Evaluating the use of concentric circular plots and mark-recapture techniques for estimating pipefish population size in a coastal marine seagrass bed

A Thesis submitted
to the Graduate School
Valdosta State University

In partial fulfillment of requirements
for the degree of

MASTER OF SCIENCE

in Biology

in the Department of Biology
of the College of Science and Mathematics

December 2024

Megan Nicole Sims

B.S. Marine Science & Biology, University of Tampa, 2018

This Thesis, “evaluating the use of concentric circular plots and mark-recapture techniques for estimating pipefish population size in a coastal marine seagrass bed”, by Megan Nicole Sims, is approved by:

Thesis Committee Chair

DocuSigned by:
Emily Rose
4BFD2383716248F...

Dr. Emily Rose
Associate Professor of Biology

Committee Members

DocuSigned by:
Ted Uyeno
2673B620695143D...

Dr. Theodore Uyeno
Professor of Biology

DocuSigned by:
John Phillips
3A01376477234DC...

Dr. John G. Phillips
Assistant Professor of Biology

Associate Provost for Graduate Studies and Research

Becky K. da Cruz

Becky K. da Cruz, Ph.D., J.D.
Professor of Criminal Justice

Defense Date: November 8th, 2024

FAIR USE

This Thesis is protected by the Copyright Laws of the United States (Public Law 94-553, revised in 1976). Consistent with fair use as defined in the Copyright Laws, brief quotations from this material are allowed with proper acknowledgement. Use of the material for financial gain without the author's expressed written permission is not allowed.

DUPLICATION

I authorize the Head of Interlibrary Loan or the Head of Archives at the Odum Library at Valdosta State University to arrange for duplication of this Thesis for educational or scholarly purposes when so requested by a library user. The duplication shall be at the user's expense.

Signature Megan Sims

I refuse permission for this Thesis to be duplicated in whole or in part.

Signature ____

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

I would like to thank John Ambrosio and the University of Tampa Marine Science Field Station for their support with field sampling and providing lab space. Also, thank you John for teaching me how to read the weather and for how to captain a boat. I would like to give my deepest gratitude to my advisors Dr. Emily Rose and Dr. Heather Mason who have supported me before, during, and after this project. Their constant support over the years has been instrumental in my personal development. Additionally, I'd like to thank my family for being a wonderful support system. To my parents for helping me sample, my dog Shiner for the mental support, and my husband Ethan for helping me in every step of this project. I'd also like to thank my lab family, the Rose Lab, for driving down from school multiple times to help me sample.

Thank you to Georgia Ambrose, Samantha Manzzullo, Dalila Sanchez, Emma Robbins, Jordan Gran, Matthew Gamache, Darshi Patel, Kenna Main, Dayten French, Ethan Sims, Anabelle Bork, Sydney Scott, Rose Gaetano, Maya McDaniel, Evan Jones, John Dorrian, Darian Blackiston, Kimberly Hernandez, Marie Hill, Jessica Jilek, Ashley Govig, Victoria Burghardt, Adriana Banuelos, Nicholle Leedom, Mary DiLalla, Sam Beemer, Daniel Wright, Mary Amato, Anja Hagenson, Anna Machado, Eileen Caro, Alana Acevedo, Brylie Ritchie, Trent Norberg, Paige Bland, Diah Brown, Don Hill, Latyr McQuarters, Ana Wade, Lamarr Wade, Alain Delrio, David Malmahann, Emma Tetreault, Lynn Tetreault, Adam Cieslik, Sydney Farrar, Paige Bathke, Miguel Correia, Elsa Camins, Samantha Kennedy, Grace Iverson, Mary, Cody Ryder, Jessica Gonzalez, Raymond Gonzalez, Sawyer Masonjones, Lisa Lefkowitz, Maggie Beemer, Sam Sims, Eric Sims, Dylan Friend, Jennie Amato, Katie Amato, Mia Olson, Campbell Bonner, Braxton Smith, Erin Kelly, Bryn Clayton, Jack Larsen, and Alydia White for help with field collection and photographing the pipefish. Thank you to Aldo Madrigal Olivarez, Kenneth Armstrong, Quinn Donahue, Dayten French, Ashley Fitzgerald, Adriana Banuelos, Rose Gaetano, Samantha Manzzullo, Jessica Jilek, Emily Cutter, Ashley Govig, and Marie Hill for your help with measuring the pipefish. Lastly, thank you to Brooke McKay, Rose Gaetano, and Thomas Wilson for visually matching the pipefish. I could not have done this project without all the friends, family, and students from Valdosta State University, The University of Tampa, and The University of South Florida who contributed their time to this project.

I'd like to thank my committee for thoughtful discussions throughout my thesis.

ABSTRACT

Concentric circular plots are widely used in terrestrial environments but are underutilized in marine environments. We tested the feasibility of using this method coupled with mark-resight and recapture designs within a Tampa Bay coastal marine seagrass bed. Two circular, 20-m diameter sites with four rings each were sampled during each event to collect adult Gulf pipefish, *Syngnathus scovelli*, a flagship species for seagrass ecosystems. In August 2022, we conducted a mark-resight experiment with 7 sampling events that collected 2,065 adult pipefish (802 individuals marked) and registered 246 recaptures (193 distinct fish). Although the population surveyed was male biased, more females were recaptured. Additionally, recaptured males disproportionally moved within the sites and were repeatedly recaptured. The immigration-emigration mixed logit-normal Mark model could not converge due to its sensitive parameters but suggested a residential superpopulation. In February 2023, we conducted a standard mark-recapture with 6 sampling events that collected 194 adult pipefish (187 individuals) and registered 6 recaptures (5 distinct fish). The POPAN Mark model estimated a superpopulation of 1,967 fish surrounding our sites and indicated a transient superpopulation. Repeated sampling disturbance within the sites had no significant impact on fish densities, but the seagrass habitat experienced significant negative effects. Data collected using concentric circular plots and linear transects during this study were similar. Therefore, our results indicate concentric circular plots are feasible in coastal marine environments and can be coupled with marking techniques to elucidate movement patterns and habitat use, but future use of this methodology should monitor habitat sampling effects.

INTRODUCTION

Concentric circular plots are a field technique to collect data where circles with varying radii are nested within one another to create rings. This methodology has been used to conceptually visualize positions, measure the distance from a centralized area, and estimate densities across research disciplines like music theory, engineering, electromagnetics, and ecology (Benadon 2007; Hao et al. 2009; Hamdi et al. 2016). Most ecological applications of concentric circular plots have been focused on forest ecosystems due to their various applications in terrestrial ecology. They have been used for censusing trees and estimating national forest inventories as well as a technique for bird censuses and assessing habitats surrounding bird nests (Nanos and de Luna 2017; Reynolds et al. 1980; Ripple et al. 1997). Other terrestrial studies have used concentric circular plots to test plant regrowth after burning and estimating deer density from pellet counts (Del Barrio et al. 1999; Smith 1968).

There has been limited use of concentric circular plots in the marine environment. Schoch et al. (2014) and Daugomah et al. (2007) utilized the idea of concentric circular plots as buffers within mapping software to classify marine habitats and grass shrimp densities. Findlay et al. (1995) had a simplified field application where they sampled sediment at fixed distances using the cardinal directions around a salmon pen. To our knowledge, only Dumont et al. (2004) has utilized concentric circular plots within the marine field to test animal density and movement. Their study used green sea urchins, *Strongylocentrotus droebachiensis*, to combine concentric circular plots with marking techniques to assess the movement of tagged individuals within their sites at 8 – 10 m depth. They recognized the potential of concentric circular plots coupled with marking techniques, like mark-recapture, for identifying a species' density, habitat usage, and small-scale movement patterns within an environment; however, this methodology needs to be tested to determine if it has wider applications than urchin barrens and can be used across coastal marine environments.

Mark-recapture techniques have long been utilized for estimating population sizes but have not been coupled with concentric circular plots in a marine ecosystem. These marking techniques within a marine seagrass environment have primarily focused on economically important species (e.g., red drum, *Sciaenops ocellatus*, Williams et al. 2016; gag grouper, *Mycteroperca microlepis*, Koenig and Coleman 1998; blue crab, *Callinectes sapidus*, Etherington et al. 2003; Johnson and Eggleston 2010) or on threatened species (e.g., green sea turtle, *Chelonia mydas*, Lelong et al. 2024; hawksbill sea turtle, *Eretmochelys imbricata*, Bjorndal and Bolten 2010; dugong, *Dugong dugon*, Lanyon et al. 2002). Common tagging methods are physical tags that are attached externally (Koenig and Coleman 1998; Lanyon et al. 2002; Bjorndal and Bolten 2010), physical internal tags (Williams et al. 2016; Etherington et al. 2003; Johnson and Eggleston 2010; Lelong et al. 2024; Lanyon et al. 2002), and body patterning unique to individuals for identification (Correia et al. 2014; Martin-Smith 2011). Although tagging methods vary between species due to their behavior and environment, assessing species with mark-recapture requires background knowledge on the natural history of the species (Cooch and White 2022).

Tampa Bay, Florida provides a model costal ecosystem to test mark-recapture techniques within concentric circular plots. Tampa Bay is a large, semi-enclosed estuary with a bay-wide mean depth of 4 m and shipping channels dredged to about 13 m depth (Greening et al. 2018). The region experiences a wet season from June to September with an increase in both rainfall and temperatures and then a dry season from October to May with decreased rainfall and cooler temperatures (Beck et al. 2024). There is a salinity gradient across the Bay with higher salinity at the mouth and lower salinity in the upper Bay due to the freshwater input, but this gradient becomes less differentiated across Tampa Bay during the dry season (Beck et al. 2024; Dusek et al. 2017). Although water temperature changes seasonally, there is no temperature gradient across and within the Bay like the salinity gradient. (Dusek et al. 2017).

Tampa Bay has shallow seagrass beds which are a primary indicator of Tampa Bay’s health (Beck et al. 2024). This vital ecosystem is dominated by *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme* (Sherwood et al. 2017). The seagrasses undergo seasonal changes with their peak growing season from July to October. The blades then die off for the winter and the short shoots begin to regrow around February (Beck et al. 2024, personal observation). Historically, Tampa Bay has been a model estuary for seagrass restoration. Nutrient pollution, anthropogenic activities (dredging), and population growth contributed to about a 50% seagrass loss between the 1950s and 1980s (Greening et al. 2018). Since then, seagrass has recovered to pre-1950s coverage thanks to public and private efforts to reduce nutrients and increase water clarity (Greening et al. 2018; Lizcano-Sandoval et al. 2023); however, there has been seagrass loss since 2016 associated with warmer water temperatures and decreased salinity within the Bay (Beck et al. 2024). Monitoring flagship species, like syngnathids, living within these seagrass beds can be used as a proxy to understand how the current seagrass loss (tied to new stressors) affects local fauna and as a tool for conservation efforts (Shokri et al. 2009).

The Gulf pipefish, *Syngnathus scovelli*, is one of seven species of syngnathids in Tampa Bay (Masonjones et al. 2010). They have a polyandrous mating system with sex-role reversal (Jones and Avise 1997). Male *S. scovelli* have a brood pouch to carry embryos full term and females have bright iridescent bands as a secondary sex trait (Jones and Avise 1997; Joseph 1957). They live within submerged aquatic vegetation ranging from Florida’s Atlantic coast, around the Gulf of Mexico, and down to Brazil (Adams et al. 2022; Joseph 1957; Flanagan et al. 2021; Díaz-Ruiz et al. 2000; Gasparini and Teixeira 1999). Recently, there have been population declines of *S. scovelli* on Florida’s east coast due to the decline in seagrass coverage (Adams et al. 2022). Considering the recent seagrass declines in Tampa Bay (Beck et al. 2024), the lack of recently published *S. scovelli* population monitoring (Masonjones et al. 2010), and their role as a flagship species for seagrass beds (Shokri et al. 2009), current Gulf pipefish population assessments are needed. Therefore, using Gulf pipefish as a model system for testing coastal concentric circular plots coupled with mark-recapture techniques would establish current population benchmarks.

In our study, we aimed to test if 1) concentric circular plots are feasible in a coastal marine environment and can be coupled with mark-recapture techniques to assess habitat usage and small-scale movement patterns; 2) mark-resight and mark-recapture models can estimate and compare population sizes of our model organism *S. scovelli*; 3) identify Gulf pipefish habitat usage, site fidelity, and differences between sexes; 4) concentric circular plot results are similar to linear transect results; and 5) the concentric circular plot methodology impacted the environment. We hypothesize concentric circular plots will be repeatable, they can elucidate movement patterns, and that *S. scovelli* populations will change seasonally with the seagrass. Additionally, we hypothesize concentric circular plot methodology will produce similar results to linear transects and its environmental impact will be dependent on the amount of disturbance during sampling.

