Quantifying Diel Change in the Metabolic Rate of

the Clearnose Skate (*Rostroraja eglanteria)*

by

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ABSTRACT

In this study, we measured the routine metabolic rate (RMR) for the Clearnose Skate (*Rostroraja eglanteria*) throughout the diel cycle to understand activity levels. Intermittent flow respirometry measured oxygen consumption of skates, a proxy for metabolic rate, during the day and night. Subtracting the day and night RMR's, two skates saw higher RMR's during the day phase (Skate 1 - average difference of 63 mg O_2/hr/kg and standard deviation of 31.1 mg O_2/hr/kg, Skate 3 - average difference of 21 mg O_2/hr/kg and standard deviation of 37.1 mg O_2/hr/kg) while one saw higher RMR's at night (Skate 2 - average difference of -50 mg O_2/hr/kg and standard deviation of 68.7 mg O_2/hr/kg). There was no significant difference between phases. To observe acclimation influence, the first RMR conducted was removed from each skate. Two skates showed contradicting patterns (Skate 2 - average difference of 9.5 mg O_2/hr/kg and standard deviation of 2.1 mg O_2/hr/kg, Skate 3 - average difference of -11 mg O_2/hr/kg and standard deviation of 4.24 mg O_2/hr/kg). No significant statistical difference was found. The first trial RMR's suggest that skates were stressed when introduced to the system, thus future applications should increase acclimation time and decrease animal handling.

Chapter 1

INTRODUCTION

1.1 Biological Rhythms

In all animals, environmental cycles influence physiology and behavior to develop endogenous (internal) patterns known as biological rhythms. Biological rhythms are entrained by behavioral patterns resulting from an exogenous cue (external influence such as temperature or sunlight). An example of environmental cues influencing behavior is the diel vertical migration (DVM). This is the large migration of zooplankton moving up in the water column at night to feed on phytoplankton and going down during the day to avoid being seen by predators (Brierley, 2014; Häfker et al, 2017). These diel patterns represent a behavioral or physiological shift between night and day over the diel cycle, thus making it a circadian rhythm (24hr cycle). Many species utilize circadian rhythms to conserve energy as a means of survival. If endogenous rhythms develop and increase fitness, the species evolves over time with these dynamics becoming an innate part in the animals' physiology (Sharma & Joshi, 2002).

A biological rhythm can be detected and analyzed by isolating an organism from its natural cyclical or periodic environmental pattern. If an endogenous rhythm continues in the absence of its coupled exogenous cue, then it is likely that a biological rhythm has been internalized as a biological clock. The organism will then continue this behavior regardless of a change in environmental cues. This is seen in bats instinctively emerging from their caves at dusk (without seeing the sun) because the bats have internal clocks that register the sunlight shifts at predictable times of day (Hope & Jones, 2013). Thus, an animal with a biological clock can exhibit cyclic activities regardless of changes in environmental cues. In elasmobranchs, the Epaulette Shark was found to demonstrate cyclic rest during a period of constant darkness, confirming the existence of diel biological clock (Wheeler et al, 2022). In contrast, a biological rhythm without a biological clock will appear as a disruption in cyclic behavior during a dramatic shift in an exogenous factor (light exposure for this study). Port Jackson sharks and Draughtsboard Sharks demonstrated this disruption as their cyclic swimming patterns were initially consistent during a 12hr:12hr dark:light exposure. Then, their pattern became more random during the constant light and constant dark exposures, confirming a biological rhythm with environmental cues and not a continued, internalized biological clock (Kelly et al, 2020). For this study, metabolic rates (calculated from oxygen consumption declines) of Clearnose Skates were used as a proxy for measuring activity level between diel phases.

1.2 Metabolic Rate

Metabolic rate is the amount of energy it takes for an organism to function and sustain itself. As animals exhibit more intense activities such as swimming or running, their energy utilization increases, thus increasing their metabolic rate. For this study, the routine metabolic rate (RMR) was used where metabolic rates were measured when the animal was fasted and left to exhibit its natural behavior (Chabot et al, 2016). Typically, the RMR is represented by heat loss resulting from an animal expending energy. Since it is difficult to measure heat loss in water, oxygen consumption is used as a proximation of metabolic rate for aquatic animals (Nelson & Chabot, 2011).

As aquatic animals use oxygen for biological activities, they extract oxygen from the water and the levels drop in the tank, allowing us to use oxygen consumption as a proxy for metabolic rate. If the animal is exhibiting swimming activity, there will be higher oxygen consumption (as well as RMR) than if it is resting (Forstner & Wieser, 1990). RMR was measured with a method called intermittent flow respirometry, in which we analyze the dissolved oxygen (DO) consumption of a marine animal in a sealed tank. This consumption can also vary depending on the intensity of the swimming activity or biological processes occurring such as stress or acclimation. Reproductive processes can also increase RMR depending on sex and maturity of an animal as reproduction can increase energetic demand.

1.3 Clearnose Skate (*Rostroraja eglanteria)*

The Clearnose Skate belongs to the subclass Elasmobranchii, which is a subclass of cartilaginous fishes that include sharks, skates, and rays. They are abundant mesopredators that inhabit Delaware Bay throughout the year and feed on bottom-dwelling creatures such as mussels, shrimps, crabs, and a variety of fishes (Packer et al, 2003; Woodland et al, 2010). In marine food webs, this family of skates (Rajidae) play an important ecological roles as mid trophic level consumers of benthic prey, and they are also prey to larger predators like the Sand Tiger Shark, *Carcharias taurus* (Lucifora et al, 2009). Within the food web, energy expenditure is crucial for the Clearnose Skate because they may be biologically unequipped to sustain swimming activity for long periods as seen in the Little Skate, *Leucoraja erinacea,* as it has a similar body shape (Di Santo & Kenaley, 2016). The lack of a propulsive caudal fin, modified pelvic fins for “walking” along the bottom, and negative buoyancy make it more energetically costly for skates to maintain swimming in the water column, thus limiting their ability to travel long distances.

Though abundant along the eastern coast of the United States, little is known about the Clearnose Skate's activity patterns or the environmental factors that impact their behavior. The species has little limitation in terms of salinity as they can be found in levels as low as 17 ppt compared to 26-27 ppt where they are typically found. Clearnose Skates are also found in a large temperature range of 6 to 20 degrees Celsius (Packer et al, 2003). This species has been observed migrating out of Delaware Bay during winter to warmer temperatures offshore (Packer et al, 2003). In terms of reproduction, it is unclear where and when this species gathers to mate, but it is known that egg deposition occurs in the spring (Fitz & Daiber, 1956).

