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IMPACT OF HUMAN DISTURBANCE ON THE BEHAVIOR AND PHYSIOLOGY OF THE  
ENDANGERED RINGED SAWBACK TURTLE (*GRAPTEMYS OCULIFERA*)

Master of Science Degree  
University of Mississippi  
Department of Biology

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12 May 2018

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## ABSTRACT

Turtles are one of the most threatened taxa worldwide. In addition to direct anthropogenic impacts such as hunting and pollution, unintentional indirect human disturbance affects poikilothermic turtles by disrupting thermoregulatory basking behavior. In this thesis I assess the behavioral and physiologic impacts of high boat traffic, reductions of basking structures, and environmental factors on basking behavior, rates of disturbance, thermoregulation, parasite load, shell condition, and population recruitment in two populations of the endangered ringed sawback (*Graptemys oculifera*), also known as the ringed map turtle, on the Pearl River outside Jackson, MS. Basking behavior was influenced by availability of basking structures, boat traffic, zone (wake or no wake), boat type, air temperature, weather, and Julian day. Mathematic simulations of anthropogenically disturbed and undisturbed adult female ringed sawbacks showed a decrease in body temperatures due to disturbance, an effect which was magnified in higher probabilities of disturbance and in the months of May and June compared to July and August. Parasite load did not differ between populations, despite apparent differences in human disturbance. Shell condition was poorer in the population near urban development, and fewer juveniles and young adults were found in the population subject to higher boat traffic. This study explores the effect of unintentional human disturbance on ectothermic riverine turtles and provides management recommendations for the conservation of an endangered, endemic species.

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**CHAPTER I:**  
**ANTHROPOGENIC AND ENVIRONMENTAL FACTORS INFLUENCING THE**  
**BASKING BEHAVIOR OF AN ENDANGERED RIVERINE TURTLE**

**Introduction**

While many studies agree on the direct negative effects of human activities such as hunting (Rosser & Mainka 2002), pollution (Trevors & Saier 2009), and habitat loss (Sekercioglu et al. 2011) on animal populations, a consensus on the possible effects of indirect forms of human disturbance has yet to be reached (Tablado & Jenni 2017). Indirect disturbance is typically unintentional (Buchholz & Hanlon 2012) and includes such activities as ecotourism (Müllner et al. 2004, Buchholz & Hanlon 2012), winter sports (Arlettaz et al. 2007), hiking (Taylor & Knight 2003), and recreational boating (Moore & Seigel 2006). These indirect forms of human disturbance may be perceived by wildlife as a predation risk and cause changes in behavior and recruitment (Blumstein 2006, Blumstein 2016). Some studies show no effect on populations (Blumstein 2006, Bejeder et al. 2006), however many more show that wild animals change their behavior in response to the presence of humans (Moore & Seigel 2006, Müllner et al. 2004, Arlettaz et al. 2007).

Because myriad variables influence an animal's behavior (Reyer 1984, Dill 1987, Eckman 1987, Morrison et al. 2004), it can be difficult to predict behavioral responses to human disturbance. Even in the absence of humans, individual animals must optimize behavior based on extrinsic factors, such as season (Jorgensen et al. 2016), temperature (Weatherhead & Robertson

1992), or habitat structure (Pittfield & Burger 2017), and intrinsic factors, such as body condition (Brodersen et al. 2008), mass (Wahle 1992), or past experience (Kelley & Magurran 2003). When disturbed by humans, animals assess the costs and benefits of an array of feasible behavioral reactions to human presence. For example, an animal must decide the distance at which a human intruder is allowed to approach before it attempts escape, commonly called the flight initiation distance (FID) (Frid & Dill 2002). Jorgensen et al. (2016) found that FID in nesting piping plovers (*Charadrius melodus*) decreased in relation to Julian day. This effect may be reflective of parent plovers' optimization of predation risk and potentially losing a nest which increases in past parental effort as the season progresses (Jorgensen et al. 2016). Pittfield and Burger (2017), on the other hand, found that cloud and canopy cover explained variation in the FID of basking turtles in the family *Kinosternidae* turtles. Increased canopy decreases perception of predation risk, resulting in shorter FID (Pittfield & Burger 2017). In small populations and in those species already vulnerable to extinction, behavioral decisions that animals make in response to humans can have cascading effects in the health and physiology of individuals, potentially resulting in population decline (Christiansen & Lusseau 2015, Nowacek et al. 2016).

Human disturbance of rivers can be particularly problematic since the spatial options available to aquatic animals is limited by the linear structures of their habitat (Bodie & Semlitsch 2000). This problem is especially acute for air-breathing animals that cannot remain below the water surface indefinitely (Kramer 1988). Air-breathing species who can remain submerged for long periods may still suffer negative physiologic effects from oxygen deprivation such as bradycardia and glycogen depletion (Penney 1974). If the species is ectothermic as well, exposure to the sun and warmer air above the river surface is essential to functional thermoregulation. Passing watercraft and the removal of deadwood from rivers to aid boat

traffic (known as de-snagging) can prevent turtles from being able to bask optimally (Moore & Seigel 2006), no doubt contributing to the fact that more than half (60.4%) of all turtle and tortoise species are threatened with extinction (Turtle Conservation Coalition 2018). Basking increases body temperatures to allow for digestion (Hammond et al. 1988), egg development (Lindeman 1999; Moore & Seigel 2006), and other metabolic activities (Lindeman 2013), as well as assisting in the maintenance of a healthy shell free from fungus (Selman et al. 2013). Additionally, basking is vital in the removal of ectoparasitic leeches (Selman & Qualls 2009) which can transmit harmful blood parasites such as the haemogregarina (Siddall & Desser 2001). Frequent boat traffic has been associated with elevated stress (Selman et al 2013) and reduced shell condition (Selman et al 2013, Galois & Ouellet 2009) in riverine turtles, ostensibly because boats disrupt basking.

The ringed sawback (*Graptemys oculifera*), also known as the ringed map turtle, is a US federally threatened species. It is listed as an endangered species by the state of Mississippi and is found solely in the Pearl River and its tributaries in Mississippi and Louisiana, USA (Jones & Selman 2009). This turtle's threatened status is mainly due to habitat modification and degradation by humans, compounded by nest predation by human commensals, such as raccoons and crows, and the invasive Argentinean fire ant (Jones & Selman 2009, Jones 2006). *Graptemys* species are known to be habitual baskers compared to other riverine turtles (Ryan & Lambert 2005) and achieve higher temperatures during basking than other riverine species: body temperature averaged 32.7°C in false map turtles (*Graptemys pseudogeographica*) compared to 30.6°C in red-eared sliders (*Trachemys scripta elegans*) (Boyer 1965, Lindeman 1999). Therefore, the consequences of interrupted basking may be higher in this species than other riverine turtles, possibly impeding recovery efforts.

The objectives of this study were to 1) characterize the indirect human disturbances occurring in the habitat of the endangered ringed sawback, and 2) investigate how human and environmental factors interact to affect basking. I hypothesize that human disturbance negatively affects basking behavior by turtles, and predict that frequent boat traffic (quantified as boats per hour) and rarity of basking structures will result in fewer basking turtles and reduced basking duration.

## **Methods**

### *Study site*

Two study sites were chosen on the Pearl River outside Jackson, MS based on suspected differences in human disturbance (Jones & Hartfield 1995, Jones 2017; Fig. 1.1). The first site, located in Canton, MS, is upstream of the Ross Barnett Reservoir (Latitude -89.86°, Longitude 32.62°). This site is comprised of the three river miles between Ratliff Ferry Trading Post and a large sandbar colloquially known as “Flag Island.” Ratliff Ferry (RF) is within the Pearl River Wildlife Management Area (PRWMA) where de-snagging is prohibited, and the river is unchannelized. However, the naturalistic setting and high numbers of sandbars make it attractive for boating and other recreational activities. Boat traffic is high, particularly on weekends and holidays (Jones 2017), presenting a significant potential disturbance to turtles. There are several no wake zones throughout the site, with one no wake zone located around the boat ramp at mile 1, and two located around large sandbars in mile 3.

The second site, located in Jackson, MS and the surrounding towns, is downstream of the Ross Barnett Reservoir (Latitude 32.33°, Longitude 90.15°). The LeFleur’s Bluff [LB, known as the Lakeland site in Jones (2017)] site is comprised of the three river miles upstream of the Pearl

River access boat ramp within LeFleur’s Bluff State Park and lacks no wake zones. Its highly variable water levels due to its location downstream of the reservoir can lead to extremely shallow areas with risk of outboard motor damage, which discourages many boaters. The channelized nature of the area and highly variable water levels may limit the availability of basking structures for turtles, presenting a different type of anthropogenic disturbance than RF.

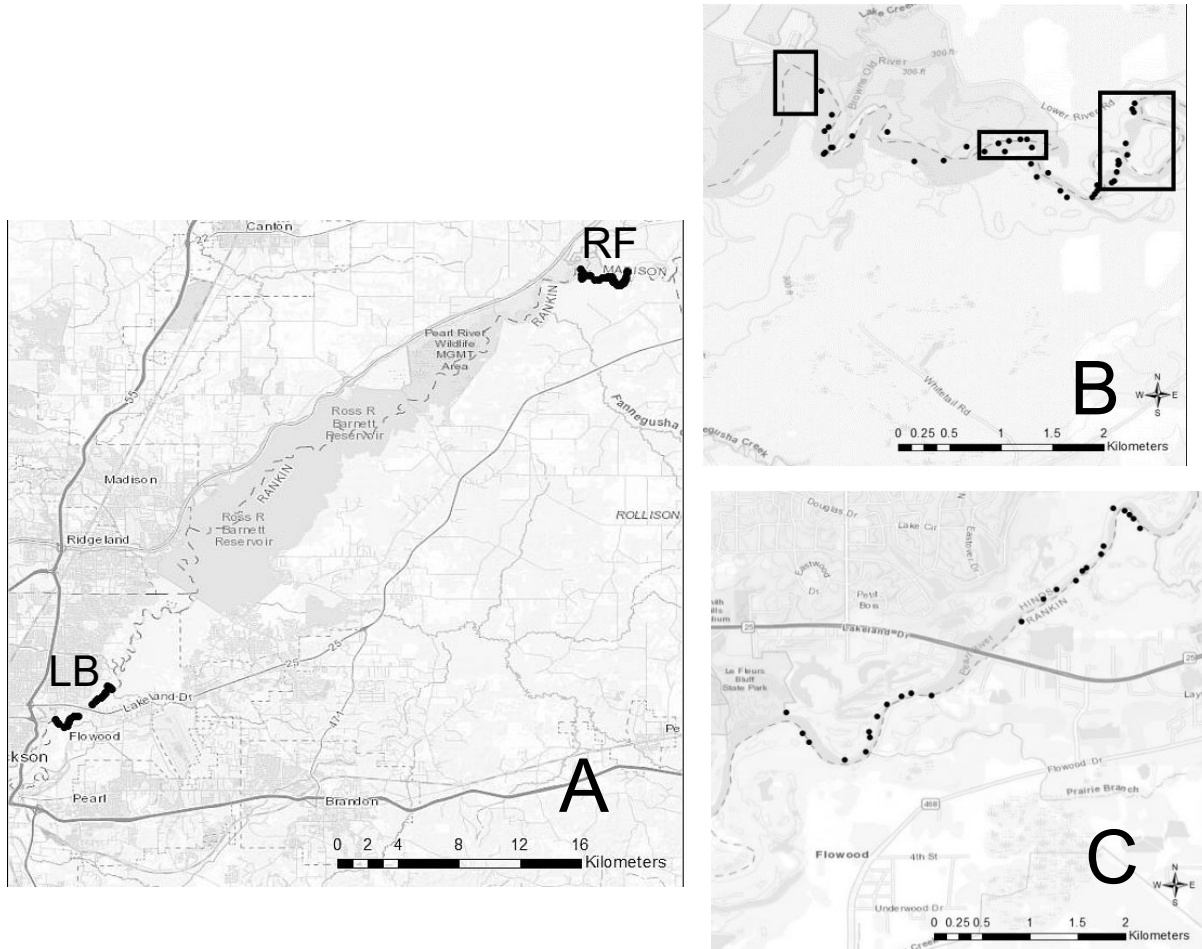


Fig. 1.1 Location of two study sites on the Pearl River outside Jackson, MS (image A). Ratliff Ferry Trading Post (RF, image B) is upstream of the Ross Barnett Reservoir in Canton, MS while LeFleur’s Bluff State Park (LB, image C) is in Jackson, MS. Basking structures are indicated by points in images B and C. Approximate location of no wake zones are indicated by boxes at RF (image B).

### *Basking site characteristics*

Assessment of behavior and disturbance took place at RF between 21 May – 2 June and 30 June – 15 July 2017. Observations took place at LB between 7 June – 14 June and 16 July – 22 July 2017. The effect of Julian day was therefore not consistent across all sites. Each site was divided into three, one river mile sections, and human disturbance was assessed separately at each river mile. A study of a related species, the yellow blotched map turtle (*Graptemys flavimaculata*), has shown average home range lengths of 1.8 km for males and 1.5 km for females (Jones 1996). Therefore, the river mile where a turtle was captured likely represents the area of disturbance experienced by an individual over time.

Basking structures with two or more turtles present during three days of assessment at the site were flagged – this eliminated any smaller structures that were potentially only accessible to juveniles and small males. If changing water levels revealed previously submerged basking structures, those additional sites were flagged subsequently. Locations of basking structures were recorded by marking waypoints using a handheld GPS (Garmin eTrex 10). The flagging used for marking basking structures did not appear to interfere with turtle behavior as turtles were observed on all flagged sites. The number of basking structures per river mile was assessed for both sites using GPS data imported into ArcGIS (ESRI, vs. 10.2.2).

### *Focal observations*

Observations of basking behavior and boat traffic were made with a spotting scope from a sandbar or jonboat located on the opposite bank of the river from a basking structure, approximately 100 – 250 m away. Basking turtles were observed for a 6-hour period between 8:30 AM and 4:30 PM, depending on the time of initial observation, that was divided into two,



three-hour morning and afternoon windows. Only one focal structure was under observation per observation period. The time frame was chosen to cover basking behavior over a range of temperatures and conditions. The duration of basking by individual turtles was observed by monitoring individuals from the point they emerged from the water until re-entry, while noting whether basking ended prematurely due to a visible disturbance. If turtles terminated basking by entering the water because of passing watercraft, the type of boat was recorded. Boat types observed included motorboats, jonboats, anglers, kayaks, personal watercraft (pwc), and airboats. Hourly boat traffic was quantified at each site by recording the number and type of passing boats. The percentage of turtles on a focal structure that were disturbed due to each passing watercraft was recorded as well. Boat traffic (number of boats per hour) was calculated separately for each three-hour morning or afternoon window. Because turtles were not individually marked, not all data are independent, although assumptions of independence were made for analyses as is common in studies of disturbance (Moore & Seigel 2006, Buchholz & Hanlon 2012, Selman et al. 2013).