METHODS

Site selection

Our study area occurred within a *Thalassia testudinum* dominated seagrass bed located in Old Tampa Bay near Port Tampa (N 27.872333, W -82.536500). The seagrass bed is semi-protected from open waters with a sandbar on the west side and is bordered by mangroves with residential areas on the east side. Fishermen often frequent the seagrass bed for bait collection or fishing (J. Ambrosio personal communication). Several syngnathid studies have occurred near this location due to its robust population of dwarf seahorses, *Hippocampus zosterae*, and Gulf pipefish (Masonjones et al. 2010; Masonjones et al. 2019; Rose et al. 2019; Tosto et al. 2023). We performed a pilot study May – July 2022 to determine *Syngnathus scovelli* densities across the seagrass bed. The highest densities occurred within the *T. testudinum* dominant middle corridor of the continuous seagrass bed. Two sites (North and South) were established within the *T. testudinum* dominant corridor (Fig. 1). Sites were selected for visibly similar habitats and seagrass coverage. At establishment, both sites had 100% *T. testudinum* coverage within the site which equates to a maximum score of 5 on the Braun-Blanquet cover-abundance scale (Poore 1955). The two sites were located 168 m apart which encompassed the furthest distance *S. scovelli* travelled in the previous mark-recapture study (Masonjones et al. 2010; Masonjones and Rose unpublished data). At each site, four concentric circular plots 1.5 m wide were centered on the site and had a 1 m walking space between each ring (Fig. 2). The sample site had a radius of 10 m and the transects covered a total area of 207.34 m^2. Ring 1, the innermost ring, was horizontally divided in half for two transects. Rings 2 and 3 were divided evenly into four transects using the cardinal directions. Ring 4, the outermost ring, was divided evenly into eight transects using the cardinal and ordinal directions. In addition to this paper's sampling, these sites were sampled once a month September 2022 – July 2023 for a yearlong population study (unpublished). All fish were collected under Florida Fish and Wildlife Conservation Commission's Special Activity License #SAL-22-2455-SR and handled in compliance with Valdosta State University's Animal Use Protocol #AUP-00082-2022.

Data collection

During a sampling event, at each site one person would stand in the center with a pole orienting the cardinal and ordinal directions. A rope would connect the center pole to a 1.5 m wide push net described by Strawn (1958) and modified with zip ties as described by Masonjones et al. (2010) to comb through the seagrass. Transect sampling would start in Ring 4 and work clockwise. Once a ring was completed, we would move inwards to the next ring. This limited fish from leaving the site while we were collecting.

Fig. 1 Location of the North Site in red and the South Site in blue within a continuous seagrass bed in Tampa Bay. Images were taken in Arc Pro software, version 3.2.1 (ArcGIS, ESRI, Redmond, CA, USA) with the light gray base layer and imagery hybrid base layer.

Gulf pipefish collected within the transects were then separated into adults and juveniles (lacking female banding or male brood pouch) and the demographics were recorded. Once the site was completely sampled, juveniles were released in the center of the site and adults were processed. Adult *S. scovelli* were then lightly anesthetized and marked on days designated for marking using Visible Implant Fluorescent Elastomer tags (VIFE; Northwest Marine Technology, Inc) following protocols in Woods and Martain-Smith (2004) in addition to Masonjones et al. (2010). Tags were placed intradermally on the ventral tail posterior to the urogenital pore in females or the brood pouch in males (Fig. 3). The position of the tags along with the tag color indicated the day and the site where the fish were collected (Fig. 4). Tag colors that looked similar to each other, identified by

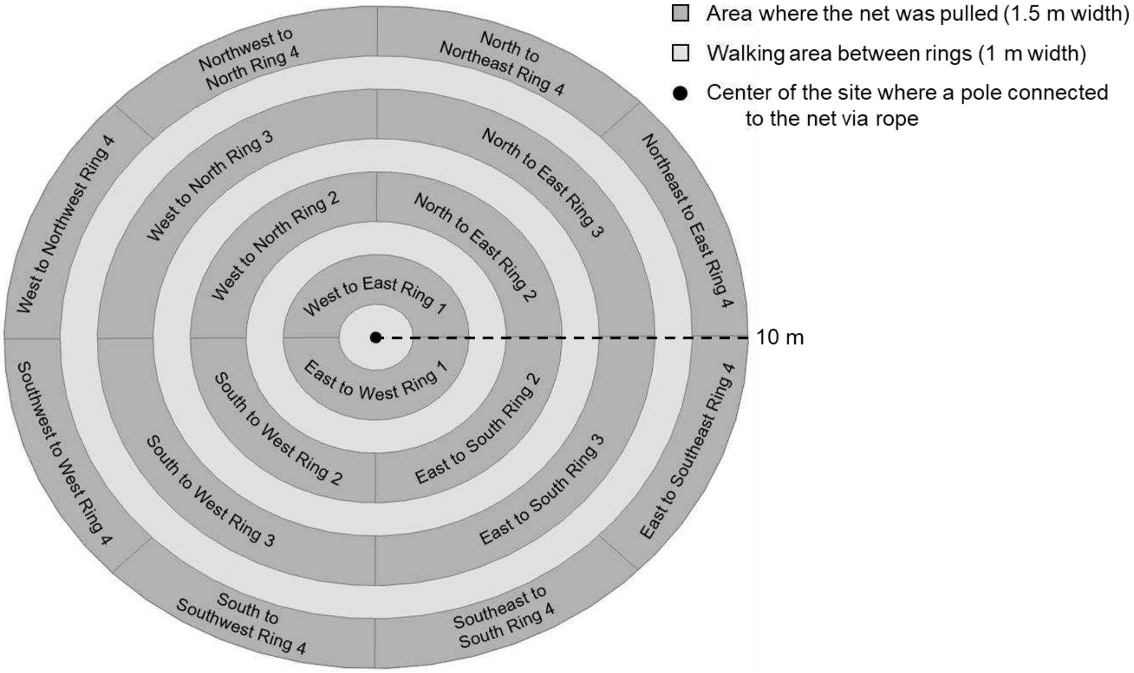

Fig. 2 Diagram of the concentric circular plots at each site with a 10 m radius. Each ring (dark gray) is 1.5 m wide with a 1 m walking buffer (light gray) in between. At the center, a pole is connected to the sampling net with a rope. Ring 4 is broken into 8-10.90 m^2 transects, Ring 3 is broken into 4-15.9 m^2 transects, Ring 2 is broken into 4-10.02 m^2 transects, and Ring 1 is broken into 2-8.25 m^2 transects. The transects cover a total of 207.34 m^2. The figure was created with Arc Pro software, version 3.2.1 (ArcGIS, ESRI, Redmond, CA, USA).

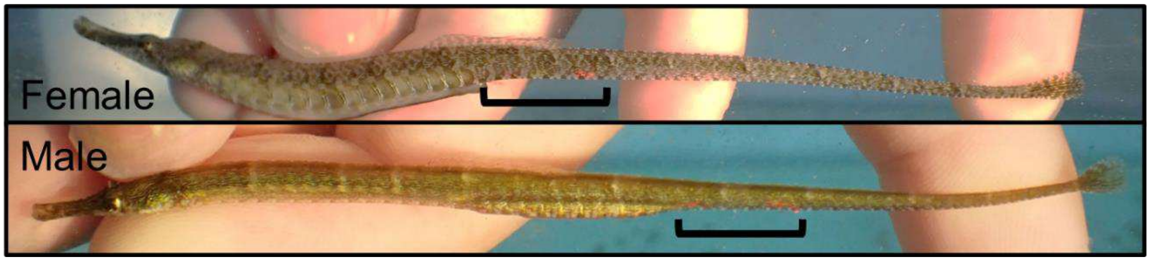

Fig. 3 Location of the subcutaneous marking area on *Syngnathus scovelli*'s ventral tail. Female (top) marks are located posterior to the urogenital pore and male (bottom) marks are located posterior to the brood pouch.

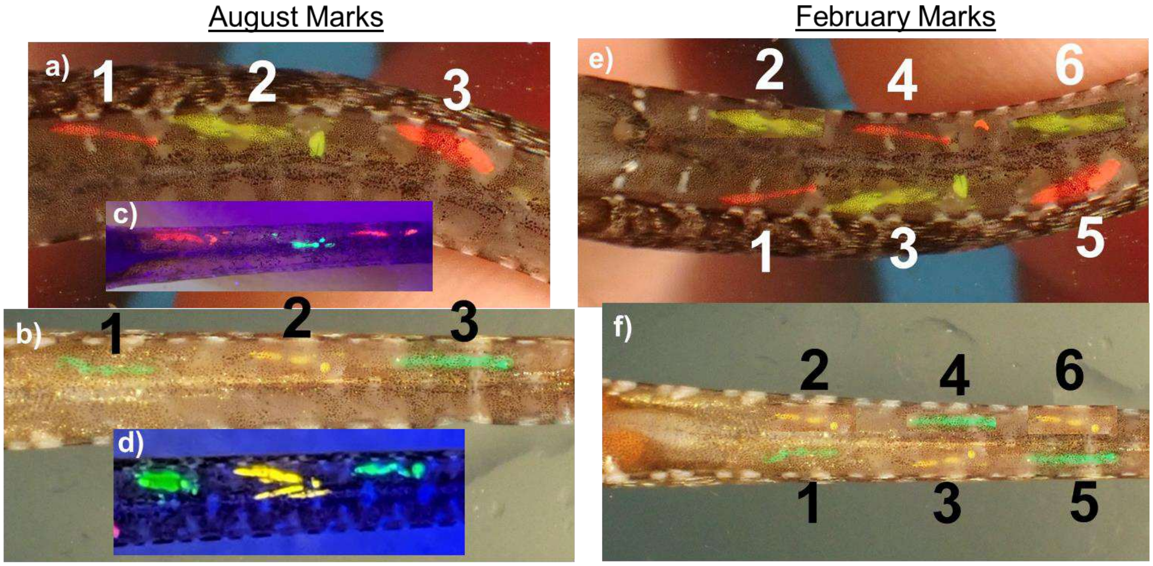

Fig. 4 Representative pictures of the position and color of marks for August (on the left) and February (on the right). All photographs are oriented with the female urogenital pore and the male brood pouch on the left. a) North Site marks with Aug. 1st red in position 1, Aug. 13th yellow in position 2, and Aug. 27th red in position 3. b) South Site marks with Aug. 1st green in position 1, Aug. 13th orange in position 2, and Aug. 27th green in position 3. c) and d) are the North Site and South Site marks, respectively, fluorescing under UV light. e) North Site marks with Jan. 29th red in position 1, Feb. 2nd yellow in position 2, Feb. 6th yellow in position 3, Feb. 10th red in position 4, Feb. 17th red in position 5, and Feb. 25th yellow in position 6. f) South Site marks with Jan. 29th green in position 1, Feb. 2nd orange in position 2, Feb. 6th orange in position 3, Feb. 10th green in position 4, Feb. 17th green in position 5, and Feb. 25th orange in position 6.

Curtis (2006), were not used in the same tag position for the North Site versus South Site color order and the August tagging pattern was different than February. The fish were then photographed with a metric scale for a full body picture, a head picture, a band picture for females, and brood pouch picture for males. These pictures were then catalogued with their sex and reproductive status. After processing, the adults were released at the center of their respective sites and there was no mortality during the marking and holding process.

Abiotic parameters were collected at each site during all sampling events. For each site, the start time, depth (cm), the amount of time it took to sample, and the number of people within the site were recorded. Water quality samples were collected mid-depth upon arrival to the sites and tested for water turbidity (Oakton T-100 Turbidimeter, measured in nephritic turbidity units, ntu), salinity (Extech refractometer, Model RF20, ppt), pH (API), phosphate (API, ppm), ammonia (API, ppm), nitrite (API, ppm), and nitrate (API, ppm). In addition, pH, water temperature (°C), and salinity (ppt) were collected with a multiparameter meter (Extech Instruments Model DO700). We tried measuring flow rate with a stream flowmeter (Geopacks MFP51), but the tidal flow was less than the meter's range of 0.05 m/s and therefore not collected. Tide height (MLLW, m), wind speed (m/s), wind direction, air temperature (°C), barometric pressure (mb), and water temperature (°C) were collected from NOAA's Old Port Tampa Station #8726607. Repeated measurements for the same tests were averaged together.

Biotic factors within the sites were measured as well. Total vegetative coverage including macroalgae, seagrass coverage and coverage by seagrass species was measured with the Braun-Blanquet System using a 0.25-m square quadrat (Poore 1955; Sherwood et al. 2017). In August and October, coverage was recorded directly outside of Ring 4 where we walked for each site's cardinal direction. Within the sites, handfuls of macroalgae collected in the net were recorded and converted into estimated dry weight. Six representative samples of macroalgae handfuls were dried and weighed using Protocol: Seagrass Macroalgae (2020). After completion of the mark-recapture studies in February 2023, we conducted a one-month and four-month post assessment seagrass survey. During these coverage surveys, each site had 60 total, evenly spaced locations that were sampled within the rings and the walking area directly outside of Ring 4. In addition, seagrass blade counts, short shoot counts, and 6 blade heights were recorded 0.75 m outside of Ring 4 in the walking area, between Rings 3 and 4, in the middle of Ring 3, between Rings 1 and 2, and in the middle of Ring 1 at evenly spaced locations in each site (Short and Coles 2001).

