

ABSTRACT

TORRES-MOLINARI, ÁMBAR. Population Characteristics and Parasitology of the American Eel in Puerto Rico. (Under the direction of Dr. Jaime A. Collazo and Dr. Thomas J. Kwak)

The American Eel (*Anguilla rostrata*) is a widespread facultative catadromous fish with a complex life history in need of better understanding. They are an economically, ecologically, and culturally important resources, but threatened species. American Eel are distributed from southeastern Greenland to Northeastern Venezuela, including the Gulf of Mexico, and the Caribbean but this species exists as a single spawning population in the Sargasso Sea of the Atlantic Ocean. The American Eel has been well documented throughout North America and Canada, yet little is known about the species in the Caribbean and South America.

Through this research we sought to understand the trends in longitudinal distribution of American Eel population characteristics, such as: density, body length, and sex ratio in rivers of Puerto Rico, and compare these to trends found in temperate regions. The second objective of our research was to document presence of parasite infections in Puerto Rico. We sampled 23 stream reaches via a 3-pass pulsed-DC electrofishing method across a longitudinal gradient in 5 rivers during 2 sampling events. We dissected 233 American Eel from 5 rivers in Puerto Rico to identify sex and assess presence of the swimbladder parasite (*Anguillicoloides crassus*) infection and evaluated presence of Monogenean parasite infections in 79 of the dissected eel. We fit a series of simple and multiple linear regression and logistic regression models to determine if American Eel population characteristics were affected by distance from the river mouth. The same process was used in developing models to determine if American Eel condition was affected by Monogenean parasite intensity and if parasite intensity varied by river and distance from the river mouth.

We found a mean density of 156.7 fish/ha and a sex ratio strongly favoring females (52:3). Female eel had a mean length of 463 mm, and male eel were twice as small with an average of 260 mm. The top linear regression model indicated that American Eel density generally decreases with increasing distances from the river mouth, and that American Eel body length increases with distance from the river mouth in Puerto Rico. Logistic regression modelling indicated that female eel are more likely to occur at sizes greater than 350 mm and at upstream environments, rather than close to the river mouth. American Eel in Puerto Rico exhibit the same longitudinal trends in density, body length, sex ratios as American Eel from temperate regions.

Our study suggests that the swim bladder parasite is absent from freshwater environments in Puerto Rico. The gills of American Eel were infected by Monogenean gill parasites. Overall Monogenean prevalence was 41.8%, while mean intensity was 10 parasites/eel. These parasites were not identified at the species level. Linear and logistic regression models indicate that American Eel remain within the bounds of good relative condition, but Monogenean intensity may decrease American Eel condition. Larger American Eel are more likely to harbor greater Monogenean intensities increased.

Our findings may provide managers with information regarding the status and longitudinal trends of American Eel in the Caribbean region to understand the overall contribution of this region to the overall spawning population. Furthermore, it will provide a better understanding of the geographic extent and level of threat of parasite infections in American Eel from Puerto Rico. This may significantly improve the reassessment of the species, help prioritize stream connectivity, and promote conservation of the American Eel in Puerto Rico to ensure the survival of this population.

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Population Characteristics and Parasitology of the American Eel in Puerto Rico

by
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DEDICATION

To my dear friend, family, and forever advisor, Dr. Thomas J. Kwak, and his family. Thank you for always supporting and believing in me. You taught me some of the best life and professional lessons that I will carry with me forever. I will count myself lucky if I am able to positively affect the life of one person, the way you did for so many of us. We miss you every day.

BIOGRAPHY

I was born in San Juan, Puerto Rico, in 1995, and resided in the island all my life, until moving to Raleigh, North Carolina. My story might be a bit different than that of a traditional fisheries biologist. Hook, line, and fish were not in my family's traditions. My upbringing revolved around music, art, and the humanities. However, throughout my educational years, I developed a deep interest and value for environmental sciences, fisheries biology, and conservation. The experiences I acquired by working with freshwater fish and the valuable mentors I have been fortunate to have inspired and led me to where I am today.

I started my undergraduate career in Environmental Sciences at the University of Puerto Rico, Rio Piedras Campus. I was interested in having a career in the ecological sciences, little did I know that fisheries biology would be an option. Until I started brainstorming research topics for my undergraduate research thesis with my then advisor Dr. Jorge Ortiz-Zayas, who briefly mentioned the *Sicydium plumieri* goby post-larval migrations in the estuary of a river in Puerto Rico. I was curious enough to decide to do field research and document this migration phenomenon that would occur 2 days after the third quarter moon phase in November and I made sure to drag my family and friends to help at those hours of the night/morning. I tirelessly worked from 11 p.m. to 7 a.m. sampling and taking measurements of these transparent and numerous fish. That night I had found my place within the ecological sciences and knew that fisheries biology and conservation was a career I would tirelessly pursue. With the help of my advisors Dr. Jorge Ortíz-Zayas and Dr. Omar Pérez-Reyes, I was able to present my research thesis at the 2018 Southern Division of the American Fisheries Society Annual Meeting in San Juan, Puerto Rico. Through this research, I was able to make connections with Dr. Augustin C. Engman,

Bonnie J. Myers, and Dr. Thomas J. Kwak, who have played an integral part in my growth as a fisheries biologist and an individual.

I graduated from the University of Puerto Rico, Rio Piedras Campus in 2018 with a B.Sc. in Environmental Sciences and went on to work as a field technician for several fisheries projects in the island. I gained extensive experience in fish sampling throughout diverse rivers in Puerto Rico with the Virginia Tech Center for Aquatic Technology Transfer and the US Forest Service. I went on to assist North Carolina Cooperative Fish and Wildlife Research unit PhD student, Bonnie J. Myers, who advised by Dr. Thomas J. Kwak, was conducting fish sampling and mesocosm experiences to evaluate fish behavior during extreme weather events in Puerto Rico. This was one of the most rewarding and challenging jobs I was a part of, where I learned everything from electric circuits to re-building and repairing complex artificial river systems. I will never forget some of the field excursions I took with Dr. Thomas J. Kwak and his students to look for fish and goby migrations throughout the island. All these experiences shaped my interests in freshwater fish biology and conservation.

After a year of continuously being in the field, on January 2020, I started my graduate career as a M.Sc. student for the North Carolina Cooperative Fish and Wildlife Research Unit at NC State University with Dr. Thomas J. Kwak. This would be the first time I would live in the United States for more than 6 days. I took the risk of moving to a new country, with all my family and friends back in Puerto Rico. A decision made less difficult by one reason: the overwhelming support of my advisor, his students, and the Applied Ecology department. Since 2020, I have been conducting research aimed at documenting the population characteristics and parasites of American Eel in the Caribbean to provide managers with valuable data where the species has been least documented. Throughout my career at NC State, I have also gained

valuable knowledge and experience in science communication by working with the National Climate Adaptation Science Center and my SciComm advisor, Michelle A. Jewell.

The experience and training I gained as a M.Sc. graduate student in fisheries, wildlife, and conservation biology at NC State University have prepared me to continue my career in fisheries conservation and science communication. My goal is to continue a career in these areas to continue improving management and conservation decisions of fish populations. I hope to continue my education by pursuing a PhD in fisheries biology or related field.

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I would like to thank my advisor and friend, Dr. Thomas J. Kwak, for his incredible support and encouragement. You helped me grow as a research biologist and a person, teaching me the importance of caring for others and letting the science follow. You were extremely proud of all the students you advised, but we were equally as proud of you. Dr. Kwak passed away on November 19, 2021, leaving an extensive legacy of knowledge in the field of fisheries conservation and aquatic ecology. He was an irreplaceable force of positive and humble leadership in the field of fisheries and in the lives of many. His valuable mentorship, passion for collaborative research, and advocacy for diversity and equality in the field will have a lasting impact on those he mentored and educated, and those who knew him.

I would also like to thank my committee members: Dr. Krishna Pacifici, Dr. Alonso Ramírez, Dr. Andrew C. Dolloff, Dr. Jaime A. Collazo, and Dr. Augustin C. Engman. Thank you all for the guidance with field plans, data analysis, and support during some challenging times. Your guidance has not only made a significant contribution to my research, but I have grown as a biologist because of it. I wish there were words bigger than “thank you” for the overwhelming support from the NC Cooperative Fish and Wildlife Research Unit, the Department of Applied Ecology, and my American Fisheries Society, Puerto Rico Chapter family. To these entities, my committee members, Dr. Nathan Hostetter, Dr. Greg W. Cope, Ruby Valetton, Michelle A. Jewell, and countless others: I reached the finish line because of your invaluable support and encouragement.

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Sánchez-Medina, for your hard work and positivity throughout those long challenging days in the field, being the best American Eel catchers, and putting up with my madness. Thank you to Mike Cush and Annika Preheim for their help with American Eel dissections. A very special thank you to my partner in crime, Bonnie J. Myers, who supported and encouraged me during those field work months for over 2 years. I could not have stayed sane through those bad weather and fishless days without your help.

I would like to thank my family and friends for their incredible support. To my family, friends, and partner in Puerto Rico, although far away, your warmth, support, and love has kept me going throughout these 3 years. I miss you all every day, but this all for you! To my friends, turned family in North Carolina, the McIver family, Clayton Lynch, the Duthie-Goodson family, Bonnie J. Myers, Ana Castillo, Augustin C. Engman, the Kwak family, Matt Damiano, and many more, thank you for always supporting me and helping me during the challenging parts of life. Your support has meant so much to me.

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CHAPTER 1

Trends in Longitudinal Distribution of American Eel (*Anguilla rostrata*)

Population Characteristics in Rivers of Puerto Rico

Abstract

American Eel (*Anguilla rostrata*) population characteristics and distribution remain drastically understudied in the Caribbean region. We conducted the first island-wide study to evaluate the distribution of density, length, and sex ratios in relation to distance from the river mouth in Puerto Rico. We sampled 23 reaches across a longitudinal gradient in 5 rivers during 2 sampling events. We found 287 American Eel and a mean density of 156.7 fish/ha. Sex ratios disproportionately favored females (52:3). Linear regression modeling and generalized linear modeling indicate that low density upstream habitats may be optimal environments for the production of large female American Eel. These findings strengthen the knowledge base on American Eel in the Caribbean and can be used to guide local and range-wide conservation and management efforts to ensure stream connectivity and access to optimal upstream habitats for the survival of the population.

Introduction

The American Eel (*Anguilla rostrata*) is a widespread facultative catadromous fish with a complex life history that needs better understanding. They are a valuable economic, biologic, and cultural resource, but threatened in the northern portion of its range (Davis et al., 2004; ASMFC, 2017). The distribution of American Eel extends from southeastern Greenland to Northeastern Venezuela, including Central America, the Gulf of Mexico, and the Caribbean but this species exists as a single, panmictic population that spawns in a single location, the Sargasso Sea of the Atlantic Ocean (Benchetrit and McCleave, 2016). Adult American Eel die after reproduction and the larvae, known as leptocephali, are carried by ocean currents to diverse estuarine and freshwater environments across the wide geographical range (McCleave and Kleckner, 1987; Tsukamoto et al., 1998; Haro et al., 2000; Benchetrit and McCleave, 2016). Leptocephali metamorphose into sexually undifferentiated glass eel during their migration into estuaries where they subsequently develop into elver and yellow eel (McCleave and Kleckner, 1982; Oliveira and McCleave, 2000). They can reside in freshwater, saltwater, and brackish environments until reaching sexual maturity as silver eel, when they begin their descent and extensive migration to the Sargasso Sea to spawn and complete their life cycle (Tsukamoto et al., 1998; Tesch, 2003; Lamson et al., 2006; Thibault et al., 2007; McCleave and Edeline, 2009). The American Eel's ability to occupy multiple habitats and their widespread dispersal has posed challenges in identifying distribution patterns in continental waters throughout its range. It is essential to understand the distribution of population characteristics of American Eel along longitudinal gradients, such as: density, length, and sex ratios. This information will aid in the management and conservation of the species by improving understanding of the importance of stream

connectivity and the effects of instream barriers, and the reproductive potential throughout the species range to the overall population.

Throughout its broad range, American Eel are an economically and ecologically valuable but threatened species. It serves as a major predator in freshwater habitats and as prey for many species in both marine and freshwater habitats (Helfman and Clark, 1986; Béguyer-Pon et al., 2012; Thompson et al., 2005; Engman et al., 2017). American Eel are also a valuable economic and cultural fisheries resource throughout its geographical distribution, where American Eel at all life stages are harvested by many indigenous tribes and other peoples (Davis et al., 2004; Arai, 2014; ASMFC, 2017). American Eel are primarily exported to Asian countries for aquaculture where they are an important nutritional source (MacGregor et al., 2008; COSEWIC, 2012; ASMFC, 2017). Despite this, American Eel are imperiled and face many threats such as: instream barriers, pollutants, climate change, environmental impacts, and parasitic diseases (Hein et al., 2014; Jessop and Lee, 2016; Kwak et al., 2016; Buttermore et al., 2018). These threats have been linked to abundance declines and high risk of extinction in North American and Canadian populations (COSEWIC, 2012; ASMFC, 2017; Jacoby et al., 2017). Species assessments in North America determined the species did not warrant protection throughout its entire range despite being listed as endangered by the International Union for Conservation of Nature Red List of Threatened Species (Shepard, 2015; Jacoby et al., 2017). Range-wide assessments are needed to understand the overall trends and conservation needs of the species (Cairns et al., 2022), but this has proven to be challenging due to knowledge gaps from southern parts of its distribution.

Studies from temperate regions indicate trends in size, sex, and density distributions along estuaries, rivers, and latitudinal gradients in the American Eel population (Goodwin and

Angermeier, 2003; Jessop, 2010). Most commonly, abundance and density decrease inland compared to environments closer to the ocean, especially in highly dammed rivers (Smogor et al., 1995; Goodwin and Angermeier, 2003; Wiley et al., 2004; Camhi et al., 2021). Low female abundance has been linked to downstream and brackish water environments with high American Eel densities and overcrowding (Krueger and Oliveira, 1999) whereas upstream and freshwater environments have been shown to harbor predominantly female eel (Helfman et al., 1984; Goodwin and Angermeier, 2003; Bonvechio et al., 2018). Females are typically longer and heavier than males. Increased frequency of females and larger individuals are associated with upstream and freshwater environments (Hansen and Eversole, 1984; Helfman et al., 1984; Goodwin and Angermeier, 2003; Oliveira and McCleave, 2002; Morrison and Secor, 2003; Hightower and Nesnow, 2006; Weeder and Hammond, 2009). Size and proportion of female American Eel have also been documented to increase with latitude (Jessop, 2010; Vélez-Espino and Koops, 2010). The longer sizes at upstream and freshwater habitats may be related to low densities resulting in less competition and higher food availability (Smogor et al., 1995; Krueger and Oliveira, 1999; Oliveira, 1999; Vélez-Espino and Koops, 2010; Goodwin and Angermeier, 2003). Although these trends have been documented for northern ranges, it is poorly understood if populations from the species' southern range exhibit similar trends.

The status and ecology of American Eel in the Caribbean, Central America, and South America is largely unknown. General presence and occurrences of commercial fishing have been noted throughout the Caribbean and South America (Benchetrit and McCleave, 2016; ASMFC, 2017; CITES, 2018). Recent descriptions of American Eel populations in the Caribbean described the species as widespread in Puerto Rico and predominantly threatened by instream barriers and habitat loss (Cooney and Kwak, 2013; Kwak et al., 2013, 2019; Roghair et al.,

2014). Preliminary evidence from island-wide data indicates that large and female eel occur in Puerto Rico and that lower densities may occur in upstream environments (Kwak et al., 2019).

Currently, no studies have documented trends in population characteristics longitudinally (downstream – upstream) in Caribbean streams. Puerto Rico presents an ideal location to address these knowledge gaps. The island is located directly South of the Sargasso Sea, research there may provide knowledge about the reproductive potential and contributions of southern portions of the population, and those located at shorter distances from the spawning location. Compared to rivers in temperate regions, the short length of rivers in Puerto Rico allows for a better understanding of population trends along longitudinal gradients covering the entirety of a river system. Our goal is to quantitatively evaluate trends in size, sex, and density of American Eel with increasing distances from the river mouth and compare these trends to those found in temperate regions.

