Vernal Migratory Behavior in Captive White-crowned Sparrows, *Zonotrichia leucophrys*

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Abstract

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Avian migration, the biannual movement of birds from non-breeding to breeding grounds, is a global phenomenon that has fascinated scientists for centuries. The presence of seasonal unrest in caged birds coincident with migration in free-living individuals led to the identification of migratory restlessness, or the expression of migration specific locomotor activity and behaviors characteristic of migration in captivity. This thesis establishes a paradigm for testing the expression of migratory restlessness in captive white-crowned sparrows (*Zonotrichia leucophrys*). First, given the role of circadian oscillators in regulating the expression of locomotor activity in other migrant species, as well as the effects of light cues on locomotor activity of migrants, I investigated the interplay of endogenous and exogenous input in the complete expression of migratory restlessness in Gambel's white-crowned sparrow (*Z.l. gambelii*). Exposing birds to constant conditions of dim and bright light, I determined that expression of intense nocturnal activity is controlled by a circadian oscillator but expression of migration specific behaviors requires dim light conditions. Second, the resident race of white-crowned sparrow (*Z.l. nuttalli*) expresses some migratory activity. However, investigating the development of this activity from late fall to spring, I showed that patterns of locomotor activity are fundamentally different from the migratory congeners. While migration specific behaviors are present in a few

individuals, *nuttalli* does not express complete migratory tendencies. Finally, considering speciation of the 3 Pacific races of *Zonotrichia leucophrys*, I tested the step-wise evolution of migration strategies from long-distance migrant to resident life history using previously established characteristics of captive migrants. I concluded that *Z.l. gambelii*, *pugetensis* and *nuttalli* express tendencies proportionate to the migratory distances covered. Thus the 3 races represent a spectrum of step-wise evolution from a long-distance migrant ancestor. In summary, my research has demonstrated that the complete expression of migratory restlessness in captive birds is a reliable measure for the analysis of multiple facets of avian migration.

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DEDICATION

Use the talents you possess,
for the woods would be a very silent place if no birds sang except for the best.
Henry Van Dyke

-- For my parents --

For always encouraging me to sing.

-- Chapter I --

Avian Migration: Expression, Evolution and Control of a Complex Life History Stage

Introduction: What is Migration?

Avian migration is a global phenomenon with billions of individuals traveling networks of routes that connect nearly every region on earth (Berthold, 1993). Migration is defined as the movement between distinct spatial locations, each with unique resources important for survival and reproduction (Baker, 1978; Gauthreaux, 1982; Dingle, 1996). The high mobility of birds allows them to cross nearly every geographical barrier on the planet and temporarily occupy ephemeral habitats. While 'migration' usually evokes a vision of highly synchronous movements to and from breeding and non-breeding habitats, the patterns and timing of movement vary greatly across and within species.

Migratory Strategies

Migrants are generally grouped into two classes: o*bligate* meaning always migrating or *facultative*, moving only in response to unpredictable events or conditions (Ramenofsky and Wingfield, 2007). The movements typically occur *annually* where round-trip journeys are composed of *seasonal* stages (Dingle and Drake, 2007). A classic example of an obligate migrant is the movement of birds to higher latitudes or altitudes during spring with a return trip to lower latitudes during autumn. In this case, the migration strategy is tightly synchronized within the annual cycle; however, not all strategies are seasonal or predictable.

Migration strategies are classified using two criteria: 1) where/how the migrants actually move and 2) what proportion of individuals within a population expresses the movement. Using this information, patterns of movement are considered *complete*, *partial*, *differential* or *irruptive*. *Complete* migration, also called *return* or *to-and-fro* migration, occurs when birds winter in one location and travel to a separate location to breed.

Individuals within a population may express variations of migratory strategy. *Partial* migration, the most common migratory pattern, occurs when some individuals within a population migrate away from breeding areas while others remain through the winter (Berthold, 1996a). In *differential* migrations, age and sex classes migrate distinct distances from the breeding grounds, i.e. adults and juvenile females tend to overwinter further distances from breeding grounds (Terrill and Able, 1988). As a result, differential migration often leads to considerable latitudinal variation in sex, age class, dominance or even body size (Salomonsen, 1955; Berthold, 1996a).

The timing at which migration occurs varies with strategy. Migrations that are not seasonally or geographically predictable are called *irruptive*. *Irruptive* movements involve large invasions or immigrations of individuals one year, without similar movements in subsequent years (Berthold, 1996a). The particular causes for *irruptive* migration vary with species, but are often correlated with increased population density and overcrowding which induce movements to find sufficient resources elsewhere

(Berthold, 1993). In great tits (*Parus major*) for example, specific reproductive strategies lead to dramatic increases in population size which necessitate the eruptive movement of at least part of the population (Kalela, 1954). Other *irruptive* movements may be triggered by food shortages or the onset of favorable conditions which allow opportunistic movements to previously inhospitable areas as is common with waxwings (*Bombycilla garrulous*) and nutcrackers (*Nucifraga caryocatactes*) (Robinson and Minton, 1989; Berthold, 1996a). It is important to note that terminologies relating to migratory strategy are not mutually exclusive. For example, *irruptive* movement of a small group of individuals can also be classified as *facultative partial migration* (Terrill and Able, 1988).

While there are numerous migration strategies, some species and even populations within a migratory species, have adopted a resident (non-migratory) strategy. For example, within white-crowned sparrows (*Zonotrichia leucophrys*), one Pacific race (*Z.l. nuttalli*) holds year round territories while other races (*Z.l. gambelii* and *Z.l. pugetensis* for instance) expresses *complete* migratory strategies (Blanchard, 1942; Cortopassi and Mewaldt, 1965). In the case of residents, individuals are able to obtain sufficient resources year round for both survival and reproduction such that the costs associated with migration outweigh the benefits (Lack, 1954).

Superimposed upon the various migratory strategies (*complete*, *partial*, etc.) there is also variation in the daily expression of migration. Alerstam (1990) places migrants into four basic categories based on whether they are diurnal or nocturnal migrants. The first category is birds which are primarily *nocturnal migrants*. Consisting mainly of small landbirds (e.g. warblers, sparrows, thrushes), this group is characterized by a sudden onset of migratory activity following sunset. Migration at night allows birds to use daytime hours to feed (Stresemann, 1934), gives them protection from diurnal predators such as raptors (Dorka, 1966), allows the use less turbulent atmospheric conditions for flight and provides celestial cues for navigation (Berthold, 1971). The second category is composed of *soaring and gliding diurnal migrants* (e.g. raptors, vultures, storks). As the sun heats the earth's surface, thermal columns of air rise thousands of meters into the sky. *Soaring and gliding* migrants circle within these columns to gain elevation, then leave the thermal and continue their migration by gliding downward to the next thermal. Having to rely on the presence of thermals, the movement of these birds is confined to warmest part of the day and to specific geographical locations for thermal development. The third category, *non-soaring diurnal migrants*, (e.g. finches, buntings, swallows) express two bouts of migratory flight, one in the earliest portion of the day and one in the evening. For this group, the mid-day hours, when temperatures are greatest and wind conditions most turbulent, are used for rest and refueling. The final category represents birds that are considered both *diurnal and nocturnal migrants*. These species (e.g. ducks, geese, waders, gulls) usually encounter significant geographical barriers (deserts, oceans, etc.) on their migration route which must be crossed in one single flight outlasting the confines of day or night.

Regardless of the strategy utilized by migrant birds, it is generally accepted that the reason animals migrate is to take advantage of the availability of resources, particularly locations which support survival and breeding (Lack, 1968). In order to justify movement from one location to another, conditions must either deteriorate such that migrants can no longer sustain life in that area, or be substantially more favorable elsewhere thus driving birds to abandon their current habitat (Berthold, 2003). Natural selection has acted upon individuals such that organisms do not wait for conditions to deteriorate prior to initiating movement (Dingle, 1996). Given the substantial energy resources required for sustained flight, birds waiting for feeding conditions to decline as a cue to leave run the risk of not having sufficient resources available for fuel acquisition. Instead, positive selection has acted on individuals to time spring arrival at breeding grounds when food is abundant, rather than time departure from wintering grounds. Thus while food availability may be the ultimate factor driving migration, the intricate timing relies on other environmental cues for the initiation of migratory behavior.

Most migrants take advantage of the relatively benign climate conditions in lower latitudes during winter and higher latitudes with bountiful resources and reduced predator pressures during spring and/or summer. While movements between habitats may at first suggest dramatic changes in resource use, functionally, migration serves to keep an organism within a window of optimal conditions throughout its life by maintaining the homogeneity of environmental conditions (Leggett, 1984). Provided the vast array of migratory strategies, natural selection has molded the characteristic behavioral and physiological properties to yield specific ecological outcomes (Dingle, 1996).

Evolution of Avian Migration

While pinpointing the exact appearance of bird migration historically is difficult, it is generally accepted that it has existed as long as modern birds (Alerstam, 1990) and that migration evolved in Saurischians prior to the adaptation of flight (Tyrberg, 1986). There are numerous theories concerning the evolution of bird migration, each involving an ancestral sedentary population and some stimulus that caused them to move (Rappole, 1995). It is unlikely however, that migration in modern birds arose from a single event and Farner (1955) suggests that it has evolved independently with successive bouts of residency and migration in diverse genera. Regardless, the major theories surrounding bird migration accept one of two opposing views, that sedentary ancestors spent their entire lives in northern home ranges (modern day temperate zones) or southern home ranges (modern day tropic zones) prior to movement to novel areas. Several of these competing ideas are briefly outlined below (summarized by Rappole, 1995):

Environmental Change / Continental Drift:

This view of the evolution of migration suggests that dramatic changes in the environment such as continental drift, advancing glaciers and/or rising tides pushed sedentary populations in the north southward (Wallace, 1874; Wolfson, 1948). At some later point when conditions were again favorable, individuals relied on 'ancestral habit' to guide them back into the north. Rappole (1995) has two main objections with this theory. First, some of the environmental changes upon which this theory is based, such as continental drift, occurred well before the appearance of most ancestral species. Second, the persistence of an underlying 'ancestral habit' suggests the inheritance of a genetic trait that may not have been positively selected for for generations. While this theory may fall short in explaining the true origin of avian migration, it is plausible that such environmental changes have forced the adaptation and speciation of modern day migrants (see Rand, 1948; Selander, 1965).

Changes in Proximate Factors / Climate:

Several theories suggest that changes in temperature, day length, or overall seasonal climate were the driving force leading to movement (Marke, 1906; Walter, 1908). Proximate factors such as temperature could very well serve as a cue for timing migratory expression, but it is unlikely that they would be the cause of movement unless, as suggested by Cohen (1967), an individual's probability of survival favored movement to another area. In this case, temperate ancestors may have moved southward in winter in efforts to increase survivability. Gauthreaux (1978, 1982) expanded upon these ideas to include aspects of social dominance interactions between individuals. As different classes within a population compete for resources (such as feeding and breeding sites), this model suggests that distance moved would be relative to an individual's ability to compete. Dominant males for instance might only move a short distance while subordinate males would be forced to move further. While these models potentially explain the evolution of partial and/or differential migration patterns, Rappole (1995) argues that they do not explain the evolution of tropical migrants where the failure to move from equatorial breeding sites would probably not threaten their existence.

Levey and Stiles (1992) emphasize that Neotropical forest birds which, due in part to the high spatial and temporal variation in their resource base, express seasonal intratropical movements that may have provided the predisposition for migration out of the tropics. This notion is supported by the fact that most Nearctic passerine migrants are derived from Neotropical taxa which express altitudinal or intratropical migrations. Conversely, most sedentary Neotropical taxa are poorly represented in temperate areas. This close relation between Nearctic and Neotropical migrants suggests an evolutionary progression of migratory tendencies out of the tropics into temperate areas (Levey and Stiles, 1992).

Migration Threshold Hypothesis:

Given advances in the understanding of underlying genetic programs and the heritability of migratory traits, Baker (1978) proposed that each individual, regardless of sedentary or migratory nature, has a genetically determined 'migration threshold.' If the availability of food or the probability of reproductive success drops below a certain threshold in an area of origin, the individual migrates (providing the costs of migration do not outweigh the benefits). Others find this theory too broad as it does not explain specific evolutionary processes, nor does it address why an animal would complete a migratory event with a return trip (Rappole, 1995; Berthold, 2003).

Partial Migration in Neotropical Species:

At the heart of the migration evolution debate is why a sedentary ancestor living in aseasonal tropical regions without an underlying genetic migratory program would leave for temperate regions. Rappole (1995) provides the following scenario as a possible explanation: First, given increased intraspecific competition and high predation rates characteristic of tropical ecosystems, annual productivity in the ancestor population was low (Fogden, 1972; Skutch, 1976). Regardless of low productivity, the longevity of adults and the number of surviving young result in decreased available space for breeding. Young individuals were forced to travel well outside parental territories in search of available habitat (partial migration), and soon evolved physiological and behavioral traits adaptive for migratory movements (hyperphagia, homing abilities, etc.; Ramos and Rappole, 1994). Once established in novel territories (temperate areas for example), increased resource abundance and decreased competition resulted in higher reproductive fitness than in tropical populations (Ricklefs, 1972). Once breeding events were completed, birds in the new territories would be expected to remain, in which the overall movement would be considered *dispersal*, not migration. If however, conditions deteriorated such that life could not be sustained through the following winter season, birds would be expected to return to their native habitat as it is 'programmed' as known suitable habitat. When competition for breeding areas arises again in the annual cycle, the new migrants should return to their temperate breeding sites. Given the increased reproductive success in northern areas and the seasonal influx of migrants back into the resident population, it is proposed that migrants would eventually flood the ancestral population and out-compete their relatives (Rappole, 1995). If movements between temperate and tropical regions were small or composed of incremental steps, it is likely that multiple populations would arise with connecting hybrid zones. Natural selection may then drive the rapid accumulation of variation in migratory tendencies (Berthold, 1996b).

Provided this scenario for the evolution of partial migration from a sedentary tropical ancestor, it can be argued that birds possessed an underlying behavioral pattern that could be easily modified into the various migratory patterns present in modern day birds (Berthold, 1996b). With time and a series of rapid microevolutionary events, short-distance partial migration could be converted into long-distance intercontinental migration or even back to pure residency if environmental conditions were favorable. Partial migration might result from young individuals moving away from parental areas as suggested by Rappole (1995), or other types of differential migration may evolve given variations in body size, dominance or date of arrival on breeding grounds (reviewed in Ketterson and Nolan, 1983). Recent work has shown that assortative mating on sympatric breeding grounds can lead to rapid changes in migratory behaviors and patterns (Bearhop et al., 2005). In this case, populations of blackcap warblers (*Syliva atricapilla*) from distinct wintering habitats mate assortatively on shared breeding grounds resulting in differential reproductive success between groups. This temporal segregation of breeding illustrates the potential evolution of a migratory divide without physical isolation of subpopulations.

In order to fully comprehend the processes governing the past and continued evolution of avian migration, we must continue to correlate both evolutionary and mechanistic theories. Analysis of genetic and behavioral adaptations may provide insight regarding the selection forces underlying potential changes in movement (Alerstam and Hedenström, 1998).

Regulation of Migration

The life cycle of all birds can be organized into a series of life history stages, each with unique behavioral and physiological characteristics (e.g. migration, molt; Figure 1.1) Each stage is composed of three phases (development, onset of mature capability, and termination) containing a series of sequential substages that represent the major phenotypic alterations in morphology, physiology and behavior at any point in the annual cycle (Wingfield and Jacobs, 1999) Based on this organization of life history stages and their underlying components, Jacobs and Wingfield (2000) classified individual vertebrates as a finite-state machine with the following properties: a) an individual's 'state' at any point in time is the combination of stages and substages expressed b) given a set number of stages and substages expressed during a life cycle, an individual can be classified as a 'machine' with a 'finite' number of expressible states. This variation in state allows for the expression of different phenotypes to match variations in environmental conditions. Since each life history stage requires a minimum amount of time and resources for complete expression, individuals with more stages can be thought of as a more complex machine with less flexibility in the timing of an annual cycle (Wingfield, 2003).

To the casual observer, expression of the migration life history stage may seem as simple as preparation, flight and arrival. Closer analysis through the application of the finite-state machine approach reveals the underlying complexity. The migration life history stage involves initial development and preparation, multiple substages involving fueling, moving and possible refueling, as well as the termination of the overall stage upon arrival (Ramenofsky and Wingfield, 2007; Figure 1.2) Each state involves the expression of different traits that must be regulated to ensure the appropriate temporal expression of migration. Given the seasonal relationship of migration within the annual cycle it is not surprising that birds rely on local environmental conditions as cues for the expression of this life history stage.

Predictable Cues:

For most seasonal migrants, changes in the environment serve as predictable cues for the regulation of the migration life history stage and can be grouped into several categories (Jacobs and Wingfield, 2000). *Initial predictive* cues are reliable signals in the environment that inform organisms of seasonal changes. The most notable is photoperiod, as the natural increase in day length during spring is consistent from year to year and serves as an important trigger for both the development of migratory behavior as well as gonadal development in anticipation of the breeding season (Follett, 1984; Gwinner, 1987). While *initial predictive* cues provide general information regarding the annual cycle, *local predictive* cues allow an organism to fine tune their response to immediate conditions in the area. *Local* cues, such as food availability or snow cover, can serve to accelerate or inhibit the initial response dictated by photoperiod. Following a particularly harsh winter for example, severe snow cover may delay a migrant's arrival on breeding sites regardless of spring advances in day length. Also, individuals may rely on social cues from other members of a flock or population (Hinde, 1965). *Synchronizing or integrating* cues are social stimuli used to ensure that regardless of environmental cues, the individual's response is consistent with that of other conspecifics (Jacobs and Wingfield, 2000). For species migrating in large flocks, social interactions between individuals serve as important modifying factors for the onset of migration.

Unpredictable Cues / ELHS:

While seasonal migration events rely on predictable cues and information regarding environmental conditions for the expression and modification of life history substages, some migratory patterns result from unpredictable events altering the life cycle of an individual or population. *Labile perturbation factors* are external events that have the potential to disrupt the natural progression of life history stages and in some cases induce migratory movements (e.g. food shortage, poor environmental conditions; Wingfield, 2003). If for instance an environmental perturbation reduces the probability of survival or significantly reduces reproductive fitness, an organism may be forced to abandon its current state and relocate to a more suitable area. Facultative migration in response to changing local conditions or social status is induced by unpredictable events and can occur at any point in an individual's life cycle (Wingfield et al., 1998; Wingfield and Romero, 2000). Irruptive, opportunistic and partial migration may be classified as responses to unpredictable cues and are most often not initiated or synchronized with seasonal predictive cues such as changes in photoperiod (Svardson, 1957; Schwabl et al., 1985; Wingfield and Silverin, 2002).

If environmental disruptions result in a negative energy balance for an individual, the expression of facultative migration abandoning a normal life history stage represents the initiation of an emergency life history stage (ELHS; Wingfield, 2003). In this instance the individual can either 1) move away from the disruption ('leave-it'), 2) conserve energy by switching to an alternate set of behavioral and physiological traits ('take-it'), or 3) attempt to conserve energy and then move away if conditions do not improve ('take-it then leave it'). While the physiological substages of irruptive movements within an ELHS are similar to those of a predictable migration strategy (foraging, fuel storage, etc.), given the difference in proximate cues (unpredictable vs. predictable), the regulatory mechanisms are most likely different (Wingfield and Kitaysky, 2002; Wingfield, 2003).

Hormonal Control:

While avian migration has been the subject of research for a long time, very little is currently known regarding the hormonal control of predictable seasonal movements. We can infer however, based on knowledge of the hormonal control of various physiological process, that at least some substages of migration are influenced by the endocrine system (Wingfield et al., 1990). Endocrine influences have been established for preparatory changes including hyperphagia, fattening and increased hematocrit, although detailed mechanisms have yet to be identified. Not surprisingly, androgens (testosterone and related metabolites) have been linked to premigratory preparations for vernal migration prior to the onset of breeding, but not autumnal migration (Weise, 1967; Stetson and Erickson, 1972; Schwabl et al., 1988). Further, thyroid hormones appear to affect muscle preparation in some species (Deaton et al., 1997) and corticosterone has been shown to be elevated with flight and arrival in bar-tailed godwits and captive white-crowned sparrows (Landys-Ciannelli et al., 2002; Landys et al., 2004b).

There is also considerable evidence that corticosterone plays an integral role in the various components of the ELHS as well as irruptive-type migration (Wingfield, 2003). It has been suggested that while corticosterone is involved in both regular and irruptive migration, the target cell control mechanisms regulating physiological changes may be different. Protein-hormone interactions, hormone concentration thresholds or variations in hormone receptors may allow glucocorticoids to have a variety of effects within several migration strategies (Wingfield et al., 1997).

Endogenous vs. Exogenous Control

The inherent difficulty of migration research is that while observable in free-living populations, migratory birds may only remain in certain locations for brief periods of time. Upon initiation of flight, birds leave for far reaching destinations. Studying migrant populations in captivity allowed scientists to further investigate migratory behavior and physiology.

Migratory Restlessness:

First identified by Naumann (1822) in golden orioles (*Oriolus oriolus*) and pied flycatchers (*Ficedula hypoleuca*), caged migrants, for which migratory flight is physically impossible, display intense nocturnal activity during periods of migration in free-living conspecifics. Described as flying, fluttering and wing-whirring at night, migratory restlessness (MR or *Zugunruhe*) has been identified as a migratory trait as these behaviors are not expressed during non-migratory life history stages (Farner, 1955; Dorka, 1966). Defined as the expression of migratory activity in caged migratory birds, MR represents sustained migratory flight of wild individuals, not simply pre-take-off restlessness (Berthold, 1996b). While MR is most conspicuous in nocturnal migrants, it has been studied in diurnal migrants such as the chaffinch (*Fringilla coelebs*) (Palmgren, 1949; Able, 1980). With the discovery of migration-specific traits in captive birds, work began to record locomotor activity and behaviors to gain insight into the movements of wild birds.

With the invention of registration cages by Szymanski (1914), various instruments involving springs, movable perches, ink pads, ultrasound devices and other equipment have been developed to record movement in captive birds (reviewed in Farner and Mewaldt, 1953). Night restlessness was first quantitatively measured as a function of MR by Wagner (1930) with the comparison of night movements in five passerine species. Through these early investigations it was discovered that not only was MR a characteristic of migrants, but that variations in MR expression was closely tied to variations in migratory strategy between species and across populations. The development of a reliable measure of MR in captive birds promoted further investigations into the general expression of MR mechanisms regulating the migration life history stage.

Endogenous Controls:

Following advances in the quantification of locomotor activity in registration cages, long term studies began to reveal seasonal patterns of expression of migratory behaviors in captive birds. In addition to MR, other seasonal traits observed in free-living birds were verified in captive individuals (such as fat deposition, molt, and gonad growth;

Gwinner, 1968; Berthold et al., 1972). Early work suggested that the timing of seasonal activities was under the control of proximate environmental factors such as photoperiod, temperature and rainfall as behavioral and physiological rhythms of captive birds would cease under constant environmental conditions (reviewed in Aschoff, 1955; Farner and Follett, 1979; Gwinner, 1981). However, by bringing willow warblers (*Phylloscopus trochilus*) into captivity and placing them on a constant 12L:12D photocycle, Gwinner (1967, 1968) first demonstrated the presence of endogenous rhythms. Under conditions of 12L:12D, birds exhibited seasonal patterns of locomotor activity and molt for 3 years without seasonal changes in photoperiod or temperature. The duration or period of the expressed rhythm deviated from exactly 1 year so they were termed 'circannual' (from *circa* = about, *annus* = year). Following the discovery of an endogenous clock regulating behavioral and physiological traits of migration, circannual rhythms have been identified in numerous migrant and resident species (Gwinner, 1986; Gwinner and Dittami, 1990; Holberton and Able, 1992; Helm and Gwinner, 2006).