Random sampling outside of sites

Outside locations within the seagrass bed were sampled using linear transects following the Masonjones et al. (2010) protocols and compared to our sites. We randomly sampled four locations in June 2022, 12 locations in July, 20 locations in October, 21 locations in March 2023, 33 locations in April, and 41 locations in July across the *T. testudinum* dominant areas surrounding our sites (Fig. 5). Location, fish density, demographics, and seagrass percent cover were recorded. In addition, blade counts, short shoot counts, and 6 blade heights were recorded for each location in April and July (Short and Coles 2001). GPS points were recorded (Garmin GPSMAP 78S) and then displayed in Arc Pro software, version 3.2.1 (ArcGIS, ESRI, Redmond, CA, USA). All maps were made with a WGS 1984 projection.

Mark models and analysis

We conducted a mark-recapture study in August 2022 and February 2023 to test if our sampling method was repeatable in addition to estimating *S. scovelli*'s population. Due to the high number of fish captured in August, we used the immigration-emigration

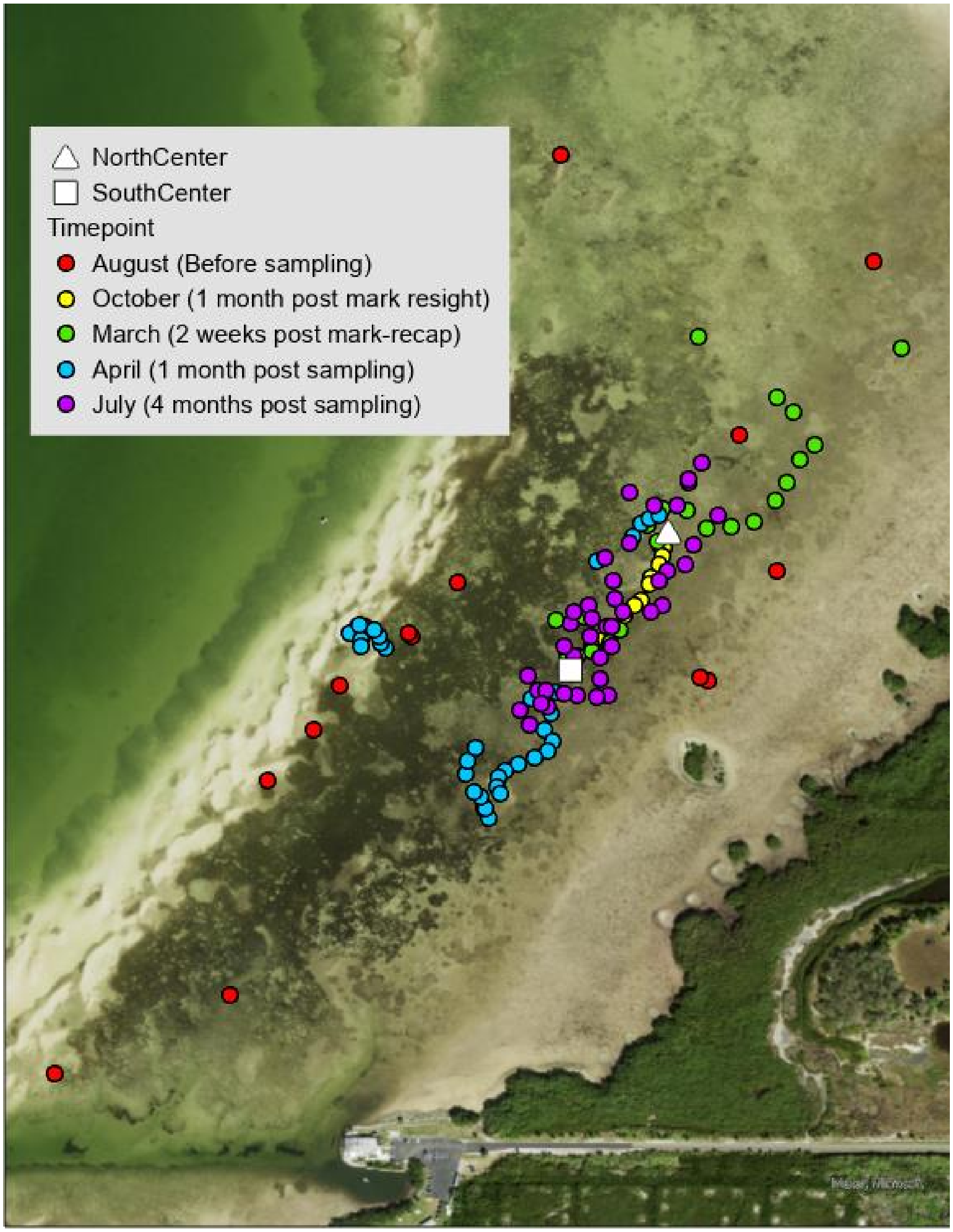

Fig. 5 Locations of random sampling outside of the North Site (white triangle) and South Site (white square) used to compare inside the sites to outside the sites. There were 16 samples for before sampling (red), 20 samples for one month post mark-resight (yellow), 21 samples for two weeks post mark-recap (green), 33 samples for one month post sampling (blue), and 41 samples for four months post sampling (purple). The image was taken in Arc Pro software, version 3.2.1 (ArcGIS, ESRI, Redmond, CA, USA) with the imagery base layer.

mixed logit-normal model for mark-resight sampling. There were three primary intervals each consisting of two secondary sampling occasions. The first primary interval had marks introduced on August 1st with secondary sampling on August 5th and 13th. The second primary interval had marks introduced on August 13th with secondary sampling on August 18th and 27th. The third primary interval had marks introduced on August 27th with secondary sampling on August 31st and September 16th. This led to seven sampling occasions with an average of 6.5 days (SE = 1.95) in between samples. During the first sampling occasion, adults from all four rings were collected and marked. In subsequent resights and markings, unmarked fish in Ring 4 were not marked and catalogued due to time and labor constraints. August mark-resight data was analyzed with Program Mark (Version 10.0; White and Burnham 1999) and the RMark package (Laake 2013) in R (R Core Team 2021). One requirement of the model we failed to meet was identifying the number of marked fish in the study area prior to resights. Since the walking areas were the only sections not sampled in the study site, and walking within the area would disturb the pipefish into relocating, we assumed the number of recaptures in the study area was the number of available marked fish in the study site. Possible abiotic and biotic covariates had their variance tested across the experiment (Table 1). If the standard deviation was over 1, then the covariate averages by day were incorporated into the Mark model's framework.

In February, we conducted a standard mark-recapture sampling scheme with 6 sampling occasions (Jan 29, Feb 2, 6, 10, 17, 25) with an average of 4.6 days (SE = 0.98) between samples. Adult *S. scovelli* collected from all four rings were marked and catalogued. The mark-recapture data were analyzed using a POPAN model in Program Mark (Version 10.0; White and Burnham 1999) and RMark (Laake 2013; R Core Team 2021). Fish were coded without individual heterogeneity since they were marked for their

Table 1 List of the possible mark-resight and mark-recapture covariates with their mean and standard deviation (± SD) for August 2022 and February 2023. The covariates used within the monthly models are designated with an asterisk (*).

	Covariate	August 2022 Mark-resight model (mean ± SD)	February 2023 Mark-recapture model (mean ± SD)
Environmental	Total seagrass Braun-Blanquet coverage	4.21 ± 1.10	2.73 ± 0.85
	Thalassia testudinum Braun-Blanquet coverage	3.58 ± 1.70	2.23 ± 1.03
	Syringodium filiforme Braun-Blanquet coverage	0.16 ± 0.55	0 ± 0
	Halodule wrightii Braun-Blanquet coverage	0.64 ± 1.38	0.3 ± 0.72
	Macroalgae Braun-Blanquet coverage	0.67 ± 0.76	0.5 ± 0.88
	Macroalgae density (estimated g/m^2)	1.09 ± 1.00*	2.59 ± 4.38*
	Time between samples (days)	6.5 ± 4.76*	4.6 ± 2.19
	Number of previous disturbances (days)	3 ± 2.16*	2.5 ± 1.87*
Fish	Total density (fish/m^2)	1.11 ± 0.73	0.18 ± 0.17
	Adult density (fish/m^2)	0.74 ± 0.51	0.08 ± 0.10
Obser-ver	Number of people in circle	4.57 ± 0.94*	4 ± 1.71*
	Total time it took to sample (minutes)	87.92 ± 26.68*	55.08 ± 18.97*
Physical	Time of day (hours)	10.52 ± 1.54*	10.73 ± 1.67*
	Depth during sampling (cm)	87.45 ± 12.96*	59.67 ± 22.97*
	Wind speed (m/s)	2.56 ± 0.70	3.33 ± 2.23
	Wind direction (degrees)	143.93 ± 36.37	182.5 ± 72.37
	Air temperature (°C)	28.55 ± 1.71	21.91 ± 1.82
	Barometric pressure (mb)	1017.86 ± 2.47*	1022.67 ± 2.45*
	Water temperature (°C)	32.67 ± 0.64*	23.23 ± 1.79*
	pH	8.10 ± 0.12	8.04 ± 0.09
	Turbidity (ntu)	2.59 ± 0.79*	1.78 ± 1.01*
	Phosphate (ppm)	0.07 ± 0.12	0.25 ± 0.1
	Ammonia (ppm)	0.02 ± 0.05	0.07 ± 0.14
	Nitrite (ppm)	0 ± 0	0 ± 0
	Nitrate (ppm)	0 ± 0	0.83 ± 1.95
	Salinity (ppt)	27.75 ± 0.87	27.83 ± 0.83

location and day. The abiotic and biotic covariates collected were assessed for their variance across the month (Table 1). If the standard deviation was over 1, the covariate averages by day were incorporated into the Mark model's framework.

During both marking studies, recaptures were identified visually in the field and by UV light while being processed. The full body photographs taken during processing were used to collect standard length (tip of snout to base of caudal fin) and body width measurements for all fish using Image J (Schneider et al. 2012). The facial photographs and female band photographs were used to identify individual fish. Gulf pipefish seem to have individually unique line patterns across the face and the females seem to have individually unique iridescent banding patterns. Both the facial photographs and female band photographs were used in I^3S Pattern (Version 4.02; Hartog and Reijns 2014) and Wild-ID (Version 1.0; Bolger et al. 2011), but both programs failed to identify individuals. Recaptured fish were then matched visually using the combination of facial and band patterning with morphometric measurements. This allowed for the creation of a more robust capture history for both months.

Statistical analysis

August recaptures were tested for an association between recapture status and sex using a Fisher's Exact Test in R (R Core Team 2021). This association could not be tested in February since only one male was recaptured. Sex ratio was analyzed across each experimental month using a Paired T-test comparing male and female counts paired by date. February counts were normalized using a square root transformation. Within August, the sex ratio was inconsistent across the month (Paired T-test, $t_6 = 3.83$, $p = 0.0087$) so an ANOVA was used to test the change in sex ratio across the experiment's duration.

To investigate movement patterns, the site rings were translated into distance away from the site's center release point. Since there was a small number of recapture events in February, only the mark-resight experiment in August was used for movement patterns within the sites. Recapture events were tested for equal distribution across the ring distances using a X^2 test. Movement outside of the sites was measured from the GPS location of the recaptured fish to its marked site's center release point in ArcPro (ArcGIS, ESRI, Redmond, CA, USA). For all recaptured fish, the change in standard length between recaptures was then divided by the days between recaptures to estimate growth rates. Caudal fin regrowth from the base of the caudal fin to the tip was also measured in ImageJ (Schneider et al. 2012).

Density changes within each site across the mark-resight and mark-recapture experiments were tested with an ANOVA. Differences in densities between inside and outside the sites were tested at 6 time points: before sampling comparing August 1st sites to June and July random samples, one month post mark-resight comparing sites and random samples in October, immediately after mark-recapture comparing February 25th sites to March random samples, two weeks after mark-recapture comparing sites and random samples in March, one month post sampling comparing sites and random samples in April, and four months post sampling comparing sites and random samples in July. Comparison across months for before sampling and immediately after mark-recapture were reasonable since previous seasonal densities were similar across the months compared (Masonjones et al. 2010). Outside random samples were categorized for North or South based on their proximity to the closest site. All data were tested for normalcy with the Shapiro-Wilk Normality Test in R (R Core Team 2021) and non-normal data were transformed to normal with a square root transformation or a natural log transformation with an added constant of 1 to correct for zeros in the data.

