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SEASCAPE GENETICS AND REHABILITATION EFFICIENCY IN THE FLORIDA MANATEE

by

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A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Conservation Biology
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

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Major Professor: Graham Worthy

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ABSTRACT

The Florida manatee (*Trichechus manatus latirostris*) was recently downlisted federally from “endangered” to “threatened” despite acknowledgments of remaining threats to long term population persistence. Challenges to future manatee conservation include, but are not limited to, increases in frequency of harmful algal blooms, intensifying anthropogenic disturbance, and loss of warm-water habitat. The goals of this dissertation were 1) to assess threats to the manatee via a comprehensive, long-term (1973-2016), retrospective analysis of the manatee rescue and rehabilitation partnership (MRRP) and 2) to use seascape genetics analysis to examine whether abiotic, biotic, or anthropogenic seascape variables could significantly describe genetic distance patterns in space for this genetically depauperate population. Results from the MRRP analysis revealed that anthropogenic threats were the most significant reason for manatees to be rescued and rehabilitated. Manatees rescued due to watercraft injuries spent long periods in recovery before succumbing or being released resulting in significant expense to the rehabilitation system. Additionally, the seascape genetics analysis indicated that watercraft activity best explained spatial genetic patterns in the manatee population. It is established that anthropogenic use of watercraft negative affects manatees through the mechanisms of sub-lethal injury and mortality, and these results suggest there may be further negative impacts via the disruption of population genetic connectivity. Future management practices should seriously consider manatee/vessel interactions as watercraft strikes are costly for management, place pressure on the manatee population, and could disrupt population gene flow with potentially dire consequences. Mitigating anthropogenic impacts on the Florida manatee population is critical for future conservation and should be a primary focus.

For Christopher McQuaid and Francesca Porri, who laid the foundation for my education as an ecologist. My days in South Africa under your mentorship will always be among my fondest memories.

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CHAPTER 1: GENERAL INTRODUCTION

The Florida manatee (*Trichechus manatus latirostris*) first received protection under the amended Marine Mammal Protection Act of 1972 and the Endangered Species Act of 1973 and has been hailed as a conservation success story after being federally downlisted from “endangered” to “threatened” in 2017. Annual manatee population estimates from synoptic surveys numbered 1,267 in 1991, but intense management has lead to increases in the population, and synoptic survey counts have been >5,000 for the 2015-2019 seasons (FWC). Despite these increases, many threats to manatee persistence remain and some threats are expected to intensify in the coming years. Major threats to future conservation include vessel interactions (Figure 1), algal blooms, cold stress (Figure 2), low genetic diversity, and loss of habitat from an increasing human population. Comparatively, the factors of disease, chemical contaminants, incidental capture, hunting, and miscellaneous threats pose relatively low risk to Florida manatees; the threat from climate change is not well understood but is of considerable concern (Marsh et al. 2011, Runge et al. 2017). Widespread occurrence of red tide blooms (*Karenia brevis*) in 2018 resulted in 224 manatee deaths, up from 73 and 67 in 2016 and 2017, respectively (FWC). Cold stress deaths for the first six months of 2018 exceeded the totals for the prior two years combined. The years of 2016-2017 experienced watercraft-induced manatee deaths exceeding 100 individuals, which had never occurred previously, and preliminary data from 2018 suggests a similar trend.

Currently, the manatee population is managed by the collaborative efforts of many different agencies. For decades, population data has been collected by using visible scars to identify manatees, and unique patterns of boat scarring from vessel strikes are so widespread within the population that they are the main feature used to identify individual manatees. Scars are marked with chalk, photographed, and entered into the Manatee Individual Photo Identification System (MIPS), a collaborative effort managed by three main partners: the United States Geological Survey (USGS), The Florida Fish and Wildlife Conservation Commission (FWC), and Mote Marine Laboratory (MML). Scars and mutilation records are continuously updated with each subsequent sighting of an animal which may occur during annual health assessments, through the efforts the Manatee Rescue and Rehabilitation Partnership (MRRP) comprising 15 different organizations (discussed extensively in Chapter 2), or when carcasses are recovered through the FWC Marine Mammal Pathobiology Lab's carcass recovery program. The considerable amount of data produced by these multiple collaborative efforts has the potential to give valuable insight into pressures on the manatee population and to inform future conservation and management efforts, if analyzed and distributed.

In this dissertation, I sought to 1) understand current and future threats to the manatee population and to improve future care via a long-term retrospective analysis of the MRRP from the beginning of rescue/rehabilitation efforts in 1973 through the end of 2016 and 2) understand variables that are important for gene flow in the manatee. In the MRRP analysis I examined seasonal trends in morbidity and injury, potential relationship between several variables of interest (i.e. sex, body length, season of rescue, and cause of rescue), survival through various points in the rescue and rehabilitation process, rehabilitation efficiency rates, and post-release survival estimates. In the seascape genetics analysis I used genetic data from the carcass recovery program and publicly available environmental data to understand the influence of the seascape on gene flow. It is important for future management to understand what variables are important for gene flow across a landscape/seascape so that conservation and restoration efforts may be directly appropriately, and management can adequately assess threats to species persistence. My aim in the present work was to use analyses of collaboratively generated data to inform future conservation efforts so that future management of the Florida manatee may have further success.

Figure 1: Example of boat scarring on an adult manatee. Scars are marked with chalk, photographed, and entered into a database to identify individuals. Photo by Madison Hall.

Figure 2: Examples of cold stress on the heads of an adult manatees. Photo by Madison Hall.

CHAPTER 2: OVERVIEW OF THE FLORIDA MANATEE RESCUE AND REHABILITATION PARTNERSHIP FROM THE ENDANGERED SPECIES ACT TO PRESENT (1973-2016).

Introduction

Rehabilitation of stressed and injured animals relies on the ethical principle that humans do not ignore suffering in wildlife, especially when the injury is anthropogenic in origin (St. Aubin et al. 1996). Yet there are both risks and benefits inherent in rehabilitating wildlife for ultimate release back into the environment (Moore et al. 2007). The risks include exposing wild populations to new diseases obtained while in captivity (Quakenbush et al. 2009), social disruption (St. Aubin et al. 1996), and potential genetic consequences of releasing less fit animals (Wilkinson and Worthy 1999). Alternatively, benefits include increasing knowledge of disease etiology and its treatment, improving individual welfare, and contributing to the demographic and/or genetic variability of wild populations (Moore et al. 2007, Adimey et al. 2012, Boede and Mujica-Jorquera 2016). The increased medical knowledge attained through rehabilitation programs benefits both common and rare species, a benefit that is often overlooked (St. Aubin et al. 1996, Aitken 2004). Further, the rehabilitation of wildlife has value by linking human empathy and the natural world, which is vital to maintain public involvement in conservation (Aitken 2004).

Reliable, specific, and well-documented stranding and rehabilitation programs coupled with post-release monitoring are required for tracking threats to animals in the marine realm

(Gulland and Hall 2007). In the United States, the Florida Manatee Recovery and Implementation Team has worked intensively to develop a conservation program that includes rescue and rehabilitation (St. Aubin et al. 1996, Adimey et al. 2012, Adimey et al. 2016). The Florida Manatee Rescue and Rehabilitation Partnership is a working group of 15 different programs (Florida Fish and Wildlife Conservation Commission, United States Geological Survey Sirenia Project, United States Fish and Wildlife Service, Lowry Park Zoo, Miami Seaquarium, Epcot Living Seas, Homossassa Springs State Park, Columbus Zoo, Cincinnati Zoo, Mote Marine Lab, Disney Conservation Fund, Sea to Shore Alliance, South Florida Museum, Save the Manatee Club and the University of Florida) that work together to rescue, rehabilitate, and release manatees back into the environment. Manatees face many challenges both anthropogenic (e.g. watercraft collision, poaching, marine debris, loss of warm water habitat, coastal development) and natural (e.g. cold stress, harmful algal blooms, loss of seagrasses, stochastic events). There are currently rehabilitation programs for the West-Indian manatee (subsp. *Trichechus manatus latriostris:* Florida, United States; *Trichechus manatus manatus:* Belize, Brazil, Columbia, Dominican Republic, Jamaica, Mexico, Puerto Rico, United States, Venezuela), and the Amazonian manatee (*Trichechus inunguis:* Brazil, Colombia, Peru) that vary in size, support, and success. These programs have advanced veterinary knowledge and resulted in successful captive reproduction for both subspecies of West Indian manatee (Adimey et al. 2012, Boede and Mujica-Jorquera 2016).

Studying the efforts of stranding and rehabilitation programs such as the Florida Manatee Rescue and Rehabilitation Partnership serve the important purpose of identifying and synthesizing anthropogenic and natural pressures on wildlife (Harris and Sleeman 2007, Molina-

López et al. 2017). For example, a study of a stranding program in the Philippines concluded that the high proportion of live-stranded animals they recovered was likely due to acoustic trauma from dynamite fishing, which was then verified via informant information (Aragones et al. 2010). Additionally, long-term retrospective studies such as the present analysis can compare variables between successful and unsuccessful outcomes and ultimately make recommendations for management and future research (Frouin et al. 2013, Adimey et al. 2016). Those results can then inform future management decisions about individuals whose success is marginal (St. Aubin et al. 1996) as well as provide information on the potential for release of captive-born individuals (Beecham et al. 2015, Adimey et al. 2016).

It is crucial for rescue and rehabilitation programs to define success and regularly evaluate program efficacy as critics have opposed investing in rehabilitation programs with unclear aims and have argued that success can be hampered by a lack of scientific evidence (Wiley et al. 2001, Moore et al. 2007, Adimey et al. 2012). Evaluations of wildlife rescue and rehabilitation programs are especially important in providing essential data for the overall goal of biodiversity conservation by clarifying threats to wild populations, providing information on ecosystem health, and informing wildlife managers of potential environmental trends (Gerber et al. 1993, Gulland et al. 2002, Lander et al. 2002, Cade et al. 2004, Cade 2007, Harris and Sleeman 2007, Sleeman 2008, Aragones et al. 2010, Bogomolni et al. 2010, Molina-López et al. 2011, Burton and Tribe 2016, Mariacher et al. 2016, Orós et al. 2016, Molina-López et al. 2017). Monitoring of released, rehabilitated California condors (*Gymnogyps californianus*) revealed the dangers of lead poisoning to both humans and wildlife alike (Cade et al. 2004) and provided evidence that mortality would prevent post-release success in rehabilitated condors until the lead poisoning issue was resolved (Cade 2007). Careful post-release monitoring of golden lion tamarins (*Leontopithecus rosalia*) showed that many animals were disoriented and not able to plot a spatial route, which led to future pre-release conditioning and training (Kleiman et al. 1986). Monitoring of a single rehabilitated and released critically endangered Mediterranean monk seal (*Monachus monachus*) provided critical new ecological data for the species and confirmed rehabilitation effectiveness, therein providing support for the continuation of the rescue program (Dendrinos et al. 2007). The study of rehabilitated animals provides a source of critical information that would be exceedingly costly and difficult to obtain from their non-rehabilitated, wild counterparts (Davis et al. 1996).

The goal of the present study was to take a comprehensive look at the Florida Manatee Rescue and Rehabilitation Partnership from its inception in 1973 through the end of 2016, a total of 1715 rescue events. We used X^2 tests to evaluate differences in season and sex between categories of rescue. We combined Kruskal-Wallis and Wilcoxon signed-rank tests to assess differences in body length between categories. We estimated rehabilitation efficiency parameters as defined in Oros et al. (2016) to analyze the outcomes of the rehabilitation process (unassisted mortality, euthanasia, or release). We examined relationships between the variables of sex, length, cause of rescue, and season of rescue and used logistic regression to clarify if any parameter was related to the ultimate rehabilitation outcome (survival or death). Specifically, we used logistic regression to examine the relationship between these variables and the outcomes of death or survival from 1) the moment of rescue to initial admission to a care facility, and 2) from moment of admission to release back into the wild. We used Welch's tests and Wilcoxon each-pair comparisons to analyze time in captivity for each cause of rescue and time to death for animals that did not survive. We also measured length of time the animal survived post-release where information was available. These results in combination illuminate trends in injury, assess treatment efficacy under various circumstances, and draw attention to problems that manatees face in the wild. Our aim was to inform future captive care and, ultimately, aid manatee conservation as a whole.

Methods

Overview (n=1715)

A retrospective analysis was performed using rehabilitation records of 1715 rescue events which equated to 1619 individual manatees since some animals were handled on more than one occasion. Data were compiled for the years 1973- 2016 utilizing medical records in the United States Fish and Wildlife Service captive manatee database. Rescue events were classified as either type A (assisted and released *in situ*) or type B (brought into captivity for treatment). Animals were divided into one of 3 age classes based on total body length: calf (< 235 cm; n=707), subadult (235-265 cm; n=325), and adult (>265 cm; n=607); 36 rescues had no recorded length information in the database. There were a total of 755 females, 642 males, and 222 rescues where no sex was recorded. Cause and number of each rescue is listed in Table 1.

Table 1: Rescue frequency for examined rescue categories. Captive births from rescued manatees, rescued companion animals, and rescues classified as other or undetermined are not included in the analyses of this paper.

Cause of Rescue	***Frequency***
Watercraft injuries	n=370 • Propeller injuries (n=99) • Impact injuries (n=201) • Propeller and impact injuries (n=58) • Unknown watercraft assoc. injuries (n=12)
Entanglement in debris	n=326
Cold stress	n=223
Entrapped	n=169 • Canals (n=8) • Culverts (n=51) • Lakes (n=27) • Natural barriers (n=2) • Powerplant intakes (n=26) • Spillways (n=5) • Tidal gates (n=8) • Water control structure (n=31) • Other entrapments (n=11)
Orphaned calves	n=168
Tidal stranding	n=126
Miscellaneous natural causes	n=80 • Birth complications (n=1) • Dehydration (n=3) • Other natural causes (n=32) • Unknown natural cause (n=44)
Other	n=75 • Companion animals (n=68) • Other misc. rescues (n=3) • Unknown cause (n=4)
Red tide poisoning (*Karenia brevis*)	n=66
Emaciation	n=38
Buoyancy issues	n=31
Unsuitable habitat	n=26
Miscellaneous human-related	n=17

Season (n=1640)

In order to study potential impacts of season, each year was divided into two seasons reflecting manatee migratory phenology: cold (November- March, 5 mo) and warm (April-October, 7 mo) (Deutsch et al. 2003). Thus, expected values for X^2 analyses are based on 5/12 mo for the cold season and 7/12 mo for the warm season. We used X^2 tests to assess whether the frequency of each type of rescue (Type A or Type B) was significantly different within each season and to assess effects of seasonality on number of rescues within each rescue category. Animals classified as *Other* rescues (*companion animals*, *unknown causes, other miscellaneous rescues*; n=75) were not included in this analysis resulting in a total of 1640 rescue events.

Sex (n=1418)

In order to determine if the distribution of males and females was equal within each cause of rescue category, we removed rescues classified as *Other* (n=75), and animals of unknown sex (n=222) leaving 1418 animals for consideration. Differences were assessed by a series of X^2 tests.

Body Length (n=1435)

In order to examine the relationship between body length and rescue categories, we removed those animals identified as *birth issues* (n=1), *orphaned calves* (n=168), animals with no length information (n=36), and those classified as *Other* (n=75) leaving a total of 1435 individuals. The relationship between body length and cause of rescue was tested for normality using a Shapiro-Wilk test, differences in mean body length were assessed by Kruskal-Wallis test and post-hoc pairwise Wilcoxon Signed-Rank tests.

Rehabilitation Efficiency (n=1085)

Several rehabilitation efficiency parameters defined in Oros et al. (2016) were used to analyze outcomes of the rehabilitation process. Final disposition (released, remain in captivity, died during rescue/rehabilitation) of animals brought into captivity was used to calculate the release rate, R_r (# released/ total # admissions), euthanasia rate, E_r (# euthanized/ total # admissions) and unassisted mortality rate, M_r (# died/ total # admissions). Time to death, (T_d, days) was calculated for animals that did not survive the rescue and rehabilitation process, and time until release (T_r, days) was evaluated for animals that recovered and were subsequently released. T_d and T_r were compared among rescue categories. This part of the analyses focused on type B rescues only (n=1165); animals that remain captive (n=22) and Type B rescues classified as *Other* rescues (n=58) were excluded from the analysis resulting in 1085 events remaining for consideration.

Death location was noted for those animals that died during each of the 5 stages of the rehabilitation process (during rescue, during transportation, at rehabilitation center but prior to treatment, during rehabilitation, or after release; n=675). T_d was compared across causes of rescue for animals that died in the first 4 stages (n=537) and T_d for animals that died after release (n=138) was examined separately.

Logistic Regressions

We used binary logistic regression analyses to evaluate whether sex, body length, season, or cause of rescue impacted survival rate from 1) the moment of rescue to admission at the rescue facility and 2) the beginning of treatment until the animal's release. We did not include Type A rescues, rescues classified as *Other* (e.g. *companion animal*s), rescues for animals that remain in captivity, animals without a recorded length, or animals with no recorded sex. Variables were individually regressed and then significant models were selected based on AICc. Variables that were significantly correlated to one another were not allowed in the same model.

Time to Death (n=532)

To evaluate factors influencing time to death (T_d), we natural log transformed $Ln(T_d+1)$ the n=532 rescues that died during rescue, transport, or rehabilitation. We did not include rescues classified as *Other*, and separately examined the n=138 animals that died after release. We assessed normality using a Shapiro-Wilk test, then evaluated differences in mean time to death (T_d) by Welch's test and Wilcoxon each-pair comparisons.

Time in Captivity (n=553)

Lastly, to determine if treatment time (T_r, days in captivity) for successfully released animals differed among rescue categories, we calculated T_r and compared among rescue categories for the n=553 successful releases of animals brought into captivity. We did not include type A rescues (n=550), rescues classified as *Other* (e.g. *companion animals)*, initial releases of captive born animals (n=25), animals that remain in captivity (n=22), or the remaining entries where animals died during the rescue and rehabilitation process (n=532). We natural-log-transformed this data (LnT_r) and assessed normality of the transformed data using a Shapiro-Wilk test. We assessed differences in T_r between groups using a Brown-Forsythe test for standard deviation and Welch's test for means, the latter allows for significantly different standard deviations between groups.