While birds held under the constant conditions of 12L:12D expressed rhythmic patterns of *approximately* 1 year, birds in captivity on natural photocycles expressed patterns of *exactly* 1 year. This difference led to the understanding that the annual photoperiodic cycle (as well as other environmental cues) entrains the endogenous rhythm to exactly 1 year (Gwinner, 1981). As variations in day length are a predictable cue from year-to-year, they entrain internal rhythms and serve as a Zeitgeber, or 'time giver' (Aschoff, 1980). Gwinner (1986) found that by artificially reducing the annual photocycle to a duration of six months, garden warblers (*Sylvia borin*), which show consistent circannual rhythms under constant conditions, would synchronize to the shortened cycle and express seasonal locomotor activity four times per calendar year as opposed to the normal two. Thus, highly synchronous expressions of seasonal activities coincident with annual cycles are the output of a tightly integrated system of control consisting of endogenous and exogenous components.

As with work on circannual rhythms, the role of circadian (*circa* = about, *diem* = day) rhythms on the expression of daily patterns of activity has received considerable attention (reviewed in Aschoff, 1962). While circannual rhythms are responsible for the seasonal expression of behaviors, such as the initiation of the migration life history stage in spring or autumn, daily rhythms are responsible for cyclic expression of nocturnal and diurnal activity within a 24h cycle (Gwinner, 1975). Similar to methods investigating annual rhythms, birds placed under constant conditions (24h dim light, or 24h bright light) express activity rhythms for many cycles (Gwinner, 1996). However, exogenous conditions can modify these patterns as is seen in captive white-crowned sparrows (*Zonotrichia leucophrys*) where dim light masks circadian outputs (Coverdill et al., 2008).

Regardless of the extensive evidence concerning the interplay of both endogenous rhythms and exogenous cues in regulating the expression of migration, little is known concerning the circadian mechanisms of photoperiodic time management. Of particular importance is the relationship between increasing photocycles in spring which induce migratory disposition, and endogenous rhythms regulating activity. Modified from initial ideas proposed by Bünning (1936), the external coincidence model suggests that a daily rhythm of photosensitivity is involved in the perception of changing photoperiodic cues. More specifically, the internal cycle is divided into two phases, the first is light-requiring and photosensitive (photophase) and the second is dark-requiring (scotophase). During periods of the annual cycle with short day lengths, light from the environment is not coincident with peak sensitivity of the photophase. As day length increases in spring however, the external cue of light becomes coincident with the internal sensitivity rhythm and photoperiodic induction occurs (Pittendrigh and Minis, 1964). While the underlying mechanisms for photoinduction have not been completely established, considerable support for this model has been provided in a number of avian species including the house finch (*Carpodacus mexicanus*) and Japanese quail (*Coturnix coturnix japonica*) (Hamner, 1964, 1965; Menaker and Eskin, 1967; Follett and Sharp, 1969).

Conclusions

While the movement of wild individuals represents the foundation for our ultimate fascination with avian migration, work in captivity has provided the means for testing inferences drawn from free-living systems. In addition to the identification of migratory restlessness and the role of endogenous and exogenous controls, laboratory studies have led to significant conclusions about the orientation of migratory flight, the heritability of migratory traits and numerous other characteristics of this life history stage (reviewed in Berthold, 1996a). While laboratory settings allow for the control of numerous environmental variables, the underlying strength of captive work relies on the successful application of these findings to the natural history of free-living species and populations. Regardless of advances in our understanding of migration, we are far from a holistic comprehension of this complex life history and many subtle links between captive behavior and free-living migration have yet to be established.

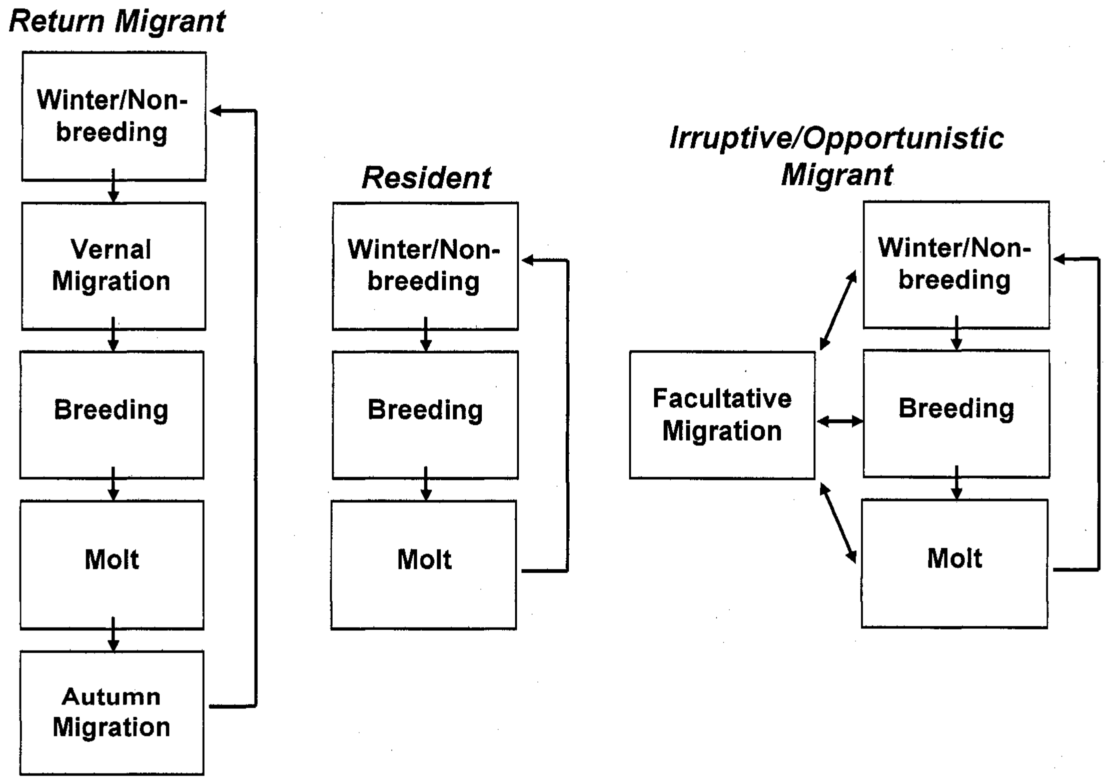

Figure 1.1 Annual Life Cycles of Migrants and Residents

Finite-state machine examples showing various life history stages for three migratory strategies. Return migrants express two seasonal migratory bouts separating breeding and non-breeding stages. Resident (non-migratory) species progress directly from non-breeding to breeding with a subsequent transition to molt. Facultative migration can be triggered during any life history stage. Following an unpredictable event, facultative migrants will return to the normal life cycle and an appropriate life history stage (adapted from Jacobs and Wingfield, 2000; Ramenofsky and Wingfield, 2007).

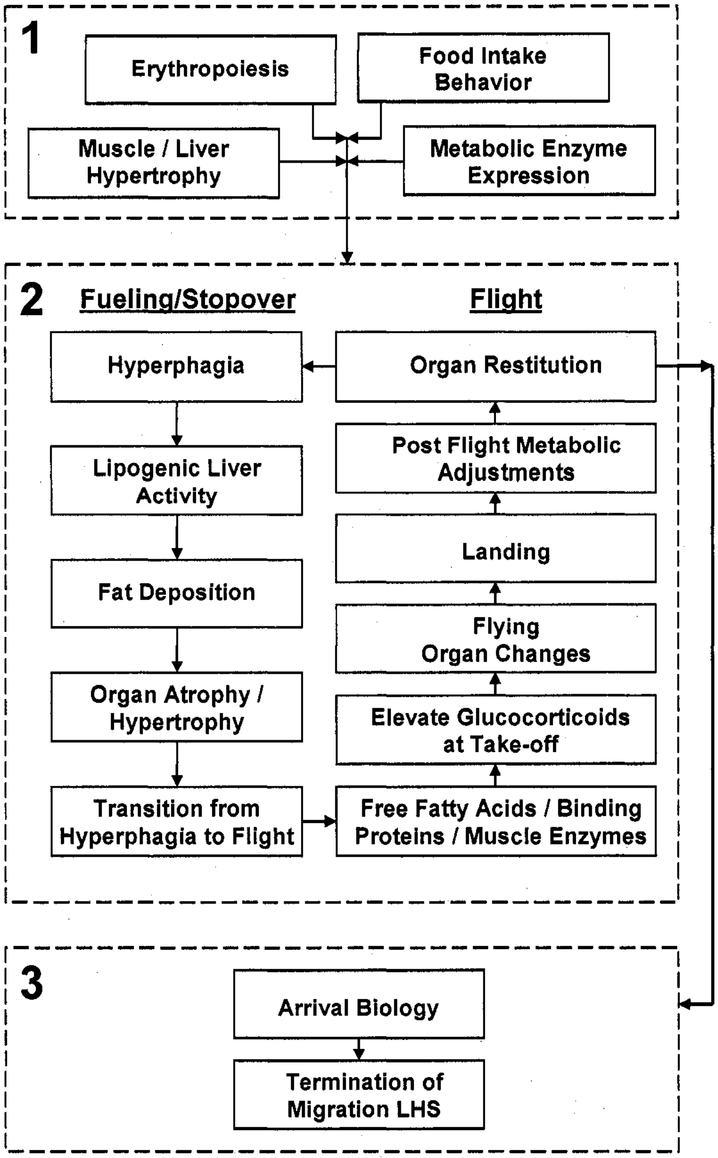

Figure 1.2 Migration Life History Stage

Each phase of the migration life history stage is composed of several substages. The developmental phase (1) is characterized by changes in physiology and behavior. Following the onset of the mature capability phase (2) fueling leads to the deposition of resources needed during flight, while substages during flight promote utilization of stored energy. Migration strategies involving multiple bouts of flight and refueling will require repeated cycling of the mature capability substages. The termination phase (3) begins when the bird arrives at or near its destination and begins the transition to subsequent life history stages (figure modified from Ramenofsky and Wingfield, 2007).

-- Chapter II --

Circadian and Masking Control of Migratory Restlessness in Gambel's White-crowned Sparrows (*Zonotrichia leucophrys gambelii*)[1]

Introduction

Circadian rhythms play a pivotal role in the timing and integration of physiological and behavioral processes. Given that seasonal activities, such as migration, require precise timing on both an annual and daily basis, it is plausible that endogenous rhythms are involved in such phenomena. There is evidence for circadian involvement in seasonal behaviors in a number of migrant species (Bartell and Gwinner, 2005; Kumar et al., 2006; McMillan et al., 1970 and McMillan, 1972). Yet, despite the depth of knowledge concerning migratory physiology and behavior of migration in Gambel's white-crowned sparrows, (*Zonotrichia leucophrys gambelii*), little is known about the role of the circadian system in regulating the daily timing of migratory behavior during this critical life history stage.

Gambel's white-crowned sparrows (GWCS) are nocturnal migrants that travel from wintering grounds in the western US and Mexico to breeding grounds from western Canada into the Arctic (Cortopassi and Mewaldt, 1965). In captivity, the vernal (spring) migratory life history stage is characterized by a stereotyped pattern of locomotor activity and associated behaviors (Ramenofsky et al., 2003). During daylight hours, birds exhibit periods of activity of intermediate intensity that include feeding and rest

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[1] A version of this chapter was published in the Journal of Biological Rhythms with coauthors George E. Bentley and Marilyn Ramenofsky (see Coverdill et al., 2008).

behaviors. Just prior to the conclusion of the light phase (approximately 90 minutes) activity levels decrease to a minimum as the birds remain still in what is called a quiescent phase (QP) (Agatsuma and Ramenofsky, 2006). With the onset of night (dark phase), birds display migratory restlessness (MR) or *Zugunruhe*, which is intense flight activity associated with migratory flight in free-living birds (Wagner, 1930; Berthold and Querner, 1988). MR is characterized in GWCS by the distinct behaviors, beak-up (BU) and beak-up flight (BUF) (Agatsuma and Ramenofsky, 2006). The particular body stances exhibited during these behaviors are thought to facilitate orientation with celestial and geomagnetic cues prior to take-off (Able and Able, 1996; Wiltschko and Wiltschko, 2002; Agatsuma and Ramenofsky, 2006).

Migratory restlessness (MR) is reported to be regulated by a circadian oscillator in white-throated sparrows, *Zonotrichia albicollis*, (McMillan, 1972) and garden warblers, *Sylvia borin*, (Bartell and Gwinner, 2005) as well as other species. With the identification of migration-specific behaviors in GWCS and the role of rhythmic oscillators in other migrant species, we hypothesized that there may be a circadian component regulating migratory behaviors in captive birds. If MR expression represents the output of an endogenous clock, then its expression should persist rhythmically in constant dim light conditions $(DD)_{dim}$ or constant bright light conditions (LL) (McMillan et al., 1970; Riker, 1977; Kumar et al., 2006). If birds require exposure to an external cue such as dawn or dusk for the maintenance of periodic MR, then exposure to constant conditions should abolish daily patterns, suggesting a behavioral response to permissive LD cycles and a lack of control by a circadian clock (Mrosovsky, 1999). In this study we sought to determine if the expression of MR locomotor activity and migration-specific behaviors can be attributed to the presence of endogenous rhythms or the result of appropriately-timed environmental input (dawn and/or dusk). While previous studies have relied primarily on the expression of high levels of locomotor activity in defining MR, the video scoring reported in the present study allows for a more detailed definition of MR, characterized by the expression of intense locomotor activity in combination with the expression of migration-specific behaviors (BU and BUF).

Based on published literature of the role of daily oscillators in the avian circadian system, we formulated the following prediction: When exposed to constant light conditions, either dim light $(DD)_{dim}$ or bright light (LL), birds in the migratory life history stage would exhibit MR similar to birds under baseline LD conditions (18L:6D). That is, we expected to observe a regular periodic pattern of intense locomotor activity combined with the expression of MR behaviors during the subjective night. Through this series of experiments we have attempted to determine the potentially complex interplay between endogenous circadian rhythms and the influence of environmental conditions on migratory behavior.

Materials and Methods

Capture of Wild Animals:

In September 2003, GWCS were captured near Sunnyside, Washington (46.1°N, 119.5°W). The birds were taken to the Department of Biology at the University of Washington, Seattle, and placed in outdoor aviaries where they were exposed to natural photoperiod and temperature conditions. All techniques involving capture, handling and experimentation were performed within the guidelines of the American Association for Accreditation of Laboratory Animal Care and approved by the Institutional Animal Care and Use Committee at the University of Washington.

Environmental Chamber Set-up:

In January, sixteen adult birds (eight for $(DD)_{dim}$, eight for LL) were randomly selected from the outdoor aviaries and placed in individual registration cages (35cmW x 40cm L x 45cm H) within one environmental chamber. Prior to their placement into the chamber, all birds were in the winter life history stage (non-migratory condition, ambient photoperiod of 9.5L:14.5D) and photosensitive (Nicholls et al., 1988). Birds were acclimated to chamber conditions of 8L:16D for 1.5 weeks. Next, the photoperiod was switched to long day conditions (18L:6D with lights off at 03:00h), thus photostimulating the birds and inducing the spring migratory life history stage (Landys et al., 2004b). Throughout the course of the study, birds were provided food (Mazuri Chow, Nutritional International, Brentwood, CA and mixed seeds, UW Custom Mix, Seed Factory NW, Kent, WA) *ad libitum* in hoppers at the front of the cages. Water for drinking and bathing was provided as well as vitamin laced grit (Bird Health Grit, Seed Factory; Ceres, CA). Previous studies have shown that dim illumination (< 1Lux) is required for captive GWCS to express MR, as birds held under complete darkness remain still and display only limited movement (Ramenofsky et al., 2003; Landys et al., 2004a). The dim light condition was achieved with 3 nightlights (Limelight Nightlight, Austin Innovations, TX) equally spaced inside the environmental chamber for illumination during the night phase of both control (18L:6D) and experimental $(DD)_{dim}$ conditions. While light intensity measured at the source of each nightlight was between 2-3 Lux, values recorded at the closest cage (<1m) were <1 Lux. Light intensity measurements during the light phase of LD and LL conditions were ~550 Lux at cage level.

When conducting the $(DD)_{dim}$ and LL trials, birds were checked and serviced on a randomized schedule to avoid entrainment to daily human disturbances. Body mass and fat score measurements were taken prior to and at the conclusion of both trials. Birds were placed in a cotton sock prior to placement on an electronic scale (Ohaus Scout Pro, model SP202) for body mass measurements to the nearest 0.1g. Fat scores were determined as the mean of the deposits in the furcular fossa and abdominal cavity using an arbitrary score of 0 to 5 (Wingfield and Farner, 1978). A score of 0 designates no fat; whereas, a score of 5 indicates bulging fat deposits.

Measurement of Locomotor Activity:

Each registration cage was equipped with one perch centrally located. One photodetector (Radio Shack Invisible Beam Alarm Entry, models #43-311 and #43-312) and a reflector were placed perpendicular to the perch in each cage as describe previously by Agatsuma and Ramenofsky (2006). Activity was catalogued by Labview software (National Instruments Corporation, Austin, TX). Further refinements of the records by D. Baldwin (NMFS Unit Seattle) calculated locomotor activity in units of average beam breaks per minute per 30 minute interval. Actograms, which track the locomotor activity of one bird during the entire trial period, were created using the time series analysis software El Temps, written by Antoni Díez-Noguera, Facultat de Farmàcia, Barcelona, Spain (version 1.192).

Behavior:

To precisely determine the behaviors displayed by birds in conjunction with the locomotor activity records, a set of focal birds in registration cages were videotaped throughout the study. Videotape analysis provided the full suite of migratory behaviors characteristic of nighttime MR expressed during the periods of increased activity as well as daytime behaviors of feeding and resting (Agatsuma and Ramenofsky, 2006). A video camera with infrared capability (Sony Handycam Vision, model CCD-TRV87) recorded activity during several intervals during the 24 h period. Taping sessions included the light and dark phases of the 18L:6D photoperiod (baseline LD), as well as the subjective day and night under constant light conditions, < 1Lux dark $(DD)_{dim}$ and light (LL) respectively. Specific periods of transition from light phase to dark or vice versa were filmed as well. To qualify activity during the filmed sessions, the behaviors of each individual were analyzed using an instantaneous scan method of Martin and Bateson (1993) and applied to captive GWCS according to methods described earlier (Agatsuma and Ramenofsky, 2006). Specifically, these involve the identification of activity at 20 second intervals for the duration of the film from a given set of 8 behaviors; rest, jump, flight, feeding, beak-up (BU) and beak-up flight (BUF), head-cock, and other (preening). The only inactive behavior was rest; jump and flight were used to categorize movement from one location to another using feet or wings respectively. Feeding included both eating seeds and drinking water. Two migratory behaviors were BU and BUF in which the bird would point its bill vertically (BU) sometimes in combination with full wing beats (BUF). Head-cock was used to categorize movement of the birds head to one side directing one eye vertically without raising the bill. Videos were categorized by photoperiod treatment and time of day during which the filming took place. The mean percentage of BU and BUF of all behaviors observed was calculated from the total recorded hours of tape.

Experiment 1: Locomotor Activity in $(DD)_{dim}$:

Birds were exposed to long day conditions (18L:6D) for 26 days. The daily locomotor patterns as well as body mass and fat scores were recorded to confirm that birds were in the vernal migratory life history stage. During the $(DD)_{dim}$ trial, 8 birds were transferred to constant dim light (<1 Lux) for 64 consecutive hours.

Experiment 2: Locomotor Activity in LL:

As with experiment 1, locomotor activity, body fat and mass measurements were collected from birds acclimated to an 18L:6D photoperiod. All birds were in the vernal migratory life history stage. During the LL trial, 8 birds were transferred to constant bright light (~550 Lux) for 140 hours, approximately 6 days. Following 140 hours under LL, the lights were switched off during the subjective night to assess the instantaneous response to darkness during this portion of the daily cycle of activity. Upon conclusion of each experimental treatment, the photoperiod was returned to 18L:6D.

Comparison of Locomotor Activity:

For both experimental trials ($(DD)_{dim}$ and LL), daytime and nighttime locomotor activity measurements were compared with control conditions (18L:6D) during discrete 3 hour time periods. Daytime measures were collected from 22:00-01:00 hours, coinciding with normal 'lights on' conditions under the control photoperiod; for $(DD)_{dim}$, this interval is considered *subjective day*. Nighttime measures were collected from 05:00-08:00 hours, coinciding with normal 'lights off' conditions under the control photoperiod; for LL, this interval is considered *subjective night*. It should be noted that these time intervals were chosen to represent daytime and nighttime conditions without potential artifacts near the transition in lighting conditions. Hence no locomotor activity measurements were analyzed within two hours of the lights on-to-off transition at 03:00h.

Given that average levels of both diurnal and nocturnal activity appear to fluctuate over several weeks or months, baseline LD measures (control) were collected two days prior to the initiation of each experimental trial for comparison of activity. Also, average levels of activity during these daytime and nighttime intervals were collected for the first two days of each trial.

Statistical Analyses:

Morphological data (mass and fat score across individuals) were first analyzed for normality using Shapiro-Wilks test, followed by parametric two-tailed Paired T-tests for both treatment groups. Within any one treatment group, values were compared prior to and following the trial (e.g. Pre-$(DD)_{dim}$ and Post-$(DD)_{dim,}$). We analyzed locomotor activity data (which Shapiro-Wilks test indicated as not normally distributed) using the non-parametric Wilcoxon Signed Ranks test with sequential Bonferroni correction for multiple comparisons (within individuals, across days) (Zar, 1999). Given that our behavior data from video analysis was collected from a subset of total birds (n=2), we did not feel confident statistically analyzing the data set.

Results

$(DD)_{dim}$ Results:

Prior to the initiation of the $(DD)_{dim}$ and LL trials, morphometric data and daily locomotor activity patterns confirmed the expression of the vernal migratory life history stage. Body mass and fat scores had increased significantly from winter condition as analyzed with Paired T-test (mass: t=-2.86, p=0.01; fat: t=-12.58, p<.001). Locomotor activity had shifted from winter patterns (Figure 2.1) resulting in the expression of intense activity and migration-specific behaviors confined to the night phase of the 24h cycle (Figure 2.2). Upon entering $(DD)_{dim}$, the patterns of locomotor activity and quiescent phase observed under 18L:6D changed. The quiescent phase appeared to be abolished and MR that was initiated at the onset of the dark phase persisted for 36.4 ± 5.3 hours (mean ± SE, n=8; Figures 2.3 and 2.4). Approximately 1 and 2 days after the initiation of the trial, locomotor activity during the subjective day was significantly greater than daytime baseline LD conditions ($(DD)_{dim}$ day 1: Z=-2.38, p=0.02; $(DD)_{dim}$ day 2: Z=-2.52, p=0.01, Figure 2.5). However, there was no significant difference in the frequency of nighttime activity between baseline and either of the first two days of $(DD)_{dim}$ ($(DD)_{dim}$ day 1: Z=-0.14, p=0.89; $(DD)_{dim}$ day 2: Z=-0.84, p=0.40, Figure 2.5). Similarly, there was no difference between baseline nighttime activity, $(DD)_{dim}$ nighttime activity or $(DD)_{dim}$ subjective day activity. Video tape analysis of the nocturnal locomotor activity confirmed the predominance of BU and BUF under dim light conditions of the subjective day (Table 2.1). Once the light phase was restored at the conclusion of the $(DD)_{dim}$, no MR was expressed. Following the trial, recovery to LD pattern was variable across individuals. Individual shown in Figure 2.3 required two nights of rest before returning to the vernal migratory life history stage, individual in Figure 2.4 required more than four nights. Both fat score and mass were reduced significantly following the $(DD)_{dim}$ exposure (Fat: t=4.31, df=7, p=0.004, n=8; Mass: t=8.45, df=7, p<0.001, n=8, Table 2.2).

