Selected Publication Abstracts of

Michaela Hau

Assistant Professor, Department of Ecology and Evolutionary Biology
Princeton University, Princeton, NJ 08544-1003

Hau, M. (2001) Timing of breeding in variable environments: Tropical birds as model systems. *Horm. & Behav.* 40: 281-290.

Animals need to adjust reproductive decisions to environmental seasonality. In contrast to species from the well-studied temperate zones, little is known for tropical birds about the environmental cues that stimulate reproductive activity and the physiological mechanisms that regulate reproduction. I am investigating the environmental and endocrine mechanisms that underlie the timing of reproduction in spotted antbirds from the near-equatorial Panamanian rainforest and in small ground finches from the equatorial and Galapagos islands. Spotted antbirds live in a fairly predictable seasonal environment and show regular changes in gonad sizes and some reproductive hormones. Despite the small annual variation in photoperiod close to the equator, these birds can measure slight photoperiodic increases and use it to initiate reproductive activity. Spotted antbirds also respond to seasonal changes in food availability, which allows them to flexibly adjust gonad growth to environmental conditions. Testosterone is involved in regulating song and aggressive behavior in these year-round territorial birds, although it can remain at low plasma levels throughout the year. In contrast, small ground finches exposed to a rather unpredictable climate on Galapagos appear to grow their gonads whenever heavy rains fall and have regressed gonads during other times of the year. The lack of a physiological preparation for the breeding season and their response to short-term cues related to rainfall indicate a striking flexibility in the regulation of breeding in small ground finches. I suggest that tropical birds can serve as model systems to study the physiological adaptations to different environments. Unraveling the neuroendocrine mechanisms behind the flexibility in reproductive timing will clarify whether differences found between temperate and tropical birds represent variations of the same basic mechanism or instead reflect a fundamental divergence in physiological control systems.

Wikelski, M., Hau, M. and Wingfield, J.C. (2000) Seasonality of reproduction in a neotropical rain forest bird. *Ecology* 81: 2458-2472.

Tropical wet forests are commonly perceived as stable and constant environments. However, many rain forest organisms reproduce seasonally. To understand the proximate regulation of life history events in tropical organisms, we asked three questions: (1) How predictable are seasonal changes in the tropical rain forest? (2) Can tropical organisms anticipate environmental seasonality, despite the presumed lack of long-term environmental cues in near-equatorial areas? (3) What environmental cues can tropical organisms use? We studied Spotted Antbirds, monogamous understory insectivores, which started breeding in Panama (9 degrees N) in May (wet season) and continued until September/October. Breeding patterns were consistent between years, indicating that tropical seasons were as predictable for Spotted Antbirds (predictability 70%) as they are for many north temperate birds. Individual Spotted Antbirds shut down reproductive capacity (i.e., decreased gonad size) from October until February. In March, during the height of the dry season and about six weeks ahead of the wet season, gonads started to grow again. The growth rate of gonads was influenced by the amount of rainfall, which has been shown to correlate with food abundance. Gonad growth was paralleled by changes in luteinizing hormone, but not in testosterone, which remained at very low plasma levels year-round. The latter contrasts with the pattern for most migratory temperate-zone birds. Seasonal changes in reproductive activity correlated strongly with changes in the tropical photoperiod, but weakly with

light intensity and rainfall, and nor with temperature. Thus, Spotted Antbirds likely anticipated rain forest seasonality using long-term cues (tropical photoperiod) and fine-tuned their reproductive activities using short-term cues (food/rainfall). The use of long-term environmental information could apply to most vertebrate species that live in the tropics.

Hau, M., Wikelski, M. and Wingfield, J.C. (2000) Visual and nutritional food cues fine-tune timing of reproduction in a neotropical rainforest bird. *J. Exp. Zool.* 286: 494-504.