Software

All statistics and graphs were generated using JMP Pro 14, R software version 3.4.1 and Microsoft Excel 2011.

Results

Descriptive Statistics

In the complete dataset of 1619 manatees there were 1556 animals that were rescued on a single occasion while 63 manatees were rescued on multiple occasions (totaling 159 rescues) for a total of 1715 rescue events. Of these, 550 events involved manatees that were assisted *in situ* (Type A) while 1165 involved cases that required captive medical treatment (Type B). Of the Type B rescues, 606 resulted in release back into the wild, 478 resulted in unassisted mortality, 59 resulted in euthanasia, and 22 remain captive as of December 2018. Additionally, 25 captive born manatees were released into the wild over the period of study.

The trend in number of rescues of each type per year (Figure 3) shows increasing rescues through time with increasing investment in and effort of the Florida Manatee Rescue and Rehabilitation Partnership. Type of rescue by cause of rescue (Figure 4) revealed that several categories were overwhelmingly or completely composed of Type B rescues that required captive care (i.e. *buoyancy, cold stress, entrapment, miscellaneous natural rescues, orphaned calves, red tide, watercraft injuries*) while the remaining categories were mixed between Type A rescues where animals were assisted *in situ* and Type B rescues requiring captive rehabilitation (i.e. *entanglement, emaciation, miscellaneous human, tidal stranding, unsuitable habitat*).

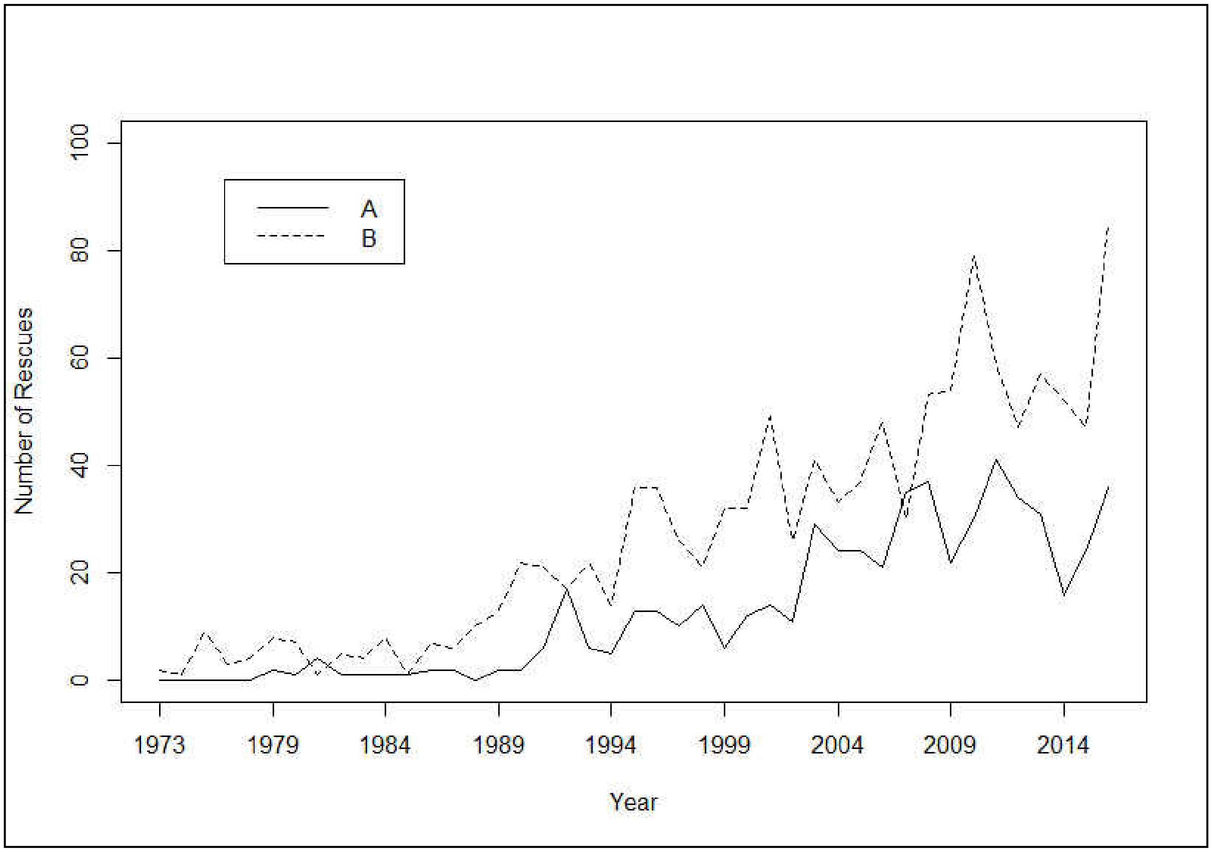

Figure 3: Number of rescues that were assisted *in situ* (Type A) or which required captive medical treatment (Type B) by year.

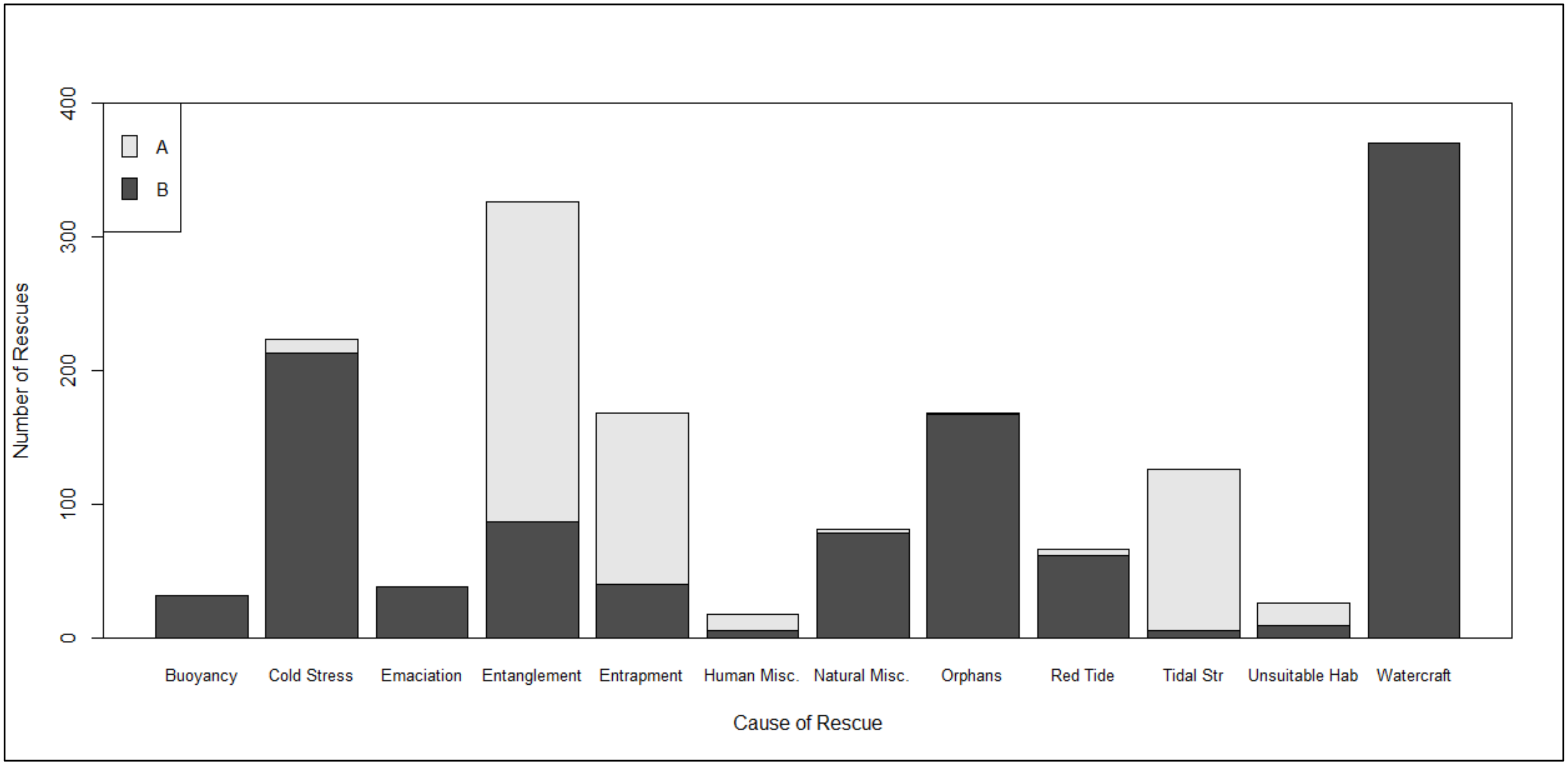

Figure 4: Cause of Rescue by rescue type (A: animals assisted in situ in light gray and B: animals brought into captivity for treatment in dark gray.

Rescue Types and Season (n=1715)

Significantly more animals received assistance *in situ* (Type A) during the warm season (X^2, p=0.003) than those requiring captive medical attention (Table 2). There were significantly more animals brought into captivity (Type B) during the cold season (X^2, p<0.0001) than animals which were assisted *in situ*.

Table 2: Differences in frequency of type A (assisted *in situ*) and type B (brought into captivity) rescues according to rescue season. The cold season was defined as November to March, the warm season as April to October.

	TYPE A- OBS	**EXPECTED**	**TYPE B- OBS**	**EXPECTED**
COLD	195	229.2	601	485.4
WARM	355	320.8	564	679.6
P-VALUE	0.003		<0.0001	

Relationship between Rescue Categories and Season (n=1640)

When each rescue category is examined, rescues due to *entanglement in marine debris* (X^2, p=0.0008), *miscellaneous natural rescues* (X^2, p=0.0197), and *tidal stranding* (X^2, p<0.0001) were significantly overrepresented in the warm season (Table 3). Rescues for *cold stress* (X^2, p<0.0001), *red tide poisoning* (X^2, p=0.0177), *emaciation* (X^2, p=0.0029), *unsuitable habitat* (X^2, p=0.0002), and *entrapment* (X^2, p=0.0334) were significantly higher in the cold season. Rescues due to *watercraft injuries* (X^2, p=0.0703), *buoyancy issues* (X^2, p=0.4850), and *rescues of orphaned animals* (X^2, p=0.8756) were not significantly different between seasons.

Table 3: significant differences in frequency of rescue causes according to season. The cold season was defined as November to March, the warm season as April to October. Significant p values are listed in bold.

CAUSE OF RESCUE	**COLD-OBS**	**EXP**	**WARM-OBS**	**EXP**	**P VALUE**
BUOYANCY	11	12.92	20	18.08	0.4850
COLD STRESS	216	92.92	7	130.08	**<0.0001**
EMACIATION	25	15.83	13	22.17	**0.0026**
ENTRAPMENT	84	70.42	85	98.58	**0.0341**
ENTANGLEMENT	106	135.83	220	190.17	**0.0008**
MISC. HUMAN	14	7.08	3	9.92	**0.0007**
MISC. NATURAL	23	33.33	57	46.67	**0.0191**
ORPHAN	69	70	99	98	0.8756
RED TIDE	37	27.5	29	38.5	**0.0177**
TIDAL STRANDING	18	52.5	108	73.5	**<0.0001**
UNSUITABLE HABITAT	20	10.83	6	15.17	**0.0003**
WATERCRAFT	137	154.17	233	215.83	0.0703

Influence of Sex (n=1418)

Females were more likely than expected to need rescue due to *entanglement* (X^2, p<5 E-11) or *tidal stranding* (X^2, p=0.03). No other significant differences were found between sexes for other rescue categories (Table 4).

Table 4: Significant differences in the distribution of sexes within each rescue category. Females were significantly more common in rescues due to *entanglements* and *tidal stranding*.

CAUSE OF RESCUE	**F-OBS**	**F- EXP**	**M-OBS**	**M- EXP**	**P VALUE**
BUOYANCY	17	15.5	14	15.5	0.5900
COLD STRESS	111.5	99	111.5	124	0.0941
EMACIATION	19.5	21	19.5	18	0.6310
ENTANGLEMENT	147	100.5	54	100.5	**5E-11**
ENTRAPMENT	63.5	60	63.5	67	0.5345
MISC. HUMAN	7.5	11	7.5	4	0.0707
MISC. NATURAL	39.5	38	39.5	41	0.7357
ORPHAN	84	84	84	84	1
RED TIDE	30.5	26	30.5	35	0.2492
TIDAL STRANDING	49	39.5	30	39.5	**0.0325**
UNSUITABLE HABITAT	12.5	9	12.5	16	0.1615
WATERCRAFT	185	197	185	173	0.2121

Differences in Length Between Rescue Categories (n=1435)

We performed a Shapiro-Wilk normality test which indicated the data were not normal (W=0.9790, p<0.0001) followed by a Brown-Forsythe test (p<0.0001) which revealed significantly different variances between groups. Because the assumptions of normality and equal variance were not met, we performed a Welch's test which indicated significant differences in length between rescue categories (p<0.0001). Mean lengths, standard deviations, and 95% confidence intervals for each group are listed in Table 5, and Wilcoxon each-pair comparisons are listed in Table 6.

Table 5: Mean length, standard deviation, and 95% confidence intervals for the rescue categories

CAUSE OF RESCUE	**MEAN LENGTH (CM)**	**STANDARD DEVIATION**	**95% CI**
MISC. NATURAL	187.8	34.5	169.4-206.2
COLD STRESS	226.6	44.4	220.8-232.5
ENTRAPMENT	243.7	43.9	236.9-250.5
EMACIATION	249.2	54.5	231.3-267.1
ENTANGLEMENT	249.7	47.6	244.3-255.0
BUOYANCY	252.36	62.9	229.3-275.4
MISC. HUMAN	254.8	64.5	221.7-287.9
RED TIDE	257.2	34.5	248.7-265.7
WATERCRAFT	260.0	43.7	255.5-264.5
UNSUITABLE HABITAT	271.1	33.4	257.6-284.6
TIDAL STRANDING	277.2	49.6	268.1-286.3

Table 6: Significant Wilcoxon-each-pair comparisons for body length between categories of rescue. When body length for the category on the X axis is larger than the category on the Y axis the box is shaded blue, when body length for the category on the X axis is smaller than the category on the Y axis the corresponding box is shaded red. Nonsignificant comparisons are represented by “ns”.

	Buoyancy	Cold stress	Emaciation	Entanglement	Entrapment	Misc. human	Misc. natural	Red Tide	Tidal stranding	Unsuitable habitat	WC injury
Buoyancy	-	0.0098	ns	ns	ns	ns	0.0005	ns	0.0394	ns	ns
Cold Stress	-	-	0.0106	<0.0001	0.0002	0.0151	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Emaciation	-	-	-	ns	ns	ns	0.0002	ns	0.0020	0.0467	ns
Entanglement	-	-	-	-	ns	ns	<0.0001	ns	<0.0001	0.0254	0.0076
Entrapment	-	-	-	-	-	ns	<0.0001	0.0434	<0.0001	0.0022	<0.0001
Misc. human	-	-	-	-	-	-	0.0036	ns	ns	ns	ns
Misc. natural	-	-	-	-	-	-	-	<0.0001	<0.0001	<0.0001	<0.0001
Red tide	-	-	-	-	-	-	-	-	0.0002	ns	ns
Tidal stranding	-	-	-	-	-	-	-	-	-	ns	<0.0001
Unsuitable habitat	-	-	-	-	-	-	-	-	-	-	ns
WC injury	-	-	-	-	-	-	-	-	-	-	-

Rehabilitation Efficiency (n=1085)

Final dispositions of the 1085 examined Type B rescues were as follows: E_r = 5.4% (n=59), M_r = 43.6% (n=473), R_r = 51.0% (n= 553). Numbers of death by location were noted for the first 4 stages of rehabilitation (n= 532; Table 7). Final dispositions (E_r, M_r, R_r) by individual rescue categories are displayed in Table 8.

Table 7: Raw numbers for death location by cause of rescue (n=532).

CAUSE OF RESCUE	RESCUE	TRANSPORT	PRIOR TO TREATMENT	REHABILITATION
BUOYANCY	1	3	2	13
COLD STRESS	10	9	7	43
EMACIATION	1	-	4	13
ENTANGLEMENT	-	1	-	13
ENTRAPMENT	-	-	-	4
MISC. HUMAN	-	-	-	2
MISC. NATURAL	2	5	18	46
ORPHAN	5	15	6	53
RED TIDE	2	2	1	6
TIDAL STRANDING	1	-	-	1
UNSUITABLE HABITAT	1	-	-	-
WATERCRAFT	22	23	21	176
SUM	45	58	59	370

Table 8: Final dispositions of manatees in Type B rescues (n=1085) according to rescue types.

CAUSE OF RESCUE	TOTAL	FINAL DISPOSITION					
		EUTHANIZED		DEAD		RELEASED	
		NUMBER	E_R(%)	NUMBER	M_R(%)	NUMBER	R_R(%)
BUOYANCY	31	2	6.5	17	54.8	12	38.7
COLD STRESS	210	2	1.0	67	31.9	141	67.1
EMACIATION	38	2	5.3	16	42.1	20	52.6
ENTANGLEMENT	86	-	-	14	16.3	72	83.7
ENTRAPMENT	39	-	-	4	10.3	35	89.7
MISC. HUMAN	5	-	-	2	40	3	60
MISC. NATURAL	78	4	5.1	67	85.9	7	9.0
ORPHAN	156	-	-	79	50.6	77	49.4
RED TIDE	61	-	-	11	18.0	50	82.0
TIDAL STRANDING	5	-	-	2	40	3	60
UNSUITABLE HABITAT	9	-	-	1	11.1	8	88.9
WATERCRAFT	367	49	13.3	193	52.6	125	34.1
OVERALL	1085	59	5.4	473	43.6	553	51.0

Time to Death (n=532)

To assess differences in time to death, measured as $Ln(T_d+1)$, we first performed a Shapiro-Wilk normality test which revealed that the distribution of the transformed data was not normal ($p<0.0001$, $W=0.8154$). We performed a Brown-Forsythe test to evaluate variances, which were unequal ($p=0.0009$). Because the data was not normal and the variances were unequal, we performed a Welch's test to assess differences in mean time to death, which was significant ($p=0.0255$) indicating that cause of rescue significantly impacts a manatee's time to death. The distribution in $Ln(T_d+1)$ by cause of rescue is displayed in Table 9 and Wilcoxon each pair comparisons are listed in Table 10.

