Sleep-swimming in Canada Geese *(Branta canadensis)*

A thesis submitted to

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Certificate

This is to certify that this dissertation entitled “Sleep-swimming in Canada Geese *(Branta canadensis)”* towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Pradeep Kumar Trimbake at Max Planck Institute for Biological Intelligence Seewiesen, Germany under the supervision of Dr. Niels C. Rattenborg, Group leader of Avian Sleep laboratory and co-supervision of Dr. Andrea Ferretti, Postdoc of Avian Sleep laboratory, during the academic year 2022-2023.

Dr. Niels C. Rattenborg

Dr. Andrea Ferretti

This thesis is dedicated to my sister, parents and my supervisors.

Declaration

I hereby declare that the matter embodied in the report entitled Sleep-swimming in Canada Geese *(Branta canadensis)* are the results of the work carried out by me at the Avian sleep group, Max Planck Institute for Biological Intelligence Seewiesen (MPIBI), Germany, under the supervision of Dr. Niels C. Rattenborg and co-supervision of Dr. Andrea Ferretti, Postdoc of Avian Sleep laboratory and the same has not been submitted elsewhere for any other degree.

Pradeep Kumar Trimbake

Date: 01/04/2023

Table of Contents

Abstract

Sleep is an imperative behavioral state that is observed in all organisms studied in the literature until now, but its functions are yet to be completely understood. Sleep is known to be homeostatically regulated and sleep deprivation over long periods can lead to severe cognitive and physiological impairments in many species. However, in specific ecological contexts, investing in sleep may be disadvantageous for an individual's fitness. Many bird species prioritize a prolonged waking phase during migration, reproduction, challenging foraging situations or under social restrictions imposed on them. Individuals engaging in such trade-offs gain fitness from such prolonged wakefulness when subjected to these restrictive conditions. Although several possibilities have been suggested, little is known about how birds obtain sleep in such situations. Great frigatebirds (*Fregata minor*) can sleep while in flight. However, these findings only pertained to passive modes of locomotion and it is unknown whether these birds are also capable of sleeping while in active motion which involve flapping of wings. Interestingly, several waterfowl species have been observed closing their eyelids while swimming, a phenomenon we term as "sleep-swimming." In this thesis project, we demonstrate the presence of “sleep-swimming” in Canada geese (*Branta canadensis*) by using a combination of accelerometers and video coupled with computer vision and characterise this novel phenomenon. We provide behavioral evidence that sleep could be compatible with locomotion. Our results show geese sleep while swimming in a manner similar to dolphins, but unlike dolphins they show bilateral sleep during motion. We also show that sleep-swimming declines in juvenile geese as they age, a characteristic of normal sleep well established in mammalian systems.

List of Tables

List of Figures

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Contributions

Contributor name	Contributor role
Niels, Andrea	Conceptualization Ideas
Niels, Andrea, Pradeep	Methodology
Andrea, Pradeep	Software
Dolores, Martina, Andrea, Pradeep	Validation
Andrea, Pradeep	Formal analysis
Andrea, Pradeep, Dolores, Martina, Niels	Investigation
Niels, Andrea	Resources
Andrea, Pradeep	Data Curation
Pradeep	Writing - original draft preparation
Andrea, Niels, Pradeep	Writing - review and editing
Andrea, Pradeep	Visualization
Niels, Andrea	Supervision
Niels, Andrea	Project administration
Niels, Andrea	Funding acquisition

Chapter 1 Introduction

1. Sleep: what is it?

From land dwelling humans to sea dwelling dolphins, from flying birds to land waddling penguins, from tiny spiders to waggle dancing bees, from clever octopuses to brainless jellyfishes, ranging across these mesmerizing myriads of organisms is a very ubiquitous phenomenon called sleep. But what is sleep in the first place? Sleep has been defined as a behavioral state during which an animal assumes a specific posture and ceases to move, almost similar to a state characterized by dormancy (Lima et al., 2005; Tobler et al., 2011). In addition, sleep is typically associated with eye closure, diminished responsiveness to environmental cues, and a complete disengagement from routine activities the animal performs when awake including anti-predator vigilance, courtship, and foraging (Lesku et al. 2012). Some animals show other kinds of behaviors that outwardly resemble sleep, such as hibernation, torpor or quiet wakefulness. However, these can be distinguished from sleep based on the sensitivity to stimuli. If we consider hibernation as the least sensitive state and quiet wakefulness as the most sensitive, then sleep lies somewhere in between these two extremes. Awakening from sleep is also quite different when compared to awakening from hibernation. When an animal is awakened from sleep, they can react quickly to stimuli coming from their immediate surroundings, whereas animals awakened from hibernation require more time before they are able to fully respond to their surroundings (Carr and Lima et al., 2013).

2. Mammalian sleep: Types and regulation mechanisms

Mammals (Rattenborg et al, 2011) are known to exhibit two primary types of sleep: non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, distinguished from one another in terms of brain activity and muscle tone. Birds (Rattenborg et al, 2011), mollusks like octopuses and cuttlefishes (Frank et al., 2012), and some reptiles (Libourel et al., 2016; Shein-Idelson et al. 2016) also show two types of sleep but birds are the only ones wherein the presence of two states (REM and NREM) is firmly established. The states of sleep observed in reptiles and cephalopods

are still an active area of research. Electroencephalograms (EEG) are commonly used to record brain activity during wakefulness and sleep. However, it should be noted that EEG only records activity from the surface of the brain. Wakefulness is characterized by low-amplitude, high frequency EEG activity (Fig 1). The characteristics of NREM and REM are as follows:

A. Non-rapid eye movement (NREM) sleep

As the name suggests, during NREM sleep, the eyes are mostly still. The EEG is characterized by high amplitude, low frequency waves (Fig 1). During NREM there is a drop-in metabolic rate, a relaxed muscle tone, lowering of brain temperature etc. Due to this characteristic EEG pattern, NREM sleep is also referred to as slow-wave sleep (SWS) (Carskadon and Dement, 2005). Research in human sleep has led to identification of sub-states of SWS sleep (Loomis et al., 1937; Dement and Kleitman, 1957a). NREM sleep is usually not divided into sub-states in other animals.

B. Rapid Eye Movement (REM) sleep

REM sleep is peculiar in the sense that the EEG pattern is very much like an awake brain state (Fig 1) (Fraigne et al., 2015). However, in contrast to an awake state, the eyes move rapidly under closed eyelids and the arousal threshold is elevated. In addition, brain temperature increases, postural muscle tone is greatly reduced, and there are occasional twitches of the limbs and face (Blumberg et al., 2020).

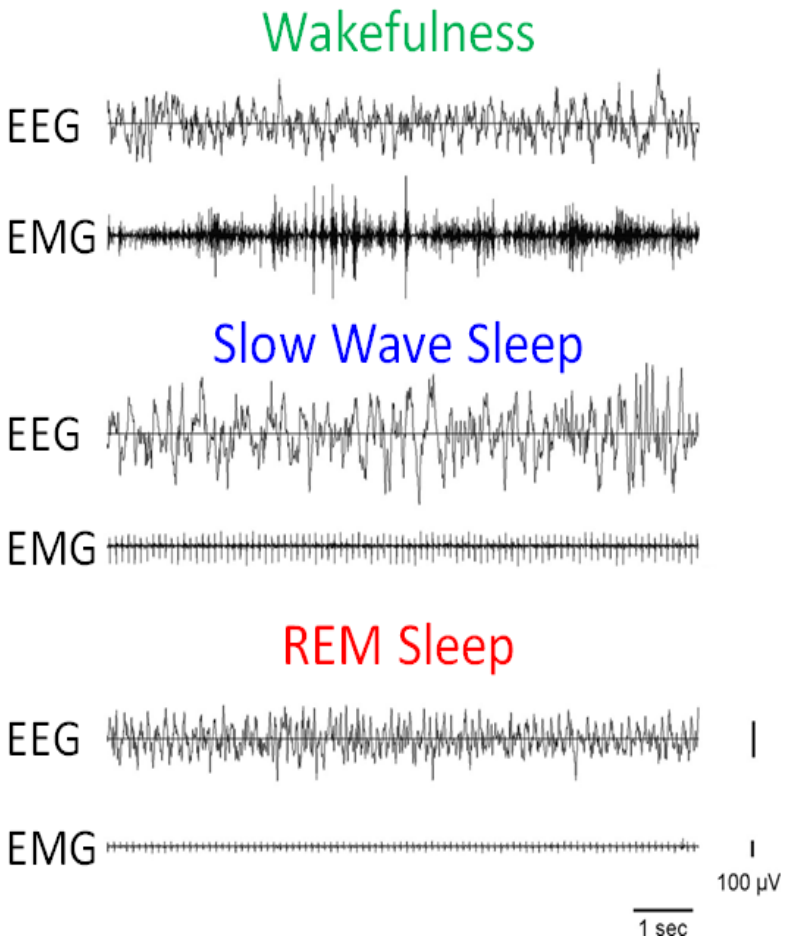


Figure 1: Recordings of EEG and EMG during wakefulness, slow wave sleep, and REM sleep.

So, how are the two types of sleep regulated? A two-process model proposed decades ago has been applied to variety of animals from different species and it still serves as the most successful conceptual framework to predict timing and intensity of sleep (Borbély, 1982; Daan *et al*., 1984). The model states that sleep is regulated by an interaction between two processes namely, "Process S" *and* "Process C". The sleep-awake homeostatic process (Process S) is gated or controlled by the circadian rhythm (Process C). Process S represents an animal's current need for sleep. It increases with time awake and decreases with time spent asleep. Process S is reflected in the amplitude and the number of slow waves, quantified as slow wave activity (SWA, 0.5 – 4 Hz spectral power) (Tobler et al., 2011; Deboer et al., 2015). SWA is highest in the initial sleep period, when the need for sleep is the highest, and lowest at the end of

sleep period, when the need for sleep is lowest. The amount of SWA is a marker of sleep intensity, as it is positively correlated with the arousal thresholds (Tobler et al., 2011; Deboer et al., 2015; Lesku et al., 2011). Given the correlation with the arousal thresholds, and the fact that SWA increases after sleep deprivation, SWA is thought to reflect the homeostatically regulated process (Process S) occurring during SWS (Tobler et al., 2011; Deboer er al., 2015; Lesku et al., 2011). In addition to making an animal sleep deeper following sleep loss, it can also make them sleep longer.

On the other hand, Process C reflects the internal biological clock that organizes the species-specific timing of activity to match that of the photoperiod. Process C interacts with Process S to determine when to let the organism fall asleep and release the sleep pressure (Borbély et al, 2016) . Process C is controlled by a specific group of neurons in the hypothalamus, called the suprachiasmatic nucleus (SCN). The SCN has an endogenous circa-24 hour rhythm that is synchronized to the day/night by environmental light received through eyes. Melatonin is a marker of process C as it is released at night from the pineal organ under the control of SCN.

Intriguingly, the two types of sleep alternate in cycles. In mammals, a cycle starts with a bout of SWS which is then followed by a bout of REM sleep. This cycle repeats throughout the main sleep period. Birds also exhibit cycles between periods mostly composed of SWS, with little REM sleep and periods with frequent bouts of REM sleep (Canavan and Margoliash, 2020). As in mammals, bouts of REM sleep become more frequent and longer later in the main sleep period (Martinez-Gonzalez et al. 2008; Low et al. 2008).

3. Function of sleep

Despite the overwhelming prevalence of sleep across different taxa of organisms, there is still much more to discover about its functions and the mechanisms governing this behavior. Although the functions of sleep are not yet fully understood, there are many theories proposed to explain its purpose. Some of the prominent ones are energy management (Schmidt et al., 2014), maintenance of immune functions (Opp

2009), and several brain-related functions. The adaptive inactivity or immobilization hypothesis (Meddis, 1975; Meddis, 1977; Siegel, 2009) proposes a largely ecological function for sleep. It suggests that sleep prevents animals from being active at times of the day when it is dangerous and unproductive for them to be awake. This includes times when their sensory systems are not adapted to effectively detect and evade predators or when food is hard to find. Although resting quietly awake could serve the same function, the hypothesis suggests that the reduction in environmental awareness keeps the animals from inadvertently responding to the environment when it would be dangerous for them to do so.

The adaptive inactivity/immobilization hypothesis has several limitations (Rechtschaffen, 1998), some of which are outlined here. It does not consider the restorative aspects of sleep. It fails to explain why there is an increase in sleep time and intensity after sleep deprivation. It does not explain why there are two types of sleep. Certain bird species and marine mammals are able to sleep with only one half of the brain at a time, an adaptation termed unihemispheric slow-wave sleep (USWS) (Rattenborg et al., 2000, 2016; Lyamin et al., 2008b, 2018). It does not explain the voluntary trade-off between being vigilant and being asleep exhibited by some animals (e.g., USWS in ducks, fur seals etc.). It predicts that there should be a seasonal variation in sleep. Although this is true for some species which sleep less during the shorter summer nights (Van Hasselt et al., 2020), birds living in the artic cycle exposed to constant daylight still spend some time sleeping (Lesku et al. 2012).

Most current theories suggest sleep has a role of central nervous system maintenance and support. Sleep is thought to play an important role in processes like learning, memory consolidation, recovery and plasticity of neurons and neural networks thereby affecting associated behaviors that lead to normal functioning of an animal in its natural surroundings (Benington et al., 1995, 2003; Blumberg, 2015). All these assumptions explain many interesting ways different animals obtain sleep and also explains why certain functions that are incompatible with wakefulness can only be carried out during sleep. The different types of sleep and the associated brain waves or oscillations have been implicated in many different functions. The characteristic

slow waves of SWS are related with local memory processing (Huber *et al.,* 2004), memory transfer from short-term to long-term storage (Havekes et al., 2015 and 2016, Klinzing *et al.,* 2019) and synaptic plasticity such as downscaling of synapses for acquiring novel information (Born et al., 2012; Tononi et al., 2014). The glymphatic system in the brain is the way through which the slow wave sleep allows the brain to remove waste products (Xie et al., 2013; Fultz *et al,* 2019; Rasmussen *et al.,* 2022). Not only does SWS affect the brain at the network and synaptic level, but also at neuronal level by providing pauses to neuronal activity (termed as slow wave off-states; Vyazovskiy and Harris, 2013) which might allow for cellular maintenance, such as DNA repair (Zada, *et al.,* 2019). REM sleep, on the other hand, has been shown to be involved in different forms of learning and memory (Boyce *et al.,* 2016; Li *et al.,* 2017; Izawa *et al.,* 2019). REM has also been proposed as a counter response to SWS related brain temperature drop, thus helping the animal to adapt and respond to the environment upon awakening (Lyamin *et al.,* 2018; Ungurean G.*, et al.,* 2020). Finally, sleep performs the function of energy conservation and redistribution through lowering of metabolic rate (Ferretti *et al.* 2019) (see Rattenborg, & Ungurean, 2022 for a detailed review). Several other hypotheses pertaining to the central nervous system are also found in literature and broadly they all can be grouped either into a category of maintenance hypotheses or into the category of synapse modulation hypotheses (Rattenborg, et al. 2017). Even though there is compelling evidence to support both of the categories of hypotheses, it is still unclear how sleep can influence so many functions. From all these debates in the literature it seems safe to conclude for now that sleep performs multiple functions and there are many more functions that might be fulfilled during sleep. Moreover, it does not seem like the brain is the only benefactor of sleep. Many recent studies have reflected the importance of sleep on muscle activity (Chen et al., 2017; Lamon et al. 2021; Pourmotabbed et al., 2020). Acute sleep deprivation has been shown to cause reduced muscle protein synthesis, increased plasma cortisol and decreased plasma testosterone (Lamon et al. 2021) suggesting that sleep affects other important bodily functions as well.