LL Results:

Birds held on 18L:6D photoperiod showed typical daily patterns of behavior . Under LL conditions for 140 hours, levels of intense activity were mostly confined to the subjective night (time between 03:00 and 09:00 when lights were formerly off) (Figures 2.6 and 2.7). There was no significant difference in the frequency of daytime locomotor activity of baseline conditions and the first two days of LL (LL day 1: Z=-1.54, p=0.12; LL day 2: Z=-1.40, p=0.16, Figure 2.8) Similarly, there was no difference in nighttime activity of baseline and subjective night conditions of LL (LL day 1: Z=0.00, p=1.00; LL day 2: Z=0.00, p=1.00, Figure 2.8). During the first 3 days under constant light, birds showed free-running patterns of activity. After which, this uniformity dissipated as most birds became arrhythmic. Behavioral analyses for the first 3 days of LL reveal that the rhythmic expression of intense activity does not contain the BU and BUF behaviors (Table 2.1). However, videotape analysis of two focal birds during the subjective night following 140 hours of LL, confirmed that reducing the illumination to <1 Lux resulted in the appearance of MR behaviors within 40 sec for one bird, and 17 minutes for the second.

Whereas fat scores significantly decreased during LL (t=4.97, df=7, p=0.002, n=8), body mass did not (Table 2.2). Comparison of the average change in both fat and mass during $(DD)_{dim}$ with that during LL indicates that $(DD)_{dim}$ treatment had significantly greater effect on the morphological measures ($p = 0.04$, and < 0.001 respectively, Table 2.3).

Discussion

Previous investigations have addressed the presence of nocturnal activity in captive migratory birds (Wagner, 1930; McMillan et al., 1970; Riker, 1977; Gwinner, 1986, 1996). We focused on the daily activity patterns, but also used behavioral characteristics associated with migratory activity to assess the possible interplay of an endogenous circadian rhythm with environmental cues on the timing of diurnal non-migratory activity and nocturnal migratory restlessness.

At the outset of this experiment, we predicted that birds exposed to constant dim light would exhibit locomotor activity patterns similar to rhythms under baseline photoperiod conditions of 18L:6D. This however was not the case. Under constant dim light, $(DD)_{dim}$, birds abolished daily cycles of activity and expressed intense locomotor activity continuously with no regularly occurring quiescent phase. Locomotor activity levels during the subjective day of $(DD)_{dim}$ were significantly greater than baseline daytime activity and more similar to those of nighttime levels of both baseline and experimental conditions. These results differ from previous studies on different migratory species where birds held under constant dim light conditions retained nocturnal *and* diurnal activity patterns for at least 24 hours (Riker, 1977) and up to 9 days (McMillan et al., 1970). While Wagner (1956) found that birds in constant dim light lost daytime activity patterns, he later realized that the retention of nocturnal patterns may have resulted from the structured feeding intervals as well as temperature and acoustic cues present in the 'constant environment.' Also, McMillan et al. (1970)

recognized that the duration of MR each night was extended under dim light conditions, suggesting that nocturnal activity is intrinsically longer than the night during which it is normally manifested. The continuation of MR for an average of 36.4 h in our study, and the complete lack of normal daytime activity patterns suggests that the expression and maintenance of MR in captive GWCS does not appear to be under circadian control, but rather is affected by the exogenous cue of dim light. We suggest that dawn maybe the required cue for termination of MR following the dark phase and the expression of daytime behaviors. Therefore, without taking our LL results into account, we might conclude that there is no involvement of the circadian system in the onset or offset of MR. Rather, once birds are in the migratory life history stage, MR is positively masked by dim light.

Our prediction that birds under constant light (LL) would continue to express rhythmic patterns of daily activity was confirmed but only during the first three days of the LL trial (Figures 2.6 and 2.7). Upon initiation of LL, intense activity was confined to the subjective night, with activity of intermediate intensity during the day. The comparison of daytime and nighttime locomotor activity under LL with that of baseline conditions supports the presence of an endogenous circadian clock regulating locomotor activity. Given the similarity of LL daytime activity with that of daytime controls, as well as increased activity during the subjective night consistent with baseline, we conclude that bright light does not immediately inhibit the persistence of locomotor activity rhythms, and that dim light is not necessary for the expression of increased locomotor activity.

While McMillan et al. (1970) found similar results in that birds exposed to constant bright light exhibit 'nocturnal restlessness' for several days prior to the patterns becoming arrhythmic, it was unclear whether the nocturnal restlessness observed was true MR involving BU and BUF or simply increased locomotor activity. In the present study, the videotape qualification of the data elucidates the behavior that accompanies the increase in activity during the subjective night in LL. Although the birds did express intense levels of activity consistent with MR during the subjective night (McMillan et al., 1970), the specific behaviors were qualitatively different and lacked the expression of either BU or BUF. These data suggest that the behavior expressed during LL was not true MR but rather a greater intensity of daytime behaviors (flight, jump, etc). Thus, we propose that LL during the subjective night acts to specifically prevent expression of BU and BUF but not increased locomotor activity. This suggests that there may be separate components of MR that are regulated differentially by a circadian oscillator and by masking by environmental conditions. These findings are consistent with Gwinner (1967), where MR was stimulated by decreased illumination in European robins (*Erithacus rubercula*) and common redstarts (*Phoenicurus phoenicurus*). Thus, we suggest that the full expression of MR was negatively masked, defined as a suppression of activity by light in a nocturnal species (Mrosovsky, 1999). This notion is supported by a dramatic change in behavior seen at the conclusion of the LL experiment. After 140 hours of constant bright light, birds were expressing intermediate to high levels of locomotor activity with no migratory behaviors during the subjective night. Yet, as soon as the light intensity was reduced to <1 Lux during the subjective night, the expression of both BU and BUF flight were observed. Thus we can conclude that not only is dim light required for the complete expression of these behaviors, it has a rapid effect on the quality and quantity of locomotor activity when applied during the subjective night. Given that nocturnal migrants rely heavily on atmospheric conditions present at night for the expression of migratory activity, it appears to be adaptive that these birds respond directly to dim light to enhance the expression of migration-specific behaviors, or positively mask them, during the night phase.

The arrhythmic patterns of activity following the first three days of LL are consistent with other studies investigating circadian processes of sighted birds under constant light (Menaker, 1971). While sighted birds do not maintain predictable patterns on a 24L:0D regime, blinded birds retain rhythmicity of activity suggesting that while the eyes are not required for entrainment, they do convey environmental information to a central clock (Menaker, 1968).

Birds in constant dim light did not feed. These findings are attributed to the fact that nocturnal migrants in the vernal migratory life history stage switch from an anabolic (feeding and resting) state during daylight hours to a catabolic (flight and MR) state at night (Agatsuma and Ramenofsky, 2006). Others found that corticosterone levels in GWCS began to increase prior to the initiation of nighttime activity, indicating a metabolic switch from energy storage during daylight hours to use during the dark phase and MR (Landys et al., 2004b; Ramenofsky and Wingfield, 2007). Field observations indicate that spring migrants at stopover sites settle in the brush in late afternoon and are quiet and aphagic prior to nocturnal flight (Morton, 1967). Energy expenditure during migratory flight is estimated to be approximately half of that expended during stopovers (Wikelski et al., 2003), suggesting dramatic changes in metabolism occur between daytime feeding and resting and nighttime migratory flight. The extended period of MR under $(DD)_{dim}$ and the lack of food intake ultimately led to the significant declines in both body fat and mass during the trial. We attribute the significant decrease in body mass and fat deposits to the fact that while migratory flight is metabolically demanding (Butler and Woakes, 1990), take-off is the most energetically expensive component of flight and captive birds in registration cages expend a great deal of energy attempting to achieve aerodynamic flight (Pennycuick, 2003; Agatsuma and Ramenofsky, 2006). Other investigations have noted a sharp increase in activity (termed hyperactivity) as a result of food deprivation (Lynn et al., 2003), but only after several days of continued food and water deprivation regardless of natural or constant dim light photoperiod conditions (Wald and Jackson, 1944; McMillan et al., 1970). In our study birds had sufficient access to both food and water *ad libitum*, so we cannot attribute the extended period of MR to hyperactivity resulting from a lack of nutrition in the dim light. In addition, videotape analysis indicated that the increased activity was true MR and not an "escape" behavior, as described by Ramenofsky et al. (2003).

As previous investigators have noted, animals approach expression of activity and/or specific behaviors by one of two routes (Mrosovsky, 1999); first, the activity may be a response to photoperiodic cues in the environment, or second, an output of a circadian oscillator. Based on our investigations we can make several conclusions regarding the activity and behavior of captive GWCS during the vernal migratory life history stage. It is now apparent that variations in light intensity play an integral role in the expression of migration-specific activity. Dim light facilitates the expression of MR and the associated behaviors and is required as an environmental cue for onset and maintenance of MR. While birds in constant bright light exhibit a circadian rhythm of overall locomotor activity with intense activity levels during subjective night, the presence of bright light negatively masks the expression of migration-specific behaviors. Given the obvious sensitivity of behavior expression to ambient light conditions, future investigations should focus on a spectrum of light intensities for masking of migratory activity and other behaviors.

Our findings do not support the notion that day-night activity rhythms may be controlled by two separate oscillators which respond differently to variations in light intensity as described by Gwinner (1967), but rather a single oscillator system that governs circadian locomotor activity. The complete expression of MR is therefore dependent on the activity oscillator in concert with environmental conditions to trigger migration-specific behaviors. Under entrained conditions, the circadian drive for nocturnal locomotor activity coincides with permissive environmental conditions for migration. When placed in $(DD)_{dim}$ conditions, the positive masking dim light may override the circadian oscillator locomotor activity output and leave the system in a fixed state. As a consequence between this interplay of circadian locomotor activity and positive masking of MR by dim light, in neither $(DD)_{dim}$ nor LL was cyclic expression of nocturnal MR present. While we are unable to conclude that BU and BUF were expressed continuously during $(DD)_{dim}$ without continuous video taping, it is unlikely that rhythmic patterns of these migration-specific behaviors occurred. A rhythmic pattern would not predict the expression of BU and BUF during several intervals of the 24h cycle including the mid subjective day. We can therefore conclude that under $(DD)_{dim}$, MR continued for extended periods of time (>24 h), and in LL, increased subjective night activity, but not true MR, was observed on a circadian basis. Future investigations on the neural bases of MR should focus on both the circadian oscillator regulating locomotor activity as well as brain areas involved in the integration of environmental and physiological cues to initiate the onset and offset of MR.

Based on this series of experiments, we can conclude that the vernal migratory life history stage of Gambel's white-crowned sparrow is a good example of the interplay between the circadian oscillator regulating locomotor activity and environmental masking, which will maximize migration efficiency. Taken collectively, our findings shed light on the importance of integrating both neural and environmental information leading to changes in activity, behavior and metabolism in this complex life history stage.

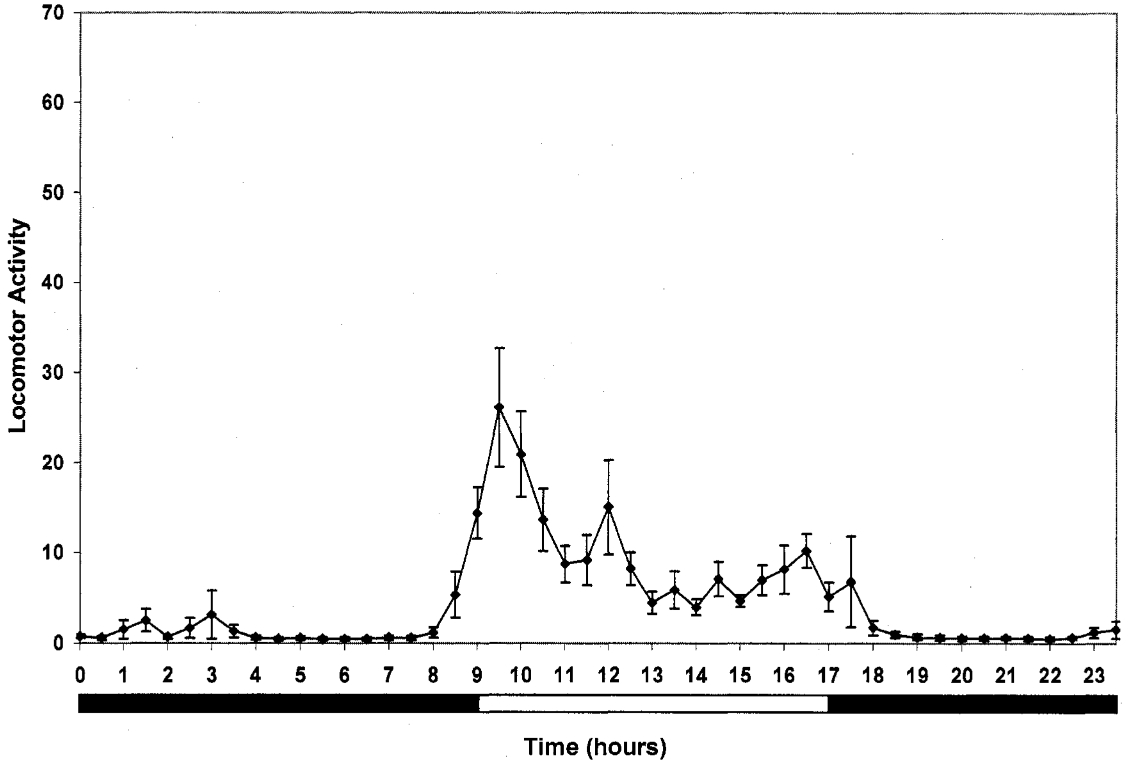

Figure 2.1: Short Day (8L:16D) Activity Waveform

24h locomotor activity waveform (average activity per min/30 min interval) of birds on short day (8L:16D) photoperiod. Closed black bars below figure indicate lights off, open white bar indicates lights on, symbols represent mean ± SE for n=8 birds.

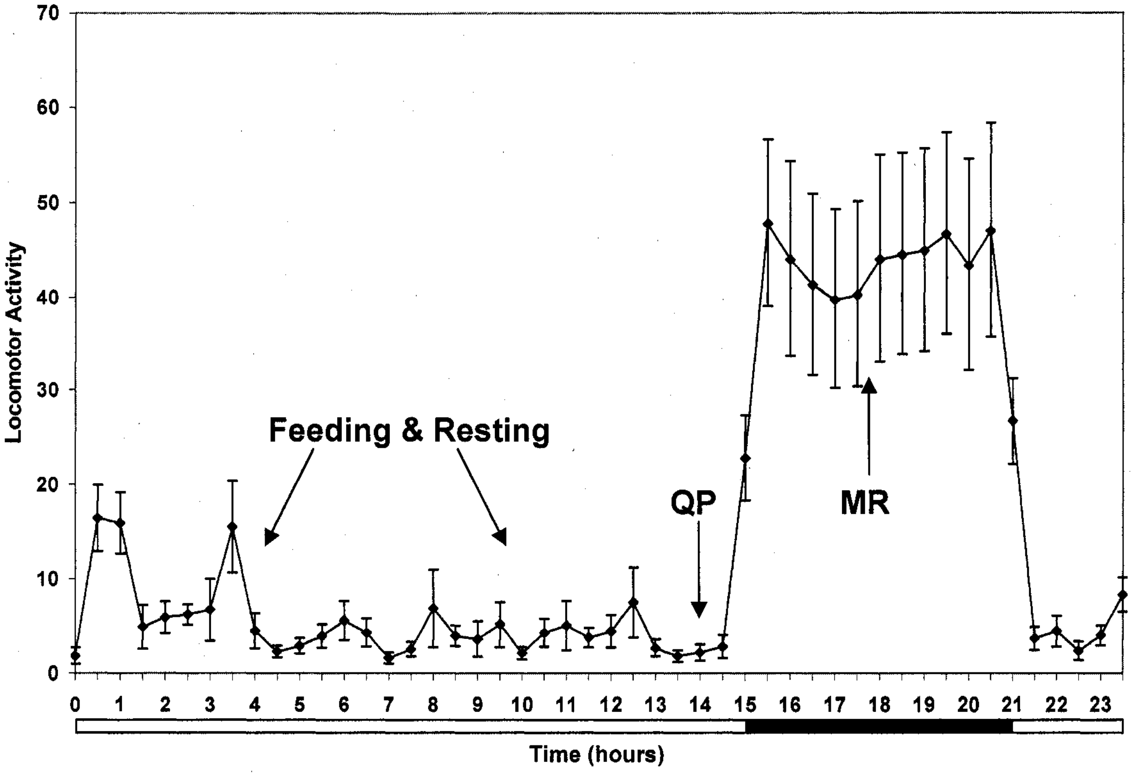

Figure 2.2: Long Day (18L:6D) Activity Waveform

24h locomotor activity waveform (average activity per min/30 min interval) of birds on long day (18L:6D) photoperiod. Closed black bar below figure indicate lights off, open white bars indicate lights on, symbols represent mean ± SE for n=8 birds. Specific behaviors indicated: QP=Quiescent Phase; MR=Migratory Restlessness.

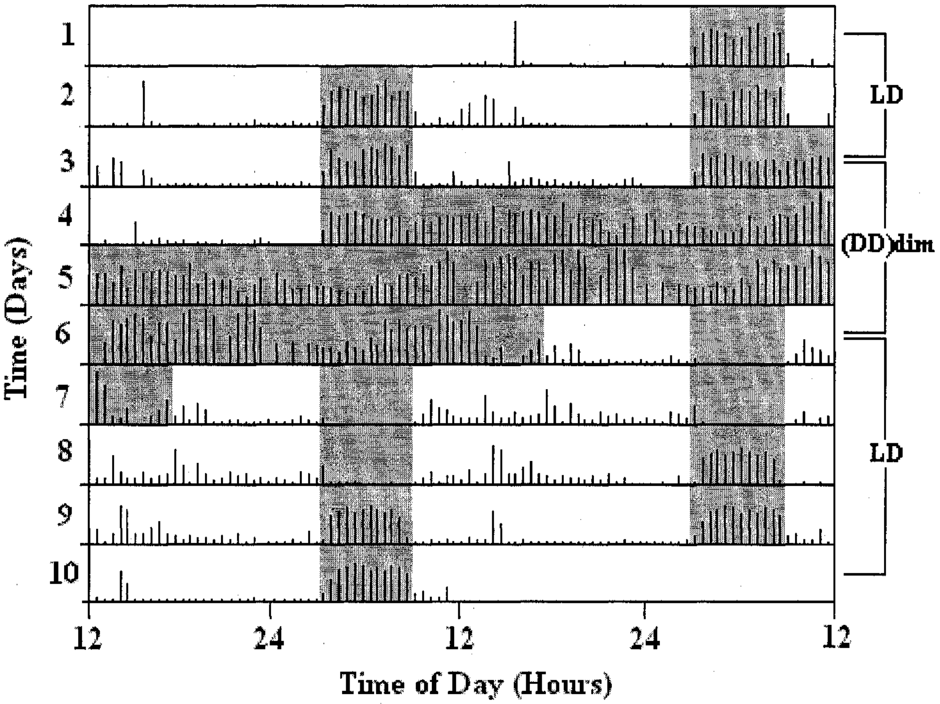

Figure 2.3: (DD)$_{\text{dim}}$ Actogram (Individual A)

Double-plot actogram of activity from one individual over a period of 216h including (DD)$_{\text{dim}}$. Each horizontal line represents two 24h days (1200 to 1200). White background shading indicates lights on, gray background shading indicates lights off. Vertical bars represent intensity of locomotor activity (actogram descriptions apply to all subsequent actograms). (DD)$_{\text{dim}}$ trial begins on Day 3 (corresponding to actogram Days 3&4) at 03:00 and continues until Day 6 at 17:30. Migratory restlessness was confined to the night phase of baseline (first two days shown), and continued uninterrupted during (DD)$_{\text{dim}}$.

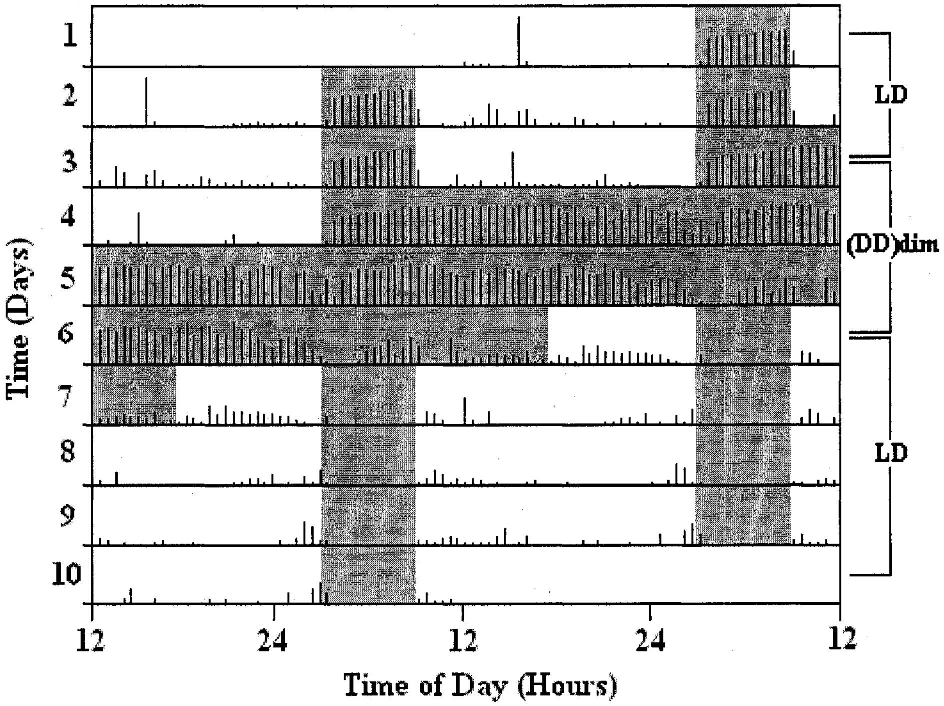

Figure 2.4: (DD)$_{\text{dim}}$ Actogram (Individual B)

Double-plot actogram of activity from second individual over a period of 216h including (DD)$_{\text{dim}}$. (DD)$_{\text{dim}}$ trial begins on Day 3 (corresponding to actogram Days 3&4) at 03:00 and continues until Day 6 at 17:30. Migratory restlessness was confined to the night phase of baseline (first two days shown), and continued uninterrupted during (DD)$_{\text{dim}}$.

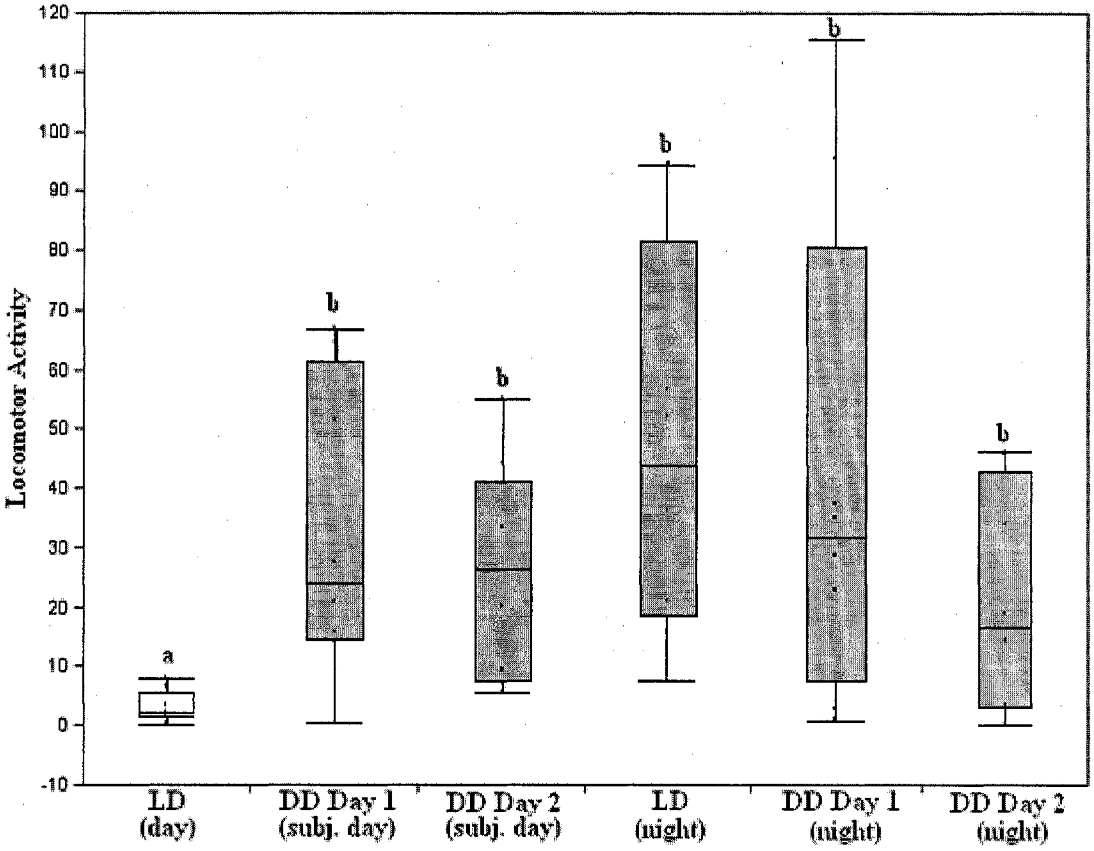

Figure 2.5: LD vs. $(DD)_{dim}$ Activity Comparison

Box-and-whisker plots of locomotor activity during six three hour periods (n=8). White shading in boxes denotes recordings during periods with lights on and gray shading indicates recordings during periods with lights off. Horizontal lines within boxes indicate median, outer limits of boxes denote upper and lower quartiles, and whiskers represent maximum and minimum values OR 1.5*interquartile range, whichever value is lower (plot descriptions apply to all subsequent box-and-whisker plots). Letters **a** and **b** signify differences of $p < 0.05$. Subjective Day: Day 1: Z=-2.38, p=0.02; Day 2: Z=-2.52, p=0.01. Subjective Night: Day 1: Z=-0.14, p=0.89; Day 2: Z=-0.84, p=0.40.