To determine the appropriate testing for comparing both inside the sites and outside random samples, North and South were compared using a Welch Two Sample T-test for normalized data or a Wilcoxon Rank Sum Test with continuity correction for non-normalized data in R (R Core Team 2021). The before sampling time point in August had a significant density difference between inside North Site and South Site (T-test, $t_{22.92} = -3.82$, $p = 0.00088$), but not within the outside samples (T-test, $t_{3.91} = 0.58$, $p = 0.59$). Due to the significance, the inside to outside comparison was analyzed with a two-way ANOVA followed by a post-hoc Tukey Honestly Significant Difference Test. All other time points had no difference between North and South inside the sites (one month after mark-resight: T-test, $t_{34} = -1.13$, $p = 0.27$; immediately after mark-recapture: Wilcoxon Test, W = 159, $p = 0.93$; two weeks after mark-recapture: Wilcoxon Test, W = 125.5, $p = 0.19$; one month post sampling: Wilcoxon Test, W = 164.5, $p = 0.95$; four months post sampling: T-test, $t_{32.46} = 0.086$, $p = 0.93$) and therefore North and South data were combined. Additionally, there was no difference between North and South within the outside samples (one month post mark-resight: T-test, $t_{9.64} = -0.89$, $p = 0.40$; immediately after and two weeks after mark-recapture: Wilcoxon Test, W = 27, $p = 0.14$; one month post sampling: Wilcoxon Test, W = 93, $p = 0.25$; four months post sampling: T-test, $t_{30.17} = -1.36$, $p = 0.18$) so the North and South data were combined. The combined densities inside the sites were compared to the combined densities outside the sites using a Welch Two Sample T-test for normalized data or a Wilcoxon Rank Sum Test for non-normalized data in R (R Core Team 2021).

Sampling effects on total vegetative coverage (including macroalgae coverage) and seagrass coverage used similar timepoints as the density comparisons at before sampling, one month post mark-resight, one month post sampling, and four months post sampling. All seagrass comparisons were tested for normality using Shapiro-Wilk

Normality Test then compared with a T-test for normalized data or a Wilcoxon Rank Sum Test for non-normalized data in R (R Core Team 2021). Inside the sites, walking area and net samples were tested for differences within disturbance type. They were then combined and compared to samples outside of the sites since both sites experienced the same amount of disturbance. Total coverage and seagrass coverage using the Braun-Blanquet system were treated as continuous data without transformations (Furman et al. 2018). Seagrass blade count, short shoot count, and averaged blade height were compared using *T. testudinum* since *Halodule wrightii* was recorded in the sites, but with low sample sizes.

RESULTS

Methodology and mark-recapture

During our August mark-resight experiment, we collected a total of 2,065 adult *Syngnathus scovelli*, cataloged 1,495 fish, and created capture histories for 1,255 individuals. There were a few fish who escaped between collection and photographing/marking, so some totals fluctuate. Each sampling averaged 295 fish (n = 7, SE = 38.84, range = 185 – 502). We collected a total of 1,373 males (average per collection date = 196.14, SE = 32.00, range = 105 – 369) and 692 females (average per collection date = 98.86, SE = 7.10, range = 80 – 133). We marked a total of 871 fish (622 male, 249 female) with 500 marks on August 1st (370 male, 130 female), 202 marks on August 13th (141 male, 61 female), and 169 marks on August 27th (111 male, 58 female) (Table 2). There were 246 registered recapture events (159 male, 87 female).

Table 2 Male (M) and female (F) recaptures across both sites during the mark-resight method in August 2022. The number of fish captured includes fish collected but not marked in Ring 4. The percentage recaptured is calculated by the number of fish recaptured divided by the total individual fish marked to date.

Day	Date	Number of Fish Captured	Total Marking Occasions to Date	Total Individual Fish Marked to Date	Number of Fish Recaptured	Percent Recaptured	Number of Marks Recaptured		
							1 mark	2 marks	3 marks
1 mark	Aug. 1	M: 370 F: 133	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -
2	Aug. 5	M: 177 F: 86	M: 370 F: 130	M: 370 F: 130	M: 24 F: 20	M: 6.49% F: 15.38%	M: 24 F: 20	M: - F: -	M: - F: -
3 mark	Aug. 13	M: 223 F: 114	M: 370 F: 130	M: 370 F: 130	M: 18 F: 10	M: 4.86% F: 7.69%	M: 18 F: 10	M: - F: -	M: - F: -
4	Aug. 18	M: 188 F: 100	M: 511 F: 191	M: 493 F: 182	M: 45 F: 19	M: 9.13% F: 10.44%	M: 39 F: 17	M: 6 F: 2	M: - F: -
5 mark	Aug. 27	M: 169 F: 93	M: 511 F: 191	M: 493 F: 182	M: 28 F: 18	M: 5.68% F: 9.89%	M: 22 F: 14	M: 4 F: 2	M: - F: -
6	Aug. 31	M: 142 F: 86	M: 622 F: 249	M: 578 F: 224	M: 24 F: 17	M: 4.15% F: 7.59%	M: 18 F: 14	M: 4 F: 3	M: 2 F: 0
7	Sept. 16	M: 105 F: 80	M: 622 F: 249	M: 578 F: 224	M: 20 F: 3	M: 3.46% F: 1.34%	M: 16 F: 3	M: 4 F: 0	M: 0 F: 0

Some fish were recaptured more than once, so the total individual fish recaptured was 193 (120 male, 73 female) and the total number of individual fish marked was 802 (578 male, 224 female) (Table 2).

Capture histories were developed with confirmed and artificial encounter data. All fish that were recaptured with the previous collection's mark (i.e., recaptures on Aug. 5th with marks from Aug. 1st) were not matched to their original marking photo and instead artificial encounter histories were created. Fish that were recaptured past the initial recapture (i.e., recaptures on Aug. 13th with marks from Aug. 1st) were visually assessed for matches with prior recaptured fish having the same mark and if they matched, a confirmed encounter history would be used. Lastly, fish with multiple recaptures were matched to create confirmed encounter histories past their initial recapture.

The highly sensitive immigration-emigration mixed logit-normal model failed to converge in both Program Mark and Rmark. The model was run with the individual fish marked superpopulation (500, 500, 675, 675, 802, 802), the unmarked seen (217, 306, 223, 215, 186, 161), 0 marks unidentified, time intervals indicating shifts between primary sampling occasions (0, 1, 0, 1, 0), and σ fixed to 0 since the fish were not marked for individual heterogeneity (Cooch and White 2022). The environmental covariates tested as individual additives to the model were macroalgae density, time between sampling, number of previous disturbances, number of people in the circle, total time it took to sample, time of day, depth during sampling, barometric pressure, water temperature, and turbidity (Table 1). Seagrass characteristics were not included in the models because they were previously statistically analyzed to determine if the variation was large enough to meet our cutoff for incorporating into Mark and barometric pressure encompassed weather variation. Although the model without covariates (32 individual models) and the model with covariates (624 individual models) could not converge, across the top models the estimated number of fish utilizing the area around the sites was similar to the average number of fish for each sampling occasion. Also, the top models suggested the time between sampling events may impact capture probability.

During the February mark-recapture, we collected a total of 194 adult *S. scovelli*, cataloged 193 fish since one male escaped before photographing/marking, and created capture histories for 187 individual fish. Each sampling averaged 32.33 fish (n = 6, SE = 7.15, range = 13 – 63). We collected a total of 107 males (average per collection date = 17.83, SE = 3.30, range = 6 – 29) and 87 females (average per collection date = 14.5, SE = 4.14, range = 7 – 34) (Table 3). We marked a total of 193 fish (106 male, 87 female) with 6 registered recapture events (1 male, 5 female) (Table 3). One fish was recaptured twice, so the total individual fish marked was 187 (105 male, 82 female). Each fish was visually matched back to their original tagging event to create the capture histories.

Table 3 Male (M) and female (F) recaptures across both sites during the mark-recapture method in February 2023. The percentage recaptured is calculated by the number of fish recaptured divided by the total individual fish marked to date.

Day	Date	Number of Fish Captured	Total Marking Occasions to Date	Total Individual Fish Marked to Date	Number of Fish Recaptured	Percent Recaptured	Number of Marks Recaptured		
							1 mark	2 marks	3 marks
1	Jan. 29	M: 29 F: 34	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -
2	Feb. 2	M: 23 F: 14	M: 28 F: 34	M: 28 F: 34	M: 0 F: 3	M: 0% F: 8.82%	M: 0 F: 3	M: - F: -	M: - F: -
3	Feb. 6	M: 18 F: 10	M: 51 F: 48	M: 51 F: 45	M: 1 F: 0	M: 1.96% F: 0%	M: 1 F: 0	M: 0 F: 0	M: - F: -
4	Feb. 10	M: 6 F: 7	M: 69 F: 58	M: 68 F: 55	M: 0 F: 1	M: 0% F: 1.82%	M: 0 F: 1	M: 0 F: 0	M: 0 F: 0
5	Feb. 17	M: 19 F: 15	M: 75 F: 65	M: 74 F: 61	M: 0 F: 1	M: 0% F: 1.64%	M: 0 F: 0	M: 0 F: 1	M: 0 F: 0
6	Feb. 25	M: 12 F: 7	M: 94 F: 80	M: 93 F: 75	M: 0 F: 0	M: 0% F: 0%	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0

The POPAN model converged in both Program Mark and Rmark with these capture histories. The covariates used in the model framework were macroalgae density, number of previous disturbances, number of people in the circle, total time it took to sample, time of day, depth during sampling, barometric pressure, water temperature, and turbidity. Seagrass characteristics were not included since they are statistically analyzed separately, the time between samples is coded into the model itself, barometric pressure represented weather variation, and nitrate was not included because it did not affect the sampling. Each covariate was added by itself to the models to identify that the number of people in the circle and the turbidity influenced the top models. The two covariates were then used for candidate models with additive effects to find the top model out of 343 models. The best fitting model was Phi(.) p(.) pent(number of people in the circle) N(.) with an AIC of 102.85 and a weight of 0.07. In this model, the probability of survival between sampling events, the probability of capture during a sampling event, and the superpopulation estimate were all time independent. Additionally, the probability of fish entering and exiting the sites was dependent on the number of people in the circle. The model estimated a density-dependent superpopulation of 1,967 fish surrounding the sites.

Recapture rates and sex ratio

In August, the overall male recapture rate was 27.51% and the overall female recapture rate was 38.84%. The recapture rate during each sampling period varied for both males and females and across sites (Tables 2, 4, and 5). Across both sites, males had an average recapture rate of 5.63% (n = 6, SE = 0.76%) and females had an average recapture rate of 8.72% (n = 6, SE = 1.74%) during the sampling periods (Table 2). During the mark-resight experiment, there was a significant association between recapture status and sex (Fisher's Exact Test, *p* = 0.031). More males were captured

Table 4 North Site male (M) and female (F) recaptures during the mark-resight method in August 2022. The number of fish captured includes fish collected in Ring 4. The percentage recaptured is calculated by the number of fish recaptured divided by the total individual fish marked to date.

Day	Date	Number of Fish Captured	Total Marking Occasions to Date	Total Individual Fish Marked to Date	Number of Fish Recaptured	Percent Recaptured	Number of Marks Recaptured		
							1 mark	2 marks	3 marks
1 mark	Aug. 1	M: 129 F: 50	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -
2	Aug. 5	M: 66 F: 24	M: 129 F: 49	M: 129 F: 49	M: 10 F: 7	M: 7.75% F: 14.29%	M: 10 F: 7	M: - F: -	M: - F: -
3 mark	Aug. 13	M: 73 F: 39	M: 129 F: 49	M: 129 F: 49	M: 5 F: 3	M: 3.88% F: 6.12%	M: 5 F: 3	M: - F: -	M: - F: -
4	Aug. 18	M: 69 F: 37	M: 180 F: 69	M: 175 F: 66	M: 14 F: 6	M: 8.0% F: 9.09%	M: 12 F: 5	M: 2 F: 1	M: - F: -
5 mark	Aug. 27	M: 51 F: 32	M: 180 F: 69	M: 175 F: 66	M: 8 F: 6	M: 4.57% F: 9.09%	M: 6 F: 5	M: 2 F: 1	M: - F: -
6	Aug. 31	M: 55 F: 39	M: 214 F: 94	M: 201 F: 85	M: 12 F: 8	M: 5.97% F: 9.41%	M: 10 F: 6	M: 0 F: 2	M: 2 F: 0
7	Sept. 16	M: 33 F: 36	M: 214 F: 94	M: 201 F: 85	M: 7 F: 0	M: 3.48% F: 0%	M: 6 F: 0	M: 1 F: 0	M: 0 F: 0

Table 5 South Site male (M) and female (F) recaptures during the mark-resight method in August 2022. The number of fish captured includes fish collected in Ring 4. The percentage recaptured is calculated by the number of fish recaptured divided by the total individual fish marked to date.