Food may act as a proximate factor in the regulation of avian seasonal breeding. Food cues could provide particularly important seasonal information to birds living in variable tropical environments, but this has not yet been tested. Spotted antbirds (Hylophylax n. naevioides) inhabiting a humid forest in central Panama (9 degrees N) Likely use changes in the tropical photoperiod to time reproduction on a long-term, seasonal basis. We predicted that these insectivorous birds also adjust reproduction to short-term cues such as food availability because the onset of the rainy season and the resulting increase in insect abundance varies considerably between years. To test this prediction, prior to their breeding season (when they had half-maximal gonads), we either exposed captive male spotted antbirds to an ad libitum standard diet only or added live crickets to this diet. Males that received live crickets significantly increased gonad sizes within 3 weeks over controls on the standard diet. Moreover, in six additional experiments cricket availability always increased song rate, usually within a few days. The stimulatory effect of live crickets on song activity may function independent of nutritional aspects: Freshly killed crickets, providing similar nutritional content as live crickets, did not stimulate the birds' song activity. However, song activity increased to intermediate levels when live crickets were shown under a clear plastic wrap, i.e., when birds could see but not eat crickets. We hypothesize that the opportunity to see and handle live insects stimulates song and reproductive activity in these birds. Our data indicate for the first time that a tropical rainforest bird can use food cues to evaluate the suitability of local environmental conditions for breeding.

Hau, M., Wikelski, M., Soma, K.K. and Wingfield, J.C. (2000) Testosterone and year-round territorial aggression in a tropical bird. *Gen. Comp. Endocrinol*. 117: 20-33.

Testosterone (T) regulates avian behaviors such as song and aggression during the breeding season. However, the role of T in year-round territorial birds is still enigmatic, especially in tropical birds. Spotted antbirds (Hylophylax n. naevioides) defend territories in the Panamanian rainforest year-round but have low plasma T levels (0.1-0.2 ng/ml), except during brief periods of social challenges. We manipulated T action in captive male Spotted antbirds to test whether this hormone is involved in the regulation of song and aggression. T-implants increased plasma androgen levels (T and dihydrotestosterone) and enhanced song in nonbreeding males. During a staged male-male encounter, T-implanted males sang more and were more aggressive than controls. In a second experiment, we blocked the two known T actions: its binding to androgen receptors and its conversion into estradiol by the enzyme aromatase. For this, we administered the androgen receptor antagonist flutamide (Flut) in combination with the aromatase inhibitor 1-4-6 androstatrien-3,17-dione (ATD) to birds in breeding condition. ATD + Flut treatment significantly elevated plasma levels of luteinizing hormone, presumably via the lack of T feedback from its receptors. ATD + Flut-treated birds gave fewer spontaneous songs than control-implanted males. During staged male-male encounters, ATD + Flut-treated males did not sing at all and showed reduced aggressive behavior. Our data indicate that T can regulate aggressive behavior in these tropical birds. Although plasma T levels can be low year-round, Spatted antbirds may use T either by secreting it briefly during social challenges, by having a high sensitivity to T action, or by enzymatically converting circulating T precursors directly at the site of action. We hypothesize that: plasma T levels are kept low in these year-round territorial birds to avoid potentially detrimental effects of tonically elevated T. Future treatment of nonbreeding birds with ATD + Flut will reveal whether T is indeed involved year-round in regulatin aggressive behavior.

Wikelski, M., Hau, M. and Wingfield, J.C. (1999) Social instability increases plasma testosterone in a year-round territorial neotropical bird. *Proc. R. Soc. Lond. Ser. B-Biol. Sci*. 266: 551-556.

In many vertebrates, elevated levels of plasma testosterone (T) are important for reproduction and territorial aggression. However, many tropical birds reproduce and defend territories while plasma T-levels are basal. We studied how aggression and T-levels are regulated in male neotropical spotted antbirds, which defend territories year-round in the Panamanian rainforest. Although spotted antbirds reproduce seasonally, T-levels of individual males often remained at baseline (0.2 ng ml(-1)) throughout the year, even in courting males. On the other hand, T-levels were elevated (maximally to 1.57 ng ml(-1)) during periods of social instability at any time of the year, even when males had entirely regressed gonads. Experimental territorial intrusions (broadcast of conspecific song) confirmed these observations by showing that T-levels increased after about two hours of playback time. Our data suggest that spotted antbirds avoided the potential costs associated with constantly high plasma T-levels (e.g. increased mortality rates). Contrary to temperate-zone birds, spotted antbirds had the potential to react to social challenges with an increase of plasma T year-round. These results are, to our knowledge, presently unique, but may apply to many vertebrate species that inhabit the tropics.