### *Surveys*

Twice daily surveys of the number of basking turtles at flagged sites were taken in the morning and afternoon between 1 and 5 days each week. These observations were taken via a passing jonboat at a speed and distance to minimize turtle disturbance, approximately 75 – 100 m and 3.2 kph. Air and water temperatures at a depth of 0.6 m were taken at three points during the survey, at the beginning, halfway point, and end, and averaged. Surveys began at one end of the site and continued until the last basking structure at the opposite end to minimize potential disturbance events due to the research vessel. Populations within the study site were assumed to be consistent throughout the study season as range lengths reported for the related yellow-

blotched sawback are within the length of the survey area (Jones 1996). Results are reported as mean number of turtles per structure.

### *Statistical tests*

All statistical tests were carried out using the statistical program R Studio (R Foundation, version 3.4.2) with an  $\alpha$  of 0.05. All factorial ANOVAs used a type II Sum of Squares model.

A multiple regression was carried out to assess the relationship between the number of basking turtles seen on surveys (dependent variable) and 8 independent variables: Julian day, weekday, weather, air temperature, water temperature, zone type (no wake or wake), density of basking structures and average boat traffic of the river mile of the basking structure.

Multicollinearity was not present between the main effects and data were normally distributed. The model with the lowest AIC value excluding predictors with RVI scores less than 0.5 was used (Appendix I: Tables 1 and 2).

To assess the effect of environmental factors and human disturbance on the duration of basking (dependent variable), a multiple regression was carried out using 7 independent variables: Julian day, weekday, air temperature, water temperature, and the zone type (wake or no wake), number of basking structures within the river mile of observation, and boats traffic from the morning or afternoon period when the turtle was observed. Multicollinearity was not present between main effects, and normality of residuals was present. The model with the lowest AIC value excluding variables with a relative variable importance (RVI) value of less than 0.5 was chosen (Appendix I: Tables 3 and 4).

The odds of turtles ceasing basking due to disturbance was calculated using a logistic regression with the same initial predictors as the previous multiple regression. The model with the lowest AIC value was used.

A chi-square analysis was used to test if type of disturbance, including non-watercraft, was related to whether a turtle was disturbed. A factorial ANOVA tested data from all passing boats to assess if zone (wake or no wake) or watercraft type (independent variables) affected percentage of basking turtles (dependent variable) disturbed per structure. Because of high numbers of boats during the Saturday of Memorial day weekend (27 May), other predictors of disturbance were not incorporated into the analysis to prevent data skew due to the high number of boats (n=388) causing disturbances not related to speed, angle, or distance of approach. Additionally, as there was no “no wake” zone at LeFleur’s Bluff to compare to at Ratliff Ferry, and few boats at LB (only 11 out of 676 total boats), the effect of location was not examined. A post hoc Tukey test was used to assess significant differences in percentages of disturbance between boat types.

While these methods were carried out on data collected in 2017, surveys, focal observations, and assessment of disturbance also took place in 2016. The results from these analyses can be found in Appendix II.

## **Results**

Results are given in mean  $\pm$  standard deviation unless otherwise noted.

### *Assessment of disturbance and behavior*

Numbers of basking structures in each river mile at RF were 8, 5, and 26 in river miles 1, 2, and 3. At LB, 10, 5, and 10 structures were found in river miles 1, 2, and 3. Structure size

appeared to be consistent across river miles but was not measured. I estimated structures to vary between 0.6 to 1.2 m in length.

One-hundred twenty-three hours of focal basking observations were made between 23 May and 22 July 2017. There were 462 instances of basking by turtles used for this analysis: 96 turtles at LB and 366 turtles at RF. Durations of basking ranged from <1 to 566 min ( $\bar{X}=34.36\pm 50.11$  min) at RF and ranged from <1 to 297 ( $\bar{X}=39.68\pm 47.55$  min) at LB.

Surveys were taken from 23 May to 21 July and were comprised of 14 morning and 12 afternoon surveys at RF and 6 morning and 2 afternoon surveys at LB. Fewer surveys were taken at LB due to continued motor malfunctions and inclement afternoon weather impeding the safe continuation of surveys. Group sizes on structures ranged from 1 to 19 ( $\bar{X}= 3.26 \pm 2.88$  turtles) at RF, and ranged from 1 to 7 ( $\bar{X}=1.84 \pm 1.56$  turtles) at LB.

Only three boat types - jonboats, motorboats, and kayaks (n=11 boats) - were seen at LB while jonboats, motorboats, anglers, pwc, airboats, and kayaks were seen at RF (n=665 boats). The highest number of boats were seen on the Saturday of Memorial day weekend at RF ( $\bar{X}=64.67\pm 19.33$  boats per hour, n=388). Boat traffic was non-existent at LB Monday through Thursday, while levels fluctuated at RF, reaching peaks on Fridays and weekends (n=288 boats, Table 1.1, excluding Memorial day weekend). Boat traffic did not vary throughout the season (Kendall's tau, tau=-0.12, p=0.32).

Table 1.1. Table of means and standard errors of measures of boat traffic across weekday and location from ANOVA model. Abbreviations are for Ratliff Ferry (RF) and LeFleur’s Bluff (LB) (n=288 boats).

RF	Mean	LB	Mean
Monday	2.00 ± 0.33	Monday	0.00 ± 0.00
Tuesday	1.25 ± 0.50	Tuesday	0.00 ± 0.00
Wednesday	2.21 ± 0.47	Wednesday	0.00 ± 0.00
Thursday	3.53 ± 0.99	Thursday	0.00 ± 0.00
Friday	2.67 ± 0.62	Friday	0.33 ± 0.34
Saturday	21.17 ± 9.17	Saturday	0.50 ± 0.50

*Survey counts*

In total, 1036 observations of group size on structures were taken during surveys, and a total of 1791 turtles were seen. After assessing competing models, the best model contained the effects of weekday, Julian day, weather, air temperature, boat traffic, and the interaction between weekday and Julian day on the number of basking turtles (multiple regression, model AIC=4825.0). Air temperature had a positive effect on the number of basking turtles (multiple regression,  $F_{(1, 1019)} = 59.04$ , slope estimate = 0.36,  $p < 0.001$ ; Fig 1.2), but fewer basking turtles were seen as the study period elapsed (multiple regression,  $F_{(1,1019)} = 62.25$ , slope estimate = -0.01,  $p < 0.001$ , Fig 1.3), and when there was higher boat traffic (multiple regression,  $F_{(1,1019)} = 8.26$ , slope estimate = -0.05,  $p = 0.004$ ; Fig 1.4). Group sizes of turtles on structures were larger on Tuesday ( $5.23 \pm 3.47$  turtles), Wednesday ( $3.04 \pm 2.21$  turtles), Thursday ( $3.06 \pm 3.13$  turtles), than Friday ( $2.56 \pm 2.52$  turtles), Saturday ( $2.67 \pm 2.62$  turtles) and Monday ( $2.04 \pm 1.32$  turtles) (multiple regression,  $F_{(5,1019)} = 3.81$ ,  $p = 0.007$ ; Fig. 1.5), and the interaction of Julian day and weekday resulted in fewer basking turtles (multiple regression,  $F_{(5,1019)} = 3.03$ , Table 1.2).

Weather had a significant effect on the number of basking turtles (multiple regression,  $F_{(3,1019)} = 5.35$ ,  $p=0.001$ ; Fig. 1.6), with more basking turtles seen per structure on sunny ( $2.66 \pm 2.49$  turtles), partly cloudy ( $3.89 \pm 3.14$  turtles), and overcast ( $3.69 \pm 3.04$  turtles) days compared to rainy ( $1.47 \pm 0.62$  turtles) days.

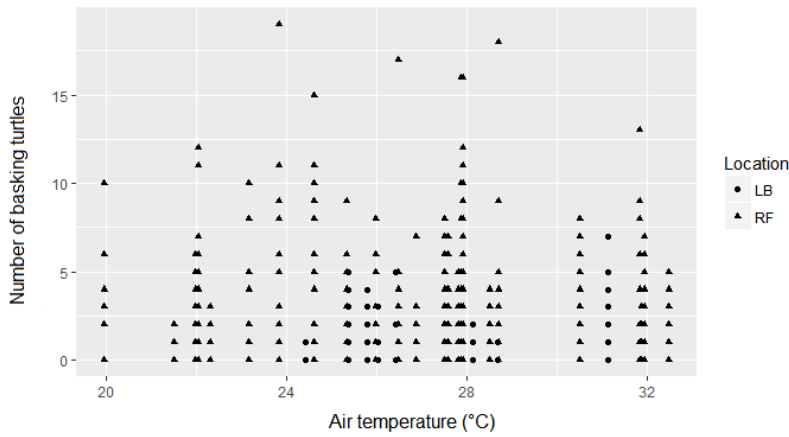


Figure 1.2 More turtles are seen basking as temperature increases (n=1791 turtles). Each point represents the group size observed on a basking structure at either LeFleur’s Bluff (LB) or Ratliff Ferry (RF). Raw data are shown. See text for results of multiple regression model results.

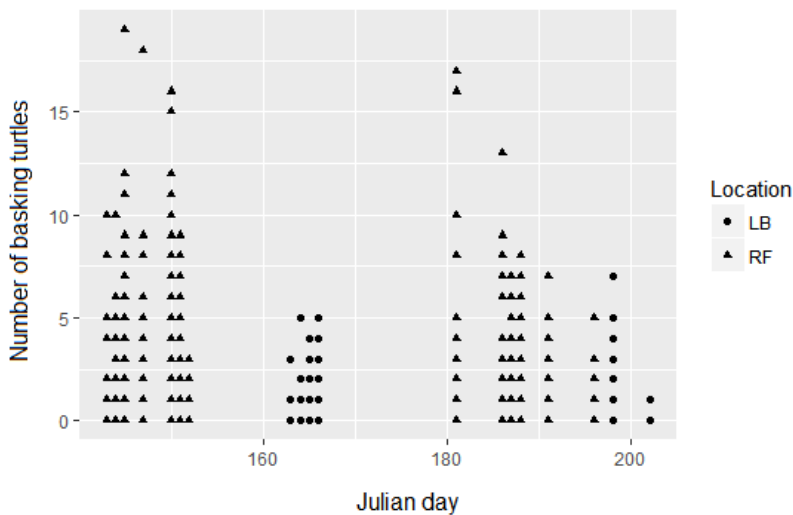


Figure 1.3 Fewer turtles were seen basking on structures as the 2017 field season progressed (n=1791 turtles). Each point represents an observation of group size on a basking structure at either LeFleur’s Bluff (LB) or Ratliff Ferry (RF). Raw data are shown. See text for results of multiple regression model results.

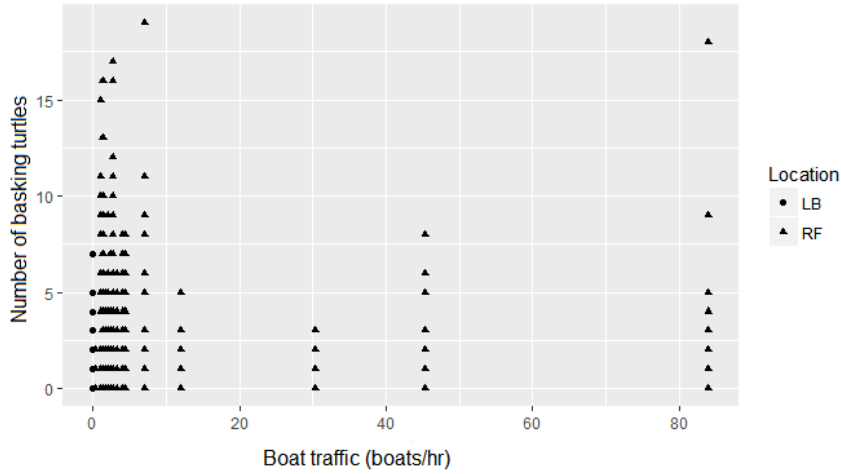


Figure 1.4 Fewer turtles are seen basking as boat traffic increases (n=1791 turtles). Each point represents an observation of group size on a basking structure at either LeFleur’s Bluff (LB) or Ratliff Ferry (RF). Raw data are shown. See text for results of multiple regression model results.

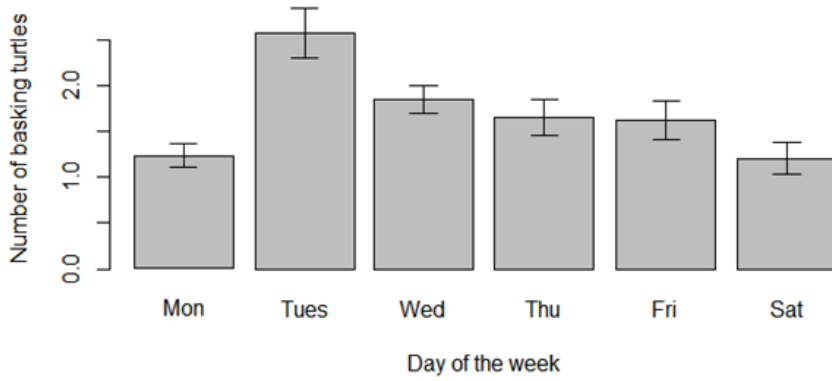


Figure 1.5 Effect of weekday on number of basking turtles seen per structure during surveys (n=1791 turtles). Bars represent standard error. No observations were made on Sunday.

Table 1.2 Effect of interaction of week day and Julian day on the number of basking turtles seen per structure during surveys on the Pearl River. Monday serves the baseline comparison for the effect of day on basking turtle numbers to compare weekdays to weekends. Slope estimates compare the effect of Julian day and other days of the week on the number of basking turtles seen on structures to the number of basking turtles seen on Mondays with the effect of Julian day. A negative slope indicates that fewer numbers of turtles are seen on those week days later in the season compared to days earlier in the field season.

Day	Slope estimate	p value
Tuesday	-0.10	0.004
Wednesday	-0.05	0.046
Thursday	-0.08	0.001
Friday	-0.09	0.024
Saturday	-0.08	0.009

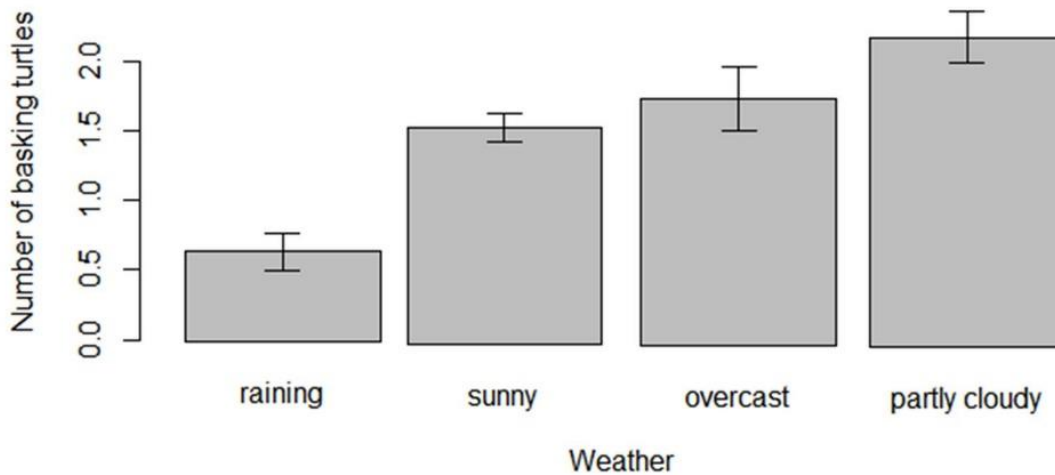


Figure 1.6 Weather had a significant effect on the number of turtles seen basking per structure during surveys (n=1791 turtles). Overcast days had complete cloud cover, partly cloudy days were mostly cloudy with some visibility of the sun, and sunny days had very few clouds. Bars represent standard error.