Methods

Study Design

We collected American Eel from November to January in 2020 (dry season) and from June to August in 2021 (wet season). During the dry season we sampled 5 rivers: Río Piedras, Río Mameyes, Río Sabana, Río Matilde, and Río Guanajibo, which are located throughout the northern, northeastern, southern, and western regions of Puerto Rico (Figure 1). We selected 4 to 6 sampling locations on each river, distributed from downstream to upstream environments (Figure 1; Table 1). We sampled a total of 23 sites, 14 of which were resampled during the wet season. These sites were located in Río Piedras, Río Mameyes, and Río Matilde and were selected based on the lack of in-stream barriers to migration, accessibility for eel (Cooney and Kwak, 2016), and known occurrence of American Eel (Roghair et al., 2014, Kwak et al., 2019). The Río Piedras was selected to evaluate any implications of urbanization on American Eel subpopulations.

We sampled American Eel using pulsed direct current backpack electrofishers and employed a 3-pass removal procedure to estimate density of American Eel at each site. We measured a 60 m long stream reach and placed block nets on each end. Upstream block nets were not placed on reaches with natural in-stream barriers or locations of high-water velocity that would impede escapement from the reach. Additionally, we measured several aspects of the physical habitat of each sampling reach including reach length, mean stream width, elevation, gradient, discharge, and distance from the river mouth. Density estimates for each of the sampling sites were calculated from population size estimates using the Carle-Strub depletion method and reach area measurements. The Carle-Strub method (via the FSA package in R) uses a maximum weighted likelihood estimation method (Carle and Strub, 1978; Hedger et al., 2013;

Ogle, 2016). American Eel density at each site was obtained by dividing population size estimates by their respective reach area measurements obtained from mean reach widths and lengths.

We took biological measurements of American Eel and sacrificed a subset of individuals from each site for gonadal sex determination. We recorded total length (mm), wet weight (g), and life stage of all collected eel. We sacrificed a subset of individuals for sex determination using a solution of MS-222, following IACUC protocol 20-216. These subsets were selected based on the abundance and lengths of eel present at each site. We predominantly used a ≥ 150 -200 mm cutoff and released all eel below 150-200mm at sites with a wide size distribution (Krueger and Oliveira, 1999; Oliveira and McCleave, 2000; Machut et al., 2007). We collected individuals < 150 mm whenever abundance of sizes greater than the cutoff were not present.

Gonadal Analysis

American Eel sacrificed from all sampling sites were transferred to the laboratory facility at North Carolina State University for sex determination. We dissected and macroscopically identified gonadal tissue to determine if eel were female, male, or undifferentiated following descriptions from Tesch (2003). Whenever macroscopic identification was not possible, we use the gonad squash technique by extracting and staining a piece of gonadal tissue with an aceto-carmine solution, pressing it between 2 microscope slides, and evaluating the tissue through a compound microscope to identify presence and absence of oocytes or spermatogonia following descriptions from Guerrero and Shelton (1974).

Models of American Eel Density and Body Length

We used Simple and Multiple Linear Regression Models (SLRs and MLRs) to assess how American Eel density and body length are influenced by geographic and temporal variables in

Puerto Rico rivers. We fit density and body length models as functions of river, distance from river mouth, season, an interaction between distance from the river mouth and river, and density (for the body length model, only). We explored all subsets of these global models to identify the best fitting model. Prior to model fitting we calculated spearman correlation values for all continuous covariates and removed density from the body length model to control for collinearity issues with distance from the river mouth. The continuous covariate, distance from the river mouth, was scaled by subtracting the mean and dividing by the standard deviation.

To select the top model explaining variation in density of American Eel we ranked all candidate models using Akaike Information Criterion, corrected for small sample sizes (AIC_{ci}), delta-AICc (Δ_i (AICc)) and weighted AICc (w_i (AICc)) (Burnham and Anderson, 2003). We calculated the 95% confidence set of linear regression models with cumulative AIC_{ci} weight ≥ 0.95 (Burnham and Anderson, 2002). In addition, we used the R^2 values to measure the predictive ability of the top model and competitive models. We evaluated the top model's coefficient (s) ($\hat{\beta}$) for a statistically significant influence on density ($\alpha=0.05$), with primary focus on distance from river mouth. The same procedure was used to select the top model that best explained variation in body length.

We generated and plotted predicted values of density and body length using their respective top models to visualize any significant relationships. We calculated and plotted predicted values of eel density and body length over new values of the covariate of interest while holding all other covariates constant at their mean values or levels, if applicable. The distance from the river mouth covariate was back-transformed, if significant, to its original scale (km) before plotting by adding the mean and multiplying by the standard deviation. The mean and standard deviation from the scaled values were obtained from the scale function in base R. A

95% confidence interval (CI) was calculated for all predicted values of eel density. The same procedure was applied to evaluate any significant trends in eel body length.

Female Eel Presence Models

We fit a logistic regression model with a binomial distribution to model probability of females as a function of river, distance from the river mouth, American Eel length, an interaction between distance from the river mouth and length, and an interaction between distance from the river mouth and river. The predictors and interactions included in the model were based on significant interactions found on the previous density and body length models and previously documented relationships (Smogor et al., 1995; Krueger and Oliveira, 1999; Jessop, 2000; Vélez-Espino and Koops, 2010; Goodwin and Angermeier, 2003, Kwak et al., 2019). The binary response variable, presence of female eel, was indicated by a value of 1 and males/undifferentiated eel were indicated by zero (denoting an absence of females).

We ranked models using the same procedure used for the density and body length models, Akaike Information Criterion, corrected for small sample sizes ($AICc_i$), delta- $AICc$ ($\Delta_i(AICc)$), and weighted $AICc$ ($w_i(AICc)$) (Burnham and Anderson, 2002). We used pseudo- R^2 values to assess goodness of fit. We conducted model averaging with the MuMIn R package using the 95% confidence set of logistic regression models to account for model selection uncertainty (Burnham and Anderson, 2002). The 95% confidence set consisted of models with a cumulative $AICc_i$ weight ≥ 0.95 (Burnham and Anderson, 2002).

We generated and plotted predicted probabilities of female eel presence using the model averaged coefficients to visualize any significant relationships with predictors of interest. First, we obtained the predicted log odd values of female presence over the predictor variable of interest while holding all other covariates in the model constant at their mean values for each

river and/or season, if included in the confidence set of models. After obtaining the predicted log odds of female presence, we converted these into probabilities by exponentiating each log odd value and dividing it by 1 minus the exponentiated log odds value. Back transformation and plotting of these values followed the same procedure applied for the density and body length models.

Results

Puerto Rico American Eel Population Characteristics

We caught 287 American Eel from 5 rivers in Puerto Rico between November 2020 and August 2021 (Table 2, Table 4). American Eel were present at 19 of 23 sampling sites (83%) during the dry season sample period and at 13 of 14 sites (93%) during the wet season (Table 2). We depleted 16 of 17 (94.1%) sites sampled during the dry season and 11 of 13 (84.6%) sites during the wet season. Densities varied between sampling locations and seasons with an overall mean of 156.7 fish/ha and maximum of 700.5 fish/ha (Table 2). American Eel densities were generally higher closer to the mouth of the rivers and decreased with distance from the river mouth (Table 2). We performed dissections on 233 American Eel for sex determination (Table 4). Of those dissected, 11 were caught outside of the sampling reaches to increase sample size for sex determination, mainly during the dry season in 2020-2021. Among the dissected fish, 55 (19.2%) had developed gonads; of these, 52 (94.5%) were female, 3 (5.5%) were males (Table 4). A total of 232 eel were classified as undifferentiated due to absent or incomplete gonadal tissue or were assumed to be undifferentiated because their lengths were less than 200 mm and were released after sampling (Table 4). Female eel had a mean length of 463 mm and mean weight of 203 g and maximum length of 702 mm. Male eel were twice as small, their mean length was 260 mm and mean weight was 28.7 g, with a maximum length of 290 mm (Table 4). American Eel from southwestern rivers (Río Matilde and Río Guanajibo) had larger mean lengths and weights than those in the north and northeastern parts of the island (Table 3).

Models of American Eel Density and Body Length

We fit 10 candidate simple and multiple linear regression models to American Eel density data and body length data, respectively (Table SI1, Table SI2). We excluded data from sampling

site 5C from our density models because we were unable to obtain an estimate of the area of the sampling reach which prevented us from obtaining density measurements. AIC_{Ci} strongly favored a model with distance from the river mouth as the independent variable, which had the lowest AIC_{Ci} score and greatest AIC_{Ci} weight, all other models' delta-AIC_{Ci} values were greater than 2, so they were not competitive (Table 5). The simple linear regression model with distance from the river mouth as the predictor indicates that eel density had a significant negative relationship ($\alpha = 0.05$) with distance from the river mouth (Table 6, Figure 2). Eel density was not included as a covariate in the body length models due to high Spearman correlations with distance from the river mouth (-0.7). For body length, the model with distance from the river mouth, river, and an interaction between the 2 variables was strongly favored with the lowest AIC_{Ci} score and the greatest AIC_{Ci} weight (Table 7). A second model containing predictors: distance from the river mouth, season, river, and an interaction between distance from the river mouth and river was a competitive model ($\text{delta-AIC}_{Ci} \leq 2$), but it was not considered for the purposes of this study, because its AIC_{Ci} weight was 42% less than the top model and inference was similar between both models (Table 7). The top body length model indicates that eel body length had a significant ($\alpha=0.05$) positive relationship with distance from the river mouth (Table 8, Figure 3). This relationship was less prominent for Río Sabana and differed from the relationship observed in Río Piedras. Río Piedras displayed a constant relationship between total length and distance from the river mouth with total lengths staying constant around 330 mm (Figure 3). Despite these observations, the model did not indicate any significant differences between the 5 rivers (Table 8). Generally, eel below 360 mm were within 2 to 10 kilometers from the river mouth while sizes began to steeply increase above 11 and 12 kilometers, except for Río Piedras (Figure 3).

Female Eel Presence Models

We fit 11 logistic regression models with a binomial distribution to female presence and absence data (Table SI3). The top logistics regression model with distance from the river mouth, length, and an interaction between the 2 predictors was favored with the lowest AIC_ci value (Table 9). There were 2 additional competitive models with delta-AIC_ci ≤ 2 (Table 9). The second best model containing distance from the river mouth, length, river, and an interaction between distance from the river mouth and length, and the third best model containing length had comparable AIC_ci weights to the top model (Table 9). Pseudo-R² values from the 3 models were between 0.70-0.75 indicating similar predictive ability (Table 9). Model averaging from the 95% confidence set of models showed length as the statistically significant predictor, while distance from the river mouth and other predictors were not statistically significant ($\alpha=0.05$) (Table 10). The coefficient for scaled length indicated that there is a 99% probability of being female as length increases (Table 10). Probability of being female increases at approximately 300 mm and reaches 99% at approximately 450 mm in all rivers, except for Río Sabana where 99% of probability is predicted to occur at lengths of 120 mm (Figure 4). Predicted probabilities for females vs. distance from the river mouth using full model averaging show probabilities of females from 0 % to 11% between 2 km and 5 km from the river mouth within 4 rivers (Figure 5). At 5 km to 10 km female probability ranges from 0.03% to 54%, and from 10 km or farther, female probability ranges from 0.8% to 81% within 4 rivers (Figure 5).

The separate coefficients from the top 3 logistics regression models show that distance from the river mouth, length, and an interaction between the 2 predictors were statistically significant ($\alpha=0.05$) (Table SI4, SI5, SI6). The scaled distance from the river mouth coefficients from the 2 top models indicated that probability of females increases between 75% - 79% with

increasing distance from the river mouth (Table SI4, Table SI5). The scaled length coefficients from the 3 top models indicated an increase in probability of females at greater lengths between 98% - 99% (Table SI4, SI5, SI6). The significant interaction between the scaled distance from the river mouth and length in the top 2 models indicate there is a 19 % - 24% decrease in the effect of distance from the river when length increases, and the same effect occurs to length when distance from the river mouth increases (Table SI4, Table SI5). The significant interaction between distance from the river mouth and body length was present in 2 of 3 top and competitive models, therefore, improving overall model performance (Table 9, SI4, SI5).

Predicted values of probability of female presence vs. total length (mm) from the top model containing distance from the river mouth, length, and an interaction between the 2 predictors showed a 0% probability of being female at sizes of 200 mm or shorter and steeply increasing in probability at sizes near 300 mm (Figure SI1). The predicted probability values of female American Eel presence was close to 0 % at lower elevations and increased gradually reaching approximately 25% at 17 km from the river mouth (Figure SI1).

Discussion

This is the first island-wide study to address longitudinal trends of American Eel population characteristics in the Caribbean and expands on preliminary information about the American Eel that are present in Puerto Rico (Roghair et al., 2014; Kwak et al., 2019). Our study shows that, in Puerto Rico, eel density decreases with distance from the river mouth and low-density upstream environments harbor larger eel, which have a greater probability of being females. Among sexually differentiated eel, females greatly outnumbered males. These trends have been observed in American Eel populations from temperate regions and agree with studies suggesting conspecific density-dependent size and sex determination (Krueger and Oliveira, 1999; Goodwin and Angermeier, 2003). These findings indicate that the Caribbean harbors American Eel with high reproductive potential that likely contribute strongly to the spawning stock in the Sargasso Sea. It is imperative to expand knowledge on the species' ecology throughout its entire range as well as implement management efforts to ensure stream connectivity to optimal upstream habitats for eel.

Our study is one of the first to collectively evaluate and generalize the commonly observed trends in American Eel density and body length in relationship to distance from the river mouth. Generally, in Puerto Rico, eel density decreases with increasing distances from the river mouth. Our findings effectively covered different regions of the island on rivers where most upstream habitats were still accessible to eel (Cooney and Kwak, 2013). Our results were consistent with those found for eel populations in temperate regions where eel densities had a negative relationship with distance upstream, consequently finding fewer individuals upstream especially for rivers with instream barriers (Smogor et al., 1995; Wiley et al., 2004). However, this trend has not been consistent throughout rivers in temperate regions. Some rivers or sections within a

drainage system can exhibit this pattern while others might lack significant correlations between density and distances inland (Oliveira and McCleave, 2000). Our study shows that American Eel body length increases with distance from the river mouth in Puerto Rico. Our results are consistent with observations from temperate regions where larger eel are found further inland or upstream of in-stream barriers and smaller individuals are commonly found in downstream and brackish water environments, especially in dammed rivers (Helfman et al., 1984; Smogor et al., 1995; Goodwin and Angermeier, 2003; Machut et al., 2007).

Our results provide insights on how urbanization and in-stream barriers may affect longitudinal trends in eel population structure. Río Piedras exhibited a noticeably truncated pattern in body length distribution compared to all other rivers in this study. Río Piedras is a highly urbanized river located in the metropolitan area of Puerto Rico, this stream is characterized by in-stream structures, pollution, and channelization (Ramirez et al., 2009). These characteristics can alter densities and population size structures (Haro et al., 2000; Cooney and Kwak, 2013). In-stream barriers are known to alter American Eel density and length throughout river systems and this trend was notable in our study. Natural in stream barriers are affecting eel distribution in the Río Sabana, where American Eel were absent from upstream sites. Río Sabana was noted by Roghair et al. (2014) to have large natural barriers in its downstream sections. Natural and artificial barriers can decrease eel density in upstream environments (Machut et al., 2007; Goodwin and Angermeier, 2003). Artificial dams have been noted to further exacerbate this and extirpate eel from suitable upstream habitats (Cooney and Kwak, 2013; Camhi et al., 2021). Like other locations in this species range, upstream freshwater habitats in Puerto Rico may harbor longer eel that are likely to be female when compared to downstream habitats. Our study showed that at greater lengths the American Eel has a high probability of being female,

with increasing probabilities starting at approximately 300 mm. Furthermore, our results also indicated that longer American Eel are mostly present at farther upstream environments. The longest eel were present at above 10 km from the river mouth. Despite distance from the river mouth not being a statistically significant predictor of female presence on the averaged model, it is considered a biologically significant predictor. The overall biological significance of distance from the river mouth is evident by considering the separate top models for female eel presence and the significant relationship between body length and distance from the river mouth. Coupled together, these models indicate that female eel are more likely to occur at distances farther upstream, rather than close to the river mouth. This can be observed at rivers with short distance sampling locations such as Rio Mameyes and Rio Sabana where the farthest points did not exceed 11 km. Throughout these distances, probabilities of female occurrence were low and shorter eel lengths were recorded, while longer eel were recorded at rivers with sampling locations farther upstream. These results agree with the overall trend of female eel occurring at low density upstream environments documented in North American and Canadian American Eel populations (Helfman et al., 1984; Oliveira, 1999; Côté et al., 2015; Bonvechio et al., 2018).

