Seasonal regulation of the circadian rhythms of behavioural temperature selection and locomotor activity in Australian sleepy lizards (*Tiliqua rugosa*, Gray; Reptilia: Scincidae)

A portfolio of publications submitted by David John Ellis*

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Abstract

This research project examined the role of environmental time cues and circadian rhythms in mediating seasonal adjustments in the body temperature and locomotor activity patterns in the Australian sleepy lizard, *Tiliqua rugosa* (Gray 1827). The first component of this study investigated whether daily rhythms of behavioural thermoregulation and locomotor activity that *T. rugosa* displays in the field are endogenous circadian rhythms. In each season, there was significant variation in each of these rhythms in lizards released on laboratory thermal gradients under prevailing light-dark (LD) cycles. Both rhythms persisted when lizards were released in constant darkness (DD) and exhibited the same free-running period suggesting a single circadian pacemaker drives both rhythms. The first component of this study revealed that seasonal changes in the expression of daily behavioural thermoregulatory and locomotor activity rhythms are mediated by the circadian system in *T. rugosa*.

The second component of this project comprised three experiments that determined the relative importance of 24 h LD and temperature cycles (TCs) in entraining the locomotor activity rhythm. In the first experiment, lizards were held under LD 12:12 and were subjected to either a TC of 33:15°C in phase with the LD cycle or a reversed TC. Following LD 12:12, lizards were maintained under the same TCs but were subjected to DD. Activity was restricted to the thermophase in LD irrespective of the lighting regime and during the period of DD that followed, suggesting entrainment by the TC. In the second experiment, lizards were held under LD 12.5:11.5 and were subjected to one of three treatments; (1) constant 30°C, (2) normal TC (30:20°C), or (3) reversed TC. Following LD, all lizards were subjected to DD and constant 30°C. Post-entrainment free-run records revealed that LD cycles and TCs both entrain locomotor activity rhythms of *T. rugosa*. Although there was large variation in the phasing of the rhythm in relation to the LD cycle in reversed TC lizards, TCs presented in phase with the LD cycle most accurately synchronised the rhythm to the photocycle. In the third experiment, lizards were held in DD at constant 30°C before being subjected to a further period of DD and one of four treatments; (1) normal TC (06:00 h to 18:00 h thermophase), (2) delayed TC (12:00 h to 00:00 h thermophase), (3) advanced TC (00:00 h to 12:00 h thermophase) or (4) control (no TC, constant

30°C). While control lizards continued to free-run in DD at constant temperature, locomotor rhythms of lizards subjected to TCs rapidly entrained to TCs irrespective of whether TCs were phase advanced or delayed by 6 h. The results of this experiment excluded the possibility that masking effects were responsible for locomotor responses of lizards to TCs. This study demonstrated that seasonal changes in the expression of this species' daily behavioural thermoregulatory and locomotor rhythms are mediated by a circadian system that is sensitive to both light and temperature. The sensitivity of the circadian system to temperature, in particular, may allow *T. rugosa* to restrict its activity to times of the year that are thermally favourable.

Original Work Declaration

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List of Publications included for the Award of Doctor of Philosophy

Ellis DJ, Firth BT, Belan I (2007) Circadian rhythms of locomotor activity and temperature selection in sleepy lizards, *Tiliqua rugosa*. Journal of Comparative Physiology [A] 193:695-701

Ellis DJ, Firth BT, Belan I (2008) Interseasonal variation in the circadian rhythms of locomotor activity and temperature selection in sleepy lizards, *Tiliqua rugosa*. Journal of Comparative Physiology [A] 194:701-712

Ellis DJ, Firth BT, Belan I (2009) Thermocyclic and photocyclic entrainment of circadian locomotor activity rhythms in sleepy lizards, *Tiliqua rugosa*. Chronobiology International 26(7):1369-1388

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Author Contributions to Publications

Publication 1

Ellis DJ, Firth BT, Belan I (2007) Circadian rhythms of locomotor activity and temperature selection in sleepy lizards, *Tiliqua rugosa*. Journal of Comparative Physiology [A] 193:695-701

Mr. David Ellis designed and conducted the experiment, analysed the data and was the corresponding author for this publication. David prepared the first draft of this manuscript and made changes to the manuscript according to suggestions made by both of his supervisors (Drs. Bruce Firth and Ingrid Belan) and by the anonymous reviewers appointed by the Journal of Comparative Physiology [A].

Dr. Bruce Firth made editorial comments and suggested corrections to the manuscript before it was submitted to the Journal of Comparative Physiology [A] for the first time. Dr. Firth made further editorial comments to the manuscript and suggestions for our response to the reviewers before it was resubmitted.

Dr. Ingrid Belan also made editorial comments and suggested revisions to the manuscript prior to its submission and after it had been accepted with revisions.

David Ellis Date

I give my consent for the abovementioned publication to be included in this thesis:

Dr. Bruce T. Firth Date

Dr. Ingrid Belan Date

Publication 2

Ellis DJ, Firth BT, Belan I (2008) Interseasonal variation in the circadian rhythms of locomotor activity and temperature selection in sleepy lizards, *Tiliqua rugosa*. Journal of Comparative Physiology [A] 194:701-712

Mr. David Ellis designed and conducted the experiments during each season, analysed and interpreted the data and was the corresponding author for this publication. David wrote the first draft of this manuscript. Prior to submission, editorial changes to the manuscript were made by David according to suggestions made by both supervisors. Following the acceptance of this publication, David made further changes under the guidance of both supervisors and in response to the reviewers.

Dr. Bruce Firth made editorial comments and suggested corrections to the manuscript before it was submitted. Dr. Firth made further editorial comments to the manuscript before it was resubmitted.

Dr. Ingrid Belan also made editorial comments and suggested revisions to the manuscript prior to its submission and after it had been accepted with revisions.

David Ellis Date

I give my consent for the abovementioned publication to be included in this thesis:

Dr. Bruce T. Firth Date

Dr. Ingrid Belan Date

Publication 3

Ellis DJ, Firth BT, Belan I (2009) Thermocyclic and photocyclic entrainment of circadian locomotor activity rhythms in sleepy lizards, *Tiliqua rugosa*. Chronobiology International 26(7): 1369-1388

Mr. David Ellis designed and conducted both experiments, analysed and interpreted the data and was the corresponding author for this publication. David wrote the first draft of this manuscript. Prior to submission, editorial changes to the manuscript were made by David according to suggestions made by both of his supervisors. Following the acceptance of this publication, David made further changes in response to comments made by both of his supervisors and the reviewers.

Dr. Bruce Firth made editorial comments and suggested corrections to the manuscript before it was submitted and made further editorial comments to the manuscript for our response to the reviewers before it was resubmitted.

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Dr. Bruce T. Firth Date

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