4. Sleep in birds: Birds offer gateway to better understand sleep functions

The majority of knowledge about sleep comes from mammalian studies as they are most intensely studied, however many previously unknown aspects of sleep and its functions have come from studies conducted on birds. Despite birds being a type of reptile in the clade Dinosauria, birds exhibit two sleep states remarkably similar to mammalian SWS *and* REM sleep (Rattenborg*, et al.* 2022). Just like in mammals, SWS sleep in birds is characterized by EEG slow-waves, relaxed muscle tone, lowering of brain temperature, etc. As in mammals, slow waves increase following sleep loss, suggesting that they reflect a homeostatically regulated process (Van Der Meij *et al.* 2019; Lesku *et al.* 2011). REM sleep also bears similar characteristics such as awake-like EEG activity, increased brain temperature (Heller *et al.* 1983; Ungurean et al., 2020), twitching and rapid eye movements. As observed in mammals, the amount of REM sleep in young birds is high which then reduces to adult levels (Scriba *et al.,* 2013). Nonetheless, several interesting differences also exist between the avian and mammalian REM sleep. Birds maintain postural muscle tone to stay standing during SWS *and* REM sleep (Dewasmes *et al.* 1985). The only consistent signs of reduced tone occur in the muscles supporting the head, which results in the head dropping when birds enter REM sleep (Lesku *et al.* 2011). In additions, episodes of avian REM sleep are very short when compared to those in mammals, typically lasting <10 s (Rattenborg *et al.,* 2022). Nonetheless, birds can have over 700 episodes of REM sleep per night (Tisdale *et al.* 2018). Finally, the pupillary behavior of birds is largely opposite that of mammals during wakefulness, SWS and REM sleep (Ungurean *et al.,* 2021). Iris sphincter muscles of birds behave like skeletal muscle unlike how muscles in mammals behave thus explaining the contrast. During wakefulness when the birds get aroused pupils constrict (for e.g., during courtship). When in SWS avian pupils are dilated and constrict rapidly during REM sleep (Ungurean *et al.,* 2021).

One very interesting aspect of sleep in birds and also some marine mammals is their adaptation to obtain sleep under extremely challenging ecological conditions. In some life history stages or under certain environmental conditions, the investment in sleep appears to be disadvantageous, and the individual would benefit from staying awake to increase their fitness. Examples include, birds that need to compete for mates around the clock (Lesku et al., 2012), birds that are constantly exposed to the risk of

predation (Rattenborg et al., 1999), birds that undergo non-stop flights lasting days to months (Rattenborg et al., 2016). Under such circumstances, birds rely on the following strategies: 1) reduce the time spent sleeping (Lesku et al. 2012; Rattenborg et al. 2016) or 2) sleep with one half of the brain at a time (Rattenborg et al. 1999; Rattenborg et al. 2016). Several bird species and marine mammals are able to sleep with only one half of the brain at a time, an adaptation termed unihemispheric slow-wave sleep (USWS) (Rattenborg et al., 2000, 2016; Lyamin et al., 2008b, 2018). Dolphins only engage in USWS, whereas fur seals and birds engage in bihemispheric slow-wave sleep (BSWS), USWS, and an intermediate state on a continuum between USWS and BSWS called asymmetric SWS (ASWS) (Fig 2).

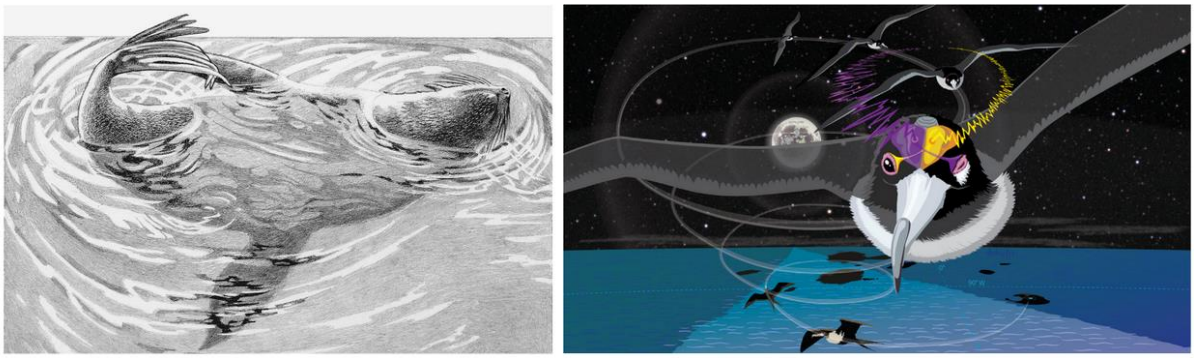


Figure 2: USWS in fur seals and great frigate birds (Rattenborg et al. (2000), Rattenborg et al. (2016)).

Behaviorally, identifying USWS and ASWS is intuitive and easy because the eye contralateral to the deeply sleeping hemisphere is closed, whereas, the ipsilateral eye remains open. This allows animals to maintain awareness about their surroundings simultaneously while the other half of the brain sleeps. Fur seals use USWS to swim and visually monitor the open sea below them to perhaps avoid shark attacks (Kendall-Bar, *et al.,* 2019) while obtaining sleep in one hemisphere, whereas dolphins use it to maintain contact with other dolphins (Lyamin, et al., 2008). Similarly, it has been shown in mallards that individuals sleeping at the outer edge of the flock engage in more USWS and direct the open eye away from the flock, as if watching for approaching predators (Rattenborg et al. 1999). In contrast, mallards sleeping inside

the flock engaged relatively less in USWS. Certainly, USWS has a profound role in anti-predator vigilance, but it has other beneficial functions.

It is known that many birds fly non-stop for several days to many months (Rattenborg, 2017). Until recently, it was unknown if and how such birds sleep in flight. An explanation for this phenomenon came from a study on great frigatebirds *(Fregata minor)* (Rattenborg et al., 2016). Great frigatebirds take foraging flights that span multiple days while taking care of their young. Despite being seabirds, frigatebirds cannot safely land on the water, and rely on predatory fish and cetaceans to drive flying fish out of the water and within their reach. EEG data loggers and accelerometers mounted on the great frigatebirds during these flights showed that the birds could sleep on the wing, primarily engaging in USWS and ASWS. However, this sleep was only observed during passive modes of flight like gliding and soaring and never during wing flapping, raising the question whether sleep in birds is possible during active modes of locomotion. If sleep is not compatible with active locomotion, then, it suggests that migratory birds, such as bar-tailed godwits (*Limosa lapponica*), which are thought to flap their wings continuously during their 8-day, non-stop flight from Alaska to New Zealand (Gill et al., 2005), might forgo sleep altogether during these marathon flights. Unfortunately, as godwits are too small to carry the available EEG loggers used to record sleep in the larger frigatebirds, it is currently not possible to determine if they can sleep during flapping flight.

5. "Sleep swimming": Is sleep compatible with locomotion?

Previously, sleep swimming has only been demonstrated in some marine mammals (Lyamin et al. 2008). Northern fur seals (*Callorhinus ursinus*) engage in USWS and keep the side facing the ocean awake and in motion to maintain posture, vigilance and breath (Fig 2). Whereas motor activity during USWS is lateralized in fur seals, dolphins can engage in complete awake-like, symmetrical locomotion while in USWS which is likely controlled by subcortical motor regions of the neuroaxis (Lyamin et al. 2008). This example, suggests that birds might be able flap their wings or paddle their feet during USWS.

Anecdotal observations in wild waterfowls, such as Canada geese (*Branta canadensis*), suggest that sleep and active locomotion might be possible in birds. Waterfowl (ducks, geese, and swans) have been observed closing an eye, apparently while actively paddling their feet, a phenomenon that we refer to as “sleep-swimming”. Eye closure is a fairly reliable behavioral correlate of sleep. EEG recordings demonstrate that closure of both eyes is associated with both BSWS and REM whereas closure of one eye is typically indicative of USWS or ASWS (Rattenborg et al. 1999; Fuchs et al. 2009; Rattenborg et al. 2000, 2019). In some species SWS is known to occur with open eyes (Rattenborg et al. 2019), which can lead to an underestimation of time spent sleeping based exclusively on eye closure. Studies have used eye closure as a proxy to gauge sleep in a variety of species that sleep in the open (Lendrem, 1983; Rattenborg et al. 1999). These studies revealed that the time spent sleeping is very susceptible to ecological conditions like the risk of predation. For example, wild mallards that are more exposed to predators opened their eyes more frequently, often keeping them closed only for a few seconds. A recent unpublished EEG study showed that chinstrap penguins obtain large quantities of sleep, but via bouts lasting on average only 4 s (Rattenborg, personal communication). Importantly, these short EEG-defined episodes of SWS corresponded to short episodes of eye closure. Even in humans, microsleeps consisting of episodes of sleep lasting <15 s are accompanied by eye closure (Nir et al. 2017). Thus, eye closure, even when it lasts just a few seconds, is a reliable indicator of sleep in birds, and other animals.

Despite having observed sleep-swimming in several waterfowl in the wild, for several reasons we are not able to fully characterize this behavior under field conditions. First, due to the lateral placement of the eyes, it is rarely possible to see both at the same time. Consequently, we are unable to determine whether this behavior occurs only with one eye closed (USWS) or also with both eyes closed (BSWS). Second, although it appears that the birds are moving through the water by paddling, we cannot determine if a foot is paddling exactly while the eye is closed. This is important because it is possible that sleep only occurs during the glides between each paddle.

Also, we cannot determine if there is a relationship between which eye is closed and which foot is padding. Based on fur seals, we would expect there to be a strong relationship, whereas based on dolphins, there should be no relationship. For these reasons, we utilize video recordings, leg accelerometers and EEG to simultaneously record all the eye closure, paddling activity and brain activity, respectively. Time synchronized output from the above experimental methods allowed us to characterize sleep-swimming.

Hypothesis:

The peculiar adaptation of sleep-swimming can be explained by the hypothesis that when swimming to different areas with better foraging options or fewer predators, waterfowl might benefit from also obtaining some sleep. The prediction from this hypothesis is that the waterfowl might then engage in more sleep-swimming when they are forced to travel frequently or over long distances as their need for sleep accumulates. It is also a well-known fact that the physiological requirements of individuals change as they develop into adults, which implies that the amount of time invested in sleeping should also decrease as the individual ages (Mander et al. 2017). This suggests that during development, when the need for sleep is high, and the parent geese might want to travel more to find better foraging conditions or fewer predators, the young goslings should engage more frequently in sleep-swimming. Moreover, geese are social animals and they move in flock. Individuals in flock may have different sleep needs due to different physiological conditions and in such cases the individuals make trade-off between sleep and staying with the flock. Such a trade-off manifests as sleep-swimming in some individuals to achieve both the demands of resting and staying with the flock. However, it is yet to be investigated whether the “sleep-swimming” phenomenon is present and whether it is modulated differently as the individual progresses through different developmental stages.

Goals of the thesis:

The aim of this thesis project is to fully characterize this previously unreported phenomenon. More specifically, we aim to determine whether the feet paddle during

eye closure. We also aim to automate sleep scoring using computer vision-based pose estimation and test the applicability of this method in semi-naturalistic conditions. If sleep swimming occurs, then we aim to answer the following questions:

1. Do the legs paddle when the eyes of the geese are closed?

2. Is there a relationship between which eye closes and which foot paddles, as suggested from fur seals?

3. Is there an effect of age on the amount of sleep-swimming the geese perform?

Chapter 2 **Materials and Methods**

Experimental subjects

For our study we established a small (n = 17) group of Canada geese (*Branta canadensis*). Several reasons guided the choice of Canada geese. First, we had observed sleep-swimming in this species in the wild. Second, eye closure is distinctly visible due to the presence of white feathers on the eyelids, which strongly contrasts against the black facial feathers of the goose and when the eye is open, it simply appears dark and blends in with the facial feathers (Fig 3). Third, this species is quite abundant and invasive in Germany.

We collected eggs from wild nests and hatched them in the lab by simulating the natural incubation conditions. The goslings were hand reared on land for ~ 6 weeks post-hatching. The geese were imprinted on experimenters wearing yellow t-shirts. The imprinting phase of the study is of immense importance because it allows us to induce the geese to follow us during experiments. At post-hatching week 8 the individuals of the population were tagged with a black and white uniquely patterned neck collar. The choice of only two colors was constrained by our ability to record in IR light at night, which records the view in shades of gray, with the darkest shade being black and the brightest shade being white. Our target species does not exhibit obvious sexual dimorphism, so we do not consider an individual's sex relevant to the project. As a result, we conducted the data analysis blind to this factor and will establish the sex genetically only at the end of the project.

The geese were housed inside a floating aviary (4m x 75m; Fig 4) on the Eβsee at the Max Planck Institute for Biological Intelligence (Seewiesen). The aviary included a partially covered 4m x 4m platform near the shore (Fig 4). The rest of the space was open water. A net kept the geese confined and potential predators out. The geese were not exposed to the floating aviary set-up until week 7 post-hatching, when they were gradually allowed to get acclimatized to the floating aviary environment.

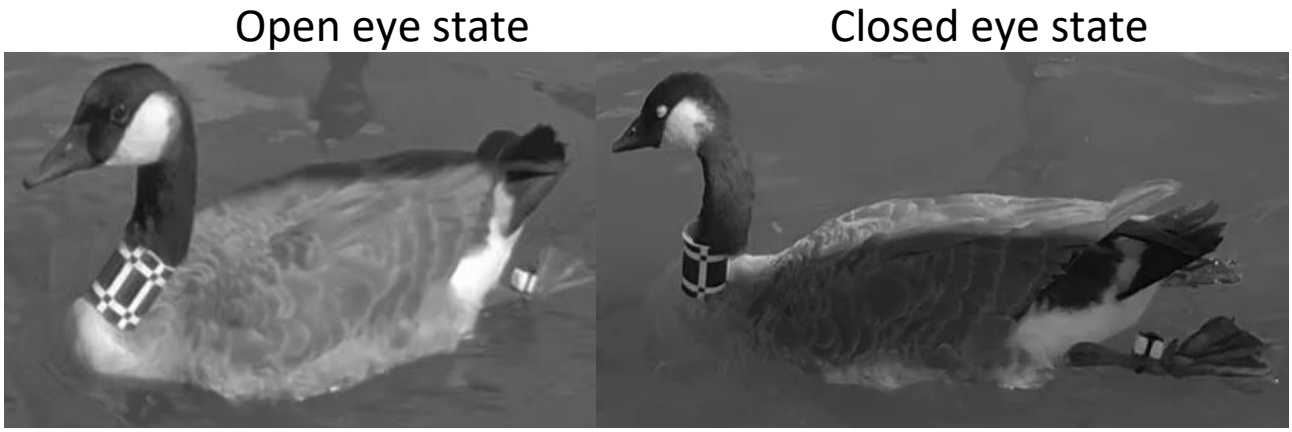


Figure 3: A goose with an open eye on the left and the same goose with a closed eye on the right. The two images are from two cameras and were taken at different time points. The image also depicts the camera system's actual view (lighting-conditions, shadows, colors, etc.).

Experimental set-up

The experiments were performed in a floating aviary (Fig 4) mentioned in the previous section. We mounted a surveillance camera system (Axis Communications GmbH) consisting of 18 offshore synchronized cameras running along the long edges of the aviary and 5 cameras on the platform of the aviary. At the very end of the floating aviary, we mounted 2 feeding stations fixed by the frame of the floating aviary at approximately 0.5 m above the water level. All the cameras were configured to record continuously throughout the day and night on the designated experimental days. The 5 cameras on the platform recorded the sleeping behavior of the geese on land, whereas the 18 offshore cameras recorded the sleeping behavior while they swam. The 18 offshore cameras were positioned such that together they created an array capable of recording the entire length of the aviary from both sides (9 for the left side and 9 for the right side of the aviary) in a continuous manner with some overlap, thus minimizing loss of information between two adjacent cameras. The 9-pair camera set-up is useful in recording all sleeping forms, namely, the bilateral sleep (both eyes closed) and the unilateral sleep (one eye closed).