Day	Date	Number of Fish Captured	Total Marking Occasions to Date	Total Individual Fish Marked to Date	Number of Fish Recaptured	Percent Recaptured	Number of Marks Recaptured		
							1 mark	2 marks	3 marks
1 mark	Aug. 1	M: 241 F: 83	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -
2	Aug. 5	M: 111 F: 62	M: 241 F: 81	M: 241 F: 81	M: 14 F: 13	M: 5.81% F: 16.05%	M: 14 F: 13	M: - F: -	M: - F: -
3 mark	Aug. 13	M: 150 F: 75	M: 241 F: 81	M: 241 F: 81	M: 13 F: 7	M: 5.39% F: 8.64%	M: 13 F: 7	M: - F: -	M: - F: -
4	Aug. 18	M: 119 F: 63	M: 331 F: 122	M: 318 F: 116	M: 31 F: 13	M: 9.75% F: 11.21%	M: 27 F: 12	M: 4 F: 1	M: - F: -
5 mark	Aug. 27	M: 118 F: 61	M: 331 F: 122	M: 318 F: 116	M: 20 F: 12	M: 6.29% F: 10.34%	M: 16 F: 9	M: 2 F: 1	M: - F: -
6	Aug. 31	M: 87 F: 47	M: 408 F: 155	M: 377 F: 139	M: 12 F: 9	M: 3.18% F: 6.47%	M: 8 F: 8	M: 4 F: 1	M: 0 F: 0
7	Sept. 16	M: 72 F: 44	M: 408 F: 155	M: 377 F: 139	M: 13 F: 3	M: 3.45% F: 2.16%	M: 10 F: 3	M: 3 F: 0	M: 0 F: 0

and marked, but females had a higher recapture rate. The sex ratio during each sampling period significantly differed across the month (Paired T-test, $t_6 = 3.81$, $p = 0.0087$) and ranged from a strong, male-biased population (0.74 on August 1) to a more even sex ratio (0.57 on September 16). The sex ratio significantly decreased over the month (ANOVA, $F_{(1, 5)} = 41.82$, $p = 0.0013$).

In February, the overall male recapture rate was 1.08% and the overall female recapture rate was 6.67%. Recapture rates across the sampling sites were low and there were no recaptured males at South Site (Tables 3, 6, and 7). Across both sites, males had an average recapture rate of 0.39% (n = 5, SE = 0.33%) and females had an average recapture rate of 2.46% (n = 5, SE = 1.38%) during the sampling periods (Table 3). The sex ratio did not significantly vary across the month (Paired T-test, $t_5 = 1.75$, $p = 0.14$). The population was slightly male-biased ranging from 0.64 (February 6) to 0.56 (February 17).

Movement patterns and recapture findings

Within all 246 recapture events in August, there was no difference in the recaptured distance from the site's center release point (X^2, $X^2_{(3, 246)} = 1.84$, $p = 0.61$). Females did not have a significant difference in recapture distance (X^2, $X^2_{(3, 87)} = 6.20$, $p = 0.1$), but males did (X^2, $X^2_{(3, 159)} = 8.22$, $p = 0.042$) with 33.33% of males being recaptured within 2.5 m of the center. There were 35 individual fish (25 male, 10 female) that were recaptured two or more times in August. Females were at most recaptured twice and males were recaptured up to four times (3 males). Individually unique facial patterns and female iridescent banding patterns were consistent across the recapture period. There were 6 fish that were recaptured outside of the sites. The distance from the fish's marked site center point to where they were recaptured ranged from 15.5 m (South marked and found outside of South Site) to at least 178 m (South marked and

Table 6 North Site male (M) and female (F) recaptures during the mark-recapture method in February 2023. The percentage recaptured is calculated by the number of fish recaptured divided by the total individual fish marked to date.

Day	Date	Number of Fish Captured	Total Marking Occasions to Date	Total Individual Fish Marked to Date	Number of Fish Recapt-ured	Percent Recaptured	Number of Marks Recaptured		
							1 mark	2 marks	3 marks
1	Jan. 29	M: 11 F: 9	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -
2	Feb. 2	M: 10 F: 7	M: 11 F: 9	M: 11 F: 9	M: 0 F: 2	M: 0% F: 22.22%	M: 0 F: 2	M: - F: -	M: - F: -
3	Feb. 6	M: 12 F: 1	M: 21 F: 16	M: 21 F: 14	M: 1 F: 0	M: 4.76% F: 0%	M: 1 F: 0	M: 0 F: 0	M: - F: -
4	Feb. 10	M: 0 F: 3	M: 33 F: 17	M: 32 F: 15	M: 0 F: 0	M: 0% F: 0%	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0
5	Feb. 17	M: 5 F: 3	M: 33 F: 20	M: 32 F: 18	M: 0 F: 0	M: 0% F: 0%	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0
6	Feb. 25	M: 6 F: 4	M: 38 F: 23	M: 37 F: 21	M: 0 F: 0	M: 0% F: 0%	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0

Table 7 South Site male (M) and female (F) recaptures during the mark-recapture method in February 2023. The percentage recaptured is calculated with the number of fish recaptured divided by the total individual fish marked to date.

Day	Date	Number of Fish Captured	Total Marking Occasions to Date	Total Individual Fish Marked to Date	Number of Fish Recapt-ured	Percent Recaptured	Number of Marks Recaptured		
							1 mark	2 marks	3 marks
1	Jan. 29	M: 18 F: 25	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -	M: - F: -
2	Feb. 2	M: 13 F: 7	M: 17 F: 25	M: 17 F: 25	M: 0 F: 1	M: 0% F: 4.0%	M: 0 F: 1	M: - F: -	M: - F: -
3	Feb. 6	M: 6 F: 9	M: 30 F: 32	M: 30 F: 31	M: 0 F: 0	M: 0% F: 0%	M: 0 F: 0	M: 0 F: 0	M: - F: -
4	Feb. 10	M: 6 F: 4	M: 36 F: 41	M: 36 F: 40	M: 0 F: 1	M: 0% F: 2.5%	M: 0 F: 1	M: 0 F: 0	M: 0 F: 0
5	Feb. 17	M: 14 F: 12	M: 42 F: 45	M: 42 F: 43	M: 0 F: 1	M: 0% F: 2.33%	M: 0 F: 0	M: 0 F: 1	M: 0 F: 0
6	Feb. 25	M: 6 F: 3	M: 56 F: 57	M: 56 F: 54	M: 0 F: 0	M: 0% F: 0%	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0

found north of North Site). Marked fish from the mark-resight experiment were last seen on November 4th, 2022 as part of the tandem monthly population study. This one male was marked on August 1st and was recorded as the longest timeframe since being marked for a recaptured individual. Across the mark-resight and mark-recapture experiments, one fish was recorded with tag loss which was identified through matching facial patterns and there was one record of a misread/unidentified tag caught using the photographs.

Fish recaptured more than once during the August mark-resight had an average growth rate of 0.17 mm per day ($n = 50$, $SE = 0.04$). Males averaged 0.15 mm per day ($n = 40$, $SE = 0.05$) and females averaged 0.26 mm per day ($n = 10$, $SE = 0.09$). In February, the four recaptured females had an average growth rate of 0.36 mm per day ($SE = 0.30$). The one recaptured male in February had a growth rate of 0.16 mm per day.

At least 13 fish were recorded with abnormally short, truncated tails. Of these fish, two had truncated tails without a caudal fin and 11 had truncated tails that regrew a caudal fin. One male was initially recorded with a truncated tail without a caudal fin on August 27th. It was then recaptured on September 16th and had grown a caudal fin where the tail truncated that measured 1.70 mm from base to tip.

Densities

Before our mark-resight experiment, *S. scovelli* densities inside and outside the sites had a significant interaction between site and location (Two-Way ANOVA, $F_{(1, 48)} = 5.07$, $p = 0.029$) as well as a significant difference in location ($p = 0.0002$) (Fig. 6). The post-hoc Tukey Honestly Significant Difference Test yielded significance only between inside South and outside South densities ($p < 0.0001$). Although preliminary analysis indicated a significant difference in densities between North and South Sites (T-test, $t_{22.92}$

= -3.82, p = 0.0009), the post-hoc Tukey Test did not identify significance with its adjusted p-value (p = 0.58). August densities started at 0.86 fish/m^2 (North Site) and 1.56 fish/m^2 (South Site) on August 1st then decreased throughout the month to 0.33 fish/m^2 (North Site) and 0.56 fish/m^2 (South Site) on September 16th (Fig. 7). The North Site densities did not significantly change across the mark-resight experiment (ANOVA, $F_{(1, 5)}$ = 5.46, p = 0.067), but the South Site significantly decreased (ANOVA, $F_{(1, 5)}$ = 8.38, p = 0.034) (Fig. 7). One month after the mark-resight experiment, densities inside the sites and outside of the sites were similar (T-test, $t_{34.56}$ = -0.50, p = 0.62) (Fig. 6).

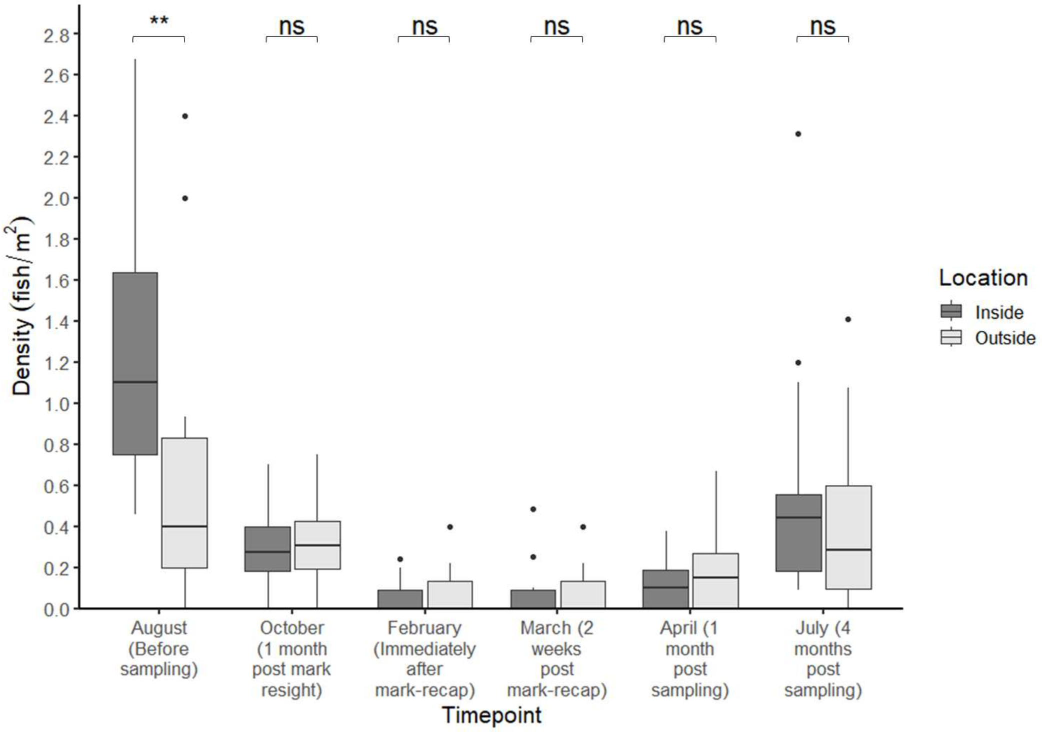

Fig. 6 Boxplots of Gulf pipefish densities inside and outside of the sites at six timepoints. Before sampling there were significantly higher densities inside the sites than outside (Wilcoxon Test, W = 443, p = 0.0022). All other timepoints had no significance between the inside and outside densities (one month post mark-resight: T-test, $t_{34.56}$ = -0.50, p = 0.62; immediately after mark-recap: Wilcoxon Test, W = 334.5, p = 0.42; two weeks post mark-recap: Wilcoxon Test, W = 327, p = 0.35; one month post sampling: Wilcoxon Test, W = 450, p = 0.08; four months post sampling: Wilcoxon Test, W = 866.5, p = 0.19).

