5-2014

Diel Patterns of Foraging Aggression and Antipredator Behavior in the Trashline Orb-weaving Spider, Cyclosa turbinata

James C. Watts
East Tennessee State University

Diel Patterns of Foraging Aggression and Antipredator Behavior in the Trashline Orb-weaving
Spider, *Cyclosa turbinata*

_____

A thesis

presented to

the faculty of the Department of Biological Sciences

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Science of Biology

_____

by

James Colton Watts

May 2014

_____

Thomas C. Jones, Chair

Darrell Moore

Lev Yampolsky

Keywords: chronoecology, behavioral trade-offs, behavioral rhythms, predator vigilance,
antipredator strategies

ABSTRACT

Diel Patterns of Foraging Aggression and Antipredator Behavior in the Trashline Orb-weaving Spider, *Cyclosa turbinata*

by

James Colton Watts

Few studies have rigorously assessed the adaptive value of diel rhythms in animals. We laid the groundwork for assessing the adaptive rhythm hypothesis by assaying diel rhythms of foraging and antipredator behavior in the orb-weaving spider *Cyclosa turbinata*. When confronted with a predator stimulus in experimental arenas, *C. turbinata* exhibited thanatosis behavior more frequently and for longer durations during the day. However, assays of antipredator response within webs revealed more complex diel patterns of avoidance behaviors and no pattern of avoidance behavior duration. Assays of prey capture behavior found that the likelihood of exhibiting prey capture behavior varied significantly across times of day and test subjects, but only test subject predicted attack latencies. Although *C. turbinata* foraging aggression changed over the diel cycle, we found no evidence of a trade-off between foraging behavior and predator vigilance. However, overall patterns of vigilance may be masked by diel changes in antipredator strategies.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

<u>The Nature of Diel Rhythms</u>

Diel rhythms of physiology and behavior are present in virtually all taxa examined to date. These daily oscillations are widely assumed to be adaptive strategies that coordinate various biological functions with ecologically appropriate times of day (Dunlap et al. 2004). Some diel rhythms arise from reactionary responses to environmental stimuli (i.e. exogenously driven rhythms), while others are proactive responses that may anticipate environmental transitions (i.e. endogenously driven rhythms). For example, the colonial orb-weaving spider *Metepeira incrassata* relies on exogenous light cues to trigger the daily replacement of the web's prey capture surface (Uetz et al. 1994). This behavior presumably ensures that the sticky capture silk is replaced sufficiently often to maintain its highly specialized and efficient function as a snare for flying insect prey (Uetz et al. 1994; Foelix 2011). In contrast, honeybees use an endogenous clock to form time-memories that coordinate collection visits to flowers with times of day during which the flowers have proven most profitable (Moore and Doherty 2009). The timing of food source visits by an endogenous clock presumably reduces the amount of energy spent visiting flowers during periods of low nectar production (Moore and Doherty 2009). In many cases diel rhythms arise from complex interactions among exogenous and endogenous cues. Take for instance the well-studied influence of light and temperature cycles on the timing of endogenous rhythms, such as the locomotor activity rhythm of the honeybee (Moore and Rankin 1985; Moore and Rankin 1993). Although the endogenous locomotor activity rhythm persists, by definition, without environmental input, the sensitivity of the rhythm to environmental input permits coordination with the current environmental cycle (Moore and

Rankin 1985; Moore and Rankin 1993). Moreover, many diel rhythms are governed by exogenously influenced endogenous rhythms that may be further modified by nonperiodic (i.e. nonentraining) environmental cues (Roth et al. 2009; Zeigler et al. 2010; Eban-Rothschild 2011). Perhaps the most notable example of this phenomenon is the well-studied diel vertical migration of plankton. For many species the diel rhythm of vertical migration persists in the absence of environmental cues, suggesting regulatory influence of an endogenous rhythm (Zeigler et al. 2010). However, the presence of predatory fish stimuli can produce substantial changes in the migratory patterns of the plankton, as can nonperiodic changes in light intensity (Zeigler et al. 2010).

<p align="center">Diel Rhythms as Targets of Selection</p>

Whether a rhythm arises from exogenous cues, endogenous cues, or an interaction between the two, the mechanisms that produce diel rhythms are likely targets of selection. Selection may shape exogenously driven rhythms by acting on genetic components underlying the sensory machinery required to detect environmental changes or the genetic, neural, and endocrine networks capable of altering physiological and behavioral processes in response to those changes. On the other hand, endogenously driven rhythms arise from mechanisms linking physiology and behavior with phases of an endogenous oscillator that, in turn, is entrained to the external environmental cycle by exogenous cues. Therefore, selection may shape endogenously driven rhythms in an analogous fashion by acting on genetic components underlying: i) the ability to detect environmental changes, ii) the mechanisms enabling the entrainment of the endogenous oscillator to those changes (e.g. Emery et al. 1998, Ceriani et al. 1999), or iii) the genetic, neural, and endocrine networks that coordinate physiological and behavioral processes with the phase of the endogenous oscillator. Consequently, selection on endogenous rhythms

<p align="center">8</p>

may affect the relationship between the endogenous oscillation and the environmental cycle, the physiological and behavioral processes that are regulated by the endogenous oscillator, and the timing of physiological and behavioral changes relative to phases of the endogenous oscillation.

Because endogenously driven rhythms are mediated by an internal timepiece, the resulting changes in physiology and behavior may be decoupled temporally from cues that define the environmental cycle and entrain the timepiece (Fleury et al. 2000). In this context selection on endogenous rhythms may explain the apparent diversity of endogenous period lengths both among and within species (Fleury et al. 2000). However, it is important to note that endogenously driven rhythms are not unique in this respect as exogenously driven rhythms may also produce responses that are temporally decoupled from environmental cues. This effect can be produced via an interval timer in which a response is activated upon reaching threshold concentrations of biochemical products that are produced or degraded in a time-dependent fashion (Bradshaw et al. 2003). These time-dependent biochemical processes are in turn reset by environmental cues that coordinate the response with the current environmental cycle (Bradshaw et al. 2003). Some authors have suggested that such biological timers might be sufficient to explain some characteristics of diel rhythms such as their role in photoperiodism (Bradshaw et al. 2003). Thus, in an evolutionary context we might consider exogenously and endogenously driven rhythms as equal recipients of selective pressures arising from diel variation in environmental conditions.

<center>The Proximate and Ultimate Causes of Diel Rhythms</center>

Since their discovery, endogenous circadian rhythms have garnered a tremendous amount of interest in the molecular mechanisms that produce environmentally sensitive self-sustaining rhythms. In all organisms studied circadian rhythms appear to arise from transcriptional-

<center>9</center>

translational feedback loops that are timed by posttranslational modification (Ceriani et al. 1999, Gallego and Virshup 2007). For example, in mammals the core oscillation derives from the activity of the transcription factors CLOCK and BMAL1 (Gallego and Virshup 2007). These transcription factors dimerize and together promote the expression of a number of genes, including their own inhibitors such as the PER and CRY proteins (Gallego and Virshup 2007). Following their translation, PER and CRY must associate in the cytoplasm before being phosphorylated and subsequently translocated into the nucleus where they inhibit the transcriptional activity of CLOCK and BMAL1 (Gallego and Virshup 2007). This time delay imparts the oscillatory nature of the circadian cycle that is then perpetuated by posttranslational modifications of PER and CRY within the nucleus that reduce inhibition of CLOCK and BMAL1 (Gallego and Virshup 2007). This basic feedback mechanism appears to be quite similar across diverse taxa despite independent evolutions of the genes involved (Gallego and Virshup 2007).

In turn, the elucidation of the genetic components and biochemical mechanisms underlying circadian oscillations has enabled extensive manipulations of rhythmic behavioral and physiological processes. These manipulations have clarified the role of a biological clock in regulating a variety of physiological and behavioral functions, including reproductive physiology (Beaver et al. 2002), oviposition (Howlader and Sharma 2006), endocrine function (Dickmeis 2009), foraging behavior and metabolism (Xu et al. 2008), structure and sensitivity of the eye (Koovor et al. 1999), and general activity levels (DeCoursey et al. 1997, 2000; Hurd and Ralph 1998). Moreover, many of these manipulative studies have provided insight into the adaptive significance of diel rhythms. For instance, Hurd and Ralph (1998) demonstrated that genetic manipulations of the endogenous clock in golden hamsters produced individuals with fragmented

locomotor activity patterns and decreased longevity. More importantly, the authors demonstrated that strong, consolidated activity rhythms and increased longevity could be induced in senescing animals by implanting sections of suprachiasmatic nuclei (SCN; the site of the primary mammalian clock) from fetal animals (Hurd and Ralph 1998). Thus, the authors concluded that intact endogenous clocks enhance the fitness of golden hamsters through their influence on longevity (Hurd and Ralph 1998).

A similar study on the effects of clock function in *Drosophila melanogaster* found that copulations between males and females with genetically disrupted clocks resulted in fewer progeny than copulations between individuals with intact clocks (Beaver et al. 2002). This effect was driven by a reduction in the number of eggs laid, possibly because oviposition in *Drosophila* is rhythmic (Howlader & Sharma 2006), as well as an increased occurrence of unfertilized eggs (Beaver et al. 2002). Upon further investigation, the authors found that males with disrupted clocks suffered a decrease in reproductive success when mated with wild-type females, an effect that appears to arise from decreased accumulation of sperm in the seminal vesicles of males with dysfunctional clock genes (Beaver et al. 2002).

In another notable study Green et al. (2002) genetically disrupted endogenous oscillations of transcription in *Arabidopsis* to determine if mutant plants lacking the ability to anticipate environmental transitions suffered fitness reductions. The researchers found that mutant plants that are incapable of anticipating environmental transitions do not alter flowering time as drastically as wild-type plants in response to changes in photoperiod (Green et al. 2002). Moreover, genetically disrupted plants suffered decreased seed viability when compared to wild-type plants but only under extreme short-day conditions (4 hours of light and 20 hours of darkness).