Hau, M., Wikelski, M. and Wingfield, J.C. (1998) A neotropical forest bird can measure the slight changes in tropical photoperiod. *Proc. R. Soc. Lond. Ser. B-Biol. Sci*. 265: 89-95.

Many tropical birds breed seasonally, but it is largely unknown which environmental cues they use to time reproduction. Changes in tropical photoperiod have been regarded as too small to be used as a proximate environmental cue. This hypothesis, however, has never been rigorously tested. Here, we report on experimental evidence that photoperiodic changes characteristic of tropical latitudes stimulate reproductive activity in a neotropical bird from the forest understory. In the central Republic of Panama (9 degrees N), photoperiod varies annually between 12 hours (December) and 13 hours (June). Free-living spotted antbirds (Hylophylax n. naevioides) had regressed gonads in December, but increased gonads ahead of the rainy (the breeding) season in May Captive spotted antbirds exposed to a 'long' photoperiod of 13 hours increased gonadal size eight-fold and song activity six-fold over that of control birds remaining on a simulated 'short' photoperiod of 12 hours of daylight. Moreover, even a photoperiod of 12 hours 17 minutes was sufficient to stimulate gonadal growth in photostimulated birds over that of controls. The dramatic changes in gonadal development were not accompanied by similar changes in hormone titres such as luteinizing hormone and testosterone as expected from temperate zone birds. We propose a more general role of the tropical photoperiod in the regulation of seasonal events in tropical organisms, or in temperate zone species migrating to the tropics.

Gwinner, E., Hau, M. and Heigl, S. (1997) Melatonin: Generation and modulation of avian circadian rhythms. *Brain Res. Bull*. 44: 439-444.

The pineal organ and its hormone melatonin are significant components of avian circadian pacemaking systems. In songbirds, pinealectomy results in the abolition or destabilization of overt circadian rhythms such as the rhythm of locomotor activity, feeding, or body temperature. A stable rhythmicity can be restored either by reimplanting a pineal organ, by periodic injections or infusions of melatonin, or by applying melatonin rhythmically through the drinking water, Several results suggest that the pineal melatonin rhythm acts on at least one other oscillator within the circadian pacemaking system, presumably the SCN, which in turn, feeds back to the pineal. As described by the "Neuroendocrine Loop" and "Internal Resonance" models, overall pacemaker output thus depends on the relative strengths of the oscillations in the pineal and the SCN. Investigations on migratory birds have shown that the amplitude of the 24-h plasma melatonin rhythm is reduced during the migratory seasons compared with the nonmigratory seasons.

According to the models mentioned above, such a reduced melatonin amplitude should result in a reduction in the degree of self-sustainment of the pacemaker as a whole. This, in turn, should facilitate adjustment to the altered Zeitgeber conditions encountered by these birds as a result of their own migratory flights, A seasonal reduction in melatonin amplitude also occurs in some high-latitude birds during midsummer and midwinter, Under such conditions a less self-sustained circadian pacemaker may enhance entrainability to weak zeitgeber conditions. These examples suggest that the properties of the circadian system may be adjusted to match the changing requirements for synchronization, and that this is achieved by altering the melatonin amplitude.

Hau, M. and Gwinner, E. ( ) Adjustment of house sparrow circadian rhythms to a simultaneously applied light and food zeitgeber. *Physiol. Behav*. 62: 973-981.