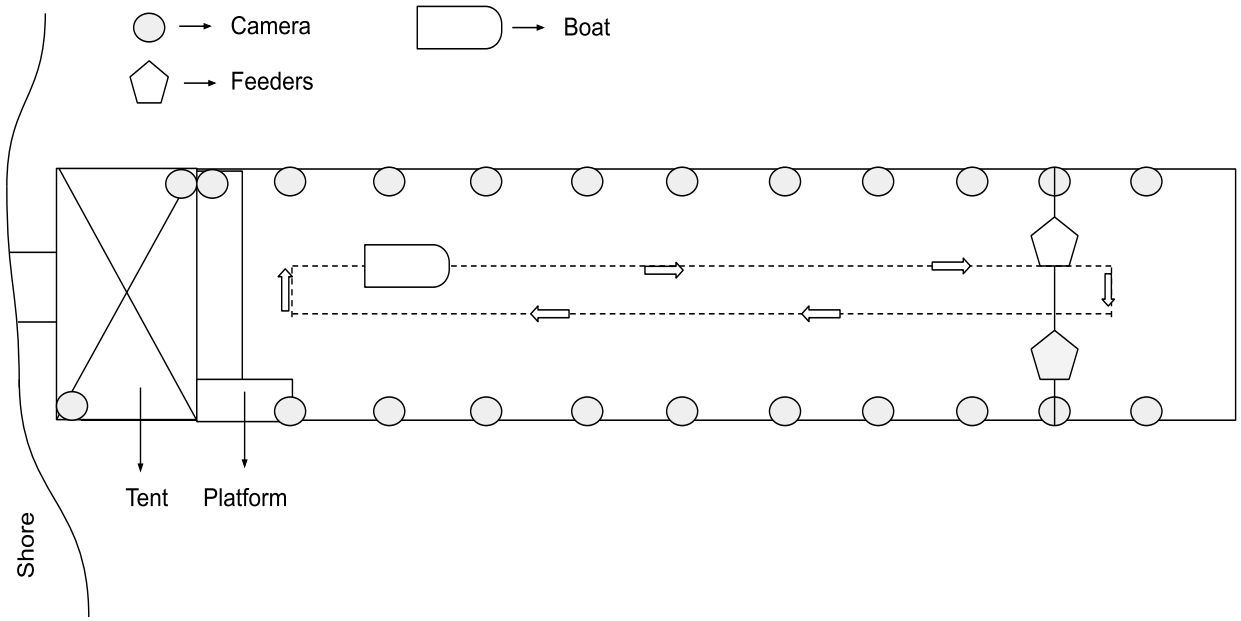


Figure 4: A schematic representation of the floating aviary

The cameras were coupled with external infrared (IR) lights to record the behavior at night. The lights are affixed at an elevated position to prevent the retinal reflection from an open eye creating ambiguity in detecting the two eye states.

Accelerometry

Imprinting of the geese onto the experimenters is one of the most important aspect of the project. Imprinted geese not only follow us during the experiment, they also make it easier to instrument them with the accelerometers as it involves animal handling for a safe affixation of the data loggers. Each goose was fitted with a pair of leg accelerometers (Fig 5). The leg accelerometers (Technosmart Europe srl) recorded the movement of the paddling feet, which then can be synchronized with behavioral data to conclusively determine whether any movement is associated with sleep. We used a combination of Axy 4S and Axy 5S models. Although the detection results were equally good with both models, the loggers were always paired and mounted on the

goose according to the model to avoid any differences in detection between the left and right legs. The accelerometers record triaxial acceleration of the legs along with other useful parameters like pressure and temperature. An accelerometer weighed 2.5 g and the dimensions of each device were 20mm x 10mm x 6mm. All the specific recording parameters are listed in Table 1. The devices were mounted on every individual at least 2 days before the start of the experiments thereby providing a 2-day period of acclimatization before the experimental session. Accelerometers were mounted on the legs of the geese using custom casings and zip-ties. Special care was taken when fastening the zip-ties so as to not hurt the animals. We mounted all the accelerometers in a particular orientation which kept the x-axis of the accelerometer pointed towards the geese' body. The specific orientation ensured that the data we collect would be consistent across all the individuals. The devices recorded the acceleration data for 2 weeks after which they were removed from the geese for data extraction and recharging battery. Data extraction and processing to comma separated values (csv) was carried out using X manager software (Technosmart Europe srl).

Sample Rate	25 Hz
G Fullscale	+/- 8g
Resolution	10 bit
Temperature and Depth logging period	5 s

Table 1: Accelerometer recording configuration

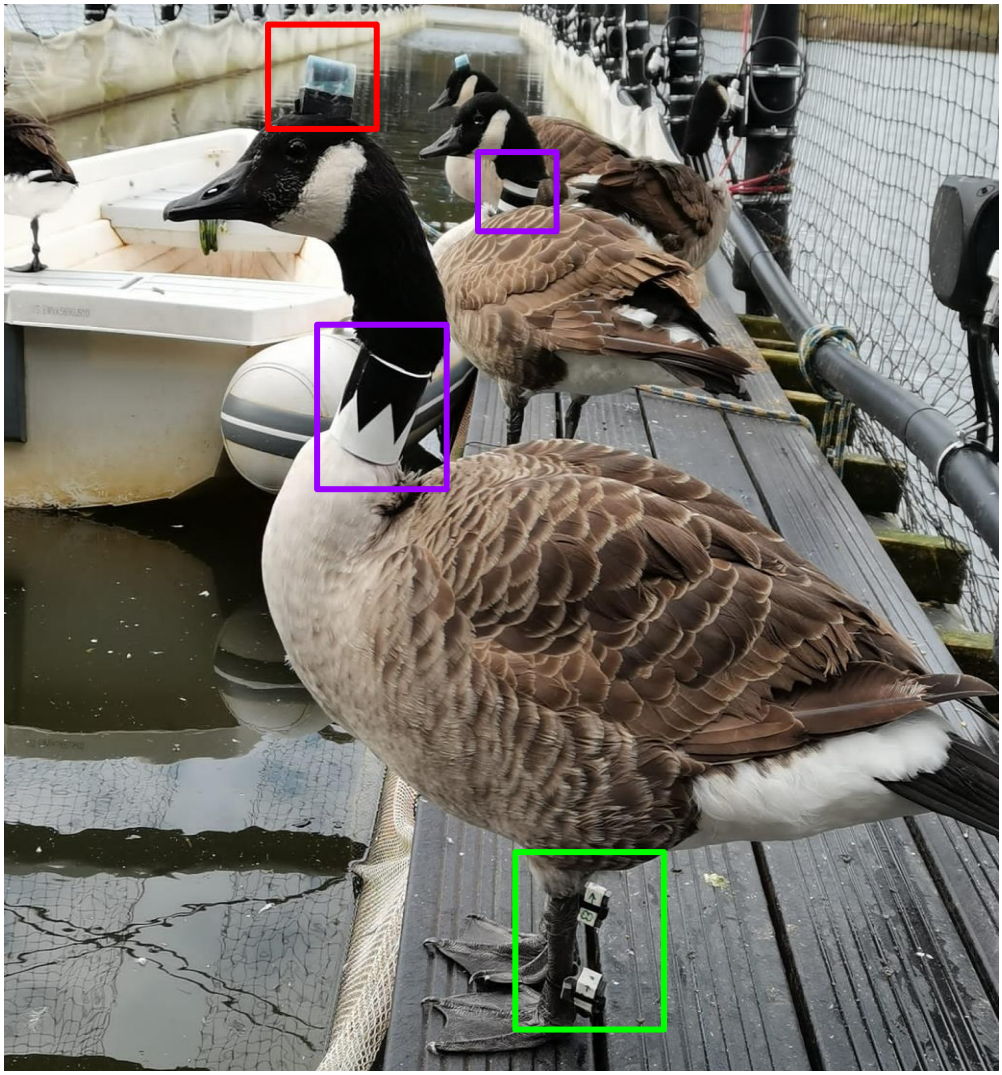


Figure 5: The most proximal instrumented goose has an electroencephalogram (EEG) data logger on the head (red box), an identification collar (purple box), and a pair of leg accelerometers (green box).

Paddling activity peak extraction

To detect the paddle movements of the geese in the accelerometer data, we first located the time points and extracted the boat session specific data. We observed that the accelerometers drift linearly. Although the amount of drift for a 2-hour session was very small, it was taken into account in order to optimize the synchronization of the loggers mounted on the left and right legs. Such a drift in time could be due to several factors, e.g. temperature or magnets used to turn them on and off. The loggers were then manually aligned with the camera time. Leg movements in the accelerometer data can be easily identified in the acceleration plot with their characteristic M-like

curve (Fig 6). The highest peak in the M-like curve represent the individual paddling events. Paddling events occurring during the boat sessions was extracted using SlipAnalysis (developed by Paul-Antoine Libourel), whereas the extraction of peaks and hypnogram construction was done using custom MATLAB scripts (coded by Andrea Ferretti and Paul-Antoine Libourel). As the accelerometers record in all (X, Y, and Z), the characteristic signal is detected in all these axes. We chose the Y axis for extracting the paddling peaks as they gave the clearest signal. The location of the peak was detected based on the parameters of minimum peak distance, minimum peak height and peak drop value. All the values that have a minimum peak distance of 12 measurements minimum peak height of 0.005 G and peaks that drop at least 0.13 G on either side before it rises again were extracted. Finally, we created an hypnogram based on such peak detection. Within each boat trip, paddling peak were marked with 2, whereas any other signals were marked with 1. Finally, the remaining signal that did not fall within the boat trip was marked with 0. We had some cases of no detection which could have been caused by several factors, e.g. spinning of the accelerometer logger on the leg. In most of these cases, we were able to get the peaks by using values from the other two axes when we observed no detections.

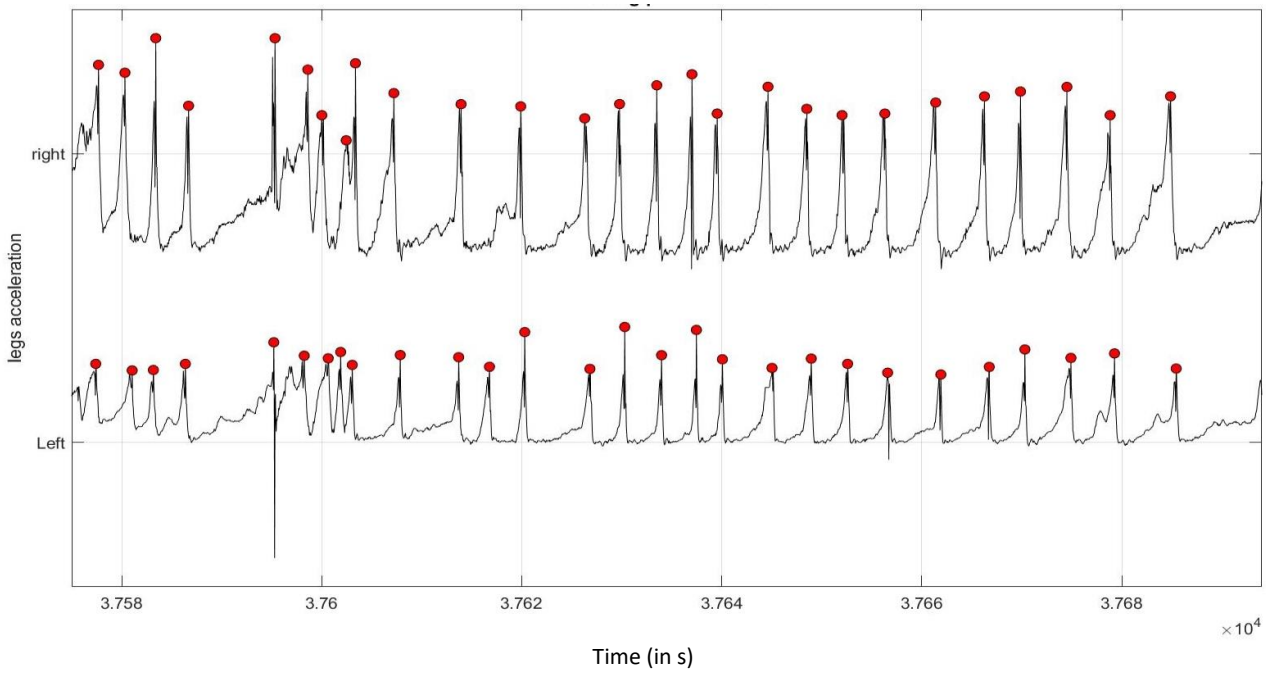


Figure 6: Acceleration of the right and left foot for goose38 on experimental day 1. The red peak marker depicts the onset of the down stroke during paddling events. A paddling event

has two dominant peaks very close to each. Due to the compressed time axis the peaks are not clear in all instances. The red dot marks the highest of the two peaks.

Experimental paradigm

Taking advantage of the fact that the goslings were imprinted on the experimenters, we devised an experimental paradigm wherein the flock leader (experimenters) would simulate a situation where the flock travels a long distance in the aviary. The experimental sessions were conducted on specific weekdays and lasted 2 hours. The experiments were conducted between 10:00am and 12:00am (CET). The session began by making the geese swim the length of the aviary with the help of an inflatable motorboat. The imprinted geese follow the experimenters driving the boat from one end of the aviary to the other. The camera array simultaneously video records the eye state from both sides and feet paddling is recorded with accelerometer data loggers. A boat lap or trip lasts for about 4 minutes and a complete two-hour session had at least 15-20 trips. The first 2 minutes were allotted as traveling time to cover the one-way length of the aviary, while the remaining 2 minutes were spent on rewarding the geese if they followed the experimenters well enough. The reward consisted of a few salad leaves per individual, mealworms, or duck food pellets. Rewarding the geese renders them excited, which might negatively affect their sleeping probability. To minimize this reward trade-off wherein if we give the reward too often, the geese reduce sleeping time versus if we do not give enough reward, the geese lose interest in following the experimenters, we settled on randomly giving rewards only when the geese faithfully followed the experimenters thereby effectively encouraging faithful following and naturalistic sleeping behavior. On experimental days, all food from the platform was removed at sunrise until sunset. At sunset, food on the platform was returned. The only source of food for the geese during this time was the offshore feeders, thus further encouraging the geese to swim back and forth in the aviary.

Video data acquisition and Object detection

All the videos were recorded using AXIS M20 Bullet network cameras. The videos were recorded for 24 hours and stored on a local server where the video files are converted to a general “MP4” format. Due to the stochasticity of goose movements and *ad libitum* feeding, it was practically impossible to predict when the geese are passing in front of each camera. The stochastic nature of the geese and the long video recordings necessitated the division of the long videos into smaller chunks. We employed a pre-existing and well-trained object detection algorithm called “YOLOv5 by Ultralytics” to extract all instances wherein the geese came across the camera. The original source code was modified to generate a custom output csv containing the object ID for birds (which is number 14 by default) and the corresponding frame number wherein the birds were detected. We ran test videos first to ascertain the accuracy of the detections and we were satisfied with the performance. The start time and end time were extracted from the frame numbers and then buffered by 20 seconds each using custom scripts written in R. The 20 seconds of buffer time allowed us to capture every single frame with a goose in it. The start time and end time were then used to chop the 24-hour video recording files into smaller chunks using *FFmpeg* video editing command line integrated into a custom PowerShell script.

Video data analysis

We employed a pose estimation software called DeepLabCut (DLC) to score the vast amount of video data. DLC uses a deep convolutional neural network made from a combination of two algorithms for object recognition and semantic segmentation: the pre-trained deep residual neural networks (ResNets) and deconvolutional layers (Mathis A. et al, 2018, Nath, T. et al, 2019). We chose DLC for several reasons:

1) It robustly performs when trained with a minimal amount of good-quality data.
2) It saves the time required to score the videos manually.
3) It can perform as accurately as a human scorer, thus allowing us to avoid the risk of experimenter-induced biases and errors creeping into our data due to the monotonous and repetitive task of scoring the eye state.

Each camera recorded the experimental sessions at a frame rate of 25 fps. To score the sleep without ambiguities, we only considered two eye states, namely, open eye state and closed eye state.