Gulf pipefish densities during the month of February had a slight decreasing trend throughout the month but did not significantly change (North Site: ANOVA, $F_{(1, 4)}$ = 2.83, p = 0.17; South Site: ANOVA, $F_{(1, 4)}$ = 2.20, p = 0.21) (Fig. 7). The highest densities for both sites were recorded on January 29th (North Site: 0.096 fish/m^2, South Site: 0.21 fish/m^2). The lowest density for North Site was 0.014 fish/m^2 (Feb. 10th) and the lowest density for South Site was 0.043 fish/m^2 (Feb. 25th) (Fig. 7). Immediately after the mark-recapture experiment, the densities inside and outside of the sites were similar (Wilcoxon Test, W = 334.5, p = 0.42). There continued to be no difference in *S. scovelli*

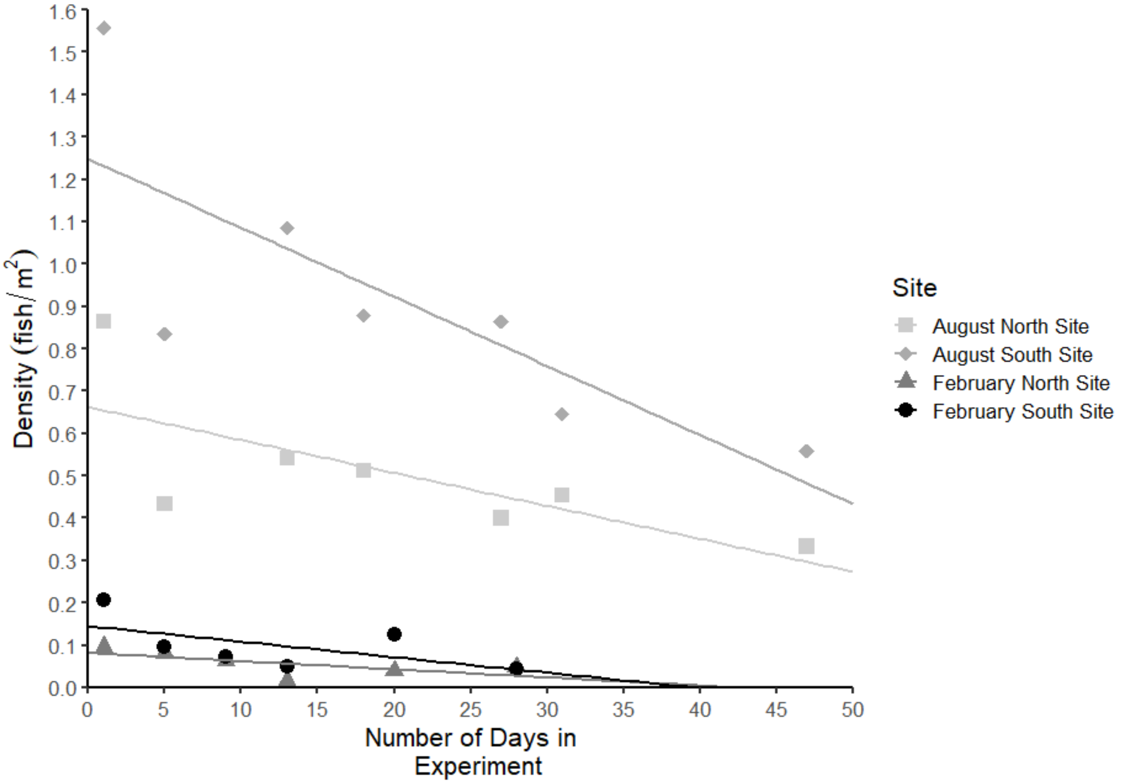

Fig. 7 Densities within each site across the mark-resight experiment in August 2022 and the mark-recapture experiment in February 2023. August's South Site significantly decreased across the experiment (ANOVA, $F_{(1, 5)}$ = 8.38, p = 0.034). All other sites did not significantly decrease across the experiments (August North Site: ANOVA, $F_{(1, 5)}$ = 5.46, p = 0.067; February North Site: ANOVA, $F_{(1, 4)}$ = 2.8, p = 0.17; February South Site: ANOVA, $F_{(1, 4)}$ = 2.20, p = 0.21).

densities inside and outside of the sites two weeks post mark-recapture (Wilcoxon Test, W = 327, *p* = 0.35), one month post sampling (Wilcoxon Test, W = 450, *p* = 0.080), and four months post sampling (Wilcoxon Test, W = 866.5, *p* = 0.19) (Fig. 6).

Seagrass comparisons

Before sampling began in August, the sites were similar to outside locations in total vegetative coverage including macroalgae (Wilcoxon Test, W = 25, *p* = 0.17) and in total seagrass coverage (Wilcoxon Test, W = 48, *p* = 0.21) (Fig. 8). There was also no difference between inside and outside of the sites one month after the mark-resight experiment (Total coverage: Wilcoxon Test, W = 69, *p* = 0.58; Seagrass coverage: Wilcoxon Test, W = 81, *p* = 0.98) (Fig. 8). One month after sampling completed (April), total coverage, seagrass coverage, and *Thalassia testudinum* blade counts were significantly lower inside the sites than outside of them (Total coverage: Wilcoxon Test, W = 1138, $p < 0.0001$; Seagrass coverage: Wilcoxon Test, W = 1398, *p* = 0.0049; Blade count: T-test, $t_{27.39} = -3.50$, *p* = 0.0016) (Fig. 8). There were no differences between inside and outside of the sites for *T. testudinum* short shoot counts (Wilcoxon Test, W = 267, *p* = 0.19) and average blade height (T-test, $t_{44.06} = 1.27$, *p* = 0.21). Within the sites, there was no difference between the walking area and the net area for *T. testudinum* blade counts (T-test, $t_{22.96} = -1.0$, *p* = 0.33), short shoot counts (T-test, $t_{28.04} = -1.06$, *p* = 0.3), and average blade height (T-test, $t_{19.32} = -0.004$, *p* = 0.997). The four-month post sampling (July) yielded similar results to the one month. In July, inside of the sites had significantly lower total coverage (Wilcoxon Test, W = 841.5, $p < 0.0001$), seagrass coverage (Wilcoxon Test, W = 814, $p < 0.0001$), *T. testudinum* blade counts (Wilcoxon Test, W = 445, *p* = 0.015), short shoot counts (Wilcoxon Test, W = 18, $p < 0.0001$), and average blade height (T-test, $t_{63.95} = -2.67$, *p* = 0.0097) than outside of the sites (Fig. 8). Within the sites, there was no difference between the walkway area and the net area for

T. testudinum blade counts (T-test, $t_{25.81} = -0.11$, $p = 0.92$), short shoot counts (Wilcoxon Test, W = 112.5, $p = 0.32$), and average blade height (T-test, $t_{21.10} = -1.62$, $p = 0.12$).

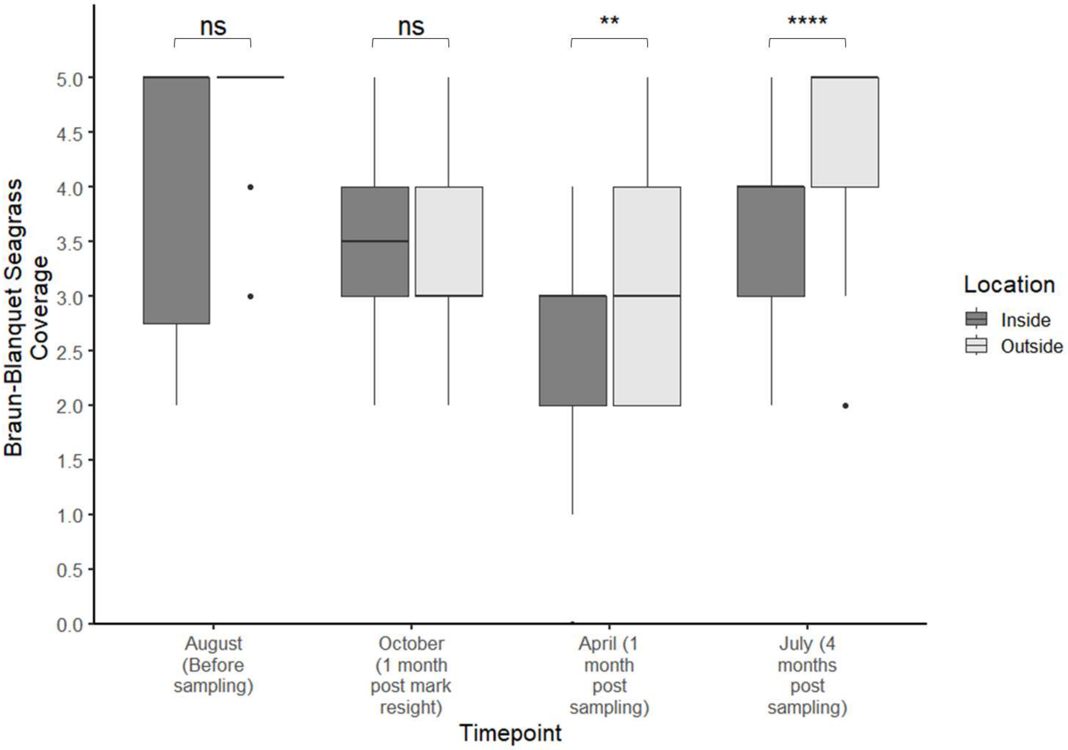

Fig. 8 Boxplot of total seagrass coverage compared at four timepoints using the Braun-Blanquet coverage scale. The scale dictates 0 = no coverage, 0.5 = < 1% coverage, 1 = 1 – 5% coverage, 2 = 5 – 25% coverage, 3 = 26 – 50% coverage, 4 = 51 – 75% coverage, and 5 = 76 – 100% coverage (Poore 1955). There was no significant difference in seagrass coverage between inside of the sites and outside of the sites before sampling began (Wilcoxon Test, W = 48, $p = 0.21$) and one month post mark-resight (Wilcoxon Test, W = 81, $p = 0.98$). There was, however, a significant difference between inside and outside of the sites at one month post sampling (Wilcoxon Test, W = 1398, $p = 0.0049$) and at four months post sampling (Wilcoxon Test, W = 814, $p < 0.0001$).

DISCUSSION

Concentric circular plot methodology is feasible and repeatable in a coastal marine seagrass environment. Assessment of concentric circular plot impacts indicated a negative sampling effect based on repeatedly sampling in the same location. Initially both sites were visually and statistically comparable in habitat complexity and seagrass coverage during *Thalassia testudinum*’s peak growing season (Beck et al. 2024). The mark-resight sampling in August had at most 40 individuals walking through the sites within a month. Similar trampling in *T. testudinum* beds in Puerto Rico had a significant decrease in seagrass coverage (Eckrich and Holmquist 2000), but we reported no difference in seagrass coverage one month after the mark-resight experiment.

Conversely, the *T. testudinum* regrowth period in February did experience similar significant seagrass coverage results as Eckrich and Holmquist (2000). During this time, the February mark-recapture experiment had at most 25 individuals walking through the sites within a month. Within the sites, there was no difference between the walkway area and the net area one month post sampling and four months post sampling indicating that walking and pulling the net are a similar level of disturbance. Inside the sites there was a significant decrease in seagrass coverage and blade counts at one month (April) and four months (July) post sampling which are similar findings to Eckrich and Holmquist (2000); however, they also experienced significant decreases in short shoot count and blade height which we saw at four months post sampling, but not at one month post sampling. It is possible that *T. testudinum* has an initial short-term recovery in short shoot density similar to *T. hemprichii* which recovered three weeks post trampling (Nurdin et al. 2019), but long-term trampling effects decreased seagrass density. It is also possible that removing macroalgae from the sites while sampling and seasonal changes in light attenuation due to macroalgae coverage and turbidity had unrecorded negative effects on the sites' seagrass growth. Eckrich and Holmquist (2000) sampled during *T. testudinum* growing and die off seasons, but comparisons were not made between the timepoints. Therefore, it seems likely that trampling effects may be dependent on the seasonality of the seagrass and its growing stage. Application of concentric circular plots in coastal marine areas should assess their sampling effects on a habitat *a priori* to determine the effects' intensity.

Additionally, some negative sampling effects were recorded with *Syngnathus scovelli* densities. At the beginning of August, South Site had significantly higher densities than North Site and outside of the sites. Although the sites were visually similar, it is possible unrecorded microhabitat differences contributed to the significance. As the sampling disturbance continued, the South Site density significantly decreased and became similar to the decreased densities in North Site and outside of the sites. Increased disturbance may have negatively impacted the densities within the site, but it does not explain the density decrease outside of the sites. More likely, a combination of disturbance influence and seasonal changes in movement patterns would explain the overall decreasing density in the fall. Prior studies have indicated that Gulf pipefish exhibit a seasonal shift in densities with high densities in the summer/rainy season and low densities in the winter/dry season (Masonjones et al. 2010; Bolland and Boettcher 2005; Díaz-Ruiz et al. 2000). The current study inadvertently captured this shift in seasonality and therefore, the intensity of disturbance effects on density cannot be disentangled from seasonal shifts during the August mark-resight experiment.

Although there is a difference in habitat utilization in February, the sampling disturbance effects can be assessed during the mark-recapture experiment. The February POPAN model indicated that the probability of fish entering and exiting the sites was dependent on the number of people sampling within the site. Sampling disturbance seemed to have slight negative non-significant effects on density since it decreased throughout the experiment, but at the end of the experiment there was no difference between densities inside and outside of the sites. Although previous studies have not indicated a drastic seasonal shift in densities during this time (Masonjones et al. 2010), density could still have unaccounted seasonal influences. These results are within a small timeframe of the population and do not account for long term seasonality variations within this population. Also, the marking process could have negatively impacted the densities. In gag grouper, *Mycteroperca microlepis*, densities declined as sampling progressed in mark-recapture transects, but did not change in transects without marking (Koenig and Coleman 1998). Conversely, in dwarf seahorses, *Hippocampus zosterae*, there were no differences for distances moved between recapture events (Masonjones et al. 2019). Sampling effects on densities from site disturbance or marking may vary by species and should be considered in future studies.