Table 9: Distribution of time to death (T_d) in days across rescue categories for n=532 animals that died in the first 4 stages of rescue/rehabilitation.

LEVEL	MINIMUM	10%	25%	MEDIAN	75%	90%	MAXIMUM
BUOYANCY	0	0	0	1	5	23	824
COLD STRESS	0	0	0	0	5	42	3949
EMACIATION	0	0	0	2.5	42.5	222.7	535
ENTANGLEMENT	0	0	0.75	26	469.5	4042	4759
ENTRAPMENT	0	0	0.5	2	16.3	21	21
MISC. HUMAN	1	1	1	24	47	47	47
MISC. NATURAL	0	0	0	2	7	27.8	449
ORPHAN	0	0	0	2	21	413	4536
RED TIDE	0	0	0	0	1	6	6
TIDAL STRANDING	0	0	0	4.5	9	9	9
UNSUITABLE HABITAT	0	0	0	0	0	0	0
WATERCRAFT	0	0	0	2	24	109.5	13748

Table 10: Significant pairwise comparisons for differences in time to death [Ln(T_d +1)] between rescue categories. When time to death for the category on the Y axis is shorter than the category on the X axis the corresponding box is shaded blue, when time to death for the category on the Y axis is longer than the category on the X axis the corresponding box is shaded red. Nonsignificant comparisons are represented by "ns".

	Buoyancy	Cold stress	Emaciation	Entanglement	Entrapment	Misc. human	Misc. natural	Orphan	Red Tide	Tidal stranding	Unsuitable habitat	WC injury
Buoyancy	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cold Stress	-	-	0.0420	0.0032	ns	ns	ns	0.0386	ns	ns	ns	0.0023
Emaciation	-	-	-	ns	ns	ns	ns	ns	0.0410	ns	ns	ns
Entanglement	-	-	-	-	ns	ns	0.0099	ns	0.0115	ns	ns	ns
Entrapment	-	-	-	-	-	ns	ns	ns	ns	ns	ns	ns
Misc. human	-	-	-	-	-	-	ns	ns	ns	ns	ns	ns
Misc. natural	-	-	-	-	-	-	-	ns	ns	ns	ns	0.0409
Orphan	-	-	-	-	-	-	-	-	ns	ns	ns	ns
Red tide	-	-	-	-	-	-	-	-	-	ns	ns	0.0332
Tidal stranding	-	-	-	-	-	-	-	-	-	-	ns	ns
Unsuitable habitat	-	-	-	-	-	-	-	-	-	-	-	ns
WC injury	-	-	-	-	-	-	-	-	-	-	-	-

Euthanasia (n=59)

Cases of euthanasia had a median T_d of 11 days (min-max: 0-3949, quartiles: 2, 62). Watercraft injuries comprised 50 of the total 59 cases that required euthanasia, followed by 5 cases attributed to *miscellaneous natural* causes, and 4 cases due to *unknown* or *other* causes.

Time in Captivity (n=553)

A Shapiro-Wilk normality test indicated the transformed dataset was not normal (W= 0.993, p=0.0201). Cause of rescue categories were shown to have unequal variances in $Ln(T_r)$ (Brown-Forsythe, p=0.0086) and significantly different means (Welch's test, p<0.0001), the latter indicating that cause of rescue significantly affects time in captivity for treatment. Distribution in $Ln(T_r)$ is displayed in Table 11, and Wilcoxon pair comparisons are listed in Table 12.

Table 11: Distribution of treatment time (T_r) in days across rescue categories for subgroup of animals released from rehabilitation (n=553).

LEVEL	MINIMUM	10%	25%	MEDIAN	75%	90%	MAXIMUM
BUOYANCY	10	13.6	47.8	96.5	169	254.8	274
COLD STRESS	6	50	87	262	399	732	2236
EMACIATION	43	63.5	91.5	221	398.3	723.9	943
ENTANGLEMENT	3	35.7	70	176	363.8	530.7	9817
ENTRAPMENT	22	29	85	147	268	576.8	5523
MISC. HUMAN	69	69	69	189	299	299	299
MISC. NATURAL	41	41	71	160	600	1311	1311
ORPHAN	41	351.6	617.5	852	1059	2158.6	7870
RED TIDE	20	35	57	90.5	133	176.8	245
UNSUITABLE HABITAT	40	40	50.3	141.5	300.8	1349	1349
TIDAL STRANDING	85	85	85	107	461	461	461
WATERCRAFT	7	58.4	103.5	171	346.5	955.2	10961

Table 12: Significant pairwise comparisons for differences in treatment time [Ln(T_r)] between rescue categories. When treatment time for the category on the Y axis is shorter than the category on the X axis the corresponding box is shaded blue, when treatment time for the category on the Y axis is longer than the category on the X axis corresponding box is shaded red. Nonsignificant comparisons are represented by “ns”.

	Buoyancy	Cold stress	Emaciation	Entanglement	Entrapment	Misc. human	Misc. natural	Orphan	Red Tide	Tidal stranding	Unsuitable habitat	WC injury
Buoyancy	-	0.0060	0.0216	ns	ns	ns	ns	<0.0001	ns	ns	ns	0.0147
Cold Stress	-	-	ns	ns	ns	ns	ns	<0.0001	<0.0001	ns	ns	ns
Emaciation	-	-	-	ns	ns	ns	ns	<0.0001	0.0003	ns	ns	ns
Entanglement	-	-	-	-	ns	ns	ns	<0.0001	0.0004	ns	ns	ns
Entrapment	-	-	-	-	-	ns	ns	<0.0001	0.0033	ns	ns	ns
Misc. human	-	-	-	-	-	-	ns	0.0105	ns	ns	ns	ns
Misc. natural	-	-	-	-	-	-	-	0.0056	ns	ns	ns	ns
Orphan	-	-	-	-	-	-	-	-	<0.0001	0.0198	0.0010	<0.0001
Red tide	-	-	-	-	-	-	-	-	-	ns	ns	<0.0001
Tidal stranding	-	-	-	-	-	-	-	-	-	-	ns	ns
Unsuitable habitat	-	-	-	-	-	-	-	-	-	-	-	ns
WC injury	-	-	-	-	-	-	-	-	-	-	-	-

Binary Logistic Regression for Survival from Rescue to Commencement of Treatment

Of the total Type B rescues examined (n=1085), 923 were alive at the start of treatment, and 162 died before treatment commenced. The logistic regression was not significant for season (p=0.7218) or sex (p=0.5568), however, body length (Whole Model Test p=0.0306, AICc=914.01) and the causes of rescue (Whole Model Test p<0.0001, AICc=882.63) were significantly related to the chances of survival until admission at facility. Length and cause of rescue were significantly related to each other (Kruskal-Wallis, p<0.0001), thus the model based on cause of rescue was chosen based on minimum AICc. Odds ratios (OR) were calculated between the rescue categories to look for differences in survival rates from rescue to commencement of treatment (Figure 5, Appendix A). Animals with *cold stress* (OR=3.34, p=0.0002), *emaciation* (OR=3.11, p=0.0347), *orphaned calves* (OR=2.36, p=0.0081), *red tide* (OR=5.28, p=0.0016), or *watercraft injuries* (OR=2.15, p=0.0059) were more likely to survive until commencement of treatment at the rehabilitation facility than animals rescued for *misc. natural* causes. Further, manatees rescued for *entanglement* injuries were more likely than those rescued for *buoyancy* (OR=20.40, p=0.0063), *cold stress* (OR=12.01, p=0.0155), *emaciation* (OR=12.88, p=0.0218), *misc. natural* (OR=40.09, p=0.0004), *orphaned calves* (OR=17.00, p=0.0059), *tidal stranding* (OR=21.25, p=0.0421), or *watercraft* injuries (OR=18.64, p=0.0040).

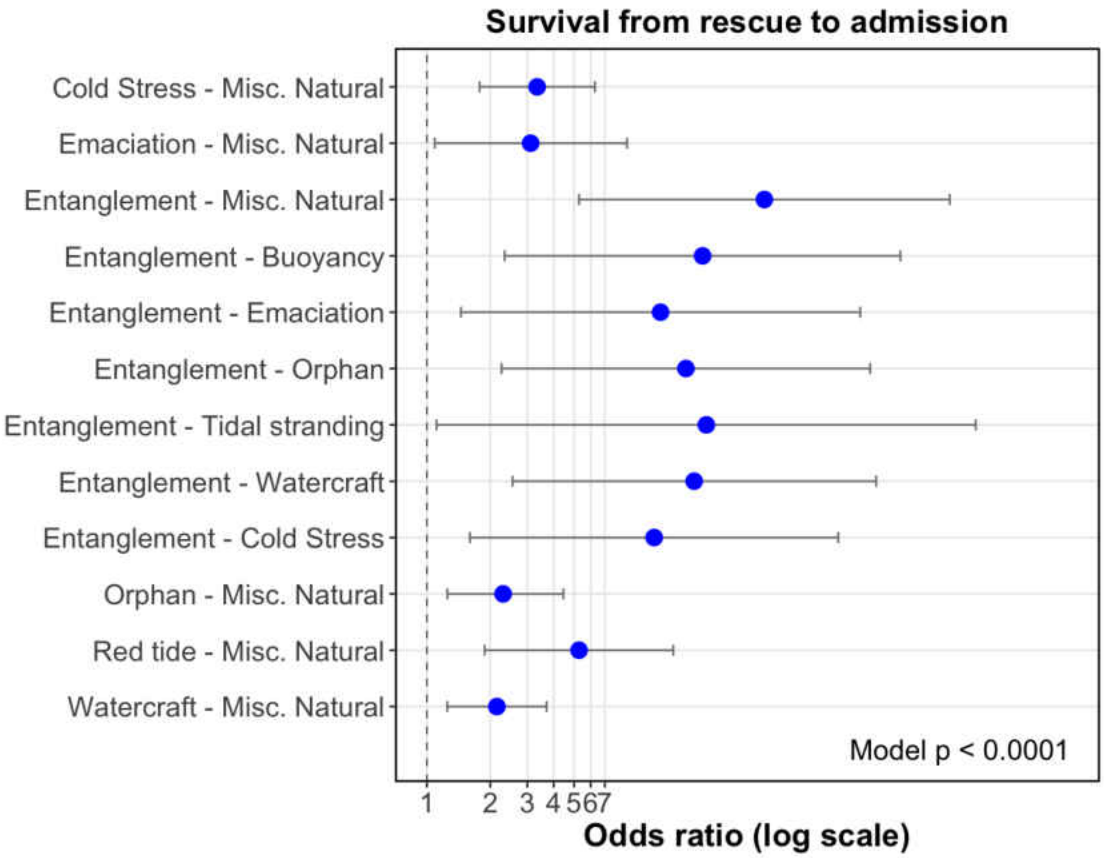

Figure 5: For each line on the Y axis, animal rescued for the 1st cause group were more likely to survive from rescue to admission than animals in the 2nd cause group by a factor equivalent to the odds ratio, displayed as a blue dot. Error bars represent 95% CI for the odds ratio estimate.

Binary Logistic Regression for Survival from Commencement of Treatment to Release

Of the 923 rescue events where live animals were admitted for treatment, 553 were ultimately released, while 370 died in treatment. Results from individual regressions of rescue category, sex and body length on survival showed that influence of rescue season (p=0.0003, AICc= 1494.8), and rescue category was significant (Whole model test p<0.0001, AICc=1297.8), but sex (p=0.6240) and body length (p=0.1899) were not. Rescue cause and season were significantly related (p<0.0001); rescue cause was selected for analysis based on minimum AICc, and odds ratios were then calculated to examine differences in survival-to-release rates between the categories of rescue (Figure 6, Appendix A). Manatees rescued for *buoyancy* issues were significantly more likely than those presenting with *misc. natural* issues to survive to release (OR=6.41, p=0.0006). *Cold stressed* animals were more likely to survive until release than manatees with *buoyancy* problems (OR=3.24, p=0.0031), *misc. natural* issues (OR=20.73, p<0.0001), *watercraft* injuries (OR=3.96, p<0.0001), or *orphaned calves* (OR=2.10, p=0.0007). Manatees with *emaciation* were significantly more likely to survive to release than those with *watercraft* injuries (OR=2.15, p=0.0256), or *misc. natural* issues (OR=11.27, p<0.0001). Manatees with *entanglement* injuries were more likely to survive to release than animals with *buoyancy* issues (OR=8.14, p<0.0001), *cold stress* (OR=2.52, p=0.0048), *emaciation* (OR=4.63, p=0.0005), *misc. natural* issues (OR=52.16, p<0.0001), *orphaned calves* (OR=5.28, p<0.0001), or *watercraft* injuries (OR=9.96, p<0.0001). Manatees rescued due to *entrapment* in man-made structures were significantly more likely to survive until release than those with *buoyancy* issues (OR=13.85, p<0.0001), *cold stress* (OR=4.28, p=0.0079), *emaciation* (OR=7.88, p=0.0009), *watercraft* injuries (OR=16.94, p<0.0001), *orphaned calves* (OR=8.98, p<0.0001), or *misc. natural* issues (OR=88.75, p<0.0001). Manatees with *misc. human* related injuries were significantly more likely to survive until release than those with *misc. natural* issues (OR=15.21, p=0.0062). Orphaned calves were significantly more likely to survive until release than manatees with *misc. natural* issues (OR=9.88, p<0.0001) or *watercraft* injuries (OR=1.89, p=0.0011). Manatees rescued due to *red tide* poisoning were more significantly more likely to survive to release than those with *emaciation* (OR=4.09, p=0.0025), *buoyancy* problems (OR=7.20, p<0.0001), *cold stress* (OR=2.22, p=0.0281), *misc. natural* issues (OR=46.10, p<0.0001), *orphaned calves* (OR=4.66, p<0.0001), or *watercraft* injuries (OR=8.8, p<0.0001). *Tidally stranded* manatees were significantly more likely to survive to release than those with *misc. natural* issues (OR=15.21, p=0.0062). Individuals rescued due to *unsuitable habitat* were significantly more likely to survive to release than manatees with *buoyancy* issues (OR=12.67, p=0.0238), *misc. natural* issues (OR=81.14, p<0.0001), *orphaned calves* (OR=8.21, p=0.0497), or *watercraft* injuries (OR=15.49, p=0.0102). Lastly, manatees with *watercraft* injuries were significantly more likely to survive until release than those rescued due to *misc. natural* issues (OR=5.24, p<0.0001).

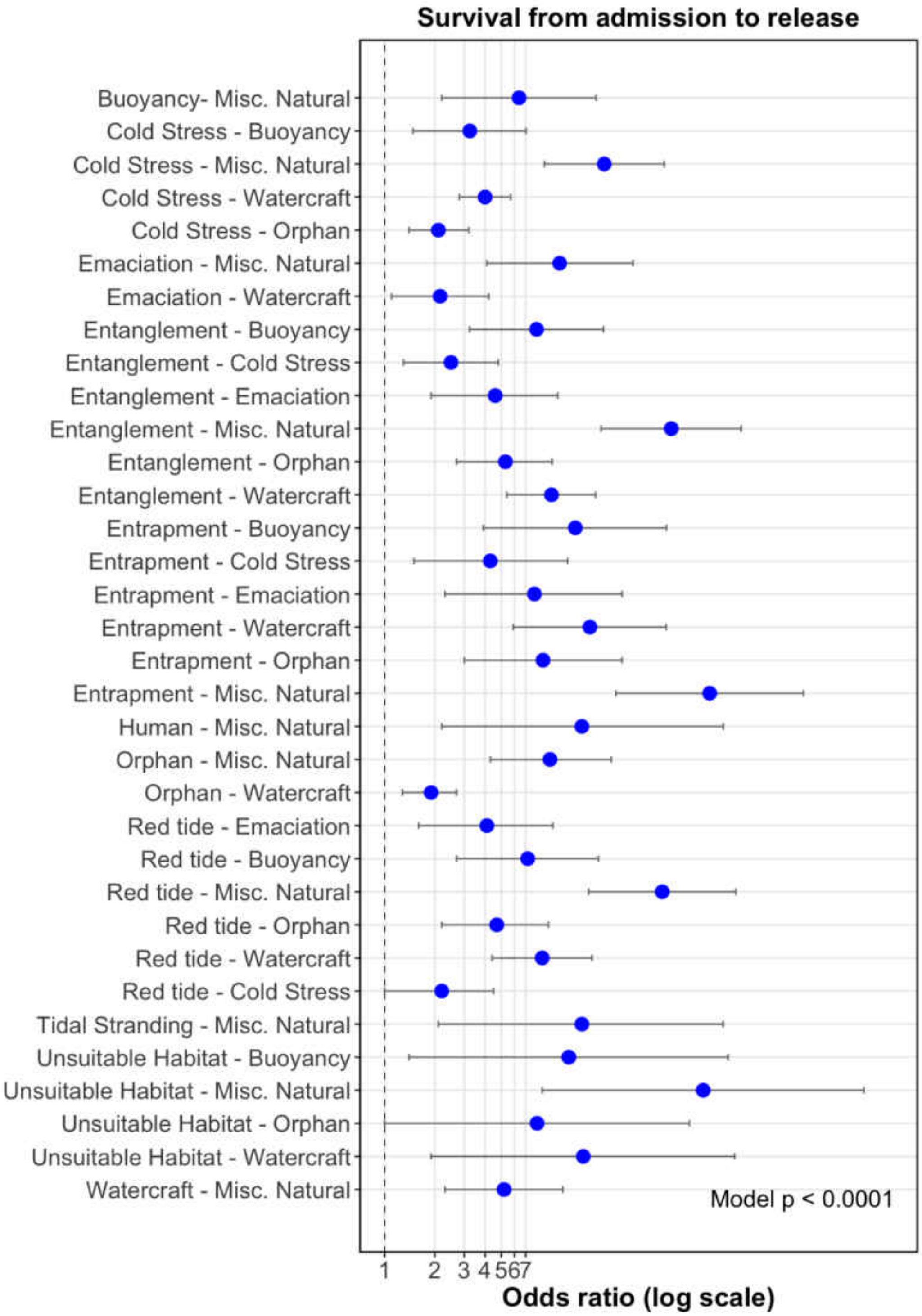

Figure 6: For each line on the Y axis, animal rescued for the 1st cause group were more likely to survive from rescue to admission than animals in the 2nd cause group by a factor equivalent to the odds ratio, displayed as a blue dot. Error bars represent 95% CI for the odds ratio estimate.