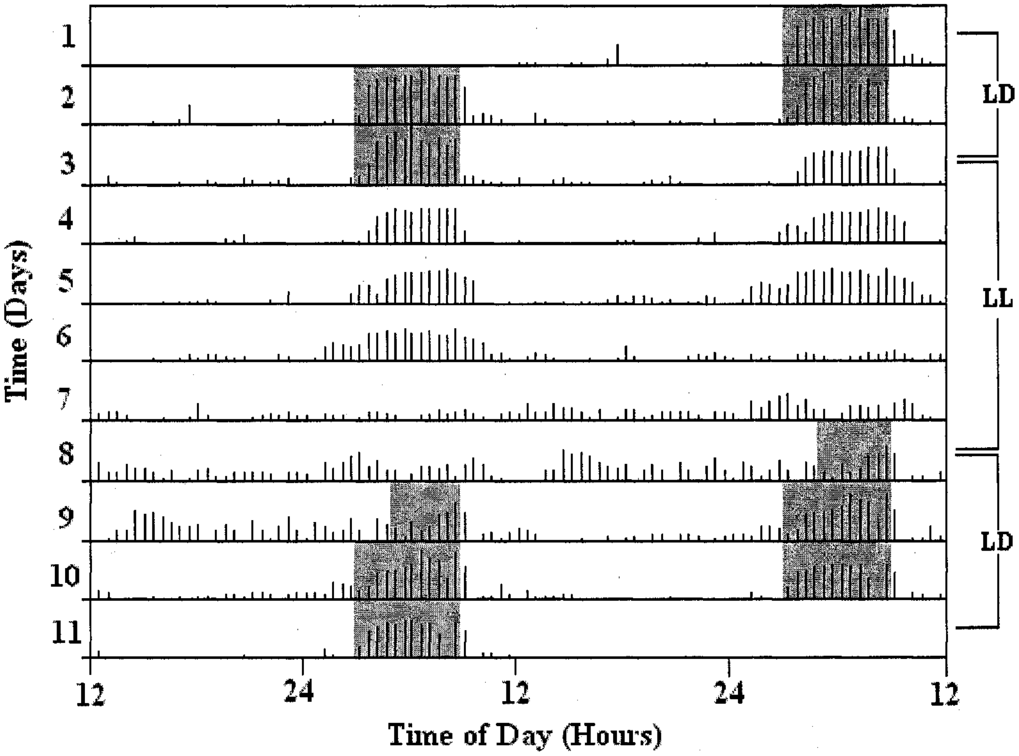

Figure 2.6: LL Actogram (Individual A)

Double-plot actogram from one individual over a period of 240h including LL. LL trial began at 09:00 on Day 2 (corresponding to actogram Days 2&3) and continued until 05:00 on Day 8 (140h). During the first 72h, the most intense locomotor activity was concentrated during the subjective night phase. All rhythms and subsequent activity patterns were lost after the initial 72h of LL.

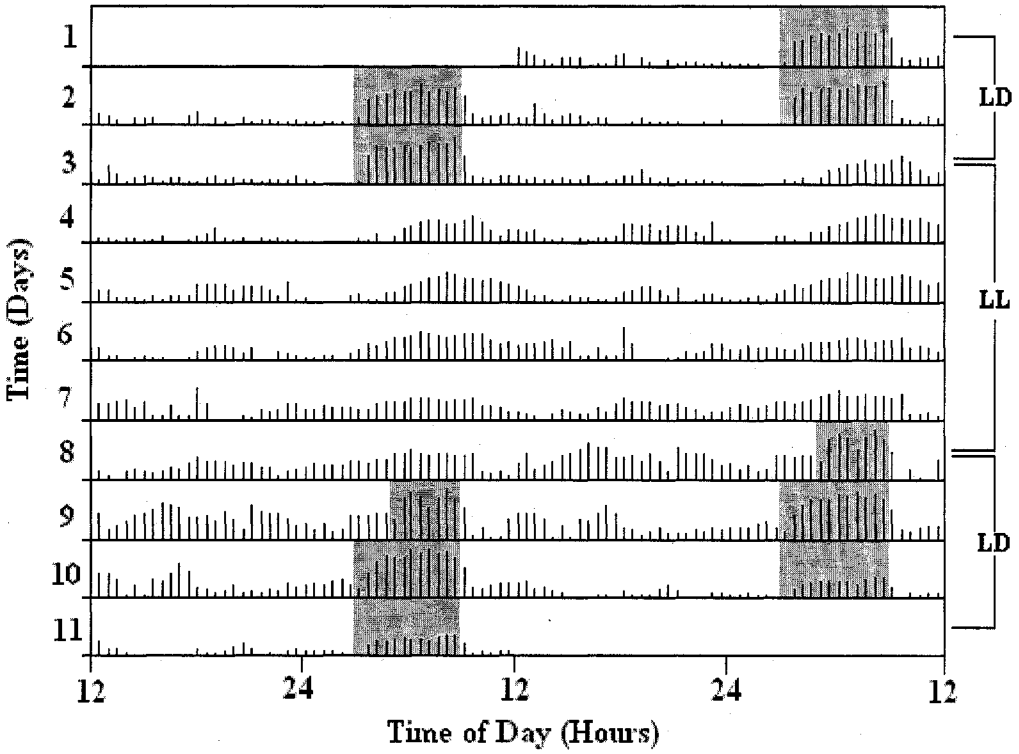

Figure 2.7: LL Actogram (Individual B)

Double-plot actogram from second individual over a period of 240h including LL. LL trial began at 09:00 on Day 2 (corresponding to actogram Days 2&3) and continued until 05:00 on Day 8 (140h). During the first 72h, the most intense locomotor activity was concentrated during the subjective night phase. All rhythms and subsequent activity patterns were lost after the initial 72h of LL.

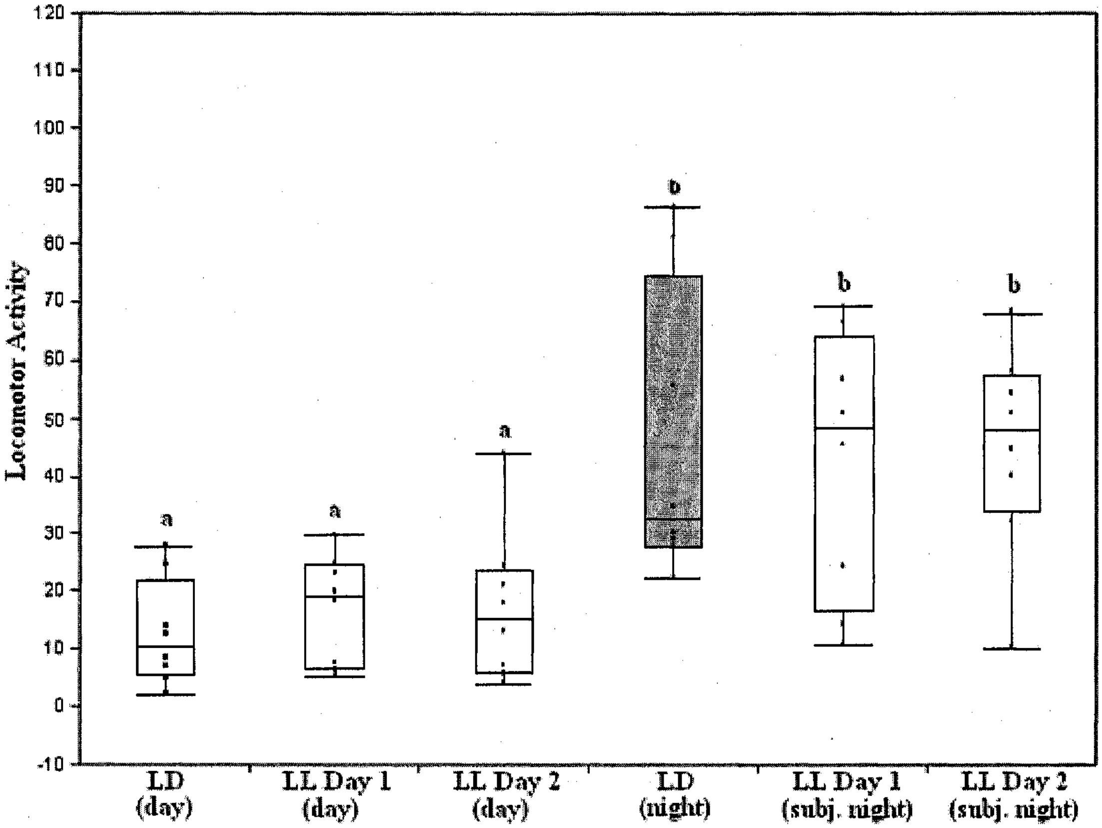

Figure 2.8: LD vs. LL Activity Comparisons

Box-and-whisker plot of locomotor activity during six three hour periods (n=8 birds). Letters **a** and **b** signify differences of $p < 0.05$. Subjective Day: Day 1: Z=-1.54, p=0.12; Day 2: Z=-1.40, p=0.16. Subjective Night: Day 1 Z=0.00, p=1.00; Day 2: Z=0.00, p=1.00.

Table 2.1: Migratory Behavior (BU/BUF) Expression

Mean percentage of migratory behaviors (BU/BUF) of all behaviors observed during subjective day, n=2 birds.

	TRIALS		
	LD (18L:6D)	**$(DD)_{dim}$**	**LL**
Lighting Conditions	On	Off (<1 Lux)	On
BU/BUF	0.06%	22.76%	0.05%

Table 2.2: Pre and Post Treatment Measures of Morphology

Comparative measures of morphology of birds in the vernal migratory condition prior to and following treatment trials, mean ± SE, n=8.

	Pre Treatment	*Post Treatment*
$(DD)_{dim}$ Fat Score	3.68 ± 0.16 *	2.25 ± 0.35
$(DD)_{dim}$ Body Mass (g)	30.30 ± 1.20*	25.10 ± 1.30
LL Fat Score	3.63 ± 0.16 *	3.06 ± 0.24
LL Body Mass (g)	28.10 ±1.10	27.60 ± 1.50

* Pre-treatment measures of body mass and fat score significantly greater prior to $(DD)_{dim}$ and LL treatments according to paired t-Tests.

- $(DD)_{dim}$ Fat Score t = 4.31, df=7, p = 0.004, n=8.
- $(DD)_{dim}$ Body Mass t = 8.45, df=7, p = <0.001, n=8.
- LL Fat Score t = 4.97, df=7, p = 0.002, n=8.

Table 2.3: Comparative Changes in Morphology

Comparative measures of change in morphology between $(DD)_{dim}$ and LL treatment groups, mean ± SE, n=8.

	$(DD)_{dim}$	**LL**
Ave Fat Score	1.40 ± 0.33 *	0.56 ± 0.11
Body Mass (g)	5.16 ± 0.6 **	0.50 ± 0.5

* Measure of change in average fat score during $(DD)_{dim}$ is significantly greater than that during LL treatment according to t-Test, $T = 2.59$, $p = 0.04$.

** Measure of change in body mass during $(DD)_{dim}$ is significantly greater than that during LL treatment according to t-Test, $T = 5.85$, $p < 0.001$.

-- Chapter III --

Diel Changes of Locomotor Activity and Migratory Restlessness in Resident White-crowned Sparrows (*Zonotrichia leucophrys nuttalli*)

Introduction

Avian migration has been the focus of research for decades as scientists have sought to understand the dramatic changes and subtle details that underlie this life history stage. Investigation of both wild and captive populations has provided insight regarding the intricate timing, physiological changes and underlying mechanisms that orchestrate migratory movements. Paradoxically, some non-migratory or resident species express certain traits thought to be characteristic of migrants (Smith et al., 1969; Chan, 1994; Helm and Gwinner, 2006). To fully assess whether a resident population is expressing complete migratory tendencies, we set out known characteristics of migrants as criteria against which to test the traits of a resident race. While the adaptive value of utilizing migrant traits by residents may be difficult to determine, comparison of closely related migrant and resident species may provide insight regarding phylogenetic relationships between the populations, as well as clues to the use of migrant traits in non-migratory birds.

Perhaps the most notable trait of migrant birds is that of movement, particularly nocturnal activity. In captivity, migrant birds express seasonally occurring night restlessness that coincides with and is a reliable measure of, biannual movements to and from breeding grounds in free-living populations (Berthold, 1973; Gwinner and

Czelschlik, 1978). Migratory restlessness (MR) or *Zugunruhe* has been the focus of previous studies investigating circannual programs, orientation, and migratory disposition in such species as blackcap warblers (*Sylvia atricapilla*), pied flycatchers (*Ficedula hypoleuca*) and garden warblers (*Sylvia boren*) (Wagner, 1930; Gwinner, 1975, 1986, 1996; Beck and Wiltschko, 1988; Berthold, 1996b). Less attention has been paid to the presence of nocturnal activity in resident populations, and while these birds might be expected to lack migratory movement, MR has been identified in several resident species. In grey-breasted silvereyes (*Zosterops lateralis*) MR was expressed in captive resident birds, especially when placed among highly night active migrants of the same species, suggesting strong social influences on this behavior (Chan, 1994). MR was also expressed in resident blackcap warblers (*Sylvia atricapilla*) and domestic Japanese quail (*Coturnix c. japonica*) although intensity of nocturnal activity was reduced in comparison with migratory congeners (Berthold, 1996b; Derégnaucourt et al., 2004). It should be noted however that these species are partial migrants, where some individuals within a population migrate while others do not.

Recently, Helm and Gwinner (2006) investigated migratory restlessness in resident stonechats (*Saxicola torquata axillaris*), suggesting MR expression is an output of an endogenous program timed by photoperiod. While MR was observed in resident stonechats, the intensity was reduced in comparison with that of a fully migratory European race (*S. t. rubicola*). Although MR expression in resident species has been attributed to juvenile dispersal, nomadism or atavistic (ancestral) traits, these authors suggest the existence of possible underlying programs for periodic movements or range expansions in response to variations in environmental conditions.

While the expression of nocturnal activity is important for any comparisons of migrant and resident species, other physiological and behavioral traits may also be considered for a more complete analysis. The Pacific races of white-crowned sparrow (*Zonotrichia leucophrys*) have been the focus of migration research for decades and are a suitable species for such comparisons (Blanchard, 1941; Farner, 1950; Farner and Mewaldt, 1953; King, 1968). Several studies have focused on the nocturnal activity of the resident race of white-crowned sparrow, *Zonotrichia leucophrys nuttalli*, particularly during vernal migration (Farner et al., 1957; Mewaldt et al., 1968; Smith et al., 1969). Located in coastal regions of central and southern California (from Humboldt to Santa Barbara counties), Nuttalls white-crowned sparrows (NWCS) hold territories year-round and come into contact seasonally with their short-distance (*Z. l. pugetensis*) and long-distance (*Z. l. gambelii*) migrant relatives (Blanchard, 1941; Cortopassi and Mewaldt, 1965; Mewaldt et al., 1968). NWCS have been shown to exhibit nocturnal activity, yet Smith et al. (1969) noted that MR was more prevalent in juveniles than adults and Farner et al. (1957) found that the intensity of nocturnal activity was less than that of locomotor activity recorded during daylight hours. Similar to Helm and Gwinner (2006), NWCS nocturnal activity appears to be less intense than that of migrant white-crowns (Mewaldt et al., 1968).

Historically, locomotor activity of captive birds during the night was synonymous with migratory restlessness. To further describe the behavioral characteristics of MR, Agatsuma and Ramenofsky (2006) identified the stereotypic behaviors beak-up (BU), beak-up flight (BUF) and quiescent phase (QP), specific components of display in captive *gambelii* during both autumn and vernal migration. Initially described as 'flying with the brakes on' (Berthold and Querner, 1988; Berthold et al., 2000), BU and BUF are thought to represent acquisition of information and take-off for migratory flight and have only been recorded in white-crowned sparrows (Agatsuma and Ramenofsky, 2006). QP, a period of low activity just prior to the onset of intense migratory restlessness, is believed to facilitate a metabolic switch from anabolic activities of feeding and fuel storage to intense activity supplemented by catabolic processes during flight (Palmgren, 1949; Morton, 1967; Agatsuma and Ramenofsky, 2006). To date, these migration specific behaviors have only been identified in migratory white-crowned sparrows (Agatsuma and Ramenofsky, 2006; Coverdill et al., 2008) and have not been studied in resident species.

An important environmental factor influencing migratory activity in both field and lab settings is intensity of light at night. While at least some light illumination is required for captive migrants to express night locomotor activity (Wagner, 1961; Helms, 1963, Ramenofsky et al., 2003), the overall intensity of moonlight directly affects MR expression. In European robins (*Erithacus rubecula*) and redstarts (*Phoenicurus phoenicurus*), higher mean MR was expressed during full moon phases compared with new moon phases (Gwinner, 1967). Similar conclusions have been found for resident NWCS, where a strong correlation between peaks in night activity with peaks of bright moon and suggests that residents as well as migrants may be affected by low level illumination at night (Smith et al., 1969).

Another well established trait for the expression of migratory disposition in both free-living and captive migrants is the deposition of fat as fuel resources during flight (reviewed in Dolnik and Blyumental, 1964; King, 1972). Not surprisingly, premigratory changes in feeding, body mass and fat deposition have been described for migrant populations of white-crowns (King and Farner, 1959; 1963). Unlike their migrant relatives, NWCS have not been shown to significantly increase body mass in spring as would be expected for long distance flight indicative of migration (Farner et al., 1957; Mewaldt et al., 1964, 1968; Smith et al., 1969).

Collectively, previous studies with migrant white-crowned sparrows have established the following characteristics of a complete migratory condition: intense nocturnal locomotor activity, expression of migration specific behaviors, increased locomotor activity coincident with increasing moonlight and premigratory increases in body mass and fat. Others have shown that resident species exhibit some seasonally occurring migratory traits. Thus, to fully assess the expression of complete migratory tendencies in resident NWCS, we tested their behavioral and physiological outputs against those of their migrant relatives. If the attendant components of migrants are present than we can confirm complete migratory expression; if all criteria are not met, we must look to the application of these traits in other life histories. Through this work we hope to further our knowledge of closely related migrant and resident species by identifying potential overlaps in behavior expression during alternate life history stages.

Materials and Methods

Capture and Handling:

In August 2005, 11 NWCS (5 males [4 juveniles, 1 adult] and 6 females [2 juveniles, 4 adults]) were captured near Monterey, California (36.6°N, 121.9°W). The birds were transported to the Department of Biology at the University of Washington, Seattle, where they were temporarily housed in an outdoor aviary exposed to natural photoperiod and temperatures. All techniques involving capture, handling and experimentation were performed within the guidelines of the American Association for Accreditation of Laboratory Animal Care and approved by the Institutional Animal Care and Use Committee at the University of Washington.

Environmental Chamber Set-up:

In November, birds were transferred from the aviary to individual cages (35cmW x 40cm L x 45cm H) within one environmental chamber. Eight birds were placed in registration cages equipped to collect locomotor activity data, while the remaining 3 were in similar cages without such equipment. Birds were provided food (Mazuri Chow, Nutritional International, Brentwood, CA and mixed seeds, UW Custom Mix, Seed Factory, NW, Kent, WA) *ad libitum* in hopper-style dishes at the front of each cage. Water for bathing and drinking, as well as vitamin laced grit (Bird Heath Grit, Seed Factory; Ceres, CA) was also provided. Birds were initially exposed to photocycle conditions of 10.5L:13.5D which was adjusted weekly to that of natural conditions of Monterey, CA (photoperiod data collected from U.S. Naval Observatory Astronomical Applications Department, http://aa.usno.navy.mil). After two months of decreasing photoperiod, the photocycle was increased to 15L:9D (maximum hours of light in Monterey), thus photostimulating the birds under long day conditions. Throughout the study, the dim light condition during the dark phase of all LD cycles, was achieved with 1 nightlight (Limelight Nightlight, Austin Innovations, TX) mounted centrally in the environmental chamber and will be referred to as 'moonlight.' Light intensity measured at the limelight source was 2-3 Lux, however values at the closest cage (<1m from source) were <1 Lux. Body mass and fat score measurements were taken weekly during the study. Birds were placed on an electronic scale (Ohaus Scout Pro, model SP202) within a cotton sock for body mass measurements to the nearest 0.1g. Fat deposits within the furcular fossa and abdominal cavity were scored using an arbitrary value of 0 (no fat) to 5 (bulging deposits) and averaged to determine overall fat score (Wingfield and Farner, 1978).

Measurement of Locomotor Activity:

Locomotor activity was measured in registration cages using one photodetector (Radio Shack Invisible Beam Alarm Entry, models #43-311 and 43-312) and a reflector, both positioned perpendicular to a centrally located perch. The output of this system is collected as average activity (beam breaks) per min per 30 minute interval, which for simplicity will be referred to as *locomotor activity*. Birds were considered 'night active' if expressing >3 beam breaks/min/30min during dark phase. 24h locomotor activity waveforms were created using Microsoft Excel. For further description of cage set-up and activity recording software, see Agatsuma and Ramenofsky (2006) and Coverdill et al. (2008).

Experimental Conditions:

To analyze potential changes in 24h patterns of locomotor activity, three discrete periods within the four month study were chosen for comparison following two weeks of acclimation to chamber conditions (Figure 3.1). Each period represents a discrete section of the continuous experimental study. The first period, Late Fall (10:20L:13:40D), was approximately 20 days *prior* to the winter solstice. The second period, Early Winter (10:20L:13:40D) began approximately 20 days *after* the winter solstice. While the photocycle for these first two periods is equal, in Late Fall the photophase was decreasing while under Early Winter it was increasing. The final period, Spring (15L:9D), occurred approximately 20 days following photostimulation. To reduce bias and limit individual day-to-day variation, 'typical' 24h waveforms of locomotor activity for each period (Late Fall, Early Winter, Spring) were created using individual activity records (n=8) averaged across four consecutive days.

Comparison of Activity Profiles:

For all three periods (Late Fall, Early Winter, Spring), the following comparisons of locomotor activity were conducted using the average activity of individuals: 1) Light phase (day) and dark phase (night) activity from an entire 24h cycle (within and between periods); 2) Evening Peak activity (EP, the increase in locomotor activity just prior to the dark phase) with Afternoon activity, the four hours immediately preceding EP (within periods); 3) Morning Peak activity (MP, the increase in locomotor activity following the initiation of the light phase) with Pre Dawn activity during the last four hours of the dark phase (within periods); and 4) Pre Dawn activity across periods (Late Fall, etc.).