With studies focused on circadian rhythms and activity patterns in elasmobranchs, the Clearnose skate is left out of the conversation. Previous studies have investigated diel vertical migrations in other skate species using data storage tags and Pop-off archival tags, which recorded depths and temperatures in the surrounding waters of the skate for a short period of time (Humphries et al, 2017; Peklova et al, 2014). These studies have indicated that skate species, such as the Arctic Skate and the Blonde Skate, frequently travel to shallower waters during the night to forage and remain in deeper waters during the day.

Only recently have scientists started examining the rhythmicity of activity levels in elasmobranchs specifically using respirometry. One such study analyzed the Draughtsboard Shark using respirometry and identified putative sleeping behavior (lower metabolic rates) in response to a 12hr:12hr light:dark period (Kelly et al, 2022). The sharks studied were filmed in the respirometer and the videos were analyzed to find indicators of sleep such as resting on the bottom of the tank and eye closure. The combination of respirometry and video analysis revealed that resting on the bottom (rather than eye closure) was an indicator of sleep with lower metabolic rates. The evidence of resting periods in this species gave new insights into previously unknown survival methods for energy conservation.

The main objective of this study was to understand when Clearnose skates are naturally most active (i.e., when metabolic rates were higher) and determine if there is a circadian rhythm attributed to resting or sleeping behavior in this species. This thesis uses respirometry to measure potential changes in metabolic rate between night and day. Based on previous literature, it is theorized that the Clearnose Skate will exhibit nocturnal behavior (higher metabolic rates at night) as seen in other skate species.

Chapter 2

METHODS AND MATERIALS

2.1 Animal Housing

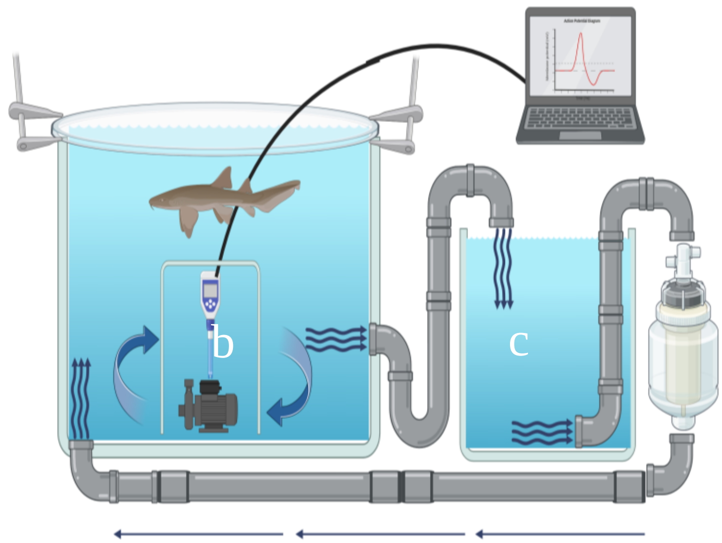
During the summer of 2024, three mature skates were collected using trawl nets from Delaware Bay off the coast of Lewes Beach, DE. They were housed in a 3000L tank within the Smith Lab at the University of Delaware campus in Lewes, DE. They were exposed to 12hr lights on (day) and 12hr night lights off (night) cycles for a month prior to testing and were fed a diet of shrimp (2-3 pieces every other day). The water was changed and water quality (salinity, pH range, temperature etc.) was monitored with the API Saltwater Master Test kit weekly. The tank was maintained at a salinity range of 28-32 ppt and temperatures between 20-21 degrees C to maintain constant experimental conditions.

After testing, skates were released back into Delaware Bay in early October of 2024 as to not subject them to freezing temperatures during the winter months. Skates were housed in the Smith Lab since the original tank system acquired a disease where the skates in the system became ill and suffered mortality.

2.2 Intermittent-flow Respirometry

To measure skate oxygen consumption rates and calculate metabolic rates, intermittent flow respirometry was employed. The system setup outlined in the studies by Rosewarne et al. (2019) and Svendsen et al. (2015) were used with specific water quality parameters (salinity, temperature, etc.) and timings as detailed in Schwieterman et al. (2019). Water was supplied from Delaware Bay (direct pipeline to lab with a sock filter attached) where it remained in large settling tanks until it reached

20 degrees Celcius and most particulates had settled out. Salinity ranged from 28 – 32 ppt during trials. The equipment and system were provided by the TRASER Lab where adjustments were made based on time and material constraints. The respiratory system was housed in the Fisheries and Aquaculture Innovation Center (FAIC).



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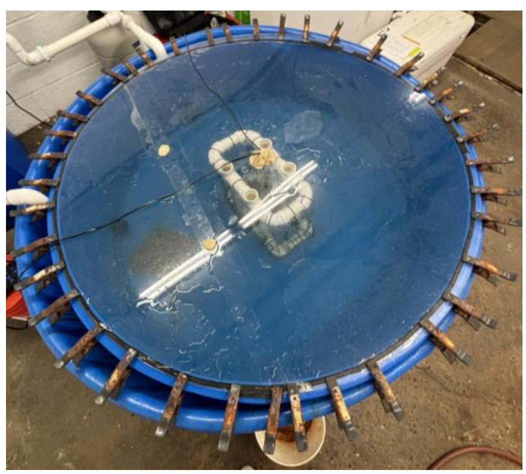

Figure 1. The design of the respirometry system (1) is utilized for data collection. The main tank (a) holds the skate (479 L) and YSI (b) reading DO percentage. The flush tank (c) holds the oxygenated water for the flushing process. Curved arrows indicate water flow during flushing process and arrows in main tank

indicate pump flow during data collection. Picture of the system during day measurement (2).

The three mature female skates had disk widths between 39.5 cm - 42 cm and masses between 1.15 kg - 1.42 kg. To eliminate the energetic effect of specific dynamic action (energetic cost of digestion processes), the skates were fasted for 48 hours and then put into the static respirometer of 479 L (large, sealed tank in Fig. 1) (Chabot. K. et al, 2016). They had an acclimation period of 12 hours (time to adjust to new environment) in the open system with an air stone to prevent DO levels from becoming hypoxic, which is less than 2-3 milligrams of oxygen per liter of water. Below this oxygen level (critical oxygen tension or Pcrit), the metabolic rate is compromised as the animal utilizes an anaerobic metabolism rather than aerobic metabolism (Rosewarne et al, 2016).