### *Duration of basking*

The best model assessing correlative effects on the duration of basking contained the effects of Julian day, weekday, air temperature, water temperature, zone, number of basking structures, boat traffic, and interaction of number of basking structures and boat traffic (multiple regression, model AIC=4859.0). Duration of basking increased across the field season (multiple regression,  $F_{(1, 449)} = 29.63$ , slope estimate = 2.41,  $p < 0.001$ ; Fig. 1.7), but turtles basked for shorter durations when air temperature was higher (multiple regression,  $F_{(1,449)} = 35.24$ , slope estimate = -22.45,  $p < 0.001$ ; Fig. 1.8). Turtles basked for longer in no wake zones ( $n=192$  turtles,  $\bar{X}=41.69 \pm 64.41$  min) than wake zones ( $n=270$  turtles,  $\bar{X}=31.03 \pm 34.95$  min) (multiple regression,  $F_{(1,449)} = 5.58$ , wake zone slope estimate = -21.72,  $p = 0.02$ ; Fig. 1.9), and when there are fewer basking structures (multiple regression,  $F_{(1,449)} = 24.16$ , slope estimate = -2.29,  $p < 0.001$ ; Fig. 1.10). Turtles basked for longer when high boat traffic was associated with high basking structure density (multiple regression,  $F_{(1, 449)} = 6.68$ , slope estimate = 0.55,  $p = 0.01$ , Fig 1.11), despite both boat traffic and basking structure density having independent negative effects on duration of basking (slopes = -2.29 and -14.82 respectively).

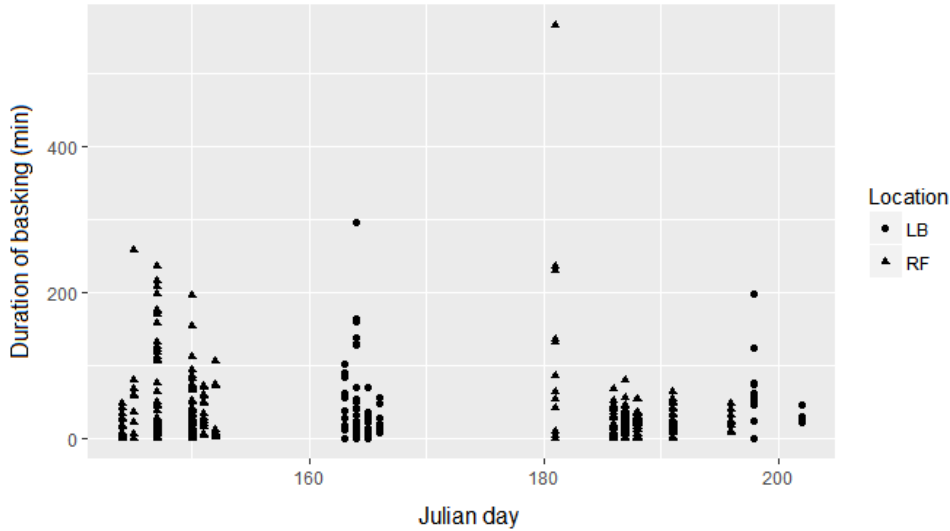


Figure 1.7 Ringed sawbacks bask for longer as the field season progressed (n=462 turtles). Each point represents an observation of a basking event of a single individual from emergence from water to re-entry at either LeFleur's Bluff (LB) or Ratliff Ferry (RF). Raw data are shown. See text for results of multiple regression model results.

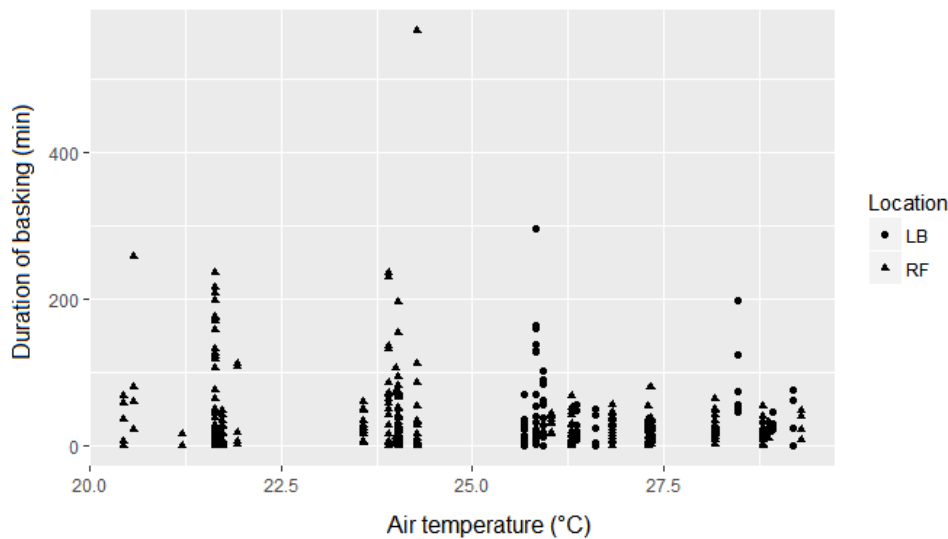


Figure 1.8 Ringed sawbacks bask for shorter durations as air temperature increases (n=462 turtles). Each point represents an observation of a basking event of a single individual from emergence from water to re-entry at either LeFleur's Bluff (LB) or Ratliff Ferry (RF). Raw data are shown. See text for results of multiple regression model results.

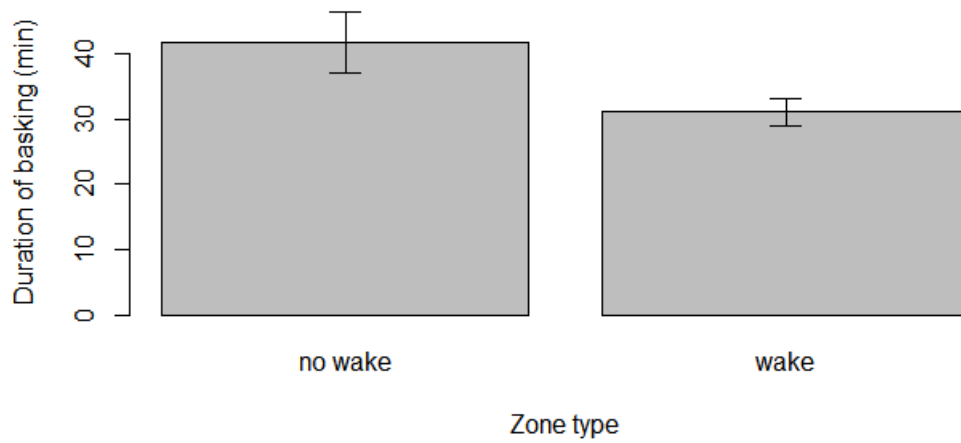


Figure 1.9 Ringed sawbacks bask longer when in a no wake zone compared to a wake zone (n=462 turtles). Bars represent standard error.

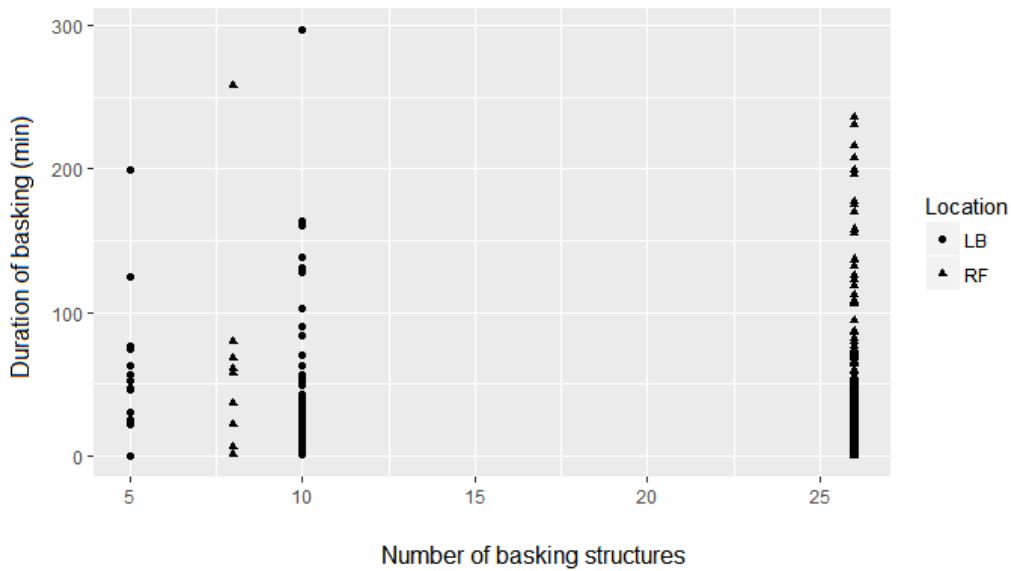


Figure 1.10 Ringed sawbacks bask for shorter durations when availability of basking structures is high (n=462 turtles). Each point represents an observation of a basking event of a single individual from emergence from water to re-entry at either LeFleur's Bluff (LB) or Ratliff Ferry (RF). An outlier point (number of basking structures = 26, duration of basking = 566 min) was removed from graph to facilitate viewing. Raw data are shown. See text for results of multiple regression model results.

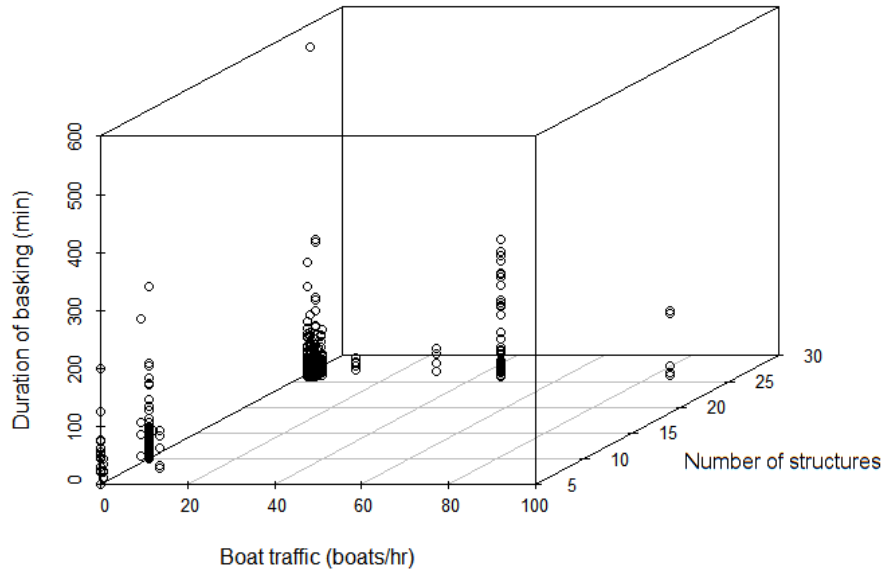


Figure 1.11 Ringed sawbacks bask for longer durations when high boat traffic is associated with high availability of basking structures (n=462 turtles). Each point represents an observation of a basking event of a single individual from emergence from water to re-entry. Raw data are shown. See text for results of multiple regression model results.

### *Odds of disturbance*

Two-hundred thirty-three basking turtles were disturbed and 229 basking turtles were undisturbed. The model with the lowest AIC value for calculating the odds of a turtle being undisturbed contained weekday, Julian day, and the density of basking structures (AIC=529.87, Cox & Snell  $R^2 = 0.24$ ). Compared to Monday, turtles were 1.4x less likely to be disturbed on a Tuesday ( $z = -3.3$ ,  $p = 0.001$ ), 1.1x less likely to be disturbed on a Wednesday ( $z = -2.7$ ,  $p = 0.006$ ), and 1.2x less likely to be disturbed on a Thursday ( $z = -3.1$ ,  $p = 0.002$ ) (Fig 1.12). Monday was chosen as the baseline day to compare weekdays to weekends. Basking turtles were less likely to be disturbed as the field season progressed (logistic regression, odds = -0.05,  $z = -5.5$ ,  $p < 0.001$ )

and are more likely to be disturbed when more basking structures are available (logistic regression, odds=0.09, z=5.2, p<0.001).

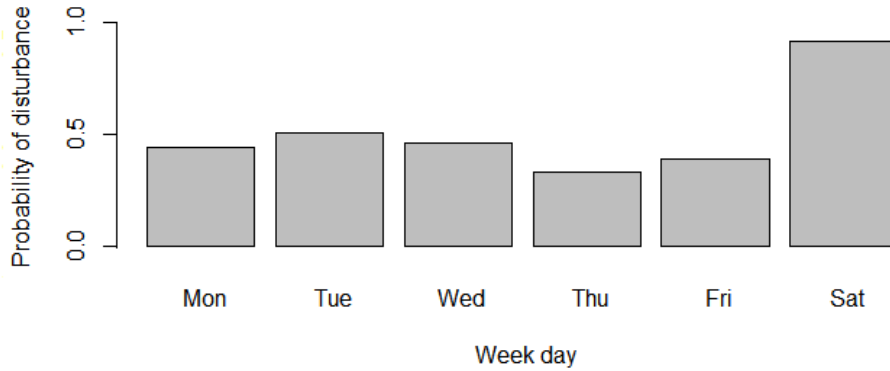


Figure 1.12 The probability of basking terminated by disturbance throughout the week (n=462 turtles).

*Disturbance type*

Type of disturbance significantly affected the number of disturbed turtles ( $\chi^2$  goodness of fit,  $\chi^2= 522.76$ , df=11, p< 0.001; Fig. 1.13), with motorboats, other turtles, and jonboats causing the most disturbances. Of the five most common sources of disturbance, four were anthropogenic.

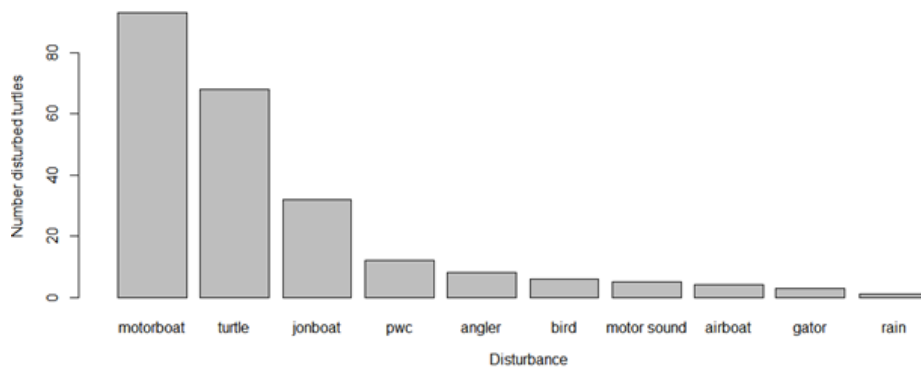


Figure 1.13 Number of turtles who were disturbed and ended basking due to 10 different observed stimuli on the Pearl River (n=233 turtles).