11

While the above studies point to an adaptive function of intact rhythm-producing mechanisms in plant and animal systems, these data must be interpreted with caution. For example, the studies of the golden hamster and *Drosophila* demonstrate an intrinsic advantage to possessing a functioning rhythm (Hurd & Ralph 1998, Beaver et al. 2002, Johnson 2005). That is, individuals derive a benefit from the temporal organization of internal events that may be entirely independent of daily variation in the environment (Johnson 2005). Consequently, these studies provide little insight into the ecological processes that may have selected for internal organization corresponding to the diel cycle as opposed to organization along any arbitrary temporal scale (Johnson 2005). Although the study by Green et al. (2002) begins to evaluate the role of 24-hour environmental cycles in producing the fitness consequences of dysfunctional rhythms, the data indicate differences between wild-type and mutant plants exist only under extremely unnatural conditions. Johnson (2005) critiques a series of experiments from the 1950s that also demonstrate increased fitness associated with a daily rhythm corresponding to environmental cycles; however, these studies relied on even less realistic manipulations of environmental cycles, namely comparisons between 24-hour and non-24-hour cycles. Moreover, these studies rely on incomplete measures of fitness to draw their primary conclusions, as do the previously described contemporary studies (Hurd & Ralph 1998, Beaver et al. 2002, Green et al. 2002, Johnson 2005). For example, a golden hamster with decreased longevity may produce an unusually large number of offspring, just as a fruit fly with decreased sperm release may be exceptionally long-lived or unusually successful in securing mating opportunities (Johnson 2005). These considerations emphasize the exceptional difficulty of rigorously assessing the adaptive value of circadian rhythms. The issue we face is not whether organisms perform better

under some types of environmental cycles than others, but whether characteristics of the 24-hour day could select for organisms that organize their physiology and behavior accordingly.

Thus, there is an unfortunate discontinuity between molecular and ecological studies examining the adaptive value of diel rhythms. The utility of molecular and genetic techniques in disrupting clock properties is well-established, yet there are few studies of this kind reaching beyond the intrinsic value of diel rhythms to consider the ecological characteristics of the 24-h day that might favor daily oscillations in physiology and behavior. In one notable effort to bridge this disjunction, Ouyang et al. (1998) staged competitive bouts among 3 strains of cyanobacteria of varying clock genotype under 24-h and non-24-h day lengths. By first characterizing the growth of each clock mutant strain in isolation under constant light and light:dark (LD) cycles, the researchers were able to rule out any inherent differences in growth among the strains. However, when placed in competition, the strain with an endogenous period most closely matching the environmental cycle excluded the other strains. While this study also exploited unnatural (i.e. non-24-h) diel cycles, the authors elegantly demonstrated a plausible ecological mechanism selecting for individuals with resonating endogenous rhythms. It remains to be seen exactly what competitive advantage is available to cyanobacteria with rhythms more closely matching those of the 24-hour day, but these data demonstrate the value of an ecologically relevant approach to understanding selection on diel rhythms.

In a similar example, Fleury et al. (2000) demonstrated that differences in endogenous oviposition rhythms among sympatric species of parasitoid wasps are sufficient to offset competitive asymmetries inherent among contemporaneously deposited offspring. These data show that diel rhythms can produce a selective advantage by enabling organisms to perform behaviors at ecologically appropriate times of day, such as those times prior to the exploitation of

a resource by a competitor (Fleury et al. 2000). These data also support hypotheses regarding the significance of intraspecific and interspecific variation in diel rhythms (Fleury et al. 2000). A species' typical diel rhythm may be shaped by selection acting on the timing of behavioral or physiological output relative to phases of the internal clock or timer, while variation in diel rhythms within a species provides the raw material upon which selection may act (Fleury et al. 2000).

Another notable example is the work of Patricia DeCoursey and her colleagues regarding adaptive activity rhythms in sciurid rodents. In one study DeCoursey et al. (1997) characterized alterations of diel rhythms of locomotor activity in antelope ground squirrels following mechanical destruction of the SCN. Destruction of the SCN disrupted the strongly diurnal locomotor activity rhythms of the squirrels in controlled laboratory settings, and SCN-lesioned squirrels returned to the field tended to experience greater predation-related mortality (DeCoursey et al. 1997). While the authors suggested that nocturnal activity of the clock-disrupted individuals was maladaptive due to increased exposure to nocturnal predators, the strength of their conclusions was limited by sample size (DeCoursey et al. 1997). In a similar study, DeCoursey et al. (2000) tracked SCN-lesioned, sham-operated, and unoperated eastern chipmunks at a field site in the Allegheny Mountains. While they did not observe any nocturnal activity outside of the burrows, they did detect nocturnal restlessness of SCN-lesioned chipmunks within their burrows. In turn, SCN-lesioned chipmunks suffered greater predation by weasels during an 80-day period after repatriation (DeCoursey et al. 2000). Thus, it seems that the circadian clock in eastern chipmunks coordinates locomotor activity with periods of reduced predation risk (DeCoursey et al. 2000). These data are of course correlative and relied heavily on survival as a proxy for reproductive fitness (DeCoursey et al. 2000). More work is needed to

evaluate whether diel variation in predation risk is sufficient to select for individuals with strongly diurnal activity patterns in antelope ground squirrels (DeCoursey et al. 2000).

Orb-Weaving Spiders as a Model System

As a model system, orb-weaving spiders are particularly well-suited for investigating the fitness consequences of diel variation in environmental conditions. Spiders are both predator and prey and, therefore, must balance the boldness needed to capture prey against the wariness needed to avoid predation (Wise 1993; Jones et al. 2011a). This trade-off may be particularly consequential for orb-weaving spiders, as these spiders are highly exposed to visual predators such as birds and wasps while foraging from the center of their aerial webs (Foelix 2011). Orb-weaving spider species also differ considerably in their daily foraging routine (Carico 1986; Herberstein and Elgar 1994). Some orb-weaving spiders forage nocturnally, constructing a web in the evening and foraging throughout the night (Carico 1986). Many of these spiders seek a retreat during the day, presumably to reduce exposure to visual predators (Foelix 2011). Other spiders appear to forage diurnally or continuously (Carico 1986; Herberstein and Elgar 1994). These species of spiders often possess additional morphological features (e.g. spines, aposematic coloration, and camouflage) that may represent adaptations for foraging during periods of heightened predation risk (Cloudsley-Thompson 1995). Taking a species-specific approach to understanding diel behavioral rhythms in spiders may provide insight into the adaptive value of diel rhythms as well as the diversity of strategies for coping with periodic environmental changes.

Additionally, behavioral syndromes of aggression-related behaviors have been described for many spider species (Pruitt and Riechert 2012) including orb-weaving spiders (Kralj-Fisher and Schneider 2012). A behavioral syndrome is a suite of correlated behavioral traits (Sih and

Bell 2008). In a population a behavioral syndrome produces both within-individual and among-individual correlations in behavior (Sih and Bell 2008). In other words, individuals exhibit consistent rank-order relationships in behavioral traits across contexts and through time (Sih and Bell 2008). In behavioral syndromes of aggression in spiders, individuals that are relatively bolder in response to predator cues tend to be relatively more aggressive toward prey, conspecifics, and inquilines (Riechert and Hedrick 1993; Pruitt et al. 2008; Pruitt and Riechert 2012; Keiser and Pruitt 2014). In some circumstances these correlations can generate additional fitness trade-offs (e.g. Pruitt et al. 2008), as any given individual cannot express the full range of phenotypic flexibility seen in the population (Sih and Bell 2008). While the potential for additional behavioral constraints may be particularly interesting in the context of the evolution of diel behavioral flexibility, a behavioral syndrome also enables the use of diel changes in a single behavioral trait as a proxy for diel changes in the relative level of aggression and boldness across contexts (Jones et al. 2011a).

For example, a recent paper by Jones et al. (2011a) characterized diel rhythms of boldness in the orb-weaving spider Larinioides cornutus by assaying antipredator behavior at different times of day. The authors found that *L. cornutus* exhibits strong diel rhythms of antipredator behavior that, according to a study on behavioral syndromes in *Larinioides* (Kralj-Fisher and Schneider 2012), may be tied to oscillations in other aggression-related behaviors (Jones et al. 2011a). The spiders assayed by Jones et al. (2011a) responded more fearfully to a predator cue during the day, as evidenced by a greater duration of thanatosis (i.e. death-feigning) behavior. The oscillation in antipredator behavior continued under constant conditions, suggesting that the rhythm is endogenously driven (Jones et al. 2011a). While previous studies have described circadian rhythms of locomotor activity and visual sensitivity in spiders (Seyfarth

1980; Suter 1993; Koovor et al. 1999), Jones et al. (2011a) are the first to describe a circadian

rhythm in a behavior directly linked with spider fitness. Consequently, the discovery of diel

rhythms of aggression-related behaviors in spiders provides a unique opportunity to place

behavioral rhythms into an ecological context.

The rhythm of antipredator behavior observed in *L. cornutus* fits intuitively with the

ecology of the species. *L. cornutus* appears to forage nocturnally, replacing its web at dusk and

remaining in the web hub throughout the night (Sherman 1994). During this time, *L. cornutus*

devotes less time to thanatosis behavior in response to predator cues (Jones et al. 2011a). The

spiders then take down their webs in the early morning and remain hidden in a retreat for the

remainder of the day (Bellmann 1997). During this period of decreased foraging effort the

spiders devote much more time to thanatosis behavior in response to predators (Jones et al.