Captive Born Animals

A total of 25 captive born manatees were released (mean T_r: 2342.88, min-max: 14-8849 days). Of those 25 manatees 15 required re-rescue, with 11 of those re-rescues occurring within the first year. Captive born animals were ultimately re-rescued for *cold stress* (4), remaining in *unsuitable habitat* (3), *emaciation* (3), *entrapment* (2), *entanglement in marine debris* (2), and *buoyancy issues* (1). Nine of the 25 released, captive born manatees were confirmed dead at a future date (mean T_d: 825.5, min-max: 11- 5251 days).

Time to Death (T_d) Post- Release

We had post-release mortality data for 138 animals released from the Florida Manatee Rescue and Rehabilitation Partnership (Figure 7; median: 1090.5; min-max: 0-9151; quartiles: 315.5, 2874.25). As these data represent the days until the carcass was recovered and not the actual days until death, these results should be regarded as approximate. Causes of death for these animals were: *watercraft injury* (31), *red tide poisoning* (17), *miscellaneous natural* (18), *cold stress* (16), *entrapment* (6), *entanglement* (5), *miscellaneous human* (2), and *other* (2). In 41 cases, recovered carcasses were too decomposed or a cause of death of was not able to be determined. A total of 69 carcasses (50%) were recovered in the first 1079 days post-release, 39 of which within the first 365 days. The remaining 50% were recovered between 1079- 9151 days, which translates to 3.0– 25.1 years post-release.

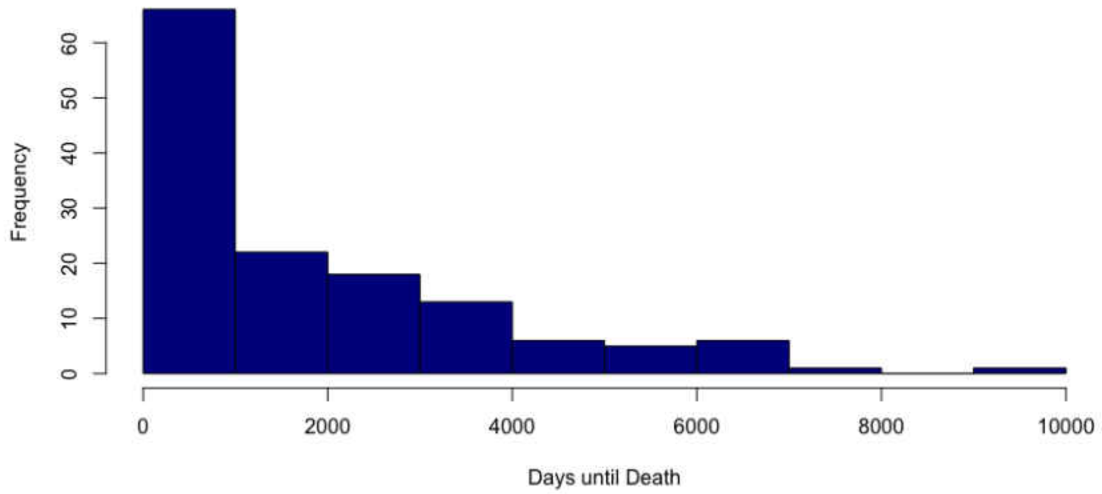

Figure 7: Histogram of approximate T_d for n=138 animals released from the Florida Manatee Rescue and Rehabilitation Partnership (MRRP) and later recovered through the carcass recovery program.

Discussion

Wildlife rescue and rehabilitation efforts provide a critical source of data for the study of diseases, threats, and treatment efficacy in free-ranging wildlife populations, but long-term analyses of these datasets are rare (Lunney et al. 2004, Zagzebski et al. 2006, Flint et al. 2010, Molina-López et al. 2011, Burton and Tribe 2016, Mariacher et al. 2016, Orós et al. 2016, Molina-López et al. 2017). This study provides the first multi-decadal (43 yr) analysis of a manatee rescue, rehabilitation and release program in the United States.

Many marine taxa are negatively impacted by the use of watercraft and traffic-related impacts remain one of the top threats to marine mammals globally (Avila et al. 2018). Off Cape

Cod, anthropogenic impacts including vessel strikes and marine debris were responsible for 45% of gray seal mortality (Bogomolni et al. 2010) and interactions with the fishing industry are a main threat to Antillean manatees in Brazil (Balensiefer et al. 2017). Watercraft traffic is a serious threat to Florida manatee health and survival. Manatees rescued for *cold stress, emaciation, entanglement, entrapment, orphaned calves, red tide,* or *unsuitable habitat* were all significantly more likely to survive from commencement of treatment to release than those rescued due to *watercraft* injuries, reflecting the high threat posed to manatees by watercraft. Overall, watercraft injuries produced the third highest mortality rate (52.6%) by rescue category, had the highest within-category euthanasia rate (13.3%) and composed 83.1% of all cases of euthanasia in the dataset. Following rescues for *misc. natural,* which had a release rate of only 9.0%, *watercraft* injuries had the second-lowest release rate at 34.1%. Time to death for manatees with *watercraft* injuries was significantly higher than those brought in for several other categories, including *misc. natural* issues. This means that while both of these categories have low rates of recovery, manatees with *watercraft* injuries are more likely to linger in rehabilitation for longer periods before succumbing to their injuries. It is apparent that *watercraft* injuries are costly to the manatee population via the mechanisms of mortality and sublethal injury. Additionally, manatees rescued due to *watercraft* injuries spend long times in recovery before release or death, imposing serious costs on the rehabilitation system.

While a previous assessment reported that *entanglement* was the most common anthropogenic cause of rescue for Florida manatees from 1993-2012 (Reinert et al. 2017), *watercraft* injuries surpassed *entanglement* in marine debris as the most numerous cause of rescue in this dataset. Rescues due to *watercraft* injuries were not only more common, but also more serious than those for *entanglements* in marine debris which is reflected in the fact that most marine debris entanglements are addressed *in situ* but all watercraft injuries required captive care. Further, manatees rescued due to *entanglement* were significantly more likely than those rescued due to watercraft injuries to survive the period from rescue to admission at rehabilitation and again from the commencement of treatment to release. Yet marine debris still poses great danger to manatees and other wildlife. Monofilament is by far the most common debris implicated in debris-related rescue or death, and ingestion of monofilament has proved to be especially lethal for manatees (Reinert et al. 2017). The frequency of entanglement in, and ingestion of, marine and aquatic debris is increasing across taxa (Moore et al. 2009, Bogomolni et al. 2010, Williams et al. 2011, Allen et al. 2012, Rodríguez et al. 2013, Udyawer et al. 2013, Baulch and Perry 2014, Boede and Mujica-Jorquera 2016, Orós et al. 2016, Stelfox et al. 2016, Currie et al. 2017, Reinert et al. 2017, Fossi et al. 2018). The number of species impacted by marine debris increased 159% from 1995-2015, and has doubled in the intervening years to over 1,465 affected species (Fossi et al. 2018).

Data from rehabilitation programs can reveal important temporal trends, including seasonal links, in the causes of morbidity in wildlife (Aragones et al. 2010, Burton and Tribe 2016, Orós et al. 2016). The results of the present study indicate seasonal differences in the frequency of Type A and Type B rescues where the former are significantly higher during the warm season and the latter are significantly higher during the cold season. When examining specific causes of rescue, *entanglement* injuries, *misc. natural* issues and *tidal stranding* were significantly higher in the warm season while *cold stress*, *red tide poisoning*, *entrapment* and *emaciation* were significantly more common in the cold season. Interestingly, *watercraft* *injuries*, *buoyancy* problems, and *orphaned calves* were consistent in frequency between seasons. The consistent rate of *watercraft* related rescues between seasons suggests a year-round saturation of watercraft in the environment; previous work also found no seasonal differences in watercraft related mortality in Florida manatees, indicating year-round environmental saturation of watercraft dating back as far as 1985 (O'Shea et al. 1985). While the rescue frequency of *orphaned calves* suggests a steady rate of maternal mortality, the causes of death and any potential connections with watercraft are not immediately discernable.

Significantly increased rescues due to red tide poisoning in the cold season are somewhat intuitive, as inshore blooms of *K. brevis* often occur in the late summer or fall (Stumpf 2001, Walsh et al. 2006). However, blooms of *K. brevis* can occur at any time of year, and some blooms can persist for a year or more (Hu et al. 2006). Additionally, red tide blooms can have delayed effects on manatees; high toxin concentrations can remain in seagrasses for months, poisoning manatees via ingestion well after the bloom has subsided (Flewelling et al. 2005), complicating the seasonal link. Increased rescues for *red tide poisoning* in the cold season may be representative of an immunosuppression cascade where immune systems can possibly be weakened by other factors (e.g. *cold stress* or *emaciation*, both overrepresented in cold season rescues) resulting in vulnerability to other diseases (e.g. harmful algal bloom toxicity) (Halvorsen and Keith 2008).

The significantly higher rates of *entrapment* in the cold season may reflect migration movements and search for warm-water refuges. It is also not unexpected that *emaciation* is more common during the cold season, as manatees are known to stay at warm-water refuges for extended periods without feeding (Bengtson 1981). Florida manatees are known to utilize several different kinds of warm-water refuge types in order to survive the cold season, and the two largest subpopulations (Southwest Florida, Atlantic coast) rely largely on effluent from power plants or on passive thermal basins (Laist et al. 2013). In fact, it has been suggested that half of all Florida manatees may use power plants to survive the coldest days of winter (Laist and Reynolds 2005, Laist et al. 2013). Natural warm water springs provide the best protection against cold stress for manatees, yet development, dams, and high anthropogenic use prevents or deters manatees from using many natural springs throughout Florida; in this respect cold stress syndrome in manatees can be considered anthropogenic (Marsh et al. 2011). A long-term plan to restore manatee access and protection to these springs will be required to prevent significant population losses as aging power plants throughout Florida are phased out of use (Bossart et al. 2004, Laist and Reynolds 2005, Laist et al. 2013).

Cold stress syndrome is a highly important threat to the persistence of the Florida manatee when compared with other species and subspecies of sirenians (Marsh et al. 2011, Owen et al. 2013) but the present analysis shows promising rates of recovery from cold stress for those animals that are brought into captivity (Rr=67.1%). T_r times for cold stress were intermediate, possibly as a result of the many complicating factors that can arise from cold stress including abscesses, enterocolitis, emaciation, and reduced immune function (Bossart et al. 2004, Walsh et al. 2005). Extreme cold weather events are expected to occur even as mean temperatures rise under climate change, and while some areas will experience fewer cold weather events, some areas may experience increased frequency (Vavrus et al. 2006, Smith and Sheridan 2018). While temperatures in Florida are characteristically mild, several studies in humans have reported higher levels of excess mortality due to cold weather in areas with typically warm climates when compared to those that are typically cold (Donaldson et al. 1998, Healy 2003, Han et al. 2017). The relatively stable year-round temperatures characteristic of the state of Florida means that a drop in temperatures results in large deviations from average (Smith and Sheridan 2018) which means that humans and wildlife have little time to prepare and can lead to excesses in mortality. Manatees begin migration in response to environmental cues, specifically when water temperatures reach approximately 20º C (Deutsch et al. 2003), and unexpected, extreme cold events leave them vulnerable to cold stress syndrome and death. Young manatees are trained to go to warm water refugia by their mothers in response to cold exposure. If a cold weather event did not occur during the nursing period then young, recently independent animals are particularly susceptible to cold stress mortality since they may not seek refuge in response to dropping water temperatures. Further, it has been documented that young and sub-adult manatees do not physiologically respond to cold like adult manatees do; adult manatees increase metabolic rate in response to temperatures <20º C, but younger manatees are apparently incapable of this, making them vulnerable to hypothermia and death from acute cold stress (Worthy 1999). Body lengths for the categories *cold stress* and *misc. natural* issues were significantly shorter (i.e. younger) than all other categories, and *misc. natural* rescues were significantly shorter than those for *cold stress*.

Females manatees were significantly more likely than males to require rescue for *entanglement*, echoing the results of a previous study (Reinert et al. 2017) and were also significantly more likely to be rescued for *tidal stranding*. Previous research indicated that females are also more likely to die of entanglement in, or ingestion of, marine debris. It has been hypothesized that this is due to intentional itching or rubbing against structures in response to swollen mammary glands (Beck and Lefebvre 1995, Reinert et al. 2017). The results of the present study are consistent with this hypothesis, as marine debris *entanglements* are significantly overrepresented during the warm season and the spring-summer peak in calving (Rathbun et al. 1995, Marsh et al. 2011).

In an effort to define and assess quality of care within wildlife rehabilitation programs, Oros et al. devised several rehabilitation efficiency parameters. Final dispositions in the present study for manatees admitted alive to rehabilitation were R_r= 51.0%, M_r= 43.6%, and E_r= 5.4%. The R_r calculated for the Florida Manatee Rescue and Rehabilitation Partnership conveys reasonable potential for recovery and release as the R_r is similar to outcomes of other wildlife rescue and rehabilitation programs with release rates at or below 50% (Punch 2001, Rodríguez et al. 2010, Mariacher et al. 2016, Sack et al. 2017). In contrast, a retrospective study of the rehabilitation of over 10,000 rescued koalas in Australia revealed an R_r of only 27%, as most koalas (66.5%) were either euthanized or died in care (Burton and Tribe 2016). In the present study, the euthanasia rate for animals brought into captivity was 5.4% and overwhelmingly (49 of 59 total) due to *watercraft* injuries. In a long-term study of rescued and rehabilitated odontocetes in California, 50% of individuals died within 24 hrs of admission and their potential for rehabilitation and release was described as minimal (Zagzebski et al. 2006). That was not the case for the manatee, where only 10.5% of animals admitted to captivity died on day 0-1 of rehabilitation in captivity.

For animals that died after commencement of treatment in captivity, categories of *red tide* poisoning and *cold stress* had significantly shorter T_d than other categories, while the categories of *watercraft* and *entanglement* injuries had significantly longer T_d values. Watercraft can inflict both blunt- and sharp- force trauma, and the thick epidermis of manatees means that more than half of watercraft related mortality is due to blunt-force (Lightsey et al. 2006, Rommel et al. 2007). During the period from 1993-2003, 73% of all manatees with watercraft-related mortality had at least one broken bone, with ribs being the most common. Broken ribs or vertebrae can further damage muscles or internal organs, including the lungs, uterus, ovaries, kidneys, heart, gastrointestinal tract and hemidiaphragms, resulting in myriad complications (Lightsey et al. 2006). Any watercraft injury that does not lead to immediate death could still potentially result in the eventual death of the animal from complications or infection. This may be the underlying force behind the significantly longer T_d seen in watercraft injuries in this study.

Orphaned calves had significantly higher T_r than all other categories, as it is standard to hold manatees until they are approximately 5-6 years old when most manatees have reached sexual maturity (Marsh et al. 2011, Adimey et al. 2012). Recent work indicates that an increased duration of time is correlated with increased post-release failure, thus perhaps this holding strategy should be reconsidered (Adimey et al. 2016).

Different programs measure success in different ways, but post-release survival has emerged as a benchmark of success across taxa to determine rehabilitation efficacy, and several studies have advocated for or employed telemetry and post-release monitoring to evaluate program success (Davis et al. 1996, Wiley et al. 2001, Gulland et al. 2002, Lander et al. 2002, Zagzebski et al. 2006, Dendrinos et al. 2007, Adimey et al. 2016, Mariacher et al. 2016). Telemetry coupled with a robust carcass recovery program has shown that many manatees survive for years after release, speaking to the value of the Florida Manatee Rescue and Rehabilitation Partnership to the conservation of the Florida manatee. In Puerto Rico, the first rehabilitated manatee that was released in 1994 was confirmed alive and thriving in the wild two decades later (Adimey et al. 2012). Many biologists argue that rehabilitation programs have greater merit when released individuals reproduce and contribute to population growth, however this idea must be balanced with the fact that many uninjured, wild animals never reproduce (Adimey et al. 2012, Aitken 2004). We must be careful not to hold rehabilitated animals to a higher standard and denigrate rehabilitation programs that do not prove reproduction in rehabilitated individuals. Releasing rehabilitated individuals back into the wild to function in the ecosystem is a measure of success in itself, and the persistence of rehabilitated individuals years after release is certainly evidence of program success.

CHAPTER 3: ANTHROPOGENIC SEASCAPE FEATURES EXPLAIN GENETIC VARIATION IN THE GENETICALLY DEPAUPERATE FLORIDA MANATEE.

Introduction

Genetic diversity is the raw material that natural selection acts upon (Frankham et al. 2002) and is crucial for ecological processes at the levels of species and community. Low levels of diversity can reduce the rate at which species mount an adaptive response to changes in the environment, making diversity especially important surrogate measure to quantify species persistence, particularly given the rate at which humans continue to alter the environment (Frankham et al. 1999, Brook et al. 2002). Thus, managing diversity in species of interest is an important management tool in conservation, and ignoring genetic diversity in practice can lead to inappropriate conservation strategies and underestimation of extinction risk (Spielman et al. 2004, Frankham 2005). It is also vitally important for conservation plans to incorporate the concept of functional connectivity, or the way that landscape characteristics promote or inhibit gene flow as a result of animal movement (Taylor et al. 1993, Tischendorf and Fahrig 2000). This intersection of population genetics and landscape ecology is known as landscape genetics when performed in terrestrial systems (Manel et al. 2003, Spear et al. 2005, Storfer et al. 2006, Balkenhol et al. 2009, Wang et al. 2009, Munshi-South 2012, Manel and Holderegger 2013, Dyer 2015, Epps and Nusha 2015, Yannic et al. 2016, van Strien 2017, Flores-Manzanero et al. 2019) or seascape genetics when undertaken in marine systems (Galindo et al. 2006, Banks et al.