An exception to the overall distribution trend was noted by the decrease in probability of female presence with distance from the river mouth in Río Guanajibo. This may be explained by the predominance of female eel throughout all the sampling locations within the river which can be seen by the statistical significance of the interaction between distance from the river mouth and length in the top 2 female presence models. This interaction indicates that despite the effect distance from the river mouth has on female eel presence, the larger eel found in downstream environments still have a high probability of being female, despite their location. The presence of

long female eel closer to the mouths of the river may occur due to downstream migrations of silvering eel (Jessop, 1987; Haro, 2003).

We are unable to definitively determine that high density downstream sites are male dominated habitats due to the low number of male eel present in our sample (Oliveira, 1999; Thibault et al., 2007). Our sample was comprised of 81% undifferentiated eel and overall sex ratio was heavily skewed towards females (52:3), which is similar to the one documented by Kwak et al. (2019). This highly disproportionate sex ratio is comparable to studies derived from North America and Canada where male occurrence was low (Gray and Andrews, 1970; Hansen and Eversole, 1984). Exceptions have been found in temperate regions where overall male dominated sex ratios occur throughout high-density river systems (Krueger and Oliveira, 1999). Our results differ from observations where rivers with high proportion of undifferentiated individuals are characteristic of male dominated sex ratios (Krueger and Oliveira, 1999).

Our study provides evidence that American Eel from the southern portion of its geographic range may strongly contribute to the reproductive potential and survival of the global population of this species. Puerto Rico, an island located directly South of the Sargasso Sea, exhibited habitats disproportionately dominated by female eel. These results differ from suggestions that the proportion of female eel increases with latitude, but generally agree with northern latitudes harboring larger female eel (Helfman et al., 1987; Barbin and McCleave, 1997). Mean female eel length recorded in this and other studies in Puerto Rico is shorter than those in Canada (Jessop, 2010; Kwak et al., 2019). Despite this, there is considerable evidence that Puerto Rico greatly contributes to the reproductive success of the population because female eel from this location must migrate shorter distances to reach their spawning location. American Eel are at high risk of predation during their long spawning migrations (Béguer-Pon et al., 2012). Short distance

migrations from optimal habitats in Puerto Rico reduce the risk of predation and increase the probability of spawning.

Our study collectively identified longitudinal trends in density, length, and sex of American Eel in Puerto Rico. Low density upstream environments may harbor predominantly large female eel, while low-density downstream environments may harbor smaller and primarily undifferentiated American Eel. This indicates that tropical American Eel subpopulations may follow the same trends as those in temperate North American regions. Based on findings from our study and those from northern latitudes, there is considerable evidence that distances from the river mouth may play a significant role in the size and sex of American Eel, which can also be affected by conspecific densities (Krueger and Oliveira, 1999; Oliveira and McCleave, 2000; Davey and Jellyman, 2005).

Understanding and identifying overall patterns in American Eel ecology, demographics, and threats is an ongoing challenge (MacGregor et al., 2008). American Eel are widely distributed throughout the Atlantic coast, and little is known about the status and trends eel populations in the southern regions (Shepard, 2015; Benchetrit and McCleave, 2016). This study expands the knowledge about American Eel population characteristics and trends in the Caribbean region. Further research is needed range-wide to understand additional habitat factors related to how downstream and upstream environments may alter size and sex in American Eel.

Conservation and Management Implications

The widespread distribution of the American Eel and its panmictic population require a range-wide assessment (Cairns et al., 2022). All species assessments are derived from the northern portions of the species' range where American Eel have declined, but do not warrant protection in the United States (COSEWIC, 2012; ASMFC, 2017; Shepard, 2015; Jacoby et al.,

2017). Our results contribute significantly to the overall management needs of American Eel population data across different regions and provide useful information to fill data gaps for future range-wide models and analytic assessments (ASMFC, 2017; Cairns et al., 2022). Possible truncated patterns in American Eel in urban streams and the importance of upstream habitats highlighted throughout this study will help in continuing bi-national collaboration between environmental, fisheries managers, and other dam companies to ensure instream passage (MacGregor et al., 2008; ASMFC, 2017; Cairns et al., 2022). Prioritizing stream connectivity is pivotal to the persistence and survival of the American Eel population by ensuring the production of large female eel near their spawning location. Similarly, understanding the reproductive contribution and status of American Eel from each region will help managers better assess the status of the population.

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Tables

Table 1. Sampling site coordinates and distances from the river mouth within 5 rivers located in the north, south, east, and west of the island of Puerto Rico. American Eel were sampled from 23 sites using a 3-pass pulsed-DC electrofishing method during the dry season (November 2020 – January 2021) and 14 sites were resampled during the wet season (June 2021-August 2021).

Site ID	River	Drainage basin	Distance from river mouth (km)	Latitude (N)	Longitude (W)	Sampling	
						Dry season 2020-2021	Wet season 2021
46C	Río Piedras	Río Piedras	4.75	18.4053657	-66.0690448	X	X
46A	Río Piedras	Río Piedras	8.61	18.3840681	-66.0586831	X	X
46B	Río Piedras	Río Piedras	11.2	18.3670476	-66.0631768	X	X
RP2	Río Piedras	Río Piedras	14.9	18.343722	-66.0560056	X	X
4Q	Río Mameyes	Río Mameyes	2.22	18.3730357	-65.7635835	X	X
4K	Tabonuco	Río Mameyes	3.97	18.361063	-65.769013	X	X
RM2	Tabonuco	Río Mamayes	5.89	18.347116	-65.7684802	X	X
4I	Río Mameyes	Río Mameyes	7.02	18.3408842	-65.7536526	X	X
4F	Río Mameyes	Río Mameyes	9.15	18.3276234	-65.7507864	X	X
4D	Río Mameyes	Río Mameyes	9.78	18.3226959	-65.7500712	X	X
5C	Río Sabana	Río Sabana	2.48	18.362925	-65.7208063	X	
5A	Río Sabana	Río Sabana	4.32	18.350457	-65.7260138	X	
RS5	Río Sabana	Río Sabana	5.35	18.3428929	-65.7300596	X	
5D	Río Sabana	Río Sabana	7.69	18.3254706	-65.7295268	X	
RS4	Río Pitahaya	Río Sabana	9.72	18.321595	-65.7164504	X	
28D	Río Canas	Río Matilde	5.5	18.0249	-66.6409652	X	X
28E	Río Pastillo	Río Matilde	7.49	18.0347752	-66.6624655	X	X

Table 1. (Continued)

Site ID	River	Drainage basin	Distance from river mouth (km)	Latitude (N)	Longitude (W)	Sampling	
						Dry season 2020-2021	Wet season 2021
28C	Río Canas	Río Matilde	7.84	18.0400361	-66.6447425	X	X
28B	Río Canas	Río Matilde	14.0	18.0802422	-66.6538603	X	X
RG3	Río Hondo	Río Guanajibo	4.71	18.1604514	-67.1497042	X	
35H	Río Rosario	Río Guanajibo	10.4	18.1267652	-67.1237605	X	
35B	Río Rosario	Río Guanajibo	14.1	18.1583217	-67.085124	X	
RG2	Río Rosario	Río Guanajibo	16.9	18.1732659	-67.0722671	X	

Table 2. Total catch and population density estimates of American Eel at 23 sites across 5 rivers in Puerto Rico during the dry and wet sampling seasons. Population density estimates were calculated using the Carle-Strub removal method, applied to 3-pass pulsed DC electrofishing data obtained from closed populations. Cells denoted with (--) indicate sites that were not sampled during the wet season.

River	Sampling site	Dry season (2020 – 2021)		Wet season (2021)	
		Total catch	Population density (fish/ha) ± SE	Total catch	Population density (fish/ha) ± SE
Río Piedras	46C	13	198.4 ± 1.072	1	25.29 ± 51.34
	46A	2	49.16 ± 25.51	6	80.28 ± 18.46
	46B	1	25.85 ± 0	2	49.54 ± 0
	RP2	6	257.1 ± 207.4	2	66.40 ± 12.75
Río Mameyes	4Q	0	0	27	328.1 ± 59.58
	4K	27	669.3 ± 43.76	29	700.5 ± 98.49
	RM2	8	327.6 ± 43.23	6	187.8 ± 31.29
	4I	13	221.5 ± 56.77	10	126.6 ± 10.87
	4F	5	54.36 ± 38.50	5	44.51 ± 1.495
	4D	5	143.5 ± 4.820	3	89.46 ± 138.9
Río Sabana	^a 5C	17	--	--	--
	5A	18	319.8 ± 14.37	--	--
	RS5	18	329.7 ± 34.71	--	--
	5D	0	0	--	--
	RS4	0	0	--	--
Río Matilde	28D	6	169.6 ± 4.027	6	175.7 ± 11.01
	28E	0	0	0	0
	28C	9	323.0 ± 100.4	2	80.13 ± 0
	28B	1	24.31 ± 0	2	49.57 ± 0
Río Guanajibo	RG3	5	148.4 ± 13.16	--	--
	35H	3	92.80 ± 8.225	--	--
	35B	8	157.1 ± 2.155	--	--
	RG2	10	124.1 ± 2.947	--	--
Total catch / mean density ± SE		175	165.3 ± 34.54	101	143.1 ± 48.41

^a Sampling site data was not used for density models.

Table 3. Mean total lengths and weights of 287 American Eel caught at each sampling site at 23 locations across 5 rivers during the dry and wet seasons. Cells denoted with (--) indicate sites that were not resampled during the wet season and cell denoted with “NP” indicate sites where eel were not present.

River	Sampling site	Dry season (2020 – 2021) (N=185)				Wet season (2021) (N=102)			
		Mean length (mm) ± SE	Min - max	Mean weight (g) ± SE	Min - max	Mean length (mm) ± SE	Min - max	Mean weight (g) ± SE	Min - max
Río Piedras	46C	337 ± 24.5	225 – 495	84.5 ± 20.7	14 – 236	214 ± 0	214 – 214	15.0 ± 0	15 – 15
	46A	375 ± 81.2	275 – 536	133 ± 86.3	34 – 305	302 ± 22.9	219 – 373	63.3 ± 14.2	23 – 105
	46B	305 ± 0	305 – 305	46 ± 0	46 – 46	372 ± 29.5	340 – 431	89.3 ± 26.9	60 – 143
	RP2	301 ± 41.6	179 – 471	67.1 ± 29.2	7 – 227	398 ± 97.0	301 – 495	145 ± 99	46 – 244
Río Mameyes	4Q	NP	NP	NP	NP	174 ± 20.1	65 – 330	20.1 ± 4.6	1 – 79
	4K	246 ± 8.7	163 – 312	29.8 ± 3.4	3 – 60	266 ± 19.1	135 – 702	58.3 ± 23.0	5 – 695
	RM2	278 ± 46.6	182 – 593	74.6 ± 52.0	8 – 438	228 ± 25.0	180 – 338	25.2 ± 10.6	10 – 76
	4I	243 ± 12.7	160 – 296	25.7 ± 4.3	5 – 47	218 ± 18.5	110 – 294	20.0 ± 4.2	2 – 41
	4F	264 ± 50.1	148 – 439	48.0 ± 21.0	3 – 132	225 ± 26.7	154 – 303	23.0 ± 8.1	5 – 50
	4D	229 ± 22.1	175 – 291	19.4 ± 5.9	8 – 36	274 ± 47.4	180 – 332	31.7 ± 12.3	8 – 49
Río Sabana	5C	251 ± 20.2	145 – 460	33.8 ± 9.4	5 – 137	--	--	--	--
	5A	223 ± 23.0	90 – 645	33.3 ± 17.5	1 – 412	--	--	--	--
	RS5	267 ± 26.6	125 – 516	41.9 ± 14.0	2 – 229	--	--	--	--
	5D	NP	NP	NP	NP	--	--	--	--
	RS4	NP	NP	NP	NP	--	--	--	--
Río Matilde	28D	312 ± 51.2	152 – 448	73.3 ± 28.2	6 – 184	316 ± 60.9	156 – 570	115 ± 62.0	19 – 418
	28E	NP	NP	NP	NP	NP	NP	NP	NP
	28C	288 ± 64.5	155 – 643	113 ± 67.2	7 – 485	314 ± 87.5	226 – 401	64 ± 46.0	18 – 110
	28B	577 ± 0	577 – 577	329 ± 0	329 – 329	526 ± 24.5	501 – 550	267 ± 29.5	237 – 296
Río Guanajibo	RG3	204 ± 38.6	153 – 358	20.2 ± 13.2	5 – 73	--	--	--	--
	35H	471 ± 7.2	462 – 485	197 ± 15.0	174 – 225	--	--	--	--
	35B	442 ± 19.7	375 – 516	141 ± 15.8	91 – 226	--	--	--	--
	RG2	468 ± 30.1	363 – 644	197 ± 43.4	84 – 475	--	--	--	--

Table 4. Overall sex ratios and respective length and weight of 287 American Eel caught at 23 sampling sites in 5 rivers in Puerto Rico during the dry and wet seasons. We dissected 233 eel and identified presence and absence of gonadal tissue types. Uncollected eel had lengths < 200 mm and were identified as undifferentiated. Female and males were identified using macroscopic examination of gonads and the gonad squash technique was applied when macroscopic identification was not possible. Undifferentiated eel were identified based on absent or incomplete gonadal tissue.

Sex	N	Mean total length (mm) \pm SE	Min-max	Mean weight (g) \pm SE	Min-max
Female	52	463 \pm 13.8	244 – 702	203 \pm 19.3	22 – 695
Male	3	260 \pm 27.7	205 – 290	28.7 \pm 7.3	14 – 36
Undifferentiated	232	231 \pm 4.8	65 – 505	27.5 \pm 1.6	1 – 149
Total	287				

Table 5. The 95% confidence set of simple and multiple linear regression models for American Eel density. The 95% confidence set of models consist of models with a cumulative $AICc_i$ weight ≥ 0.95 . Models were fit to data obtained from pulsed DC-electroshocking surveys in 5 rivers of Puerto Rico during the dry season (November-January 2020) and the wet season (June-August 2021). The top model was selected using $AICc_i$, Δ_iAICc , and $w_i(AICc)$. Models with $\Delta_iAICc \geq 2$ were not considered as competing models. P-values were calculated at $\alpha=0.05$. Distance from river mouth is a numerical predictor abbreviated as ‘dtm’, ‘river’ and ‘season’ are categorical predictors representing the 5 rivers included in this study, and the dry and wet seasons, respectively. Interactions between ‘dtm’ and ‘river’ are denoted by (:).

Response	Model description	$AICc_i$	Δ_iAICc	$W_i(AICc)$	Adjusted R^2	k
density	dtm	470.4	0.0	0.639	0.133	1
	dtm + season	472.6	2.22	0.211	0.115	2
	1 (null)	474.2	3.81	0.095	--	0

Table 6. Coefficients (β) from the top simple linear model for density of American Eel with ‘distance from the river mouth’ as the single numerical predictor. This top model was selected based on AIC_c values, delta-AIC_c values, and weighted AIC_c values. P-values were calculated at $\alpha=0.05$. The coefficients for the numerical predictor is scaled and distance from river mouth is abbreviated as ‘dtm’.