DeepLabCut pipeline

A) DeepLabCut Workflow

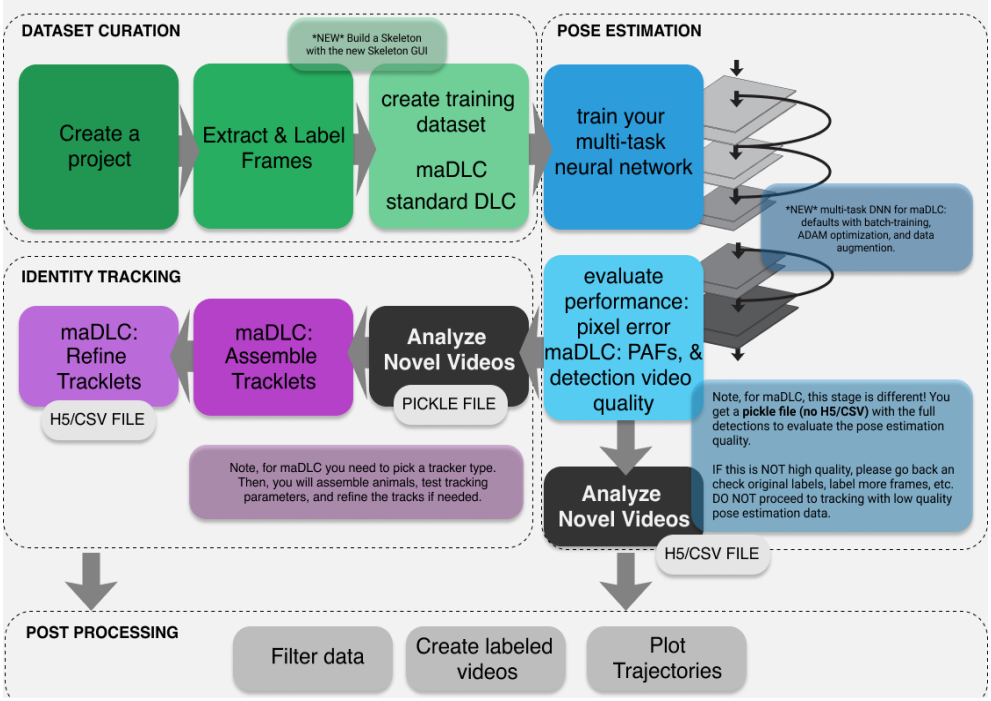


Figure 7: DeepLabCut Workflow (adapted from "https://deeplabcut.github.io/DeepLabCut/README.html")

B) Model Training

The multi-animal DeepLabCut (DLC) (version: 2.2.1.1) was used since we have multiple individuals to track (Lauer et al. 2022). To train a robust model capable of identifying sleeping states and the pose of the animals, we took 84 videos, out of which 42 videos were from experimental sessions conducted during the day. The other 42 videos were nocturnal recordings. These 84 videos were from different cameras in the array. At least one video was included from all the cameras, ensuring that all the different viewing conditions, lighting, shadows, and other aspects that could potentially confuse the model were incorporated into the training dataset. Even though we only

analyse the daytime experimental sessions in this report, the future aim of the project is to also analyse nocturnal sleeping behaviour. We extracted 12 frames from each of the 84 videos for training the model and manually labeled these frames for a total of 9 body parts, namely, 1) bill, 2) cheek, 3) eye closed state (labeled only the closed white eyelid), 4) head, 5) collar (a black and white unique pattern assigned to each individual goose), 6) the proximal end of the spine, 7) middle of the spine, 8) the distal end of spine and finally 9) the tail base (Fig 8).

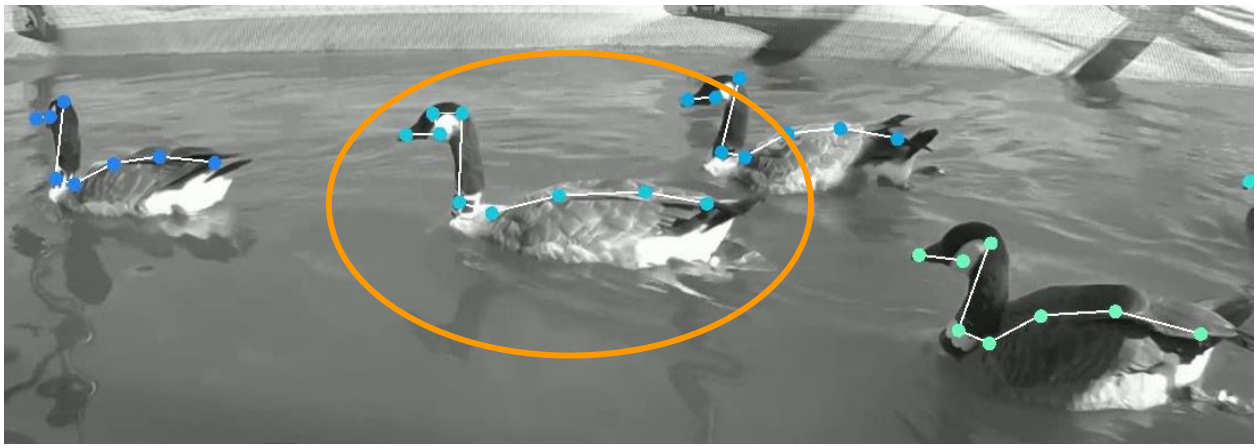


Figure 8: A frame extracted from an analyzed video depicting all the different body parts labeled to train the model. The orange box highlights one such fully marked goose (cyan dots), and these marked body parts appear in the video because the model detects them with a likelihood of at least 60%.

The model was trained for 200,000 iterations on reshuffled data with 95% training and a 5% test dataset split. It was ensured that loss had converged and training was stopped to avoid overtraining of the model.

C) Model Evaluation

The model evaluation step is extremely crucial for our study because at this stage the final checks for model accuracy, performance speed, precision can be finely tuned and optimized to our use case. To evaluate the performance of the trained network we used the built-in command to compute the Mean Average Euclidean error (MAE): deeplabcut.evaluate_network(config_path, plotting=True). MAE is proportional to average root mean squared error between manual labels and the labels that are predicted by DLC. The above command also generates frames of the labeled videos

with both manual and predicted DLC labels (Fig 9). All the frames are manually examined to identify any potential outlier. At this step we looked for any major body part swap or exchange of body parts between adjacent individuals. All such cases were identified and corrected to retrain the model with more accurate labels. We performed one iteration of training again after the correction. After every iteration of training we carried out the model evaluation step to ensure that we had a fairly robust model.

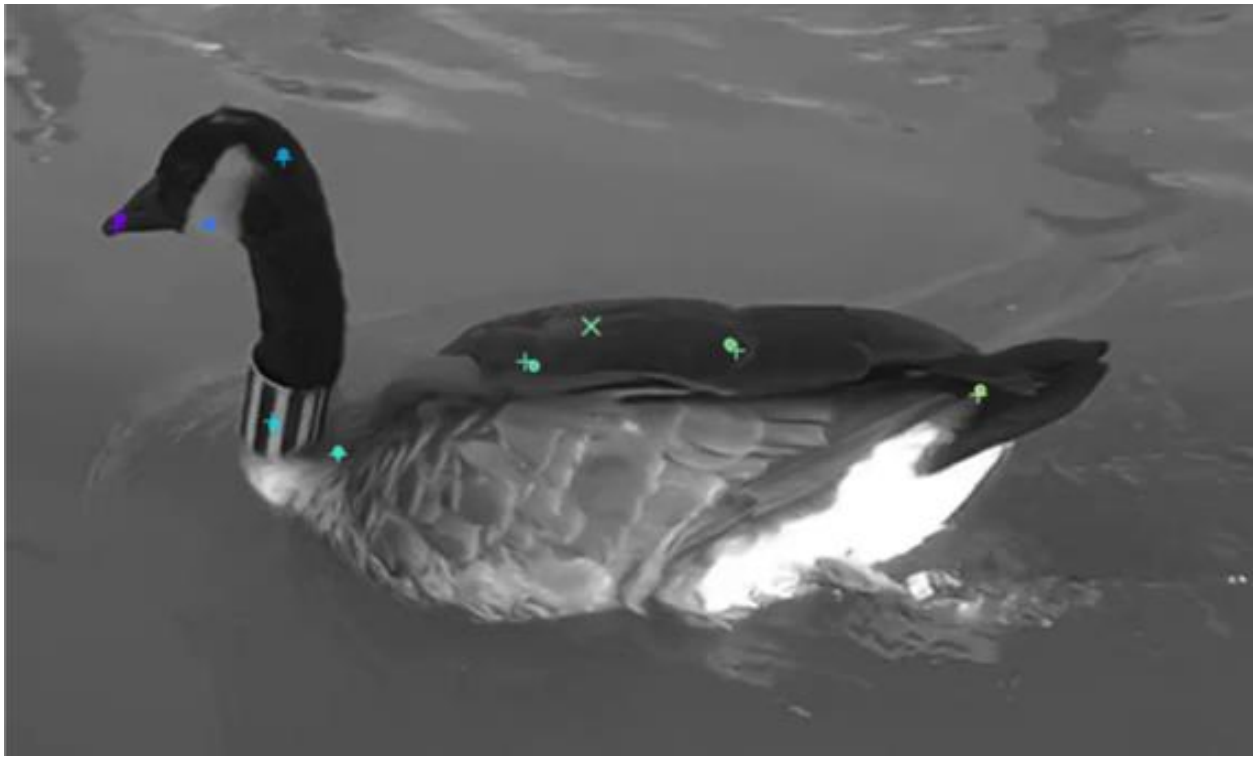


Figure 9: An evaluation frame depicting the different body parts marked with labels. The "+" markers are the ground truths or human marked labels. The "●" markers are body parts predicted by the model with a likelihood > p-cutoff which is 0.6 for our model. The "x" markers are the body predicted by the model with a likelihood <= p-cutoff.

D) Optimal model Identification to quantify eye state based on pose estimation

The model trained for 200,000 iterations on 1004 frames (1920 x 1080 px) and had a pixel error of 3.01px for the train dataset and 8.05px for the test dataset, further improving to 2.95px and 6.04px, respectively, for the train and test dataset when the p-cutoff of 0.6 was applied. We tested the DLC model in two configurations to identify an optimal model that performs well on our data set, as listed below:

a) Identity learning: In this configuration, we employed the built-in identity learning capability of the software. We consistently labelled the individuals according to the collar patterns so that the model could learn the identity of the individuals based on the collar. Our model did not do well in this configuration and failed to track the correct identity due to several probable reasons, of which the major ones are: 1) The collar patterns appear similar in certain viewing conditions, 2) The collars may not always be visible in a given frame, 3) The amount of sunlight, direction of sunlight, shadows, and water reflection further made it challenging, 4) The non-continuous arrangement of cameras made it difficult for the model to keep track of the number of individuals present in the scene or view frame of the camera, individuals that left the scene, and individuals that newly entered the scene, 5) Re-entries of previously scored individuals made it even harder for the model to precisely distribute the number of individuals to all the geese present. The model also frequently misidentified individuals in a frame and erratically switched from one individual to another (Fig 10 and Fig 11).

b) Traditional pose estimation (without identity learning): Next, we trained the DLC model to label all the geese without considering their identities. Furthermore, during the inference of pose the code was also modified such that the time taken to fixate and retain an individual entirely with all the body parts as marked or scored was increased to avoid major swaps between individuals. The model, therefore, tracked individuals based on a mix of the order in which they entered the frame and the information that the model had from the training dataset. For example, in a group of 5 geese, if goose 5 enters the frame as the first goose, it is labeled as goose 1, and if goose 1 enters very last in the frame, it is marked as goose 5. But occasionally as a rule this did not apply because the model learns the order of entry that occurred in the training dataset. The configuration worked well with minimal swaps between individuals, and we used this configuration for the analysis (Fig 10 and Fig 11). However, with this configuration, we had to manually correct and reassign the labels to the correct individuals.

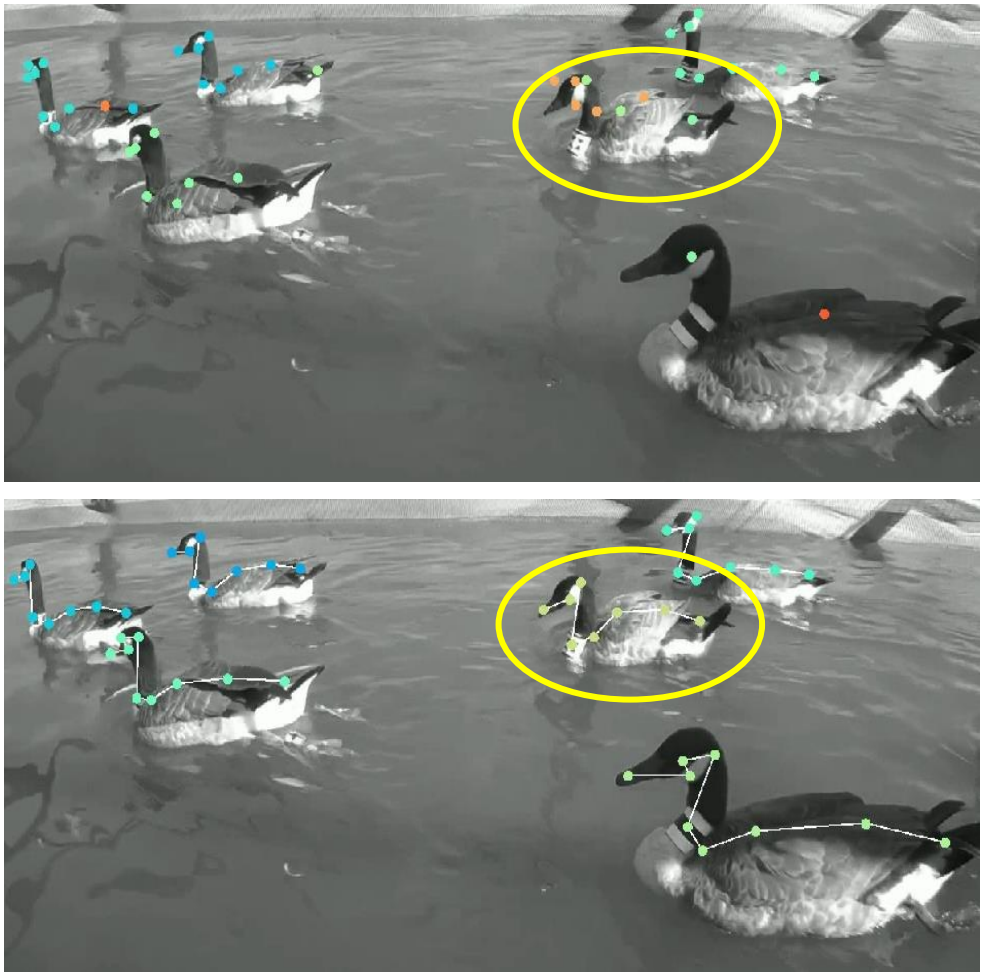


Figure 10: Observe the goose highlighted with a yellow oval shape. The top frame is from the model incorporating the animal's identity, whereas the same frame at the bottom is analyzed using the second model. Note that the colors of the dots marking the goose in the top frame are mixed, implying that the body parts marked are a mixture of points from two geese, one with a green marker and the other with an orange marker. In comparison, the bottom frame shows a single dot color consistently marked throughout the video on this particular goose implying no identity swap.