2011a). This pattern may reflect a trade-off between foraging aggression and predator vigilance

for *L. cornutus* (Jones et al. 2011a). Flying prey caught in webs are often able to free themselves

after some time, constraining the duration of time a spider may spend out of the web in response

to predators (Nentwig 1982, Rypstra 1982). Thus, during periods of active foraging, spiders may

be required to reduce predator vigilance to be present in the web hub sufficiently often to catch

flying prey (Jones et al. 2011a). However, during the day, when the spider does not actively

forage, there is no cost in lost prey capture associated with maximizing defensiveness in

response to predators (Jones et al. 2011a). Consequently, patterns of antipredator behavior and

thus aggression in *L. cornutus* appear to reflect the daily switch in the foraging mode of the

spider and differences in behavioral trade-offs that may exist between the foraging and

nonforaging period. Whether the daily foraging routine in itself is an adaptive strategy for coping

with daily changes in environmental conditions (e.g. prey and predator abundance) remains to be tested.

Hypotheses and Design

The present study represents the next step in assessing the adaptive value of diel behavioral rhythms in orb-weaving spiders. We hypothesize that diel rhythms of aggression-related behaviors in orb-weaving spiders represent adaptive strategies for maximizing foraging gain relative to predation risk over the diel cycle. If our hypothesis is correct, we expect to observe diel rhythms of aggression-related behaviors even in spiders that forage continuously throughout the day. In other words, if diel variation in environmental factors is sufficient to provide an advantage to individuals that modulate their aggression over the diel cycle, then we should observed diel rhythms of aggression even in spiders that to do not transition between foraging and nonforaging states. Alternatively, diel rhythms of aggression may simply reflect daily changes in foraging mode. If this is true, then continuously foraging spiders should not modulate their aggression over the course of the day because behavioral trade-offs remain essentially constant.

We further hypothesize that diel rhythms of aggression-related behaviors arise from trade-offs between predator vigilance and foraging efficiency. If our hypothesis is correct, aggression towards prey and wariness of predators should be negatively correlated through time and across individuals. In other words, periods of increased foraging intensity should be characterized by a decreased tendency to leave the foraging area (i.e. the web center) in response to predator cues. Any link between these behaviors should also manifest at the individual level, with individuals that generally tend to respond more aggressively toward prey also tending to be less wary of predators. In this case individuals are not optimally flexible and must balance

conflicting ecological roles through time and across contexts. Alternatively, aggression toward prey and wariness of predators may be positively correlated through time and across individuals. Such a mechanism would enable spiders to evade predators with great efficiency while maintaining high levels of responsiveness to prey, but the advantage of this strategy depends upon the ability of the spider to reliably discriminate predator and prey cues. A spider that inappropriately exhibits aggressive prey capture behavior in response to a predator cue likely suffers a severe fitness cost, as would a spider exhibiting antipredator behavior in response to flying prey entering its web. It is also possible that foraging and antipredator behavior are decoupled in *C. turbinata*, indicating a remarkable degree of sensory discrimination and behavioral flexibility that may decrease behavioral time-budget trade-offs (Sih et al. 2012).

To assess these hypotheses, we assayed diel rhythms of foraging and antipredator behavior in a continuously foraging orb-weaving spider, *Cyclosa turbinata* (Araneae: Araneidae). We first assayed diel rhythms of antipredator behavior of *C. turbinata* in experimental arenas. These observations provided a reduced, baseline impression of diel rhythms of defensive behavior in *C. turbinata* under controlled conditions. Moreover, these data are comparable with previous studies examining antipredator behavior of other spider species within experimental arenas (Pruitt et al. 2008; Jones et al. 2011a). We then assayed diel rhythms of antipredator behavior of spiders maintained in webs to assess the reliability of antipredator behavior data collected under reduced conditions as compared to a more natural setting. Finally, we assayed diel rhythms of foraging behavior for spiders maintained in webs to determine if foraging behavior changed over the diel cycle. To our knowledge, this is the first study quantitatively assessing diel rhythms of foraging behavior in spiders.

In addition to investigating hypothesis concerning diel behavioral rhythms in a continuously foraging spider, this study provides a framework for rigorous assessments of the adaptive significance of diel rhythms. By quantifying diel variation in environmental conditions and the role of spider behavior in modifying exposure to temporally variable risks, we can model the optimal behavioral policy for spiders over the diel cycle. More importantly, we can use pharmacological manipulations of spider behavior to demonstrate fitness costs that might be associated with deviations from the optimal daily routine. Ultimately, this work will contribute to a comparative study aimed at understanding the diversity of diel behavioral routines observed across closely related spider taxa. Thus, the consequences of the work described here reach well beyond the scope of this study.

CHAPTER 2

DIEL PATTERNS OF FORAGING AGGRESSION AND ANTIPREDATOR BEHAVIOUR IN THE TRASHLINE ORB-WEAVING SPIDER, *CYCLOSA TURBINATA*

J. Colton Watts, Ashley Herrig, William D. Allen, Thomas C. Jones*

Department of Biological Sciences, East Tennessee State University, U.S.A.

Diel rhythms of physiology and behaviour are present in virtually all taxa examined to date. However, few studies have rigorously assessed the adaptive value of physiological and behavioural rhythms in animals. We laid the groundwork for an assessment of the adaptive rhythm hypothesis by assaying diel rhythms of foraging and antipredator behaviour in the trashline orb-weaver Cyclosa turbinata (Araneae: Araneidae). When confronted with a predator stimulus in experimental arenas, C. turbinata exhibited thanatosis behaviour more frequently and for longer durations during the day. However, assays of antipredator response within webs revealed more complex diel patterns of avoidance behaviours and no pattern of avoidance behaviour duration. A preliminary assay of prey capture behaviour suggested that C. turbinata also exhibits nocturnal patterns of foraging aggression. A refined foraging experiment, in which we randomized prey stimulus frequency, found that time of day and spider ID strongly predicted the likelihood of exhibiting prey capture behaviour. Only spider ID predicted latency to attack the prey stimulus despite low individual repeatability ($r = 0.10$). These data support our prediction that C. turbinata modulate foraging aggression over the diel cycle, but we found no evidence of a trade-off between foraging behaviour and predator vigilance. However, overall patterns of vigilance may be masked by diel changes in antipredator strategies that correspond to fluctuations in the relative abundances of predator types.

Keywords: chronoecology; behavioural trade-offs; behavioural rhythm; predator vigilance; antipredator strategies

Diel rhythms are widely assumed to enhance individual fitness by coordinating various behaviours and physiological processes with periods of favorable environmental conditions. Some diel rhythms arise from reactionary responses to changing environmental stimuli, while others are regulated by an endogenous (circadian) clock and may anticipate environmental transitions. For example, zooplankton exhibit entrained rhythms of vertical migration that reduce exposure to diurnal visual predators (Enright & Hammer, 1967; Lampert, 1989). Honeybees utilize a circadian time-memory to exploit food sources during times of day when they have proven most profitable (Moore & Doherty, 2009). The colonial orb-weaving spider *Metepeira incrassata* relies on changing light intensity to signal the web-replacement behaviour necessary for daily renewal of the prey capture surface (Uetz et al., 1994). The diversity of rhythmic outputs and their physiological bases demands rigorous exploration of the adaptive significance of diel rhythms. While many authors have demonstrated fitness costs associated with genetically and mechanically disrupted biological rhythms (Green et al., 2002; Beaver et al., 2002; DeCoursey et al., 1997, 2000) few have demonstrated ecological mechanisms through which heritable rhythmicity may enhance individual fitness (Ouyang et al., 1998; but see Johnson, 2005).

As both predator and prey, spiders offer a robust opportunity to examine trade-offs between the aggression needed to capture prey and the wariness needed to avoid predation (Jones et al., 2011a). Several studies of spider taxa have provided evidence that aggression toward prey and wariness of predators are negatively correlated as part of a behavioural syndrome aggression (Riechert & Hedrick, 1993; Pruitt et al., 2008; Pruitt et al., 2012a). This "aggressive spillover" presumably constrains behavioural flexibility (Pruitt et al., 2008) and would require individuals to balance the consequences of exhibiting bold or docile behaviour across ecological contexts

23

(Pruitt et al., 2008). Behavioural syndromes in spiders appear to be heritable (Riechert & Maynard Smith, 1989), suggesting that selection may act to optimize correlated suites of behaviours in the natural environment.

According to the Risk Spreading Theorem (RST), individuals should devote the minimum amount of time to foraging (i.e. decreased vigilance) that meets the energetic requirements of survival and reproduction (Houston et al., 1993). This model assumes temporal homogeneity of risk and reward which is unrealistic in most environmental settings. We have unpublished data demonstrating considerable diel fluctuations in the abundance of spider prey and threat species (i.e. flying insects and parasitoid wasps) in the natural habitat. If fluctuations in prey and threat abundances produce diel changes in the relative intensities of potential foraging gain and predation risk, selection should favor individuals that modulate their aggression level over the course of the day (Lima & Bednekoff, 1999). We hypothesize that selection acts on suites of aggression-related behaviours in spiders and that diel patterns of foraging and antipredator behaviour are adaptive behavioural strategies that maximize foraging gain relative to predation risk.

A recent study provided evidence of diel rhythms of antipredator behaviour in a nocturnal orb-weaving spider that appear to reflect daily transitions from refuge use to active foraging (Jones et al., 2011a). If diel variation in environmental conditions selects for rhythms of aggression-related behaviours, we expect to see rhythms of antipredator behaviour and foraging aggression in spider species that do not entirely forego foraging during certain periods of the day.

We assessed our prediction by assaying diel variability of antipredator and foraging behaviour in the trashline orb-weaver *Cyclosa turbinata* (Araneae: Araneidae) (Walckenaer). These spiders continuously occupy the web hub, leaving only to replace the web prior to sunrise,

and appear to forage during day and night (unpublished data). We first determined if antipredator behaviour varied over the diel cycle using a modification of the assay described in Jones et al. (2011a). We later assayed antipredator behaviour and prey capture behaviour concurrently for spiders maintained in webs to determine i) if antipredator behaviour and foraging aggression vary over the diel cycle, ii) if patterns of antipredator behaviour in the web reflect those seen in experimental arenas, and iii) if aggression toward prey stimuli and boldness towards predator cues are negatively correlated with respect to time of day and within individuals in *C. turbinata*. Finally, we conducted a refined assessment of diel rhythms of foraging behaviour to verify the data from our concurrent antipredator and foraging behaviour experiment.