Predictor variable	$\hat{\beta}$	SE	P-value
intercept	156.7	25.99	7.92e ⁻⁰⁷ ***
dtm	-66.58	26.36	0.0164 *

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Table 7. The 95% confidence set of simple and multiple linear regression models for American Eel density. The 95% confidence set of models consist of models with a cumulative $AICc_i$ weight ≥ 0.95 . Models were fit to data obtained from pulsed DC-electroshocking surveys in 5 rivers of Puerto Rico during the dry season (November-January 2020) and the wet season (June-August 2021). The top model was selected using $AICc_i$, $\Delta_i AICc$, and $w_i(AICc)$. Models with $\Delta_i AICc \geq 2$ were not considered as competing models. P-values were calculated at $\alpha=0.05$. Distance from river mouth is a numerical predictor abbreviated as ‘dtm’, ‘river’ and ‘season’ are categorical predictors representing the 5 rivers included in this study, and the dry and wet seasons, respectively. Interactions between ‘dtm’ and ‘river’ are denoted by (:).

Response	Model description	$AICc_i$	$\Delta_i AICc$	$w_i(AICc)$	Adjusted R^2	k
body length	dtm + river + dtm:river	3469.2	0.00	0.705	0.295	3
	dtm + season + river + dtm:river	3471.0	1.81	0.285	0.294	4

Table 8. Coefficients (β) from the top multiple linear regression model for length of American Eel. The top model contained the numerical predictor distance from the river mouth abbreviated as ‘dtm’ and the categorical predictor ‘river’ representing the 5 sampled rivers. Interaction between ‘dtm’ and ‘river’ was denoted by (:). P-values were calculated at $\alpha=0.05$. All coefficients for levels of each river and interaction are based on comparison to the reference level ‘Río Guanajibo’. Coefficients for numerical predictors are scaled.

Predictor variable	$\hat{\beta}$	SE	p-value
Intercept	276.0	34.83	5.68e ⁻¹⁴ ***
distance from river mouth (dtm)	76.46	16.52	5.70e ⁻⁰⁶ ***
river (Río Mameyes)	-33.58	36.13	0.3535
river (Río Matilde)	26.77	40.87	0.5131
river (Río Piedras)	53.65	40.11	0.1821
river (Río Sabana)	-25.10	45.16	0.5788
dtm: river (Río Mameyes)	-53.86	21.58	0.0132 *
dtm: river (Río Matilde)	18.40	32.99	0.5775
dtm: river (Río Piedras)	-77.95	22.45	0.0006 ***
dtm: river (Río Sabana)	-66.72	46.37	0.1513

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Table 9. The 95% confidence set of logistic regression models for presence of female eel. The 95% confidence set of models consist of models with a cumulative $AICc_i$ weight ≥ 0.95 . Models were fit to data obtained from pulsed DC-electroshocking surveys in 5 rivers of Puerto Rico during the dry season (November-January 2020) and the wet season (June-August 2021). The top model was selected using $AICc_i$, $\Delta_i AICc$, and $w_i(AICc)$. Models with $\Delta_i AICc \geq 2$ were not considered as competing models. The numerical predictor distance from river mouth is abbreviated as ‘dtm’ in the model description. The numerical predictor ‘length’ represents total length of American Eel, the categorical predictor ‘river’ represents the 5 sampled rivers, and interactions between ‘dtm’ and ‘length’, and ‘dtm’ and ‘river’ are denoted by (:). Coefficients for numerical predictors are scaled.

Response	Model description	$AICc_i$	$\Delta_i AICc$	$W_i(AICc)$	Pseudo R^2	k
presence of females	dtm + length + (dtm:length)	83.2	0.00	0.369	0.724	3
	dtm + length + river + (dtm:length)	83.7	0.51	0.286	0.753	4
	length	85.1	1.92	0.141	0.701	1
	dtm + length	86.0	2.82	0.090	0.706	2
	dtm + river + (dtm:river) + length + (dtm:length)	87.1	3.88	0.053	0.772	5
	dtm + river + length	87.2	4.02	0.049	0.732	3

Table 10. Full model averaged coefficients ($\hat{\beta}$) from the 95% confidence set of logistic regression models for presence of female American Eel. P-values were calculated at $\alpha=0.05$. The full model average contains distance to the mouth abbreviated as ‘dtm’ and ‘length’ as numerical predictors. The categorical predictor ‘river’ is represented by the 5 sampled rivers. Interactions between ‘dtm’ and ‘length’, and between ‘dtm’ and ‘river’ are denoted by (:). Coefficients for numerical predictors are scaled.

Predictor variable	$\hat{\beta}$	SE	Log-odds probability	p-value
intercept	-3.97	1.09	0.019	0.00029***
dtm	0.893	0.68	0.74	0.19
length	4.89	1.28	0.99	0.00014***
dtm:length	-0.932	0.79	0.21	0.24
Río Mameyes	0.0351	0.90	0.52	0.97
Río Matilde	1.08	1.74	0.94	0.54
Río Piedras	-0.191	0.87	0.38	0.83
Río Sabana	-0.952	2.48	0.081	0.70
dtm:Río Mameyes	-0.0175	0.29	0.42	0.95
dtm:Río Matilde	0.378	1.83	1.00	0.84
dtm:Río Piedras	0.0527	0.33	0.73	0.87
dtm:Río Sabana	-0.203	2.05	0.022	0.92

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Figures

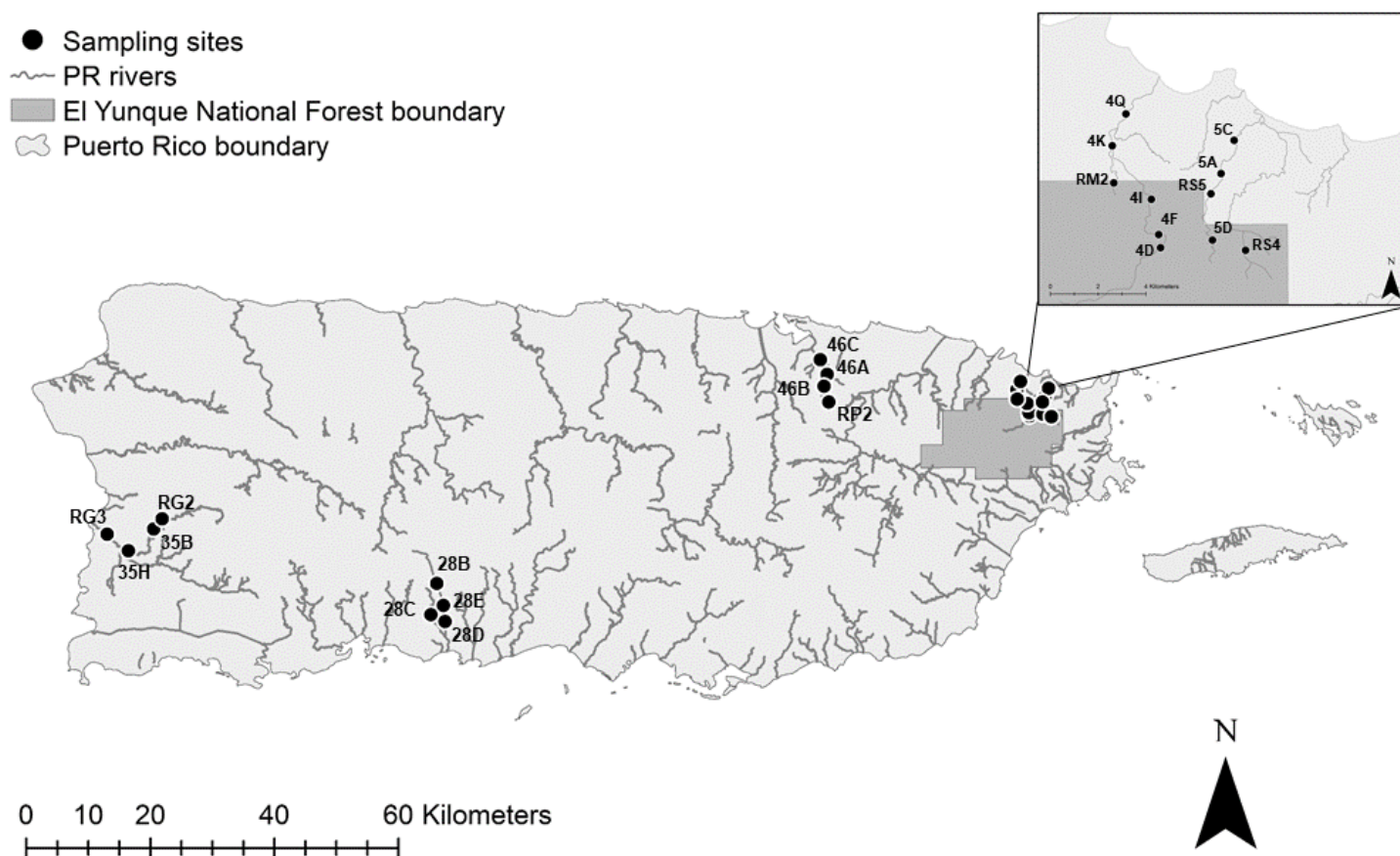


Figure 1. Map of American Eel sampling reaches (n = 23) located in 5 rivers throughout the north, south, east, and west of the island of Puerto Rico. All sites were sampled using a pulsed-DC electroshocking method on 60-m long reaches.

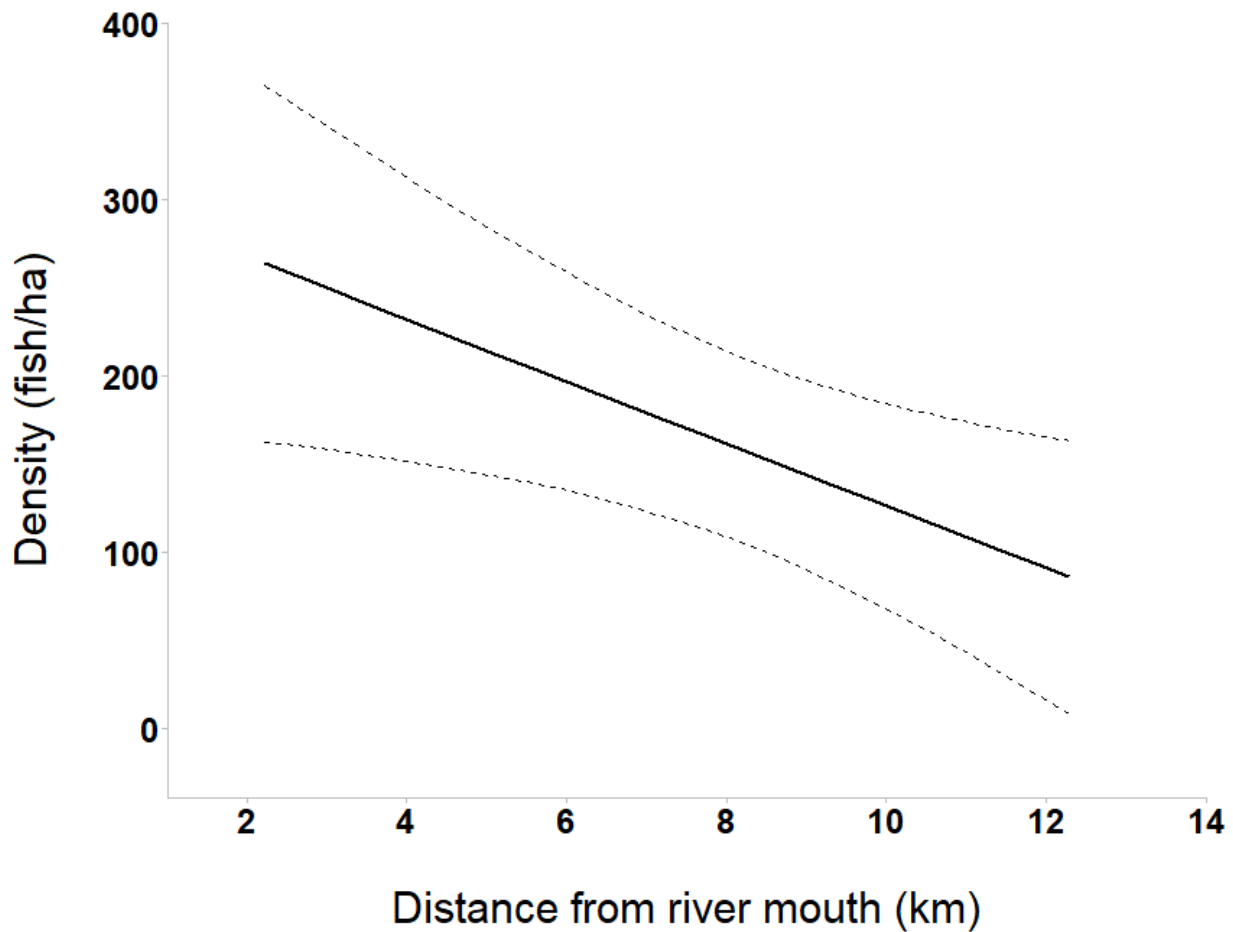


Figure 2. Predicted density values vs. distance from river mouth predicted using the simple linear regression model with distance from the river mouth (numerical predictor). This top model was developed by fitting a multiple linear regression (MLR) to 36 data points of scaled distance from the river mouth and eel density from 21 sampling sites (dry season) of which 12 were resampled (wet season). Predicted values are given by the solid line and the 95% confidence intervals is given by the dashed lines. Scaled distance to mouth values displayed on the x-axis were back transformed to their original scale in kilometers.

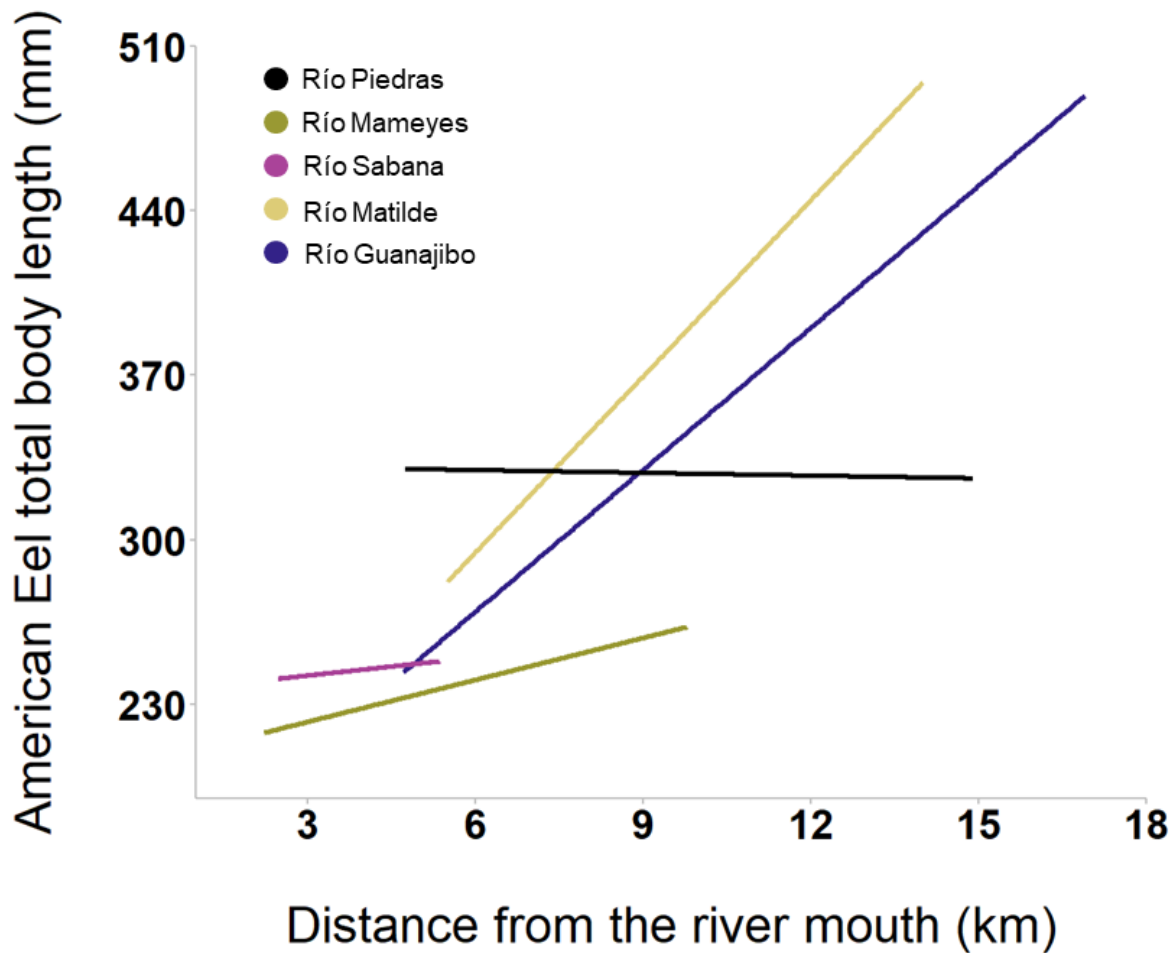


Figure 3. Predicted values of American Eel length given the distance from the river mouth (solid line) and 95% confidence intervals (dashed lines) for each of the 5 rivers. These values were calculated by fitting the model containing distance from the river mouth, river, and an interaction between the 2 predictors to 287 observations of American Eel length and scaled distance from river mouth from 21 sampling sites (dry season) of which 12 were resampled (wet season). Scaled distance to mouth values displayed on the x-axis were back-transformed to their original scale in kilometers.