2007, Schultz et al. 2008, Selkoe et al. 2008, Galindo et al. 2010, Amaral et al. 2012, Selkoe et al. 2016).

Researchers have developed many ways to conceptualize how landscape/seascape characteristics affect gene flow. Isolation by distance (IBD) describes a positives relationship between genetic differentiation and Euclidean distance where the cost surface is uniform across the landscape (Wright 1943, Cassens et al. 2000, Schultz et al. 2008). Least cost path analysis (LCP) incorporates habitat heterogeneity into the resistance landscape, predicting increasing genetic differentiation with increasing effective distance based on optimal movement via a single pathway (Adriaensen et al. 2003, Beier et al. 2003, Walker et al. 2007, LaRue and Nielsen 2008, Sawyer et al. 2011, van Strien et al. 2012, Marrotte and Bowman 2017). Isolation by resistance, or circuit theory (CT), also incorporates habitat features into resistance distances and simultaneously considers all possible pathways when attempting to explain genetic variation in space (McRae 2006, McRae and Beier 2007, McRae et al. 2008). When initially developed, CT was argued to be more robust to heterogeneity and more theoretically justified than other methods, and while it has performed better than other methods in many studies it is unlikely that any one model describing landscape connectivity will be optimal in all possible systems (McRae 2006, McRae and Beier 2007, McRae et al. 2008, Kershenbaum et al. 2014, Haase et al. 2017, Khimoun et al. 2017, Flores-Manzanero et al. 2019)

Important drivers of seascape genetics patterns can be difficult to ascertain, as marine systems are diffusive and ephemeral and populations of marine organisms are often large and highly mobile (Selkoe et al. 2008, Selkoe et al. 2010, Amaral et al. 2012). The Florida manatee (*T. m. latirostris*) presents an especially difficult case. A previous study on Florida manatee population genetics revealed that the statewide population possesses levels of diversity that are lower than averages reported for other placental mammals, including fragmented and nonideal populations (Garner et al. 2005, Tucker et al. 2012). Most genetic variation (95%) was between individuals, with only minor portions of variance explained by defined conservation management units or between the East and West coast populations (Tucker et al. 2012). However even in populations with weak differentiation, low diversity, or chaotic structure, landscape/seascape genetics has proven useful in understanding the distribution of genetic variation in space (Spear et al. 2005, Banks et al. 2007, Selkoe et al. 2010, Amaral et al. 2012, Diopere et al. 2017) and may be able to explain more of the Florida manatee genetic structure than the model-based clustering methods used previously.

The goal of the present study was to elucidate whether any hypothesis based on abiotic (bathymetry, temperature, salinity), biotic (chlorophyll-a concentration, seagrass cover), anthropogenic (boat activity, coastal habitat disturbance), or a combination of seascape factors could explain genetic distance (GDis) in manatees better than distance alone. I approached this question by generating a GDis matrix for all pairs of individual manatees in the dataset, using mantel tests to select informative ecological distance (IBD, LCP, CT) hypotheses based on objective transformations of seascape layers, and using maximum-likelihood population-effects mixed modelling to select the model that best explained GDis based on measures of model fit (AICc). Results of this study can inform managers on what conducts, or conversely inhibits, gene flow in the Florida manatee, and in turn this information can be used to implement conservation and recovery plans as anthropogenic pressure on and other environmental threats to Florida manatee populations increase.

Methods

Genetic Data

I obtained microsatellite data at 20 loci from 319 manatee carcasses analyzed by the Florida Fish and Wildlife Conservation Commission/ Florida Wildlife Research Institute. Carcasses were collected in the months of April - October between the years 2009 – 2015 and fell into 8 populations: 4 east coast populations and 4 west coast populations. I tested for deviations from Hardy-Weinberg equilibrium at each locus within each population using the "pegas" package (Paradis 2010), and applied a sequential Holm-Bonferroni correction for multiple comparisons using the "stats" package (R Core Team 2017). I computed the total number of alleles (A), the effective number of alleles (A_e), expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficient (F_{IS}) at each locus using the package "gstudio" (Dyer 2014). I estimated individual-based GDis between all pairs of samples using the proportion of shared alleles (Dps) in the package "adegenet" (Jombart 2008) and then produced a GDis matrix by subtracting 1-Dps.

Seascape Genetic Analyses

Information on data sources for the seascape layers is listed in Appendix B. I obtained monthly marine layers for bathymetry, chlorophyll-a, salinity, and temperature from MARSPEC (Sbrocco and Barber 2013); these layers have a 30 arcsecond (~ 1 km) spatial resolution and span the years 1955-2010. Using the R package “raster” (Hijmans 2019). I calculated minimum salinity, mean chlorophyll-a, mean temperature, mean bathymetry for the months of April to October corresponding to the warm season when manatees typically mate. Additionally, I obtained freshwater data from the State of Florida Department of Environmental Protection Impaired Waters Rule Database (IWR- Run 53) for the months of April to October from 1974-2015. I averaged measurements at each unique monitoring station to obtain a single value for the warm season each year. Then, I checked the resulting dataset for errors and eliminated data points that indicated errors in measurement or recording based on the following criteria: salinity entries were removed if negative or >45 psu (39 entries), chlorophyll-a entries were removed if negative or >1000 mg/m^3 (50 entries), temperature entries were removed if <13º C or >40º C (121 entries), and a single bathymetry entry of 2055.4 m was removed. I calculated the minimum salinity, mean chlorophyll-a, mean temperature, mean bathymetry at each unique DEP monitoring station and then mapped these values to an empty raster (84 -79.75º W, 24.75- 31.25º N) with 30 arcsecond (~1km) spatial resolution. Each DEP data raster was then merged with the corresponding satellite raster.

Additionally, I gathered categorical information on seagrasses, coastal habitat disturbance, and boat activity from FWC/FWRI. Prior to transformation, I reclassified the layer describing areas of continuous seagrass cover and discontinuous/patchy seagrass cover (scale 1: 25000 m, layer did not identify species composition). Areas with continuous seagrass were given a value of 2, areas with discontinuous seagrass cover were assigned a value of 3, and open ocean was assigned a value of 6. Prior to transformation, the layer containing the coastal habitat classifications 43 different categories (scale 1: 25000 m) was reclassified to 2 categories (natural or disturbed). New categories were then extracted and rasterized at the locations of coastal DEP monitoring stations in Florida. Further information about the coastal habitat classifications data layer is contained in Appendix B. Thus, 3 final coastal habitat categories were defined and assigned a numeric weight: open ocean was given a value of 1.5, habitats classified as "natural" were given a value of 2, and habitats classified as "disturbed" were given a value of 9. Lastly, I constructed a boat activity feature layer by rasterizing boat ramp inventory information combined with map information for official and unofficial commonly used waterways. Prior to transformation, cells in the boat activity raster were given 1 of 3 possible numeric weights: open ocean cells were given a value of 0.5, cells with either a boat ramp or a defined waterway were given a value of 1.5, and cells with both a boat ramp and a defined waterway were given a value of 2.5. All categorical layers were cropped to match the extent of the continuous data layers, resampled to a 30 arcsecond spatial resolution, and cropped to the study area plus a 50km border. Minimum and maximum values for continuous layers and levels for categorical layers are listed in Table 13.

Table 13: Descriptions of examined seascape layers. Min- max values and categorical levels are for the final rasters masked within 50km of the Florida coastline.

Seascape layer	Data type	Min- max/ levels
Bathymetry	Continuous	0.61 – 850 m
Boat activity	Categorical	Open ocean, boat ramp or waterway, boat ramp and waterway
Chlorophyll-a conc.	Continuous	0 – 135 mg/m^3
Coastal habitat classification	Categorical	Open ocean, disturbed habitat, natural habitat
Salinity	Continuous	0.31 – 36.06 psu
Seagrass cover	Categorical	Open ocean, continuous seagrass, discontinuous seagrass
Temperature	Continuous	21.51 – 32.28 ℃

Each variable layer was transformed using the “ResistanceGA” package (Peterman 2018) which uses eight transformations (M, monomolecular; IM, inverse monomolecular; RM, reverse monomolecular; IRM, inverse reverse monomolecular; R, ricker; IR, inverse ricker; RR, reverse ricker; IRR, inverse reverse ricker) to objectively parameterize resistance surfaces. The benefit of this method is that it removes the role of expert opinion in surface parameterization, as opinion is often not adequate in describing the biological and ecological processes that structure populations (Shirk et al. 2010, Spear et al. 2010, Peterman 2018). Using these transformations I produced 48 resistance layers scaled from 0-100. After transformation, the peninsula of Florida was assigned a resistance value of 5000. I produced 2 distance matrices (least cost distance and circuit-theory distance) for each resistance layer in addition to a single isolation-by-distance matrix, resulting in 97 individual variable distance matrices. I produced least cost distances using the costDistance() function and produced circuit theory distances using the commuteDistance() function in the R package “gdistance” (van Etten 2018).

Individual variable distance matrices were compared with the GDis matrix using mantel tests (Mantel 1967) with the mantel() function in the R package "vegan" (Oksanen et al. 2018) under 10,000 permutations. The use of mantel tests has been challenged in recent years (Balkenhol et al. 2009, Cushman and Landguth 2010, Legendre and Fortin 2010, Legendre et al. 2015) but recent simulation studies support use of the mantel test statistic for landscape genetic studies, as long as results are not based on p-values (Cushman et al. 2013, Zeller et al. 2016, Shirk et al. 2018). Following mantel tests measuring correlations between ecological distance and GDis for individual layers, I selected the models representing the transformation and distance type with the highest mantel r statistic (rM) for each individual seascape variable and used the package "raster" to compute Moran's I, a measure of spatial autocorrelation, for each of the univariate layers. I then assessed the models for correlation in ecological resistance distances using mantel tests and eliminated layers that were highly correlated (rM >0.9) with one another. Highly correlated resistance hypotheses are a major source of model selection error in landscape resistance studies and reduce the ability to correctly identify the resistance surface that gave rise to the observed genetic pattern; retaining models with ecological distance correlations <0.9 markedly improves chances of correctly identifying the resistance surface that gave rise to the observed genetic pattern and decreases the chance of making spurious correlations (Cushman et al. 2013, Zeller et al. 2016, Shirk et al. 2018). In order to eliminate highly correlated models, I sequentially eliminated the model with the top number of problematic correlations with the other considered seascape variables until all remaining variables were below the threshold. When 2 models possessed the same number of problematic correlations with other remaining models, I retained the one that was more highly correlated (i.e. higher rM) with the GDis matrix. After I eliminated highly correlated models, I summed the remaining models to form a composite layer. Lastly, I produced a least cost distance matrix and a circuit theory distance matrix from the composite layer for a final total of 99 considered ecological distance hypotheses, and measured Moran's I for the composite model.

In order the select the resistance model that best explained the GDis, I compiled ecological distance matrices generated by 1) isolation by distance 2) the uncorrelated individual variable models from the previous step and 3) the distance type most highly correlated (maximum rM) with GDis for the composite layer. I evaluated these candidate models using maximum-likelihood population-effects (MLPE) mixed modelling in the package "ResistanceGA" using the resist.boot() function under 10,000 permutations and selected the best model using minimum corrected Aikike's Information Criterion (AICc) as a measure of model-fit (Akaike 1974, Sugiura 1978).

Results

Genetic Data

After checking that all carcass locations had values in all data layers, I eliminated data from 26 carcasses because the carcass recovery locations were not captured by the spatial data layers, leaving data from 293 for analysis (Figure 8).

Initially, 44 of 160 tests for deviations from HWE were significant (Table 14); however, after the Bonferroni- Holm correction for multiple comparisons (Holm 1979) only 7 of 160 tests were significant (Table 15), thus I considered all 20 loci to be independent and included all in subsequent analyses. Statewide measures of genetic diversity for this dataset (Table 16; A_e= 1.93, H_e= 0.449, H_o= 0.445) were similar to previous measures as calculated by Tucker et al. 2012 (A_e=2.082, H_e=0.478, H_o=0.455), with the exceptions of A and F_{IS} which were lower in this dataset than previously estimated (A= 2.95 vs A=4.8; F_{IS}=0.0095 vs F_{IS}=0.045).

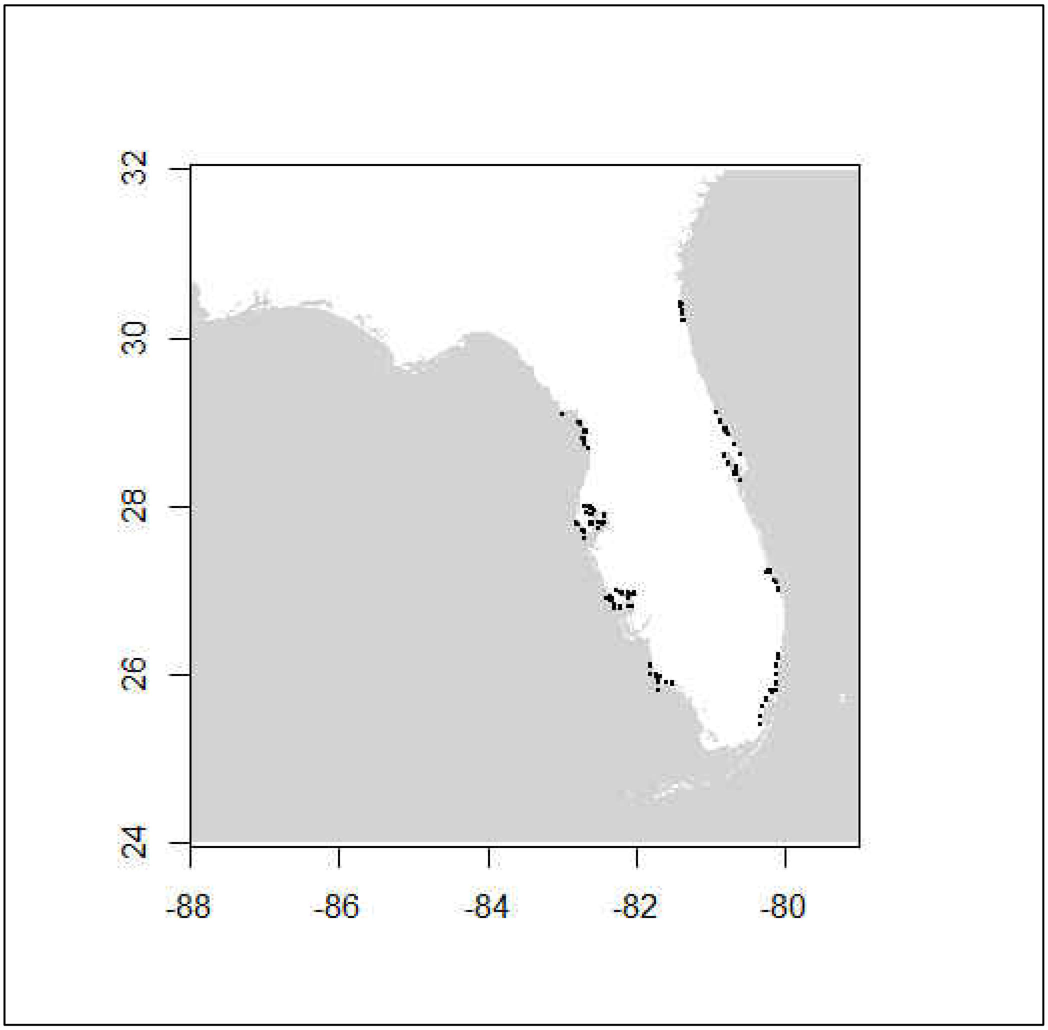

Figure 8: Sampling locations for 293 carcasses used in this study.

Table 14: Deviations from Hardy-Weinberg equilibrium (α=0.05). Significant p-values are indicated in bold.

	E1	E2	E3	E4	W1	W2	W3	W4
TMAA03	**0.038**	0.442	**0.023**	**0.019**	1	0.081	**0**	**0.012**
TMAE01	0.052	**0.043**	**0.003**	**0.027**	**0.001**	**0.003**	**0**	0.079
TMAE02	0.528	0.179	1	0.458	0.102	0.772	0.101	0.53
TMAE04	0.564	**0**	0.246	**0.001**	0.321	1	1	1
TMAE07	0.181	0.745	0.424	0.631	0.305	0.059	**0.039**	0.091
TMAE08	0.121	0.296	0.173	0.422	0.65	0.793	0.003	0.339
TMAE11	0.882	0.473	0.499	0.098	0.308	0.248	0.126	**0.025**
TMAE14	0.054	0.728	0.4	0.476	**0.001**	**0.012**	**0.001**	0.102
TMAFWC01	0.149	0.588	0.663	0.585	1	0.609	0.592	0.714
TMAFWC02	0.647	0.579	0.177	0.113	**0.027**	0.073	0.204	0.07
TMAFWC06	1	0.322	1	**0.023**	0.057	**0.019**	**0.031**	0.373
TMAFWC09	0.77	0.683	0.918	0.918	0.343	0.276	0.351	0.725
TMAFWC10	0.647	**0.001**	**0.021**	**0.002**	0.358	**0.021**	**0.014**	**0**
TMAFWC11	1	0.053	0.633	0.238	0.371	0.167	0.587	0.769
TMAFWC12	0.352	**0.016**	**0.029**	**0.025**	**0.016**	0.7	0.084	0.369
TMAFWC13	1	0.3	0.339	**0.001**	0.841	**0.013**	**0.039**	**0.004**
TMAFWC18	0.516	1	1	**0.031**	0.168	0.347	0.702	1
TMAH13	0.134	0.057	0.265	0.059	0.085	0.191	**0.018**	1
TMAK01	**0.004**	**0**	**0.037**	0.067	0.138	0.126	**0**	0.12
TMASC13	0.338	**0**	1	0.05	1	1	0.32	**0.015**

Table 15: Deviations from Hardy-Weinberg equilibrium using the Bonferroni- Holm adjusted p-values for multiple comparisons (α=0.05). Significant p-values are italicized and in bold.