Behavior:

To identify the behaviors of captive birds corresponding with locomotor activity at night, 4h infrared videos were recorded for 9 birds (5 juveniles, 4 adults) during one month of the Spring period. Each video was analyzed using an instantaneous scan method (Martin and Bateson, 1993; modified by Agatsuma and Ramenofsky, 2006) which classifies behavior at 20 second intervals from a subset of 8 behaviors; rest, jump, flight, feeding, beak-up (BU), beak-up flight (BUF), head-cock and other (typically preening). The two migratory behaviors, BU and BUF, are typically associated with MR in white-crowned sparrows. All other behaviors were considered non-migratory activity as they occur during daytime activity. The total percentage of BU and BUF (combined) and Rest for each individual 4h recording was calculated from the total behavioral observations (e.g. 25% BU/BUF and 70% Rest from 100% total observations).

Moonlight Manipulation:

The moonlight manipulations took place following approximately 45 days of photostimulation (Figure 3.1). To test the effects of variation in moonlight intensity on dark phase locomotor activity, birds were subjected to one night each of 0, ½, and 2 limelights or 'moons' (each <1Lux intensity). As the birds had been acclimated to 1 moon throughout this study, activity during the dark phase with 1 moon served as the control. Order of exposure to various treatments was randomized. Each treatment night was followed by two control nights (1 moon). It should be noted that while we refer to these lights as 'moons,' light intensity measured at cage level under 1 moon is <1Lux and does not represent the intensity at Earth's surface of a natural full moon under summer conditions (~2 Lux, Austin et al., 1976). Only birds that showed nocturnal activity during this manipulation period were used in the analysis of locomotor activity (n=5).

Statistical Analyses:

Shapiro-Wilks tests indicated that locomotor activity data collected for this study did not follow a pattern of normal distribution and therefore required non-parametric tests for analysis. For comparisons across all periods (Late Fall, etc.), Friedman test was used to analyze change over time. Given significant changes via Friedman analysis, pair-wise analyses within or across groups were conducted using Wilcoxon Signed Ranks tests, integrating a Sequential Bonferroni correction for multiple comparisons (Rice, 1989; Zar, 1999). For all comparisons, significant differences indicate $p<0.05$.

Results

Locomotor Activity:

For each experimental period (Late Fall, Early Winter, Spring) locomotor activity was similar during the four days used to create each 'typical' profile (Late Fall: $\chi^2_{27,3}=.60$, $p=0.90$; Early Winter: $\chi^2_{27,3}=.91$, $p=0.82$; Spring: $\chi^2_{18,3}=5.13$, $p=0.16$). Locomotor activity waveforms representing typical 24h patterns of activity are illustrated in Figures 3.2, 3.3 and 3.4 (Late Fall, Early Winter and Spring, respectively). Light phase activity (lights on) was significantly greater than dark phase activity (lights off) for Late Fall ($Z=-2.24$, $p=0.03$) and Early Winter ($Z=-2.52$, $p=0.01$), but not during Spring ($Z=-0.28$, $p=0.78$, Table 3.1). Only 3 of the 8 individuals (2 adult females, 1 juvenile male) expressed dark phase activity during Late Fall (Figure 3.2 inset). Following photostimulation of 15L:9D (Spring), 7 of the 8 birds expressed dark phase activity while one (adult male) showed no dark phase activity throughout the study.

Locomotor activity during the light phase did not change across experimental periods ($\chi^2_{8,2}=1.75$, $p=0.42$, Figure 3.5) while dark phase activity was significantly greater during Spring than during Early Winter conditions ($Z=-2.38$, $p=0.02$, Figure 3.6). All three waveforms are characterized by intermediate levels of activity during the light phase with a significant peak of activity (EP) preceding the dark phase (Z=-2.52, p=0.01, Table 3.2, Figures 3.2-4).

While Late Fall and Early Winter waveforms show very little activity at night, during Spring birds elevate night activity with a peak apparent at 5am (Figure 3.4). Therefore, for Late Fall and Early Winter (but not Spring), activity during the last four hours of the night (Pre Dawn) is significantly less than the morning peak (MP) initiated at the onset of the light phase (Late Fall: Z=-2.38, p=0.01; Early Winter: Z=-2.52, p=0.01; Spring: Z=-1.12, p=0.26; Figure 3.7). Comparison of predawn activity across experimental periods reveals a significant increase in Spring over Late Fall (Z=-2.10, p=0.04) and Early Winter (Z=-2.38, p=0.02, Figure 3.8).

Behavior Scoring:

Inter-individual expression of BU and BUF and Rest was high (Table 3.3). Some birds were active but not expressing migration specific behaviors (primarily flight and jump, see 202, 208, Table 3.3), while other birds (203, 205) expressed high percentages of BU and BUF (39% and 34% of all observed behaviors, respectively).

Moonlight:

Friedman analysis indicated that night activity did not change during control nights (1 Moon) separating treatment nights (0, ½, 2 Moons, $\chi^2_{5,6}$=10.63, p=0.10). Analysis of treatment nights with moonlight (½, 1 or 2 moons) did not reveal significant differences in activity ($\chi^2_{5,2}$=0.40, p=0.82), while comparison of treatment night with no moonlight (0 moon) with control indicates significantly greater dark phase activity when some dim light is present in the chamber (Z=-2.02, p=0.04, Figure 3.9).

Body Mass and Fat Score:

Pair-wise comparison of the five measurements before and after photostimulation (Day 0, Figure 3.10) reveled no significant difference in either body mass or fat score (mass: Z=-1.28, p=0.20; fat: Z=-1.42, p=0.16).

Discussion

Previous studies have focused on the nocturnal activity and behaviors of captive migrants (Wagner, 1930; Gwinner 1986, 1996; Berthold, 1996b; Agatsuma and Ramenofsky, 2006). Given the paradoxical findings of Mewaldt et al. (1968), Smith et al. (1969) and Helm and Gwinner (2006), that resident species show night activity thought to be part of migratory restlessness, we set out criteria characteristic of migrants, against which to compare a resident species. Based on research with migratory Gambel's white-crowned sparrow (*Z.l. gambelii,* GWCS), we investigated changes in locomotor activity, the expression of migratory behaviors, responses to changes in moonlight intensity and patterns of body mass and fat gain in resident Nuttall's white-crowned sparrow (*Z.l. nuttalli*, NWCS).

Throughout, the general relationship between light and dark phase locomotor activity remained relatively constant although the intensity of activity varied. During the Early Fall and Late Winter periods, light phase activity exceeded that during the dark. Following photostimulation (Spring), dark phase activity increased significantly but did not reach peak light phase intensities. These findings are in stark contrast to activity patterns of the long distance migrant GWCS, where spring dark phase activity levels represent the maximum for a 24h cycle (Ramenofsky et al., 2003; Coverdill et al., 2008). During all periods, locomotor activity showed a marked increase just prior to the initiation of the dark phase (EP). This peak is similar to that observed in autumn migrating and wintering GWCS (Morton, 1967; Ramenofsky et al., 2003). One could speculate that in these cases, foraging behavior increases in preparation for night roosting (winter) and the quiescent phase within the dark phase (autumn). In spring GWCS however, QP is expressed just prior to the dark phase with no peak in activity preceding it (Agatsuma and Ramenofsky, 2006).

The most significant change in locomotor activity across Late Fall, Early Winter and Spring conditions was that of night activity. During the first period, only 3 individuals exhibited limited activity at night and the movement was confined to the predawn hours just before the light phase. Given the significant increase in predawn activity between Early Winter and Spring, we can conclude that photostimulation affects an increase in predawn activity. Interestingly, this development of night activity during the predawn hours with gradual progression towards dusk is consistent with GWCS. As GWCS transition from winter to spring life history stages, night activity begins as predawn activity and gradually spreads to include the entire dark phase (Ramenofsky, unpublished results). The similar development of nocturnal activity in both races might suggest that photostimulation acts to synchronize birds during the transition to spring life history stages. The overall shift in night activity expression in NWCS (7 birds registering activity during Spring, opposed to only 3 under previous photoperiod conditions) led to a noticeable shift in the 24h profile of activity (Figure 3.4).

Much like the 24h locomotor activity pattern of wintering GWCS (Coverdill et al., 2008), NWCS showed a significant peak in activity (MP) upon entering the light phase during Late Fall and Early Winter. Under Spring conditions however, activity levels did not change when transitioning from dark phase to light. This suggests that unlike migrating GWCS which show an immediate decrease in activity following long bouts of migratory restlessness, activity expressed during the dark phase was not energetically taxing and most likely represents the expression of behaviors other than migration.

Unlike Smith et al. (1969), the expression of nocturnal activity was not confined to juvenile birds. Although all four juveniles expressed night activity, three out of the four adults were night active, two of which showed night movement under short-day conditions as opposed to only one juvenile. While prebreeding juvenile dispersal has been documented for a number of resident species (Odum, 1942; Johnston, 1956), it is unlikely that such behavior would occur during the spring, and at night (Smith et al., 1969). Given that NWCS begin nesting in March (Blanchard, 1941; Mewaldt et al., 1968), one would expect potential dispersal of juvenile birds and the establishment of territories to take place in the months prior, and only one juvenile in our study exhibited night activity during this time. The presence of night activity in juveniles and adults, and its coincidence with the expression of migratory restlessness in migrant white-crowned sparrows (Mewaldt et al., 1968) leads us to conclude that nocturnal activity in these resident birds should not be attributed to dispersal of juveniles.

While numerous studies have relied solely on the expression of nocturnal locomotor activity for the classification of migratory restlessness, the identification of migration specific behaviors (BU/BUF) in white-crowned sparrows has provided additional confirmation. Interestingly, two birds in this study (one juvenile [203], one adult [205]; Table 3.3) expressed percentages of BU and BUF during filmed sessions consistent with values recorded in migratory GWCS (see Chapter IV). The combined expression of increased locomotor activity during the dark phase of the Spring period and the presence of BU/BUF behaviors lead us to conclude that at least some NWCS do exhibit behavior consistent with migratory restlessness. This combined expression of activity and behavior was variable across individuals with most birds exhibiting little or no BU/BUF and mostly rest during filming. In addition, two juveniles expressed very little BU/BUF and only 31% and 53% rest (202, 208, Table 3.3), indicating that these birds were quite active but not expressing migratory behaviors (mainly jump and flight). Thus, we can not conclude that increased activity is always the complete expression of migratory restlessness, again suggesting that perhaps this activity may represent something other than migratory tendencies.

Nocturnal illumination has been shown to influence migratory activity in free-living and captive birds (Ramenofsky et al., 2003; Tokuda and Ramenofsky, in prep) However, in this study dark phase locomotor activity did not change with increases in moonlight illumination. We attribute the similarity of activity under ½, 1 and 2 moonlights to the fact that while the number of lights in the chamber increased, the actual intensity of light reaching each cage was not significantly different across treatments. Regardless of the number of lights, intensity measured at each cage was <1Lux for all treatments. While previous investigations have noted increases in night activity during brighter phases of the moon, or when the moon was more prevalent in the sky (Gwinner, 1967; Smith et al., 1969) these observations may be correlated more with the presence of the moon itself, rather than the intensity of available light. Acting as a visual cue, the moon may indicate that atmospheric conditions are suitable for flight and thus induce a stronger urge for migratory restlessness. The significant decrease in night activity under moonless conditions is consistent with previous investigations of migrants that show that at least some dim light is required for movement at night (Ramenofsky et al., 2003; Landys et al., 2004b).

Unlike migratory congeners, NWCS did not exhibit significant increases in body mass or fat, particularly following photostimulation, consistent with previous work (Farner et al., 1957; Mewaldt et al., 1968). The subtle variations seen in these measures are not biologically relevant when compared with hyperphagic migrants in preparation for long distance flight. More importantly, there was no difference prior to and following photostimulation as is seen in numerous migrant species including white-crowned sparrows (King and Farner, 1959, 1963; Ramenofsky et al., 2003).

Others have shown that resident species exhibit seasonally occurring nocturnal locomotor activity and have attributed this to migratory tendencies (Smith et al., 1969; Chan, 1994; Helm and Gwinner, 2006). Captive studies with GWCS have established characteristics of complete migratory condition (intense nocturnal locomotor activity, premigratory fattening, etc.) to which we compared resident NWCS.

The combined expression of migration specific behaviors with increased activity is consistent with the expression of migratory restlessness in at least some individuals. While exhibiting some nocturnal activity, the general 24h pattern of activity differed from that of migrants. The similarity of EP in NWCS with wintering migrants and the complete lack of QP during a 24h cycle, suggests that these residents do not prepare for metabolically demanding activity during the dark phase. As hyperphagia leading to increased fuel stores is a necessary characteristic for the development and mature capability of migration, we can conclude that NWCS are not physiologically prepared for long distance flight.

The presence of night activity in these resident birds with a development pattern similar to migrating relatives supports the notion that activity expression may be the result of remnant ancestral characteristics. Taking into account NWCS close relation to migratory white-crowns and the general position of *Zonotrichia leucophrys* within other migratory *Zonotrichia* taxa (Zink et al., 1991), it is possible that migratory restlessness in these resident birds is an atavistic expression of these behaviors. While this expression may facilitate a readiness to move in response to environmental change (Helm and Gwinner, 2006), the limited range of Nuttalls white-crowned sparrows within a confined habitat does not support this notion.

Collectively, these findings allow us to conclude that NWCS do not express the full suite of characteristics required for complete migratory condition. Given the lack of migratory movement in free-living NWCS, it is more likely that this expression represents the application of this activity for new purposes. In the wild, NWCS appear to move about their territories and occasionally sing at night (J Wingfield, field observ.). It is likely that the night activity of these birds manifests itself as territory maintenance or other functions pertaining to breeding. Field studies with particular attention to night movements of wild birds may resolve this issue.

By comparing resident birds to a suite of traits characteristic of captive migrants, we have demonstrated that the expression of one migration component may not be indicative of overall migratory condition. Future investigations of resident species must consider not only nocturnal locomotor activity, but also key behavioral and physiological aspects for the complete identification of a migration life history stage.

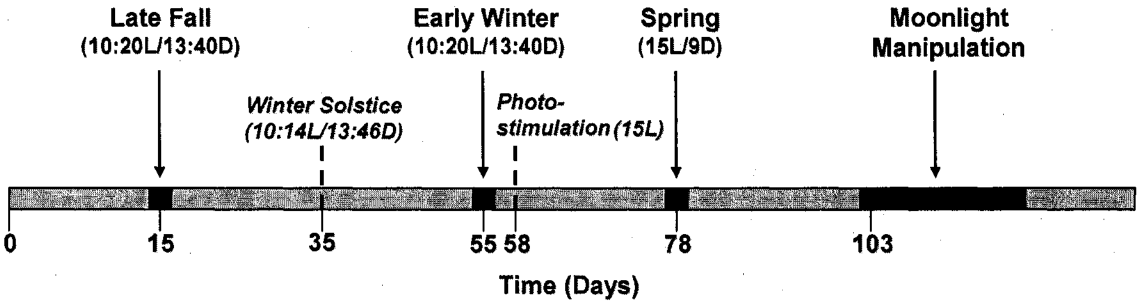

Figure 3.1: Experimental Timeline

Timeline for experimental periods. Late Fall refers to records collected following two weeks of chamber acclimation and 20 days prior to Winter Solstice. Early Winter was 20 days following the Winter Solstice. Spring was 20 days after photostimulation of 15L:9D. Moonlight manipulation measures of activity (under 0, 1, ½ and 2 moonlights) were conducted following 45 days of 15L:9D.

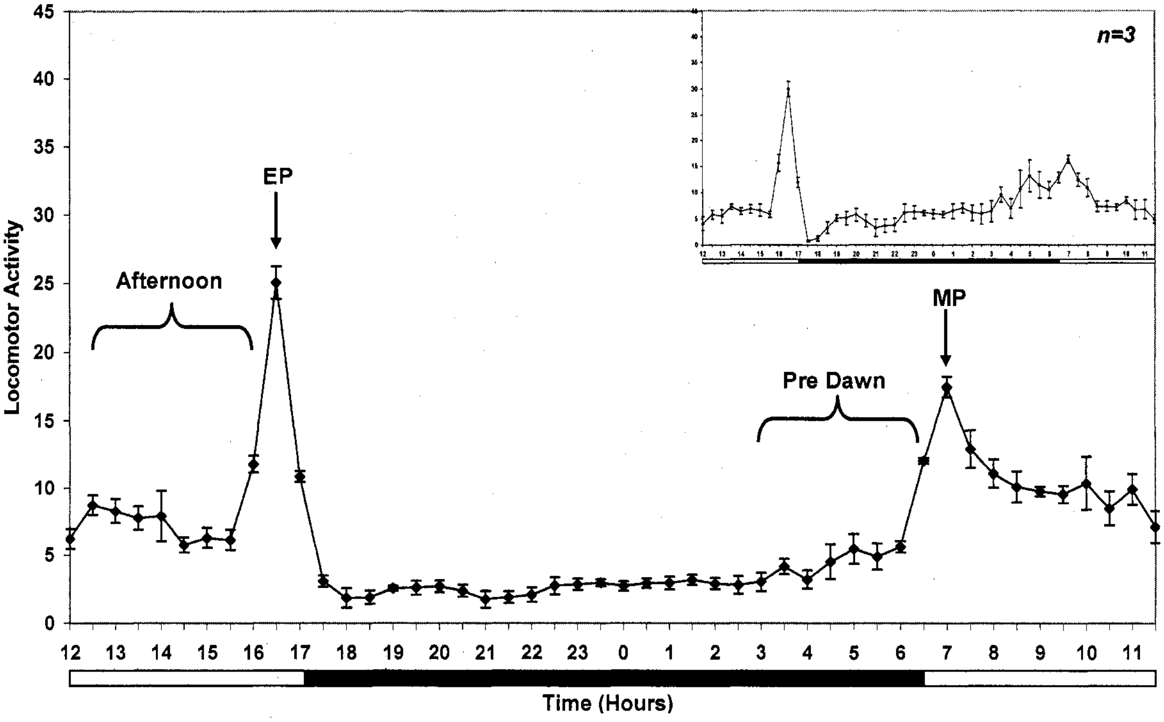

Figure 3.2: Late Fall Activity Waveform

24h locomotor activity waveform (average activity per min/30 min interval) of birds during the Late Fall (10:20L/13:40D) experimental period. Closed black bars below figures indicate lights off, open white bars indicate lights on, symbols represent mean ± SE from 4 consecutive days, n=8. Specific periods of activity indicated: Afternoon; EP=Evening Peak; Pre Dawn; MP=Morning Peak. Inset waveform represents activity pattern of night active birds (n=3) during this period.

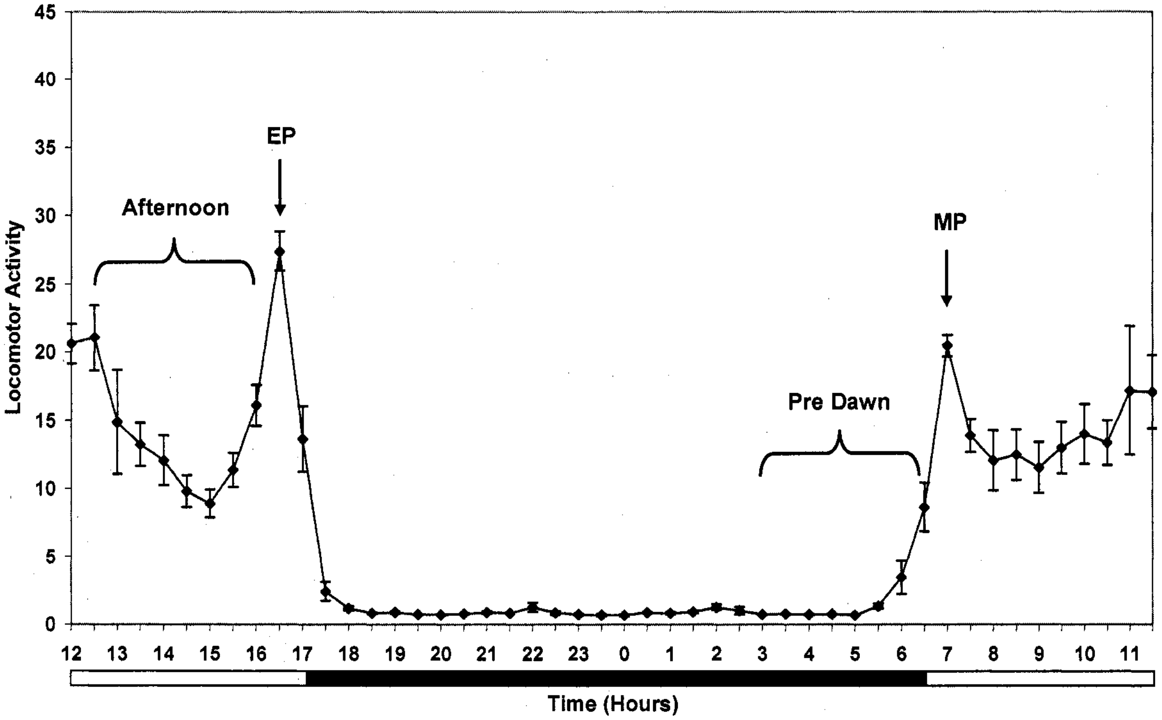

Figure 3.3: Early Winter Activity Waveform

24h locomotor activity waveform (average activity per min/30 min interval) of birds during the Early Winter (10:20L/13:40D) experimental period. Closed black bars below figures indicate lights off, open white bars indicate lights on, symbols represent mean ± SE from 4 consecutive days, n=8. Specific periods of activity indicated: Afternoon; EP=Evening Peak; Pre Dawn; MP=Morning Peak.

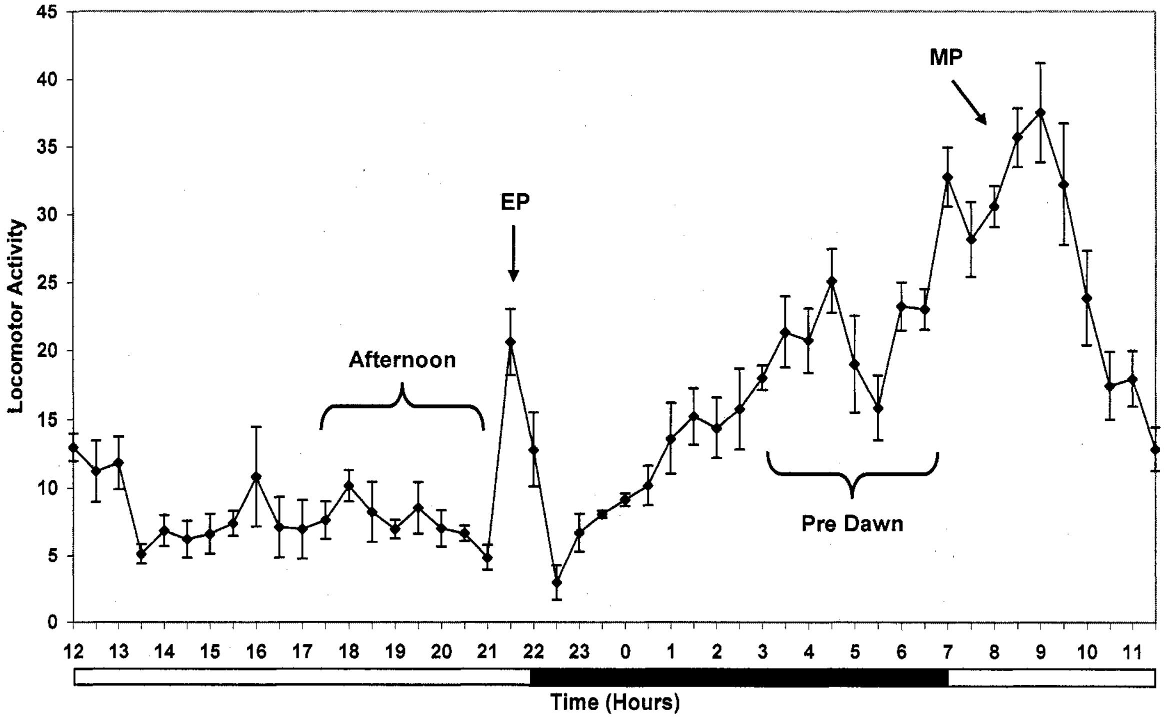

Figure 3.4: Spring Activity Waveform

24h locomotor activity waveform (average activity per min/30 min interval) of birds during the Spring (15L/9D) experimental period. Closed black bars below figures indicate lights off, open white bars indicate lights on, symbols represent mean ± SE from 4 consecutive days, n=8. Specific periods of activity indicated: Afternoon; EP=Evening Peak; Pre Dawn; MP=Morning Peak.