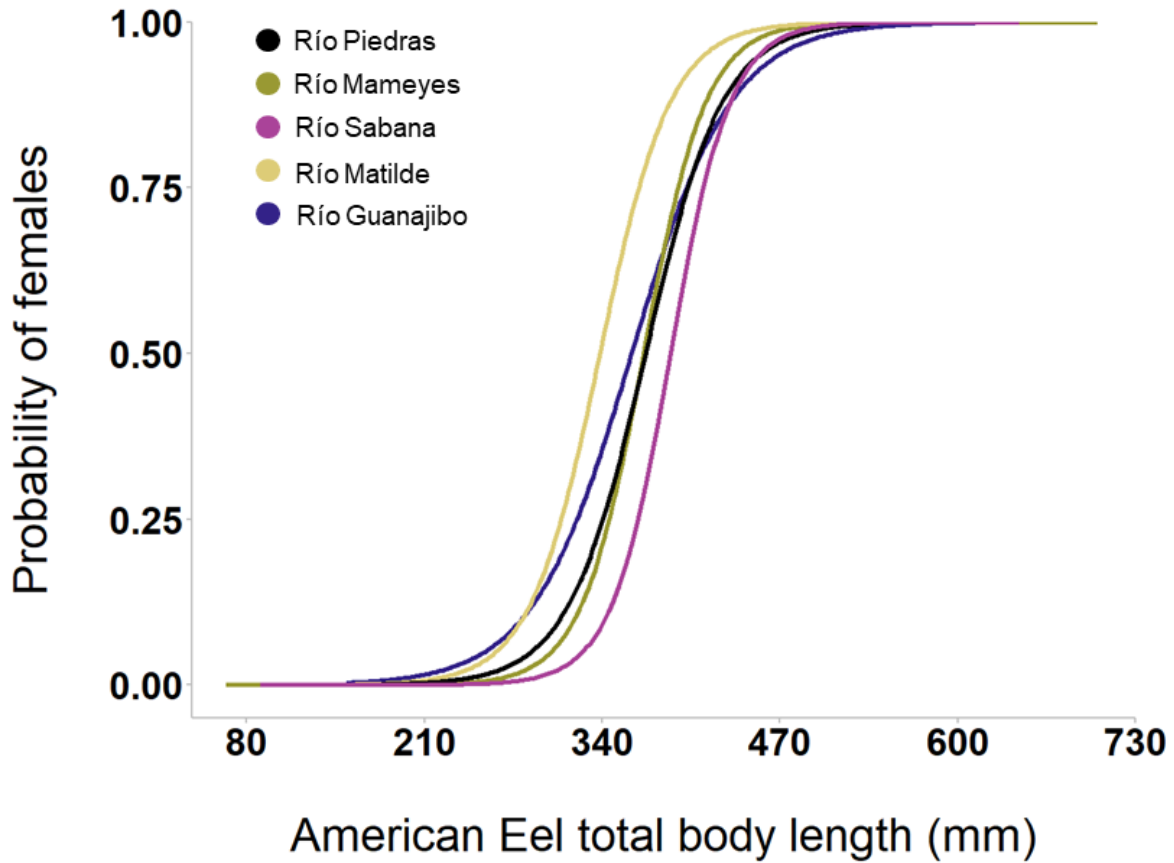


Figure 4. Probability of female eel presence given American Eel length. These predicted values were calculated by using the full model averaged coefficients from the 95% confidence set of logistics regression models containing the predictors: distance from the river mouth (numerical), American Eel length (numerical), river (categorical), and an interaction between distance from the river mouth and American Eel length. Scaled American Eel length values displayed on the x-axis were back-transformed to their original scale in millimeters.

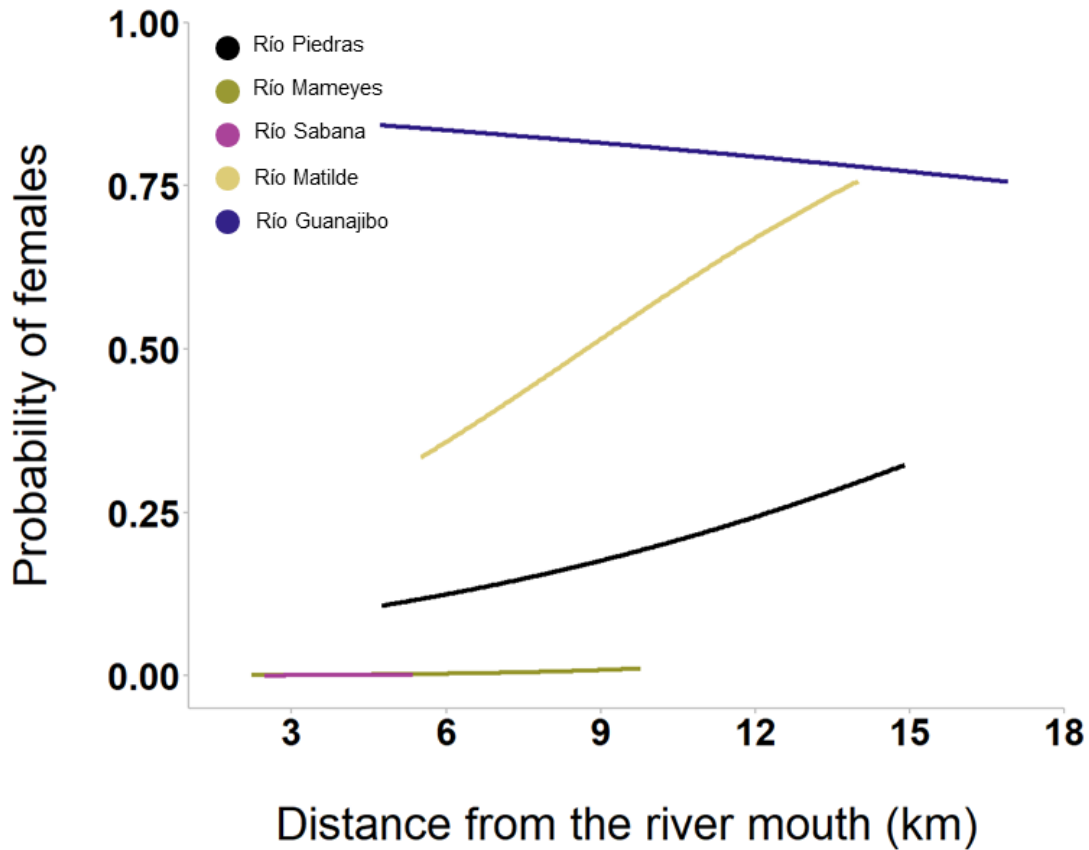


Figure 5. Probability of female eel presence given the distance from the river mouth. These predicted values were calculated by using the full model averaged coefficients from the 95% confidence set of logistics regression models containing the predictors: distance from the river mouth ‘dtm’ (numerical), American Eel length (numerical), river (categorical), and an interaction between distance from the river mouth ‘dtm’ and American Eel length. Scaled distance from the river mouth values displayed on the x-axis were back-transformed to their original scale in kilometers.

CHAPTER 2

Parasites of the American Eel (*Anguilla rostrata*) in Puerto Rico

Abstract

Invasive parasite infections are thought to be one of the possible causes contributing to declines of American Eel (*Anguilla rostrata*) from Canada and North America. The geographical extent of the swim bladder parasite (*Anguillicoloides crassus*) and Monogenean gill parasites in the American Eel is relatively unknown. We evaluated infection from these parasites in American Eel from Puerto Rico. We dissected 233 American Eel from 5 rivers in Puerto Rico to assess presence of *A. crassus* infection. We evaluated 79 of 233 American Eel for Monogenean parasite infection. *A. crassus*, is not present in Puerto Rico, while Monogeneans were detected. Overall Monogenean prevalence was 41.8%, while mean intensity was 10 parasites/eel. Linear and logistic regression models indicate that Monogenean intensity may not decrease American Eel condition. American Eel remain within good relative condition. Monogenean intensities increased with American Eel length and varied by river. Our findings may help managers assess the level of threat these parasites present for American Eel and the importance of stream connectivity to reduce the probabilities of reinfection.

Introduction

American Eel (*Anguilla rostrata*) are in decline throughout the United States and Canada and introduced parasites may be one of the drivers (ASMFC 2017; COSEWIC 2012). Reductions in indexes of the American Eel population have been attributed to overfishing barriers to migration, pollution, climate change, and most recently, to non-native parasites (Hein et al., 2014; Jessop and Lee, 2016; Cooney and Kwak, 2013; Buttermore et al., 2018). The recent introduction and spread of the exotic swim bladder parasite (*Anguillicoloides crassus*) (Kuwahara et al., 1974), and monogenean gill worms, such as *Pseudodactylogyrus sp.* (Kikuchi, 1929; Yin and Sproston, 1948). Documenting information regarding these parasites and any effects on American Eel in Puerto Rico may provide important information related to the geographical extent of infection for the conservation and management of the species in the Caribbean and throughout its range.

Anguillicoloides crassus is a parasitic nematode that affects the swim bladder of anguillid eels. They can become infected by *A. crassus* at all life stages by ingesting intermediate and paratenic hosts, such as: copepods, fish, amphibians, and freshwater invertebrates (Thomas and Ollevier, 1992; Moravec, 1996; Li et al., 2015; Hubbard et al., 2016). These nematodes migrate across the body cavity to the swim bladder lumen and wall where they molt, feed, reproduce, and lay tens of thousands of eggs (De Charleroy et al., 1990). The eggs and larvae exit the eel's body through the intestines and repeat the cycle by attracting hosts. This life cycle can be completed in as little as 2 months under ideal temperature and salinity conditions (De Charleroy et al., 1990).

The swim bladder parasite, *A. crassus*, rapidly spread from Japan to Europe, and most recently, to North America and Canada, while little information is available regarding its presence through the Caribbean and South America. *A. crassus* originated in Japan where it is an

endemic parasite of the Japanese Eel (Kennedy, 2007). The parasite spread to Europe in the 1980's and subsequently reached the east coast of the United States where it was initially detected in 1995 in wild and farmed American Eel in Texas and South Carolina (Neumann, 1985; Fries et al., 1996; Kirk et al., 2003). Since then, it has rapidly spread throughout eastern North America and has reached as far north as Nova Scotia and as far south as Florida (Rockwell et al., 2009; Bonvechio et al., 2018). High parasite prevalence and intensities have been reported for American Eel in North America and Canada (Barse, et al., 2001; Denny, et al., 2013). Introduction and spread of the parasite are thought to occur through human transport and imports of live eel and the ability of the American Eel to spread widely (Lefebvre et al., 2012). Despite this, there have not been reports of *A. crassus* in South America, while absence of the parasite has been documented in Puerto Rico (Kwak et al. 2019). Continued research is needed to monitor and document the status and extent of *A. crassus* spread.

Pathogenic effects of *A. crassus* on the eel are unclear and largely dependent on the number of parasites present, the host, and environmental conditions. Due to its recent introduction, most of the knowledge regarding these effects on the American Eel are derived from studies with the European Eel. It is unknown if *A. crassus* infection may impact population dynamics (Warshafsky et al., 2019). However, in aquaculture settings, American Eel and European Eel that were infected with *A. crassus* have exhibited hemorrhaging, an opaque swim bladder, and dilated blood vessels. In the European Eel, damage can result in degradation and loss of swim bladder function (Würtz et al., 1996; Pelster, 2015). These damages are inferred to alter silvering, feeding, growth, reproductive potential, and the ability to survive spawning migrations, but has yet to be tested (Kennedy, 2007).

Monogenean gill parasites known to infect American Eel have followed the same pattern of spread as the swim bladder parasite, but the geographical extent of Monogenean infection in the American Eel is poorly understood. It is thought that *Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini* originated from Japan where they were first recorded on the Japanese Eel (Kikuchi, 1929). They spread to Europe in the 1980s causing disease on European Eel farms (Buchmann et al., 1987; Buchmann, 1988). It is thought that *P. anguillae* spread to Canada in the 1990s and *P. bini* to the United States in the 2000s in wild American Eel, although some believe they are endemic (Cone and Marcogliese, 1995; Hayward et al., 2001). Few studies have begun to record and provide prevalence and intensity data for these monogeneans in the American Eel in Canada and the US. These studies have documented Monogenean prevalence of up to 70% - 100% (Hayward et al., 2001; Larrat et al., 2012). These parasites have not been recorded throughout most of the United States, the Gulf of Mexico, the Caribbean, and South America (Hayward et al., 2001). It is essential to identify the extent and degree of Monogenean infection in the American Eel throughout its entire geographical range.

Monogeneans gill parasites known to infect Anguillid eels can rapidly spread and cause a wide array of effects. Eels can become infected from direct contact with another infected eel (Reed et al., 2013). Monogeneans do not require intermediate hosts to complete their life cycles which allows them to rapidly reproduce and reach great numbers (Kennedy, 2007; Buchmann, 2012). As a result, the ciliated free-swimming larvae needs to attach to a host within 6 hours (Golovin, 1977; Imada and Muroga, 1978). They attach to the head and gills of eel and each species move to their preferred microhabitats in the host's gills where they feed on epithelia and mucus (Buchmann, 2012). High infection intensity of Monogenean gill parasites can cause morbidity and mortality in eel from farms and may produce few complications in wild eel

populations (Buchmann, 2012; Kennedy, 2007; Reed et al., 2013). The effects of these infections are largely dependent on parasite intensity compared to the host's body size (Buchmann, 2012). Low parasite intensities are not known to be pathogenic, while high parasite intensities can cause hemorrhaging, hyperplasia of gill tissue, gill surface area reduction, and impaired respiration (Chan and Wu, 1984; Abdelmonem et al., 2010; Buchmann, 2012; Newbold et al., 2015). Eels may be more susceptible to infection and have higher parasite intensities in low water quality environments, drought conditions, and captive environments (Reed et al., 2013).

It is crucial to understand the relationship between these parasites, their host, and the environment throughout the American Eel's entire range. Although these parasites have not been found to cause detrimental effects in wild eel populations, it is important to monitor these relationships. It has been found that American Eel condition is not affected by *A. crassus* infection (Machut and Limburg, 2008) and studies derived from European Eel found no correlation between eel length and *A. crassus* abundance (Thomas and Ollevier, 1992). Additional studies with the European Eel and Japanese Eel indicated that abundance of *P. anguillae* and *P. bini* monogeneans was negatively correlated with body condition (Gérard et al. 2013), while having a positive correlation with body length (Hun-Yun et al. 1984; Buchmann, 1989). These infections can be dependent on environmental conditions where low water quality and urbanization increase the vulnerability to infection (Machut and Limburg, 2008). Monitoring these relationships throughout the American Eel's entire range will improve understanding of the effects caused by these infections throughout diverse environments.