	E1 adj	E2 adj	E3 adj	E4 adj	W1 adj	W2 adj	W3 adj	W4 adj
TMAA03	1	1	1	1	1	1	**0**	1
TMAE01	1	1	0.438	1	0.153	0.438	**0**	1
TMAE02	1	1	1	1	1	1	1	1
TMAE04	1	**0**	1	0.153	1	1	1	1
TMAE07	1	1	1	1	1	1	1	1
TMAE08	1	1	1	1	1	1	0.438	1
TMAE11	1	1	1	1	1	1	1	1
TMAE14	1	1	1	1	0.153	1	0.153	1
TMAFWC01	1	1	1	1	1	1	1	1
TMAFWC02	1	1	1	1	1	1	1	1
TMAFWC06	1	1	1	1	1	1	1	1
TMAFWC09	1	1	1	1	1	1	1	1
TMAFWC10	1	0.153	1	0.294	1	1	1	**0**
TMAFWC11	1	1	1	1	1	1	1	1
TMAFWC12	1	1	1	1	1	1	1	1
TMAFWC13	1	1	1	0.153	1	1	1	0.572
TMAFWC18	1	1	1	1	1	1	1	1
TMAH13	1	1	1	1	1	1	1	1
TMAK01	0.572	**0**	1	1	1	1	**0**	1
TMASC13	1	**0**	1	1	1	1	1	1

Table 16: Measure of manatee genetic diversity at each locus. Values represent averages across the entire examined dataset (n=293 manatee carcasses). A = number of alleles, Ae= effective number of alleles, He= expected heterozygosity, Ho= observed heterozygosity, FIS= inbreeding coefficient.

Locus	A	A_e	H_e	H_o	F_{is}
TMAA03	2	1.2490	0.1993	0.1684	0.1551
TMAE01	5	2.2078	0.5471	0.5216	0.0466
TMAE02	2	1.9116	0.4769	0.4369	0.0839
TMAE04	2	1.5128	0.3390	0.3356	0.0099
TMAE07	3	2.8357	0.6473	0.6069	0.0624
TMAE08	3	2.1374	0.5321	0.5034	0.0539
TMAE11	5	2.4981	0.5997	0.6503	-0.0845
TMAE14	3	2.6606	0.6241	0.5719	0.0837
TMAFWC01	2	1.4520	0.3113	0.3106	0.0023
TMAFWC02	5	2.1495	0.5348	0.4467	0.1646
TMAFWC06	3	1.4557	0.3130	0.2897	0.0747
TMAFWC09	4	3.1638	0.6839	0.6894	-0.0080
TMAFWC10	2	1.8113	0.4479	0.4316	0.0364
TMAFWC11	3	1.4929	0.3302	0.3368	-0.0200
TMAFWC12	2	1.6111	0.3793	0.3910	-0.0309
TMAFWC13	3	1.7738	0.4362	0.4740	-0.0866
TMAFWC18	2	1.6521	0.3947	0.3767	0.0456
TMAH13	3	1.4669	0.3183	0.2907	0.0868
TMAK01	3	1.9275	0.4812	0.6517	-0.3544
TMASC13	2	1.6	0.375	0.4241	-0.1310
Average	2.95	1.9285	0.4486	0.4454	0.0095

Seascape Genetic Analyses

The combined data layers captured most of the manatee carcass locations (293 of a total 319) for the continuous variables of bathymetry, salinity, and temperature, however very few carcass locations were captured with the combined chlorophyll-a data layer, thus, I eliminated chlorophyll-a data from further consideration.

Based on simple mantel tests, univariate models with the highest correlation to GDis were all CT distances: IRM coastal habitat disturbace (rM= 0.0407, Moran's I= 0.4276); IRM boat activity (rM= 0.0334, Moran's I= 0.2470); IRM bathymetry (rM=0.0326, Moran's I= 0.9455); IRR salinity (rM=0.0313, Moran's I= 0.8035); IRM temperature (rM=0.0305, Moran's I= 0.6888); IRM grasses (rM=0.0208, Moran's I=0.8214) (Appendix C). However, mantel tests between ecological distances based on these competing univariate hypotheses showed correlation problems (Table 17). Thus, I eliminated three models based on high correlation with other seascape models combined with low correlation with GDis: IRR salinity, IRM bathymetry, and IRM temperature. IRR salinity and IRM bathymetry were highly correlated with 4 of the 5 other models; IRR salinity was eliminated first based on low correlation with GDis. Following elimination of IRR salinity, IRM bathymetry was highly correlated with 3 of the 4 other remaining models and eliminated second. Following the elimination of IRR Salinity and IRM bathymetry, IRM temperature was highly correlated with 1 of the 3 other remaining models and was eliminated third. Following the elimination of these 3 univariate resistance hypotheses, there were no remaining problematic correlations between ecological distance matrices. The models of IRM boat activity, IRM grass cover, and IRM coastal habitat disturbance were then combined into a composite layer and compared with GDis; circuit theory distance outperformed least-cost distance for the composite model (rM= 0.0269, Moran's I= 0.6763). The final suite of hypotheses for evaluation by MLPE were 1) Isolation by distance, 2) circuit theory distance for IRM boat activity, 3) circuit theory distance for IRM grass cover, 4) circuit theory distance for IRM coastal habitat disturbance and 5) circuit theory distance for the composite model.

Table 17: Simple mantel correlations (rM) between ecological distances for univariate resistance distance models and genetic distance (Gdis). High correlation (rM > 0.9) between ecological distances are in bold. E represents the elimination order based on a) problematic correlations with competing models and b) low correlation with Gdis; "-" indicates the model was retained for the MLPE bootstrap analysis.

	E	Gdis	IRM Bathymetry	IRM Boat Activity	IRM Grass Cover	IRR Salinity	IRM Coastal Disturbance	IRM Temperature
IRM Bathymetry	2	0.0326	-	-	-	-	-	-
IRM Boat Activity	-	0.0334	**0.9589**	-	-	-	-	-
IRM Grass Cover	-	0.0208	**0.9197**	0.8679	-	-	-	-
IRR Salinity	1	0.0313	**0.9481**	**0.9356**	**0.9217**	-	-	-
IRM Coastal Disturbance	-	0.0407	0.4373	0.5027	0.4237	0.5392	-	-
IRM Temperature	3	0.0305	**0.9769**	**0.9798**	0.8862	**0.9243**	0.4493	-

Results from the maximum-likelihood population-effects (MLPE) mixed modelling bootstrap revealed that boat activity was selected as the top model most often (86.52%, Table 18), followed by the coastal habitats model (13.42%). The IRM transformation of boat activity (Figures 9-10) assigned low resistance (≤ 5) to cells with open ocean, slightly higher resistance (5-15) to areas with either a boat ramp or an official waterway, and high resistance (95-100) to cells with both a boat ramp and an official waterway. Values in the boat activity layer ranged from 1- 100.4, but high resistance values were exceedingly rare (Figure 11). The IRM transformation of the coastal habitats layer (Figures 12-14) assigned low resistance (≤ 5) to cells with open ocean or natural habitat and high resistance (95-100) to cells with disturbed coastal habitat. The layers representing isolation by distance (0.06%), the composite model (0%), and the seagrass coverage model (0%) had poor support.

Table 18: Results from bootstrap for maximum-likelihood population-effects (MLPE) mixed modelling under 10,000 permutations. Type= distance type, CT= circuit theory, IBD= isolation by distance, IRM= inverse-reverse monomolecular. Composite layer is the sum of IRM Grass cover, IRM Boat activity, and IRM Coastal habitat disturbance.

Surface	Type	Avg AIC	Avg AICc	Avg weight	Avg rank	Avg R^2	Avg LL	n	Percent top
IRM Boat Activity	CT	-77964.12	-77964.07	0.6320	1.1348	0.0017	38986.06	8652	86.52
IRM Coastal Habitat Disturbance	CT	-77960.72	-77960.67	0.2023	2.459	0.0080	38984.36	1342	13.42
Distance	IBD	-77960.69	-77960.64	0.1292	2.5512	0.0015	38984.35	6	0.06
Composite	CT	-77956.95	-77956.90	0.0264	3.8777	0.0015	38982.48	0	0
IRM Grass Cover	CT	-77954.20	-77954.14	0.0101	4.9773	0.0015	38981.10	0	0

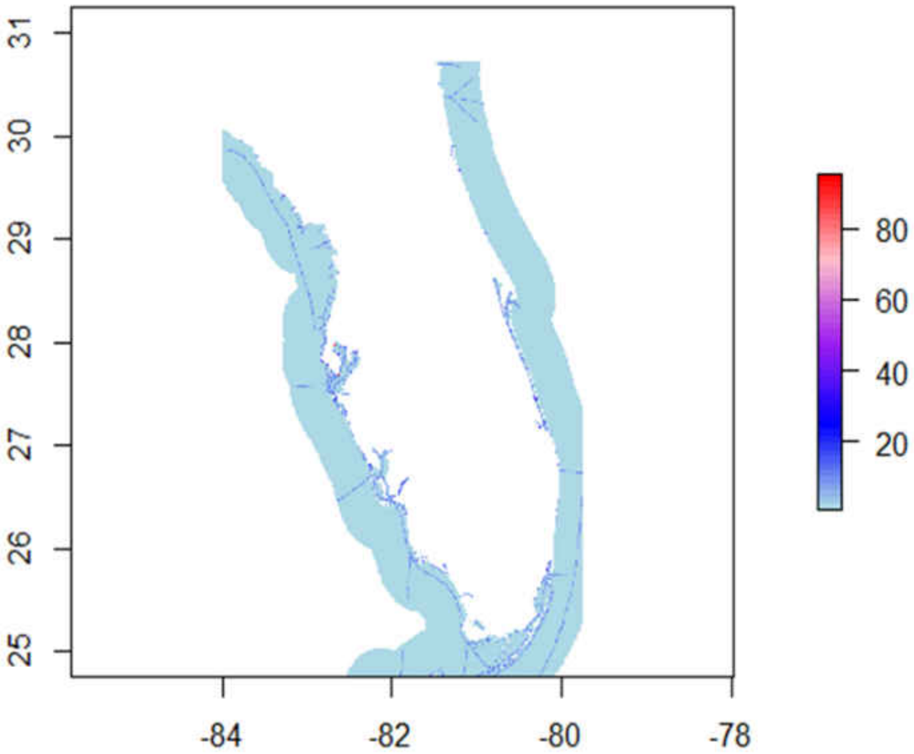

Figure 9: Boat activity under IRM transformation where resistance was low in cells without boat ramps and/or waterways and resistance increased in cells with boat ramps and/or waterways.

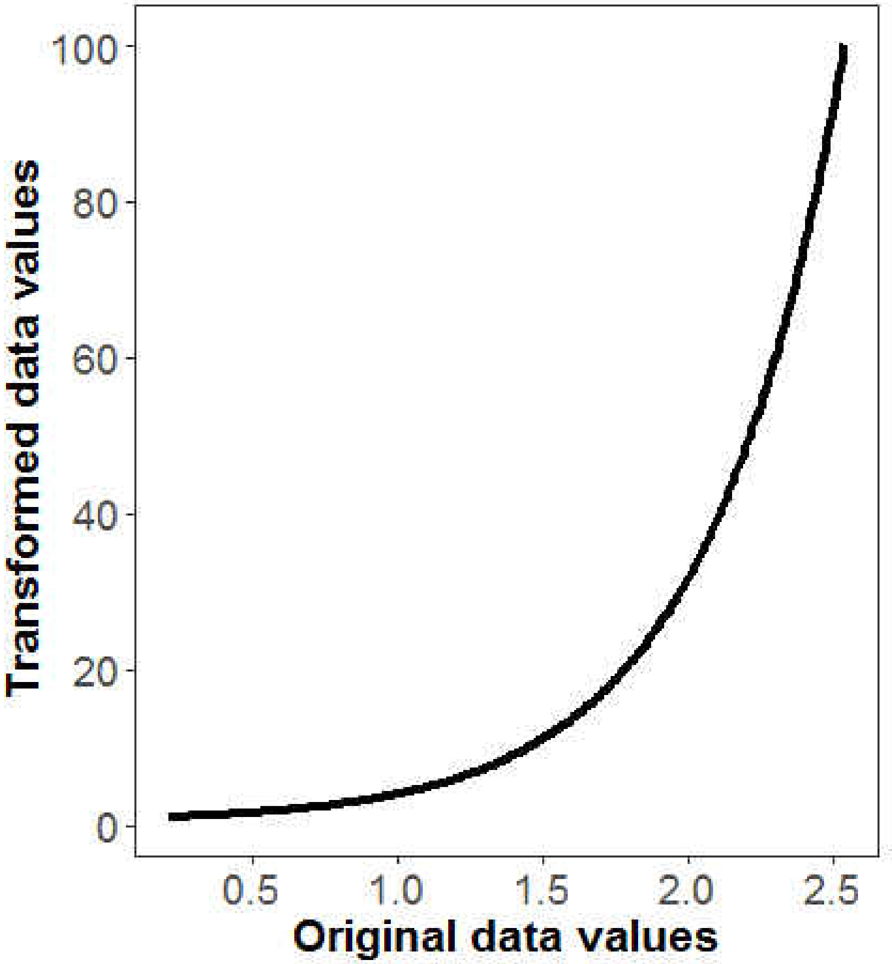

Figure 10: Inverse-Reverse Monomolecular transformation of boat activity values. Cells with open ocean (original value 0.5) were assigned very low resistance values, cells with either a boat ramp or an waterway (original value 1.5) were assigned slightly higher resistance values, and rare cells with both a boat ramp and an waterway (original value 2.5) were assigned very high resistance values.

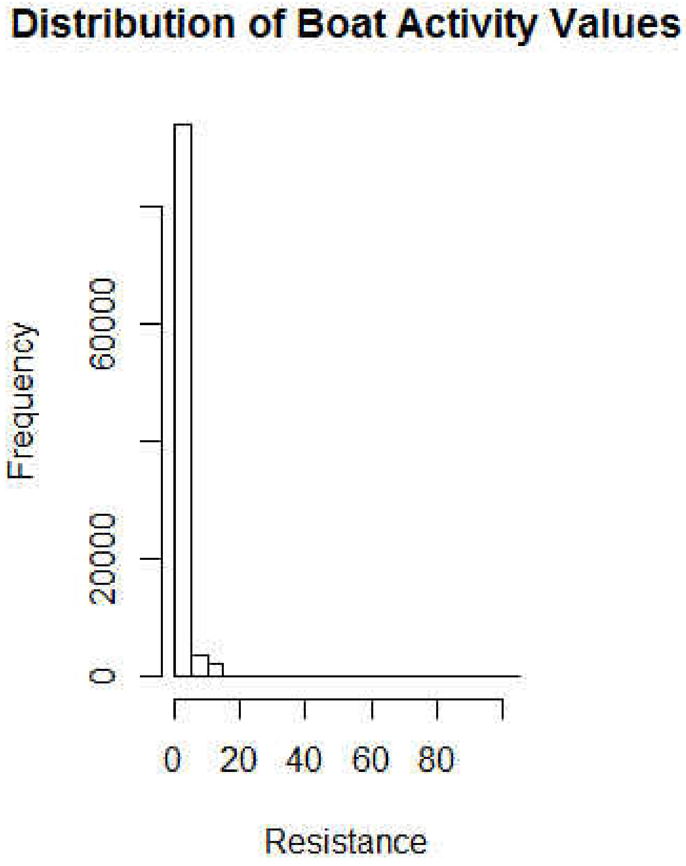

Figure 11: Histogram of resistance values in the Boat Activity layer. Cells with open ocean were assigned very low resistance values, cells with either a boat ramp or an official waterway were assigned slightly higher resistance values, and rare cells with both a boat ramp and an official waterway were assigned very high resistance values.

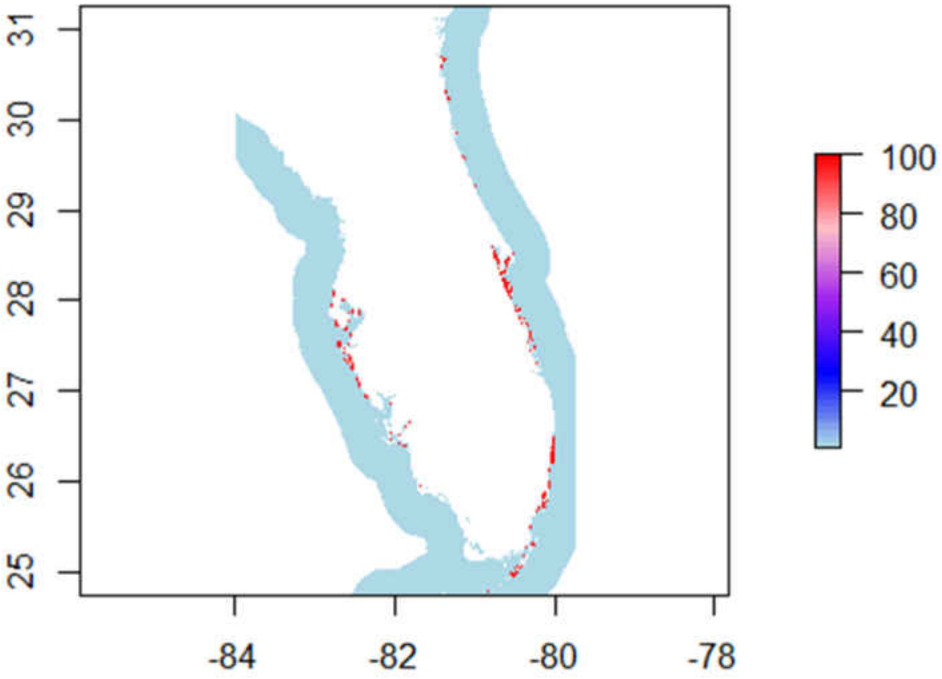

Figure 12: Coastal habitat disturbance under IRM transformation. Cells with open ocean or natural habitat (low resistance) are displayed as light blue, while areas with disturbed coastal habitat (high resistance) are displayed as red.