Before the trial began, the air stone was removed, and the system was flushed with oxygenated water, sealed with an acrylic lid, and clamped to block gas exchange between water and the atmosphere. The DO% in the water was recorded using a logging YSI multiparameter meter over a 1.5 hour period, then the system was flushed with fresh water to reoxygenate the system for five minutes (maintain a dissolved oxygen value above 80%). DO% measurements were taken every minute for a total of 3 hours during the light hours (collected between 9:30am – 1:30pm) and during the dark hours (collected between 9:30pm - 1:30am), thus resulting in 2 metabolic rates (trials) per experiment with one flush in the beginning and in the middle (Table 1). This allowed for equal exposure to diel conditions during the trial since lights were turned on at 7am and turned off at 7pm.

To account and correct for additional respiration within the tank such as algae photosynthesis and microbial respiration, a background measurement was collected for 6 hours without the skate in the system. At the end of the trials, the skates were returned to their holding tank. Due to its agitated behavior, Skate 3 was observed every 15 minutes during the trial to check for outside stressors beyond light presence

2.3 Day Night Procedure

Skate	Acclimation	First Experiment	Break	Second Experiment
1	12 hr	Day Trials (D1- 1.5 hr, D2- 1.5 hr)	8 hr	Night Trials (N1)
2	12 hr	Night Trials (N1- 1.5 hr, N2- 1.5 hr)	8 hr	Day Trials (D1- 1.5 hr, D2- 1.5 hr)
3	12 hr	Day Trials (D1- 1.5 hr, D2- 1.5 hr)	8 hr	Night Trials (N1- 1.5 hr, N2- 1.5 hr)

Table 1. The design of the data collection during the two weeks. Skate 2's first experiment was switched with night experiments starting first to account for acclimation time. Skate 1 lost a rate due to a leak of air into the tank.

Data were collected over the period of two weeks. To test each of the three skates, Skate 2's experiment was conducted with the first experiment during the night (9:30pm - 1:30am), and Skate 1 and 3's experiments were conducted with the first experiment during the day (9:30am – 1:30pm). A twelve-hour acclimation occurred before the experiment, and an eight-hour break occurred between each experiment duration (Table 1). Skate 1 only acquired one rate for the night experiment due to a leak of air into the respirometer during testing.

2.4 Statistical Analysis

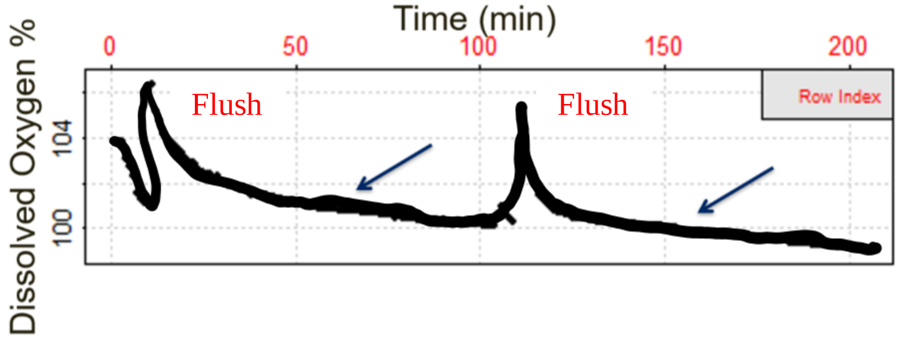

Figure 2. Example of the oxygen consumption rates data collected during respirometry trials. Arrows indicate the slopes in which respR analyzed to calculate metabolic rate. The peaks in dissolved oxygen percentages are the flushes that occur to reoxygenate system (all data listed in the Appendix).

Data were organized into a spreadsheet based on each skate's DO percentage over time and analyzed using the package respR in Rstudio (version 4.3.0) to calculate a mass-specific respiration rate for each animal (Harianto et al, 2019). Only data from the last 30 minutes of each trial period were used to mitigate the effect of the flush on the skate (Fig. 2). The last 30 minutes were the most steady, linear slopes of the data points which indicated less stress from the skate in the system.

Metabolic rates were plotted by the diel phase to determine if there was any trend between RMR and diel phase. A parametric t-test was used to test for differences between the diel phases after being run through a Shapiro test for normal distribution, and the Levene test for homogeneity of variance. To compare how metabolic rate changed between day and night in each individual skate, the metabolic rate from each day measurement period was subtracted from each night measurement period and plotted per individual. Positive values showed more activity during the day, while negative values showed more activity at night.

It was also investigated that the impact that the order and timing of experiments may influence metabolic rate. The first RMR recorded for each skate (D1 for Skates 1 and 3, and N1 for Skate 2) were removed from the dataset, and reevaluated in the same manner as the original dataset. The same comparisons were calculated and plotted with the removal of the first RMR. These measurements assessed the potential impact that stress associated with handling and initiation of the experiments may have had on our measurements (regardless of diel effect). A Wilcoxon Ranked Sign test was used to test for differences between the day and night experiment since the removed point dataset did not pass the Shapiro test.