A total of 676 boats were observed, and 571 of these boats passed structures occupied by turtles. Watercraft type significantly affected the percentage of disturbed turtles (ANOVA,  $F_{(5,562)}=8.4806$ ,  $p<0.0001$ ; Fig. 1.14). Higher percentages of basking turtles were disturbed due to kayaks and anglers than other watercraft (Table 1.3). Boats also disturbed more turtles when passing in wake zone ( $n=154$  boats,  $\bar{X}=19.78 \pm 34.20\%$ ) than in a no wake zone ( $n=417$  boats,  $\bar{X}=2.28 \pm 10.01\%$ ,  $F_{(1,562)}=67.13$ ,  $p<0.001$ ; Fig. 1.15).

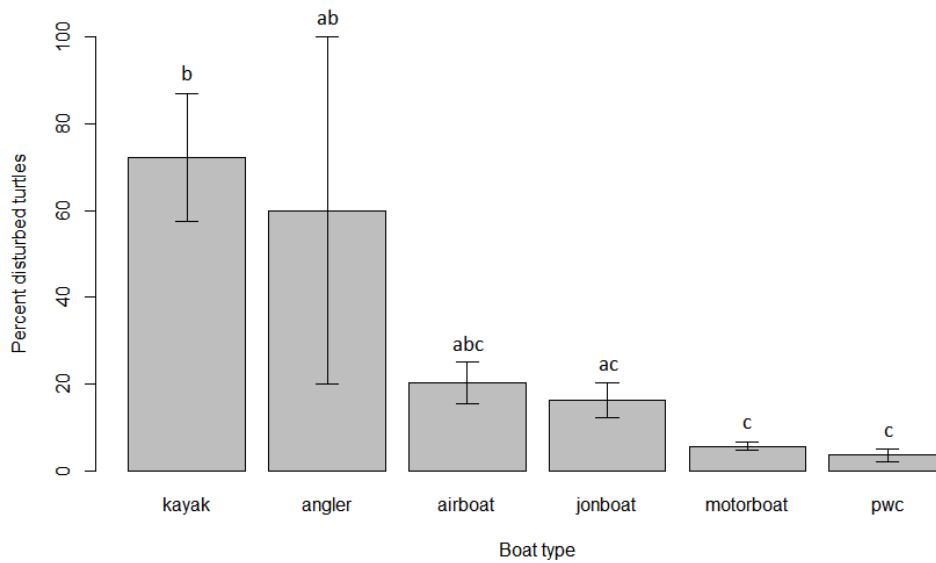


Figure 1.14 Percentage of basking turtles on a structure disturbed due to passing boats ( $n=571$  boats). Columns with different letters are significantly different from each other. Error bars represent standard error.

Table 1.3 Means and standard error of significant differences between percentages of basking turtles disturbed by passing watercraft (n=571 boats).

Disturbance	Means (%)	Disturbance	Means (%)	p value
motorboat	5.58 ±0.92	angler	60.00 ± 40.00	0.03
pwc	3.50 ± 1.57	angler	60.00 ± 40.00	0.018
jonboat	16.26 ± 4.07	kayak	72.22 ± 14.70	< 0.001
motorboat	5.58 ±0.92	kayak	72.22 ± 14.70	< 0.001
pwc	3.50 ± 1.57	kayak	72.22 ± 14.70	< 0.001

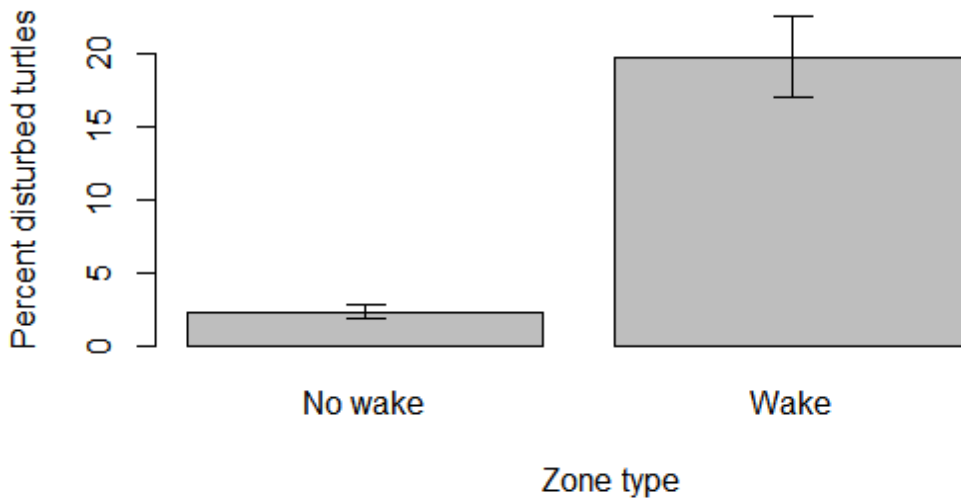


Figure 1.15 Percentage of basking turtles on a structure disturbed due to passing boats (n=571 boats) in a wake and no wake zone. Error bars represent standard error.

## **Discussion**

Human and environmental effects interact to affect behavioral decisions made by wildlife. Poikilothermic turtles must incorporate information about predation risk, thermal efficiency, and competition from other turtles when choosing when to bask and when to terminate basking. Achieving a balance between these factors and optimal temperatures for

metabolic activities is necessary for the health and survival of individual turtles (Huey 1982, Dill 1987, Nowacek et al. 2016).

My study incorporated human and environmental effects into analyses on the basking behavior of ringed sawbacks. Environmental factors of air temperature and Julian day significantly affected basking behavior of ringed sawbacks. High air temperatures independent of water temperatures are associated with shorter basking durations. This suggests that turtles are willing to terminate basking when conditions are thermally favorable for rapid re-warming. Unexpectedly, this appears to conflict with the effect of Julian day, as turtles basked for longer periods as the study season progressed when air temperatures would be the highest. However, the summer of 2017 was one of the wettest on record (NOAA 2017), and there were many days when water temperatures were higher than air temperatures even into mid-June. High rainfall has been shown to lower water temperature and affect basking in turtles (Pittfield & Burger 2017). Because temperature can be important in mediating escape related behaviors in poikilotherms (Weatherhead & Robertson 1992, Martín & López 1999) such as turtles, sawbacks react behaviorally to optimize predation risk. Cooler animals may move slower due to lowered metabolisms, reducing escape efficiency (Weatherhead & Robertson 1992, Martín & López 1999), and may react to reduced escape efficiency by shortening FIDs (Pittfield & Burger 2017), as has been seen in northern water snakes (*Nerodia sipedon*) (Weatherhead & Robertson 1992), or increasing reluctance to leave shelters as has been seen in the Iberian rock lizard (*Lacerta monticola*) (Martín & López 1999). As more turtles were seen basking in higher air temperatures (Fig. 1.2), turtles are likely reacting to lower temperatures by a decreased proclivity to leave the relative safety of the water to bask due to susceptibility to predation (Hertz et al. 1982). Air temperatures did not consistently remain several degrees higher than water temperatures until



July, so it is possible that turtles also did not bask earlier in the season to conserve heat that would have been lost due to low air temperatures and evaporative cooling (Boyer 1965, Brattstrom 1965). These factors taken together indicate that ringed sawbacks are likely basking at temperatures both conducive to thermal gain and escape efficiency.

In terms of human effects on disturbance, turtles terminate basking sooner when there are more basking structures, the opposite of my prediction. If turtles face lower competition for access to basking structures in areas with high structure availability, then they may show an increased willingness to terminate basking. Competition has been shown to negatively affect successful basking by turtles (Polo-Cavia et al. 2010, Cadi & Joly 2003). Polo-Cavia et al. (2010) found that aggressive, invasive red-eared sliders reduced the basking activity and structure usage of native, endangered Spanish terrapins (*Mauremys leprosa*). In my study, other turtles were the second most common reason for terminated basking (Fig. 1.12), therefore turtles in areas with low density of basking structures may be optimizing basking opportunities in relation to perceived competition.

Considering the longer basking durations in no wake zones, the higher percentage of basking turtles that are disturbed by boats passing in wake zones, and the lack of effect of number of passing boats, boat wakes may impact basking more than the mere presence of boats. This effect may be particularly true of turtles at RF, which may be habituated to and tolerant of passing watercraft (Blumstein 2006) but are still swept off structures due to large wakes. During high traffic days such as the weekends and Memorial Day weekend, many turtles were observed to be washed off structures due to passing wakes. I observed that the effect of boat wake is exacerbated by multiple passing boats as waves compound each other to sometimes completely submerge the basking structure. Additionally, large, slow moving boats are known to cause more

disturbance to turtles than small, fast moving boats (Moore & Seigel 2006). This effect could be due to the larger wakes produced by such slow moving boats (Selman et al. 2013). While habituation could result in the tolerance of passing boats, turtles may still fail to successfully bask due to forcible termination of basking by wakes.

Unexpectedly, the interaction of boat traffic and basking structures increased duration of basking, even though individually each had a negative effect. This may be due to the fact that the river mile with the highest boat traffic, mile 3 at RF, contained many no wake zones. Therefore, despite high numbers of boats, turtles were potentially less likely to be disturbed because of passing boat wakes. This effect may have been magnified due to basking observations from Memorial day weekend, when hourly boat traffic reached 84 boats per hour in the afternoon at RF river mile 3. However, due to large crowds and the presence of Mississippi Department of Wildlife, Fisheries, and Parks (MDWFP) personnel on law enforcement vessels, boaters carefully observed the no wake zone. The cases of basking from this day, combined with other cases of high boat traffic observations in the same river mile's no wake zone, may explain the combined positive effect of boat traffic and basking structures on basking durations.

Day significantly affected the odds of a turtle being disturbed, as they were less likely to be disturbed on weekdays than weekends. This could be related to the number of passing boats, which are fewer on weekdays, although the amount of boat traffic did not significantly affect the odds of being disturbed. This lack of effect could potentially be due to the very few boats at LB (11 out of 676 total), where many turtles were instead disturbed by other turtles. In keeping with previous findings, high numbers of basking structures increase the odds of turtles being disturbed. This is again likely due to optimization of basking opportunity and thermal efficiency.

Outside of turtles impeding each others' basking, motorboats, anglers, and pwc disturbed the most number of turtles (Fig. 1.12). However, kayaks, and anglers disturbed the highest percentage of turtles basking on a structure (Fig. 1.13), a difference possibly due to cases when only a few turtles out of many terminated basking. Moore and Seigel (2006) found that slow moving water craft, such as the kayaks in this study which are limited in speed by the kayaker, and anglers who remained in the same area for long durations of time, move slowly primarily using a trawling motor, and may remain out of the main channel close to basking structures, disturbed the greatest number of turtles, as they are potentially more likely to be perceived as a predator threat (Blumstein 2006). Increased human approach speed is associated with longer FID in the stripefoot anole (*Anolis lineatopus*), (Cooper 2006) and pedestrians who stop to watch basking snakes cause more snakes to flee than pedestrians who walk past (Burger 2001). These studies suggest that speed of approach plays an important role in perceived predation risk by wildlife.

More turtles were seen basking in higher air temperatures and when the weather was not raining. Temperature and weather are both correlated to basking efficiency, as turtles are shown to heat quicker in warm air temperatures (Boyer 1965, Crawford et al. 1983), Red-eared sliders (Crawford et al. 1983, Schwarzkopf & Brooks 1985) have been observed to bask more when air temperatures are closer to optimal operating temperatures of the turtle species. Though basking efficiency is higher in direct sunlight, even in heavy cloud cover basking turtles can warm up 3 degrees Celsius higher than ambient temperatures (Boyer 1965, Brattstrom 1965). Turtles may choose to bask under thermally favorable conditions to increase efficiency while minimizing predation risk due to terrestrial predators (Huey 1982). Increased boat traffic was associated with fewer basking turtles, likely due to disturbance. While the number of turtles varied with day

of the week, the combined effect with Julian day was associated with significantly fewer basking turtles. When variation in boat traffic is combined with the higher temperature seen as the season progresses, turtles may increase their reactions to perceived predation risk when shorter basking times are needed to achieve optimal temperatures.

To mitigate the effect of human disturbance on basking, increasing the number of no wake zones at RF could reduce the number of turtles being swept off structures. This could also reduce boat traffic if boaters decide to utilize the unrestricted boating activities of the Ross Barnett Reservoir. Managers could also reduce boat traffic by limiting the number of access points to the Pearl River in the PRWMA under the management of the MDWFP. In comparison to LB, RF has several access points along the river, including the Ratliff Ferry Trading Post, Coal Bluff State Park, and other boat ramps along the Natchez Trace. By closing lesser used boat ramps, boaters may move their recreational activities to other locations rather than compete for access to boat ramps or endure the greater distances required to reach their desired recreational areas

## **Conclusion**

To fully predict the effect of human disturbance on animal behavior and populations, researchers must incorporate factors, both intrinsic and extrinsic to the individuals under study, that may influence behavior. Managers are better able to create plans to conserve threatened species after accounting for variations in environmental factors or human disturbance. Recovering the ringed sawback will require multiple approaches, and a greater understanding of the relationship between humans, turtles, and the environment will be vital to the preservation of this and other riverine species.

**CHAPTER II:**  
**CONSEQUENCES OF ANTHROPOGENIC DISTURBANCE OF BASKING ON THE  
BODY TEMPERATURE OF THE RINGED SAWBACK (*GRAPTEMYS OCULIFERA*)**

**Introduction**

Organisms have two possible thermal responses to the environment – thermoconformity and thermoregulation (Seebacher 2005, Flouris 2011). Thermoconformity occurs when an animal’s internal body temperature conforms to match the temperature of the environment (Huey & Slatkin 1976), while thermoregulation occurs when an animal maintains a consistent or nearly consistent body temperature regardless of changing environmental temperatures (Seebacher 2005, Flouris 2011). Poikilotherms have the option to thermoregulate or thermoconform based on intrinsic body condition and external environmental factors (Huey & Pianka 1977, Mathies & Andrews 1997). Thermoconformity may be favorable when environmental temperatures and optimal body temperatures are close (Seebacher & Grigg 1997), when energetic costs of seeking out thermally optimal habitat are high (Hoekstra 2015), or when predation risk makes limited movement favorable (Hertz et al. 1982, Huey 1982). Thermoregulation to achieve a desired temperature is favorable when certain metabolic processes are necessary, such as digestion (Gatten 1974, Greenwald & Kanter 1979, Blouin-Demers & Weatherhead 2001), egg development (Charland & Gregory 1990, Gregory et al. 1999, Lourdais et al. 2008), immune function (Merchant et al. 2007, do Amaral et al. 2002), or when attempting to remove ectoparasites (Selman & Qualls 2009). Because of the many factors determining whether an

organism thermoconforms or thermoregulates, poikilotherms may be sensitive to permutations in habitat structure that would influence the favorability of one tactic or another.