METHODS

Study Species

Cyclosa turbinata (Araneae: Araneidae)(Walckenaer) is a small (4-7 mm) orb-weaving spider common along forest edges and fencing in the southeastern United States. They construct vertical orb webs containing a stabilimentum lined with prey carcasses, detritus, and egg cases, the so called 'trashline' (Fig. 1). The stabilimentum appears to interfere with ability of predators to locate the spider within the web (Chou et al., 2005; Tseng & Tso, 2009). Spiller (1984) reported that *C. turbinata* spiders are bivoltine, reproducing in the late spring and fall; however we are not aware of any studies describing the life history of *C. turbinata* in the southeastern United States. *C. turbinata* are easily collected and readily construct webs within wooden frames in the lab. We collected individuals from the field at the start of each of our three experiments.

25

Fig. 1. A female *Cyclosa turbinata* as seen in the web hub. The so-called 'trashline' composed of prey remains and detritus is visible above and below the spider. Photo courtesy of Richard A. Bradley.

Antipredator Behaviour Assay in the Arena

Collection and maintenance

In August, 2012 we collected 13 adult female *C. turbinata* from fences and hedges in Johnson City, TN, USA. We maintained spiders individually in 59 ml deli containers under a 12:12 h light:dark cycle and approximately 23°C for 5 days. All spiders refused prey while in deli containers. After 5 days of acclimation, we placed each spider into a 30 cm X 30 cm X 10 cm four-sided wooden frame which was then wrapped in plastic food wrap and left overnight to

promote web construction. On day 7 we unwrapped each frame, gave each spider two

Drosophila hydei or two termite workers, and misted the web with distilled water. We left the

spiders in the temperature- and light-controlled room for four more days with only daily misting.

On day 12, we again misted the webs and gave each spider 2 *Drosophila hydei* or 2 termite

workers. We left the spiders overnight to permit feeding before returning the spiders to 59 ml

deli containers the next day. We permitted one day of acclimation to deli containers before

beginning antipredator behaviour assays on August 25 at 1100 h (3 h after onset of photophase).

Behavioural assay

To determine whether antipredator behaviour varies over the diel cycle in *C. turbinata*, we used

the 'huddle response' assay described in Jones et al. (2011b). We coaxed spiders from their

containers into a clean glass dish (15 cm diameter, 6cm high). We gave each spider 30 s to

acclimate before delivering a gentle puff of air from approximately 10 cm away. The puff of air

initiates the well-known 'huddle response,' or thanatosis, that many spiders exhibit when

threatened. We timed the duration of the response with a stopwatch to the nearest second. We

delivered the puff of air 3 times or until an active response was observed. Since individuals did

not always exhibit thanatosis behaviour, we noted the type of behaviour observed. Individuals

not actively responding after 3 stimulus deliveries were scored as "non-responsive." Spiders that

huddled in response to our coaxing them from their containers were given up to 5 min to break

from the huddle before beginning the 30 s acclimation interval. Spiders that did not break from

an initial huddle within 5 min were given a score of 500 s. We repeated this assay every 4 h for 5

days under 12:12 h light:dark and 23°C.

Collection, maintenance, and design

We collected 35 adult female *C. turbinata* and their trashlines from fences and hedges in Johnson City, TN, USA during the last week of July, 2013. We haphazardly selected 20 individuals to place immediately into 30 cm X 30 cm X 10 cm four-sided wooden frames backed with canvas. We permitted spiders to retain their original trashlines during the experiment. We used removable fiberglass screens to enclose the spider within the frames before leaving them overnight under a ramping (1 h) 12:12 h light:dark cycle and 23 °C. All remaining spiders were maintained in 110 ml deli containers in the same temperature- and light-controlled room. Any spider that had not built a web by the following morning was removed and replaced with a haphazardly selected spider from the same collection group. We repeated this process until 20 frames were occupied by a spider in its web. We then began a 5 day acclimation period during which each spider was misted daily at irregular intervals. We provided 2 termite workers daily for the first 4 days of this period and starved the spiders on day 5. We haphazardly assigned each spider to one of two groups before beginning assays at 0300 h (4 h prior to beginning of 1 h photophase ramp) on day 6. We arbitrarily designated one group to receive the predator stimulus during the first trial while the other group received the prey stimulus (stimuli described below). To ensure that all 20 spiders were tested for both stimuli at each time of day, we alternated the stimulus received by each group throughout the remaining trials. We tested both groups every 4 h for 6 consecutive trials before allowing a 20 h break. This break shifted the group-specific schedule by 4 h so that responses to both stimuli could be captured for both groups at all 6 times

of day. We repeated this process 4 times in order to gather 2 responses of each spider to each

stimulus for 6 times of day. We fed each spider 2 termite workers midway through each 20 h

break in an attempt to reduce hunger and/or habituation bias. However, we observed such strong

habituation to the prey stimulus that we lengthened the duration of the second 20 h break to 116

h in an attempt to regain the foraging behaviour initially exhibited by the spiders.

Prey stimulus and assay

Our prey stimulus consisted of a small (2-inch diameter) audio speaker wired to a

function generator (PI-9598, PASCO Scientific, Roseville, CA, U.S.A.). An 8 cm section of an

approximately 2.5 mm diameter wooden dowel was fixed to the speaker cone perpendicular to

the plane of the speaker using hot-glue. A 10 cm section of plastic twist tie was then fixed to the

end of the wooden dowel using hot-glue. The twist tie was topped with a 5 cm length of 0.30mm

diameter monofilament to prevent the stimulus from penetrating and destroying the web. Finally,

hot-glue was used to fix a small, matte-black plastic bead to the monofilament. The bead served

to add weight to the end of the apparatus as well as to provide surface area for adhesion to the

capture spiral. We used data on web-borne vibrations of prey in *C. turbinata* webs from Suter

(1978) to select our prey stimulus frequency of 250 Hz. We set the tone generator to a low

amplitude output. We took a wooden frame from the shelf it was housed upon and placed it upon

the lab bench. We then removed the screen enclosing the front of the frame and the spider was

allowed to acclimate while we prepared a second frame. The first spider was then tested by

applying the stimulus to the web at approximately 3cm to the left or right of the spider and

recording the time elapsed between contact of the bead with the web and contact of the spider

with the bead (hereafter "latency to attack"). Spiders that did not attack within 2 min were recorded as "non-responsive." This process was repeated during each trial until the entire group (n = 10) had been tested. Unfortunately, the stimulus frequency was inadvertently shifted to approximately 150 Hz at some unknown point in the experiment. Consequently, we used these data only to test for behavioral correlations in *C. turbinata* and for an initial, qualitative assessment of diel rhythms of foraging behavior.

Predator stimulus and assay

The predator stimulus consisted of a 10 cm section of plastic-coated wire affixed by tape to a waterproof handheld vibratory stimulation device (*Mini Neon Vibes, California Exotic Novelties*) (procedure modified from Keiser & Pruitt 2014). We wrapped the free end of the wire tightly around the centre of a craft puff ball. The vibratory stimulation device was incidentally broken during the 1100 h trials on the third test day, after which the wire and puff ball were affixed to a backup device (*First Time Mini Vibe, California Exotic Novelties*). During trials, the wooden frames were taken from the shelves and opened following the procedure described for prey stimulus trials. After the acclimation period, we turned the vibratory device to its lowest setting and then gently brushed the spider's abdomen with the craft puff ball. We applied the stimulus to the spider three times or until an "active response" was elicited. The active responses we recorded consisted of fleeing to the web edge, dropping from the web, shaking the web, and rearing (raising 1st pair of legs). We timed the duration of absences from the web hub arising from a response to the predator stimulus. Spiders that did not elicit one of these four behaviours within three applications of the stimulus were recorded as "non-responsive".

Foraging Behaviour in the Web – Refined Analysis

Collection and maintenance

We collected 15 adult female *C. turbinata* from fences and hedges in Johnson City, TN, USA on October 9, 2013. We immediately placed individuals in web frames under conditions identical to those described above in *Foraging and Antipredator Behaviour in the Web*. We allowed a 5 day acclimation period to lab conditions, during which we fed the spiders two termite workers every other day and misted webs daily at irregular intervals. We began foraging behaviour trials at 0700 h on day 6.

Prey stimulus and assay

We modified the protocol and stimulus described above to conduct a second round of foraging assays. We circumvented issues of habituation and incidental variation in stimulus frequency by randomizing the prey stimulus frequency across applications. We again consulted Suter (1978) in identifying the range of 150-250 Hz within which we randomized the stimulus frequency. This range conveniently encompassed the original stimulus frequency as well as the frequency to which the stimulus had been shifted in our previous experiment. We divided the range into 10 Hz increments and used a random number table to determine the number of increments by which the frequency would be raised above 150 Hz for each application of the stimulus. Our assay protocol was identical to that described above, however we did not assay

antipredator behaviour in parallel. This relaxed the need for a complex alternating schedule. We

assayed all 15 spiders every 4 h for 5 days, with no feeding breaks or misting of webs. We

provided supplemental humidity with a small humidifier filled with distilled water.