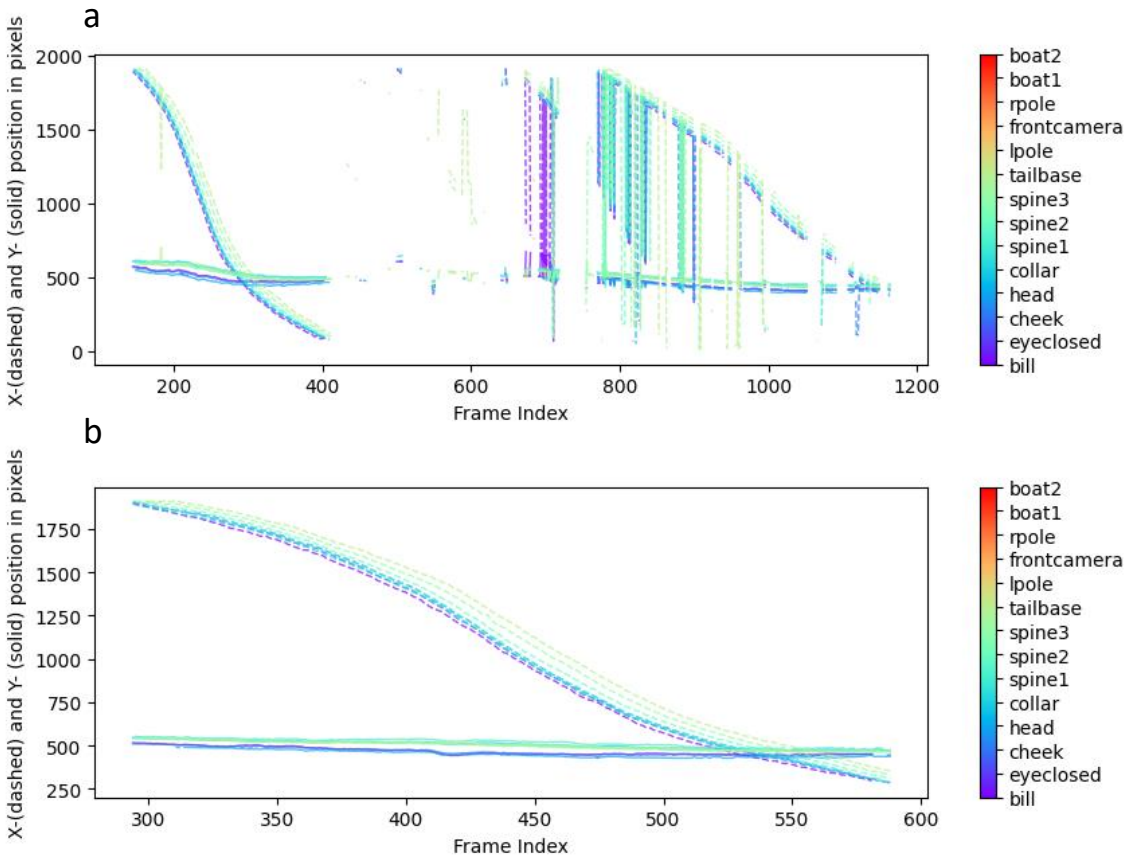


Figure 11: The above two graphs depict the change in x- and y-coordinates of the different body parts with respect to the frame or time (dividing frame index by 25 to get time) for a single goose #23. The top panel is for configuration (a), and the bottom is for configuration (b). The line breaks in (a) are due to identity swaps that happen quite frequently in the first configuration, in contrast with a smooth curve for (b).

The performance of the above two configurations can be assessed by comparing the exact same video analyzed by these two model configurations: video-1 configuration (a) vs. video-2 configuration (b). Figures (Fig 10 and Fig 11) from the above two videos concisely depicts the difference in performance.

Statistical approach

We used the DLC model for pose estimation on three experimental day video recordings from a total of 16 cameras and estimated the body parts of interest for all

17 birds. To optimize and obtain relevant data in the time frame of the thesis project we reduced our sample size to 5 geese recorded during each experimental day on 5 pairs of cameras. Trip number was defined as the boat trip wherein the geese of interest followed the boat. All the other instances of trips were discarded from the analysis. We considered an individual trip as the fundamental unit of measurement. We did not resort to dividing the trips based on camera view. For all the trips where the camera recorded both the sides of the geese, we defined the eye states as both eyes open (BO), both eyes closed (BC), unilateral right eye closed (URC) and unilateral left eye closed (ULC). Next, we defined a paddling event in any of the eye closure states described above, as sleep-swimming when the paddling event (i.e., a single paddle of a single foot) is observed to happen simultaneously with an eye closure state

All the statistical analysis was done in R statistical software. We modelled different dependent variables of interest using the package glmmTMB (Brooks et al. 2017). We had various fixed effects depending on our models and question of interest but we always kept identity of the geese as random effect. Individual models and their characteristics are as follows:

Model 1: Preference of feet

We examined if the total amount of paddling events is affected by the fixed effects eye state and leg when ID of the geese was a random factor. We extracted total paddling events that occur during each trip for each leg separately. Then we grouped the paddle events according to eye state. Next, to check pairwise differences between each interaction terms of eye state and leg we performed a post-hoc test (Holm method).

Model 2: Effect of experimental day and trip number on sleep proportion

We extracted the total proportion of the sleep from the percentage eye closure for all the eye states (inclusive of BC, URC and ULC) when both the eyes of the geese were visible to the camera array. We use proportions because we needed the amount of sleep to be normalised and to separate out the cases when we only had one side of the geese visible, and this ensured that we performed a more complete analysis. The sleep proportion was calculated as:

$$\frac{\text{Total amount of sleep in each eye state (in percentage)}}{\text{Total amount of time both eyes were visible (in percentage)}}$$

Sleep proportion was kept as the dependent variable and the predictors were the experimental day and trip number. We did not increase the complexity of the model further, as including more parameters to a limited amount of data caused the model convergence to fail. We made use of post-hoc test with Bonferroni correction to extract individual interactions between the days. Using the same modelling parameters, we made a partial model with reduced number of trips. The reasons for constructing a partial model are discussed in the results under the section Modeling sleep proportion and ontogeny of sleep.

Model 3: Effect of day and trip number on sleep proportion in each eye state

Sleep proportions for each individual eye state was calculated from the total sleep proportion described in model 2. The sleep proportion for each eye state was calculated as:

$$\frac{\text{Sleep proportion in an eye state (both eyes visible)}}{\text{Total sleep proportion in all the eye states (both eyes visible)}}$$

The three eye states individually were modelled against the trip number and day as the fixed effect to make predictions based on our data. In all the cases, we made use of post-hoc test with Bonferroni correction to extract individual interactions between the days.

Chapter 3 Results

Eye states during swimming behaviour

A total of 72 trips spanned the experimental days of which 29 trips occurred on the first experimental day, 18 trips occurred on second experimental day and 25 trips occurred on the third experimental day in the months of July, August and September respectively. Of the total eye state detections, we found 59% of these were BO, 13% were BC, 12% were of ULC and, 16% were URC (Fig 12). The percentage composition for individual geese were quite variable but generally had similar trend of BO being the most abundant, followed by the unilateral eye closures and the least for BC (Fig 13).

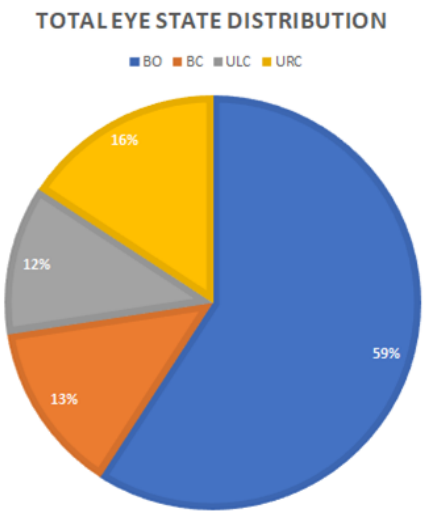


Figure 12: Total distribution of eye states for all trips across experimental day and across all individuals when geese were visible from both sides and detected by the DLC model.

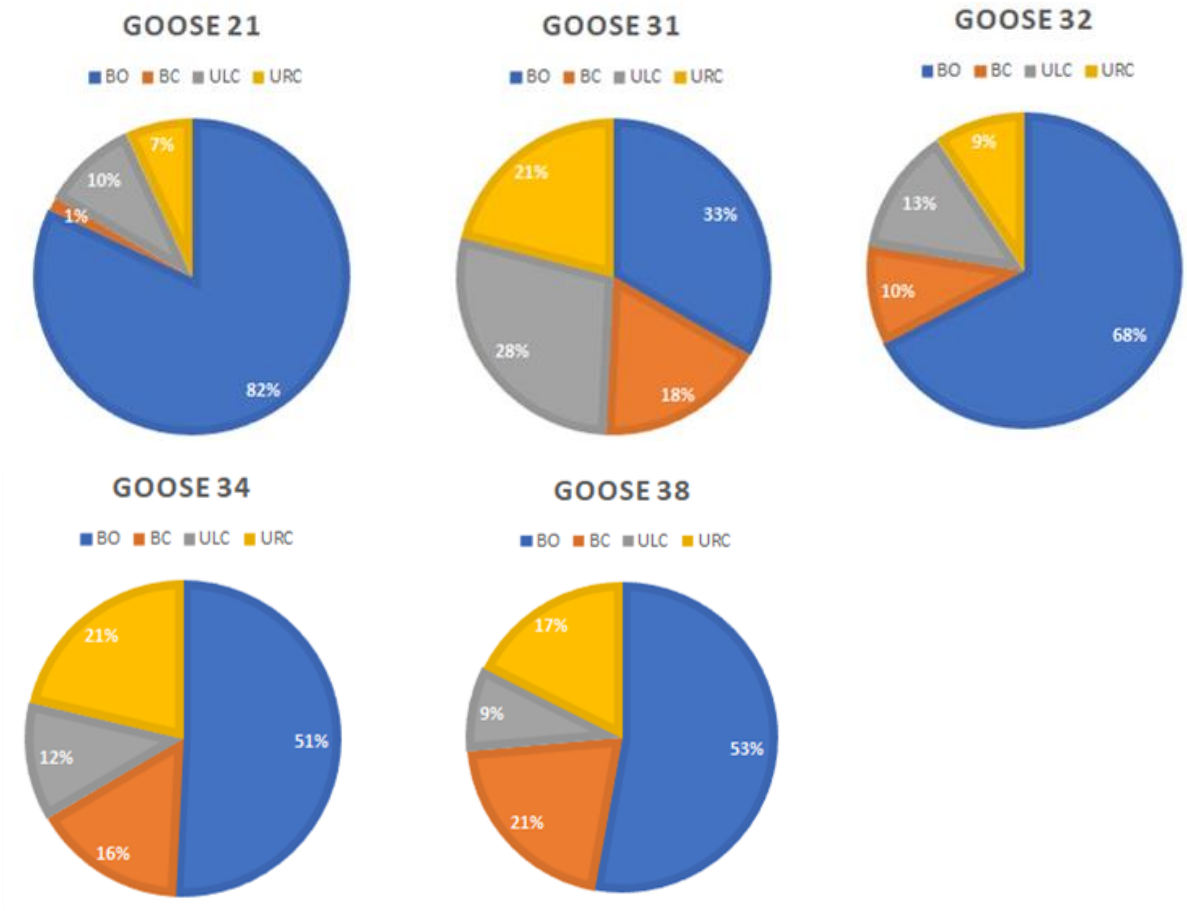
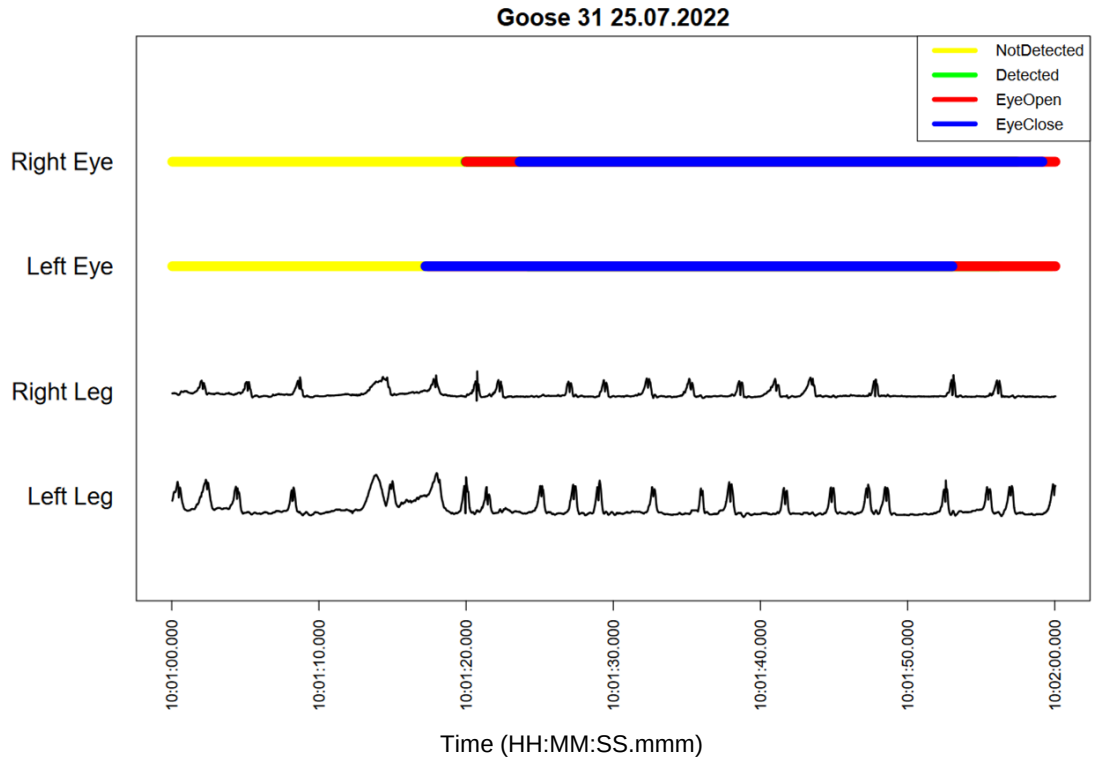


Figure 13: Distribution of eye states when geese were visible from both sides for each individual and detected by the DLC model.

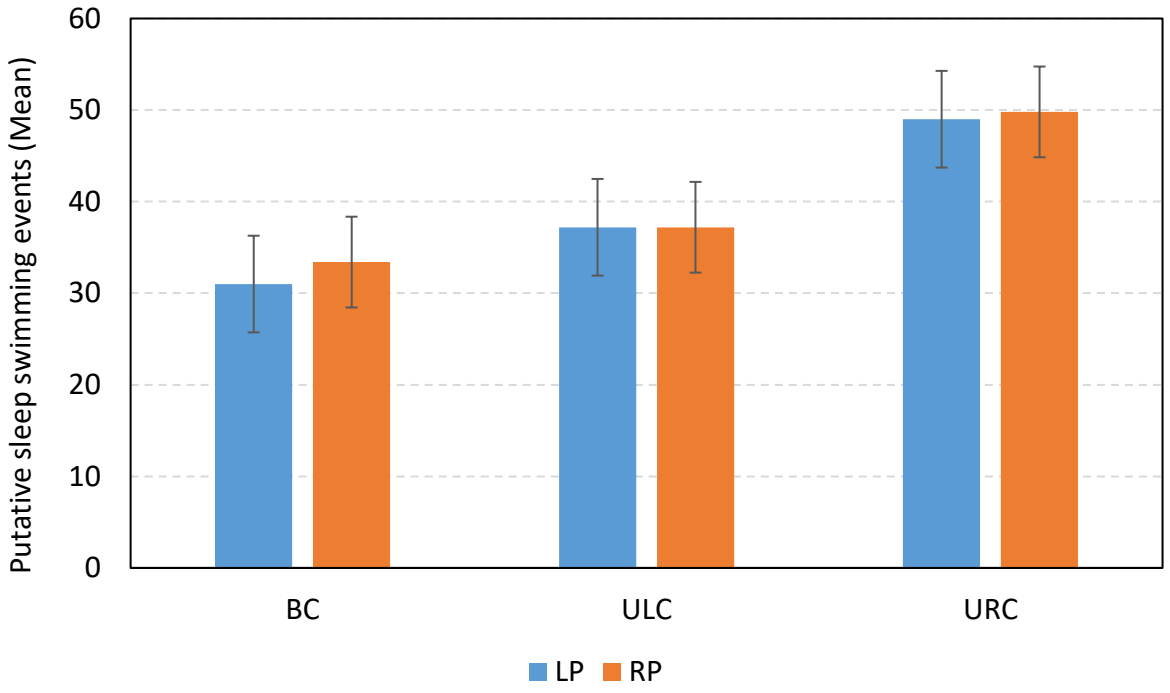
Presence of "Sleep-swimming"

We synchronized the accelerometer data and DLC pose estimation output with video chunks' timestamp for each trip to extract sleep-swimming events. Sleep-swimming events were observed in all the eye closure states (Fig 14 a). The majority of events for both left and right legs were observed in URC eye state (494; LP = 245 and RP = 249), followed by ULC eye state (372; LP= 186 and RP = 186) and least in BC (322; LP=155 and RP = 167), where LP and RP stand for left and right paddling respectively (Fig 14 b). There was no preference for either of the leg (see paddling feet preference during eye state section).

