J.J. Kovatch. Measuring metabolism and energetics of darters (*Etheostoma*) to better understand their life history. ΣΞ Research Day, Marshall University. Huntington, WV. May 1, 2015. (Poster).

Kovatch, J.J, E. Kist, T. Tuggle, M. Castle, S. Legg, J. Becker, M. Bernot, and T. Lauer. Pharmaceutical and personal care product concentration in the Mud and Ohio Rivers compared to national data. ΣΞ Research Day, Marshall University. Huntington, WV. May 1, 2015. (Poster).

Levin-Nielsen, Emma, A. Jackson, and G. LaFata. Comparison of plant height, leaf surface area, and leaf length to width ratio between two populations of *Aster prenanthoides* in Upshur County, WV. Association of Southeastern Biologists 74th Annual Meeting, Marshall University. Huntington, WV. April 10-13, 2013.

Levin-Nielsen, Emma, A. Marcelo, and R. Egleton. Regulation of Vascular Endothelial Growth Factor Signaling (VEGF) in the Choroid Plexus (CP) in Diabetes. 10th Annual WV-INBRE Summer Research Symposium, Marshall University. Huntington, WV. July 28, 2011. (Poster).

Jackson, A., E. Levin-Nielsen, and G. Lafata. Duration of courtship behavior in reproductively successful and unsuccessful male budgerigars, (*Melopsittacus undulates*). West Virginia Wesleyan College Campus Research Day. Buckhannon, WV. December 2011. (Poster).