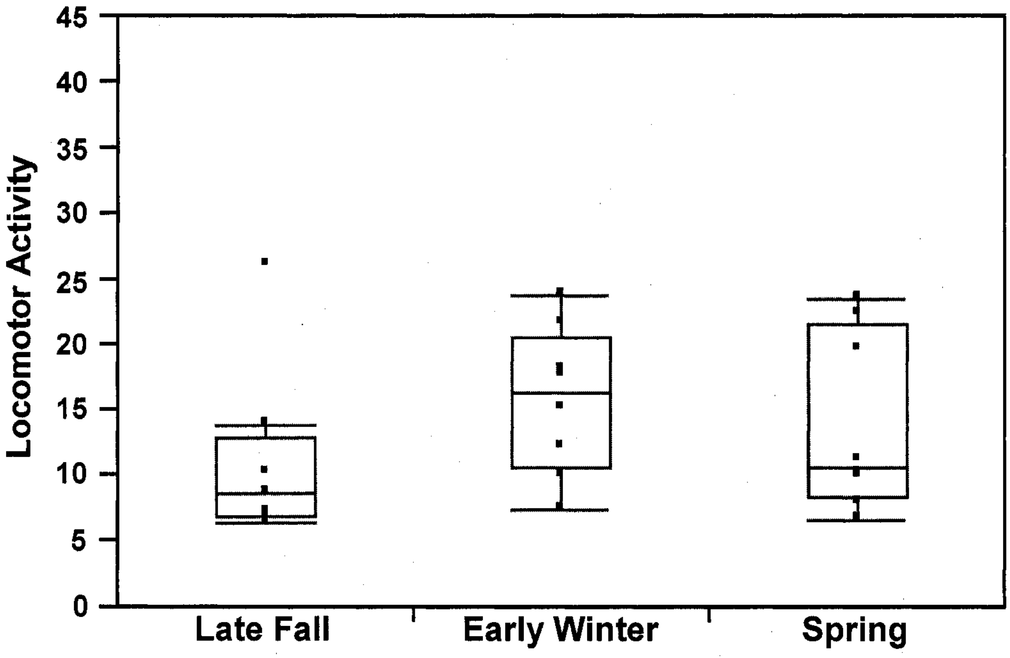

Figure 3.5: Light Phase Activity

Box-and-whisker plot comparison of average light phase locomotor activity during Late Fall, Early Winter and Spring conditions (n=8). Horizontal lines within boxes indicate median, outer limits of boxes denote upper and lower quartiles, and whiskers represent maximum and minimum values or 1.5*interquartile range, whichever value is lower. White shading in boxes indicates periods with lights on, gray shading in boxes are from periods with lights off (plot descriptions apply to all subsequent box-and-whisker plots).

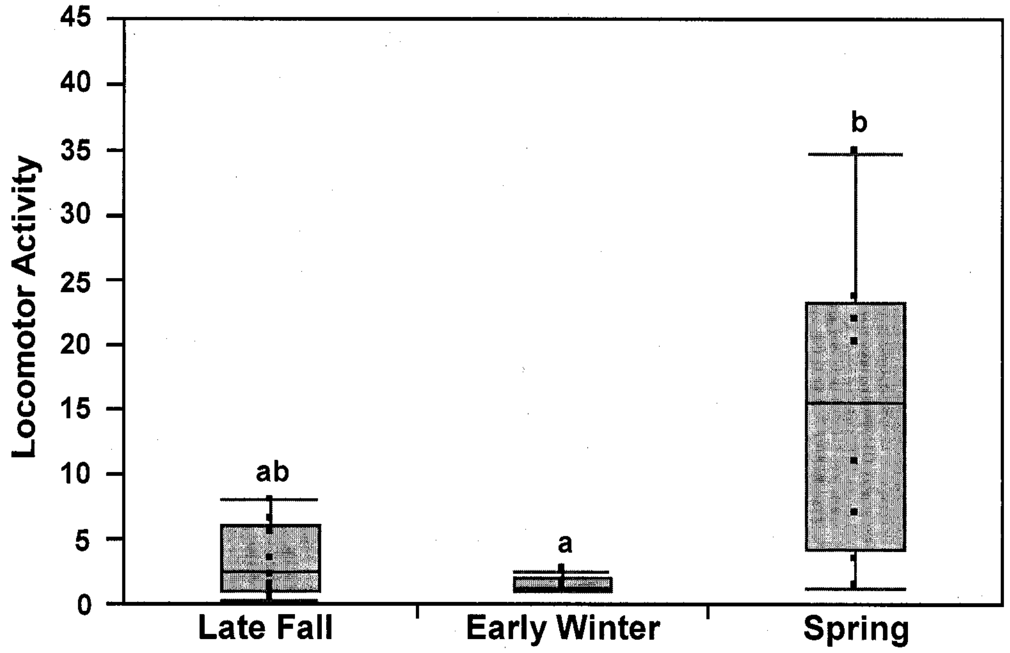

Figure 3.6: Dark Phase Activity

Box-and-whisker plot comparison of average dark phase locomotor activity during Late Fall, Early Winter and Spring conditions (n=8). Letters **a** and **b** signify differences between groups. Spring vs. Early Winter dark phase activity: Z = -2.38, p = 0.02.

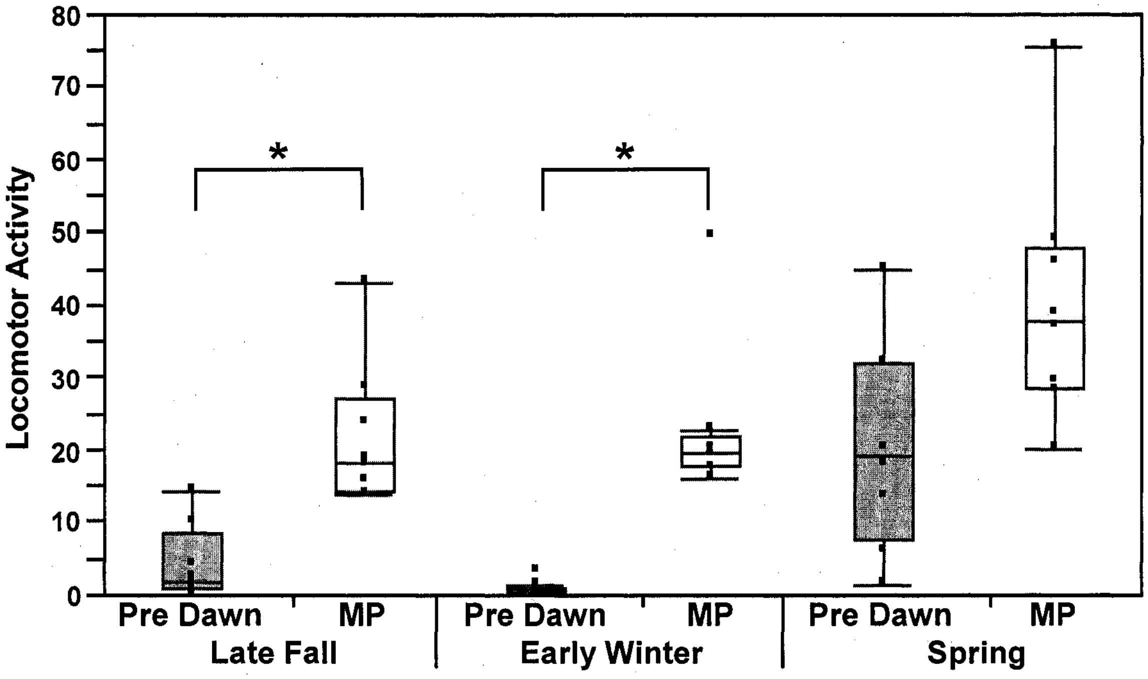

Figure 3.7: Predawn and MP Activity

Box-and-whisker plot comparison of average predawn and morning peak (MP) locomotor activity from Late Fall, Early Winter and Spring conditions (n=8). Symbol * denotes significant pair-wise difference. Late Fall: Z = -2.38, p = 0.02. Early Winter: Z = -2.52, p = 0.01.

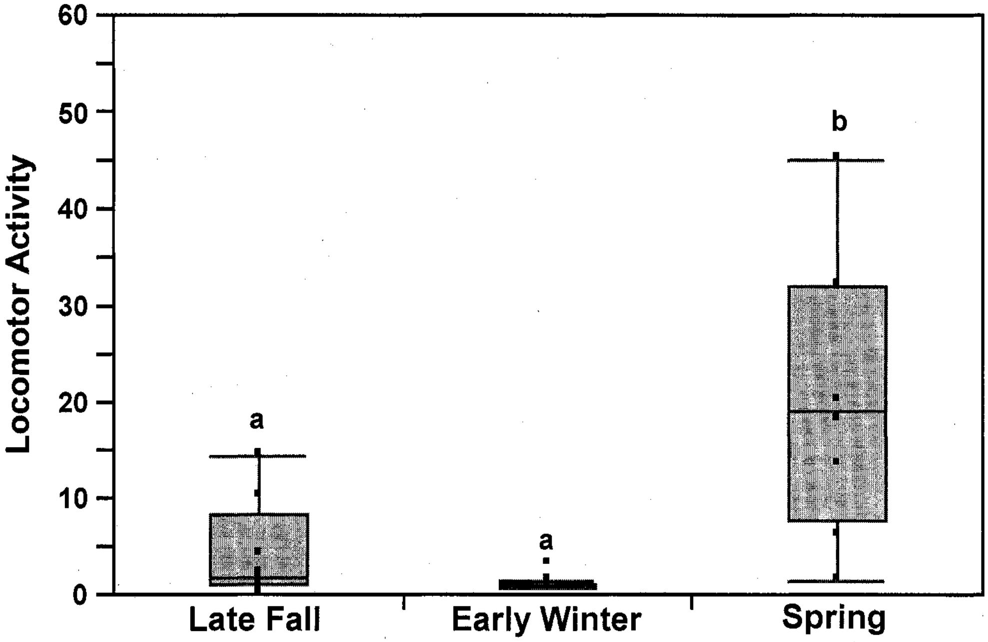

Figure 3.8: Predawn Activity

Box-and-whisker plot comparison of average predawn locomotor activity from Late Fall, Early Winter and Spring experimental periods (n=8). Letters **a** and **b** signify differences between groups. Late Fall/Spring: Z = -2.10, p = 0.04, Early Winter/Spring: Z = -2.38, p = 0.02.

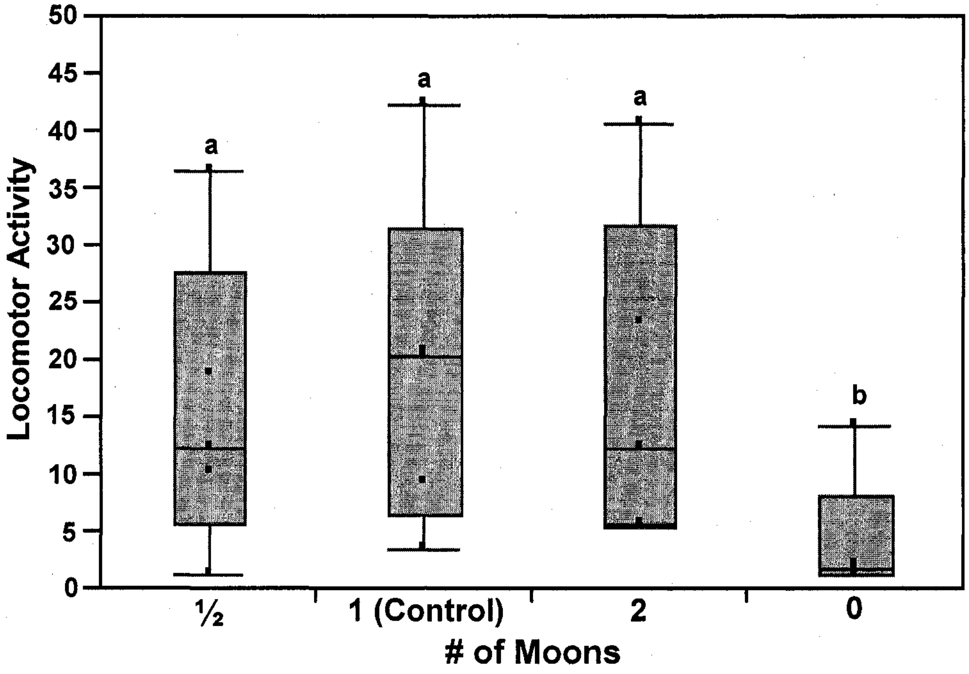

Figure 3.9: Moonlight Manipulation (*nuttalli*)

Box-and-whisker plot comparison of locomotor activity during nights with ½, 1, 2 and 0 moonlights (Moons) in the environmental chamber (n=8). Letters **a** and **b** signify differences between groups. Z = -2.02, p = 0.04.

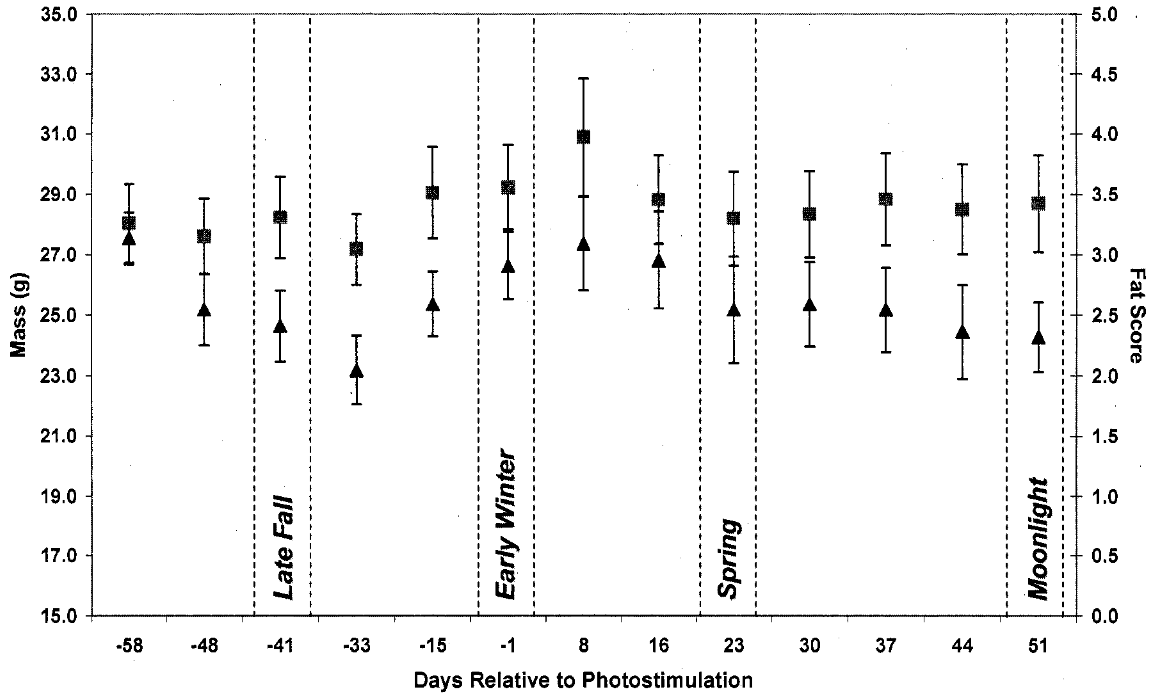

Figure 3.10: Morphology Measures

Average mass (squares) and fat scores (triangles) of captive birds throughout the study (n=11). Day 0 represents switch to photostimulating photocycle of 15L:9D. Dashed lines denote approximate timing of experimental periods (Late Fall, etc.). There was no significant change in either measure following photostimulation (mass: Z=-1.28, p=0.20; fat: Z=-1.42, p=0.16).

Table 3.1: Light vs. Dark Phase Activity

Locomotor activity during the light and dark phases of three experimental periods, median (min, max), n = 8.

	Light Phase Activity	*Dark Phase Activity*
Late Fall	8.55 (6.37, 26.04)	2.58 (0.31, 7.89) * [a]
Early Winter	16.23 (7.24, 23.71)	1.37 (0.90, 2.46) * [b]
Spring	10.48 (6.47, 23.60)	15.42 (1.26, 34.87)

* Day activity significantly greater than night activity according to Wilcoxon Signed Ranks test with Sequential Bonferroni correction.

a. Late Fall Z = -2.24, p = 0.03

b. Early Winter Z = -2.52, p = 0.01

Table 3.2: Afternoon vs. EP Activity

Locomotor activity during the Afternoon and Evening Peak phases of the three experimental periods, median (min, max), n=8.

	Afternoon	*Evening Peak*
Late Fall	5.14 (2.64, 20.50)	28.26 (9.00, 35.83) *
Early Winter	13.39 (4.78, 21.35)	27.69 (15.55, 38.76) *
Spring	4.94 (3.18, 17.28)	18.17 (13.97, 45.01) *

* Evening Peak activity significantly greater than Afternoon activity according to Wilcoxon Signed Ranks test with Sequential Bonferroni correction (Z=-2.52, p=0.01 for all comparisons)

Table 3.3: Migratory Behavior and Rest Expression

Percentage of migratory behaviors (BU and BUF) and Rest of all behaviors observed for juvenile and adult birds filmed during Spring period (15L:9D).

	Bird ID	*% BU and BUF*	*% Rest*
JUVENILES	201	0.1	87.9
	202	0.0	31.0
	205	34.3	44.0
	208	4.3	52.6
	210	1.7	97.0
ADULTS	203	39.1	45.0
	204	1.7	95.4
	207	0.0	90.0
	209	0.0	93.4

-- Chapter IV --

Comparison of Vernal Migratory Behavior in Pacific White-crowned Sparrows (*Zonotrichia leucophrys*)

Introduction

Avian migration, the biannual movement of birds to and from breeding and wintering habitats, has captivated enthusiasts for centuries. Prior to recent advances in telemetry, tracking physical movements of birds was difficult to study in the field. Studying captive migrants, von Hoymer (1881) and Wachs (1926) noted that while physically unable to migrate, caged birds expressed activity and behavior that was indicative of migration in free-living populations. Since these early discoveries, numerous investigations of captive species have provided insight regarding the physiology, timing, and overall expression of the migration life history stage (reviewed in Berthold, 1975, 1996; Gwinner, 1986). Using a distinct set of traits characteristic of migrants in captivity, we compared closely related populations which express a spectrum of migration strategies (long and short-distance migrants, and resident [non-migrant]). This experimental paradigm provides the means for studying the stepwise evolutionary departure from long-distance migration to a resident life history.

White-crowned sparrows (*Zonotrichia leucophrys*) have been the focus of captive migration research for over 50 years (Farner, 1950; Farner and Mewaldt, 1953; King, 1963; Ramenofsky et al., 2003), and the Pacific races serve as a model for comparisons of captive behavior and migration strategy. Gambel's white-crowned sparrow (*Z.l.*

gambelii) is considered a long-distance migrant as it travels from wintering grounds in the western US and Mexico to breeding grounds of western Canada into the Arctic (Cortopassi & Mewaldt, 1965). The Puget Sound white-crowned sparrow (*Z.l. pugetensis*) migrates from wintering areas in California and Oregon to breeding territories in the northwest US and southern British Columbia, and is therefore classified as a short-distance migrant (Mewaldt et al., 1968). Both *gambelii* and *pugetensis*, however, are considered short-bout migrants as their total migration distance is traveled in short, nightly bouts, unlike long-bout migrants that cross such barriers as open water, inhospitable deserts and vast mountain ranges in a single bout. For mechanistic purposes it is important to distinguish long versus short-distance migrants and whether they move by long or short bouts. The resident race, Nuttall's white-crowned sparrow (*Z.l. nuttalli*), holds year-round territories in coastal regions of central and southern California (Blanchard, 1941; Cortopassi and Mewaldt, 1965). Separation of the ancestral migratory white-crowned sparrow populations into the various Pacific races probably occurred during multiple glaciations of the Pleistocene (Rand, 1948; Banks, 1964). Advance of the Laurentian ice sheet most likely resulted in allopatric breeding conditions. Presumably *gambelii* became isolated in the Yukon-Bering Sea region while *pugetensis* and *nuttalli* along the Pacific coastal region of their current ranges (Selander, 1965; Mewaldt et al., 1968). Positive selection for distinct breeding sites would have led to variation in migratory patterns. The current populations of white-crowned sparrows represent a spectrum of migration strategies from migratory to resident. Thus we used this array of congeners to test the step-wise evolution of migration strategies from long-distance to residency.

For migratory birds in captivity, in which actual flight is impossible, the expression of migratory behaviors are manifested as intense activity called migratory restlessness (MR) or *Zugunruhe* (Wagner, 1930). Comparisons of migratory populations of European warblers during autumn relate the intensity and duration of MR expressed in captive birds to the relative distance traveled in free-living populations (Gwinner, 1968; Berthold, 1978; Berthold and Querner, 1981). Thus MR activity measured in captivity is considered a reliable measure of migration tendency. Given this relationship in European migrants, it is possible that 24h cycles of MR may relate to the spectrum of migration strategies of the Pacific white-crowned sparrows. Therefore, we predicted that *gambelii* would show night activity of greater intensity than *pugetensis*. While seemingly paradoxical, previous research has shown limited night activity typically attributed to migrants in *nuttalli* (Mewaldt et al., 1968; Smith et al., 1969; Coverdill et al., in prep) which we predicted would be of less intensity than either of its migratory relatives.

Moonlight intensity has been shown to have positive modifying effects on night activity in several species of both migrants and residents (Wagner, 1961; Gwinner, 1967; Smith et al., 1969). Given that captive migrants show little or no activity in complete darkness (Helms, 1963; Ramenofsky et al., 2003) we predicted that both *pugetensis* and *nuttalli* would increase night activity with increases in moonlight intensity and remain inactive in complete darkness.

Although early investigations of MR focused solely on locomotor activity, expression of unique behaviors and body postures has become recognized as an integral component of MR. In nocturnal migrants, which typically show minimal activity at night during non-migratory stages (Farner, 1955; Ramenofsky et al., 2003), MR has been described as continued wing-whirring or 'fluttering of the wings at high frequency but low amplitude while sitting on a perch' (Berthold and Querner, 1988). Recently, the identification of the specific migratory behaviors 'beak-up' (BU) and 'beak-up flight' (BUF) expressed during night locomotor activity in *gambelii*, has provided an additional tool for quantifying migratory activity (Ramenofsky et al., 2003; Agatsuma and Ramenofsky, 2006). Prior to video taping night phase activity of all three subspecies, we predicted that *gambelii* and *pugetensis* would express BU and BUF consistent with migratory restlessness, while *nuttalli* would not.