a)


Goose 31 25.07.2022
NotDetected
Detected
EyeOpen
EyeClose
Right Eye
Left Eye
Right Leg
Left Leg
10:01:00.000
10:01:10.000
10:01:20.000
10:01:30.000
10:01:40.000
10:01:50.000
10:02:00.000
Time (HH:MM:SS.mmm)

b)


Putative sleep swimming events (Mean)
60
50
40
30
20
10
0
BC
ULC
URC
LP
RP

c)

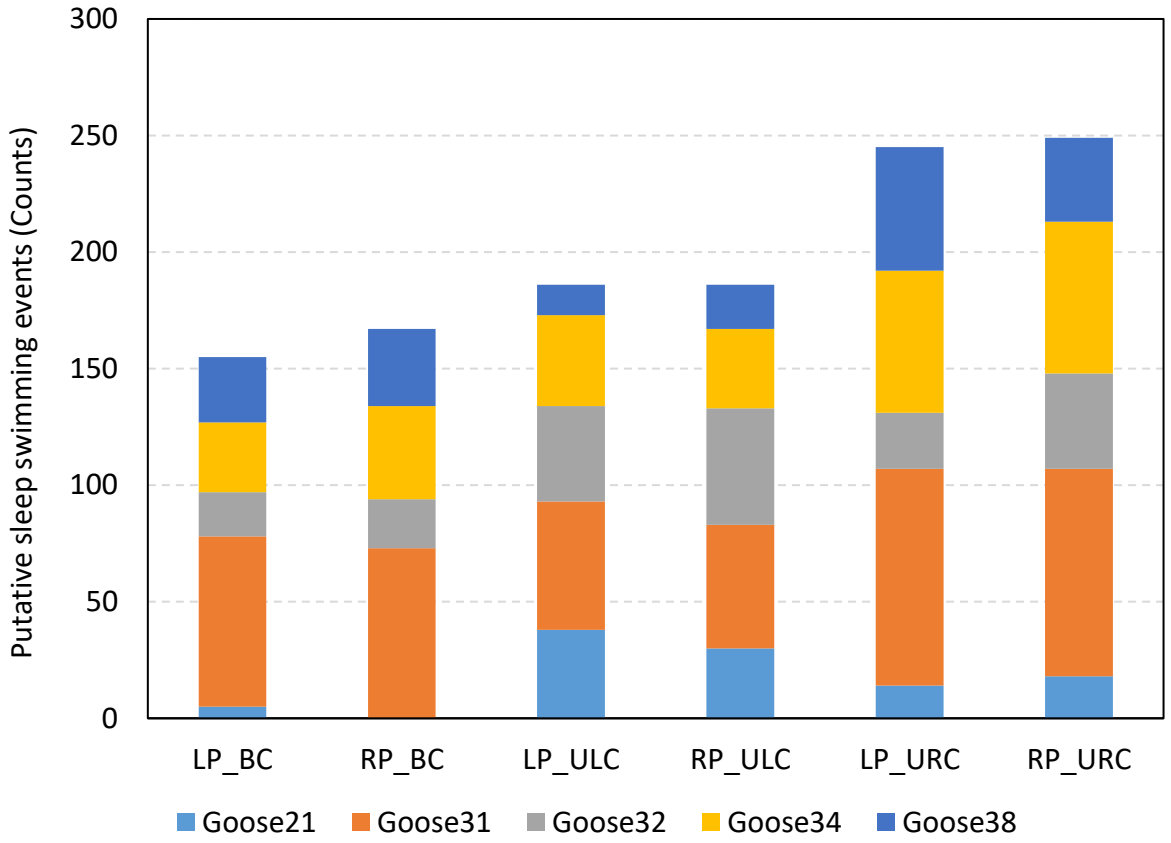


Figure 14: a) An example plot of time synched eye state detections and leg accelerometer recording for goose #31. The peaks in accelerometer recording can be seen to occur simultaneously during eye closure. The simultaneous occurrence of peaks and eye closure refers to sleep-swimming. b) Mean putative sleep-swimming events for each eye closure state across all individuals (n = 5) and all trips. The sleep swimming events are depicted for each leg separately (LP is for left leg paddling events and RP is for right leg paddling events). The whiskers depict the standard error of mean. c) Putative number of sleep swimming events for each individual for each eye state (BC, ULC and URC) separately for each leg (LP and RP).

Paddling feet preference during eye states

Observations from the data show the presence of feet activity during eye closure. However, geese are able to undergo unilateral slow wave sleep, suggesting that there might be a preference of feet contralateral to the side of the goose that shows eye closure. We used poisson mixed model (poisson family with a log link) to predict the total paddling events with the interaction between leg and eye state as fixed effect.

The explanatory power of the model is modest with a conditional R^2 of 0.31 (marginal R^2 = 0.29). We found that the effect of eye state BO is statistically significant and positive on the total paddling events and intercept (beta = 0.65, 95% CI [0.48, 0.82], p < .001; Std. beta = 0.65, 95% CI [0.48, 0.82]). This result is expected because paddling events would be more frequent when geese are awake. On the contrary, the effect of eye states ULC and URC was found to be non-significant (Fig 15). This suggest that the paddling events occurred more often in BO state. The interaction terms between eye state and leg were all non-significant (BO on Leg R: beta = 0.05, 95% CI [-0.19, 0.28], p = 0.691; Std. beta = 0.05, 95% CI [-0.19, 0.28]; ULC on Leg R: beta = -0.03, 95% CI [-0.33, 0.27], p = 0.826; Std. beta = -0.03, 95% CI [-0.33, 0.27] and URC on Leg R: beta = -0.04, 95% CI [-0.32, 0.24], p = 0.801; Std. beta = -0.04, 95% CI [-0.32, 0.24]). Post-hoc test for each eye state to leg interaction revealed no significant preference for any of the legs. (Table 2 summarizes the model formula, contrasts which are the pairwise interactions, the standard error of mean SE, and p values associated with each interaction).

Model Formula	Total paddling events ~ Leg * Eye state + (1 | ID)				
contrast	**estimate**	**SE**	**df**	**z.ratio**	**p.value**
L BC - R BC	-0.0084722	0.1115834	Inf	-0.0759270	1.0000000
L BO - R BO	-0.0563470	0.0453199	Inf	-1.2433182	1.0000000
L ULC - R ULC	0.0249433	0.1037117	Inf	0.2405063	1.0000000
L URC - R URC	0.0277524	0.0900527	Inf	0.3081794	1.0000000

Table 2: Pairwise differences between the eye states

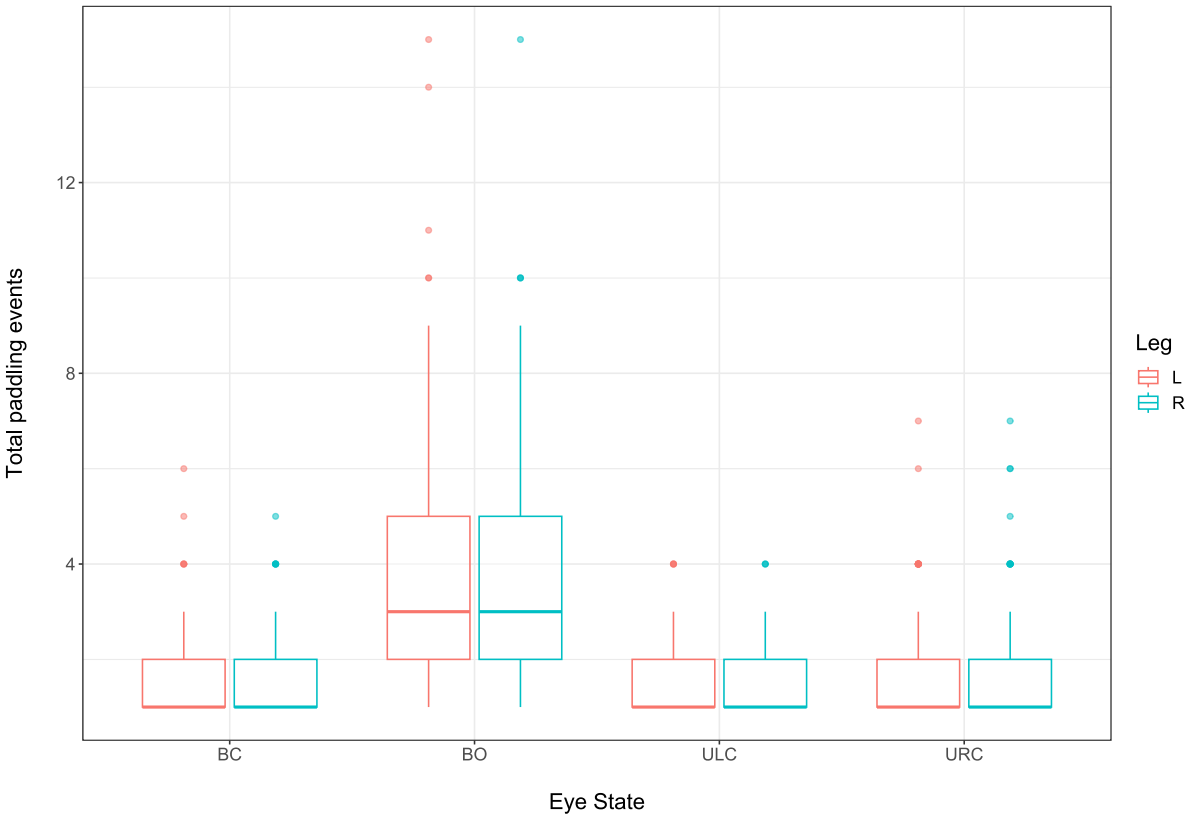


Figure 15: Boxplot of left and right total paddling events for each eye state plotted as mean across all trips and days. L and R in the legend stands for left leg and right leg respectively. The horizontal lines in the box represent median, the boxes represent the first and third quantiles, and the points depict outliers.

Modeling sleep proportion and ontogeny of sleep

a) Full model with all trips included

We expected sleep proportion to increase as the trip number increases. To test this, we fitted a zero-inflated logistic mixed effect model (beta family with a logit link) to predict sleep proportion against the trip number and day as the fixed effects (formula: Sleep proportion ~ “Trip number” + Day + (1 | ID)). The model’s total explanatory power was strong (conditional R^2 = 0.73 and marginal R^2 = 0.15). Interestingly, the “trip number” had a statistically non-significant effect on the proportion of sleep and the estimate for beta was negative (beta = -2.77e-03, 95% CI [-0.02, 0.01], p = 0.732; Std. beta = -0.02, 95% CI [-0.14, 0.10]). The effect of days, on the other hand, was

significant. Day 2 (2022/08/22) had a statistically significant and negative effect on the sleep proportion (beta = -0.56, 95% CI [-0.83, -0.28], p < .001; Std. beta = -0.56, 95% CI [-0.83, -0.28]), similarly day 3 (2022/09/19) had a statistically significant negative effect on sleep proportion (beta = -0.57, 95% CI [-0.86, -0.28], p < .001; Std. beta = -0.57, 95% CI [-0.86, -0.28]). Post-hoc analysis revealed a significant difference between day 1 and day 2, and day 1 and day 3 but no significant difference between day 2 and day 3 (Table 3 summarizes the model formula, contrasts which are the pairwise interactions, the standard error of mean SE, and p values associated with each interaction) (Fig 16).

b) Partial model with reduced trip number

The negative trend is a rather unexpected result from our data. We expected the relationship to be positive due to a build-up of a need for sleep as the experiment progresses. We reduced the number of trips to the first 20 for each day as the trips that occur later during an experimental session are not natural. These latter trips are, in a way, coerced and occur due to the presence of reward. If this is the case we would expect a positive relationship between sleep proportion and trips when we confine our analysis for the initial 20 trips of each day. We fitted a zero-inflated logistic mixed model to test the effect of reduced trip number on sleep proportion. The trip number still did not have a significant effect on sleep proportion (beta = 0.02, 95% CI [-5.57e-03, 0.04], p = 0.140; Std. beta = 0.09, 95% CI [-0.03, 0.22]). The effect of days was significant. Day 2 (2022/08/22) had a statistically significant and negative effect on the sleep proportion (beta = -0.69, 95% CI [-0.99, -0.38], p <.001; Std. beta = -0.69, 95% CI [-0.99, -0.38]), similarly day 3 (2022/09/19) had a statistically significant negative effect on sleep proportion (beta = -0.68, 95% CI [-0.98, -0.38], p <.001; Std. beta = -0.68, 95% CI [-0.98, -0.38]). Although the effect of trip number is not significant, the model had a strong positive R^2 of 0.77 and the R^2 associated with the fixed effect is 0.22. We found a significant difference between day 1 and day 2, and day 1 and day 3 but no significant difference between day 2 and day 3 (Table 4 summarizes the model formula, contrasts which are the pairwise interactions, the standard error of mean SE, and p values associated with each interaction) (Fig 17).

Independent of the number of trips, we found a consistent negative effect of the experimental days on the sleep proportion. This suggests age of the geese might affect the amount of sleep that they undertake.

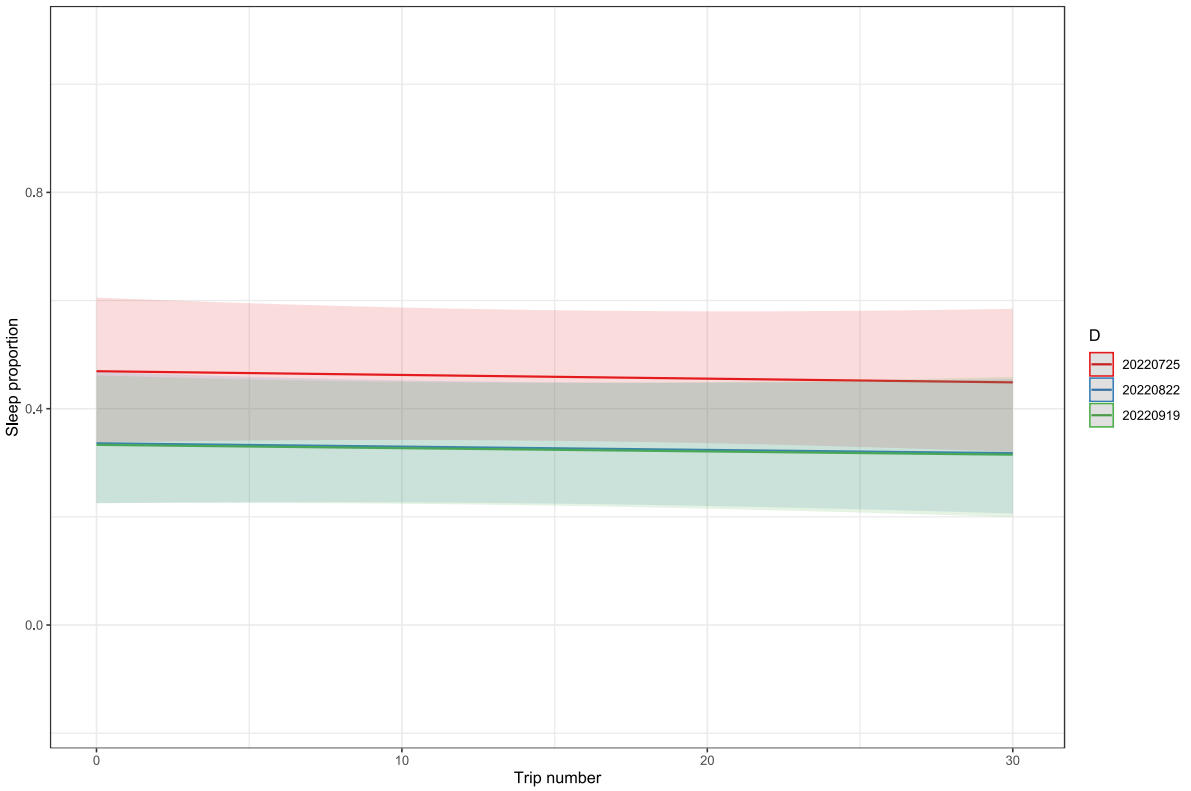


Figure 16: Sleep proportion (all eye states) against trip number shows a slight negative trend when all the trips are modelled. Note the overlap between day 2 and day 3 trendlines. The two days as indicated by the pairwise comparison are remarkably similar.