Basking by riverine turtles is an important physiologic activity (Boyer 1965) and is the purposeful placement and orientation by an animal on a substrate in the sunlight to enable many metabolic functions associated with thermoregulation (Lindeman 2013). Basking has been shown to be important in turtles to aid in digestion (Gatten 1974, Greenwald & Kanter 1979, Hammond et al. 1988, Blouin-Demers & Weatherhead 2001), immune function (do Amaral et al. 2002, Merchant et al. 2007), and has been theorized to play a role in egg development in female turtles (Gregory et al. 1999, Lindeman 1999, Moore & Seigel 2006, Lourdais et al. 2008). Basking is vital for raising body temperatures above environmental temperatures, presumably to reach a more optimal body temperature (Litzgus & Brooks 2000, Lindeman 2013). However, the fitness consequences of reduced basking due to disturbance have not been well-studied, as few studies incorporate fitness measures or fitness proxies (Moore & Seigel 2006, Selman et al. 2013, Jain-Schlaepfer et al. 2017).

Human disturbance has significant, negative effects on wildlife, primarily through the perception by wildlife that humans are predators (Blumstein 2006). Many of the studies on the effect of human disturbance focus on endotherms (Buchholz & Hanlon 2012), ignoring the potential for disturbance affecting thermoregulation and physiologic functions in poikilotherms. As predation risk is a strong factor in decisions to thermoregulate, human disturbance may serve to discourage optimal basking behavior in poikilotherms (Moore & Seigel 2006, Selman et al. 2013, Jain-Schlaepfer et al. 2017). Passing boats are shown to negatively affect the basking behavior of yellow-blotched sawbacks (Moore & Seigel 2006) and common map turtles (*Graptemys geographica*) (Jain-Schlaepfer et al. 2017) by triggering escape behavior in basking

turtles, causing them to enter the water. While the behavioral consequences of human disturbance are documented (Moore & Seigel 2006, Selman et al. 2013, Polich & Borazowski 2016, Pittfield & Burger 2017), the thermal consequence is less explored (Jain-Schlaepfer et al. 2017). Whether such behavioral disturbance translates into a negative physiologic effect, such as reduced metabolism or clutch size merits further examination (Jain-Schlaepfer et al. 2017).

Thermal modeling and simulations can give important insight into thermal ecology of organisms when it is difficult or impossible to extract the same information from live organisms (Dzialowski 2005, Dubois et al. 2009, Yagi & Litzgus 2013, Jain-Schlaepfer et al. 2017).

Temperature change in water and air is proportional to the difference in body temperature and the surrounding environment (Dzialowski & O'Connor 2001, Jain-Schlaepfer et al. 2017). This proportion is a thermal constant (Dzialowski & O'Connor 2001, Jain-Schlaepfer et al. 2017).

When change in body temperature over time is regressed against body temperature ( $T_b$ ), air temperature ( $T_{air}$ ), and water temperature ( $T_w$ ), the values of thermal constants can be obtained.

This information has been used in simulations of disturbance on common map turtles to assess reductions in body temperature and metabolic rate of disturbed adult female turtles (Jain-Schlaepfer et al. 2017). As obtaining accurate body temperature data can be difficult without implanting temperature recording devices (Boyer 1965, Dubois et al. 2009), model simulations are an excellent method for assessing thermal condition without invasive methods, particularly for threatened and endangered taxa.

In this chapter, I use the results of my disturbance chapter (Chapter 1) to examine a potential consequence of interrupted thermoregulatory behavior to draw greater connections between anthropogenic disturbance and fitness consequences. My objective is to simulate the thermal cost of anthropogenic disturbance from observational field data to assess the impact of

reduced basking on an endangered species. I hypothesize that anthropogenic interruptions to basking will negatively affect thermoregulation and predict that simulation will show that adult female ringed sawbacks who are disturbed due to anthropogenic sources while basking will have a lower average body temperature than undisturbed turtles, and that the effect of disturbance will decrease as water and air temperatures increase.

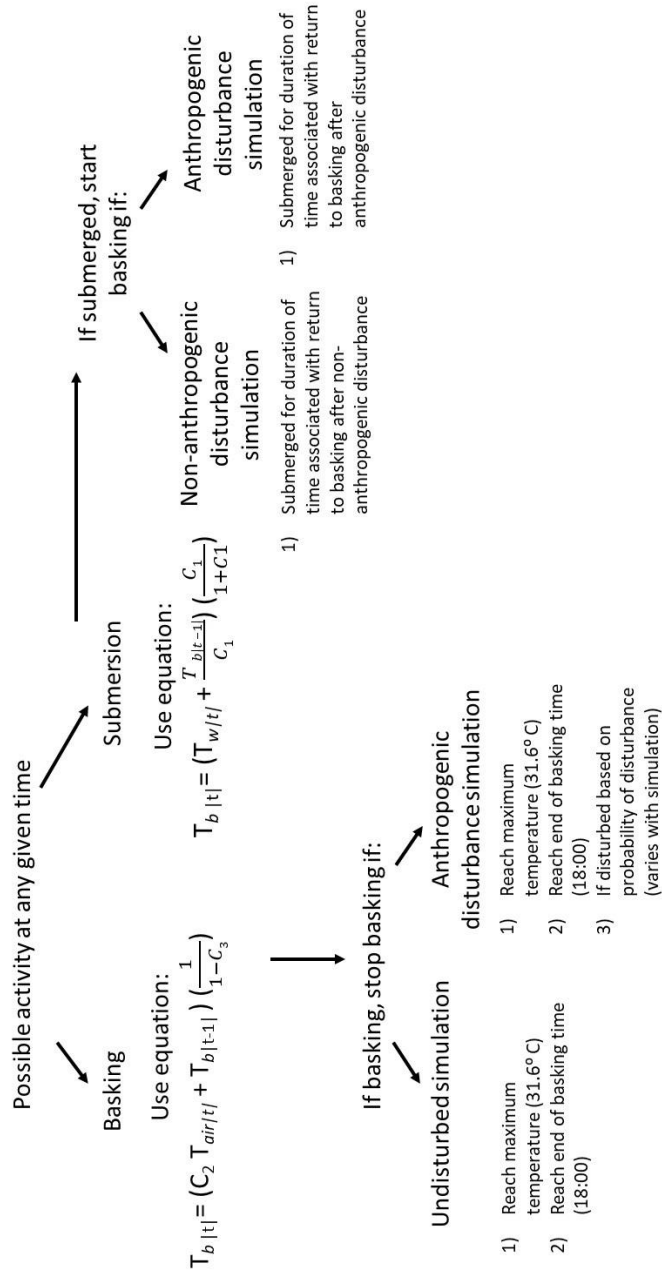
## **Methods**

### *Simulation of disturbance*

To simulate the effect of disturbance on the body temperatures of turtles, methods were adapted from Jain-Schlaepfer et al. (2017) as seen in Figure 2.1. Body temperature was calculated every minute based on the behavior at that moment, either basking or submersion. The average body temperature over the course of a day for adult female ringed sawbacks was simulated 100 times each for both anthropogenically disturbed and undisturbed turtles at various probabilities of disturbance. The variables needed were 1) values for thermal constants for temperature change during basking and submersion, 2) time of day when basking ceased, 3) probabilities of disturbance for simulations, 4) average water and air temperature for time period of simulation, 5) optimal body temperatures of ringed sawbacks, and 6) behavioral observations of the number of minutes to return to basking after a disturbance.



Figure 2.1 Chart showing the steps in behavioral simulations of anthropogenically and undisturbed turtles based on methods described by Jain-Schlaepfer et al. (2017). Thermal constants from Jain-Schlaepfer et al. (2017) for common map turtles are 0.0591, 0.00624, and 0.0149 for  $C_1$ ,  $C_2$ , and  $C_3$  respectively.



### *Values of thermal constants*

Thermal constants reported for adult females from a related species, the common map turtle, were used (Jain-Schlaepfer et al. 2017). While female common map turtles and ringed sawbacks differ in average mass (1500 g versus 800 g [Jain-Schlaepfer et al. 2017, Heppard Chapter 3]), Jones (2006) reports gravid female ringed sawbacks reaching up to 1457 g. Other turtle species with known warming constants differ in both size and shell shape (ornate box turtle (*Terrapene ornata*), mass=250 g [Actams & Decarvalho Jr 1984], spiny softshell turtle (*Apalone spinifera*), mass=7400g [Smith et al. 1981], Table 2.1). Although rate of heat gain is generally negatively correlated to organism size (Jain-Schlaepfer et al. 2017), shell shape can impact efficiency of basking (Polo-Cavia et al. 2009), so constants from a related species with a similar shell structure (the common map turtle) were used. Therefore,  $T_b$  calculated using the thermal constants from common map turtles may overestimate of the effect on smaller ringed sawbacks. These potential differences in warming constants were accounted for by running statistical tests on body temperature differences between undisturbed and disturbed turtles, rather than calculated  $T_b$ . Constant 1 ( $C_1$ ) was found to be 0.0591, constant 2 ( $C_2$ ) was found to be 0.00624, and constant 3 ( $C_3$ ) was found to be 0.0149 (Jain-Schlaepfer et al. 2017).

Table 2.1 Selection of reported thermal constants from turtle species.

Species	$C_1$	Mass (g)	Publication
<i>Terrapene ornata</i>	0.3934	250	Actams & Decarvalho 1984
<i>Apalone spinifera</i>	0.023	7400	Smith et al. 1981
<i>Graptemys geographica</i>	0.0591	1500	Jain-Schlaepfer et al. 2017

### *Time of basking end and probabilities of disturbance*

Turtles were assumed to cease basking at 18:00 (R. Jones, pers. comm). The probability of disturbance in each hour was run under several conditions: 0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.5, 0.7, 1. This range of probabilities covered the range of hourly disturbances seen in behavioral observations (see below) and simulated the effect of extremely high disturbances.

### *Air and water temperatures*

Four time periods were chosen for simulations based on activity periods from Jones (2006): 27-31 May (early nesting, primarily for large females), 13-17 June (peak nesting), 13-17 July (late nesting, primarily for large females with second clutch), and 1-5 August (end nesting). By using time frames based on nesting activities, inferences can be made on the effect of lowered  $T_b$  on egg development, an important physiologic activity that could have consequences for the recovery of the species.

Hourly air and water temperatures from each of the four given activity periods were averaged over five days in 2016 and 2017 from data taken from the United States Geologic Survey (USGS) NSTL Station on the Pearl River, located in Hancock County, MS (Lat 30°21'08", Long 89°38'45" NAD27) (USGS 2017). Table 2.2 shows the air and water temperature averages for each of the four activity periods used in the simulation. Water temperatures were higher than air temperatures across activity periods.

Table 2.2 Mean and standard error of hourly air and water temperatures for four time frames in 2016 and 2017 from the United States Geologic Survey NSTL station located in Hancock County, MS.

Time frame	Air temperature (°C)	Water temperature (°C)
May (27-31)	24.38 ± 0.20	25.39 ± 0.90
June (15-17)	26.28 ± 0.21	27.38 ± 0.08
July (15-17)	27.09 ± 1.19	28.80 ± 0.08
August (1-5)	26.38 ± 0.21	28.21 ± 0.12

#### *Optimal body temperatures*

The optimal basking temperature represents the optimal operating temperature for a basking turtle; any temperature higher than it should result in cessation of basking (Dubois et al. 2008, Jain-Schlaepfer et al. 2017). Average basking temperatures were calculated from four captive ringed sawbacks housed at the Mississippi Museum of Natural Science (Jackson, MS). Heat lamps hanging above wooden basking structures provided warmth, water temperature was approximately 29° C, and air temperature was approximately 26° C. Two adult male and two adult female ringed sawbacks were equipped with Thermochron iButtons (Model # DS1921H, Maxim Integrated San Jose, CA) attached to carapaces via marine epoxy and observed over the course of four days. Past study has shown that optimal body temperature is similar between females, males, and juveniles (Bulté & Blouin-Demers 2010, Jain-Schlaepfer 2017). The average carapace temperature during the observed basking period was assumed to be the optimal temperature, and was calculated to be 31.6° C.

### *Behavioral observations*

Behavioral and disturbance observations took place between 27 June and 6 August in 2016 and 23 May and 15 July in 2017, and are explained fully in Chapter 1.

Because individual turtles were not identified, the time to return after an anthropogenic disturbance was calculated from the time when a passing watercraft disturbed all turtles on a structure to the time of the first turtle to return to basking. As time to return from a watercraft disturbance did not vary between months (ANOVA,  $F_{(3,42)}=0.542$ ,  $p=0.656$ ), all times to return from anthropogenic disturbance were combined for use in the simulation. The time to return to basking after non-anthropogenic disturbance was calculated from the time when a turtle was disturbed by another turtle to the time when the next turtle surfaced, as it was assumed to be the same disturbed turtle as this was observed to be the case many times. The use of this time to return served as a comparison between natural disturbance and voluntary cessation of basking compared to anthropogenic disturbance. The time to return from anthropogenically disturbed basking was  $15.91 \pm 17.29$  min ( $n=22$ ) and nonanthropogenic disturbance was  $6.73 \pm 6.81$  min ( $n=46$ ).

Percentage of observed basking turtles disturbed each month can be found in Table 2.3. Observed probability of disturbance per hour was 0.02 in May, 0.01 in June, 0.004 in July, and 0.02 in August. The observed probability of disturbance each hour was 0.16 during the day of highest observed hourly boat traffic ( $64.67 \pm 19.33$  boats per hour) which was the Saturday of Memorial day weekend (27 May in 2017).

Table 2.3 Number of basking turtles on the Pearl River observed and percent disturbed for four months in 2016 and 2017.

Month	Total basking turtles	Percent disturbed
May	194	75.77
June	226	39.82
July	481	37.21
August	163	52.76

*Limitations of the simulations*

This study was limited by several factors. By using thermal constants from a different, larger species, accurate simulated  $T_b$  cannot be determined, limiting comparisons to the difference in temperatures between simulations. As individual turtles were not marked for observation, the time between cessation of and return to basking may not reflect behavior of individual turtles, and time to return from a disturbance may differ between adult male and female turtles and juveniles.

*Statistical tests*

All statistical tests were carried out using the statistical program R Studio (R Foundation, version 3.4.2) with an  $\alpha$  of 0.05.

A multiple regression was used to test if differences in body temperatures (dependent variable) varied between anthropogenically disturbed and undisturbed simulations across probabilities of disturbance (independent variables). Multicollinearity was not present between variables, and the model with the lowest AIC value and variables with relative variable importance factors over 0.5 was used (see Appendix Tables 5 and 6).

## **Results**

### *Model simulations*

The interaction between activity period and probability of hourly disturbance significantly affected the difference in simulated body temperature between anthropogenically disturbed and undisturbed turtles (multiple regression,  $F_{(3,3592)}=283.67$ ,  $p < 0.001$ , Fig. 2.2).

Difference between  $T_b$  of anthropogenically disturbed and undisturbed turtles increased as the probability of disturbance increased, and were highest in May, followed by June, August, and July. Average body temperatures across probabilities of disturbance and activity periods can be seen in Table 2.4.