Statistical Methods

The nature of the data posed considerable statistical challenges. To analyse diel patterns

of antipredator behaviour in the arena, we used a Chi-square analysis to test the distribution of

pooled thanatosis occurrences among times of day against a uniform distribution. The small

number of observations of antipredator behaviour in the web precluded analysis of individual

behaviours. We lumped response types according to whether the response resulted in absence

from the web hub (the area from which individuals forage) and used a Chi-square test to

determine if spiders were equally likely to flee the web hub (i.e., flee to the web edge or drop

from the web) across all times of day. We used Scheirer-Ray-Hare tests (Dytham, 2011) to test

the effects of time of day, individual, and the interaction of time of day and individual on huddle

duration in the arena. We used an identical Scheirer-Ray-Hare model to predict duration of

absence from the web hub for all instances in which a spider left the web hub. We constructed a

GLM testing the association between individuals' tendencies to flee the foraging area and to

attack simulated prey using data from our combined foraging and antipredator behaviour

experiment. Since stimulus variation and habituation confounded the foraging data from the

combined experiment, we excluded these data from subsequent statistical analyses. We used the

data from the second foraging experiment to construct a binary logistic regression model testing

the effects of time of day and individual on the likelihood of attacking the prey stimulus. We

32

included the number of prior stimulus experiences and the frequency of the stimulus at each

delivery (hereafter "trial number" and "stimulus frequency," respectively) in the regression

model to test for effects of habituation/hunger and frequency bias. We also used a Scheirer-Ray-

Hare test to determine the effects of time of day, individual, and time by individual interaction on

latency to attack the prey stimulus for all instances in which attacks occurred. Any individual

that died during the experiment or did not exhibit any prey capture behaviour was excluded from

the analyses (n = 2). We calculated repeatability as described by Lessells and Boag (1987) for

huddle duration in the arena and latency to attack in the web to evaluate the individual

consistency of *C. turbinata* behaviours. Since huddle duration and latency to attack data could

not be transformed to normality, we used ANOVAs on rank-ordered data to partition the

variance components. We used MS Excel 2013 to calculate descriptive statistics and create

figures. We used SPSS (version 21, IBM Corp., Armonk, NY, U.S.A.) for our binary logistic

regression model and for the rank ANOVAs necessary for Scheirer-Ray-Hare tests and

partitioning of variance components.

RESULTS

Diel Patterns of Antipredator Behaviour in the Arena

When assayed in experimental arenas under 12:12 light:dark conditions, *C. turbinata*

exhibited strong diel patterns of thanatosis behaviour (Fig. 2). The number of spiders "huddling"

in response to our predator stimulus rose gradually throughout the early morning and increased

sharply between 0700 h and 1100 h (Fig. 2). Between 1900 h and 2300 h the number of spiders

huddling decreased sharply. Our analysis indicated that the distribution of pooled thanatosis

occurrences among times of day differed significantly from a uniform distribution (Chi-square

test: $X^2_4 = 27.34$, $P < 0.0001$). We found similar patterns of thanatosis duration, with individuals

exhibiting thanatosis huddling longer during the day (Fig. 2). Transition times and magnitudes

closely resembled those seen in thanatosis occurrence. We found significant associations of time

of day and individual with the duration of the huddle response (Scheirer-Ray-Hare test: $df = 5$, H

$= 69.74$, $P < 0.0001$; $df = 12$, $H = 47.78$, $P < 0.0001$, respectively). However, there was no effect

of time of day by individual interaction (Scheirer-Ray-Hare test: $df = 54$, $H = 38.98$, $P = 0.94$).

Repeatability of individual huddle durations was relatively low (r = 0.196). We observed very

few instances of aggressive behaviour (i.e. rearing, n = 8) when spiders were tested in arenas.
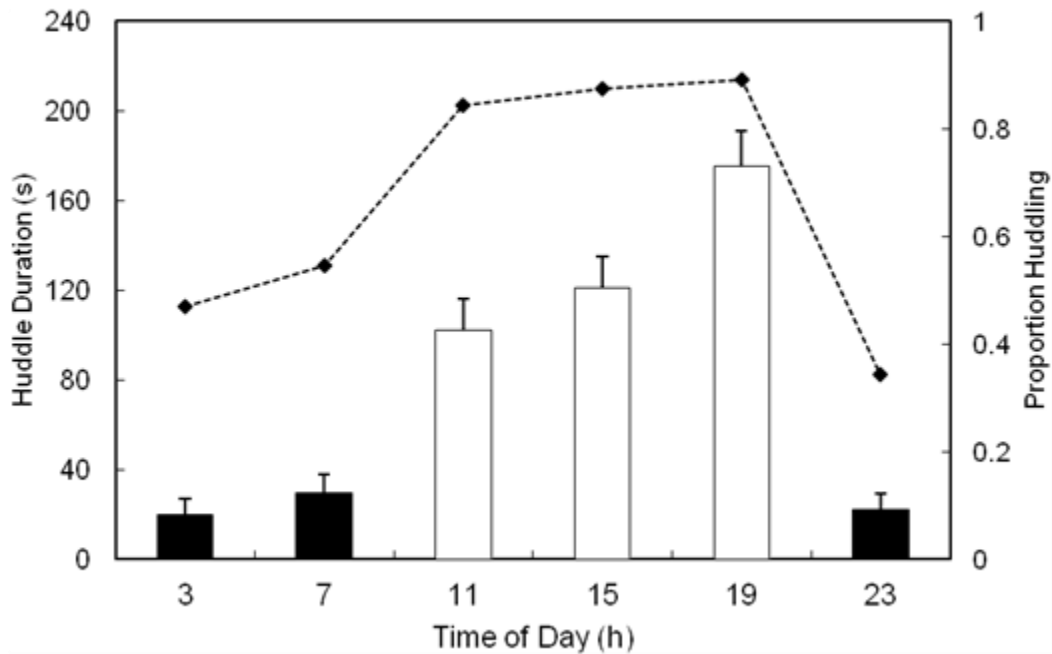
Fig. 2. Diel rhythms in antipredator response of *C. turbinata* assayed in arenas under light:dark 12:12 h. The dotted line represents the proportion of trials at each time of day in which spiders exhibited thanatosis. Columns represent the means and standard errors of thanatosis durations for 13 spiders tested at 6 times of day for 5 days. Open bars: spiders tested with lights on; solid bars: spiders tested with lights off.

Diel Patterns of Foraging and Antipredator Behaviour in the Web

When we assayed spiders in their webs under a ramping 12:12 light:dark cycle, we again observed diel patterns of antipredator behaviour. The variety of behaviours exhibited within webs greatly exceeded that seen within arenas; however, several behaviours were only observed during certain times of day (Fig. 3). For instance, we never observed spiders running to the web edge at 1500 h or 1900 h (Fig. 3a). Similarly, spiders never dropped from the web hub when

35

assayed at 0700 h, 1100 h, or 1500 h (Fig. 3b). Web-shaking behaviour, on the other hand,

occurred at all times of day except 2300 h (Fig. 3c). We observed rearing behaviour and non-

responsiveness at all times of day (Fig. 3d&e). When we combined antipredator responses types

into those which involved leaving the foraging position of the web hub and those which did not,

we found significant diel patterns in the occurrence of absence from the web hub (Chi-square

test: $X^2_4 = 57.14$, $P < 0.0001$) (Fig. 4). We found no significant effect of time of day, individual,

or time of day by individual interaction on the duration of absences from the hub (Scheirer-Ray-

Hare test: $df = 4$, $H = 5.24$, $P = 0.263$; $df = 17$, $H = 20.78$, $P = 0.236$; $df = 12$, $H = 6.68$, $P = 0.878$, respectively). Although habituation and uncontrolled variation in prey stimulus frequency

confounded our initial foraging data, the data were suggestive of nocturnal patterns of foraging

aggression (Fig. 5a). However, we observed attacks at all times of day at which we assayed

individuals. We did not find a significant association between individuals' tendency to leave the

web hub and their tendency to attack the prey stimulus (GLM: $t = 1.30$, $P = 0.211$, $R^2 = 0.085$).

Our second, refined assessment of foraging behaviour in the web corroborated our initial

findings (Fig. 5). When we randomized the prey stimulus frequency and tested individuals every

4 h for 5 days, we again found nocturnal patterns of foraging aggression (Fig. 5b). More spiders

attacked the prey stimulus during the night and early morning than during the day and early

evening (Fig. 5b). Moreover, we found that time of day strongly predicted the likelihood of

attack ($Wald = 18.702$, $df = 5$, $P = 0.002$). We also detected a strong effect of individual on the

likelihood of attack ($Wald = 44.22$, $df = 10$, $P = 0.0001$). While stimulus frequency did not

predict the likelihood of attack ($Wald = 1.294$, $df = 1$, $P = 0.255$), the effect of trial number was

marginally non-significant ($Wald = 3.539$, $df = 1$, $P = 0.060$). Although latency to attack the prey

stimulus tended to be greater during the day and early evening, this pattern was non-significant