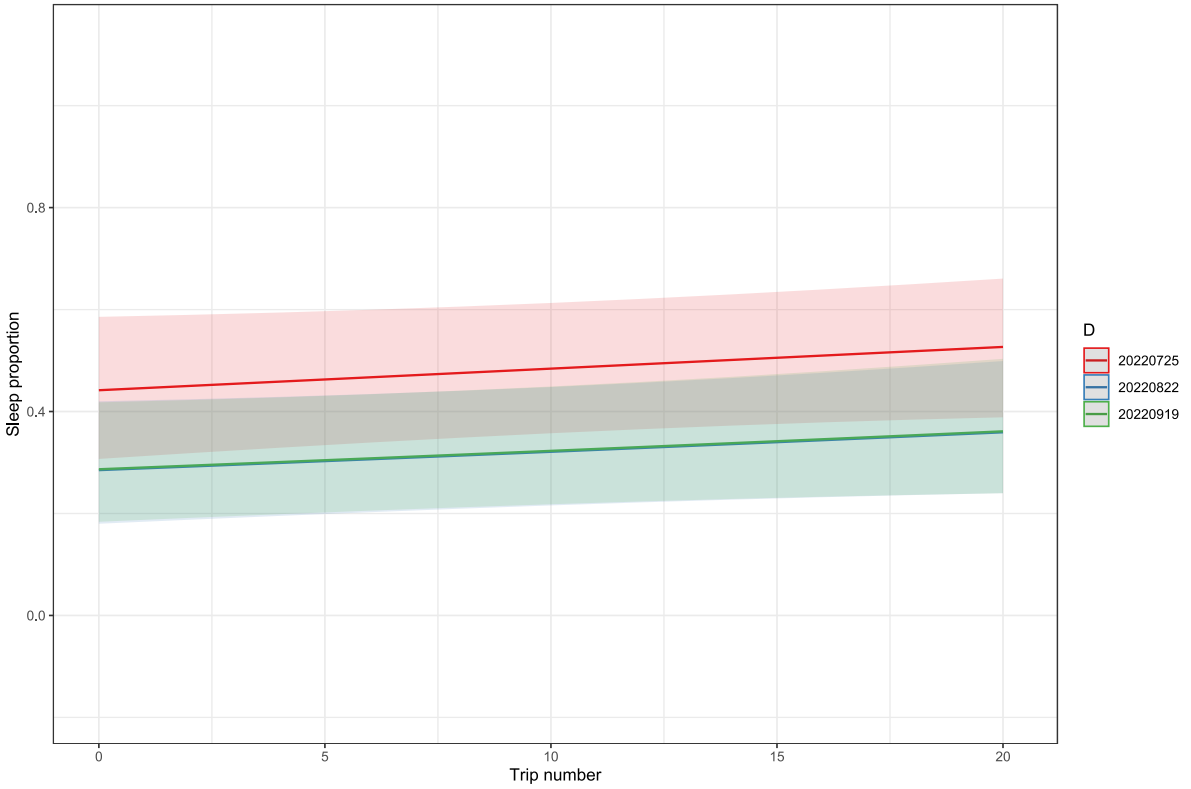


Figure 17: Sleep proportion (all eye states) against partial number of trips (n = 20) shows a slight positive trend. Note the overlap between day 2 and day 3 trendlines. The two days as indicated by the pairwise comparison are remarkably similar.

Model Formula	Sleep proportion ~ “Trip number” + Day + (1 | ID)				
contrast	**estimate**	**SE**	**df**	**z.ratio**	**p.value**
D20220725 - D20220822	0.5581453	0.1396255	Inf	3.9974455	**0.0001921***
D20220725 - D20220919	0.5711110	0.1485688	Inf	3.8440843	**0.0003630***

D20220822 - D20220919	0.0129657	0.1608739	Inf	0.0805955	1.0000000

Table 3: Pairwise differences between the days of experiment for full model with all trips.

Model Formula	Sleep proportion ~ “Trip number (first 20 trips)” + Day + (1 | ID)				
contrast	**estimate**	**SE**	**df**	**z.ratio**	**p.value**
D20220725 - D20220822	0.7580991	0.1827149	Inf	4.1490819	**0.0001001***
D20220725 - D20220919	0.6309698	0.1734104	Inf	3.6385934	**0.0008224***
D20220822 - D20220919	-0.1271293	0.1912906	Inf	-0.6645871	1.0000000

Table 4: Pairwise differences between the days of experiment for partial model with 20 trips.

Sleep proportion during each eye state

Interval plots between the sleep proportion in different eye states and the experimental days show a decrease with the days (Fig 18). To understand how the proportion of sleep in each eye state (BC, ULC, URC) varies with the trip number, we calculated the

proportion of BC, ULC and URC and modelled the three eye states individually to make predictions based on our data.

BC eye state:

We used a zero-inflated logistic mixed model (beta family with a logit link). BC sleep proportion was kept as the dependent variable, the fixed effects are trip number and day and the random effects are the identity of the animals. The model had a substantial explanatory power with a conditional R^2 value of 0.44 and the marginal R^2 value of 0.05. The effect of trip number was positive but not statistically significant (beta = 0.01, 95% CI [-3.28e-03, 0.03], p = 0.118; Std. beta = 0.10, 95% CI [-0.02, 0.22]). Effect of day 2 and day 3 was negative and statistically significant (day2: beta = -0.10, 95% CI [-0.39, 0.19], p = 0.506; Std. beta = -0.10, 95% CI [-0.39, 0.19]; day3: (beta = -0.25, 95% CI [-0.57, 0.07], p = 0.126; Std. beta = -0.25, 95% CI [-0.57, 0.07]). Across the experimental days, we found no significant difference (Table 5 summarizes the model formula, contrasts which are the pairwise interactions, the standard error of mean SE, and p values associated with each interaction) (Fig19).

Model Formula	BC sleep proportion ~ “Trip number” + Day + (1 | ID)				
contrast	**estimate**	**SE**	**df**	**z.ratio**	**p.value**
D20220725 - D20220822	0.0980799	0.1476172	Inf	0.6644201	1.0000000
D20220725 - D20220919	0.2506083	0.1638865	Inf	1.5291576	0.3786761
D20220822 - D20220919	0.1525284	0.1856797	Inf	0.8214598	1.0000000

Table 5: Pairwise differences between the days of experiment for BC sleep proportion model.

ULC eye state:

We fitted a zero-inflated logistic mixed model (beta family with a logit link). ULC sleep proportion was kept as the dependent variable, the fixed effects are trip number and day and the random effects are the identity of the animals. The model had a strong explanatory power with a conditional R^2 value of 0.56 but a marginal R^2 value of 0.24. The effect of trip number was negative but not statistically significant (beta = -0.01, 95% CI [-0.03, 8.62e-03], p = 0.292; Std. beta = -0.07, 95% CI [-0.21, 0.06]). Effect of day 2 was positive and non-significant (beta =0.29, 95% CI [-0.03, 0.61], p = 0.071; Std. beta = 0.29, 95% CI [-0.03, 0.61]), whereas the effect of day 3 was negative and significant (beta = -0.59, 95% CI [-0.93, -0.24], p < .001; Std. beta = -0.59, 95% CI [-0.93, -0.24]). Interestingly for ULC sleep proportion there was significant difference between day 1 and day 3, and day 2 and day 3 but no significant difference between day 1 and day 2, which is in contrast to BC eye state (Table 6 summarizes the model formula, contrasts which are the pairwise interactions, the standard error of mean SE, and p values associated with each interaction) (Fig 19).

Model Formula	ULC sleep proportion ~ “Trip number” + Day + (1 | ID)				
contrast	**estimate**	**SE**	**df**	**z.ratio**	**p.value**
D20220725 - D20220822	-0.2919587	0.1618079	Inf	-1.804355	0.2135272
D20220725 - D20220919	0.5886879	0.1760637	Inf	3.343608	**0.0024809***
D20220822 - D20220919	0.8806466	0.1900420	Inf	4.633957	**0.0000108***

Table 6: Pairwise differences between the days of experiment for ULC sleep proportion model.

URC eye state:

We fitted a zero-inflated logistic mixed model (beta family with a logit link). URC sleep proportion was kept as the dependent variable, the fixed effects are trip number and day and the random effects are the identity of the animals. The model had a strong explanatory power with a conditional R^2 value of 0.65 but a marginal R^2 value of 0.48. The effect of trip number was negative but not statistically significant (beta =-1.07e-03, 95% CI [-0.02, 0.02], p = 0.911; Std. beta = -8.00e-03, 95% CI [-0.15, 0.13]). Effect of day 2 was negative and non-significant (beta = -0.04, 95% CI [-0.36, 0.28], p = 0.809; Std. beta = -0.04, 95% CI [-0.36, 0.28]), whereas the effect of day 3 was positive and significant (beta = 0.71, 95% CI [0.37, 1.06], p < .001; Std. beta = 0.71, 95% CI [0.37, 1.06]). For ULC sleep proportion there was significant difference between day 1 and day 3, and day 2 and day 3 but no significant difference between day 1 and day 2 (Table 7 ULC sleep proportion there was significant difference between day 1 and day 3, and day 2 and day 3 but no significant difference between day 1 and day 2, which is in contrast to BC eye state.) (Fig 19).

Model Formula	URC sleep proportion ~ “Trip number” + Day + (1 | ID)				
contrast	**estimate**	**SE**	**df**	**z.ratio**	**p.value**
D20220725 - D20220822	0.0396777	0.1642292	Inf	0.2415994	1.0000000
D20220725 - D20220919	**-0.7136264**	**0.1771212**	**Inf**	**-4.0290282**	**0.0001680***
D20220822 - D20220919	**-0.7533041**	**0.1887984**	**Inf**	**-3.9899917**	**0.0001982***

Table 7: Pairwise differences between the days of experiment for URC sleep proportion model.

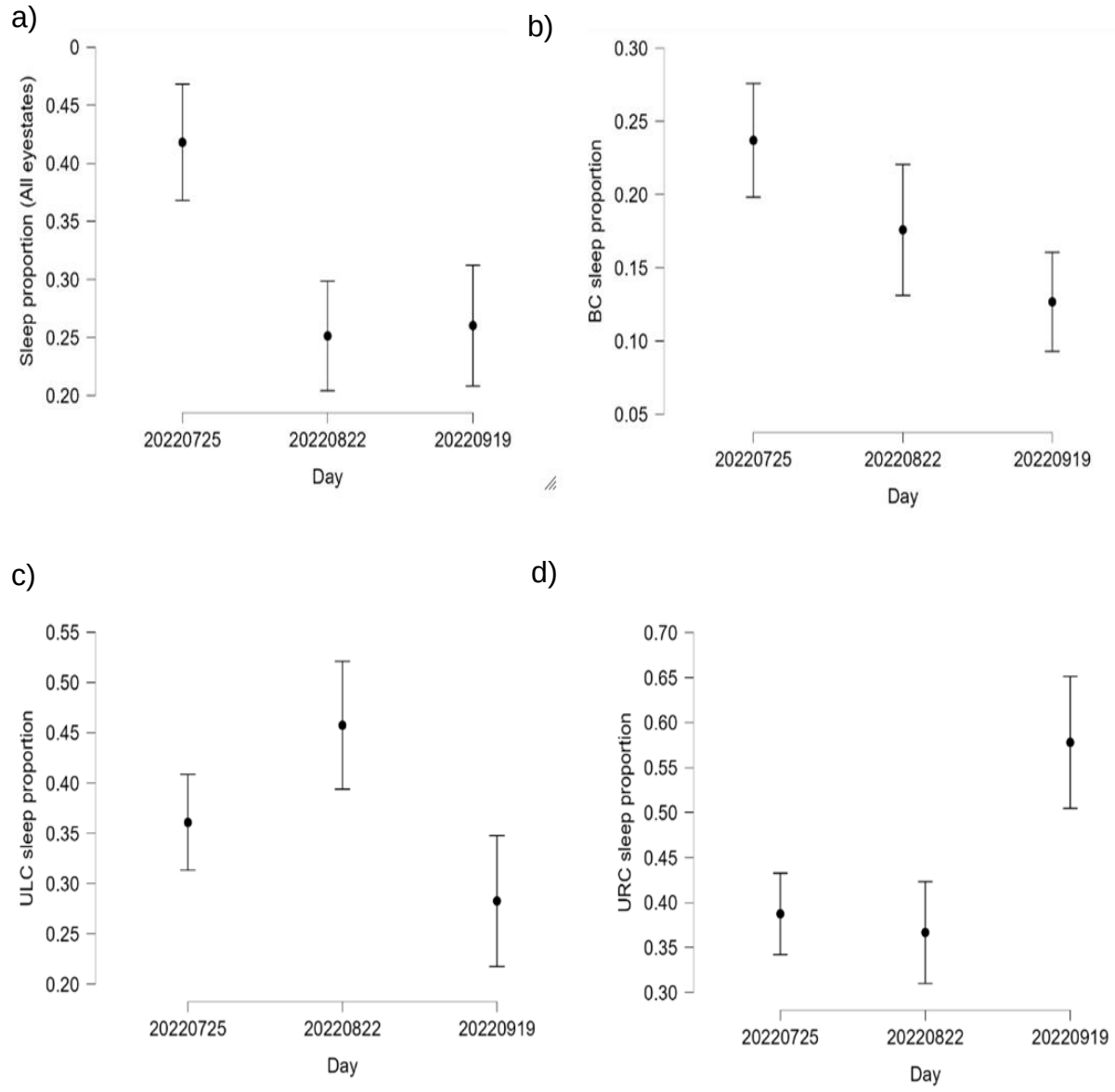
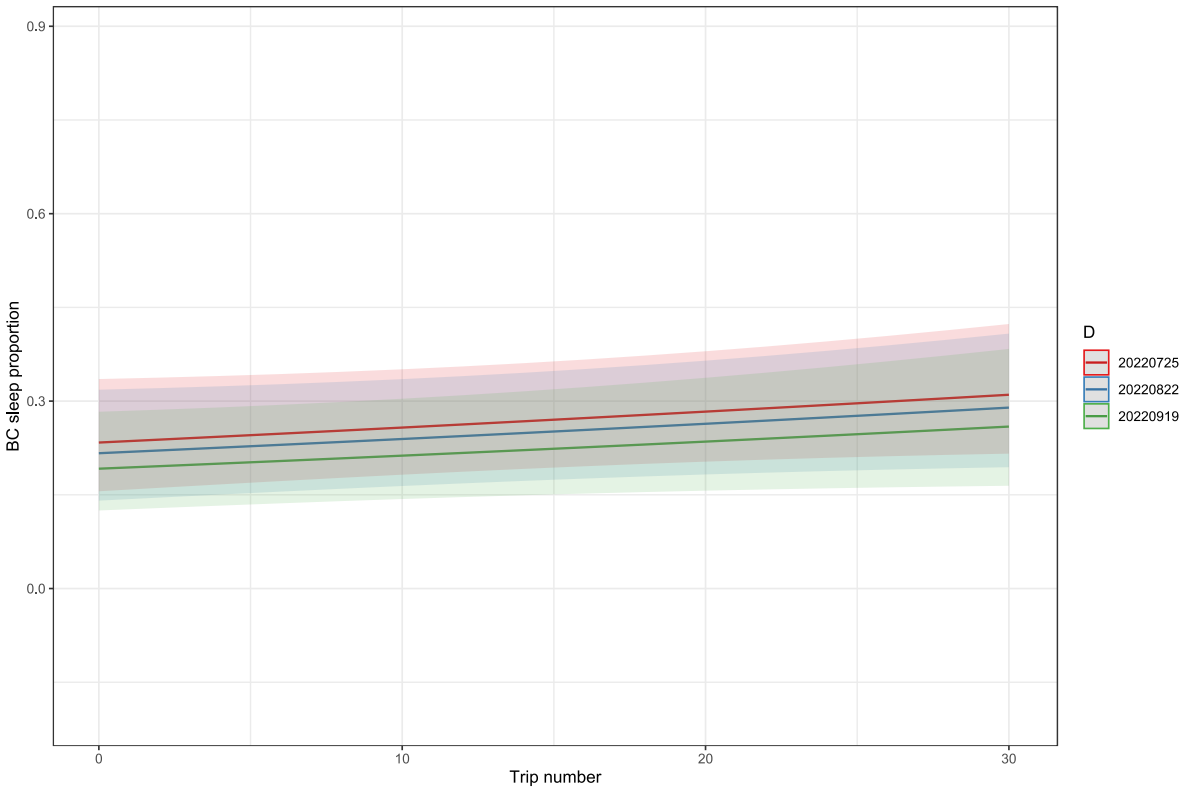
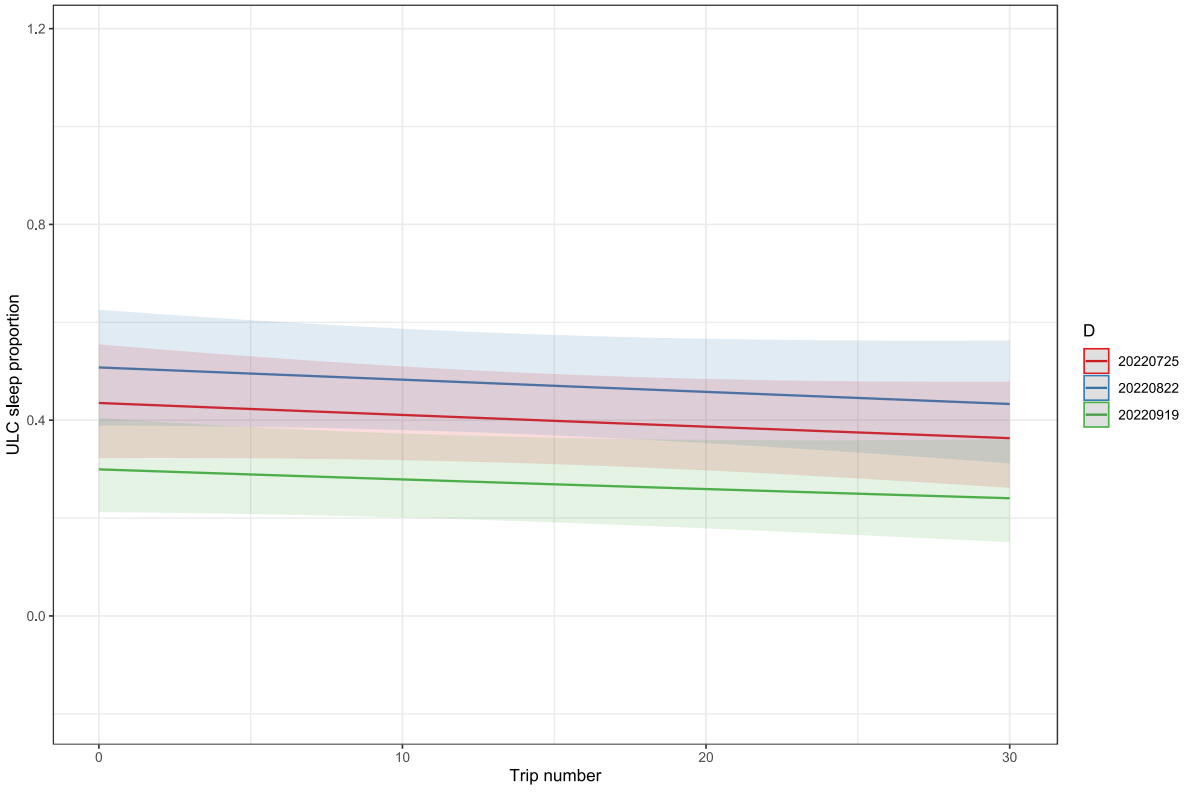