This is the first study to evaluate the presence of both *A. crassus* and Monogenean gill parasites in American Eel in the Caribbean. Despite their rapid spread due to their short life cycle and high reproductive outcome not much is known about the geographical extent and effects of

these parasites (De Charleroy et al., 1990; Reed et al., 2013). At present, there is 1 documented study in Puerto Rico where *A. crassus* was reported to be absent (Kwak et al., 2019), while various species of Monogeneans have been documented for sport fishes in Puerto Rico, but these have not been reported for American Eel in the island (Williams and Williams, 1994). Our research expands on these previous results from Kwak et al. (2019) by providing current information on the status of the swim bladder parasite and documented general Monogenean gill parasite presence in American Eel. We provided prevalence, intensity, and distribution data, if detected, in rivers of Puerto Rico. We evaluated relationships between American Eel condition, body length, geographical variables, and parasite intensity.

Methods

Sampling Design

We collected a total of 233 American Eel using pulsed direct current backpack electrofishing during 2 sampling events from November to January in 2020 and from June to August in 2021. They were caught at 19 sampling locations along five rivers: Río Piedras, Río Mameyes, Río Sabana, Río Matilde, and Río Guanajibo, which are located throughout the northern, northeastern, southern, and western regions of Puerto Rico (Table 1; Figure 1). After capture, we took biological measurements of wet weight (g) and total length (mm). We euthanized American Eel using a MS-222 solution, following IACUC protocol 20-216. We froze and transported them to the laboratory facility at North Carolina State University for parasite analysis.

Necropsy and Parasite Dissection

We examined eel for *A. crassus* and Monogenean gill parasite infection. Eel collected during the November 2020 to January 2021 (n = 154) sampling event were examined only for *A. crassus* infection and eel collected in from June to August 2021 (n = 79) were examined for *A. crassus* and Monogenean gill parasite infection. We dissected partially thawed American Eel and extracted the viscera. We macroscopically examined the swim bladder wall and lumen for any visual signs of *A. crassus* infection. We extracted gills and mucus from both cavities, placed them in a glass dish, and rinsed with water. We examined gill arches, filaments, mucus, and water content for Monogenean gill parasites under compound and dissecting microscopes. We counted, extracted, and preserved Monogenean parasites from each eel in 95% ethanol. Parasites were not identified at the species level and all specimens were pooled because visual

differentiation of Monogeneans is not reliable (Kennedy, 2007). We calculated prevalence and intensity of monogeneans following descriptions by Brush et al. (1997).

American Eel Condition and Body Length Models

We fit a length-weight relationship (LWR) curve to 1825 measurements of American Eel from Puerto Rico sampled by Kwak et al. (2019), Roghair et al. (2018), and Torres-Molinari, et al. (2023) during 2005 – 2021. We developed a linear equation in log form of the allometric growth equation $W = aL^b$ for American Eel in Puerto Rico, where W is wet weight (g), L is total body length (mm), and a and b are parameters describing relationships between total length and weight (Blakeslee et al., 2018). We used the Puerto Rico LWR equation to calculate the relative condition factor (K_n) for each American Eel evaluated for Monogenean gill parasites ($n = 79$) in the June to August 2021 collected sample (Le Cren, 1951; Neumann et al., 2013).

We fit a multiple linear regression model to American Eel condition as a function of distance from the river mouth, river, and Monogenean parasite intensity, and an interaction between distance from the river mouth and river. The numerical predictors, distance from the river mouth and monogenean count, were scaled by subtracting the mean and dividing by the standard deviation. We explored all subsets of the full model to evaluate if Monogenean parasite intensity affected American Eel condition. We calculated Spearman correlation values for all numerical predictors to control for collinearity issues in the models.

We ranked all candidate models using the Akaike information criterion, corrected for small sample sizes ($AICc_i$), delta- $AICc$ ($\Delta_i(AICc)$), and weighted $AICc$ ($w_i(AICc)$) to select the top model explaining variation in American Eel condition (Burnham and Anderson, 2003). We used the adjusted R^2 values to measure the predictive ability of the top model and competitive models. We conducted full model averaging using the 95% confidence set of models. We

evaluated the full model averaged coefficient (s) ($\hat{\beta}_i$) for a statistically significant influence on American Eel condition ($\alpha=0.05$), with primary focus on monogenean parasite count. We evaluated coefficients from the top and competing linear regression models for any significant relationships. We generated and plotted predicted values for American Eel condition values as a function of Monogenean parasite intensity.

Parasite Intensity Models

We fit a logistic regression model with a negative binomial distribution to assess how parasite infection is influenced by geographic and biological variables. We considered the following covariates for inclusion in the model: distance from the river mouth, river, length, and an interaction between distance from the river mouth and river. We ranked and selected models using the same procedure used for the condition models, Akaike Information Criterion, corrected for small sample sizes ($AICc_i$), delta- $AICc$ ($\Delta_i(AICc)$) and weighted $AICc$ ($w_i(AICc)$) (Burnham and Anderson, 2003). We used the pseudo - R^2 values to measure the predictive ability of the top model and competitive models. We evaluated the top model coefficient (s) ($\hat{\beta}_i$) for a statistically significant influence on American Eel condition ($\alpha=0.05$), focusing on river and length. We calculated a 95% confidence interval for all top model coefficients. We exponentiated model coefficients to obtain incident rate ratios (IRR).

Results

Parasite Presence, Prevalence, and Intensity

A. crassus was not detected in any American Eel in Puerto Rico, while Monogenean gill parasites were present in eel from all rivers sampled. Monogenean parasites were detected in the gills of American Eel at 10 of 13 sites (77.0%) (Table 3; Figure 3). These parasites were present at all sites in Río Piedras and absent from 1 site located in a tributary of Río Matilde (Figure 3). They were present throughout most of the main stem in Río Mameyes but were not detected at sites farther inland (Table 3; Figure 3). The island-wide prevalence of Monogeneans was 41.8% while site-specific prevalence ranged from 0 % – 100% (Table 3). Mean intensity of Monogeneans was 10 parasites per eel with mean intensity at each site ranging from 0 – 113 parasites per eel (Table 3). The most infected eel was found in Río Matilde with 166 monogeneans. Mean length and weight of American Eel was 288 mm and 62 g, respectively (Table 2).

American Eel Condition Models

The length-weight relationship equation from Puerto Rico indicated a fish growth parameter $b = 3.1172$. This indicates positive allometric growth. Overall relative condition averaged 1.07 and ranged from 0.340 – 13.1 (Table 2). Relative condition of American Eel sampled during the wet season averaged 1.07 and ranged from 0.730 – 13.1 (Table 2). We fit 9 candidate simple and multiple linear regression models to American Eel condition data. The Akaike Information Criterion ($AICc_i$) favored the model with distance from the river mouth (Table 4). Three additional models were considered competitive due to their $\Delta AICc_i \leq 2$ and have comparable $AICc_i$ weight values to the top model (Table 4). The first competitive model had American Eel condition as a function of distance from the river mouth and river (Table 4).

The second competitive model contained American Eel condition as a function of distance from the river mouth, Monogenean parasite count, and river (Table 4). The third competitive model contained American Eel condition as a function of distance from the river mouth and Monogenean parasite count (Table 4). The full model average of the 95% confidence set of models indicated that Monogenean parasite intensity was not statistically significant ($\alpha = 0.05$) (Table 6). The model averaged results indicated a statistically significant decrease in American Eel condition with increasing distance from the river mouth ($\alpha = 0.05$) (Table 6). The Monogenean parasite predictor was either not present or not significant in the top and competitive multiple regression models ($\alpha = 0.05$) (Table SI1, SI2, SI3, SI4). Adjusted R^2 values for each of the models were 0.04 – 0.08 f indicating that Monogenean parasite count, distance from the river mouth, and differences between rivers do not adequately explaining variation in American Eel body condition.

Monogenean Intensity Model

We fit 9 generalized linear regression models with a negative binomial distribution to parasite count data. The Akaike Information Criterion ($AICc_i$) strongly favored the model with Monogenean parasite intensity as a function of river and American Eel length (Table 5). This model had the greatest $AICc_i$ weight of 57%. There was an additional competitive model indicating Monogenean parasite intensity as a function of river, distance from the river mouth, and American Eel length (Table 5). Pseudo R^2 values for these 2 models ranged from 0.49 – 0.50 (Table 5). The top logistic regression model indicated that Río Matilde significantly differed from Río Mameyes and Río Piedras (the reference rivers) (Table 7). The incidence rate ratio (IRR) indicates that length-corrected parasite intensity increases by 20.9 in Río Matilde, compared to Río Mameyes, and increases by 9.78, compared to Río Piedras (Table 7). Log

Monogenean parasite counts in Río Piedras did not significantly differ from those in Río Mameyes. The top logistic regression model also indicated that length has a significant positive relationship with parasite count (Table 7). This indicates that Monogenean parasite intensity increases by 2.31 with increasing American Eel length (Table 7).

Discussion

The swim bladder parasite (*Anguillicoloides crassus*) is absent from freshwater environments in Puerto Rico. Our results and those obtained from previous island-wide studies have noted the absence of the swim bladder parasite in the island. American Eel collected in this study and those from Kwak et al. (2019) included eel from Río Piedras. The Río Piedras is the most susceptible river to exotic parasite invasion due to large shipping vessels requiring access to the San Juan Bay (Lugo et al., 2011). The absence of *A. crassus* even on this river may be due to high salinity and temperature environments and no records of anthropogenic introduction of the species in Puerto Rico (De Charleroy et al., 1990; Barse et al., 2001; Kwak et al., 2019).

This is the first report describing Monogenean gill parasite infection in American Eel from Puerto Rico. The length of time these parasites have been present in American Eel on the island is unknown. We are not aware of reports regarding imports of American Eel on the island and these parasites are not able to survive high salinities which may support arguments about Monogenean parasites to be endemic on American Eel (Cone and Marcogliese, 1995; Kennedy, 2007). Various species of Monogenean parasites, not known to have infected American Eel, have been reported on the gills of native and stocked sport fishes in Puerto Rico (Williams and Williams, 1994). Therefore, possible invasion of one of these species of gill parasites could have occurred with stocking of sport fish species and may present a new host record for American Eel found in Puerto Rico, as seen with other fish in Europe (Ondračková et al., 2020). However, the Monogenean parasites reported on sport fishes in Puerto Rico have not been reported to infect American Eel on the island or in other parts of the United States and Canada (Williams and Williams, 1994). There is evidence to suggest the *Pseudodactylogyrus* sp. may be infecting the gills of American Eel in Puerto Rico, as Monogenean parasites species can be host specific

(Buchmann and Lindenstrøm, 2002; Bychowsky, 1957). However, further evaluation is needed to identify the specimens at the species level.

Monogenean parasites can reach high prevalence and intensity in Anguillid eels. Monogenean prevalence in Puerto Rico was comparable to those from eels infected with *P. anguillae* and *P. bini* Monogeneans in the United States and Portugal (Rodrigues and Saraiva, 1996; Hayward et al., 2001). Maximum parasite intensity in our sample was significantly lower than those indicated in American Eel by Hayward et al. (2001), who reported intensities > 200 parasites per eel. However, Monogenean parasite intensities from Puerto Rico and the United States were greater compared to those from Nova Scotia (Cone and Marcogliese, 1995). One possible explanation may be that Monogeneans produce less eggs and mature at slower rates at lower temperatures (Reed et al., 2003; Kennedy, 2007). Maximum parasite intensity found in our study was greater than those reported for wild European Eel in Poland, where parasite intensity reached 16 and 56 parasite per eel, respectively (Cone and Marcogliese, 1995; Sobocka and Pilecka-Rapacz, 2003). These differences in Monogenean intensity may be due to a wide array of possible causes ranging from differences in environmental conditions to differences between hosts (Huu-Yun et al., 1984; Sobocka and Pilecka-Rapacz, 2003; Reed et al., 2003). Despite these differences among locations, our results, and those from multiple regions, show that Monogenean parasites can rapidly spread and reach great intensities in eels once it invades freshwater environments.

Monogenean parasite intensity may not affect American Eel body condition. American Eel, on average, display good relative condition in Puerto Rico. The slope indicating American Eel growth in Puerto Rico from the LWR equation was $b = 3.1172$. This slope was within the range (3.06 – 3.29) recorded for American Eel throughout the United State Atlantic coast (ASMFC,

2017). The mean relative condition factor ($K_n = 1.1$) indicated that American Eel from our sample are in good relative condition. Our results indicated no significant effects of Monogenean parasite intensity on American Eel body condition. These results are not comparable to those found in the European Eel where Monogenean parasite abundance was negatively correlated with body condition of females (Gérard, et al., 2013). This indicates that wild populations of American Eel in Puerto Rico may not be significantly affected by these infections, as noted by Kennedy (2007) for Atlantic eels. It is possible that American Eel in the sampled rivers of Puerto Rico remain within the bounds of good relative condition, despite Monogenean parasite infection, due to their ability to move freely throughout the river system, as the rivers are either free flowing or still had accessibility to upstream reaches (Cooney and Kwak, 2013). This causes less densities of American Eel to accumulate downstream and may prevent reinfection of individuals (Reed et al., 2013). However, this can quickly change with increasing in-stream barriers, water diversions, and drought conditions where environments can become crowded and induce stress, resulting in greater parasite intensities that may lead to clinical effects (Larrat, et al., 2012; Reed et al., 2013).

Monogenean parasite intensity in American Eel varies by river in Puerto Rico. American Eel from Río Matilde are more likely to have greater parasite intensities than other rivers included in this study. Greater parasite intensities in Río Matilde may be associated with run-off from predominantly high populated rural residential areas in the riparian zone (Rodríguez-Martínez et al., 2005) and further aggravated by its narrow channel (Table 7). This may lead to stress inducing environments compromising the eel's immune response to the parasite making it possible for transmissions to occur at higher rates (Buchmann, 1993; Larrat et al., 2012; Reed et al., 2013). Cone and Marcogliese (1995) observed similar infection patterns when monogeneans

were first described in American Eel in Nova Scotia to occur in streams passing through agricultural lands and domestic areas. On the other hand, Río Mameyes had the lowest Monogenean parasite intensities with an absence of the parasite in the river's upstream environments. This may be due to Río Mameyes being one of the most pristine free-flowing rivers in Puerto Rico and protected by the United States Forest Service in upstream reaches (Kwak et al., 2013; Smith and Kwak, 2014). However, even low intensities of monogeneans should require continued monitoring for the overall health of American Eel and possible spread of the parasite to upstream environments.

Monogenean parasite intensity may increase with American Eel body length in Puerto Rico. Similar to other studies with Anguillid eels, we found that American Eel with greater total length have a greater probability of harboring greater Monogenean parasite intensities (Huu-Yun, et al., 1984; Buchmann, 1989; Sobecka and Pilecka-Rapacz, 2003). This was also observed by Aguilar et al. (2005) in Spain where body length of European Eel had a positive relationship with presence of *Pseudodactylogyrus sp.* This may be explained by greater gill surface area available for parasite attachment in larger eel (Hughes, 1966; Buchmann, 2012). Although greater parasite intensities can be found in longer American Eel, the probability of any detrimental effects to occur on the individual may be lower, compared to shorter American Eel (Buchmann, 2012).

This is the first step towards understanding the geographical extent of parasite infections and threats caused by these in American Eel. This study has expanded the known range of Monogenean gill parasite infection in American Eel. It has further validated the absence of the swim bladder parasite, *A. crassus*, in Puerto Rico. Our findings indicate that Monogenean parasite intensity may decrease American Eel condition in Puerto Rico, but the species remains within the overall bounds of good relative condition. Our study suggests that Monogenean

parasite intensities may vary between rivers. It is possible that different environmental conditions that may alter the host's ability to withstand greater infections (Cone and Marcogliese, 1995; Barker and Cone, 2000). These parasite intensities appear to be dependent on host length. Mean intensities found in the sample were low, compared to other regions. However, there is a possibility this could change with increasing in-stream barriers and extreme drought events in Puerto Rico (Reed et al., 2013). Our findings provide natural resource managers with crucial information to improve monitoring efforts, water quality, and in-stream connectivity to reduce the vulnerability of infection which will benefit both wild and farmed American Eel.