Chapter 3

RESULTS

3.1 Diel Phases

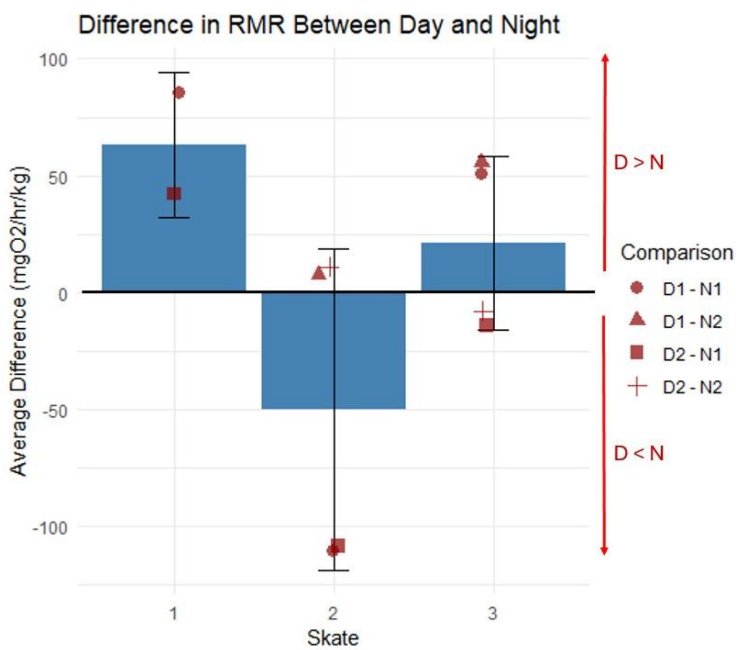

Figure 3. **Difference in RMR of Clearnose Skates between diel phases (D - N).** The comparison of subtracting the day trial rates by the night trial rates and plotting the average differences (blue) with differences (red). Skate 1 had an average difference of 63 mg O_2/hr/kg with a standard deviation of 31.1 mg O_2/hr/kg. Skate 3 had an average difference of 21 mg O_2/hr/kg with a standard deviation of 37.1 mg O_2/hr/kg. Skate 2 had an average difference of -50 mg O_2/hr/kg with a standard deviation of 68.7 mg O_2/hr/kg. There was no significant difference between diel phases (p-value 0.95).

The comparisons between the day RMR's and the night RMR's were calculated as such. The first day RMR (D1) was subtracted by the first night RMR (N1), then the second day RMR (D2) was subtracted by N1. The RMR for D1 was subtracted by second night RMR (N2), and D2 was subtracted by N2. Thus, Skates 2 and 3 had four differences while Skate 2 had two differences due to only one night RMR (N1). These comparisons were plotted with the average difference of each skate highlighted in blue (Fig. 3). As such, positive averages in difference showed more activity during the day, while negative averages showed more activity at night.

After determining the difference in RMR between day and night, Skates 1 and 3 demonstrated higher RMR's during the day than at night. Skate 1 had an average difference of 63 mg O_2/hr/kg with a standard deviation of 31.1 mg O_2/hr/kg, while Skate 3 had an average difference of 21 mg O_2/hr/kg with a standard deviation of 37.1 mg O_2/hr/kg (Fig. 3). In contrast, Skate 2 demonstrated an opposite trend of lower rates during the day than at night. Skate 2 had an average difference of -50 mg O_2/hr/kg with a standard deviation of 68.7 mg O_2/hr/kg. However, the total average metabolic rates for the diel phases were similar. Night period rates were slightly higher with an average of 193.8 mg O_2/hr/kg with a standard deviation of 78.7 mg O_2/hr/kg. Day period rates averaged 190.2 mg O_2/hr/kg with a standard deviation of 81.8 mg O_2/hr/kg. Overall, the total average rates were too close determine if the skates had higher RMR's during the day or night, but the average differences indicated higher RMR's for the day in Skates 1 and 3.

Though there was an observed increase in RMR during the day experiments for Skate 1 and 3, there was no statistically significant difference between the raw night and day RMR's. To meet the requirements for a parametric t-test, the data was tested for normality using the Shapiro and the Levene test for homogeneity. Since the data was normally distributed and showed equal variance (p values > 0.05), a parametric t-test was run and resulted in a p-value of 0.95. Thus, the null hypothesis of the metabolic rates being the same between diel phases failed to be rejected.

3.2 Acclimation Factor

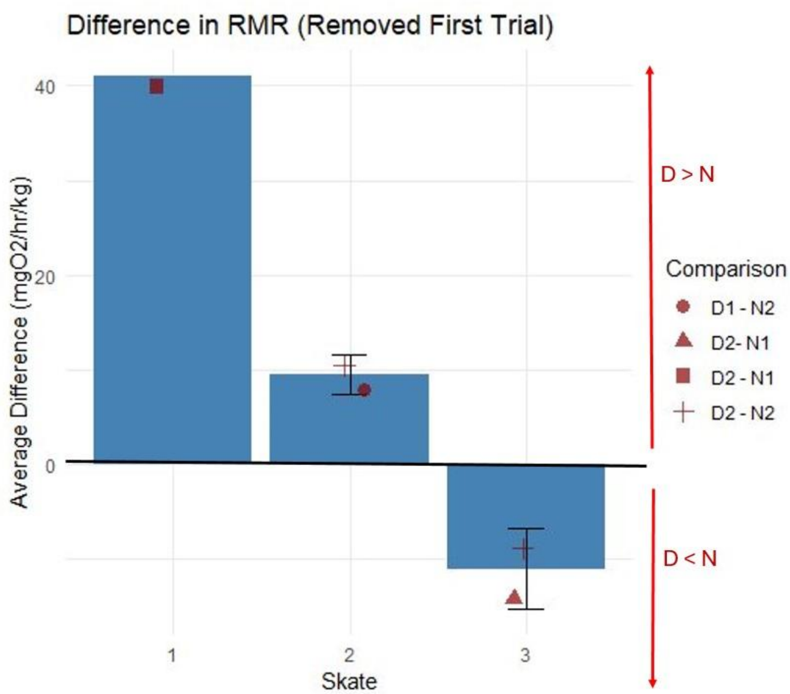

Figure 4. **Acclimation factor in the RMR comparisons of Clearnose Skates (D - N)**. The comparison of subtracting the day trial rates by the night trial rates and plotting the average differences (blue) with differences (red) without the first trials of all skates. Skate 1 had an average difference of 41 mg O_2/hr/kg with a standard deviation of 0 mg O_2/hr/kg. Skate 2 had an average difference of 9.5 mg O_2/hr/kg with a standard deviation of 2.1 mg O_2/hr/kg. Skate 3 had an average difference of -11 mg

O_2/hr/kg with a standard deviation of 4.24 mg O_2/hr/kg. There was no significant difference (p-value 0.79).

There was the possibility for the skate's duration within the respirometer may have increased the metabolic rates between experiments one and two of testing (Table 1). Independent of the diel phase, the first trial of data collection demonstrated higher RMR's than the other rates. The metabolic rate of the first trial in the first experiment (D1 for Skates 1 and 3, N1 for Skate 2) was removed and Figure 3 was regenerated with the remaining comparisons. Only Skate 1 showed a consistent pattern of higher metabolic rates during the day in both plots with an average difference of 41 mg O_2/hr/kg with a standard deviation of 0 mg O_2/hr/kg (Fig. 4). Interestingly Skates 2 and 3 demonstrated opposite patterns in metabolic rate compared to previously seen in Figure 3. Skate 2 had higher RMR's during the night with an average difference of 9.5 mg O_2/hr/kg and a standard deviation of 2.1 mg O_2/hr/kg. Skate 3 had higher RMR's during the night than during the day with an average difference of -11 mg O_2/hr/kg and a standard deviation of 4.24 mg O_2/hr/kg.