Figure 18: a) Mean sleep proportion against experimental day for all geese for a) all eye states, b) both eyes closed, c) unilateral left eye closed, and d) unilateral right eye closed. The whiskers represent the 95% confidence interval of the mean.

a)


0.9
0.6
0.3
0.0
BC sleep proportion
0
10
20
30
Trip number
D
20220725
20220822
20220919

b)


1.2
0.8
0.4
0.0
ULC sleep proportion
0
10
20
30
Trip number
D
20220725
20220822
20220919

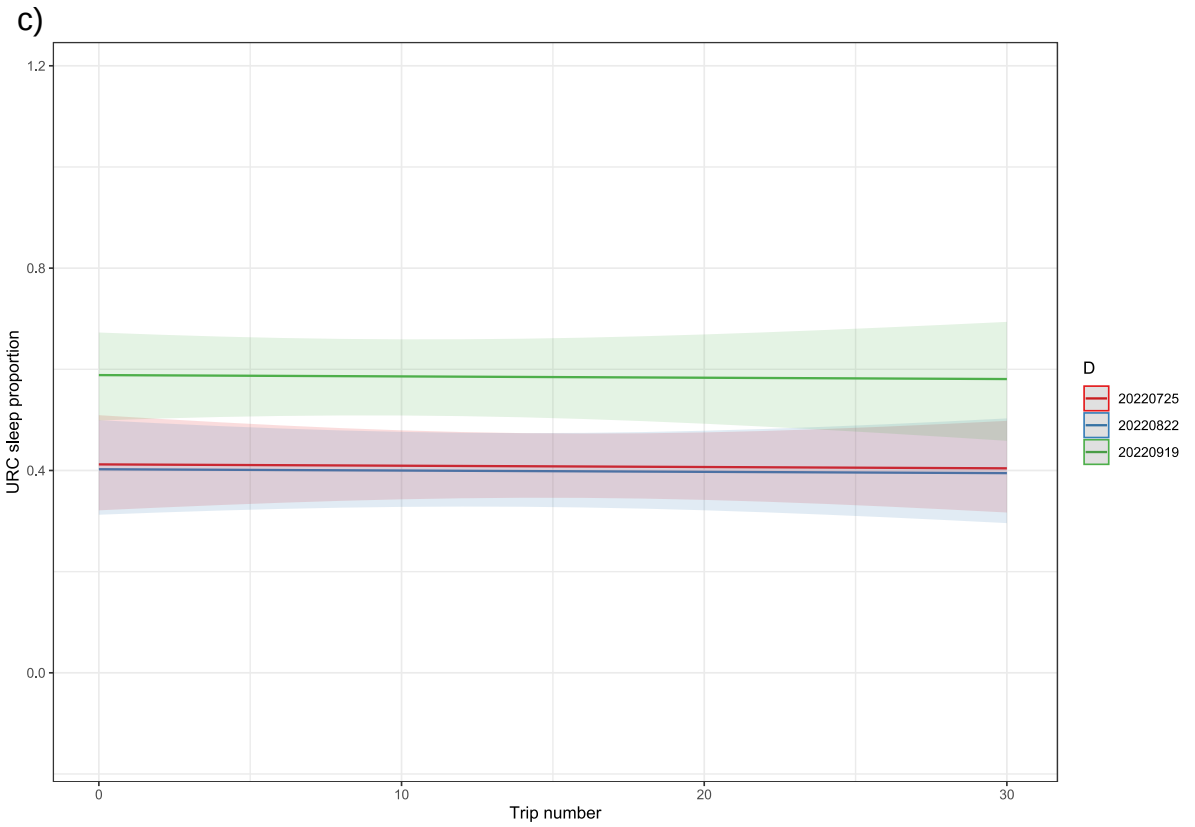


Figure 19: a) BC sleep proportion against trip number shows a slight positive trend. The trend is observable in all the three days. b) ULC sleep proportion against trip number shows a slight negative trend. c) ULR sleep proportion against trip number shows a slight negative trend as well.

Chapter 4 Discussion

Preliminary observations and the results of the project clearly indicate that Canada geese are able to sleep while they paddle their feet. Paddling events occurred concurrently in all states of eye closure. Interestingly, the model generated from our data could not show a preference in the sleep states. A parsimonious expectation for leg preference considering the literature (Lyamin et al., 2008, 2018) would be that the geese would prefer the leg contralateral to the eye that is closed. The locomotory activity of geese during unilateral sleep states as well as bilateral sleep states seems to be symmetrical from our data. The result is interesting in relation with marine mammals. Unlike fur seals there was no relationship between the side of the eye closure and paddling of the feet. Instead, geese performed swimming with both their feet to a similar extent regardless of which eye is closed and this resembles the way dolphins swim (Lyamin et al., 2018). Being bilateral swimmers, dolphins swim by using both of their flippers despite having one brain hemisphere asleep. What is more interesting about geese is that geese perform bilateral paddling while having both of their eyes closed unlike dolphins that rarely sleep with both eyes closed. It seems that although one or both brain hemispheres are asleep motor control is somehow uncoupled from them and motor centres remain active.

Presumably, it might be that visual brain hemispheres and related motor control regions could undergo a local neuronal lapse (Nir et al., 2017), which could manifest as eye closure, and the cortical motor regions associated with feet might be kept awake to control paddling. These explanations can be tested in bird brains non-invasively with the use of functional ultrasound imaging (Mace et al., 2013) for instance. However, the major limitation of this technique is that it cannot be used in the wild when such behaviours are more relevant. Flight simulation in wind tunnels and more accessible ways to measure the brain activity during extended fight can help relate the findings that we observed here, with distance forager like great frigate birds and migrating birds. Such experiments may help answer the long-standing question of how exactly birds that migrate over the ocean for days or birds that fly over the ocean to forage might sleep. Identifying mechanisms underlying the sleep-swimming

behaviour would be the first step to probe the mechanisms of sleep in flight. One potential mechanism could be that of a central pattern generator (CPG) which might be in play and one of the most likely locations of such neurons or networks would be in the brain stem. But this mechanism by itself fails to explain the absence of a regular pattern of left – right – left – right, if a CPG was in place. It might also be possible that there is no inherent pattern in which the geese prefer to paddle and individual differences might get lost due to cumulative nature of the analysis that we performed.

On an ecological standpoint for the geese, it seems that they are most benefitted by staying within the ranks of their flock and they appear to make compromises to achieve this. Being in a flock provides them with safety and dilution effect also comes into play further increasing their chances of survival. Sleep-swimming might be the way that allows the geese to sleep while traveling thereby coping with both social pressures and individual physiological pressures.

Our data does not show a huge reduction in the number of paddling events when the goose has both of its eyes closed, however the overall number of paddling events are lesser in the case of both eyes closed (Fig 14b). Birds, like mammals, show muscle atonia when they undergo REM sleep (Dewasmes et al., 1985, Rattenborg et al. 2019)), but unlike mammals the muscle atonia is partial and not every muscle of the body goes into atonia. Notably, waterfowls like geese are known to balance on one foot during REM sleep. However, a reduction in paddling events hints at the possibility that the geese might also undertake REM sleep. But it might also be possible that REM only occurs rarely during both eyes closed state and it rather occurs during gliding motion. The results from EEG data are needed to clarify if and how the REM sleep relates with paddling activity during the both eye closed state.

A consistent trend in our partial model with fewer trips has been a positive one across days, although not significant due to sample size. Our data does not support the most plausible hypothesis that the geese accumulate a sleep deficit during the session and therefore start to sleep swim at the expense of visual vigilance. We observe no

significant effect of trip number on the sleep proportion, suggesting that the geese show no preference towards sleeping in one state over the other. It would be expected for the geese to accumulate a need for sleep as the experiment progresses and, along the experiment, a transition towards a bilateral sleep state over a unilateral one. Although the trend from the data supports this explanation, it is not statistically significant. The result suggests that geese can fulfil their need for sleep in either bilateral or unilateral sleep states. However, this contradicts the assumption that bilateral sleep would provide a more efficient way to recoup the losses made by staying awake to stay within the ranks of the flock, vigilance or obtaining food. Moreover, the presence of a reward in our study might affect the expectation of increased sleep with an accumulation of sleep with trip number as the reward promotes competition within the hierarchical arrangement of the flock demanding each goose to be vigilant to maximize their chances of acquiring the reward. These explanations are not necessarily mutually exclusive and can occur all at once.

Across days the eye closure proportion differed and the trend was observed to be negative. Although for a sample size of 3 day does not provide the full picture but the geese in our experiment reached the full adult size by the day 3 of experimental day. Furthermore, our complete data is interspersed with more observations that we made within the three months. We aim to analyse the data that lies between the extremes of day 1 and day 3 which would provide us with a more complete picture. In humans, it is known that as the age of the individual increases the amount of deep sleep (or SWS) goes on decreasing until adulthood where it stabilizes to a certain level (Ohayon et al., 2004; Li et al., 2018). In mammals, other than humans this trend has also been observed (Jouvet-Mounier et al, 1969; Frank, 2020). However, it must be noted that the decrease is strongly associated with REM sleep and the SWS whereas the NREM stage 1 and stage 2 proportion of sleep is observed to increase with age (Li et al., 2018). The models from our data suggest the presence of a similar pattern in the geese. The major limitation is that the sample size across days is small. Nonetheless, the effect of day has been consistent in all our sleep proportion models and it has been significantly negative in two of them.

A natural question in this kind of study would be the reliability of eye closure as the proxy for sleep. Sleep in mammals and birds has been studied across many species. A number of studies on mammals and birds alike have examined the relationship between eye state and sleep-related brain activity as measured with EEG (Ogilvie and Wilkinson, 1988; Nir et al. 2017). The studies show a strong relationship between eye closure state and the brain waves corresponding to sleep. Therefore, the claim that the birds close their eyes just to blink can be safely refuted. Moreover, birds clean and moisten their eyes by rapidly sweeping the transparent nictitating membrane across the surface of the lens. In this way, birds minimize the time during which visual vigilance is compromised. By contrast, closure of the eyelid during sleep, clearly sacrifices visual vigilance. A related concern is that geese prefer to close their eyes to avoid direct exposure to the sun. However, the presence of both eye closure states simultaneously does not explain the hypothesis of sun exposure avoidance. Moreover, the amount of sleep in both eye closure states and the unilateral closure for either of the eyes is found to be comparable in our study if not more in some individuals scored in our study. Another concern would be relatively short duration of eye closure that we interpret as sleep. The average duration of eye closure during active paddling events was observed to be 7.25 $\pm$ 0.45 s for the right eye and 6.41 $\pm$ 0.39 s for the left eye. The duration of episodes of SWS during the flight of frigate birds as measured using EEG were found to be very close to what we observed, 10.89 $\pm$ 0.81 s (Rattenborg et al., 2016). EEG data from Canada geese is absolutely essential to support the presence of sleep but behaviourally our study demonstrates a strong possibility for the presence of the phenomenon.

The use of Deeplabcut is feasible and our trained model is able to detect eye states in experimental conditions. Anecdotal observations of the videos suggest that the model does a good job of detecting eye closure. However, the model needs validation with human labeled data to support the pose estimates made by the model. Deeplabcut was useful in reducing the analysis time, but the lack of bird identity tracking considerably increased the post processing time of correcting identities. A relatively simple test called the Bland-Altman test (Lecomte et al., 2021; Giavarina, D, 2015) can be performed to compare the sleep durations measured by deeplabcut model

predictions and the manual annotations made with a behavior examination software like Solomon coder and we are well underway with this process.

Our study lays the basic groundwork to test the presence of sleep-swimming unequivocally using techniques involving EEG, head accelerometer and EMG. The subjects in our study were instrumented with the EEG data logger and we are in the data extraction phase of this experiment. As discussed earlier, eye closure is a very good proxy for sleep but the credibility of the proxy will only be strengthened if EEG data is also used. In addition to providing a reading of the brain waves during awake and sleep states, the EEG recordings also provide us ways to determine if REM sleep occurs in these relatively small bouts of sleep-swimming. The accuracy, accessibility and robustness provided by the computer vision algorithm deeplabcut combined with precise physiological measurements done using EEG, EMG and head accelerometers will provide us novel insights into the sleep-swimming behavior. The implications from the study on a clinical standpoint are broad and can help us unravel the unknown mechanisms that affect sleep in humans. An example of such an implication is the case of sleep-walking in humans, the study of which lacks a suitable model organism and as a result the phenomenon remains understudied in humans, and lacks effective treatment.

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