Periodic food availability has been shown to be an effective circadian zeitgeber in many vertebrates. It is still unclear, however, i) whether light active species like most birds can synchronize with food cycles in the presence of a strong light-dark (LD) cycle and ii) whether it is common among non-mammalian vertebrates to use a separate circadian oscillator to synchronize with food cycles as most mammals do. We investigated these questions experimentally by exposing house sparrows simultaneously to two zeitgebers: light and food. The LD cycle was set at 1410 h; food was always available for 12 hour per day, but at different phases of the LD cycle. The effects of the two zeitgebers were analyzed by observing two behavioral outputs of the birds' circadian system, the rhythms of locomotion and feeding. The data revealed that light acted as the dominant zeitgeber in most conditions. Food cycles affected the phase of the behavioral rhythms of the birds only when the food was presented no later than 3 h after the onset of light. Apart from their synchronizing actions both light and food cycles also exerted direct (masking) effects on the behavioral rhythms of the birds. The results suggest that the circadian system of house sparrows can indeed adjust to two simultaneous environmental periodicities, i.e. light and food. We propose that light is the stronger zeitgeber and plays a 'permissive' role in determining the phases at which synchronization with food cycles comes into effect. We did not find evidence that the house sparrows' behavioral rhythms are controlled by a food-entrainable circadian oscillator that is distinct from the light-entrainable system as is the case in most mammals. The differences in the patterns of food synchronization and organization of the circadian system that appear to exist between different species can be interpreted in two ways: i) species of different phylogenetic origin (e.g., mammals versus birds) evolved different circadian system or ii) regardless of phylogeny, species with different ecological requirements show a specialization in their circadian organization which is adjusted to the importance of zeitgebers in nature.

Hau, M. and Gwinner, E. (1996) Food as a circadian zeitgeber for house sparrows: The effect of different food access durations. *J. Biol. Rhythms* 11: 196-207.

House sparrows (Passer domesticus) can synchronize their circadian rhythms of locomotion and feeding with times of periodic food availability. In contrast to most mammals, which synchronize only a specific part of their circadian system with feeding cycles and thus express two distinct activity components, house sparrows synchronize their circadian activity rhythms as a whole with the food zeitgeber. Previous results had indicated that feeding cycles act as comparatively weak zeitgebers for house sparrows. In the present study, therefore, we investigate whether feeding schedules are weak zeitgebers in general or whether their impact on the circadian system of the birds depends on the degree of food restriction. A detailed analysis of the synchronization pattern under the different experimental conditions should help to clarify whether house sparrows use a different mechanism for food-synchronization than mammals. House sparrows were kept in continuous dim light and exposed to different feeding schedules with daily food access durations ranging from 8 to 20 h. Many birds lost synchronization and exhibited free-running rhythms in locomotor and feeding activity when the daily food access

duration was lengthened but became synchronized when the feeding duration was shortened. The interpretation that short food access durations represent stronger zeitgebers than long food access durations was supported by the occurrence of large negative phase-angle differences during long daily feeding schedules, contrasting with small and sometimes positive phase-angle differences under short food access durations. There were no indications that house sparrows possess a specific food-entrainable circadian oscillator as mammals do. Rather, periodic food availability seems to be a zeitgeber for the whole circadian system, which, hence, can be synchronized both by light and food. An explanation for such different mechanisms of food-synchronization is offered in the feeding ecology of these animals. Birds may evaluate the importance of a specific feeding schedule as a zeitgeber either from temporal information on the duration of the daily food access time or from energetic considerations. The phase-angle differences associated with the different feeding schedules and the maintenance of daily activity times may ensure an appropriate temporal integration of behavior with specific conditions. Nonsynchronized birds exhibited masking-induced feeding activity, which might represent an alternative means of adjusting to feeding cycles when synchronization cannot occur.

Wikelski, M. and Hau, M. (1995) Is there an endogenous tidal foraging rhythm in marine iguanas*? J. Biol. Rhythms* 10: 335-350.