The averages between the day and night experiment demonstrated similar increased averages in RMR during the night as seen in the original dataset. Without the first trial RMR's, the night RMR's averaged 188 mg O_2/hr/kg with a standard deviation of 100.5 mg O_2/hr/kg, while the day RMR's averaged 156.3 mg O_2/hr/kg with a standard deviation of 73.9 mg O_2/hr/kg. Again, there was no statistically significant difference between the day and night experiments. Only the first experiment of rates was normally distributed in the Shapiro test, and both groups had homogeneity of variance in the Levene test. Since only one test was passed, a nonparametric Wilcoxon Ranked Sign test was performed resulting in a p-value of 0.79. Thus, removing the first trial rates did not change the statistical analysis results; the null hypothesis of similar metabolic rates between the day and night experiments failed to be rejected.

3.3 Skate Three

Throughout trials, it was evident that Skate 3 had consistently higher rates (>260 mg O_2/hr/kg) compared to the other two tested (mean =138.3 mg O_2/hr/kg). Skate 3's mean day measurements were 328 and 264 mg O_2/hr/kg while the night measurements were 278 and 272 mg O_2/hr/kg. During the 15 minute checks of the first experiment, Skate 3 was observed swimming in circles at the top of the tank. Unlike the other two skates, it did not settle at the bottom until the end of the second trial in the first experiment. However, during the second experiment of rates, Skate 3 settled on the bottom/slowed its swimming speed after 30 minutes of DO% measurement as exhibited by the other skates.

Chapter 4

DISCUSSION

4.1 Diel Influence

The raw metabolic rates were not significantly different during different diel phases without accounting for individuals. This suggests that skates have some biological rhythm occurring based on general diel shifts in metabolic rate and skate activity. However, when accounting for individuals and comparing metabolic rates during the night and day within the same skate, results showed more activity during the day in two out of three skates. The individual increase in RMR during the day phase of the trials in two skates could suggest that the Clearnose Skate is diurnal rather than nocturnal or crepuscular (active during dusk and dawn).

This behavioral pattern contradicts the activity cycles of other bottom-dwelling skates such as the Flapper Skate, the Yellow Nose Skate, and the Rough Skin Skate (Lavender et al, 2021; Ahumada et al, 2025). These species may exhibited vertical migration in response to diel changes in biotic and abiotic parameters, including prey availability which shifts over diel cycles (DVM). According to their studies, these skates frequently move to shallower waters during the night to hunt prey and remain in deeper waters during the day to avoid larger predators. The findings presented here are interesting as the Flapper Skate, the Yellow Nose Skate, and the Rough Skin Skate are also coastal elasmobranchs similar to the Clearnose Skate. It is then expected to see similar behaviors in the Clearnose Skate rather than the opposite behavior in this study. The diurnal pattern may be the result of specific ecological factors (such as prey species' cyclic behaviors) or environmental fluctuations which would influence the species to be more active during the day. If so, it would maximize their chances of survival and reproductive success, resulting in a biological rhythm that syncs with diel phases. However, our varying individual results show that it could be skate-specific, and more work is needed to determine if skates are typically more active during the day or at night.

This study could not determine a circadian rhythm as time constraints prevented testing disruptions (such as a 24hour light conditions) to the Clearnose Skate's biological rhythm. Additionally, only three skates were tested without replicating the process, so testing other skates with repeated trials may reveal an opposite nocturnal effect or no effect. With more trials, it is likely that the Clearnose Skate will exhibit increased individual variation in rhythms and possibly nocturnal activity as observed in other skate species.

4.2 Acclimation during Respirometry Procedures

After trials, it was discovered that the acclimation conditions may have limited the accuracy of this study. Acclimation period and exposure may not have been long enough to eliminate the effect of stress associated with handling. This could potentially bias our results and mask their true cyclic behavior. Though not statistically significant in their difference, the first experiment of DO% measurements for all skates were nearly double the results for the second experiment. This pattern suggests that skates exhibited increased stress and activity levels when the skates were first introduced to the respirometer.

The acclimation period prior to testing was 12 hours, but skates were not yet subjected to the increased water flow during the flushing process (oxygen replenishment from flush tank with loud pump system and water pressure).

Acclimation is crucial to obtaining accurate measurements of metabolic using respirometers, especially for larger species such as skates. In a similar procedural study, the stress levels (measured using cortisol levels in blood samples) of rainbow trout were recorded to compare the stressors of being chased within a tank and sitting in a respirometer (Murray et al, 2017). Scientists found that the fish had similar stress levels during both the chase procedure and respirometry procedure. Though cortisol was not measured for this study, the skates tested in the respirometer likely had higher stress levels influencing their biological systems (increased heart rhythm and breathing) and behavior (swimming activity). As a result, their RMRs were likely inflated and may have confounded any potential diel patterns in RMR.

4.3 Individual Variation

Skate 3's increased RMR compared to the other skates demonstrates the potential high degree of individual physiological variability among organisms. Variability between individuals' metabolic rates is based on an organism's biological traits (sex, mass, maturity, etc.) and experiences (aggressive handling, exposure to air, captivity duration, etc.). In this study, the skates had disk lengths ranging between 39.5 to 42 cm (standard deviation of 1.32 cm), and their masses ranged from 1.15 to 1.42 kg (standard deviation of 0.14 kg). Since the skates were similar sized mature females with low standard deviations, the potential variation in RMR due to sex as well as in size was removed.

Therefore, Skate 3's increased RMR was possibly the result of its exacerbated reaction to the respirometry system, sensitivity to handling, or other unknown effects (e.g., reproductive status). An investigation involving Senegalese Sole used respirometry to determine that fish had different coping styles (passive and active) when faced with a stressor and exhibited varying metabolic rates based on these styles (Martin et al, 2011). Due to its prolonged swimming pattern, Skate 3's coping mechanism was assumed to be "escape" rather than "freeze" like Skates 1 and 2. Thus, Skate 3's metabolic rates would be higher as that skate utilized more energy in its response to stress.

4.4 Future Directions

To determine if the Clearnose Skate has a biological clock versus a biological rhythm, future research should include a second experiment of trials to disrupt circadian rhythm. Specifically, the skate would be subjected to 24hr light or 24hr dark conditions to disrupt brain signaling and observe variations in rhythm. Time constraints limited this study to only include a 12hr:12hr light: dark treatment.