Management and Conservation Implications

Our results provide management and conservation agencies with a better understanding of the extent of *A. crassus* infection in Puerto Rico and the Caribbean. It is necessary to continuously monitor American Eel for infection throughout its known range, given the possible effects *A. crassus* infections may cause in the eel's resistance to environmental stressors, spawning migration, and reproduction (Lefebvre et al., 2007, 2012; Kennedy, 2007; Palstra et al. 2007; Sjoberg et al., 2009). The absence of the swim bladder parasite on American Eel in Puerto Rico may increase the probability of completing spawning migrations, which suggests this region is significantly contributing to the survival and reproductive success of the species. This information can aid in continuing efforts to monitor parasite infection in the American Eel in Puerto Rico and the Caribbean.

This study has considerably expanded knowledge regarding the geographical extent of Monogenean gill parasite infection in American Eel. Although it is not considered a major threat to the health of wild American Eel, exports of these infected fish from Puerto Rico may cause

complications in aquaculture facilities (Møllergaard, 1990; Kennedy, 2007). This information may help natural resource managers to prioritize stream connectivity to decrease environmental stress and rapid increments of parasite intensity that may cause wild populations to experience adverse effects (Reed et al., 2013).

Little is known regarding parasitic infections of American Eel throughout its entire range. Our findings present a first step towards understanding the level of risk associated with parasite infection. Despite this, there is need for research aimed at analyzing and quantifying the direct effects of parasite intensity on American Eel health as well as increasing monitoring for *A. crassus* and Monogenean gill parasite infections throughout the United States and Canada. Increasing sample size and parasite identification at the species level will aid in monitoring and management decisions. Improving monitoring, water quality, and in-stream connectivity will decrease vulnerability to greater intensity of infection and ensure the health and persistence of wild and farmed American Eel.

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Tables

Table 1. Sampling site coordinates and their respective distances from the river mouth within 5 rivers located in the north, south, east, and west of the island of Puerto Rico. American Eel were collected from 20 sites using a 3-pass pulsed-DC electrofishing method during the dry season (November 2020 – January 2021) and 14 sites were resampled during the wet season (June 2021-August 2021).

Site ID	River	Drainage basin	Distance from river mouth (km)	Latitude (N)	Longitude (W)	Sampling	
						Dry season 2020-2021	Wet season 2021
46C	Río Piedras	Río Piedras	4.75	18.4053657	-66.0690448	X	X
46A	Río Piedras	Río Piedras	8.61	18.3840681	-66.0586831	X	X
46B	Río Piedras	Río Piedras	11.2	18.3670476	-66.0631768	X	X
RP2	Río Piedras	Río Piedras	14.9	18.343722	-66.0560056	X	X
4Q	Río Mameyes	Río Mameyes	2.22	18.3730357	-65.7635835	X	X
4K	Tabonuco	Río Mameyes	3.97	18.361063	-65.769013	X	X
RM2	Tabonuco	Río Mamayas	5.89	18.347116	-65.7684802	X	X
4I	Río Mameyes	Río Mameyes	7.02	18.3408842	-65.7536526	X	X
4F	Río Mameyes	Río Mameyes	9.15	18.3276234	-65.7507864	X	X
4D	Río Mameyes	Río Mameyes	9.78	18.3226959	-65.7500712	X	X
5C	Río Sabana	Río Sabana	2.48	18.362925	-65.7208063	X	
5A	Río Sabana	Río Sabana	4.32	18.350457	-65.7260138	X	
RS5	Río Sabana	Río Sabana	5.35	18.3428929	-65.7300596	X	
5D	Río Sabana	Río Sabana	7.69	18.3254706	-65.7295268	X	
RS4	Río Pitahaya	Río Sabana	9.72	18.321595	-65.7164504	X	
28D	Río Canas	Río Matilde	5.5	18.0249	-66.6409652	X	X
28E	Río Pastillo	Río Matilde	7.49	18.0347752	-66.6624655	X	X

Table 1. (Continued)

Site ID	River	Drainage basin	Distance from river mouth (km)	Latitude (N)	Longitude (W)	Sampling	
						Dry season 2020-2021	Wet season 2021
28C	Río Canas	Río Matilde	7.84	18.0400361	-66.6447425	X	X
28B	Río Canas	Río Matilde	14.0	18.0802422	-66.6538603	X	X
RG3	Río Hondo	Río Guanajibo	4.71	18.1604514	-67.1497042	X	
35H	Río Rosario	Río Guanajibo	10.4	18.1267652	-67.1237605	X	
35B	Río Rosario	Río Guanajibo	14.1	18.1583217	-67.085124	X	
RG2	Río Rosario	Río Guanajibo	16.9	18.1732659	-67.0722671	X	

Table 2. Mean length, weight, and relative condition (K_n) measurements for American Eel collected from 5 rivers in Puerto Rico during the dry and wet seasons. Sampling sites denoted with (--) indicate the absence of American Eel. “n” denotes the total number of American Eel caught at each site.

River	Site ID	n	Mean length (mm) ± SE	Min – max	Mean weight (g) ± SE	Min – max	Mean K_n ± SE	Min – max
Dry season (2020 – 2021)								
Río Piedras	46C	13	337 ± 24.5	225 – 495	84.5 ± 20.7	14 – 236	1.01 ± 0.04	0.637 – 1.16
	46A	3	375 ± 81.2	275 – 536	133 ± 86.3	34 – 305	1.04 ± 0.04	0.952 – 1.10
	46B	1	305 ± 0	305 – 305	46 ± 0	46 – 46	0.933 ± 0.0	0.933 – 0.933
	RP2	8	301 ± 41.6	179 – 471	67.1 ± 29.2	7 – 227	0.877 ± 0.07	0.490 – 1.19
Río Mameyes	4Q	0	--	--	--	--	--	--
	4K	27	246 ± 8.72	163 – 312	29.8 ± 3.4	3 – 60	1.03 ± 0.03	0.429 – 1.33
	RM2	8	278 ± 46.6	182 – 593	74.6 ± 52.0	8 – 438	1.01 ± 0.05	0.811 – 1.21
	4I	13	243 ± 12.7	160 – 296	25.7 ± 4.3	5 – 47	0.906 ± 0.03	0.736 – 1.06
	4F	6	264 ± 50.1	148 – 439	48.0 ± 21.0	3 – 132	0.947 ± 0.10	0.533 – 1.19
	4D	5	229 ± 22.1	175 – 291	19.4 ± 5.9	8 – 36	0.843 ± 0.05	0.653 – 0.974
Río Sabana	5C	18	251 ± 20.2	145 – 460	33.8 ± 9.4	5 – 137	0.912 ± 0.03	0.631 – 1.24
	5A	23	223 ± 23.0	90 – 645	33.3 ± 17.5	1 – 412	0.842 ± 0.03	0.448 – 1.26
	RS5	18	267 ± 26.6	125 – 516	41.9 ± 14.0	2 – 229	0.821 ± 0.04	0.349 – 1.03
	5D	0	--	--	--	--	--	--
	RS4	0	--	--	--	--	--	--
Río Matilde	28D	6	312 ± 51.2	152 – 448	73.3 ± 28.2	6 – 184	0.947 ± 0.05	0.817 – 1.13
	28E	0	--	--	--	--	--	--
	28C	9	288 ± 64.5	155 – 643	113 ± 67.2	7 – 485	0.990 ± 0.04	0.792 – 1.17
	28B	1	577 ± 0	577 – 577	329 ± 0	329 – 329	0.914 ± 0.00	0.914 – 0.914
Río Guanajibo	RG3	5	204 ± 38.6	153 – 358	20.2 ± 13.2	5 – 73	0.933 ± 0.07	0.688 – 1.05
	35H	3	471 ± 7.2	462 – 485	197 ± 15.0	174 – 225	1.03 ± 0.03	0.967 – 1.07
	35B	8	442 ± 19.7	375 – 516	141 ± 15.8	91 – 226	0.899 ± 0.06	0.627 – 1.08
	RG2	10	468 ± 30.1	363 – 644	197 ± 43.4	84 – 475	0.929 ± 0.03	0.790 – 1.04

Table 2. (Continued)

River	Site ID	n	Mean length (mm) \pm SE	Min – max	Mean weight (g) \pm SE	Min – max	Mean K_n \pm SE	Min – max
Wet season (2021)								
Río Piedras	46C	1	215 \pm 0.0	215 – 215	15 \pm 0.0	15 – 15	0.918 \pm 0.00	0.918 – 0.918
	46A	6	302 \pm 22.9	219 – 373	63 \pm 14.2	23 – 105	1.21 \pm 0.07	0.998 – 1.42
	46B	3	372 \pm 29.5	340 – 431	89 \pm 26.9	60 – 143	0.917 \pm 0.04	0.867 – 0.987
	RP2	2	398 \pm 97.0	301 – 495	145 \pm 99.0	46 – 244	1.03 \pm 0.06	0.972 – 1.09
Río Mameyes	4Q	27	174 \pm 20.1	65 – 330	20 \pm 4.6	1 – 79	1.56 \pm 0.14	0.789 – 3.79
	4K	29	266 \pm 19.1	135 – 702	58 \pm 23.0	5 – 695	1.17 \pm 0.03	0.795 – 1.61
	RM2	6	228 \pm 25.0	180 – 338	25 \pm 10.6	10 – 76	0.991 \pm 0.03	0.899 – 1.12
	4I	10	218 \pm 18.5	110 – 294	20 \pm 4.2	2 – 41	0.976 \pm 0.04	0.730 – 1.12
	4F	5	225 \pm 26.7	154 – 303	23 \pm 8.1	5 – 50	0.994 \pm 0.04	0.853 – 1.06
	4D	3	274 \pm 47.4	180 – 332	32 \pm 12.3	8 – 49	0.778 \pm 0.03	0.733 – 0.840
Río Matilde	28D	6	316 \pm 60.9	156 – 570	115 \pm 62.0	19 – 418	3.04 \pm 2.02	0.885 – 13.1
	28E	0	--	--	--	--	--	--
	28C	2	314 \pm 87.5	226 – 401	64 \pm 46.0	18 – 110	0.940 \pm 0.01	0.929 – 0.950
	28B	2	526 \pm 24.5	501 – 550	267 \pm 29.5	237 – 296	0.989 \pm 0.03	0.956 – 1.02
<i>Island-wide statistics</i>								
Mean K_n \pm SE					1.07 \pm 0.05			

Table 3. Site specific and site-wide prevalence and mean intensity of Monogenean gill parasites from 79 American Eel collected by pulsed-DC backpack electrofishing from 13 sites in Puerto Rico from June - August 2021. The swim bladder parasite, *A. crassus*, was absent from all American Eel in this sample. Sampling sites denoted by (--) indicate the absence of American Eel. “n” denotes the number of American Eel.

River	Site ID	n	^a Prevalence (%) ± SE	Mean Intensity (parasites/eel) ± SE	Min - max
Río Piedras	46C	1	100 ± 0	1 ± 0	1 – 1
	46A	6	83.3 ± 15.2	4 ± 2.0	0 – 13
	46B	3	66.7 ± 27.2	13 ± 12.2	0 – 37
	RP2	2	50.0 ± 35.4	17 ± 16.5	0 – 33
Río Mameyes	4Q	13	53.8 ± 13.8	3 ± 1.6	0 – 19
	4K	28	17.9 ± 7.2	1 ± 0.2	0 – 4
	RM2	3	0 ± 0	0 ± 0	0
	4I	8	37.5 ± 17.1	3 ± 1.6	0 – 11
	4F	3	0 ± 0	0 ± 0	0
	4D	3	0 ± 0	0 ± 0	0
Río Matilde	28D	5	100 ± 0	70 ± 30.7	3 – 166
	28E	--	--	--	--
	28C	2	100 ± 0	6 ± 3.0	3 – 9
	28B	2	100 ± 0	113 ± 24.0	89 – 137
<i>island-wide statistics</i>					
prevalence (%) ± SE			41.8 ± 5.6		
mean intensity (parasites/eel) ± SE			10 ± 3.3		

^aProportion of infected eel

Table 4. The 95% confidence set of simple and multiple linear regression models for American Eel condition. Competing models have $\Delta AIC_{ci} \leq 2$. American Eel condition was calculated using the relative condition factor (K_n) using a LWR equation for Puerto Rico. Models included the numerical predictors: distance from the river mouth ‘dtm’ and Monogenean parasite counts ‘mpcount’, the categorical predictors ‘river’ representing the 3 rivers included in the study, and an interaction between distance from the river mouth and river, denoted by (:). All numerical predictors were scaled.

Response	Model description	AIC _{ci}	ΔAIC_{ci}	W_i (AIC _c)	Adjusted R ²	k
Condition (K_n)	dtm	-51.3	0.00	0.314	0.05	1
	dtm + river	-50.7	0.59	0.234	0.08	2
	dtm + mpcount + river	-50.3	0.96	0.194	0.09	3
	dtm + mpcount	-49.3	1.98	0.117	0.04	2
	dtm + river + dtm:river	-48.1	3.23	0.062	0.08	3
	dtm + mpcount + river + dtm:river	-46.8	4.53	0.033	0.08	4

Table 5. The 95% confidence set of logistic regression models with a negative binomial distribution for Monogenean parasite counts. Competing models have $\Delta AICc_i \leq 2$. Models included the numerical predictors: distance from the river mouth ‘dtm’ and Monogenean parasite counts ‘mpcount’, the categorical predictors ‘river’ representing the 3 rivers included in the study, and an interaction between distance from the river mouth and river. All numerical predictors were scaled.

Response	Model description	AICc _i	$\Delta AICc_i$	W _i (AICc)	Pseudo R ²	k
Monogenean intensity (mpcount)	river + length	316.8	0.00	0.571	0.492	2
	river + dtm + length	318.1	1.28	0.301	0.502	3
	river	321.2	4.33	0.066	0.425	1
	river + dtm + length + dtm:river	322.7	5.82	0.031	0.505	4

Table 6. Full model averaged coefficients ($\hat{\beta}$) from the 95% confidence set of simple and multiple linear regression models for American Eel condition. Models included the numerical predictors: distance from the river mouth ‘dtm’ and Monogenean parasite counts ‘mpcount’. The categorical predictor ‘river’ represents the 3 rivers included in the study. The interaction between distance from the river mouth and river is denoted by (:). Coefficients for numerical predictors are scaled. P-values were calculated at $\alpha=0.05$.

Predictor variable	$\hat{\beta}$	SE	P-value
intercept	1.06	0.02	<2.0e-16***
distance from the river mouth ‘dtm’	-0.0619	0.03	0.030*
Río Matilde	-0.0234	0.07	0.72
Río Piedras	0.0642	0.08	0.42
Monogenean count ‘mpcount’	0.00960	0.02	0.65
dtm:Río Matilde	0.00761	0.03	0.80
dtm:Río Piedras	0.00491	0.02	0.85

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Table 7. Coefficients ($\hat{\beta}$) from the top logistic regression model for Monogenean gill parasite count as a function of the categorical predictor ‘river’ and the numerical predictor ‘length’ which represent the 3 rivers in the study and the total length of American Eel, respectively. The coefficient for the numerical predictor is scaled. P-values were calculated at $\alpha=0.05$. The incidence rate ratio (IRR) values were calculated by exponentiating each of the coefficients.