(Scheirer-Ray-Hare test: $df = 5$, $H = 4.66$, $P = 0.459$) (Fig. 5). We found a strong effect of individual on latency to attack the prey stimulus (Scheirer-Ray-Hare test: $df = 9$, $H = 19.80$, $P = 0.019$) but found no evidence of time of day by individual interaction (Scheirer-Ray-Hare test: $df = 23$, $H = 25.15$, $P = 0.834$). Interestingly, repeatability of attack latencies for individuals was relatively low (r = 0.11).
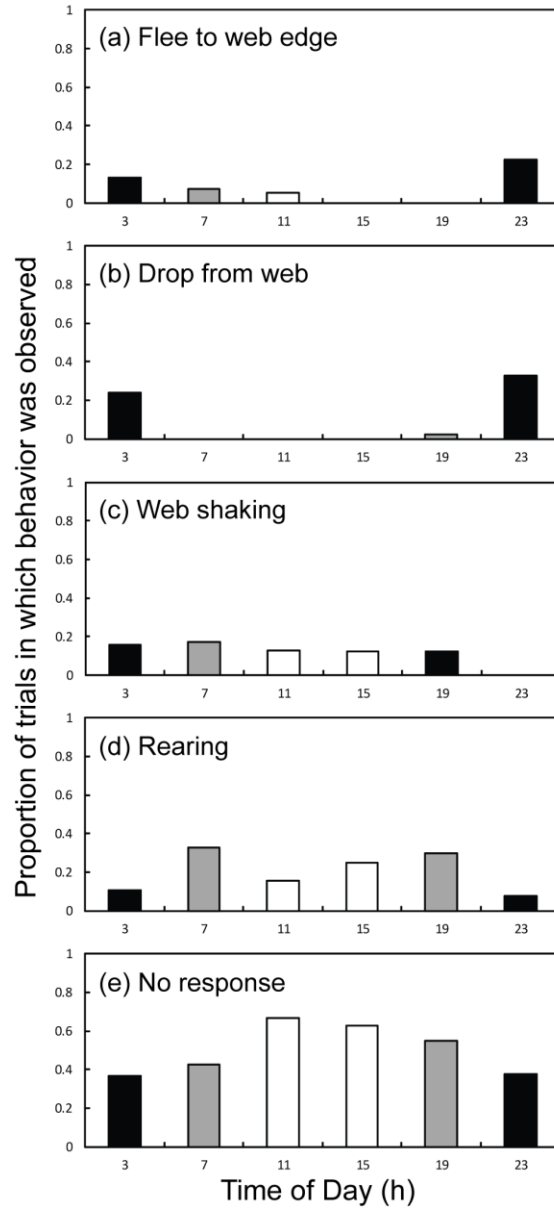
Fig. 3. Occurrence of *C. turbinata* antipredator response types by time of day for individuals assayed in webs under ramping light:dark 12:12 h. Individuals responded to simulated predator attacks by a) fleeing to the web edge, b) dropping from the web, c) shaking the web, d) rearing, or e) remaining motionless in the web hub (no response). Bars represent the proportion of trials at each time of day in which each response was elicited. Open bars: spiders tested with lights on; shaded bars: spiders tested during crepuscule; solid bars: spiders tested with lights off.

Fig. 4. Diel rhythms of absence from the foraging area of *C. turbinata* assayed in webs under ramping light:dark 12:12 h. The dotted line represents the proportion of trials at each time of day in which spiders left the web hub. Columns represent the means and standard errors of web hub absence durations for 20 spiders tested at 6 times of day for 2 days. Open bars: spiders tested with lights on; shaded bars: spiders tested during crepuscule; solid bars: spiders tested with lights off.
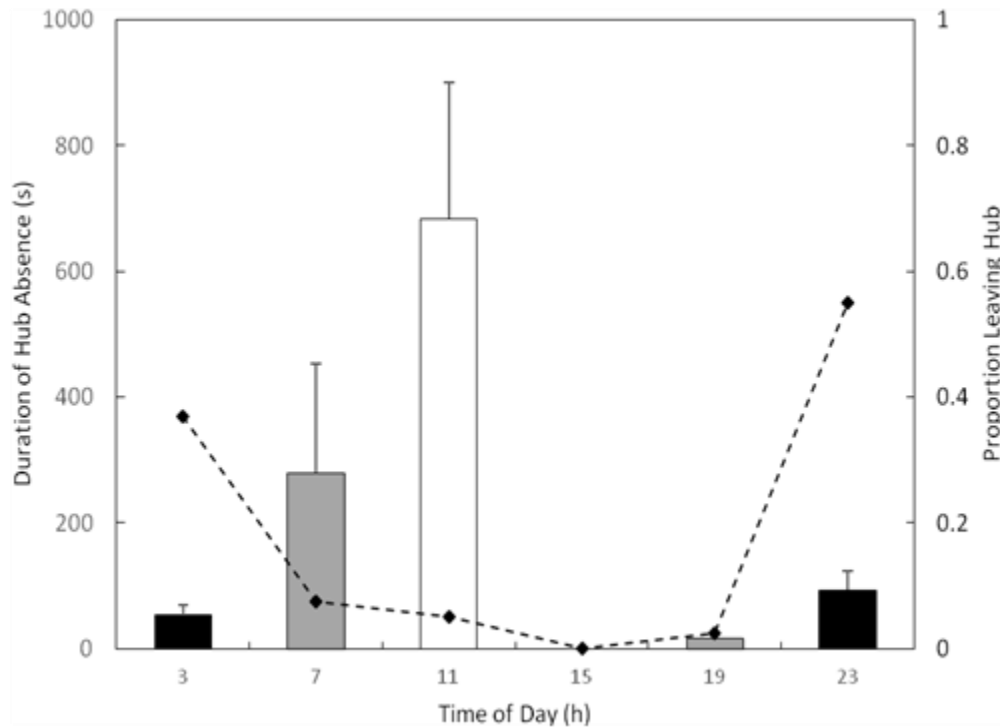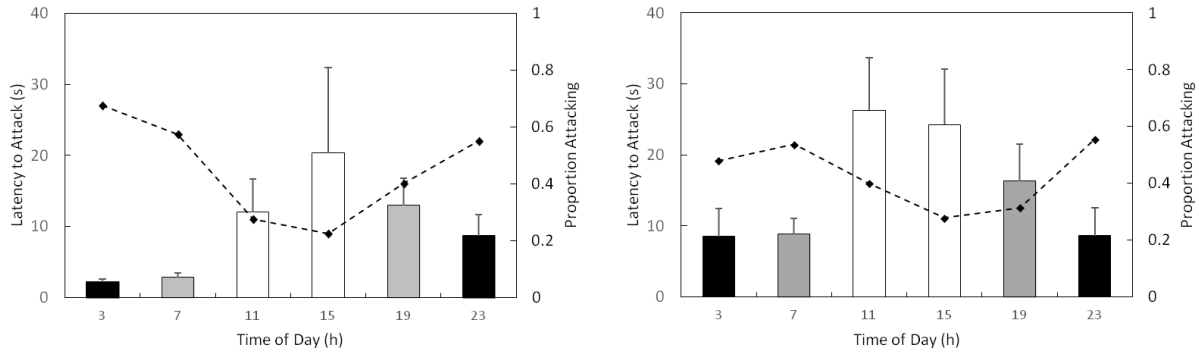
Fig. 5. Diel rhythms of foraging aggression of *C. turbinata* assayed in webs under ramping light:dark 12:12 h. Dotted lines represent the proportion of trials at each time of day in which spiders attacked the prey stimulus. Columns represent the means and standard errors of latency to attack the prey stimulus. a) Initial data collected from 20 spiders assayed at 6 times of day for 2 days using a fixed stimulus frequency; b) data collected from 10 spiders tested at 6 times of day for 5 days using a randomized stimulus frequency. Open bars: spiders tested with lights on; shaded bars: spiders tested during crepuscule; solid bars: spiders tested with lights off.

DISCUSSION

Diel rhythms have been widely touted as adaptations that enhance individual fitness by coordinating physiological and behavioural functions with periods of favorable environmental conditions. However, little evidence exists in support of this assumption, particularly in animal systems (Johnson, 2005). As both predator and prey, spiders provide a tractable model system for investigating the adaptive value of diel behavioural variation. Our findings contribute to a growing body of literature suggesting that spiders modulate their antipredator behaviour over the diel cycle (Jones et al., 2011a; Watts et al., unpublished data). Moreover, we provide evidence of diel rhythms of foraging aggression in a continuously foraging spider. These results support our

prediction that foraging aggression and predator wariness vary over the diel cycle even in spider species that do not transition from refuge use to active foraging.

Contradictory patterns of antipredator response types in our two assays suggest that antipredator behaviour is highly context-dependent in *C. turbinata* (compare Fig. 2 & Fig. 4). When we assayed *C. turbinata* in arenas, we observed more instances and longer durations of antipredator behaviour (i.e. thanatosis) during the day (Fig. 2). These patterns are similar to those reported in another Araneid spider, *Larinioides cornutus* (Jones et al., 2011a). Jones et al. (2011a) posited that increased thanatosis behaviour during the day may correspond to an increased fitness cost associated with fleeing the web hub during the evening and night, when flying insect prey are most abundant (unpublished data). The authors also noted that such patterns of vigilance may arise from diurnal patterns of risk of predation by visual predators (e.g. birds and wasps) (Jones et al., 2011a). However, when we assayed *C. turbinata* in webs, we found that absences from the foraging area occurred predominately during the night (Fig. 3). It may be that the contradictory and non-intuitive nature of our findings arises from the commitment of *C. turbinata* to a cryptic predator avoidance strategy during the day (Tseng & Tso, 2009). If individuals rely on camouflage provided by the trashline to avoid detection by diurnal visual predators, then non-responsiveness in the web may constitute a predator avoidance strategy (Tseng & Tso, 2009). Consequently, the diel patterns of antipredator behaviour we described for spiders in webs may not reflect overall predator vigilance but may instead reflect transitions between a passive avoidance strategy (i.e. non-responsiveness to maximize camouflage) and an active avoidance strategy (i.e. dropping from or fleeing the foraging area). Therefore it is possible that rhythms of antipredator behaviour in arenas do in fact represent patterns of overall vigilance, as has been suggested by studies of behavioural syndromes in other

41

spider taxa (Pruitt & Riechert, 2012). Alternatively, *C. turbinata* may simply modify their behavioural responses in context. Our contradictory findings emphasize the caution with which behavioural assays in unnatural settings should be interpreted.