Observations of free-living *gambelii* by Morton (1967) noted that birds that would depart on migration following sunset became aphagic and would roost during twilight hours. Extending this finding in the laboratory, Agatsuma and Ramenofsky (2006) identified a period of inactivity prior to the onset of MR subsequently termed the quiescent phase (QP). While Morton (1967) described this period as a time when birds could reduce overall weight by fully digesting gut contents, it can generally be hypothesized that QP represents a metabolic switch from the consumption of food during daylight hours (anabolic) to the use of stored energy during flight (catabolic). Much like BU and BUF, QP can be classified as a migration-specific trait as it is only expressed during migration life history stages and has yet to be identified in resident species. Thus we predicted that QP would be expressed in *gambelii* (as shown by Agatsuma and Ramenofsky, 2006) and *pugetensis*, but not *nuttalli.*

The final migratory trait documented in captive birds that we tested across the races of white-crowned sparrow is a premigratory increase in mass. Natural increases in photoperiod during spring trigger the onset of hyperphagia that results in deposition of fat stores and eventual weight gain (Weise, 1963; Stetson and Erickson, 1971; Lewis and Farner, 1973; Ramenofsky, 1990). This phenomena has been well documented (for reviews see Odum, 1960; Berthold, 2001) yet is not required for the complete expression of MR in *gambelii* (King and Farner, 1963; Landys et al., 2004a). Given previous work with this species (King and Farner, 1959, 1963; Smith et al., 1969; Lewis, 1975) as well as other migrant-resident comparisons (Wolfson, 1942; Koch and de Bont, 1952), we predicted that only *gambelii* and *pugetensis* would exhibit significant increases in body mass following photostimulation.

Given previous work on the expression of migratory activity and behavior in captive birds (Palmgren, 1949; Gwinner, 1968; Berthold and Querner, 1981), we hoped to identify differences in captive behavior and physiology in closely related populations representing a spectrum of migration strategies. While many of these traits have previously been documented as present and/or absent in migrants and residents, analyses of differential expression over a range of migration strategies will help further our ability to apply information from captive studies to interpret the movements of free-living birds. Similarly, given the geographical segregation of the Pacific races of white-crowned sparrow, the captive measures elucidate step-wise progression in evolution as populations depart from a migration strategy towards a resident life history.

Materials and Methods

Capture and Handling:

The work conducted with the three subspecies in this study was not performed simultaneously. Variations in methodology for each group (particularly different photocycle treatments reflecting latitudinal effects of each subspecies' range) are due in part to the fact that both *gambelii* and *nuttalli* were part of other primary research projects prior to this comparison (See Chapters II & III). All birds were transported from capture sites (outlined below) to the Department of Biology at the University of Washington, Seattle and housed in outdoor aviaries exposed to natural photoperiod and temperature conditions. All techniques involving capture, handling and experimentation were performed within the guidelines of the American Association for Accreditation of Laboratory Animal Care and approved by the Institutional Animal Care and Use Committee at the University of Washington.

Gambel's white-crowned sparrows (*gambelii*) were captured near Sunnyside, WA (46.1°N, 119.5°W) in September 2003. In January, 8 birds were transferred from the aviary to an environmental chamber and placed in individual registration cages (35cmW x 40cm L x 45cm H). Birds were acclimated to chamber conditions for approximately 1.5 weeks under a short day photocycle (8L:16D) after which the photoperiod was increased to 18L:6D for photostimulation. Locomotor activity data used in this study were collected following 20 days of photostimulation.

Puget Sound white-crowns (*pugetensis*) were captured at the Union Bay Natural Area in Seattle, WA (47.6°N, 122.3°W) in June of 2005 and housed in an outdoor aviary for ~8 months. In March, 11 birds were transferred to individual cages within an environmental chamber (8 in registration cages) and exposed to a photocycle of 12.5L:11.5D. Prior to this move, birds had not yet been photostimulated as the natural photoperiod had not yet reached 12L. The photocycle was increased 30 minutes each week, so birds were exposed to 14L:10D when locomotor activity data were collected for this study (20 days post photostimulation, as with *gambelii*).

Nuttall's white-crowned sparrows (*nuttalli*) were captured near Monterey, CA (36.6°N, 121.9°W) in August 2005. In November, 11 birds were transferred to an environmental chamber (8 in registration cages) with a photocycle of 10.5L:13.5D. The photoperiod was adjusted weekly to that of natural conditions of Monterey, CA (photoperiod schedule collected from U.S. Naval Observatory Astronomical Applications

Department, http://aa.usno.navy.mil). Following two months of weekly increases in day length, the photocycle was increased from 10:20L:13:40D to 15L:9D (maximum hours of light in Monterey). Locomotor activity data for this study was collected 20 days later.

Environmental Chamber Conditions:

All birds were provided food (Mazuri Chow, Nutritional International , Brentwood, CA and mixed seeds, UW Custom Mix, Seed Factory, NW, Kent, WA) *ad libitum* in hopper style dishes along with water for drinking and bathing and vitamin laced grit (Bird Health Grit, Seed Factory; Ceres, CA). The dim light condition during the dark phase of all LD cycles was achieved with 1 nightlight simulating moonlight (Limelight Nightlight, Austin Innovations, TX) centrally mounted within the environmental chamber (light intensity measured at each cage [<1m from source] was <1 Lux). Light intensity during the light phase was ~550 Lux and was achieved with four 48" fluorescent tube lights. Body mass measurements were obtained by placing birds within a cotton sock on an electronic scale (Ohaus Scout Pro, model SP202) accurate to the nearest 0.1g.

Locomotor Activity Collection:

For all three subspecies, locomotor activity data were collected following approximately 20 days of photostimulation (18L:6D for *gambelii*, 14L:10D for *pugetensis*, 15L:9D for *nuttalli*). Locomotor activity was measured in each registration cage using one infrared photodetector (Radio Shack Invisible Beam Alarm Entry, models #43-311 and 43-312) and a reflector, both mounted perpendicular to a centrally located perch. The photodetector/reflector create a continuous beam which, when broken by a moving bird, is registered as a 'beam break.' The output of this set-up is collected as average activity (beam breaks) per min per 30 min interval which will be referred to simply as *locomotor activity*. To reduce day-to-day variations in activity and limit bias, locomotor activity waveforms for each subspecies (created in Microsoft Excel) are composed of individual averages for each bird (n=8 for all subspecies) across four consecutive days.

Waveform Model:

A race specific model illustrating locomotor activity was derived from activity recorded for each subspecies. Using the approximate average locomotor activity during key periods of the 24h cycle (such as the night activity plateau), generalized patterns were created for each subspecies. This model allows for the visual comparison of activity during specific points in the 24h cycle (such as lights on or off) from activity data collected on subspecies specific lighting regimes of 18L:6D, 14L:10D and 15L:9D.

Locomotor Activity Comparison:

Peak night locomotor activity was compared across each of the three subspecies. For *gambelii* and *pugetensis*, average peak night activity was defined as the average activity per 30 min during the dark phase plateau seen in their respective waveforms (hours 22.5-3.5 for *gambelii*; hours 0.5-7.5 for *pugetensis*). For *nuttalli*, which lack a plateau of activity during the dark phase, peak activity was defined as any night activity (per 30 min) greater than two SD above baseline activity (lowest point during dark phase) and corresponds with hours 3.5-6.5. Due to large variation in intensity of activity, peak activity was calculated for each subspecies as the average all birds on each of the four consecutive nights (n=4).

To investigate the presence of a quiescent phase in each subspecies, locomotor activity was compared prior to and following the light to dark transition within each group. Three specific 60 min periods of activity were analyzed; period I: 60 minutes just prior to the dark phase (lights on); period II: 60 minutes following the initiation of the dark phase (lights off); period III: 60 min immediately following period II (lights off).

Behavior:

To identify the specific behaviors expressed during locomotor activity at night, several 4h videos were recorded for focal birds of each subspecies (n=4 *gambelii*, n=8 *pugetensis*, n=9 *nuttalli*) using a camcorder with infrared capabilities (Sony Handycam Vision, model CCD-TRV87). Each video was analyzed using an instantaneous scan method of Martin and Bateson (1993) further modified by Agatsuma and Ramenofsky (2006). The method, which uses an established subset of 8 behaviors, classifies activity at 20 second intervals for the duration of each film. The behaviors include: rest, jump, flight, feeding, beak-up (BU), beak-up flight (BUF), head-cock and other (typically preening). BU and BUF have been associated with migratory restlessness in white-crowned sparrows (*gambelii*) and are therefore considered migratory behaviors, while the others are not. The total percentage of BU and BUF (combined), rest, and all other behaviors (combined) were then calculated from the total observed counts.

In order to identify qualitative differences in BUF expression between subspecies, videos of the two individuals with the highest total percentage of observed BU and BUF from each subspecies were examined further. Using the instantaneous scan data, we identified the 10 min period of each film where BUF expression was greatest and then subsequently analyzed the film by tabulating the number of BUF bouts, the duration of each bout, and the average wing beat frequency. From these data we also calculated the average BUF bout duration and the total amount of time BUF was expressed during the 10 min interval.

Moonlight Manipulation:

As with our previous work on *nuttalli* (see Chapter III), we tested the effects of variations in dim light intensity on the locomotor activity of *pugetensis* during the dark phase. Birds were exposed to one night each of ½, 2 and 0 moonlight nightlights (moons). Given that birds had been acclimated to 1 moon throughout the project, this condition served as the control. To control for natural variations in activity over time as well as treatment effects, each treatment night was separated by 2 nights of 1 moon (control) and the order of treatment conditions (½, 2, etc.) was randomized. Of the 8 birds in registration cages, only 5 birds were used in this portion of the study as they were the only individuals exhibiting significant locomotor activity (>5 beam breaks per min per 30 min) during the dark phase.

Statistical Analysis:

Peak night activity data (n=4) were normally distributed and were analyzed using a one-way ANOVA followed by a Tukey post hoc test. Quiescent phase data within each species required non-parametric analysis so Friedman test was used followed by subsequent pair-wise comparison (Wilcoxon Signed Ranks test with Sequential Bonferroni correction). Moonlight activity data for *pugetensis* (normal distribution) were analyzed with a repeated measures ANOVA. Given the large variation in sample sizes for the total behavior analysis and the low sample sizes for quantitative BUF analysis (n=2 for each group), we did not feel confident statistically analyzing behavioral data. Pair-wise analysis of body mass measurements for *gambelii* and *nuttalli* were conducted with non-parametric Wilcoxon Signed Ranks tests. All statistical analyses were conducted using SPSS 14.0 and box-and-whisker plot figures were created using JMP 6.0.

Results

Locomotor Activity:

For each subspecies, locomotor activity did not change significantly during the four days used to create each waveform. Locomotor activity waveforms representing typical patterns of activity during a 24h cycle can be seen in Figures 4.1, 4.2 and 4.3 (*gambelii*, *pugetensis* and *nuttalli*, respectively). Generalizing the patterns of activity seen in each subspecies 24h waveform, a simplified model waveform was derived for the direct comparison of activity at specific periods within the 24h cycle (Figure 4.4). This model elucidates the following distinctions: All subspecies exhibit low levels of activity during the afternoon hours of light phase. Prior to the dark phase (lights off) *gambelii* decrease activity with the expression of a quiescent phase, while *pugetensis* and *nuttalli* both increase activity. Upon initiation of the dark phase, *gambelii* exhibit an immediate increase in activity to maximum levels for a 24h cycle. For p*ugetensis* and *nuttalli* however, activity decreases at the onset of night with *pugetensis* exhibiting an apparent quiescent phase prior to a rise in activity (24h maximum) and *nuttalli* slowly increasing activity during the night which peaks with dawn. With the initiation of the light phase (lights on), both *gambelii* and *pugetensis* show an immediate decrease in activity to low/intermediate daytime intensities. In contrast, *nuttalli* continue to increase activity during the first hours of the light phase (reaching their 24h maximum) before gradually decreasing levels to an intensity consistent across subspecies.

Peak night activity was significantly higher in *gambelii*, followed by *pugetensis* and subsequently *nuttalli* ($F_{4,2}=79.36$, $p<0.001$, Figure 4.5). Quiescent phase analysis of locomotor activity surrounding the light-to-dark transition, indicates that in *gambelii*, activity during period I (last 60 minutes of light phase) is significantly less than either of the first two periods of the dark phase (I/II and I/III: $Z=-2.52$, $p=0.01$; II/III: $Z=-0.280$, $p=0.78$, Figure 4.6A). For *pugetensis*, activity following the initiation of the dark phase (period II) is significantly less than activity during periods I or III (I/II: Z=-2.51, p=0.01; II/III: Z=-2.380, p=0.02; I/III: Z=-0.56, p=0.58, Figure 4.6B). Locomotor activity for *nuttalli* did not change during any of the three periods ($X^2_{8,2}$=3.25, p=0.20, Figure 4.6C).

Behavior:

Variation in expression of behavior across individuals was high in all subspecies (Table 4.1). Of the 8 categorized behaviors, only 5 were expressed during any dark phase video (BU, BUF, rest, flight and jump). The total percentage of migratory behaviors (BU and BUF) observed from all observations varied within and across subspecies (*gambelii* range: 88-37%, *pugetensis* range: 37.8-0.2%, *nuttalli* range: 39.1-0.0%, Table 4.1) although *gambelii* was the only group in which all individuals expressed high levels of BU/BUF.

Quantitative analysis of BUF suggests a differential expression of this migratory behavior between migrant (*gambelii* and *pugetensis*) and resident (*nuttalli*) birds. Most notably, the average duration of individual BUF bouts as well as the total duration of BUF expressed during each 10 min video segment appears greater in migrants (Table 4.2). While *gambelii* and *pugetensis* have combined ranges of 44.9-3.2 sec (average bout duration) and 583-282 sec (total duration), *nuttalli* appear to be less (1.0-0.8 sec average duration, 106-69 sec total duration).

Moonlight:

Analysis of control nights (1 moon) separating treatment nights indicated that *pugetensis* night activity was not affected by time ($F_{8,7}$=0.83, p=0.57). Comparison of activity across treatment nights indicated that night activity was not affected by different intensities of light (½, 1 or 2 moons), however a significant decrease was seen with no light present (0 moon, $F_{8,3}$=6.12, p=0.01 Figure 4.7A). The same pattern was found for *nuttalli* in our previous work (see chapter III, summarized in Figure 4.7B).

Body Mass:

Pair-wise comparison of body mass values prior to and following photostimulation (Day 0, Figure 4.8) revealed a significant increase for *gambelii* (Z=-3.35, p=0.001) but no significant change for *nuttalli* (Z=-1.28, p=0.20). With no data prior to photostimulation for *pugetensis*, a similar comparison could not be made; however, mass values following photostimulation for *pugetensis* are included in Figure 4.8 and suggest a general decrease in body mass following placement in the environmental chamber.

Discussion

Given the geographical segregation of ancestral white-crowned sparrow populations, the Pacific races present today represent the step-wise evolutionary progression from migrant to resident life histories. Using a distinct set of traits documented in captive migrants, we set out to determine if populations of birds representing a spectrum of migratory strategies would express these traits proportionately.

Comparing long-distance migrant (*gambelii*), short-distance migrant (*pugetensis*) and resident (*nuttalli*) races of Pacific white-crowned sparrows, we predicted that variations in these migration strategies would be reflected in the expression of locomotor activity during a 24h cycle. The most striking difference between these subspecies, as seen in our general comparison model of locomotor activity (Figure 4.4), is that unlike the migratory subspecies, *nuttalli* do not express a dramatic increase in night activity. Historically, the expression of intense migratory restlessness (MR) has been synonymous with migration in captive birds (Wagner, 1930). While *nuttalli* did express night activity consistent with previous work (Smith et al., 1969), the deviation from migrant patterns and the predominance of activity towards dawn, suggest that activity may be the expression of local movements within a territory, rather than intense migratory flight to a distant site.

Comparison of 24h activity waveforms (Figures 4.1-4.3) and the significant difference in peak activity confirm that *gambelii* express night activity of greater intensity than *pugetensis*, followed by *nuttalli*. Decreases in locomotor activity upon initiation of the light phase in both *gambelii* and *pugetensis*, suggest that night activity contains unique behaviors confined to the dark phase that are fundamentally different than light phase activity. We can also speculate that night phase activity in the migrants was energetically demanding and necessitated high amounts of rest (low activity) during the early light phase. The continued increase in activity following lights on in *nuttalli*, leads to the conclusion that behaviors expressed were not confined to either lighting condition and that the activity during the dark phase was not sufficiently taxing to require recuperation at dawn.

While previous work has suggested that free-living white-crowned sparrows (including the resident *nuttalli*) increase night activity in response to increases in moonlight intensity (Smith et al., 1969), we were unable to duplicate these results with *pugetensis*. Our prediction that activity during the dark phase would follow a dose-response to increasing intensity of dim light was not confirmed as there was no difference in activity expressed under any conditions with some dim light. We attribute these results to the fact that regardless of increases in the number of moonlights within the environmental chamber, light intensity was <1 Lux for all treatments. In complete darkness however, night activity was significantly decreased. While not consistent with results from outdoor birds, these results are consistent with findings in *nuttalli* and *gambelii* following similar methods (see chapter III; Ramenofsky et al., in prep) suggesting that white-crowned sparrows respond to gradients of dim light from 0 to <1Lux.

As predicted, both migratory races expressed a quiescent phase (QP) just prior to the onset of intense MR at night. Consistent with previous findings (Agatsuma and

Ramenofsky, 2006) the QP for *gambelii* occurred prior to the initiation of the dark phase (lights on), correlating with Morton's (1967) observations that birds in the field roost during twilight hours prior to migratory flight at night. The QP for *pugetensis* however, occurred during the first 60 min of the dark phase when lights were off. As the onset of the dark phase has been shown to be an important cue regulating migratory behaviors (Coverdill et al., 2008), it is possible that *pugetensis* may use the exogenous cue of dim light to trigger the QP prior to departure. As QP is a migration-specific trait, the lack of a QP in *nuttalli* is not surprising and is consistent with our predictions.

Some migration specific behaviors (BU and BUF) were identified in *nuttalli* as well as both migrant subspecies. For *gambelii*, high percentages of migratory behaviors were expressed in all individuals filmed. This finding, in combination with the increased average BUF bout duration and total duration of BUF expressed, suggests that these behaviors are an integral part of night activity coinciding with intense locomotor movement. For *pugetensis*, the expression of BU and BUF was similar to *gambelii* with the exception that they were not expressed in all individuals. The fact that not all *pugetensis* expressed these migration specific behaviors during filmed periods might indicate that night movements may not be synchronous across individuals. At first, the presence of increased BU and BUF in a two *nuttalli* might imply the expression of migratory tendencies. However, with most individuals not exhibiting these traits, the decreased average BUF bout duration, and the decreased total duration of BUF, we suggest that they do not represent the primary behaviors expressed during night activity and are not consistent with expression patterns of migrants.

Following photostimulation, *gambelii* exhibited a significant increase in body mass consistent with the development of migratory disposition (Weise, 1956; King and Farner, 1959, 1963; Mattocks, 1976; Schwabl and Farner, 1989). Not requiring physiological changes in preparation for migratory flight, *nuttalli* did not exhibit changes in body mass following an increase in day length. Without body mass data prior to photostimulation for pugetensis, we can not make similar comparisons. Monitoring changes in body weight for all three subspecies in captivity, Lewis (1975) noted a significant increase for all subspecies, but only a 'pronounced increase' for *gambelii*. This finding was attributed to the idea that only the long-distance migration strategy of this race would require significant energy reserves.

Taken collectively, our findings can be related directly to the natural histories and migration strategies of these races of white-crowned sparrow. For *gambelii*, who not only face a greater migration route, the breeding season in the Arctic is short, in turn requiring individuals of a population to be highly synchronous in the timing of vernal migration and the initiation of breeding. Responding to these conditions, captive *gambelii* have an increased expression of migratory flight as is seen with the high intensity of nocturnal activity and the unanimous expression of migration specific behaviors. Similarly, the amount of migratory flight accomplished at night appears to be maximized by *gambelii* by preparing metabolically during the last few hours of daylight (QP expressed with lights on). Finally, as the expression of continued MR throughout the night is energetically demanding, *gambelii* prepare for vernal migration through the deposition of fat deposits resulting in increases in body mass following photostimulation in spring.

For the short-distance migrant *pugetensis*, we suggest that the decreased physical distance traversed and the decreased temporal constraints of breeding in temperate regions with relatively long summers, place less demands on the timing of migration. The decreased intensity of nocturnal activity compared with *gambelii* and the high variation in BU and BUF expression suggest that this life history stage may be less synchronous in this subspecies. Interestingly, as these birds are not faced with short nights during which to fly, their QP is expressed within the dark phase. Expression of this aphagic period following the onset of night could possibly allow for continued foraging during twilight hours. Similarly, we propose that the combination of extended feeding prior to the dark phase in concert with nocturnal activity of moderate intensity at night does not require significant premigratory depositions of fat in spring. It is possible that *pugetensis* are able to gather sufficient daily fuel resources for MR expression each night.

The captive behaviors expressed by *nuttalli* are noticeably different than their migratory congeners and are consistent with their more sedentary natural history. While *nuttalli* do express some night activity, the intensity of this activity and the decreased expression of BUF within it, are inconsistent with measures in migrants. The complete absence of a QP and a lack of an increase in body mass following photostimulation suggest the absence of attendant processes for migration. The presence of night activity in concert with at least some low intensity migration specific behaviors however, is notable. Previous investigators have deemed this expression an atavistic trait from a migratory ancestor (Smith et al., 1969), which may suggest a lack of negative selection on this trait allowing for its persistence in at least some individuals. Others have classified migratory behavior in resident species as an underlying program which would allow movement from an area when conditions within a habitat are no longer favorable (Helm and Gwinner, 2006). We suggest that regardless of the mechanisms underlying the presence of migratory behaviors in this resident race, birds may now apply these traits for novel purposes. It is possible that these once migratory behaviors are used for the maintenance of breeding territories and other social interactions in this resident race.

The evolution of the various migration strategies of the Pacific races of white-crowned sparrow can also be superimposed upon the Pleistocene speciation proposed by Rand (1948), Banks (1964) and Selander (1965). It is generally believed that the Pacific races originated from a long-distance migrant ancestor occupying most of western North America. With the advance of significant glaciation into the interior of North America, *gambelii* breeding sites were isolated in the northwest corner of their historic range. As glaciers eventually receded, *gambelii* expanded their breeding range encompassing areas used today. Having maintained breeding grounds in the Arctic, *gambelii* continued long-distance migration during glacial periods, a strategy consistent with current populations.

For *pugetensis*, glaciation pushed breeding territories into coastal regions of British Columbia and the Northwest US, within the southern range of the ancestral population. Following glacier recession, *pugetensis* expanded their range south although to a much less extent than *gambelii*. Given the close proximity of their wintering and breeding habitats, the subspecies adapted a short-distance migration strategy. The southern isolation of *nuttalli* most likely forced this population to utilize ancestral wintering habitat as breeding territories during glacial events. Following the loss of glacial cover, *nuttalli* most likely did not expand their range as they had established breeding grounds within previous winter areas. Through these geographically isolating events, conditions of allopatry may have been initiated, promoted by rapid selection for intermediate states of migratory strategy and in the case of *nuttalli*, its disappearance. Such cases for rapid selection have previously been demonstrated in blackcap warblers (*Sylvia atricapilla*) (Berthold and Pulido, 1994; Berthold and Querner, 1995).

Through this series of experiments, we have shown that several traits expressed by birds held in captivity are not only a reliable measure of migratory activity in wild populations, but can also be used to distinguish subtle differences between closely related populations with separate migration strategies. While advances in technology are greatly improving our knowledge concerning the movements of free-living birds, the development of tools and ideas used to distinguish and quantify captive behaviors for nearly 200 years have greatly influenced our understanding of this complex life history stage.