Chapter 5

CONCLUSION

Due to time constraints, technical limitations (skate death and disease, releasing test subjects prior to winter), and the resulting low sample size, this investigation could not determine whether the Clearnose Skate's metabolic rates are influenced by a biological rhythm. However, our results suggest that skate RMR may be higher during the day in some individuals, suggesting that this species may naturally be more active during the day, but more trials are needed to thoroughly address this question. Other research indicates that skates are nocturnal, so interestingly, our results are not consistent with previous research regarding skate rhythm in activity level. Additionally, the observed increase in RMR during the first experiment suggests that skates were not acclimated long enough to obtain accurate measurements of unstressed skates. Future trials need to be modified with increased acclimation periods (24 - 48hours) and decreased handling time to minimize the potential impact of stress on oxygen consumption measurements.

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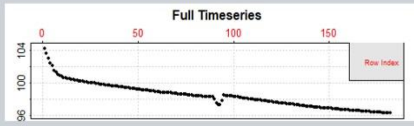
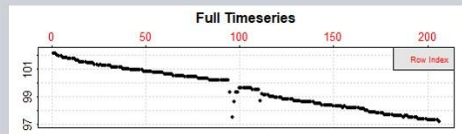
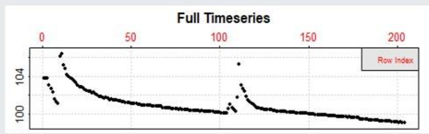
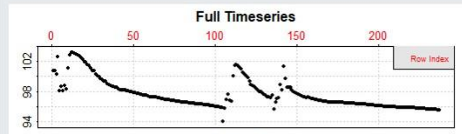
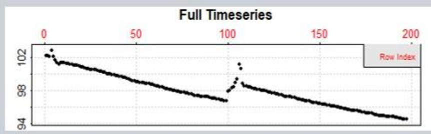
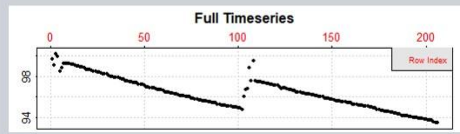
APPENDIX

Skate	**Day (mgO$_2$/hr/kg)**	**Night (mgO$_2$/hr/kg)**
1	**189**	**104**
	145	
2	**106**	**217**
	109	**98**
3	**328**	**278**
	264	**272**

Table 2. RMR's of each skate during diel phases and trials. These results were collected using respR and represented in Figure 3.

Skate	Day Series	Night Series
1		
2		
3		

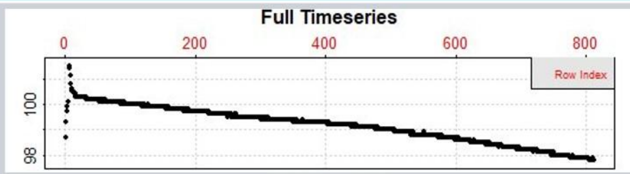
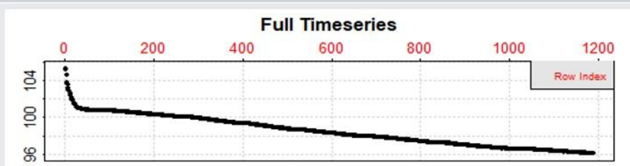
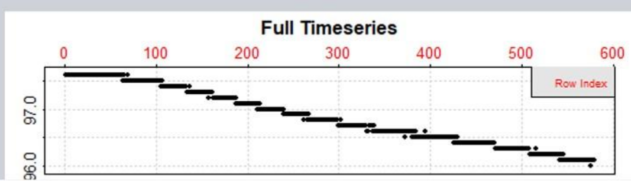
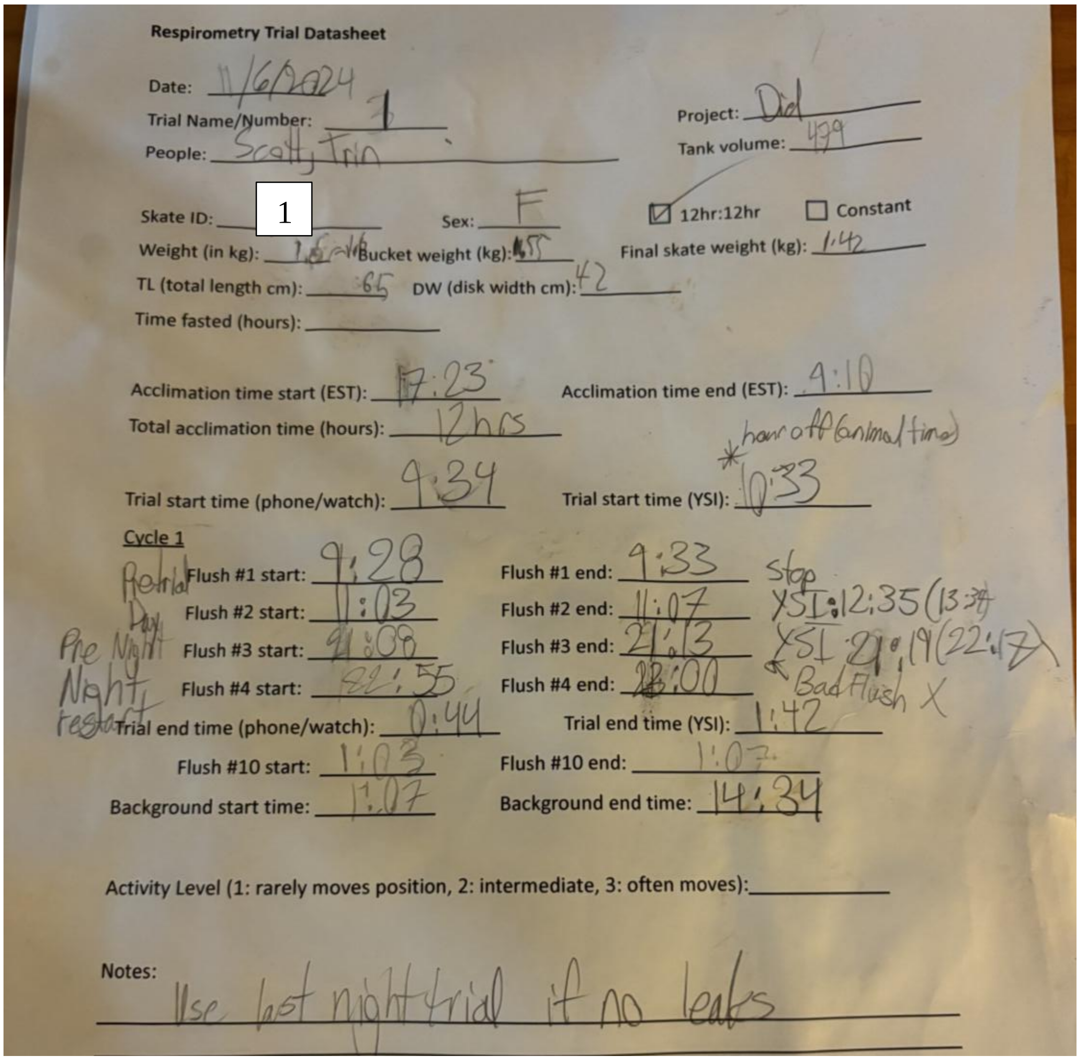
Skate	Background Series
1	
2	
3	