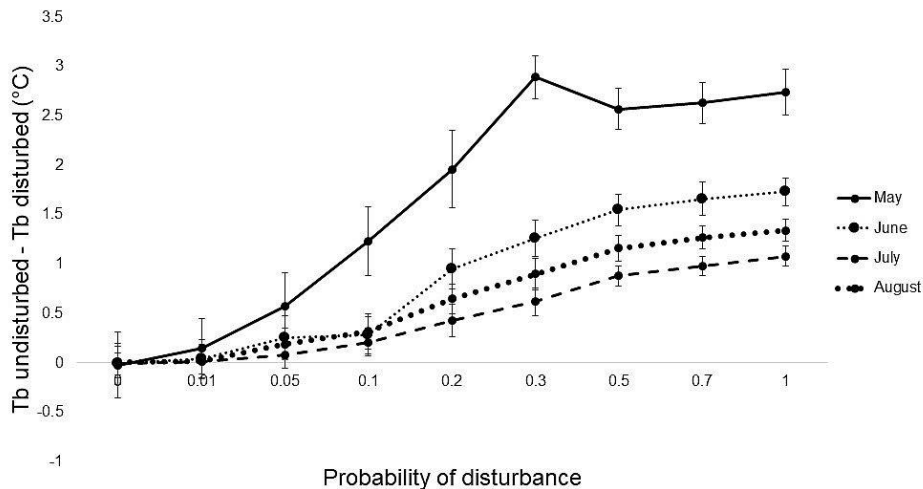


Figure 2.2 Differences in simulated body temperatures of undisturbed and anthropogenically disturbed adult female ringed sawbacks in the Pearl River over different probabilities of disturbance during four activity periods: 27-31 May (early nesting, primarily for large females), 13-17 June (peak nesting), 13-17 July (late nesting, primarily for large females with second clutch), and 1-5 August (end nesting).

Table 2.4 Average and standard deviations of simulated daily body temperature differences between undisturbed and anthropogenically disturbed adult female ringed sawbacks across four summer months.

Probability of disturbance	May	June	July	August
0	-0.02±0.33	-0.01±0.21	-0.01±0.11	0.01±0.16
0.01	0.15±0.3	0.04±0.2	0.01±0.12	0.02±0.17
0.05	0.57±0.34	0.26±0.22	0.08±0.13	0.19±0.16
0.1	1.23±0.35	0.28±0.19	0.21±0.14	0.32 ±0.18
0.2	1.96±0.39	0.95±0.20	0.43±0.16	0.65±0.15
0.2	2.89±0.22	1.26±0.18	0.62±0.14	0.90±0.16
0.5	2.57±0.21	1.55±0.16	0.88±0.10	1.16±0.13
0.7	2.63±0.21	1.66±0.17	0.98±0.10	1.27±0.12
1	2.74±0.23	1.73±0.14	1.08±0.10	1.34±0.11

## **Discussion**

This study sought to quantify the difference in body temperatures between simulations of thermoregulation in anthropogenically interrupted and uninterrupted basking adult female ringed sawback turtles across four activity periods relevant to egg development and nesting. While ringed sawbacks are the model for this study, if species exist in a similar climate or habitat structure, such as the related yellow-blotched sawback which has already been shown to suffer from high amounts of boat traffic that impact basking and nesting (Moore & Seigel 2006), then it is likely that my findings may be applicable to those other riverine species.

While body temperatures significantly differed between month and probability of disturbance, as the effect of disturbance was most pronounced in May, whether this difference is ecologically relevant is unknown. Disturbed turtles at the highest natural disturbance rate (0.20)



in May had a temperature difference of  $\sim 2$  °C in May and  $\sim 1$  °C in June. While body temperatures in gravid versus non-gravid turtles has not been assessed, previous studies of temperature differences have shown temperature increases of  $\sim 1$  °C in eastern box turtles (*Terrapene carolina*) injected with bacterial lipopolysaccharide to elicit an immune response (do Amaral et al. 2002) and  $\sim 1.5$ °C to facilitate digestion in recently fed ornate box turtles compared to unfed turtles (Gatten 1974). However, the largest temperature difference between disturbed and undisturbed turtles at a probability seen on a non-holiday across months (0.01) was only 0.15° C in May. On the other hand, reduced daily  $T_b$  of 0.11° C in disturbed common map turtles corresponded to an average reduction in standard metabolic rate of 2.7% (Jain-Schlaepfer et al. 2017). Persistent, lowered metabolic rate could result in reduced growth in juveniles (Williamson et al. 1989, Litzgus & Hopkins 2003), making them more susceptible to predation (Jones 2017), impede immune function (Smith et al. 2017), and reduce metabolic rate and embryonic growth in nestlings due to maternal effects (Steyermark & Spotila 2000, Rowe et al. 2017). This chronic reduction in metabolism could impede individual survival and population growth in disturbed populations and obstruct recovery efforts.

Additionally, variation in body temperature, rather than average lower  $T_b$ , during gravidity could affect reproduction in adult female ringed sawbacks. Body temperatures were found to be less variable in gravid children's pythons (*Antaresia children*) (Lourdais et al. 2008) and prairie rattlesnakes (*Crotalus viridis*) (Charland & Gregory 1990) compared to non-gravid snakes. Maintaining a consistent body temperature may be more favorable than an elevated  $T_b$ , particularly as Charland and Gregory (1990) found that average  $T_b$  did not differ between gravid and non-gravid snakes. Studies on a similar potential pattern in turtles would be valuable for predicting if frequent changes in body temperatures due to terminating basking could correspond

to reduced reproductive output (Jain-Schlaepfer et al. 2017), potentially impeding recovery in this species.

Persistent lowered body temperatures may represent a possible mechanism responsible for observed differences in health and physiology between populations of turtles. Selman et al. (2013) found that yellow-blotched sawbacks had poorer shell quality (assessed by prevalence of fungal and bacterial infections on the carapace) and higher heterophil:lymphocyte (H:L) counts at a recreationally disturbed site on the Pascagoula River in comparison to a undisturbed reach of the Leaf River in Mississippi. A lowered ability to thermoregulate may be responsible for increased stress of individuals, as H:L counts are often used as indices of stress and immune function. Bennett et al (2009) investigated differences in common map turtle populations in disturbed and intact areas in Ontario, Canada and found that turtles in riverine areas with high concentrations of locks, dams, and other construction projects were smaller than those in uninterrupted stretches of the river. Reduced metabolic function due to lower body temperatures could account for this effect (Litzgus & Hopkins 2003).

Future studies should seek to quantify ecologically relevant temperature changes in turtles for physiologic activities such as digestion, escape behavior, and egg development to assess critical decreases in body temperature. If boat traffic affects body temperatures in an ecologically relevant way, measures should be taken to reduce anthropogenic effect on basking, such as increasing the number of no wake zones and reducing river access as discussed in Chapter 1. Since environmental temperatures play a role in efficiency of thermoregulation (Boyer 1965), as shown by the reduced effect of disturbance as the summer progresses, future studies could examine the effect of body temperature reduction across climate zones.

Conservationists may then target specific regions with environmental temperatures which exacerbate the effect of anthropogenic disturbance.

### **Conclusion**

This study demonstrates a significant difference in average body temperatures between anthropogenically disturbed and undisturbed turtles. While the ecological relevance of body temperature reduction has not been studied fully Jones (2017) showed that the population of ringed sawbacks at RF is in decline. Considering other studies which show consequences of boat traffic (Moore & Seigel 2006, Selman et al. 2013), efforts to minimize anthropogenic disturbance should be considered.

**CHAPTER III:**  
**OTHER OBSERVATIONS ON THE RINGED SAWBACK (*GRAPTEMYS OCULIFERA*)**  
**AND MANAGEMENT RECOMMENDATIONS**

**General Introduction**

The previous two chapters of this thesis have attempted to elucidate the effect of human disturbance on the behavior and physiology of the endangered ringed sawback. This final chapter is a collection of observations that were made during the course of this study, but do not warrant individual chapters. While limited in scope, this information is worth documentation as preliminary data that will draw attention to areas suitable for future research.

Human disturbance can affect riverine turtles via removal of substrate and interruption of basking activities by passing watercraft, as outlined in Chapter 1, and as seen in Moore & Seigel (2006), Selman et al. (2013), and Jain-Schlaepfer et al. (2016). I have shown the effects of human disturbance on ringed sawback basking behavior in Chapter 1, and effects on thermal regulation in Chapter 2. Here, I will use indices of individual health (parasite load, shell quality), and population health (age structure) to compare my two study sites that vary in the amount of human disturbance present; site differences are summarized in Table 3.1. Details on the study sites, Ratliff Ferry (RF) and LeFleur's Bluff (LB) can be seen in Chapter 1 and Fig. 1.1.

Table 3.1 Comparison of assumed human disturbance at two sites, Ratliff Ferry (RF) and LeFleur’s Bluff (LB), on the Pearl River outside Jackson, MS

Site	Hourly boat traffic	Effect on population	Availability of basking structures	Effect on population
RF	High	Negative	High	Positive
LB	Low	Positive	Low	Negative

The objectives of this chapter are to describe variation in parasitism across sites and types of human disturbance, to describe variation in age classes and shell condition, and to provide management recommendations drawing from this thesis and previous related studies.

All collections and animal handling were carried out in accordance to IACUC protocol #16-022, MDWFP Scientific Collecting Permit #0430171, and USFWS Endangered Species Permit #TE98486B-0.

## **Parasitism**

### ***Introduction***

Turtles have many different endo- and ecto-parasites (Telford 1984, Telford 2009). One of the most common ectoparasites are leeches (*Placobdella*, spp.), which will attach to turtles and draw blood meals both from soft tissues (Readel et al. 2008) and from bony tissue (Siddall & Gaffney 2004). Leeches have been recorded in many species of freshwater turtles, including common map turtles (Ryan & Lambert 2005), common musk turtles (*Sternotherus odoratus*) (Ryan & Lambert 2005, Readel et al. 2008), and common snapping turtles (*Chelydra serpentina*) (Brown et al. 1994, Siddall & Gaffney 2004, Readel et al. 2008) in the United States. Leeches present a physiological cost both through their taking of blood meals (Berven & Boltz 2001),

which can lead to anemia (Readel et al. 2008), and because they may transmit haematozoa, such as haemogregarina and trypanosomes to the host (Telford 1984, Siddall & Dessler 2001, Telford 2009).

Infections of haemogregarines have been noted in Blanding's turtles (*Emydoidea blandingii*) (Lacroix et al. 2012), common snapping turtles (Paterson & Dessler 1976), and Sicilian pond turtles (*Emys trinacris*) (Arizza et al. 2016), and the genus *Haemogregarina* has been noted to occur in reptiles on most continents (Telford 2009). While haemogregarine infection is thought to cause no significant harm to turtle species (Brown et al. 1994, Davis & Sterrett 2011, Arizza et al. 2016) infection of weak individuals may have consequences. Özvegy et al. (2015) associated haemogregarina infections with skin lesions on European pond turtles (*Emys orbicularis*). Mihalca et al (2002) noted reduced lymphocyte and increased eosinophil counts in European pond turtles infected with haemogregarina, which could affect immune responses to other stressors.

Because basking removes leeches via desiccation (Selman & Qualls 2009) and basking has been shown to be important in immune function (do Amaral et al. 2002, Ibáñez et al. 2015), human disturbances that reduce basking may result in higher parasite loads. Therefore, quantifying ecto- and endo-parasite load both by site and disturbance quantified as average hourly boat traffic and number of basking structures in the river mile of capture (see Methods in Chapter 1) could offer clues on the impact of human disturbance on individual health. I hypothesize that human disturbance will be associated with higher parasite counts and predict that areas with high boat traffic and low structures per river mile will have turtles with higher parasite loads.

## ***Methods***

### *Assessment of disturbance*

Sampling and assessment of disturbance were made in the two sites, RF and LB, discussed in the general introduction to this chapter. Assessment of boat traffic and basking structures are covered in detail in Chapter 1.

### *Sampling methods*

Sampling of adult and juvenile ringed sawbacks took place between May and August 2017. Turtles were captured via basking traps attached to flagged deadwood structures (excluding structures currently under boat traffic observation). Traps were made of crawfish wire formed into an open-topped box or made from modified hoop nets (after Lindeman 2014) and were checked at least once a day.

The shells of captured turtles were cleaned and marked uniquely with holes drilled into marginal scutes (Cagle 1939). These populations were previously marked by Jones and Hartfield (1995) between 1988 and 1990, by Jones (2006) 1995-1996, and intermittently by Jones (2017) between 1994-2004. The capture location, number of leeches (*Placobdella* spp.), and a blood sample (0.2 mL) was taken from adult turtles with a 27 gauge needle via the dorsal coccygeal vein (Hughe 2010, McAuliffe 1977, Siddall & Desser 2001). Individuals who were recaptured during the course of the study (n=3) were not resampled. A thin smear was prepared from a drop of blood on a glass slide, which was air-dried, and fixed with methanol for five minutes before being stored for staining in the laboratory at a later date (Wood & Ebanks 1984, Barta & Desser 1984).

### *Quantification of haematozoa*

Fixed blood smears were stained with 1:10 Giemsa (Fisher Scientific Wright's Giemsa, pH = 7) in buffered water for fifty-five minutes, rinsed with buffered water, and air dried (McAuliffe 1977, Wood & Ebanks 1984, Barta & Dessler 1984). Blood smears were examined under 1,000x magnification for the presence of haematozoa per 1,000 erythrocytes (McAuliffe 1977, Barta & Dessler 1984). Haematozoa were compared to images in Telford (2009) for preliminary identification.

### *Statistical methods*

All statistical tests were carried out using the statistical program R Studio (R Foundation, version 3.4.2) with an  $\alpha$  of 0.05.

To assess if parasitism (dependent variable) differs between site and indices of human disturbance, a MANOVA was carried out using boat traffic and number of structures for the river mile where an individual was caught, and location (RF or LB) as independent variables. Leech load and haematozoa infections were given a Z score to place them within the same unit of comparison. Assumptions of normality of residuals and equal variance for were not met despite transformations, but significance of the tests was not likely affected by the violations.

A logistic regression was carried out to assess if boat traffic and number of basking structures per river mile, or location predicted the likelihood of a turtle being infected with parasites, including haematozoa or leeches.



## **Results**

### *Assessment of disturbance*

Of the 288 boats observed in total, more were seen at RF (n=277 boats) than LB (n=11 boats). Number of basking structures per river mile was similar at both RF ( $13.0 \pm 9.27$  structures, total structures=39) and LB ( $8.33 \pm 2.89$  structures, total structures=25). Average hourly boat traffic and basking structures per river mile can be found in Table 3.2. Boat traffic was not assessed at mile 2 at RF because of lack of availability of observation locations for monitoring structures. However, this deficiency did not affect analyses as no turtles were captured within that river mile.

Table 3.2 Measurements of human disturbance in 3 river miles at Ratliff Ferry (RF) and LeFleur's Bluff (LB) on the Pearl River. Boat traffic (average boats per hour) was not assessed at RF due to lack of available observation site.

Location	River mile	Boat traffic	Number of basking structures
RF	1	4.83	8
RF	2	N/A	5
RF	3	8.43	26
LB	1	1.00	10
LB	2	1.17	5
LB	3	0	10

Nineteen adult ringed sawbacks and 0 juveniles were captured and sampled at Ratliff Ferry and 21 adults and 8 juveniles were sampled at LeFleur's Bluff. At RF all turtles were captured within the same river mile (mile 3). At LB, 5 were captured at river mile 1, 4 were

captured at river mile 2, and 11 turtles were trapped at river mile 3. An adult male was sampled at RF that had been first captured and marked by Dr. Robert Jones in 1988, with an estimated age now of at least 34 years.