As strictly herbivorous reptiles, Galapagos marine iguanas graze on algae in the intertidal areas during low tide. Daily foraging rhythms were observed on two islands during 3 years to determine the proximate factors underlying behavioral synchrony with the tides. Marine iguanas walked to their intertidal foraging grounds from far-off resting areas in anticipation of the time of low tide. Foraging activity was restricted to daytime, resulting in a complex bitidal rhythm including conspicuous switches from afternoon foraging to foraging during the subsequent morning when low tide occurred after dusk. The animals anticipated the daily low tide by a maximum of 4 h. The degree of anticipation depended on environmental parameters such as wave action and food supply. "Early foragers" survived in greater numbers than did animals arriving later at foraging sites, a result indicating selection pressure on the timing of anticipation. The timing of foraging trips was better predicted by the daily changes in tabulated low tide than it was by the daily changes in actual exposure of the intertidal foraging flats, suggesting an endogenous nature of the foraging rhythms. Endogenous rhythmicity would also explain why iguanas that had spontaneously fasted for several days nevertheless went foraging at the "right" time of day. A potential lunar component of the foraging rhythmicity of marine iguanas showed up in their assemblage on intertidal rocks during neap tide nights. This may indicate that iguanas possessed information on the semi-monthly rhythms in tide heights. Enclosure experiments showed that bitidal foraging rhythms of iguanas may free run in the absence of direct cues from the intertidal areas and operate independent of the light:dark cycle and social stimuli. Therefore, the existence of a circatidal oscillator in marine iguanas is proposed. The bitidal foraging pattern may result from an interaction of a circadian system with a circatidal system. Food intake or related stimuli may be used as tidal zeitgebers in synchronizing the foraging rhythms of these reptiles under natural conditions.

Hau, M. and Gwinner, E. (1995) Continuous melatonin adminstration accelerates resynchronization following phase-shifts of a light-dark cycle. *Physiol. Behav*. 58: 89-95.

Circadian rhythms of most passerine birds and some reptiles depend to a considerable extent on the presence of periodic melatonin, which is considered to be part of the central pacemaking system. Recent results on house sparrows have suggested that melatonin, apart from its periodic effects on the circadian system, may also exert effects derived from a constant action: Continuous melatonin applied through subcutaneously implanted silastic tubing enhanced the synchronization response to a low-amplitude light-dark zeitgeber, indicating some kind of sensitization to zeitgeber stimuli. In the present study we tested the prediction derived from these

results, that speed of resynchronization after a phase shift of a light-cycle should be enhanced if melatonin is continuously administered. We found that, indeed house sparrows required less time to resynchronize to an 8 h advance or delay phase shift of a low-amplitude light-dark cycle while carrying a silastic implant filled with melatonin than while carrying an empty implant. The effect is suggested to result from either one or a combination of the following mechanisms: (i) An increased circadian visual sensitivity, (ii) a diminished amplitude of the circadian oscillation, iii) an altered feedback of the locomotor activity to the oscillatory system.

Hau, M. and Gwinner, E. (1994) Melatonin facilitates synchronization of sparrow circadian-rhythms to light. *J. Comp. Physiol. A* 175: 343-347.

We recorded circadian locomotor activity rhythms of house sparrows (Passer domesticus) exposed to low-amplitude light-dark cycles (2:1 lux) with periods of 22.5 or 24.5 h. Under these conditions the circadian rhythms of the majority of the birds were not synchronized by the light cycle but either free-ran or showed relative coordination. However, when melatonin was administered continuously via subcutaneous silastic implants the rhythms became synchronized. It is proposed that melatonin facilitates synchronization either by weakening the circadian oscillatory system thereby in creasing its range of entrainment, or by enhancing circadian sensitivity to the light zeitgeber. In general, the re suits suggest that melatonin, besides its well-known phasic effects on the circadian system also has important tonic effects modifying the ease with which circadian systems can be entrained.

Hau, M. and Gwinner, E. (1992) Circadian entrainment by feeding cycles in house sparrows, passer-domesticus. *J. Comp. Physiol. A* 170: 403-409.

We studied the potential zeitgeber qualities of periodic food availability on the circadian rhythms of locomotor and feeding activity of house sparrows. The birds were initially held in a LD-cycle of 12:12 h, with food restricted to the light phase. After transfer to constant dim light, the birds remained entrained by the restricted feeding schedule. Following an exposure to food ad libitum conditions, the rhythms could be resynchronized by the feeding cycle. Shortening of the zeitgeber period to 23.5 h resulted in the loss of entrainment in most birds, whereas a longer zeitgeber period of 25 h re-entrained the rhythms of most birds. Although these results prove that periodic food availability can act as a zeitgeber for the circadian rhythms of house sparrows, several features of our data indicate that restricted feeding is only a weak zeitgeber. The pattern of feeding activity prior to the daily time of food access shown under some experimental conditions suggests that anticipation is due to a positive phase-angle difference of the birds' normal circadian system rather than being caused by a separate pacemaker.