Table 3. **Time series of raw data from each skate**. The first table reflects the DO% declines that were measured during the day and night sessions. The second table reflects the DO% decline during the background recording.



Respirometry Trial Datasheet

Date: 11/6/2024

Trial Name/Number: 1

Project: Diel

People: Scott, Erin

Tank volume: 479

Skate ID: 1

Sex: F

☑ 12hr:12hr ☐ Constant

Weight (in kg): 1.5

Bucket weight (kg): .455

Final skate weight (kg): 1.42

TL (total length cm): 65

DW (disk width cm): 42

Time fasted (hours):

Acclimation time start (EST): 17:23

Acclimation time end (EST): 9:10

Total acclimation time (hours): 12hrs

*hour off (animal time)

Trial start time (phone/watch): 9:34

Trial start time (YSI): 10:33

Cycle 1

Flush #1 start: 9:28

Flush #1 end: 9:33

Stop

Restart Day

Flush #2 start: 11:03

Flush #2 end: 11:07

YSI: 12:35 (13:34)

Pre Night

Flush #3 start: 21:08

Flush #3 end: 21:13

YSI 21:19 (22:17)

Night restart

Flush #4 start: 22:55

Flush #4 end: 23:00

Bad Flush X

Trial end time (phone/watch): 0:44

Trial end time (YSI): 1:42

Flush #10 start: 1:03

Flush #10 end: 1:07

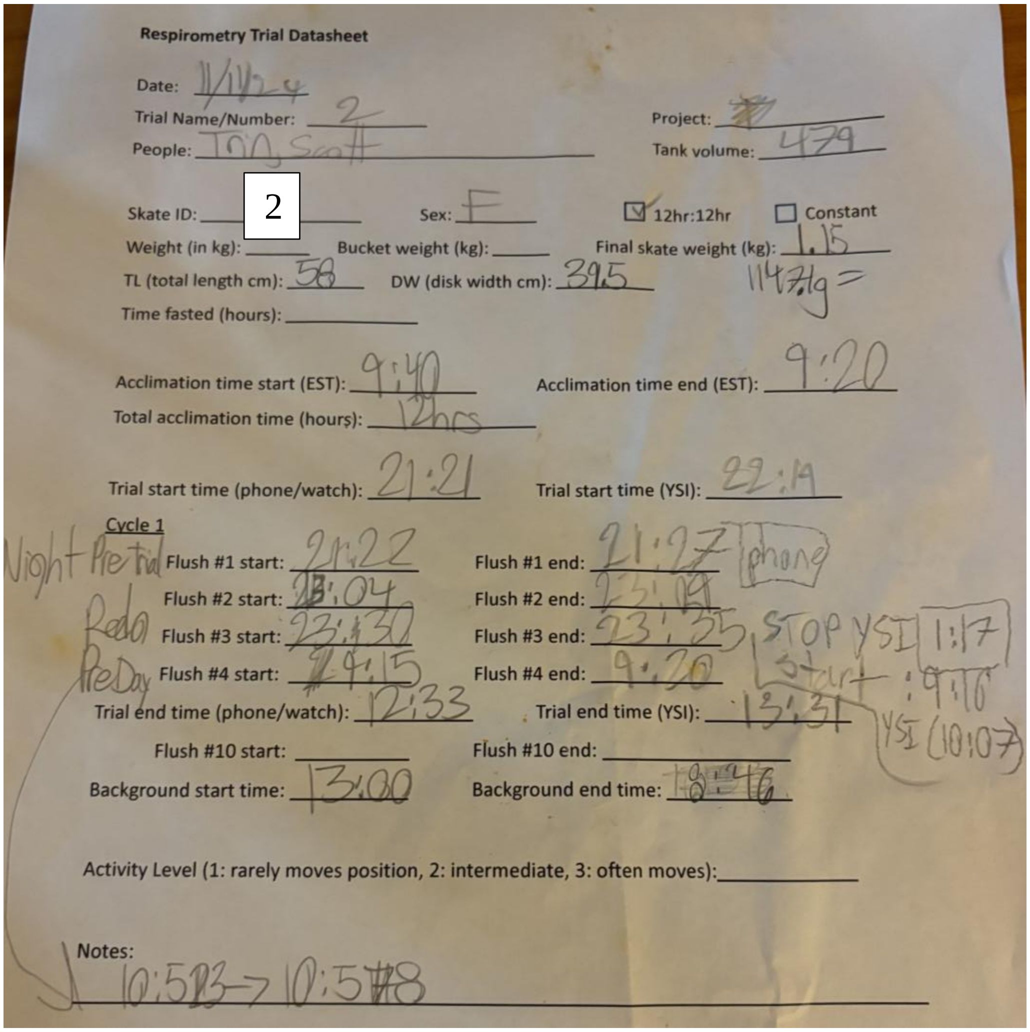
Background start time: 1:07

Background end time: 14:34

Activity Level (1: rarely moves position, 2: intermediate, 3: often moves):

Notes: Use last night trial if no leaks

Notes 1. Data sheet for Skate 1. Times the YSI was turned on and off are listed next to flush numbers and the skate's trait are listed in the tope section. Skate 1 was the largest skate at 1.42 kg.