Four of the 40 adult captured turtles had one or more leeches ( $\bar{X}=1.50\pm 0.50$  leeches). Leeches were not observed on basking turtles as all leeches were found attached to skin connecting to the carapace or plastron. Twenty turtles were infected with haematozoa; of these, 2 turtles carried leeches. Prevalence of haematozoan infection was similar at LB (10 infected out of 21 total) and RF (10 infected out of 19 total). In infected turtles, haematozoa infections ranged from 1 (0.1% cells infected) to 28 (2.8% cells infected) haematozoa per 1,000 RBCs, with an average infection of  $4.78 \pm 6.50$  RBCs. These haematozoa infections were comprised of haemogregarina (Apicomplexa: Adeleiorina) and an unknown haematozoa that could not be identified (Fig 3.1).

Neither boat traffic, number of basking structures per river mile, nor location was related to leech load or haematozoa infection rate (MANOVA, all  $p > 0.15$ ).

After comparing AICs of possible logistic regression models predicting the likelihood of infection, the best model included boat traffic alone. However, boat traffic did not significantly predict the likelihood of an individual being infected with haematozoa (logistic regression, AIC=59.3, Z=-0.387,  $p=0.699$ ).

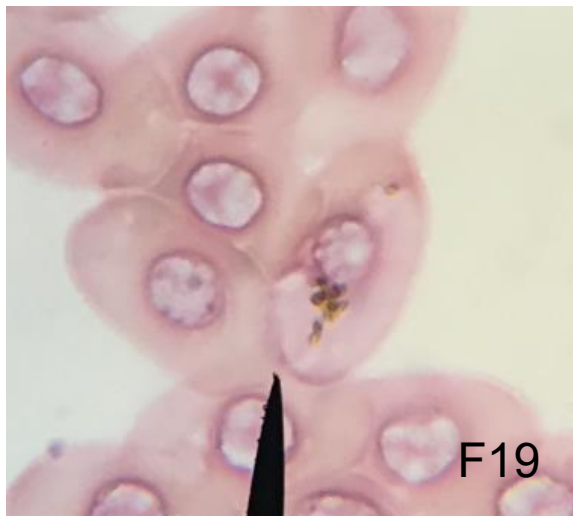
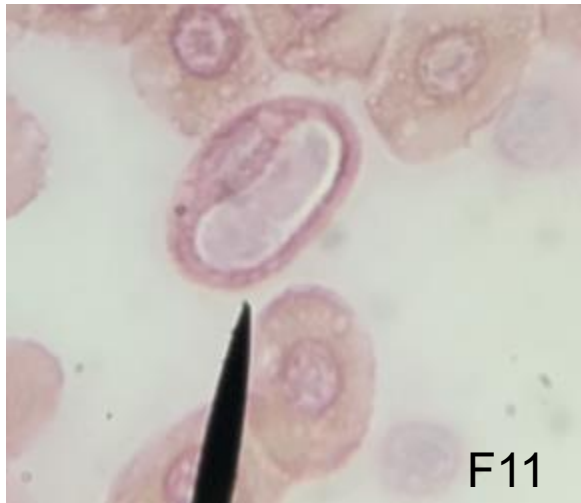


Figure 3.1 Representatives of haematozoa at 1000x magnification found in adult ringed sawbacks (*Graptemys oculifera*) collected from the Pearl River outside Jackson, MS. The top photo is the gamont stage of haemogregarine from an adult female (F11). The bottom photo is an unknown haematozoa from an adult female (F19).

## *Discussion*

Human disturbance can have wide reaching consequences on individual health, causing cascading effects that may impact species populations (Moore & Seigel 2006, Nowacek et al. 2016, Jones 2017). The measure of individual health used in this study, parasite load, was not correlated to the measures of human disturbance used in this analysis – number of basking structures and average hourly boat traffic. Indeed, haematozoa load was close to equal at both RF and LB despite apparent differences in amount of human disturbance. While I predicted that differences in basking due to disturbance would lead to higher leech loads, and thus higher haematozoa counts, leech load and haematozoa infection did not appear to be related in the few captured turtles that were parasitized by leeches. To my knowledge, the only study directly examining differences in parasite load based on human disturbance was by Lacroix (2012), who found that haematozoa infections were highest in Blanding’s turtles near wetlands compared to more modified habitats. This difference was attributed to prevalence of leeches in wetlands which may transmit haematozoa. However, very few turtles were found to be infected with leeches in this study.

There are a few possible explanations for the lack of relationship between human disturbance, leech parasitism, and haematozoa infection. Potential interruptions to basking because of disturbance may not impact leech attachment and feeding. This is counter to the “desiccating leech hypothesis” (Shealy 1976, Ryan & Lambert 2005) which posits that basking by turtles serves to desiccate and remove leeches. However, the low number of turtles found with leeches (10% of captures) is in keeping with Ryan and Lambert (2005) who found that leeches will preferentially feed on common snapping turtles rather than common map turtles even when basking is denied. Leeches may preferentially avoid feeding on ringed sawbacks in favor of more

benthic turtle species, or may not stay attached long enough to either transmit haematozoa or pose a prolonged cost through the drawing of blood meals. A second explanation could be that haematozoa transmission in this species occurs primarily through another vector, such as a mosquito (Paterson & Desser 1976), indicating that interruptions to basking and leech attachment do not influence haematozoa transmission, or may suggest that map turtles are resistant to infection via leeches. Finally, as species of leeches differ in their attachment behavior (Maloney & Chandler 1976), leeches that transmit haematozoa may not remain attached long enough to be recorded. *Placobdella parasitica* has been shown to remain attached to riverine turtles long after feeding, while *Placobdella ornata* releases the host shortly after feeding (Maloney & Chandler 1976). If ringed sawbacks are primarily parasitized by *P. ornata*, evidence of their attachment may not be obvious.

Levels of human disturbance do not appear to influence parasite load in ringed sawbacks, although further research should explore the reasons for the absence of this relationship, as it presents an interesting case study for parasite-host relationships.

## **Differences in population structure and shell condition**

### ***Introduction***

The southeastern United States has been identified as a hotspot for turtle biodiversity, containing representatives from 42 species, 11 of which are endemic (Mittermeier et al 2015). Unfortunately, turtles across the United States are threatened, with 26 out of the 57 species found in the US listed in Appendix 3 on CITES by the US Fish and Wildlife Service (USFWS, The IUCN Red List). Recovery of these listed species may be impeded by direct and indirect effects of human disturbance.

Selman et al. (2013) found that yellow-blotched sawbacks in more disturbed areas had poorer shell condition than those in undisturbed areas. Shell condition was assessed by the presence or absence of white spots on the carapace which occur when marginal scutes are not shed properly and indicate the presence of a bacterial or fungal infection (Selman et al. 2013). As this failure to shed marginal scutes with poor shell condition is seen in captive turtles with improper light and heat sources (Hernandez-Divers et al. 2009), improper basking likely results in the same condition. Shell condition then may serve as an index for basking ability and individual health between populations.

Examining age structure and recruitment between sites can also indicate potential effects of human disturbance. Passing watercraft, and the humans they carry, can directly reduce recruitment in populations. Boat traffic interferes with nesting activities by startling gravid, nesting turtles from sandbars into the water (Moore & Seigel 2006), and human occupation of sandbars for camping and other recreational activities unintentionally destroy nests (Moore & Seigel 2006). Food and trash left on sandbars attract human commensals such as raccoons, armadillos, and fish crows (Jones 2017). These vertebrate predators represent a serious threat to nests, destroying up to 86% of nests within days of laying (Jones 2006, Jones 2017). A twenty-year study by Jones (2017) concluded that the population at RF decreased while the ringed sawback population at LB increased. The last year of sampling for the study was in 2014 and continued assessment of these trends would be valuable in the assessment of conservation efforts (USFWS 2005).

I hypothesize that sites of increased human disturbance will be linked to poor individual population health and predict that high human disturbance will be linked to poor shell condition and fewer juveniles and young adults.

## ***Methods***

### *Capture methods*

Collection of adult and juvenile ringed sawbacks took place between May and August 2017 at the two sites, RF and LB addressed in Chapter 1. Capture methods are addressed earlier in this chapter.

The shells of captured turtles were cleaned and marked uniquely with holes drilled into marginal scutes (Cagle 1939). These populations were previously marked by R. Jones and P. Hartfield (1995) between 1988 and 1990, by R. Jones (2006) 1995-1996, and intermittently by R. Jones (2017) between 1994-2004. The capture location, plastron length (PL), plastron width (PW), plastron height (PH), carapace length (CL), carapace width (CW), mass, sex, gravidity via palpation (if female), and age were recorded (Richards-Dimitrie 2011). Carapaces were examined visually for the presence or absence of white spots as an assessment of shell condition.

### *Statistical tests*

Differences in population age and composition between sites (RF and LB) were assessed using a Fisher's exact test, first between number of adults and juveniles per site, then between age classes of adults as assessed by the presence or absence of annuli. While present in young turtles, annuli become worn over time and are not visible in older adult turtles (Huntzinger & Brown, pers. comm). As age ranges were not even amongst captured turtles and age is correlated with body size (Cagle 1946; Halliday & Verrell 1988), inferential statistics were not run between body sizes to prevent conclusions which could be attributed to age class differences.

A Chi-square contingency analysis was used to assess if shell condition, as measured by the presence or absence of white spots on carapaces of adult turtles (dependent variable) differed between sites (independent variable).

**Results**

White spot fungus was present on carapaces of more adult turtles at LB (n=13 turtles, 62% of captures) than at RF (n=5 turtles, 26% of captures) ( $\chi^2= 5.10$ , df=1, p=0.02).

More juveniles were caught at LeFleur’s Bluff (n=8) than at Ratliff Ferry (n=0) (Fisher’s exact test, p=0.017). More adult turtles had visible annuli at LeFleur’s Bluff (n=8) than Ratliff Ferry (n=1) (Fisher’s exact test, p=0.021), indicating a younger population overall at LeFleur’s Bluff. A summation of body measurement differences between captured adult turtles can be seen in Table 3.3.

Table 3.3 Mean and ( $\pm$  SD) of adult ringed sawback body measurements between two sites on the Pearl River; Ratliff Ferry (RF) and LeFleur’s Bluff (LB) (n=40). Abbreviations indicate carapace length (CL), carapace width (CW), carapace height (CH), plastron length (PL), plastron width (PW).

Sex	Site	CL (cm)	CW (cm)	CH (cm)	PL (cm)	PW (cm)	Mass (g)	Sample size
Female	RF	17.2 $\pm$ 0.3	14.6 $\pm$ 0.3	7.6 $\pm$ 0.2	15.8 $\pm$ 0.3	11.2 $\pm$ 0.3	847.7 $\pm$ 55.9	11
	LB	15.8 $\pm$ 0.3	13.2 $\pm$ 0.3	7.1 $\pm$ 0.3	14.5 $\pm$ 0.3	10.5 $\pm$ 0.4	590.0 $\pm$ 28.4	10
Male	RF	8.9 $\pm$ 0.1	7.5 $\pm$ 0.0	3.6 $\pm$ 0.0	8.1 $\pm$ 0.1	5.6 $\pm$ 0.1	103.1 $\pm$ 7.4	8
	LB	8.5 $\pm$ 0.2	6.8 $\pm$ 0.2	3.5 $\pm$ 0.1	7.6 $\pm$ 0.1	5.4 $\pm$ 0.1	88.6 $\pm$ 5.2	11



## *Discussion*

By examining differences between individual health (shell condition) and population structure between sites, I have identified trends which may be of interest to managers seeking to conserve ringed sawbacks or other riverine turtle species.

Turtles at LB had a higher prevalence of white spots on carapaces, due to fungal and bacterial infections which can develop in between scutes that are not shed properly (Hernandez-Divers et al. 2009, Selman et al. 2013). As duration of basking did not differ between RF and LB (J. Heppard, see Chapter 1), there is likely another explanation for differences in shell condition. As LB is below the Ross Barnett reservoir and receives drainage both from the reservoir and from the greater Jackson area, water quality is likely worse there than at RF. I observed trash and chemical discharge at LB during field work, particularly after high rains and high water events, where water was released from the reservoir. Poor water quality could nurture shell infections more readily at LB than the less contaminated RF site (Garner et al. 1997).

There were significantly fewer juveniles and young adults found at RF compared to LB. This is in keeping with Jones (2017) who found a decreasing population trend at RF but not at LB. There are several likely explanations for differences in recruitment and population age structure, all of which are likely due to human disturbance effects. First, human usage of nesting beaches may impact nesting success. RF had higher boat traffic throughout the week compared to LB where boats were few and restricted to the weekend (Chapter 1). Boaters at RF were also observed to stop and park boats at sandbars while boaters at LB were primarily kayakers passing between boat ramps. Passing boaters can interrupt females in the process of scouting and creation of a nest and the laying of eggs (Moore & Seigel 2006, Jones 2006) and may destroy nests unwittingly through the course of picnicking or camping (Moore & Seigel 2006).

A second potential explanation could be reduction in recruitment if human disturbance alters choice of nesting location to areas where they are easily accessed by predators. During the study, many empty nests were observed at RF surrounded by egg shells (judged to be from *Graptemys* species based on their oblong shape and location on sandbars) and predator (raccoon) tracks at the edges of sandbars close to the tree line. Human occupation of “prime” sandbars may force females to nest close to forested areas which are within easier access of vertebrate nest predators such as raccoons and armadillos (Jones & Selman 2009).

A third explanation is that high human presence at RF attracts vertebrate predators of nests such as raccoons and armadillos. These vertebrate nest predators are supplemented by human presence and are attracted to camping and picnicking areas both by the scraps of food left by humans and the removal of apex predators that can control their numbers (Jones 2017). One or more of these human effects may be critical in reducing recruitment, and therefore the maintenance and recovery of the population, at RF.

Human disturbance appears to be a significant cause of site differences of shell condition and poorer recruitment. Efforts to mitigate the effects of human disturbance will be vital to ensuring the maintenance of healthy populations

### **Thesis conclusions and management recommendations**

Long-lived species such as turtles pose unique challenges when assessing population sizes (Jones 2017), which can confound assumptions about the effect of disturbance if a population appears to be stable when a large population of adults exists (Nowacek et al. 2016, Jones 2017). Riverine turtles are in a unique position as they are limited by the confines of the riverine system (Bodie & Semlitsch 2000), must thermoregulate through basking behavior

(Boyer 1965, Seebacher 2005), and have a reproductive strategy that results in a long time to maturity and very few eggs laid per season (Jones 2006). Studies such as this one which factor individual health and the presence of juveniles and young adults into assessments of disturbance and population assessments that are taken over many years are vital for understanding the effects of disturbance and predicting future population shifts (Jones 2017).

Throughout this thesis I have examined the effect of two aspects of human disturbance, reduction of basking structures and increased boat traffic, on the ringed sawback. Based on behavioral changes, thermal consequences, and reduced recruitment likely from high boat traffic, I suggest the following measures.