Concentric circular plots had slight negative sampling effects on site density, but linear transects yielded similar results to the concentric circular plots. At one month post mark-resight (October), immediately after mark-recapture (February), two weeks post mark-recapture (March), one month post sampling (April) and four months post sampling (July), there were no differences in densities between the circular plots and the linear transects indicating both methods produce comparable results. This was also the case for a study focused on forest sampling (Jones et al. 2022). Jones et al. (2022) suggested that since both concentric circular plot and linear transect methods were similar, ease of implementation would dictate which method to use. Conversely, linear transects by Nakajima et al. (2011) had more accurate estimations of woody vegetation variables in dense forests than concentric circular plots. Another concept in comparing the methods is tracking movement. Concentric circular plots are more accurate for small scale movement patterns (Dumont et al. 2004), while linear transects are more reasonable for identifying large scale movements (Masonjones and Rose, unpublished data; Bjorndal and Bolten 2010).

We were able to successfully couple mark-recapture techniques with the concentric circular plots, but it was difficult for the mark-resight model to converge. The August data did not fully fit the immigration-emigration resight model because we could not identify the number of marked individuals that could be recaptured prior to sampling. We had to assume that we captured all marked individuals within the sites, but that may not have been the case. It is possible that marked fish were in the site, but just outside of the net area, or that they were in the net area but swam out of it before the net was pulled.

Fish density ultimately dictated our sampling effort and therefore the Mark modeling. While both mark-resight and mark-recapture models can yield a superpopulation, they are incomparable to each other. The August mark-resight model's superpopulation is density-independent and only estimates the number of animals utilizing the area, unlike the February density-dependent mark-recapture model which estimates the total number of animals in the area (Cooch and White 2022). Estimating densities *ad hoc* from mark-resight models may overestimate or underestimate the density; however, Rich et. al (2014) had no significant difference between mark-recapture and *ad hoc* mark-resight density estimations. If pattern recognition programs like I^{3}S could identify patterning from a similar colored background, then we could use our August mark-resight photos as photographic mark-recapture and directly estimate densities (Martin-Smith 2011). We could then identify if unmarked fish or newly marked fish were previously captured during resight only samples and compare the density-dependent output to February. February models indicated fish were leaving the sites which may have resulted in underestimating the density. A mark-recapture study on *Nerophis lumbriciformis* experienced an underestimation in their Jolly-Seber capture recapture for an open population model due to fish traveling to other habitats (Monteiro et al. 2005). Occupancy sampling, as Erb et al. (2015) termed the immigration-emigration mark-resight model, should only be exercised over mark-recapture when an animal's habitat usage, and not density, is the main focus of a study.

Mark-recapture studies yield more information than a snapshot study. Both mark-recapture studies and snapshot studies can record demographics and morphometrics at a moment in time, but mark-recapture allows researchers to assess how animals utilize their environment (Lelong et al. 2024; Masonjones et al. 2019). In addition, recapturing the same individual across time points reveals growth rates, sexual development, and the ability to heal from injuries (Mellado et al. 2022). Without conducting a mark-recapture study on flagship species, novel information like growth rates and caudal fin regrowth would have never been identified in previous snapshot studies (Bolland and Boettcher 2005, Masonjones et al. 2010). Additionally, running mark-recapture studies at different timepoints in the year can elucidate seasonal habitat usage. In August, Gulf pipefish had a low superpopulation estimate and high recapture rates suggesting that pipefish are utilizing the area. Females had higher recapture rates, but males had more multiple recaptures. Although small scale movement patterns of Gulf pipefish might be influenced by tidal driven currents at their release, the currents were undetectable by a stream flow meter within the sites. Future studies where slow flow currents might impact behavior should use methods with better detection such as dye crystals (Ismail 1970; Wiegel et al. 1986) or hot wire anemometers (Melani et al. 2008).

At the South Site, a larger amount of *S. scovelli* were captured than the North Site possibly due to unidentified microhabitat differences. Adult densities in August were similar to the highest recorded total density (including juveniles) in the Indian River Lagoon system (0.69 fish/m^2 in 1998, Adams et al. 2022). August adult densities also were higher than the maximum recorded total densities in Tamiahua Lagoon, Mexico (0.0058 fish/m^2 in October 1994, Díaz-Ruiz et al. 2000) and in the same seagrass bed (1.04 fish/m^2 in 2006 wet season; Masonjones et al. 2010). More males were captured in August, but more females were recaptured. Females might be more area specific similar to *N. lumbriciformis* where lekking behavior was female specific and not site specific (Monteiro et al. 2017). Male counts decreased across study which could be related to a change in habitat usage or as a result of the disturbance within the site. Although males had lower recapture rates, there was a higher proportion of males captured multiple times. It is possible that overall, males travel shorter distances than females. This would support why we saw a larger proportion of recaptured males in Ring 1, although this could be biased towards the one outlier. We recaptured 17 males from South Site's Ring 1 on August 18th, 16 of them were marked on August 13th. Additionally, males might reduce activity and utilize a smaller habitat area when they are pregnant because they are not actively seeking mates, reducing feeding behaviors, or reducing the risk of predation (Paczolt and Jones 2015; Berglund 1993). Accurate assessment of habitat usage between pregnant and nonpregnant *S. scovelli* could not be analyzed due to the high percentage of pregnancy (Sims, Masonjones, and Rose, unpublished data), but future studies are encouraged to identify if pregnancy status changes male habitat use. Unlike *N. lumbriciformis*, it is unlikely *S. scovelli* have site fidelity but rather use areas within the seagrass bed as a home range (Monteiro et al. 2005). Broadening the scope of future studies could elucidate long range movement patterns and if these patterns differ by sex.

Although the immigration-emigration mark-resight model did not converge, themes from the top models give insights into the population. The top models estimated a low density-independent superpopulation suggesting that Gulf pipefish are utilizing the area around the sites. This is supported by the high recapture rates and multiple recaptures during the experiment. Although we cannot directly compare the mark-resight model to the mark-recapture model, it seems the habitat usage changed. In February, the density-dependent estimated superpopulation was similar to the density seen within the sites. Since the only time dependent parameter was the probability of entry/exit and the density decreased throughout the mark-recapture experiment, Gulf pipefish seemed to be transient throughout February. Additionally, the low recapture rates support that the population was passing through the sites. This transient habitat usage contrasts the August residential population suggesting habitat usage shifts throughout the year. The shift between habitat ranges could be for food availability, mate availability, or predator avoidance due to the shift in seagrass height and coverage.

Gulf pipefish facial patterning and female banding patterns were individually identifiable and consistent across three months. Picture lighting was not consistent, but visual matching indicated there are possible facial pigmentation changes like *N. lumbriciformis* (Monteiro et al. 2017). Further investigation similar to Monteiro et. al (2005 and 2017) is needed to determine if Gulf pipefish facial patterning is consistent long term, if facial patterns are asymmetrical between sides, and if facial pigmentation changes as well as signals for an individual’s readiness to mate.

Concentric circular plots were successfully implemented within a coastal marine seagrass bed. We were able to couple this sampling method with mark-resight and mark-recapture techniques using the model system *S. scovelli* to estimate population size and investigate small scale movement patterns after capture. Considering recent seagrass declines in Tampa Bay, this study reports the current baseline for Gulf pipefish. With the mark-resight experiment in August 2022 and the mark-recapture experiment in February 2023, we were able to identify that Gulf pipefish are more residential during the wet season, more transient during the dry season, and they likely do not have site fidelity. Although the mark-resight model did not converge, the density-independent August superpopulation estimated that the fish utilizing the area around our sites were similar to what was recorded within our sites. The February mark-recapture model was able to converge and estimated the density-dependent superpopulation to be 1,967 fish. Within both experiments, more males were captured, but proportionally more females were recaptured. In August, recaptured males disproportionally were collected within 2.5 m of their release location and were collected up to five times.

Some negative sampling effects occurred with the concentric circular plot method. Over the course of two experiments, repeated site disturbance negatively affected density, but there was no significant difference when comparing site density to outside locations. Additionally, this comparison indicated that there is no difference in data collected between concentric circular plots and linear transects. There was, however, a significant sampling effect on the habitat. Repeated trampling of the sites affected seagrass coverage, *T. testudinum* blade counts, short shoot counts, and average blade height. Both the net area and the walkway area yielded the same amount of disturbance. Based on these results, concentric circular plots are feasible in coastal communities, but researchers should be cautious about negative sampling effects. When studying a cryptic species, some level of disturbance is necessary to accurately assess the population. This disturbance could impact mark-recapture estimates, but the results are applicable for habitat and animal population management practices.

REFERENCES

Adams, D.H., A. Sebastian, and R. Paperno. 2022. Population decline of Gulf pipefish (*Syngnathus scovelli*) in a subtropical estuary: ecosystem changes and habitat loss. *Marine Biodiversity* 52 (5): 57.

Beck, M.W., K. Flaherty-Walia, S. Scolaro, M.C. Burke, B.T. Furman, D.J. Karlen, C. Pratt, C.J. Anastasiou, and E.T. Sherwood. 2024. Hot and Fresh: Evidence of Climate-Related Suboptimal Water Conditions for Seagrass in a Large Gulf Coast Estuary. *Estuaries and Coasts* 47 (6): 1475–1497.

Benadon, F. 2007. A circular plot for rhythm visualization and analysis. *Music Theory Online* 13 (3).

Berglund, A. (1993). Risky sex: male pipefishes mate at random in the presence of a predator. *Animal Behaviour 46*(1): 169–175.

Bjorndal, K.A., and A.B. Bolten. 2010. Hawksbill sea turtles in seagrass pastures: success in a peripheral habitat. *Marine Biology* 157: 135–145.

Bolger, D.T., B. Vance, T.A. Morrison, and H. Farid. 2011. Wild-ID user guide: pattern extraction and matching software for computer-assisted photographic mark-recapture analysis. Dartmouth College, Hanover, NH: 1–12.

Bolland, J., and A. Boettcher. 2005. Population structure and reproductive characteristics of the Gulf pipefish, *Syngnathus scovelli*, in Mobile Bay, Alabama. *Estuaries* 28: 957–965.

Cooch, E. and G.C, White. 2022. *Program MARK," A gentle introduction"*. http://www.phidot.org/software/mark/docs/book/. Accessed 4 Apr 2023.

Correia, M., J. Palma, H. Koldewey, and J.P. Andrade. 2014. The use of a non-invasive tool for capture–recapture studies on a seahorse *Hippocampus guttulatus* population. *Journal of Fish Biology* 84 (4): 872–884.

Curtis, J.M. 2006. Visible implant elastomer color determination, tag visibility, and tag loss: potential sources of error for mark–recapture studies. *North American Journal of Fisheries Management* 26 (2): 327–337.

Daugomah, J.W., T.C. Siewicki, D.E. Porter, and G.I. Scott. 2007. The effects of urbanization on populations of grass shrimp *Palaemonetes spp*. in small, high salinity estuaries. NOAA Technical Memorandum NOS NCCOS 54. https://aquadocs.org/handle/1834/30496. Accessed 17 Sept 2024.

Del Barrio, J., E. Luis-Calabuig, and R. Tárrega. 1999. Vegetative response of *Arctostaphylos uva-ursi* to experimental cutting and burning. *Plant Ecology* 145: 191–195.

Díaz-Ruiz, S., A. Aguirre-León, and O. Pérez-Soli. 2000. Distribution and abundance of *Syngnathus louisianae* and *Syngnathus scovelli* (Syngnathidae) in Tamiahua Lagoon, Gulf of Mexico. *Ciencias Marinas* 26 (1): 125–143.

Dumont, C., J.H. Himmelman, and M.P. Russell. 2004. Size-specific movement of green sea urchins *Strongylocentrotus droebachiensis* on urchin barrens in eastern Canada. *Marine Ecology Progress Series* 276: 93–101.

Dusek, G., J. Park, and C. Paternostro. 2017. Seasonal variability of tidal currents in Tampa Bay, Florida. *Journal of Waterway, Port, Coastal, and Ocean Engineering* 143 (3): 04016023.

Eckrich, C.E., and J.G. Holmquist. 2000. Trampling in a seagrass assemblage: direct effects, response of associated fauna, and the role of substrate characteristics. *Marine Ecology Progress Series* 201: 199–209.

Erb, L.A., L.L. Willey, L.M. Johnson, J.E. Hines, and R.P. Cook. 2015. Detecting long-term population trends for an elusive reptile species. *The Journal of Wildlife Management* 79 (7): 1062–1071.

Etherington, L.L., D.B. Eggleston, and W.T. Stockhausen. 2003. Partitioning loss rates of early juvenile blue crabs from seagrass habitats into mortality and emigration. *Bulletin of Marine Science* 72 (2): 371–391.

Flanagan, S.P., E. Rose, and A.G. Jones. 2021. The population genomics of repeated freshwater colonizations by Gulf pipefish. *Molecular Ecology* 30 (7): 1672–1687.