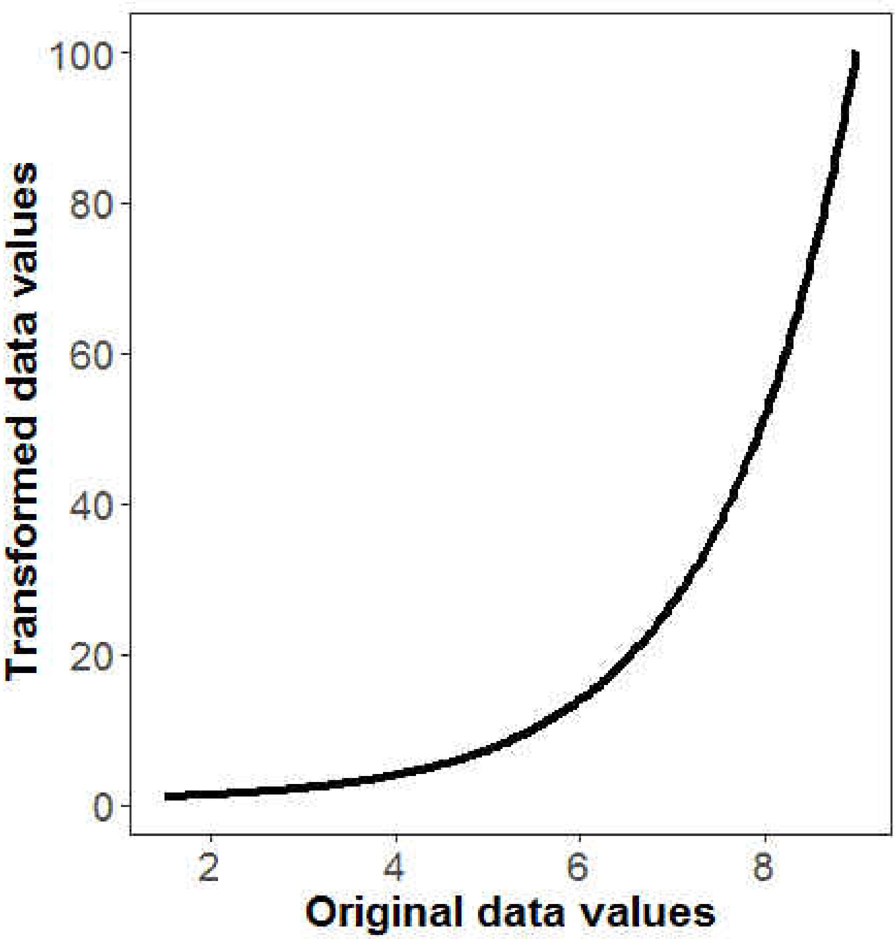

Figure 13: Inverse-Reverse Monomolecular transformation of the Coastal Habitat Disturbance layer. Cells with open ocean (original value 1.5) or natural coastal habitat (original value 2) were assigned low resistance values, while cells with disturbed coastal habitat (original value 9) were assigned high resistance values.

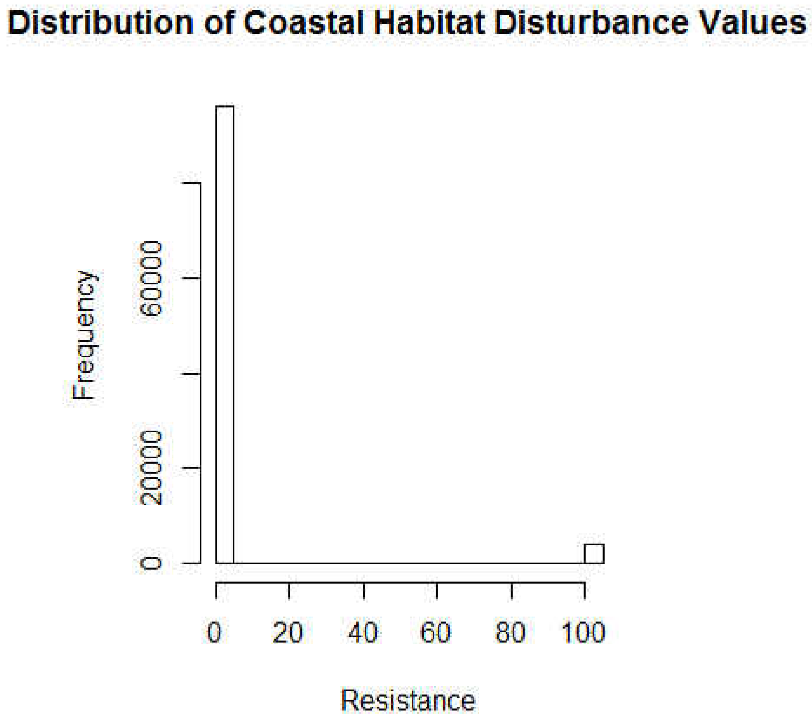

Figure 14: Histogram of resistance values in the coastal habitat disturbance layer. Cells with open ocean or natural coastal habitat were assigned low resistance values, while cells with disturbed natural habitat were assigned high resistance values.

Discussion

The results of the present study indicate that barriers from anthropogenic boat activity and coastal disturbance have disrupted gene flow and created spatial genetic structuring in the Florida manatee population. Sea surface temperature has been linked to genetic variation in many marine species including the New Zealand greenshell mussel, (Wei et al. 2013), sea urchins (Banks et al. 2007), sole (Diopere et al. 2017), and harbor porpoises (Fontaine et al. 2007), yet surface temperature was not especially important here. Bathymetry has been important in understanding geographical genetic variation in studies of several fishes (D'Aloia et al. 2014, Saha et al. 2015, Gubili et al. 2016, Liggins et al. 2016, Diopere et al. 2017), but was not very informative for the manatee. Food availability has also been shown to be important in describing spatial genetic variation in some of these studies (Fontaine et al. 2007, Diopere et al. 2017), but seagrass cover was not important here. Instead, anthropogenic seascape variables were selected in >99% of iterations.

This study is unique in the inclusion of anthropogenic variables in the seascape and their subsequent importance in explaining genetic variation. While anthropogenic traffic has been shown to disrupt genetic connectivity in many terrestrial populations including beetles (Keller et al. 2004), bears (Proctor et al. 2005), sheep (Epps et al. 2005), bobcats and coyotes (Riley et al. 2003, Riley et al. 2006) this result is heretofore rare in the world of seascape genetics.

Tucker et al. (2012) demonstrated that manatee genetic diversity is very low, albeit not immediately dire, but stated that further disruption in gene flow could drastically change the situation. The negative effects of boats and coastal development on gene flow in the Florida manatee are cause for concern but these barriers present opportunities for management, as understanding how landscape/seascape variables affect population connectivity is crucial in targeting conservation and restoration action (McRae et al. 2012, Tscharntke et al. 2012)

Even though genetic patterns can take hundreds of generations to equilibrate, landscape resistance to gene flow can produce detectable genetic patterns in space very quickly (Cushman and Landguth 2010). It is compelling that the two anthropogenic seascape layers (boat activity and coastal habitat disturbance) had higher simple mantel correlations than all other examined seascape hypotheses and were consistently selected before the IBD hypothesis in the bootstrap. The low performance of IBD with respect to model fit (selected in <1% of iterations) suggests there is some seascape factor structuring genetic distance, but it is possible the factor generating the observed structure was not examined here. Additionally, it is important to note that CT distance was more informative than LCP distance in all individual variables in this study, giving further credence to considering multiple pathways when modelling connectivity (McRae 2006, McRae and Beier 2007, McRae et al. 2008, Khimoun et al. 2017).

The results of the present study are distinct from previous assessments on manatees in the seascape. On a smaller temporal and spatial scale, seascape resistance was important for manatee habitat selection during winter but boat channels were not found have a negative or positive effect on manatee movement (Haase et al. 2017). Similarly, dugongs show little avoidance behavior in response to boat traffic (Hodgson and Marsh 2007). There is, however, additional evidence to the contrary i.e. that manatees attempt to avoid boat traffic and human activity (Provancha and Provancha 1988, Buckingham et al. 1999, Nowacek et al. 2004, Rycyk et al. 2018). A previous manatee occupancy model indicated manatees might not avoid developed areas, but authors state this might be an artifact of the study location (Bauduin et al. 2013). The effects of boats and anthropogenic development on manatee gene flow may be therefore be a result of reduction in genetic connectivity via mortality instead of a reduction of manatee movement per se.

The relatively high performance of the boat activity hypothesis in this study is intuitive. There is a large body of literature reinforcing the idea that fragmentation via terrestrial road traffic can have negative effects on terrestrial populations especially for large, highly mobile mammals animals (Riley et al. 2003, Dickson et al. 2005, Clevenger and Wierzchowski 2006, Riley et al. 2006, Strasburg 2006, Balkenhol and Waits 2009, Fahrig and Rytwinski 2009, Benítez-López et al. 2010, Taylor and Goldingay 2010, Lesbarrères and Fahrig 2012, Rytwinski and Fahrig 2012). Strikes from watercraft traffic are a risk for many marine vertebrates including large whales (Jensen and Silber 2003, Panigada et al. 2006, Berman-Kowalewski et al. 2010, van der Hoop et al. 2013), dolphins (Wells and Scott 1997, Warren-Smith and Dunn 2006, Donaldson et al. 2010), dugongs (Limpus et al. 2003, Hodgson 2004), sea turtles (Casale et al. 2010, Denkinger et al. 2013, Orós et al. 2016, Shimada et al. 2017), sharks (Speed et al. 2008, Towner et al. 2011, Ramírez-Macías et al. 2012) and seals (Colegrove et al. 2005, Bexton et al. 2012). It has been recognized for decades that boats have major, detrimental effects on the manatee population in Florida and have dominated as a major cause of manatee rescue (Chapter 1 in this dissertation) and mortality (O'Shea et al. 1985, Ackerman et al. 1995, Lightsey et al. 2006, Runge et al. 2017). Despite collision risk and increased stress from boats, some marine mammals may not be able to totally avoid watercraft traffic. Authors of a recent study in Panama showed dolphins do not avoid areas as boat numbers increase, suggesting the cost of leaving may outweigh the cost of staying (Barragán-Barrera et al. 2017).

The direct effects of watercraft on manatees are widespread and obvious; scars from sub-lethal watercraft injuries have long been used to identify individual manatees, and many manatees have scars from multiple, separate collisions with boats (O'Shea 1995, O'Shea et al. 2001, Langtimm et al. 2004) After a collision, fatal injuries can result from sharp-force trauma from propeller wounds, from massive internal injuries from blunt-force trauma, or from infections from lesser wounds (Lightsey et al. 2006). While some manatees can survive for long periods after being struck, watercraft injuries may also have sub-lethal population effects as females mutilated and disfigured by watercraft may produce fewer calves (O'Shea et al. 2001). Taken together, some combination of mortality from, injury by and/or avoidance of boats may create meaningful barriers to gene flow.

The most recent status and threats analysis for the Florida manatee, the Core Biological Model (CBM) lists watercraft mortality as a primary threat to manatee persistence, alongside loss of warm-water sites and red tide (*Karenia brevis*) mortality (Runge et al. 2017). Of the three major threats, only an increase in watercraft mortality is projected to substantially increase the chances of quasi-extinction (<500 adults on either coast); any increase in watercraft-related mortality exponentially increases the chances of quasi-extinction, and if watercraft mortality were to double, the chances of quasi-extinction of the Florida manatee would increase tenfold (Runge et al. 2017). The risk of population declines increases when considering the potential for multiple emerging threats. In combination, the CBM estimates multiple threats could increase the chance of quasi-extinction by 14 fold (Runge et al. 2017). Interestingly, the emerging threats scenario in the CBM assumes that watercraft mortality will increase by 50% over the next 30 years and then stabilize, but the authors offer no argument as to why this would occur. Being that watercraft is the only individual threat that substantially increases the chance of quasi-extinction, and the Florida human population may increase by >44% by 2045 (Rayer and Wang 2019), the threat to manatee persistence from watercraft mortality may be higher than the authors estimated, with potentially negative consequences for Florida manatee persistence.

While serious, there are several opportunities for mitigation of watercraft induced injury and mortality in Florida manatees. One such opportunity is the enforcement of existing reduced speed zones. Previous work has argued that speed zones are reasonable, appropriate, and defensible management action (Calleson and Frohlich 2007, Jett and Thapa 2010, Runge et al. 2017). Slower boat speeds have been shows to reduce risk to manatees by giving both boat operators and manatees more time to react, and by reducing the severity of the injury to manatee in the event of a collision, but boater compliance with speed zones is variable (Nowacek et al. 2004, Laist and Shaw 2006, Calleson and Frohlich 2007, Jett and Thapa 2010). The presence of law enforcement increases compliance with reduced speed zones, yet law-enforcement is incapable of consistently enforcing reduced speed zones statewide (Gorzelany 2004, Jett and Thapa 2010). Thus at present, boater compliance with regulations and reporting of collisions is primarily voluntary (Calleson and Frohlich 2007, Jett and Thapa 2010). Projections in the manatee CBM assume protections such as reduced speed zones will stay in place indefinitely (Runge et al. 2017) but existing protections may not suffice without proper enforcement as human coastal populations and numbers of watercrafts increase. Attempts to quantify the effects of existing speed zones found that relative lethal collision risk was reduced by 51.5-70.0% compared to a scenario where no speed protections existed, but authors stated that the effectiveness would be reduced with less than full compliance by boaters (Udell et al.).

There is also opportunity to improve the rescue response to collisions. While Flamm and Braunsberger (2014) found that >90% of survey respondents expressed willingness to call authorities if they struck a manatee, this high rate is not reflected in actual calls to authorities. A 2006 study reported only 21 of >1400 collisions resulted in self or eye-witness reporting of the responsible watercrafts to (Calleson and Frohlich 2007). Follow-up interviews suggested that many boaters simply do not have the information on who to call when a collision occurs (Flamm and Braunsberger 2014). The authors suggest the implementation of an informational decal could increase reports of vessel strikes, improve the knowledge of situations that lead to collisions, and promote rapid rescue response for injured manatees. This simple action could alleviate some of the anthropogenic pressure placed on manatee populations and contribute to population recovery and sustainability (Flamm and Braunsberger 2014).

There is also an opportunity to improve boater education. A previous study indicated that boating experience as measured by years of boating and number of manatee sightings was negatively correlated with attitudes towards safe boating behavior (Morris et al. 2007) which may reflect boater's perceptions that protections are unnecessary for an animal they frequently encounter. However, giving boaters ownership over the problem and reinforcing the Florida manatee as a unique feature of Floridian identity may improve efforts to protect manatees and their habitat (Morris et al. 2007, Rodgers and Pienaar 2017). Additional effort in studying compliance, educating boaters, and enforcing manatee protection zones is important to the future of the Florida manatee.

The manatee CBM states that reduced vigilance in management would increase the risk of manatee population decline, and watercraft mortality is a primary threat to manatee persistence (Runge et al. 2017). Results of this study indicate that boat traffic and coastal development should be a priority to managers, as reduction in gene flow can have major negative effects in fragmented populations (Frankham 2005). The barriers to gene flow from anthropogenic activity in the seascape identified in this study provide opportunities to focus and improve future habitat restoration and Florida manatee conservation.

CHAPTER 4: GENERAL DISCUSSION

In the most recent analysis of threats to the manatee, the CBM, three threats had profound effects on the chances of quasi-extinction of the manatee: red tide, cold stress, and watercraft (Runge et al. 2017). The impacts of cold stress will be through a reduction in carrying capacity, while red tide and watercraft through mortality. Of these three, only watercraft has the potential to substantially increase the risk of extinction; any increase in watercraft mortality increase the chances of quasi-extinction exponentially. The way we deal with watercraft in the future is of great consequence. In Chapter two, watercraft injury was identified as the most frequent reason for rescue and overwhelmingly composed the cases where euthanasia was required. Cases of watercraft injury had significantly longer stays in rehabilitation and were less likely to survive until release than cases of red tide poisoning or cold stress. Current management plans assume the MRRP will continue to function, and this study provides data on trends and rehabilitation efficiency that can be used to improve care. Additionally, this dissertation provided evidence that as much as half of the manatees released from rehabilitation are still alive 3-25 years after release, which speaks to program success.

While chances of quasi-extinction of the Florida manatee were estimated to be low in the CBM, the authors did acknowledge a great amount of uncertainty. Surprisingly, the CBM did not take into account the effects of climate change despite longstanding scientific discussion of the challenges it poses to biodiversity conservation (Araújo and Rahbek 2006, Thuiller 2007, Cheung et al. 2009, Heller and Zavaleta 2009, Jones and Cheung 2015, Runge et al. 2017). The emerging threats scenario examined assumes that watercraft mortality will increase by 50% and then stabilize, but provide no justification as to why stabilization would occur. This is especially troubling given the unique ability of increases in watercraft mortality to increase quasi-extinction. It is suggested that manatees will find new warm-water habitat when industrial warm-water sources are phased out, despite the fact that manatees are known to show high fidelity to seasonal habitats (Deutsch et al. 2003) and the authors acknowledge that this may result in higher mortality than what was estimated in the CBM. Previous assessments acknowledged the negative effects of low genetic diversity on the future of the Florida manatee (Marsh et al. 2011, Tucker et al. 2012), yet the CBM did not consider genetic diversity. Taken together, the risks to manatee persistence may be higher than what has been recently estimated.