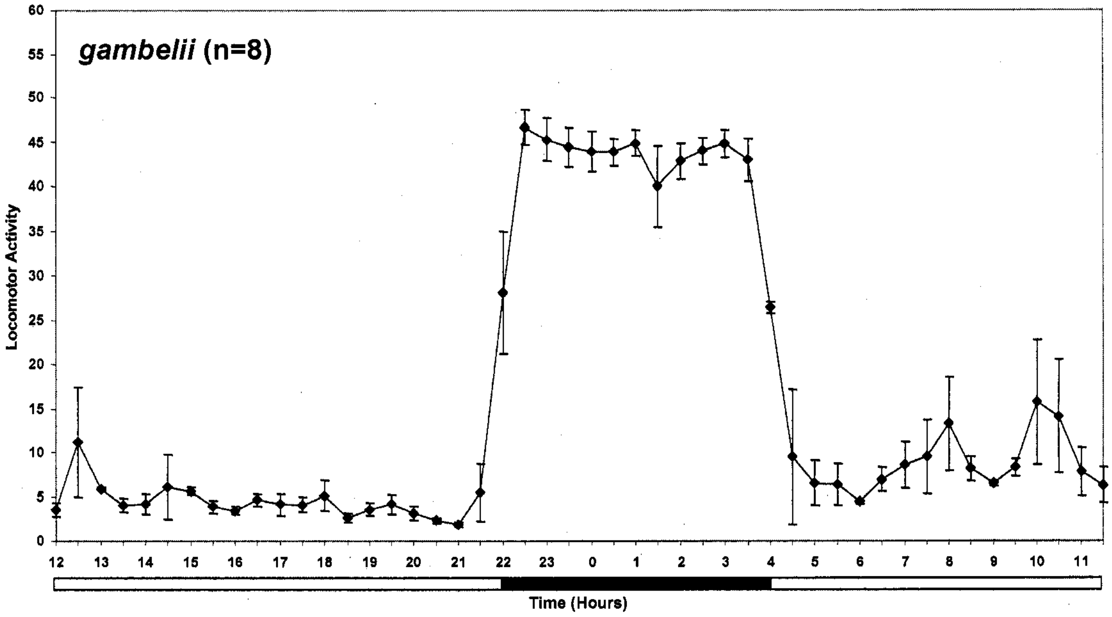

Figure 4.1: Locomotor Activity Waveform (*gambelii*)

24h locomotor activity waveform (average activity per min/30 min interval) for captive *gambelii* on 18L:6D photocycle. Closed black bar below figure indicates lights off, open white bars indicate lights on, symbols represent mean ± SE from 4 consecutive days, n=8.

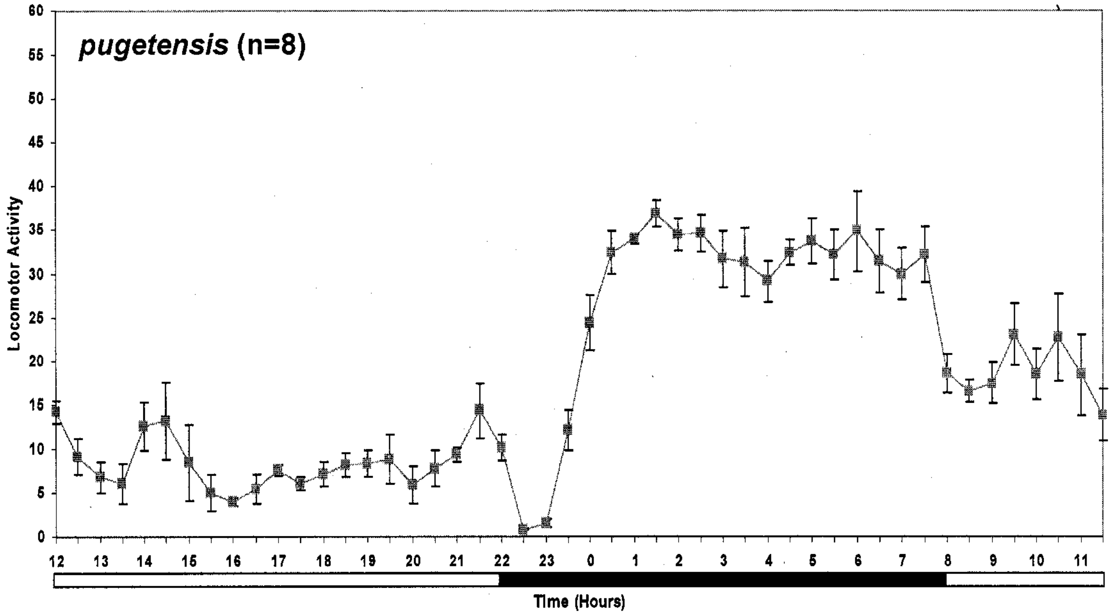

Figure 4.2: Locomotor Activity Waveform (*pugetensis*)

24h locomotor activity waveform (average activity per min/30 min interval) for captive *pugetensis* on 14L:10D photocycle. Closed black bar below figure indicates lights off, open white bars indicate lights on, symbols represent mean ± SE from 4 consecutive days, n=8.

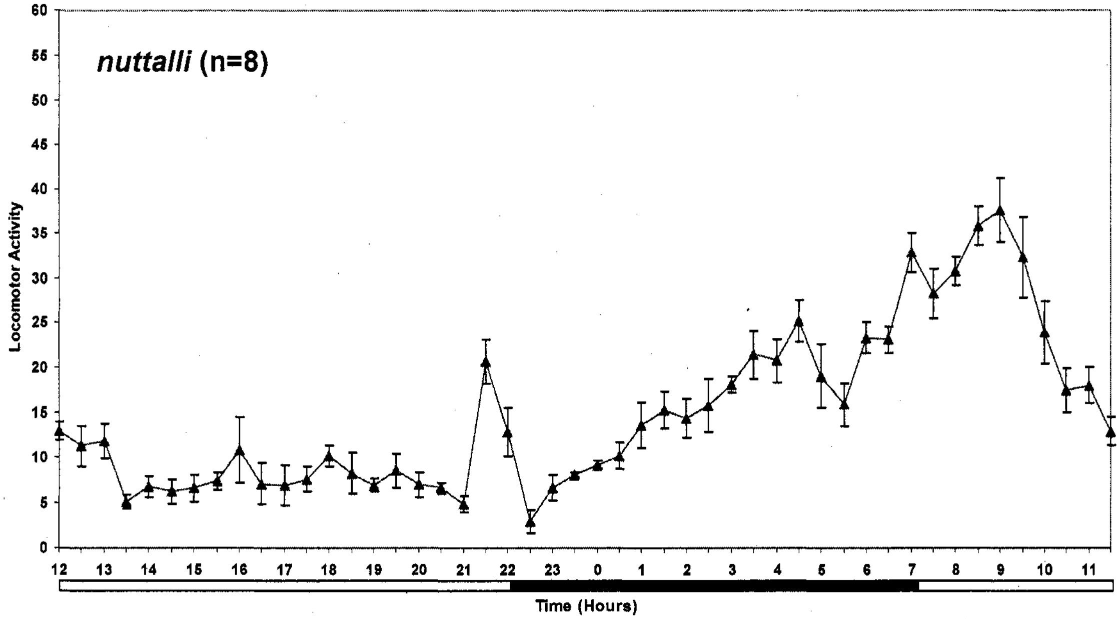

Figure 4.3: Locomotor Activity Waveform (*nuttalli*)

24h locomotor activity waveform (average activity per min/30 min interval) for captive *nuttalli* on 15L:9D photocycle. Closed black bar below figure indicates lights off, open white bars indicate lights on, symbols represent mean ± SE from 4 consecutive days, n=8.

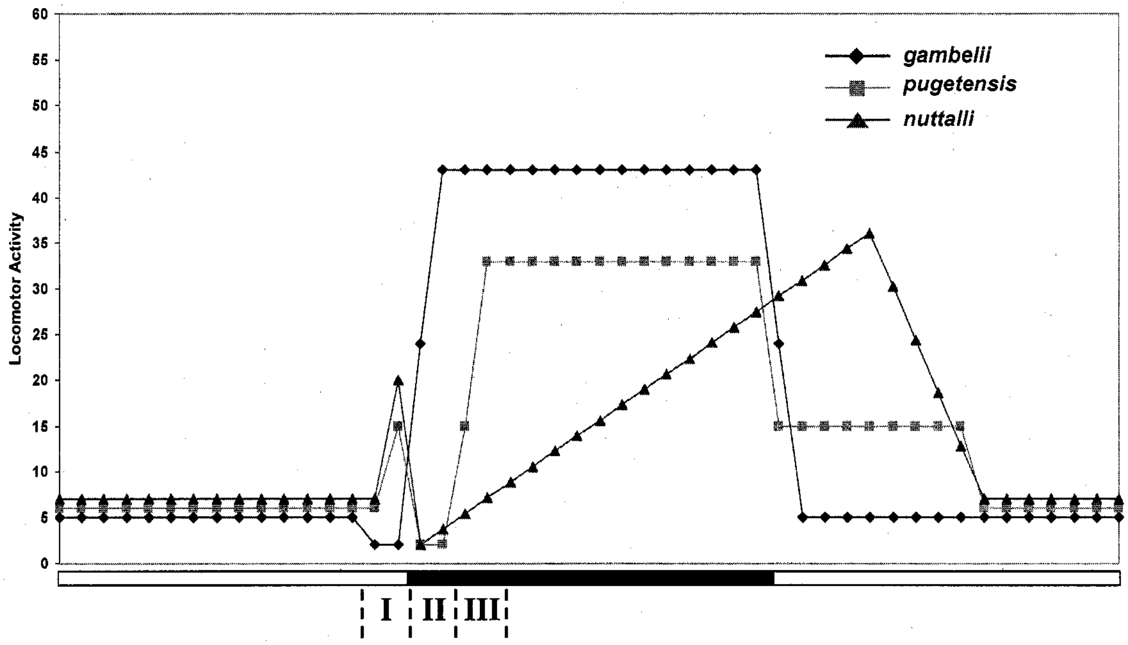

Figure 4.4: Derived Waveform Model

General waveform model derived from patterns of locomotor activity for each subspecies during a 24h cycle (**Figures 4.1-4.3**). Closed black bar below figure indicates lights off, open white bars indicate lights on. Relative position of quiescent phase analysis periods **I**, **II** and **III** are noted below figure.

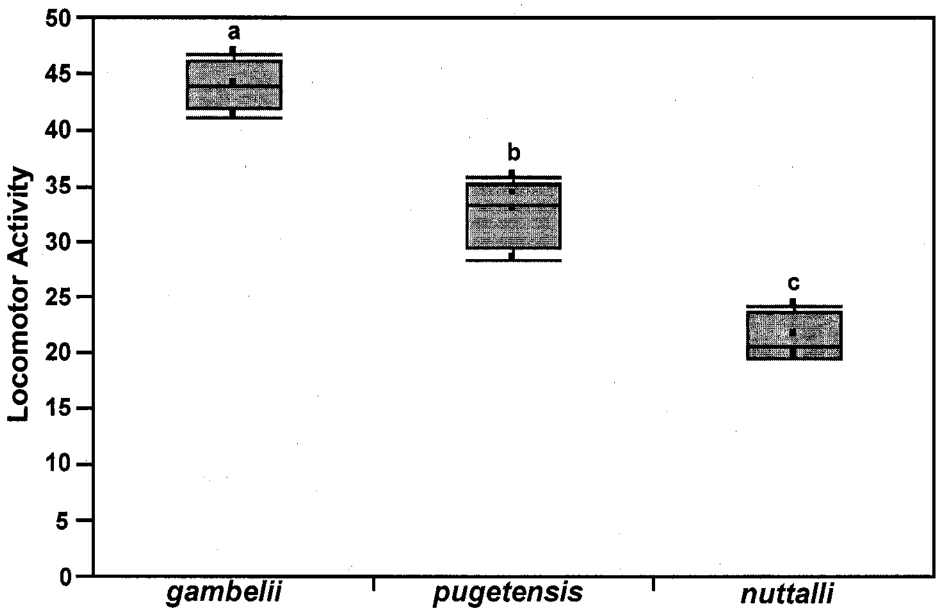

Figure 4.5: Peak Night Activity

Box-and-whisker plot of peak night locomotor activity for 4 consecutive days (n=4) for *gambelii*, *pugetensis* and *nuttalli*. Horizontal lines within boxes indicate median, outer limits of boxes denote upper and lower quartiles, and whiskers represent maximum and minimum values OR 1.5*interquartile range, whichever value is lower. White shading in boxes indicates data from periods with lights on, gray shading in boxes indicates data from periods with lights off (box descriptions apply to all subsequent box-and-whisker plots). Letters **a**, **b** and **c** signify differences between groups of $p<0.05$.

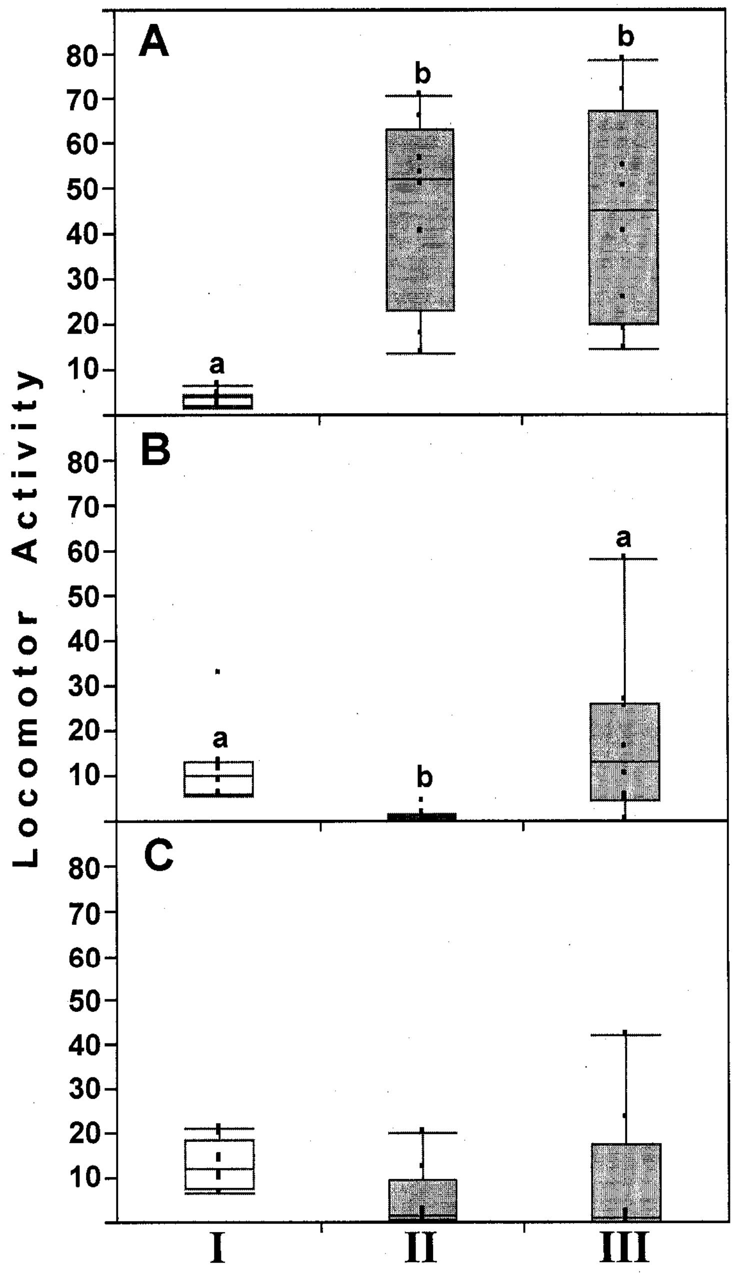

Figure 4.6: Quiescent Phase Analysis

Box-and-whisker plots of average locomotor activity prior to and following the light-to-dark phase transition for *gambelii* (**A**), *pugetensis* (**B**) and *nuttalli* (**C**). Period **I** includes activity during the last 60 min of the light phase; Period **II** includes activity during the first 60 min of the dark phase; Period **III** includes activity during the second 60 min of the dark phase. Letters **a** and **b** signify differences between periods of $p<0.05$: *gambelii* period **I/II** and **I/III**: $Z=-2.51$, $p=0.01$; p*ugetensis* periods **I/II**: $Z=-2.51$, $p=0.01$, **II/III**: $Z=-2.38$, $p=0.02$.

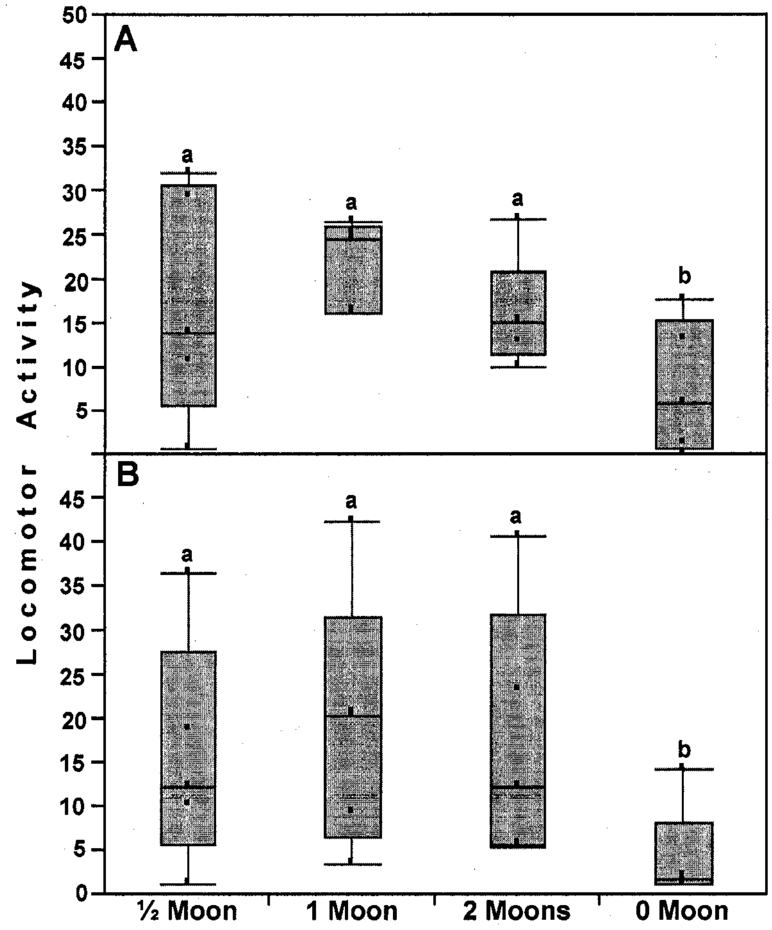

Figure 4.7: Moonlight Manipulation (*pugetensis*)

Box-and-whisker plot comparison of average locomotor activity during nights with ½, 1, 2 and 0 moonlights (Moons) in the environmental chamber for *pugetensis* (**A**, n=8) and *nuttalli* (**B**, n=8). Letters **a** and **b** signify differences between groups of $p<0.05$: *pugetensis*: $F_{8,3}=6.12$, $p=0.01$; *nuttalli*: $Z = -2.02$, $p = 0.04$.

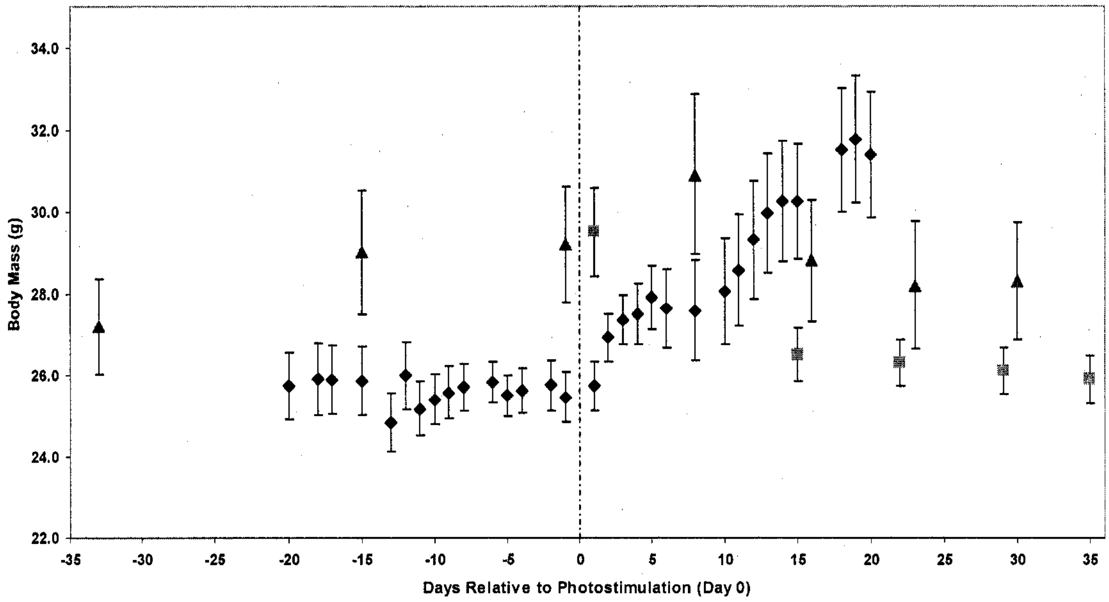

Figure 4.8: Body Mass Comparison

Average body mass of captive *gambelii* (♦, n=8), *pugetensis* (■, n=11) and *nuttalli* (▲, n=11). Symbols represent group means ± SE. Day 0 represents photostimulation as birds were switched to long day photocycle conditions. Following photostimulation, body mass significantly increased in *gambelii* (Z=-3.35, p=0.001), but not *nuttalli* (Z=-1.28, p=0.20). Data were not collected prior to day 0 for *pugetensis*.

Table 4.1: Subspecies Behavior Expression
Percentage of migratory (BU & BUF), Rest and non-migratory (Flight & Jump) behaviors expressed by individuals during dark phase filming. Each horizontal line represents data from one individual. Two individuals from each subspecies with the highest expression of migratory behaviors (**bolded**) were used in quantitative BUF analysis (**Table 4.2**)

	% BU & BUF	*% Rest*	*% Flight & Jump*
gambelii **(n=4)**	**88.0**	**5.0**	**7.0**
	80.0	**20.0**	**0.0**
	37.0	62.0	1.0
	37.0	47.6	15.4
pugetensis **(n=8)**	**37.8**	**61.7**	**0.5**
	16.0	**74.0**	**10.0**
	14.9	81.0	4.1
	11.5	75.0	13.5
	9.4	58.7	31.9
	3.7	89.0	7.3
	0.8	98.6	0.6
	0.2	91.5	8.3
nuttalli **(n=9)**	**39.1**	**45.0**	**15.9**
	34.3	**44.0**	**21.7**
	4.3	52.6	43.1
	1.7	97.0	1.3
	1.7	95.4	2.9
	0.1	87.9	12.0
	0.0	93.4	6.6
	0.0	90.0	10.0
	0.0	31.0	69.0

Table 4.2: BUF Quantitative Analysis
Quantitative measures of BUF collected from 10 min video (600 sec) segments of the two individuals of each subspecies with the highest expression of migratory behaviors. Generally, the two migratory species (*gambelii* and *pugetensis*) appear to express BUF in longer bouts (ave bout duration) and for a greater majority of the 10 min period (total duration).

	# BUF Bouts	***Ave Bout Duration (sec)***	***Total Duration (sec)***	***Wing Beat Freq. (Hz)***
***gambelii* (n=2)**	13	44.9	583	10.4
	103	3.5	358	4.0
***pugetensis* (n=2)**	16	36.2	579	9.3
	87	3.2	282	6.1
***nuttalli* (n=2)**	70	1.0	69	3.4
	135	0.8	106	3.2

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VITA

Alexander (Alex) J. Coverdill was born in Detroit, Michigan on June 23, 1980. During grade school he moved with his family to North Carolina, and again to Boring, Oregon. He attended Sandy High School in Oregon where he graduated Valedictorian of his class while developing a love for both biology and drama. In 1998 he began his undergraduate work at the University of Portland in Portland, Oregon where he divided his attention between classes and the stage. He was given the extraordinary opportunity to travel to Kent Island, New Brunswick where he conducted his senior thesis research project on breeding Leach's storm-petrels. In the theater he worked as a scenic carpenter while earning acting roles in several productions including *You Can't Take It With You* and Shakespeare's *Henry V*. In 2002 he completed his bachelor's degree in biology, including a minor in theater.

Following a move to Seattle, Washington, Alex began work on his doctorate, focused on the migration physiology of songbirds. Apart from his captive work on white-crowned sparrows and research trips to Alaska and Greenland, he traveled to Iceland to investigate migratory and resident populations of snow buntings. Having found a passion for teaching, in 2007 he was awarded the University of Washington's Excellence in Teaching Award. In June of 2008, he earned a Doctor of Philosophy degree in Zoology and the following August was married to his best friend Jessica Vick.