Findlay, R.H., L. Watling, and L.M. Mayer. 1995. Environmental impact of salmon net-pen culture on marine benthic communities in Maine: a case study. *Estuaries* 18: 145–179.

Furman, B.T., E.H. Leone, S.S. Bell, M.J. Durako, and M.O. Hall. 2018. Braun-Blanquet data in ANOVA designs: comparisons with percent cover and transformations using simulated data. *Marine Ecology Progress Series* 597: 13–22.

Gasparini, J.L., and R.L. Teixeira. 1999. Reproductive aspects of the gulf pipefish, *Syngnathus scovelli* (Teleostei: Syngnathidae), from Southeastern Brazil. *Revista Brasileira de Biologia* 59: 87–90.

Greening, H., A. Janicki, and E.T. Sherwood. 2018. Seagrass recovery in Tampa Bay, Florida (USA). *The Wetland Book: II: Distribution, Description, and Conservation* 495–506.

Hamdi, B., S. Limam, and T. Aguili. 2016. Uniform and concentric circular antenna arrays synthesis for smart antenna systems using artificial neural network algorithm. *Progress In Electromagnetics Research B* 67: 91–105.

Hao, L.K., A.R. Ghani, P. Edi, and K. Yusuf. 2009. Impact energy absorption of concentric circular tubes. *WSEAS Transactions on Applied and Theoretical Mechanics* 4 (3): 95–104.

Hartog, J., and R. Reijns. 2014. Interactive individual identification system (I^3S) pattern. Reijns Free Software Foundation Inc, Boston.

Ismail, N.M. 1970. Effect of wave-current induced circulation on coastal structure design. *WIT Transactions on The Built Environment* 70.

Johnson, E.G., and D.B. Eggleston. 2010. Population density, survival and movement of blue crabs in estuarine salt marsh nurseries. *Marine Ecology Progress Series* 407: 135–147.

Jones, A.G., and J.C. Avise. 1997. Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role reversal. *Molecular Ecology* 6 (3): 203–213.

Jones, C., A. Van Dongen, J. Harvey, and D. Degenhardt. 2022. Field sampling methods on seismic lines: a comparison between circular plots and belt transects. *Restoration Ecology* 30 (7): e13619.

Joseph, E.B. 1957. A study of the systematics and life history of the gulf pipefish, *Syngnathus scovelli* (Evermann and Kendall). Dissertation. Florida State University.

Koenig, C.C., and F.C. Coleman. 1998. Absolute abundance and survival of juvenile gags in sea grass beds of the northeastern Gulf of Mexico. *Transactions of the American Fisheries Society* 127 (1): 44–55.

Laake, J. 2013. RMark: An R Interface for Analysis of Capture-Recapture Data with MARK. AFSC Processed Report 2013-01, Alaska Fisheries Science Center, NOAA, National Marine Fisheries Service, Seattle, WA. https://apps-afsc.fisheries.noaa.gov/Publications/ProcRpt/PR2013-01.pdf. Accessed 26 Sept 2023.

Lanyon, J.M., H.L. Sneath, J.M. Kirkwood, and R.W. Slade. 2002. Establishing a mark-recapture program for dugongs in Moreton Bay, south-east Queensland. *Australian Mammalogy* 24: 51–56.

Lelong, P., A. Besnard, M. Girondot, C. Habold, F. Priam, M. Giraudeau, G. Le Loc'h, A. Le Loc'h, P. Fournier, C. Fournier-Chambrillon, P. Bustamante, S.M. Dupont, O. Vincze, J. Gros-Desormeaux, J. Martin, O. Bourgeois, M. Lepori, S. Régis, N. Lecerf, F. Lefebvre, N. Aubert, C. Frouin, F. Flora, E. Pimentel, M. Pimentel, F. Siegwalt, L. Jeantet, P. Chambault, G. Hielard, A. Arqué, M. Arthus, L. Louis-Jean, A. Brador, P. Giannasi, D. Etienne, N. Lecerf, P. Chevallier, T. Chevallier, S. Meslier, A. Landreau, M. Maceno, E. Larcher, Y. Le Maho, and D. Chevallier. 2024. Demography of endangered juvenile green turtles in face of environmental changes: 10 years of capture-mark-recapture efforts in Martinique. *Biological Conservation* 291: 110471.

Lizcano-Sandoval, L., D. Otis, E. Montes, and F. Muller-Karger. 2023. Seagrass areal cover in Tampa Bay over the last 30 years (1990–2021) observed by satellites. *Florida Scientist* 86 (2): 331–338.

Martin-Smith, K.M. 2011. Photo-identification of individual weedy seadragons *Phyllopteryx taeniolatus* and its application in estimating population dynamics. *Journal of Fish Biology* 78 (6): 1757–1768.

Masonjones, H.D., E. Rose, L.B. McRae, and D.L. Dixson. 2010. An examination of the population dynamics of syngnathid fishes within Tampa Bay, Florida, USA. *Current Zoology* 56 (1): 118–133.

Masonjones, H.D., E. Rose, and M.C. Masonjones. 2019. Techniques used to increase recapture rates of dwarf seahorses (*Hippocampus zosterae*) in Tampa Bay: Implications for population estimates and movement patterns. *Gulf and Caribbean Research* 30 (1): 10–19.

Melani, M., L. Bertini, M. De Marinis, P. Lange, F. D'Ascoli, and L. Fanucci. 2008. Hot wire anemometric MEMS sensor for water flow monitoring. *Proceedings of the conference on design, automation and test in Europe* 342–347.

Mellado, B., L.D.O. Carneiro, M.R. Nogueira, and L.R. Monteiro. 2022. The impacts of marking on bats: mark-recapture models for assessing injury rates and tag loss. *Journal of Mammalogy* 103 (1): 100–110.

Monteiro, N.M., M. da Natividade Vieira, and V. Almada. 2005. Homing behaviour and individual identification of the pipefish *Nerophis lumbriciformis* (Pisces; Syngnathidae): a true intertidal resident?. *Estuarine, Coastal and Shelf Science* 63(1–2): 93–99.

Monteiro, N.M., D. Carneiro, A. Antunes, N. Queiroz, M.N. Vieira, and A.G. Jones. 2017. The lek mating system of the worm pipefish (*Nerophis lumbriciformis*): a molecular maternity analysis and test of the phenotype-linked fertility hypothesis. *Molecular Ecology* 26 (5): 1371–1385.

Nakajima, N.Y., S.P. Netto, J.P. Druszcz, and M. Yoshitani Junior. 2011. Precision comparison between line sampling and concentric circular plot sampling methods in *Pinus elliottii* stands. Revista Acadêmica Ciências Agrárias e Ambientais 9 (2): 131–139.

Nanos, N., and S.S. de Luna. 2017. Fitting diameter distribution models to data from forest inventories with concentric plot design. *Forest Systems* 26 (2): e01S–e01S.

Nurdin, N., Y. La Nafie, M.T. Umar, M. Jamal, and A. Moore. 2019. Preliminary study: human trampling effects on seagrass density. *IOP Conference Series: Earth and Environmental Science* 370 (1): 012050.

Paczolt, K.A., and A.G. Jones. 2015. The effects of food limitation on life history tradeoffs in pregnant male Gulf pipefish. *PLoS One* 10 (5): e0124147.

Poore, M.E.D. 1955. The use of phytosociological methods in ecological investigations: I. The Braun-Blanquet system. *Journal of Ecology* 43 (1): 226–244.

Protocol: Seagrass Macroalgae. 2020. Tennenbaum Marine Observatories Network, MarineGEO, Smithsonian Institution. https://marinegeo.github.io/assets/modules/seagrass-macroalgae/marinegeo_protocol_seagrass_macroalgae.pdf. Accessed 4 June 2022.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reynolds, R.T., J.M. Scott, and R.A. Nussbaum. 1980. A variable circular-plot method for estimating bird numbers. *The Condor* 82 (3): 309–313.

Rich, L.N., M.J. Kelly, R. Sollmann, A.J. Noss, L. Maffei, R.L. Arispe, A.P. Paviolo, C.D. De Angelo, Y.E. Di Blanco, and M.S. Di Bitetti. 2014. Comparing capture-recapture, mark-resight, and spatial mark-resight models for estimating puma densities via camera traps. *Journal of Mammalogy* 95 (2): 382–391.

Ripple, W.J., P.D. Lattin, K.T. Hershey, F.F. Wagner, and E.C. Meslow. 1997. Landscape composition and pattern around northern spotted owl nest sites in southwest Oregon. *The Journal of Wildlife Management*: 151–158.

Rose, E., M. Simmonds, A.L. Hayashida-Boyles, and H.D. Masonjones. 2019. Seasonal and spatial variation in the reproductive biology of the dwarf seahorse *Hippocampus zosterae*. *Journal of Fish Biology* 95 (2): 357–366.

Schoch, G.C., D.M. Albert, and C.S. Shanley. 2014. An estuarine habitat classification for a complex fjordal island archipelago. *Estuaries and Coasts* 37: 160–176.

Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis". *Nature Methods* 9: 671–675.

Sherwood, E.T., H.S. Greening, J.R. Johansson, K. Kaufman, and G.E. Raulerson. 2017. Tampa Bay (Florida, USA) documenting seagrass recovery since the 1980's and reviewing the benefits. *Southeastern Geographer* 57 (3): 294–319.

Shokri, M.R., W. Gladstone, and J. Jelbart. 2009. The effectiveness of seahorses and pipefish (Pisces: Syngnathidae) as a flagship group to evaluate the conservation value of estuarine seagrass beds. *Aquatic Conservation: Marine and Freshwater Ecosystems* 19 (5): 588–595.

Short, F.T., and R.G. Coles. 2001. Global seagrass research methods. Elsevier.

Smith, R.H. 1968. A comparison of several sizes of circular plots for estimating deer pellet-group density. *The Journal of Wildlife Management* 585–591.

Strawn, K. 1958. Life history of the pigmy seahorse, *Hippocampus zosterae* Jordan and Gilbert, at Cedar Key, Florida. *Copeia* 1958 (1): 16–22.

Tosto, N.M., E. Rose, S.P. Flanagan, and H.D. Mason. 2023. The development of a quantification method for measuring iridescence using sexually selected traits in the Gulf pipefish (*Syngnathus scovelli*). *Frontiers in Marine Science* 10: 1127790.

White, G.C. and K.P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. *Bird Study* 46 (sup 1): 120–138.

Wiegel, R.L., J.T. Wells, and M.A. Murdoch. 1986. Cooling Water Recirculation in the Ocean. *Coastal Engineering 1986*: 2694–2705.

Williams, J.A., G.J. Holt, M.M.R. Robillard, S.A. Holt, G. Hensgen, and G.W. Stunz. 2016. Seagrass fragmentation impacts recruitment dynamics of estuarine-dependent fish. *Journal of Experimental Marine Biology and Ecology* 479: 97–105.

Woods, C.M., and K.M. Martin-Smith. 2004. Visible implant fluorescent elastomer tagging of the big-bellied seahorse, *Hippocampus abdominalis*. *Fisheries Research* 66 (2-3): 363–371.

APPENDIX A

Institutional Animal Care and Use Committee (IACUC) Approval

Institutional Animal Care and Use Committee (IACUC)

ANIMAL USE PROTOCOL APPROVAL

August 30, 2022

Dr. Emily Rose
Department of Biology
Valdosta State University

Dear Dr. Rose;

Animal Use Protocol (AUP) *"Spatial Analysis of the "Syngnathus scovelli Population in Tampa Bay"* (AUP-00082-2022) has been approved by the Institutional Animal Care and Use Committee (IACUC). This approval is from 08.25.2022 – 08.25.2025. Each year, an animal report must be submitted to the IACUC to keep your protocol active. You will be contacted by the Office of Sponsored Programs and Research Administration approximately one month before the annual report is due.

Please remember that you must obtain IACUC approval before amending, or altering the scope, or procedures of the protocol. You are also required to report to attending Veterinarian, the IACUC Chair, and the IACUC Administrator any unanticipated problems with the animals that become apparent during the course, or as a result of the research, or teaching activity.

Should you have questions concerning your approved research, please contact Tina Wright, Research Compliance Specialist, at 229.253.2947, or email IACUC at jacuc@valdosta.edu.

Sincerely,

Ann

Elizabeth "Ann" Olphie
IACUC Administrator

cc: Dr. Becky da Cruz, Associate Provost for Graduate Studies and Research
Dr. Teresa Doscher, Attending Veterinarian
Dr. Robert L. Gannon, Department Head

OFFICE of SPONSORED PROGRAMS & RESEARCH ADMINISTRATION (OSPRA)
PHONE 229.259.5045 **FAX** 229.245.3853 **WEB** www.valdosta.edu/opsra
ADDRESS 1500 N. Patterson St. Valdosta, GA 31698 **LOCATION** Third Floor – Psychology Building
A Regional University of the University System of Georgia and an Equal Opportunity Institution