While we observed prey capture behaviour at all times of day, we found that *C. turbinata* was more likely to attack the prey stimulus during the night and early morning (Fig. 5). These observations indicate that *C. turbinata* forages across the diel cycle, yet modulates foraging aggression in a periodic fashion. The rhythm of aggression seen in *C. turbinata* resembles previously collected data on diel patterns of flying insect abundance (unpublished data). However, the period of greatest foraging aggression coincides with period during which *C. turbinata* is most likely to leave the foraging area in response to simulated predator attacks (Fig. 4 & Fig. 5). This suggests that *C. turbinata* does not sacrifice predator vigilance to increase foraging effort, but our uncertainty regarding rhythms of overall vigilance casts doubt on this interpretation. As mentioned above, diel shifts in predator avoidance strategies may mask overall rhythms of vigilance of spiders in their webs. If we accept that diel rhythms of antipredator behaviour in arenas are representative of rhythms of overall vigilance, then foraging aggression and predator vigilance may be negatively correlated with respect to time of day (Fig. 2 & Fig. 5). Alternatively, if rhythms of wariness in arenas are artifacts of unnatural conditions, aggression toward prey and wariness of predators while in the web may in fact be positively correlated. Such a relationship could arise if aggression and wariness are mutually affected by rhythms of general neurological arousal (i.e. individuals are generally more sensitive to stimuli during certain times of day). However, we did not detect a correlation between individuals' tendency to attack and their tendency to leave the foraging area in response to simulated predation attempts. This suggests that foraging aggression and predator vigilance are not correlated in *C. turbinata*

42

and contrasts with recent evidence that antipredator behaviour is negatively correlated with aggression toward prey in other spider taxa (Pruitt & Riechert, 2012). It is important to note, however, that our ability to detect any association between aggression toward prey and boldness toward predators in *C. turbinata* is likely limited by individual consistency as evidenced indirectly by our analysis of antipredator response duration and latency to attack prey (r = 0.196, r = 0.11, respectively). Furthermore, we used rough proxies for individual foraging aggression and predator wariness that differ from those used in previous studies (Pruitt & Riechert, 2012). If aggression and boldness are in fact unrelated in *C. turbinata*, this would represent an example of remarkable behavioural flexibility in a spider that may permit nearly optimal responses to concurrent risks (i.e. predation and lost foraging).

We are still uncertain whether diel variation in foraging effort and predator vigilance represents an adaptive strategy to maximize foraging gain relative to predation risk across the diel cycle. A rigorous test of this adaptive hypothesis will require i) quantifying diel fluctuations in foraging gain and predation risk and ii) determining whether/how different behavioural responses alter the outcomes of interspecific interactions. We have recently begun quantifying diel environmental variability for *C. turbinata* (unpublished data), and several recent studies have shown that the behavioural tendency of individuals affects the outcome of interspecific interactions in other taxa (Keiser & Pruitt, 2014; Pruitt et al., 2012b). We believe that the framework developed here will facilitate future investigations of the adaptive significance of diel rhythms of behaviour. In particular, an enhanced understanding of the environmental cues and neurochemical processes mediating behavioural rhythmicity will enable the manipulations necessary for rigorous evaluations of the adaptive rhythm hypothesis.

ACKNOWLEDGMENTS

REFERENCES

Beaver, L. M., Gvakharia, B. O., Vollintine, T. S., Hege, D. M., Stanewsky, R., Giebultowicz, J. M. (2002) Loss of circadian clock function decreases reproductive fitness in males of Drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America*, *99(4)*, 2134-2139.

Chou, I., Wang, P., Shen, P., Tso, I. (2005) A test of prey-attracting and predator defence functions of prey carcass decorations built by *Cyclosa* spiders. *Animal Behaviour*, *69*, 1055-1061.

DeCoursey, P. J., Krulas, J. R., Mele, G., Holley, D. C. (1997) Circadian performance of SCN-lesioned antelope ground squirrels in a desert enclosure. *Physiology and Behavior*, *62(5)*, 1099-1108.

DeCoursey, P. J., Walker, J. K., Smith, S. A. (2000) A circadian pacemaker in free-living chipmunks: essential for survival? *Journal of Comparative Physiology A*, *186*, 169-180.

Dytham, C. (2011) *Choosing and Using Statistics: A Biologist's Guide* (3rd ed.). West Sussex, UK: Wiley-Blackwell.

Enright, J. T. & Hammer, W. M. (1967) Vertical diurnal migration and endogenous rhythmicity. *Science, New Series*, *157(3791)*, 937-941.

Green, R. M., Tingay, S., Wang, Z., Tobin, E. M. (2002) Circadian rhythms confer a higher level of fitness to Arabidopsis plants. *Plant Physiology*, *129(2)*, 576-584.

Houston, A. I., McNamara, J. M., Hutchinson, J. M. C. (1993) General results concerning the trade-off between gaining energy and avoiding predation. *Philosophical Transactions: Biological Sciences*, *341(1298)*, 375-397.

Johnson, C. H. (2005) Testing the adaptive value of circadian rhythms. *Methods in Enzymology*, *393*, 818-837.

Jones, T. C., Akoury, T. S., Hauser, C.K., Moore, D. (2011a) Evidence of circadian rhythm in antipredator behavior in the orb-weaving spider *Larinioides cornutus*. *Animal Behavior*, *82(3)*, 549-555.

Jones, T. C., Akoury, T. S., Hauser, C. K., Neblett II, M.F., Linville, B. J., Edge, A. A., Weber, N. O. (2011b) Octopamine and serotonin have opposite effects on antipredator behavior in the orb-weaving spider, *Larinioides cornutus*. *Journal of Comparative Physiology A*, *197*, 819-825.

Keiser, C. N. & Pruitt, J. N. (2014) Spider aggressiveness determines the bidirectional consequences of host-inquiline interactions. *Behavioral Ecology*, *25*, 142-151.

Lampert, W. (1989) The adaptive significance of diel vertical migration of zooplankton. *Functional Ecology*, *3*, 21-27.

Lessels, C. M. & Boag, P. T. (1987) Unrepeatable repeatabilities: a common mistake. *The Auk*, *104(1)*, 116-121.

Lima, S. L. & Bednekoff, P. A. (1999) Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *The American Naturalist*, *153(6)*, 649-659.

Moore, D. & Doherty, P. (2009) Acquisition of a time-memory in forager honey bees. *Journal of Comparative Physiology A*, *195*, 741-751.

Ouyang, Y., Anderson, C. R., Kondo, T., Golden, S. S., Johnson C. H. (1998) Resonating circadian clocks enhance fitness in cyanobacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *95(15)*, 8660-8664.

Pruitt, J. N., Riechert, S. E., Jones, T. C. (2008) Behavioural syndromes and their fitness consequences in a socially polymorphic spider, *Anelosimus studiosus*. *Behavioral Ecology*, *76*, 871-879.

Pruitt, J. N., Oufiero, C. E., Avilés, L., Riechert, S. E. (2012) Iterative evolution of increased behavioral variation characterizes the transition to sociality in spiders and proves advantageous. *The American Naturalist*, *180(4)*, 496-510.

Pruitt, J. N., Stachowicz, J. J., Sih, A. (2012) Behavioral types of predator and prey jointly determine prey survival: potential implications for the maintenance of within-species behavioral variation. *The American Naturalist*, *179(2)*, 217-227.

Pruitt, J. N. & Riechert, S. E. (2012) The ecological consequences of temperament in spiders. *Current Zoology*, *58(4)*, 589-596.

Riechert, S. E. & Maynard Smith, J. (1989) Genetic analyses of two behavioral traits linked to individual fitness in the desert spider *Agelenopsis aperta*. *Animal Behaviour 37*, 624-637.

Riechert, S. E. & Hedrick, A. N. (1993) A test for correlations among fitness-linked behavioral traits in the spider *Agelenopsis aperta* (Araneae: Agelenidae). *Animal Behaviour 46*, 669-675.

Spiller, D. A. (1984) Competition between two spider species: experimental field study. *Ecology*, *65(3)*, 909-919.

Suter, R. B. (1978) *Cyclosa turbinata* (Araneae: Araneidae): prey discrimination via web-borne vibrations. *Behavioral Ecology and Sociobiology*, *3(3)*, 283-296.

Tseng, L. & Tso, I. (2009) A risky defence by a spider using conspicuous decoys resembling itself in appearance. *Animal Behaviour*, *78*, 425-431.

Uetz G. W., Heiber, C. S., Jakob, E. M., Wilcox, R. S., Kroeger, D., McCrate, A., Mostrum, A. M. (1994) Behavior of colonial orb-weaving spiders during a solar eclipse. *Ethology*, *96*, 24-32.

REFERENCES

Beaver LM, Gvakharia BO, Vollintine TS, Hege DM, Stanewsky R, Giebultowicz JM. 2002. Loss of circadian clock function decreases reproductive fitness in males of *Drosophila melanogaster*. Proc Natl Acad Sci USA. 99(4): 2134-2139.

Bellmann H. 1997. Kosmos-atlas spinnentiere. Stuttgart: Frankh-Kosmos Verlag.

Bradshaw WE, Quebodeaux MC, Holzapfel CM. 2003. The contribution of an hourglass timer to the evolution of photoperiodic response in the pitcher-plant mosquito, *Wyeomyia smithii*. Evolution 57(10): 2342-2349.

Carico JE. 1986. Web removal patterns in orb-weaving spiders. In: Shear, WA, editor. Spiders: webs, behavior, and evolution. 1st ed. Stanford: Stanford University Press. p 306-318.

Ceriani MF, Darlington TK, Staknis D, Mas P, Petti AA, Weitz CJ, Kay SA. 1999. Light-dependent sequestration of timeless by cryptochrome. Science 285: 553-556.

Chou I, Wang P, Shen P, Tso I. 2005. A test of prey-attracting and predator defence functions of prey carcass decorations built by *Cyclosa* spiders. Anim Behav. 69(5): 1055-1061.

Cloudsley-Thompson JL. 1995. A review of the anti-predator devices of spiders. Bull Br Arach Soc. 10(3): 81-96.

DeCoursey PJ, Krulas JR, Mele G, Holley DC. 1997. Circadian performance of SCN-lesioned antelope ground squirrels in a desert enclosure. Physiol Behav. 62(5): 1099-1108.

DeCoursey PJ, Walker JK, Smith SA. 2000. A circadian pacemaker in free-living chipmunks: essential for survival? J Comp Physiol A. 186: 169-180.

Dickmeis T. 2009. Glucocorticoids and the circadian clock. J Endocrinol. 200: 3-22.

Dunlap JC, Loros JJ, DeCoursey PJ, editors. 2004. Chronobiology: biological timekeeping. Sunderland: Sinauer Associates.