Tucker et al. (2012) stated that while the lack of genetic diversity in the Florida manatee is not immediately dire, any further reduction in gene flow could have serious consequences for the population. The results of Chapter three in this dissertation posit that watercraft traffic and/or coastal habitat disturbance may be the mechanisms by which gene flow is reduced in the future. Thus, management must seriously consider how to reduce the impacts of watercraft on the manatee. The results of the present study also provide evidence of marine traffic as a barrier to gene flow in the marine realm. This is a rare and important result in the field of seascape genetics that holds larger implications for the study of the factors that influence spatial genetic variation as well as future manatee conservation efforts. This dissertation describes the costs of watercraft injuries to the rescue/rehabilitation system, confirms the severity of the threat of watercraft to Florida manatee conservation, and supports the primacy of anthropogenic threats to manatee persistence.

APPENDIX A: ODDS RATIOS FROM LOGISTIC REGRESSIONS

Table 19: Significant odds ratios for survival from rescue to commencement of medical treatment. In this table, animals in the 1st group were more likely to survive until admission than animals in the 2nd group by a factor equivalent to the odds ratio.

1st group	**2nd group**	**ODDS RATIO**	**P value**
RED TIDE	MISC. NATURAL	5.28	0.0016
COLD STRESS	MISC. NATURAL	3.34	0.0002
EMACIATION	MISC. NATURAL	3.11	0.0347
ENTANGLEMENT	MISC. NATURAL	40.09	0.0004
ENTANGLEMENT	BUOYANCY	20.40	0.0063
ENTANGLEMENT	EMACIATION	12.88	0.0218
ENTANGLEMENT	ORPHAN	17.00	0.0059
ENTANGLEMENT	TIDAL STRANDING	21.25	0.0421
ENTANGLEMENT	WATERCRAFT	18.64	0.0040
ENTANGLEMENT	COLD STRESS	12.01	0.0155
ORPHAN	MISC. NATURAL	2.36	0.0081
WATERCRAFT	MISC. NATURAL	2.15	0.0059

Table 20: Significant odds ratios for survival from admission to rehabilitation to release. In this table, animals in the 1st group were more likely to survive until release than animals in the 2nd group by a factor equivalent to the odds ratio.

1st group	**2nd group**	**ODDS RATIO**	**P value**
BUOYANCY	MISC. NATURAL	6.41	0.0006
COLD STRESS	BUOYANCY	3.24	0.0031
COLD STRESS	MISC. NATURAL	20.73	<0.0001
COLD STRESS	WATERCRAFT	3.96	<0.0001
COLD STRESS	ORPHAN	2.10	0.0007
EMACIATION	MISC. NATURAL	11.27	<0.0001
EMACIATION	WATERCRAFT	2.15	0.0256
ENTANGLEMENT	BUOYANCY	8.14	<0.0001
ENTANGLEMENT	COLD STRESS	2.52	0.0048
ENTANGLEMENT	EMACIATION	4.63	0.0005
ENTANGLEMENT	MISC. NATURAL	52.16	<0.0001
ENTANGLEMENT	ORPHAN	5.28	<0.0001
ENTANGLEMENT	WATERCRAFT	9.96	<0.0001
ENTRAPMENT	BUOYANCY	13.85	<0.0001
ENTRAPMENT	COLD STRESS	4.28	0.0079
ENTRAPMENT	EMACIATION	7.88	0.0009
ENTRAPMENT	WATERCRAFT	16.94	<0.0001
ENTRAPMENT	ORPHAN	8.98	<0.0001
ENTRAPMENT	MISC. NATURAL	88.75	<0.0001
HUMAN	MISC. NATURAL	15.21	0.0062
ORPHAN	MISC. NATURAL	9.88	<0.0001

1st group	**2nd group**	**ODDS RATIO**	**P value**
ORPHAN	WATERCRAFT	1.89	0.0011
RED TIDE	EMACIATION	4.09	0.0025
RED TIDE	BUOYANCY	7.20	<0.0001
RED TIDE	MISC. NATURAL	46.10	<0.0001
RED TIDE	ORPHAN	4.66	<0.0001
RED TIDE	WATERCRAFT	8.8	<0.0001
RED TIDE	COLD STRESS	2.22	0.0281
TIDAL STRANDING	MISC. NATURAL	15.21	0.0062
UNSUITABLE HABITAT	BUOYANCY	12.67	0.0238
UNSUITABLE HABITAT	MISC. NATURAL	81.14	0.0001
UNSUITABLE HABITAT	ORPHAN	8.21	0.0497
UNSUITABLE HABITAT	WATERCRAFT	15.49	0.0102
WATERCRAFT	MISC. NATURAL	5.24	<0.0001

APPENDIX B: ADDITIONAL INFORMATION ON DATA USED IN CHAPTER 3

Table 21: Sources and descriptions for considered environmental variables.

Data description	Data source	Temporal resolution	Spatial resolution
Bathymetry, m	MARSPEC - derived from SRTM30 PLUS	1955- 2010	30 arcseconds (~1km)
Bathymetry, m	FL DEP	Periodic monitoring, April- October, 1974- 2015	N/A
Point coordinates of boat ramps	FWC/FWRI	2005- present	N/A
Officially designated and unofficial navigational routes	FWC/FWRI- digitized from NOAA	Various	Various
Chlorophyll-a, mg/m^3	NASA-NEO	Monthly, April- October, 2002-2015	4 km resampled to 30 arcseconds (~1 km)
Chlorophyll-a, mg/m^3	FL DEP	Periodic monitoring, April- October, 1974- 2015	N/A
Habitat classification	FWC/FWRI	2004-2008	30 m resampled to 30 arcseconds (~1 km)
Salinity, psu	MARSPEC	Monthly, April- October, 1955-2010	30 arcseconds (~1 km)
Salinity, psu	FL DEP	Periodic monitoring, April- October, 1974- 2015	N/A
Seagrass cover	FWC/FWRI	1987-2016	Polygons rasterized to 30 arcseconds (~ 1km)
Temperature, ℃	MARSPEC	Monthly, April- October, 1955-2010	30 arcseconds (~1km)
Temperature, ℃	FL DEP	Periodic monitoring, April- October, 1974- 2015	N/A

Table 22: Original and final classes for the coastal habitat disturbance raster

Original habitat class	Final raster class
1. Coastal Strand 2. Sand/Beach 3. Xeric Oak Scrub 4. Sand Pine Scrub 5. Sandhill 6. Dry Prarie 7. Mixed-Pine Hardwood 8. Hardwood Hammock 9. Pinelands 10. Cabbage Palm/ Live Oak Hammock 11. Tropical Hardwood Hammock 12. Freshwater Marsh/ Wet Prarie 13. Sawgrass Marsh 14. Cattail Marsh 15. Shrub Swamp 16. Bay Swamp 17. Cypress Swamp 18. Cypress/ Pine/ Cabbage Palm 19. Mixed Wetland Forest 20. Hardwood Swamp 21. Hydric Hammock 22. Bottomland Hardwood Forest 23. Salt Marsh 24. Mangrove Swamp 25. Scrub Mangrove 26. Tidal Flat 27. Open Water 28. Shrub and Brushland 29. Grassland	Natural
1. Bare Soil/ Clearcut 2. Improved Pasture 3. Unimproved Pasture 4. Sugar Cane 5. Citrus 6. Row/Field Crops 7. Other Agriculture 8. Exotic Plants (Australian Pine, Melaleuca, Brazilian Pepper, Other) 9. High Urban Impact 10. Low Urban Impact 11. Extractive	Disturbed

APPENDIX C: MANTEL TEST RESULTS

Table 23: Results from mantel tests comparing environmental resistance distance matrices with the genetic distance matrix (Dps) performed under 10,000 permutations. Each variable was transformed 8 times (M= monomolecular, IM= inverse monomolecular, RM= reverse monomolecular, IRM= inverse reverse monomolecular, R= ricker, IR= inverse ricker, RR= reverse ricker, IRR= inverse reverse monomolecular). The layers with the highest mantel statistic (rM) for each variable were summed to form a composite layer. The 6 models annotated with * were examined for cost distance correlation.

	Variable	Transformation	Type	Mantel statistic (rM)
1	Distance	-	Isolation by distance	0.0263
2	Bathymetry	M	Least cost	0.0207
3	Bathymetry	M	Circuit theory	0.0271
4	Boat activity	M	Least cost	0.0267
5	Boat activity	M	Circuit theory	0.0282
6	Grasses	M	Least cost	0.0198
7	Grasses	M	Circuit theory	0.0195
8	Salinity	M	Least cost	0.0268
9	Salinity	M	Circuit theory	0.0275
10	Habitat	M	Least cost	0.0383
11	Habitat	M	Circuit theory	0.0345
12	Temperature	M	Least cost	0.0267
13	Temperature	M	Circuit theory	0.0243
14	Bathymetry	IM	Least cost	0.0218
15	Bathymetry	IM	Circuit theory	0.0184
16	Boat activity	IM	Least cost	0.0111
17	Boat activity	IM	Circuit theory	0.0220
18	Grasses	IM	Least cost	0.0301
19	Grasses	IM	Circuit theory	0.0169

	Variable	Transformation	Type	Mantel statistic (rM)
20	Salinity	IM	Least cost	0.0268
21	Salinity	IM	Circuit theory	0.0303
22	Habitat	IM	Least cost	0.0245
23	Habitat	IM	Circuit theory	0.0146
24	Temperature	IM	Least cost	0.0212
25	Temperature	IM	Circuit theory	0.0236
26	Bathymetry	RM	Least cost	0.0261
27	Bathymetry	RM	Circuit theory	0.0246
28	Boat activity	RM	Least cost	0.0259
29	Boat activity	RM	Circuit theory	0.0236
30	Grasses	RM	Least cost	0.0251
31	Grasses	RM	Circuit theory	0.0153
32	Salinity	RM	Least cost	0.0268
33	Salinity	RM	Circuit theory	0.0282
34	Habitat	RM	Least cost	0.0227
35	Habitat	RM	Circuit theory	0.0143
36	Temperature	RM	Least cost	0.0257
37	Temperature	RM	Circuit theory	0.0240
38	Bathymetry	IRM	Least cost	0.0261
39	Bathymetry	IRM	Circuit theory	0.0326*
40	Boat activity	IRM	Least cost	0.0272

	Variable	Transformation	Type	Mantel statistic (rM)
41	Boat activity	IRM	Circuit theory	0.0334*
42	Grasses	IRM	Least cost	0.0176
43	Grasses	IRM	Circuit theory	0.0208*
44	Salinity	IRM	Least cost	0.0267
45	Salinity	IRM	Circuit theory	0.0296
46	Habitat	IRM	Least cost	0.0319
47	Habitat	IRM	Circuit theory	0.0407*
48	Temperature	IRM	Least cost	0.0303
49	Temperature	IRM	Circuit theory	0.0305*
50	Bathymetry	R	Least cost	0.0195
51	Bathymetry	R	Circuit theory	0.0246
52	Boat activity	R	Least cost	0.0226
53	Boat activity	R	Circuit theory	0.0230
54	Grasses	R	Least cost	0.0211
55	Grasses	R	Circuit theory	0.0249
56	Salinity	R	Least cost	0.0268
57	Salinity	R	Circuit theory	0.0300
58	Habitat	R	Least cost	0.0320
59	Habitat	R	Circuit theory	0.0247
60	Temperature	R	Least cost	0.0231
61	Temperature	R	Circuit theory	0.0242
62	Bathymetry	IR	Least cost	0.0222

	Variable	Transformation	Type	Mantel statistic (rM)
63	Bathymetry	IR	Circuit theory	0.0185
64	Boat activity	IR	Least cost	0.0244
65	Boat activity	IR	Circuit theory	0.0318
66	Grasses	IR	Least cost	0.0192
67	Grasses	IR	Circuit theory	0.0261
68	Salinity	IR	Least cost	0.0267
69	Salinity	IR	Circuit theory	0.0288
70	Habitat	IR	Least cost	0.0360
71	Habitat	IR	Circuit theory	0.0313
72	Temperature	IR	Least cost	0.0292
73	Temperature	IR	Circuit theory	0.0283
74	Bathymetry	RR	Least cost	0.0261
75	Bathymetry	RR	Circuit theory	0.0321
76	Boat activity	RR	Least cost	0.0270
77	Boat activity	RR	Circuit theory	0.0320
78	Grasses	RR	Least cost	0.0279
79	Grasses	RR	Circuit theory	0.0282
80	Salinity	RR	Least cost	0.0268
81	Salinity	RR	Circuit theory	0.0279
82	Habitat	RR	Least cost	0.0237
83	Habitat	RR	Circuit theory	0.0239
84	Temperature	RR	Least cost	0.0288

	Variable	Transformation	Type	Mantel statistic (rM)
85	Temperature	RR	Circuit theory	0.0276
86	Bathymetry	IRR	Least cost	0.0237
87	Bathymetry	IRR	Circuit theory	0.0234
88	Boat activity	IRR	Least cost	0.0244
89	Boat activity	IRR	Circuit theory	0.0231
90	Grasses	IRR	Least cost	0.0193
91	Grasses	IRR	Circuit theory	0.0273
92	Salinity	IRR	Least cost	0.0257
93	Salinity	IRR	Circuit theory	0.0313*
94	Habitat	IRR	Least cost	0.0292
95	Habitat	IRR	Circuit theory	0.0304
96	Temperature	IRR	Least cost	0.0211
97	Temperature	IRR	Circuit theory	0.0235
98	Composite (IRM Grass cover + IRM Boat activity + IRM Habitat Disturbance)	-	Least cost	0.0207
99	Composite (IRM Grass cover + IRM Boat activity + IRM Habitat Disturbance)	-	Circuit theory	0.0269

APPENDIX D: TRANSFORMATIONS FOR GRASS COVER AND COMPOSITE LAYERS

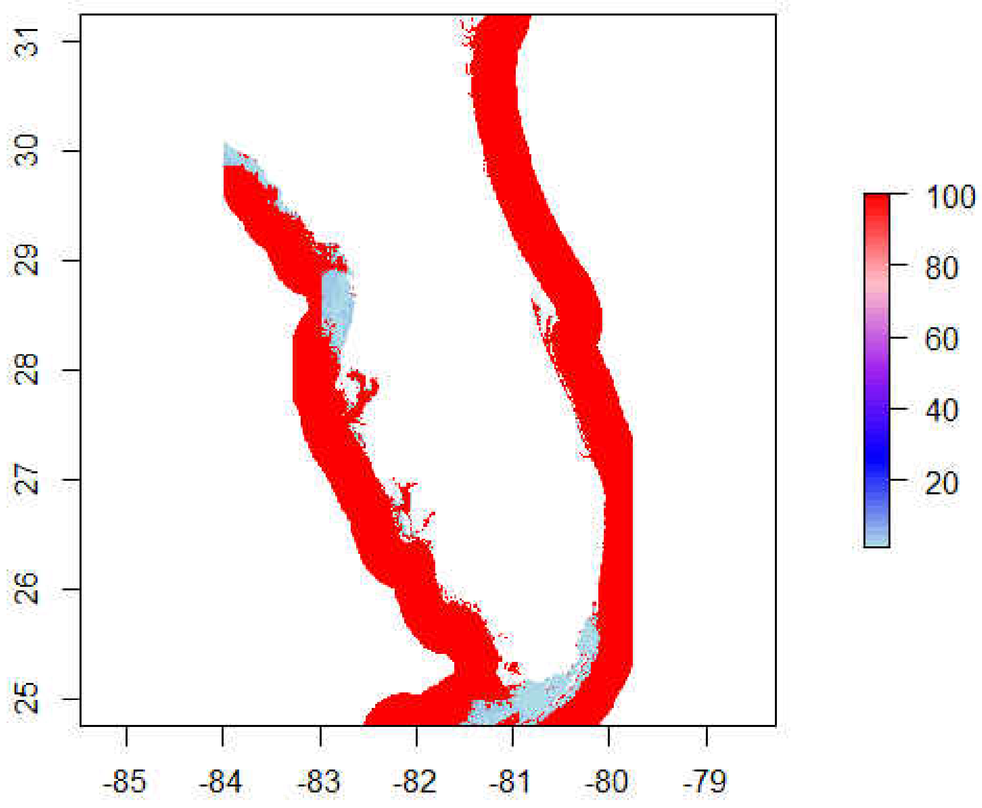

Figure 15: Grasses under IRM transformation

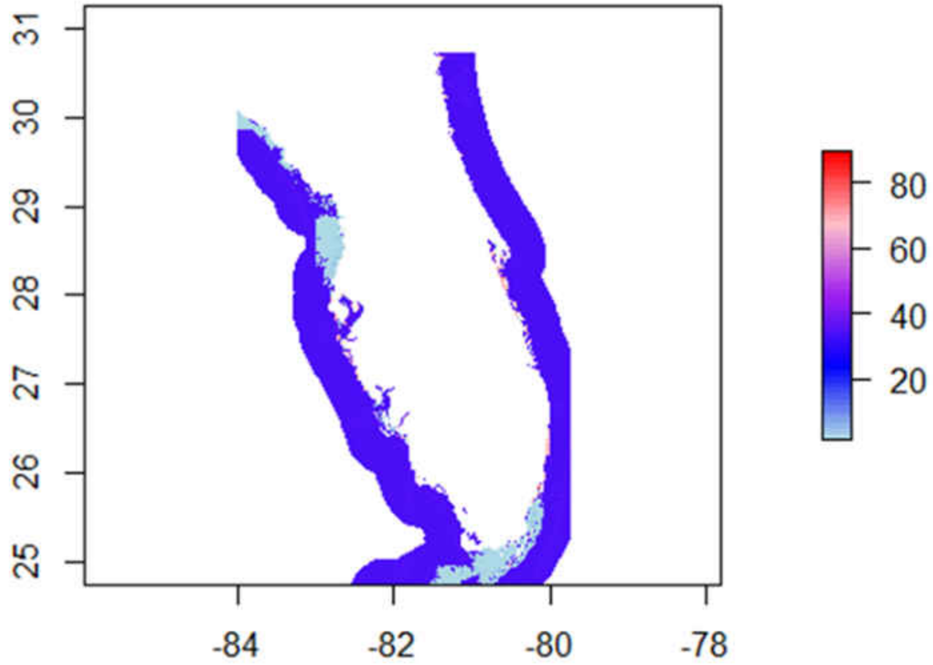

Figure 16: Composite layer composed of IRM grass cover, IRM boat activity, and IRM coastal disturbance.

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