Respirometry Trial Datasheet

Date: 1/11/24

Trial Name/Number: 2

Project:

People: Tom, Scott

Tank volume: 479

Skate ID: 2

Sex: F

☑ 12hr:12hr ☐ Constant

Weight (in kg):

Bucket weight (kg):

Final skate weight (kg): 1.15

TL (total length cm): 58

DW (disk width cm): 39.5

1147.1g =

Time fasted (hours):

Acclimation time start (EST): 9:40

Acclimation time end (EST): 9:20

Total acclimation time (hours): 12hrs

Trial start time (phone/watch): 21:21

Trial start time (YSI): 22:19

Cycle 1

Night Pre trial

Flush #1 start: 21:22

Flush #1 end: 21:27

phone

Flush #2 start: 23:04

Flush #2 end: 23:09

Redo

Flush #3 start: 23:30

Flush #3 end: 23:35

STOP YSI 1:17

PreDay

Flush #4 start: 9:15

Flush #4 end: 9:20

start: 9:16

Trial end time (phone/watch): 12:33

Trial end time (YSI): 13:31

YSI (10:07)

Flush #10 start:

Flush #10 end:

Background start time: 13:00

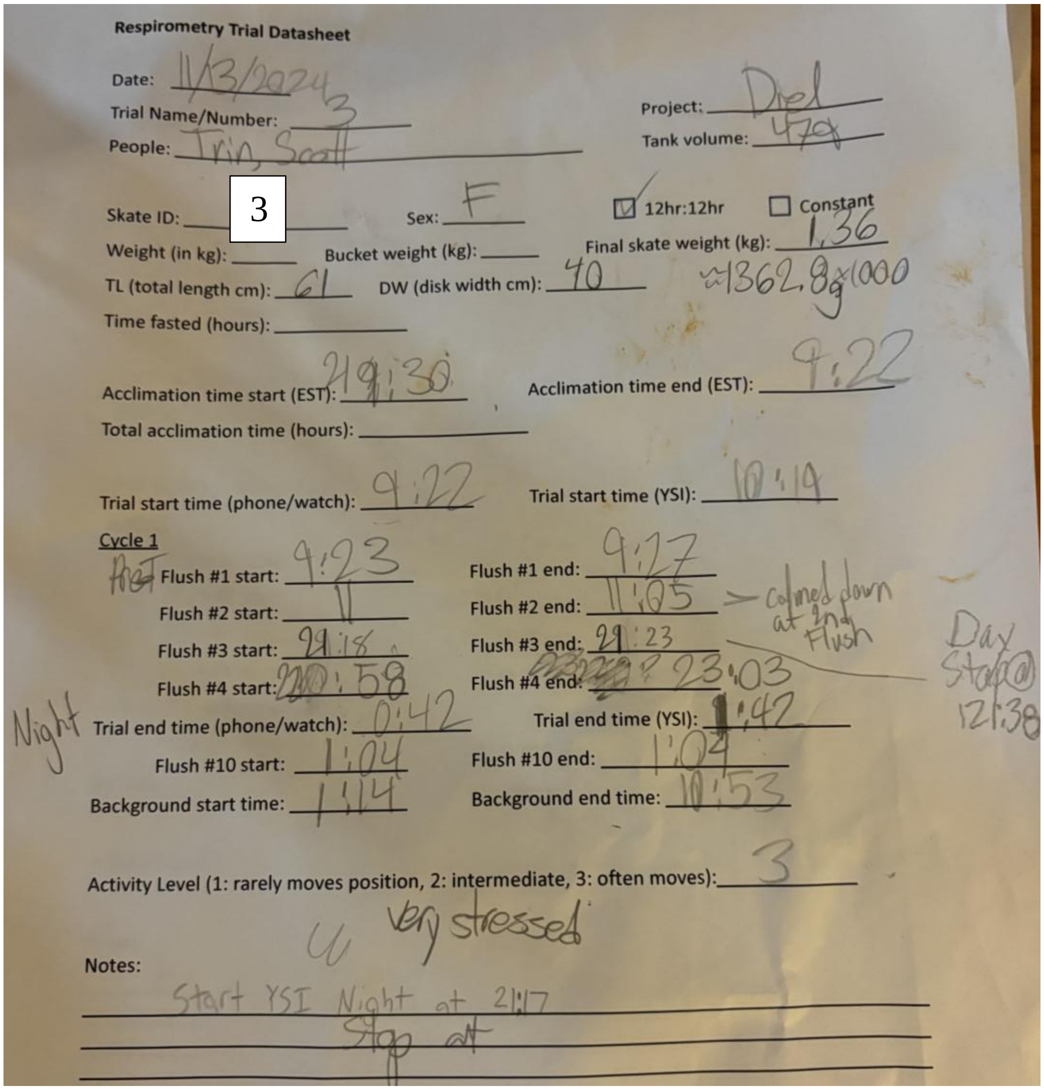
Background end time: 13:46

Activity Level (1: rarely moves position, 2: intermediate, 3: often moves):

Notes:

10:53 → 10:58

Notes 2. Data sheet for Skate 2. Times the YSI was turned on and off are listed next to flush numbers and the skate's trait are listed in the tope section. Skate 2 was the smallest skate at 1.15kg.


Respirometry Trial Datasheet

Date: 11/13/2024

Trial Name/Number: 3

People: Trin, Scott

Project: Dred

Tank volume: 479

Skate ID: 3

Sex: F

☑ 12hr:12hr ☐ Constant

Weight (in kg):

Bucket weight (kg):

Final skate weight (kg): 1.36

TL (total length cm): 61

DW (disk width cm): 40

~1362.8g x1000

Time fasted (hours):

Acclimation time start (EST): 19:30

Acclimation time end (EST): 9:22

Total acclimation time (hours):

Trial start time (phone/watch): 9:22

Trial start time (YSI): 10:19

Cycle 1

Flush #1 start: 9:23

Flush #1 end: 9:27

Flush #2 start: 11

Flush #2 end: 11:05

calmed down at 2nd Flush

Flush #3 start: 21:18

Flush #3 end: 21:23

Flush #4 start: 20:58

Flush #4 end: 23:03

Day Stop @ 12:38

Night

Trial end time (phone/watch): 0:42

Trial end time (YSI): 1:42

Flush #10 start: 1:04

Flush #10 end: 1:04

Background start time: 1:14

Background end time: 10:53

Activity Level (1: rarely moves position, 2: intermediate, 3: often moves): 3

very stressed

Notes:

Start YSI Night at 21:17

Stop at

Notes 3. Data sheet for Skate 3. Times the YSI was turned on and off are listed next to flush numbers and the skate's trait are listed in the tope section. Skate 3 had the highest rates overall during this study.