Dytham C. 2011. Choosing and using statistics: a biologist's guide. 3rd ed. West Sussex, UK: Wiley-Blackwell.

Eban-Rothschild A, Belluci S, Bloch G. 2011. Maternity-related plasticity in circadian rhythms of bumble-bee queens. Proc R Soc B 287: 3510-3516.

Emery P, So WV, Kaneko M, Hall JC, Rosbash M. 1998. CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95: 669-679.

Enright JT, Hammer WM. 1967. Vertical diurnal migration and endogenous rhythmicity. Science, New Series 157: 937-941.

Fleury F, Allemand R, Vavre F, Fouillet P, Bouletreau M. 2000. Adaptive significance of a circadian clock: temporal segregation of activities reduces intrinsic competitive inferiority in *Drosophila* parasitoids. Proc R Soc Lond B. 267: 1005-1010.

Foelix RF. 2011. Biology of spiders. 3rd ed. New York: Oxford University Press.

Gallego M, Virshup DM. 2007. Post-translational modifications regulate the ticking of the circadian clock. Nat Rev Mol Cell Bio. 8(2): 139-148.

Green RM, Tingay S, Wang Z, Tobin EM. 2002. Circadian rhythms confer a higher level of fitness to Arabidopsis plants. Plant Physiol. 129(2): 576-584.

Herberstein ME, Elgar MA. 1994. Foraging strategies of *Eriophora transmarina* and *Nephila plumipes* (Araneae: Araneoidea): nocturnal and diurnal orb-weaving spiders. Aust J Ecol. 19: 451-457.

Houston AI, McNamara JM, Hutchinson JMC. 1993. General results concerning the trade-off between gaining energy and avoiding predation. Phil Trans R Soc Lond B. 341: 375-397.

Howlader G, Sharma VK. 2006. Circadian regulation of egg-laying behavior in fruit flies *Drosophila melanogaster*. J Insect Physiol. 52: 779-785.

Hurd MW, Ralph MR. 1998. The significance of circadian organization for longevity in the golden hamster. J Biol Rhythms 13(5): 430-436.

Johnson CH. 2005. Testing the adaptive value of circadian rhythms. Method Enzymol. 393: 818-837.

Jones TC, Akoury TS, Hauser CK, Moore D. 2011a. Evidence of circadian rhythm in antipredator behavior in the orb-weaving spider *Larinioides cornutus*. Anim Behav. 82(3): 549-555.

Jones TC, Akoury TS, Hauser CK, Neblett II MF, Linville BJ, Edge AA, Weber NO. 2011b. Octopamine and serotonin have opposite effects on antipredator behavior in the orb-weaving spider, *Larinioides cornutus*. J Comp Physiol A. 197: 819-825.

Keiser CN, Pruitt JN. 2014. Spider aggressiveness determines the bidirectional consequences of host-inquiline interactions. Behav Ecol. 25: 142-151.

Koovor J, Ortega-Escobar J, Munoz-Cuevas A. 1999. Circadian structural changes in the retina of *Lycosa tarantula* (Araneae: Lycosidae). Biol Rhythm Res 30(4): 407-423.

Kralj-Fisher S, Schneider JM. 2012. Individual behavioural consistency and plasticity in an urban spider. Anim. Behav. 84(1): 197-204.

Lampert W. 1989. The adaptive significance of diel vertical migration of zooplankton. Funct Ecol. 3: 21-27.

Lessels CM, Boag PT. 1987. Unrepeatable repeatabilities: a common mistake. The Auk 104(1): 116-121.

Lima SL, Bednekoff PA. 1999. Temporal variation in danger drives antipredator behavior: the

    predation risk allocation hypothesis. Am Nat. 153(6): 649-659.

Moore D, Doherty P. 2009. Acquisition of a time-memory in forager honey bees. J Comp

    Physiol A. 195: 741-751.

Moore D, Rankin MA. 1985. Circadian locomotor rhythms in individual honeybees. Physiol

    Entomol. 10(2): 191-197.

Moore D, Rankin MA. 1993. Light and temperature entrainment of a locomotor rhythm in

    honeybees. Physiol Entomol. 18(3): 271-278.

Nentwig W. 1982. Why do only certain insects escape from a spider's web? Oecologia 53(3):

    412-417.

Ouyang Y, Anderson CR, Kondo T, Golden SS, Johnson CH. 1998. Resonating circadian clocks

    enhance fitness in cyanobacteria. Proc Natl Acad Sci USA. 95(15): 8660-8664.

Pruitt JN, Riechert SE, Jones TC 2008. Behavioural syndromes and their fitness consequences in

    a socially polymorphic spider, *Anelosimus studiosus*. Behav Ecol. 76: 871-879.

Pruitt JN, Oufiero CE, Avilés L, Riechert SE. 2012. Iterative evolution of increased behavioral

    variation characterizes the transition to sociality in spiders and proves advantageous. Am

    Nat. 180(4): 496-510.

Pruitt JN, Stachowicz JJ, Sih A. 2012. Behavioral types of predator and prey jointly determine

    prey survival: potential implications for the maintenance of within-species behavioral

    variation. Am Nat. 179(2): 217-227.

Pruitt JN, Riechert SE. 2012. The ecological consequences of temperament in spiders. Curr Zool.

    58(4): 589-596.

Riechert SE, Maynard Smith J. 1989. Genetic analyses of two behavioral traits linked to

      individual fitness in the desert spider *Agelenopsis aperta*. Anim Behav. 37(4): 624-637.

Riechert SE, Hedrick AN. 1993. A test for correlations among fitness-linked behavioral traits in

      the spider *Agelenopsis aperta* (Araneae: Agelenidae). Anim Behav. 46(4): 669-675.

Roth T, Sprau P, Schmidt R, Naguib M, Amrhein V. 2009. Sex-specific timing of mate searching

      and territory prospecting in the nightingale: nocturnal life of females. Proc R Soc B. 276:

      2045-2050.

Rypstra AL. 1982. Building a better insect trap: an experimental investigation of prey capture in

      a variety of spider webs. Oecologia 52(1): 31-36.

Seyfarth EA. 1980. Daily patterns of locomotor activity in a wandering spider. Physiol Entomol.

      5: 199-206.

Sherman PM. 1994. The orb-web: and energetic and behavioural estimator of a spider's dynamic

      foraging and reproductive strategies. Anim Behav. 48(1): 19-34.

Sih A, Cote J, Evans M, Fogarty S, Pruitt JN. 2012. Ecological implications of behavioural

      syndromes. Ecol Lett. 15: 278-289.

Sih A, Bell AM. 2008. Insights for behavioral ecology from behavioral syndromes. Adv Stud

      Behav. 38: 227-281.

Spiller DA. 1984. Competition between two spider species: experimental field study. Ecology,

      65(3): 909-919.

Suter RB. 1978. *Cyclosa turbinata* (Araneae: Araneidae): prey discrimination via web-borne

      vibrations. Behav Ecol Sociobiol. 3(3): 283-296.

Suter RB. 1993. Circadian rhythmicity and other patterns of spontaneous motor activity in

    Frontinella pyramitela (Linyphiidae) and *Argyrodes trigonum* (Theridiidae). J Arachnol.

    21(1): 6-22.

Tseng L, Tso I. 2009. A risky defence by a spider using conspicuous decoys resembling itself in

    appearance. Anim Behav. 78: 425-431.

Uetz GW, Heiber CS, Jakob EM, Wilcox RS, Kroeger D, McCrate A, Mostrum AM. 1994.

    Behavior of colonial orb-weaving spiders during a solar eclipse. Ethology 96: 24-32.

Wise DH. 1993. *Spiders in Ecological Webs*. Cambridge: Cambridge University Press.

Xu K, Zheng X, Sehgal A. 2008. Regulation of feeding and metabolism by neuronal and

    peripheral clocks in *Drosophila*. Cell Metab. 8: 289-300.

Ziegler TA, Cohen JH, Forward Jr. RB. 2010. Proximate control of diel vertical migration in

    phyllosoma larvae of the Caribbean spiny lobster *Panulirus argus*. Biol Bull. 219: 207-

    219.

VITA

JAMES COLTON WATTS

Education:	Cookeville High School, Cookeville, Tennessee, 2008
	B.S. Biology, East Tennessee State University, Johnson City, Tennessee, 2012
	M.S. Biology, East Tennessee State University, Johnson City, Tennessee, 2014
Professional Experience:	Graduate Assistant, East Tennessee State University, College of Arts and Sciences, 2012-2014
Publications:	Joyner ML, Ross CR, Watts JC, Jones TC (submitted Nov. 16, 2013) A Stochastic Simulation Model for *Anelosimus studiosus* During Prey Capture: A Case Study for Determination of Optimal Spacing. *Mathematical Biosciences and Engineering*.
	Watts JC, Herrig A, Allen WD, Jones TC (submitted Dec. 18, 2013) Diel Patterns of Foraging Aggression and Antipredator Behaviour in the Trashline Orb-weaving Spider, *Cyclosa turbinata*. *Animal Behaviour*.
Presentations:	Ecological Implications of Diel Rhythms of Aggression in the Subsocial Spider Anelosimus studiosus (Araneae: Theridiidae). Oral Presentation, Entomological Society of America Meeting, 2012
	Ecological Implications of Diel Rhythms of Aggression in the Subsocial Spider Anelosimus studiosus (Araneae: Theridiidae). Poster Presentation, Appalachian Student Research Forum, 2013
	Chronoethology of Cyclosa turbinata. Oral Presentation, American Arachnological Society Meeting, 2013
Honors and Awards:	James H. Quillen Graduate Scholar, 2013-2014
	Runner-up Best Student Talk, American Arachnological Society Meeting, 2013
	1st Place Graduate Poster Presentation in Natural Sciences, Appalachian Student Research Forum, 2013
	National SMART Grant, 2011