- 1) Reduce boat traffic at RF in order to increase recruitment and decrease disturbances to basking.

- a) Reduce river access to RF. In contrast to LB, which only has two access points at LeFleur's Bluff State Park and the spillway below the Ross Barnett Reservoir 10.4 river mi upstream, boaters can access RF directly upstream of the reservoir, at various access points on the Natchez Trace, at Ratliff Ferry Trading Post, and at Coal Bluff State Park. Reducing the number of access points to the river may encourage boaters to utilize recreational areas at the Ross Barnett reservoir rather than enter the river and travel further to reach desired sand bars.

- b) Increase the number of no wake zones at RF to include all the PRWMA, as this has been shown to reduce disturbance and may reduce erosion to sandbars that can destroy nests (Selman et al. 2013). These expanded no wake zones could also be in place only during the early summer

months (May and June) in order to reduce impacts on basking behavior during egg development.

c) Restrict boat access to areas of RF. There are many inlets off the main river channel at RF; constructing barriers out of deadwood or posting signs to restrict boat traffic to the main river channel could allow ringed sawbacks undisturbed places to bask and nest.

2) Improve nesting habitats at RF.

a) Jones (2006) found most nests approximately 18m from the water line and within 1m of the vegetation line. If gravid turtles make nest placement decisions based on distance to the vegetation line, managers could reduce vegetation on minor sandbars (those with approximately a meter between the water and vegetation lines) to increase overall depth. Increasing the distance between the river and the vegetation line could allow the presence of viable nests on minor sandbars throughout the river. These minor sandbars would hopefully pass the notice of recreational boaters and increase the amount of available nesting substrate.

b) While PRWMA has prohibited long-term camping on sandbars and all camping on minor sandbars, placing informative signs or restrictions on sandbars could change behavior of campers and encourage them to utilize the larger sandbars that would not be used by ringed sawbacks.

3) Improve water quality at LB.

a. Increase monitoring to locate chemical leaks more quickly and improve clean-up efforts, particularly after heavy rain events

By examining consequences of human disturbance, I have outlined possible short-term (behavioral, thermal) and long-term (body condition, recruitment) effects on the behavior and physiology of an endangered riverine turtle. Seemingly innocuous human disturbance may in fact have far reaching consequences on ringed sawbacks, and careful action must be taken to prevent extirpation of populations. Scientists and managers must strike a balance between encouraging the public to engage with and enjoy nature and creating sustainable habitats for threatened populations.

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## **APPENDICES**

## **APPENDIX I**

Table 1. Measures of relative variable importance (RVI) of the predictor variables Julian day, air temperature, week day, weather, the interaction of day and Julian day, boat traffic, number of basking structures, the interaction of day and weather, and the interaction of air temperature and number of structures on number of basking turtles seen on surveys. Variables with RVI scores greater than 0.5 have a moderate to strong effect on the response variable.

	Julian Day	Air temperature	Day	Weather	Day:Julian day	Boat traffic	Num. structures	Day:Weather	Air temperature: Num. structures
RVI	1	1	1	0.99	0.92	0.74	0.41	0.37	0.2

Table 2. Akaike information criterion (AIC) scores for the multiple regression models with lowest AIC values of the predictor variables Julian day, air temperature, week day, weather, the interaction of day and Julian day, boat traffic, number of basking structures, the interaction of day and weather, and the interaction of air temperature and number of structures on number of basking turtles seen on surveys. A plus (+) underneath a variable indicates its inclusion in the model.

	Julian day	Air temperature	Day	Weather	Day:Julian day	Boat traffic	Boat traffic:Julian day	Boat traffic:Zone	Water temperature	AIC score
Inclusion	+	+	+	+	+	+				4856.4
	+	+	+	+	+		+	+		4856.8
	+	+	+	+	+	+				4857.1

Table 3. Measures of relative variable importance (RVI) of the predictor variables Julian day, air temperature, number of basking structures, zone, boat traffic, the interaction of boat traffic and number of structures, the interaction of boat traffic and Julian day, the interaction of boat traffic and zone, the interaction of boat traffic and number of structures, the interaction of boat traffic and Julian day, the interaction of boat traffic and zone, and water temperature on duration of basking by turtles. Variables with RVI scores greater than 0.5 have a moderate to strong effect on the response variable.

	Julian day	Air temperature	Num. structures	Zone	Boat traffic	Boat traffic:Num.structures	Boat traffic:Julian day	Boat traffic:Zone	Water temperature
RVI	1	1	1	1	0.76	0.41	0.39	0.37	0.31

Table 4. Akaike information criterion (AIC) scores for the multiple regression models with lowest AIC values of the predictor variables Julian day, air temperature, number of basking structures, zone, boat traffic, the interaction of boat traffic and number of structures, the interaction of boat traffic and Julian day, the interaction of boat traffic and zone, the interaction of boat traffic and number of structures, the interaction of boat traffic and Julian day, the interaction of boat traffic and zone, and water temperature on duration of basking by turtles. A plus (+) underneath a variable indicates its inclusion in the model.

	Julian day	Air temperature	Num. structures	Zone	Boat traffic	Boat traffic:Num.structures	Boat traffic:Julian day	Boat traffic:Zone	Water temperature	AIC score
Inclusion	+	+	+	+						4856.4
	+	+	+	+	+		+	+		4856.8
	+	+	+	+	+	+				4857.1

Table 5. Measures of relative variable importance (RVI) of the predictor variables month, probability of disturbance, and the interaction of month and probability of disturbance on body temperature differences between simulations of anthropogenically disturbed and undisturbed basking. Variables with RVI scores greater than 0.5 have a moderate to strong effect on the response variable.

	Month	Probability of Disturbance	Month:Probability of Disturbance
RVI	1	1	1

Table 6. Akaike information criterion (AIC) scores for the multiple regression models with the lowest AIC values of the predictor variables month, probability of disturbance, and the interaction of month and probability of disturbance on body temperature differences between simulations of anthropogenically disturbed and undisturbed basking. A plus (+) underneath a variable indicates its inclusion in the model. Only a single model was generated.

	Month	Probability of Disturbance	Month:Probability of Disturbance	AIC score
Inclusion	+	+	+	3393.6

## **APPENDIX II**

## **Introduction**

While basking surveys and focal observations were measured at RF (but not LB) in 2016, high degrees of multicollinearity between variables precluded survey counts and basking durations from inclusion in models seen in Chapter 1. Data on boat traffic and rates of disturbance at RF, however, were combined from 2017 and 2016 for the models below.

## **Methods**

Focal basking observations in 2016 and assessment of boat traffic took place between 8 July and 6 August at RF. Methods of focal observations and boat traffic assessment are discussed in Chapter 1.

A chi-square analysis was used to test if type of disturbance, including non-watercraft, was related to whether a turtle was disturbed at RF. A factorial ANOVA tested data from all passing boats to assess if zone (wake or no wake) or watercraft type (independent variables) affected percentage of basking turtles (dependent variable) disturbed per structure. Because of high numbers of boats during the Saturday of Memorial day weekend (27 May 2017), other predictors of disturbance were not incorporated into the analysis to prevent data skew due to the high number of boats (n=388) causing disturbances not related to speed, angle, or distance of approach. A post hoc Tukey test was used to assess significant differences in percentages of disturbance between boat types.

## **Results**

Descriptive statistics are given in mean  $\pm$  standard deviation unless otherwise noted.

A total of 977 iterations of basking were observed; of these, 547 were undisturbed and 430 were disturbed. Type of disturbance significantly affected the number of disturbed turtles ( $\chi^2$  goodness of fit,  $\chi^2= 1473.9$ ,  $df=12$ ,  $p< 0.001$ ; Fig. 1), with motorboats, other turtles, and jonboats causing the most disturbances. Of the five most common sources of disturbance, three were anthropogenic.

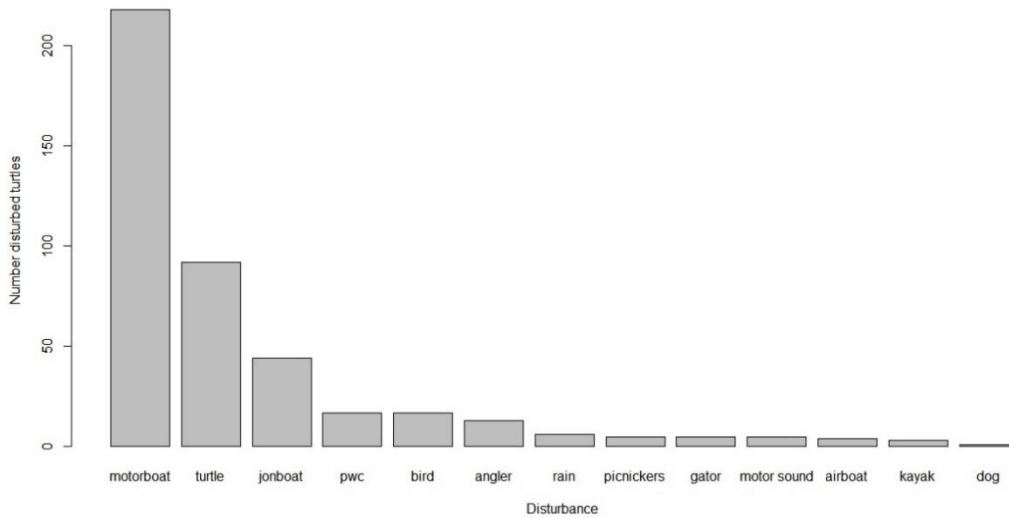


Figure 1. Number of turtles in 2016 and 2017 who were disturbed and ended basking due to 13 different observed stimuli on the Pearl River (n=430 turtles).

A total of 1308 boats were observed, and 1051 of these boats passed structures occupied by turtles. Watercraft type significantly affected the percentage of disturbed turtles (ANOVA,  $F_{(5,1044)}=16.84$ ,  $p<0.0001$ ; Fig. 2). Higher percentages of basking turtles were disturbed due to anglers and kayaks than other watercraft (Table 1). Boats also disturbed more turtles when passing in wake zone ( $n=543$  boats,  $\bar{X}=4.97\pm 16.26\%$ ) than in a no wake zone ( $n=508$  boats,  $\bar{X}=14.31 \pm 30.12\%$ ,  $F_{(1, 1044)}=37.86$ ; Fig 3).



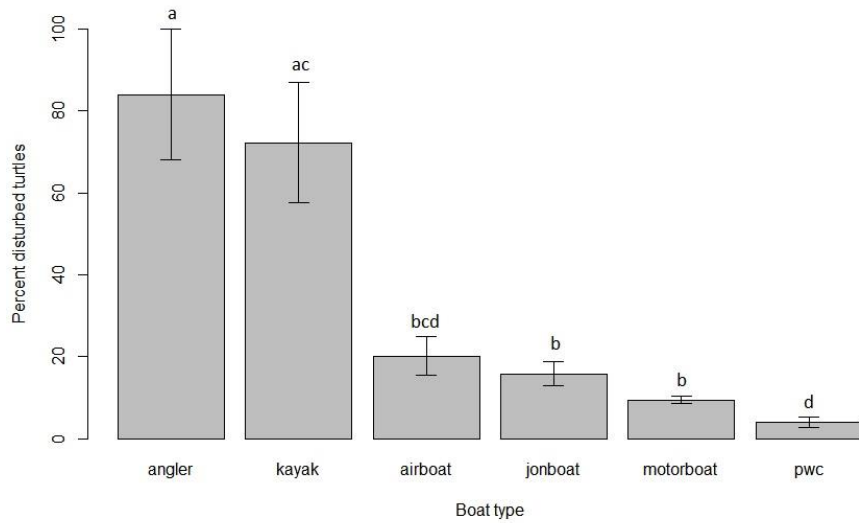


Figure 2. Percentage of basking turtles on a structure disturbed due to passing boats in 2016 and 2017 (n=1051 boats). Similar letters indicate no statistical difference between categories. Error bars represent standard error.

Table 1. Means and standard error of significant differences between percentages of basking turtles disturbed by passing watercraft (n=1051 boats).

Disturbance	Mean %	Disturbance	Mean %	p
angler	84.0±16.0	jonboat	15.85 ± 3.05	< 0.001
angler	84.0±16.0	motorboat	9.41±0.87	<0.001
angler	84.0±16.0	pwc	3.96±1.24	<0.001
angler	84.0±16.0	airboat	20.19±4.81	0.049
jonboat	15.85 ± 3.05	kayak	72.22±14.70	0.002
jonboat	15.85 ± 3.05	pwc	3.96±1.24	<0.001
kayak	72.22±14.70	motorboat	9.41±0.87	<0.001
kayak	72.22±14.70	pwc	3.96±1.24	<0.001
motorboat	9.41±0.87	pwc	3.96±1.24	0.008

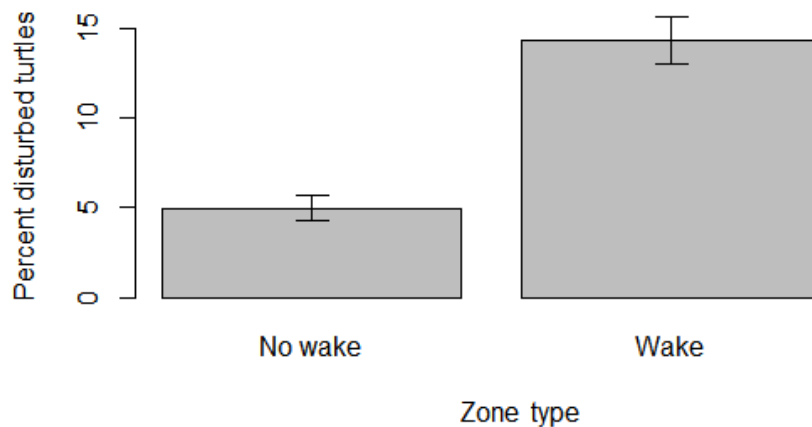


Figure 3. Percentage of basking turtles on a structure disturbed due to passing boats (n=1051 boats) in a wake and no wake zone. Error bars represent standard error.

## Discussion

More types of disturbances were seen in basking observations from combined years 2016 and 2017 at RF as disturbances from birds, dogs, and picnickers were not observed in 2017 at RF and LB. Interestingly, this means that at RF in 2016 and 2017 two of the top five disturbances were due to non-anthropogenic causes, namely other turtles and birds. However, anthropogenic disturbance accounts for a larger percentage of disturbances (73%) compared to natural disturbances (27%), indicating that high amounts of anthropogenic disturbance occur in this population. Anglers, kayaks, and airboats disturbed the highest percentage of turtles. While kayaks disturbed a higher percentage of turtles in 2017 than anglers at RF and LB, this could be due to either more observations of anglers or of fewer kayaks at RF. These results still support the conclusions in Chapter 1 – that slow moving water craft disturb a higher percentage of turtles than faster moving water craft, presumably due to perception of predation risk (Moore & Seigel 2006, Selman et al. 2013, Blumstein 2006).

Boats passing in wake zones disturbed more turtles than those in no wake zones, again in agreement with results from Chapter 1. Again, this could be due to turtles being washed off structures in no wake zones. While turtles at RF may habituate or become tolerant of passing boats, the physical removal of turtles from basking structures due to boat wake may still reduce basking in the population.

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