Predictor variable	$\hat{\beta}$	IRR	SE	P-value
Reference level = Río Mameyes				
intercept	0.341	1.41	0.28	0.23
river (Río Matilde)	3.04	20.9	0.73	3.3e-5***
river (Río Piedras)	0.760	2.14	0.64	0.23
length	0.839	2.31	0.24	0.00056***
Reference level = Río Piedras				
intercept	1.10	3.00	0.57	0.052 .
river (Río Mameyes)	-0.760	0.4677	0.64	0.23
river (Río Matilde)	2.28	9.78	0.84	0.0069**
length	0.839	2.31	0.24	0.00056***

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Figures

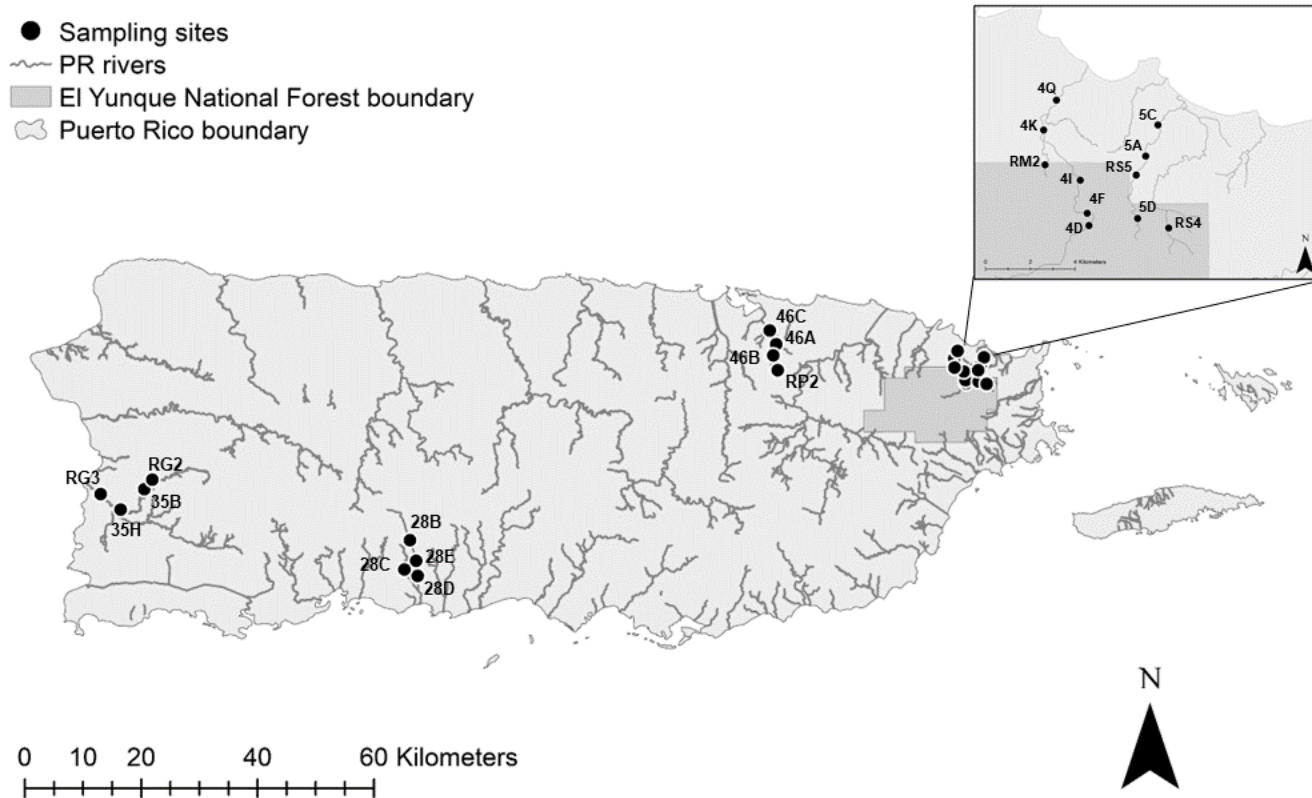


Figure 1. — Map of American Eel sampling reaches (n = 23) located in 5 rivers throughout the north, south, east, and west of the island of Puerto Rico. All sites were sampled for eel collection using a pulsed-DC electroshocking method on 60-m long reaches.

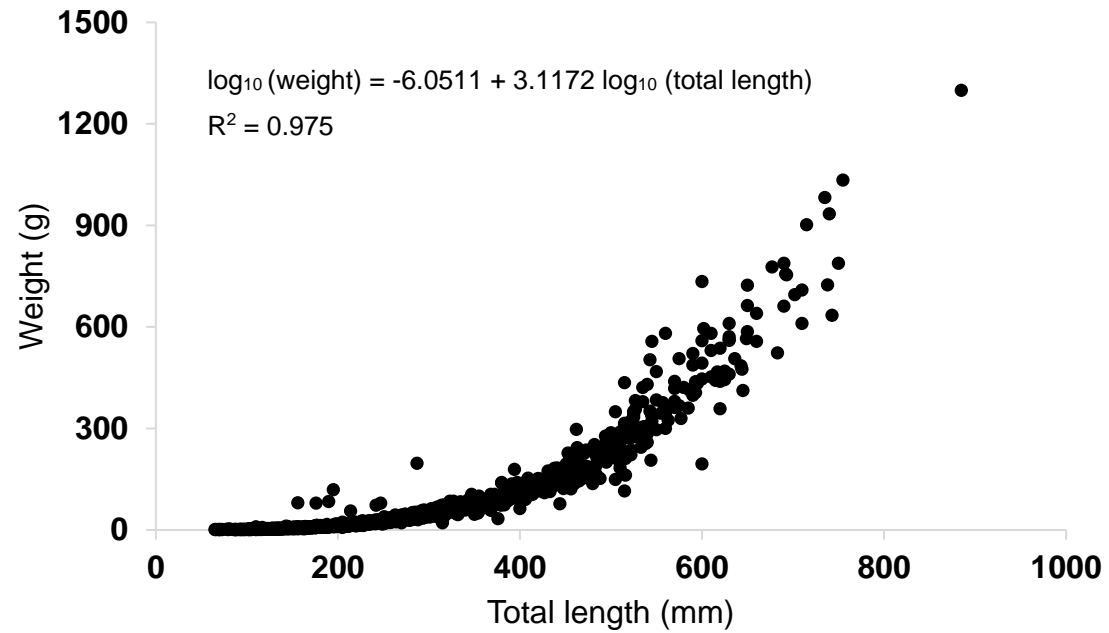


Figure 2. Length-weight relationship of 1825 American Eel sampled island-wide in Puerto Rico from 2005 – 2021 by Kwak et al. (2019), Roghair et al. (2018), and Torres-Molinari et al. The relationship is indicated by the linear equation displayed in the plot.

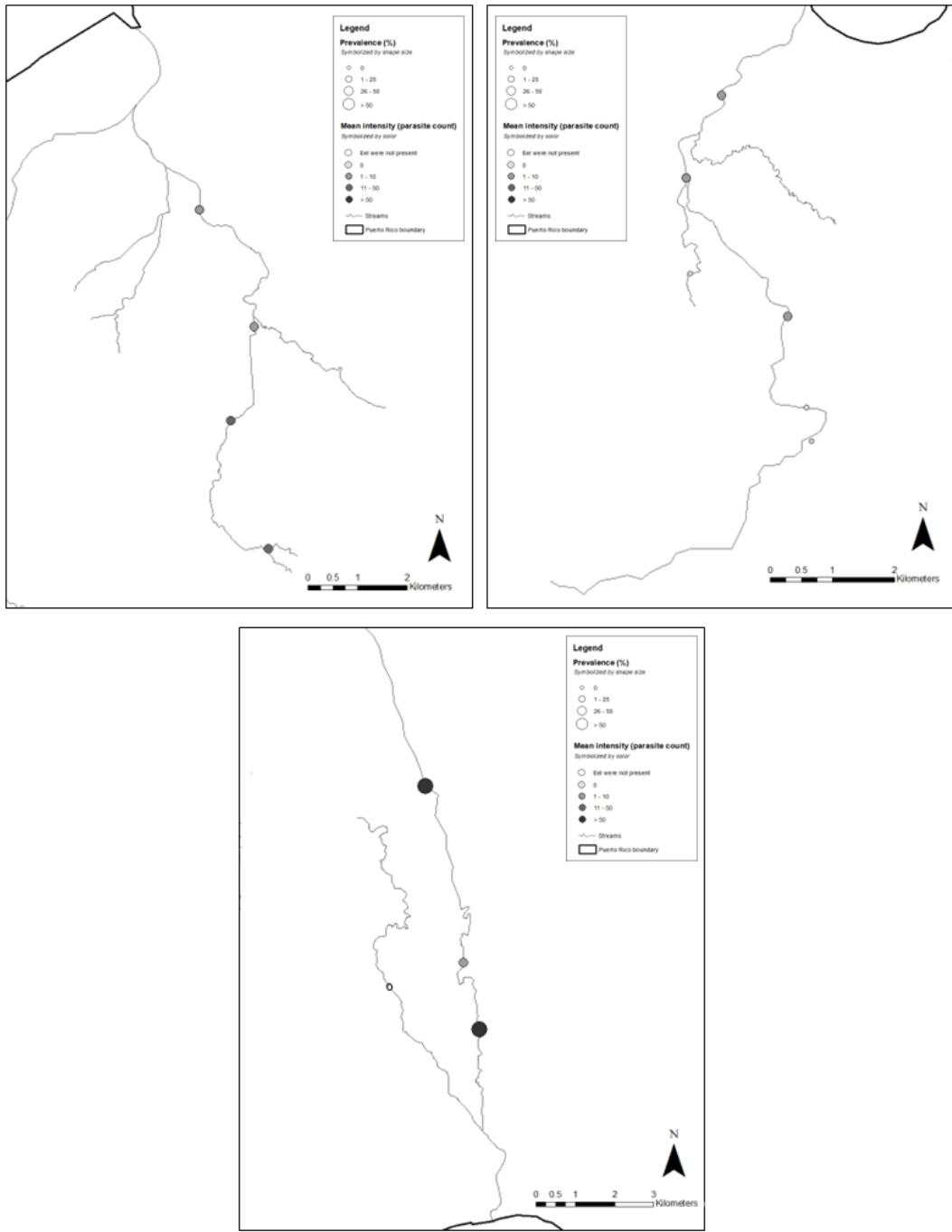


Figure 3. Prevalence and mean intensity of Monogenean gill parasites in American Eel collected from 3 rivers located in the north and south regions of Puerto Rico.

APPENDICES

Appendix A

Table SI 1. Candidate set of simple and multiple linear regression models for density of American Eel. These models are ordered by ascending AIC_c and delta-AIC_c values and descending AIC_c weights. The first model represents the top model explaining variation in density of American Eel. The term ‘dtm’ represents the numerical predictor of distance from the river mouth and the term ‘dtm:river’ represents an interaction between the 2 predictor variables. The terms ‘season’ and ‘river’ are categorical predictors representing the dry and wet seasons, and the 5 rivers included in this study, respectively.

Response	Model description	AIC _c	Δ _i AIC _c	W _i (AIC _c)	Adjusted R ²	k
density	dtm	470.4	0	0.639	0.133	1
	dtm + season	472.6	2.22	0.211	0.115	2
	1 (null)	474.2	3.81	0.095	--	0
	season	476.4	6.04	0.031	-0.0250	1
	dtm + river	478.6	8.23	0.010	0.0968	2
	river	479.1	8.67	0.008	0.0355	1
	dtm + river + season	481.6	11.20	0.002	0.0749	3
	river + season	481.9	11.45	0.002	0.0123	2
	dtm + river + dtm:river	484.6	14.15	0.001	0.190	3
	dtm + river + dtm:river + season	488.7	18.25	0.000	0.169	4

Table SI 2. Candidate set of simple and multiple linear regression models for American Eel body length. These models are ordered by ascending $AICc_i$ and delta- $AICc_i$ values and descending $AICc_i$ weights. The first model represents the top model explaining variation in American Eel body length. Models with $\Delta_i AICc \leq 2$ are considered competitive models. The term ‘dtm’ represents the numerical predictor of distance from the river mouth and the term ‘dtm:river’ represents an interaction between the 2 predictor variables. The terms ‘season’ and ‘river’ are categorical predictors representing the dry and wet seasons, and the 5 rivers included in this study, respectively.

Response	Model description	$AICc_i$	$\Delta_i AICc$	$W_i(AICc)$	Adjusted R^2	k
body length	dtm + river + dtm:river	3469.2	0.00	0.705	0.295	3
	dtm + season + river + dtm:river	3471.0	1.81	0.285	0.294	4
	dtm + river	3478.3	9.10	0.007	0.261	2
	dtm + season + river	3480.2	11.02	0.003	0.259	3
	dtm	3490.4	21.20	0.000	0.218	1
	dtm + season	3490.5	21.36	0.000	0.221	2
	river	3492.8	23.63	0.000	0.220	1
	season + river	3494.1	24.92	0.000	0.220	2
	season	3555.1	85.93	0.000	0.0203	1
	1 (null)	3559.9	90.76	0.000	--	0

Table SI 3. Candidate set of logistic regression models with a binomial distribution for presence of American Eel. These models are ordered by ascending AIC_c and delta-AIC_c values and descending AIC_c weights. The first model represents the top model explaining variation in the probability of female presence. Models with $\Delta_i\text{AIC}_c \leq 2$ are considered competitive models. The term ‘dtm’ represents the numerical predictor of distance from the river mouth, ‘river’ is a categorical variable representing the 5 sampled rivers, and the term ‘dtm:river’ represents an interaction between the 2 predictor variables.

Response	Model description	AIC _c	$\Delta_i\text{AIC}_c$	$W_i(\text{AIC}_c)$	Pseudo- R ²	k
presence of females	dtm + length + dtm:length	83.2	0.00	0.369	0.724	3
	dtm + river + length + dtm:length	83.7	0.51	0.286	0.753	4
	length	85.1	1.92	0.141	0.701	1
	dtm + length	86.0	2.82	0.090	0.706	2
	dtm + river + dtm:river + length + dtm:length	87.1	3.88	0.053	0.772	5
	dtm + river + length	87.2	4.02	0.049	0.732	3
	dtm + river + dtm:river + length	90.2	6.98	0.011	0.752	4
	dtm + river	199.7	116.5	0.000	0.310	2
	dtm + river + dtm:river	206.4	123.2	0.000	0.317	3
	dtm	218.6	135.4	0.000	0.210	1
	1 (null)	273.6	190.4	0.000	4.4e ⁻¹⁶	0

Table SI 4. — Coefficients ($\hat{\beta}$) from the top logistics regression model for presence of female American Eel. The top model contains the numerical predictors distance from the river mouth abbreviated as ‘dtm’, ‘length’ of American Eel, and an interaction between the 2 predictors. P-values were calculated at $\alpha=0.05$. Coefficients for continuous predictors are scaled and interactions are denoted by (:).

Predictor variable	$\hat{\beta}$	Log-odds probability	SE	P-value
intercept	-3.937	0.01913	0.62	$1.90e^{-10}$ ***
distance from river mouth (dtm)	1.086	0.7476	0.43	0.0114 *
length	4.855	0.9923	0.99	$8.97e^{-07}$ ***
dtm:length	-1.131	0.2440	0.51	0.0280 *

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Table SI 5. — Coefficients ($\hat{\beta}$) from the competing logistic regression model for presence of female American Eel with $\Delta\text{AICc}_i \leq 2$. This model includes the numerical predictors distance from the river mouth ‘dtm’ and ‘length’, the categorical predictor ‘river’, and an interaction between distance from the river mouth ‘dtm’ and ‘length’. P-values were calculated at $\alpha=0.05$. Coefficients for numerical predictors are scaled and interactions are denoted by (:).

Predictor variable	$\hat{\beta}$	Log-odds probability	SE	P-value
intercept	-4.27	0.01377	1.40	0.0023**
dtm	1.32	0.7889	0.57	0.021*
Río Mameyes	0.121	0.5302	1.32	0.93
Río Matilde	2.80	0.9428	1.70	0.10 .
Río Piedras	-0.396	0.4023	1.02	0.70
Río Sabana	-2.07	0.1123	2.19	0.34
length	5.43	0.9956	1.30	2.8e ⁻⁰⁵ ***
dtm:length	-1.43	0.1931	0.63	0.022*

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Table SI 6. — Coefficients ($\hat{\beta}$) from the competing logistic regression model for presence of female American Eel with $\text{delta-AICc}_i \leq 2$. This model includes the numerical predictor ‘length’. P-values were calculated at $\alpha=0.05$. Coefficients for the numerical predictor is scaled.

Predictor variable	$\hat{\beta}$	Log-odds probability	SE	P-value
intercept	-3.56	0.02763	0.48	$8.3e^{-14}$ ***
length	4.16	0.9847	0.64	$6.8e^{-11}$ ***

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

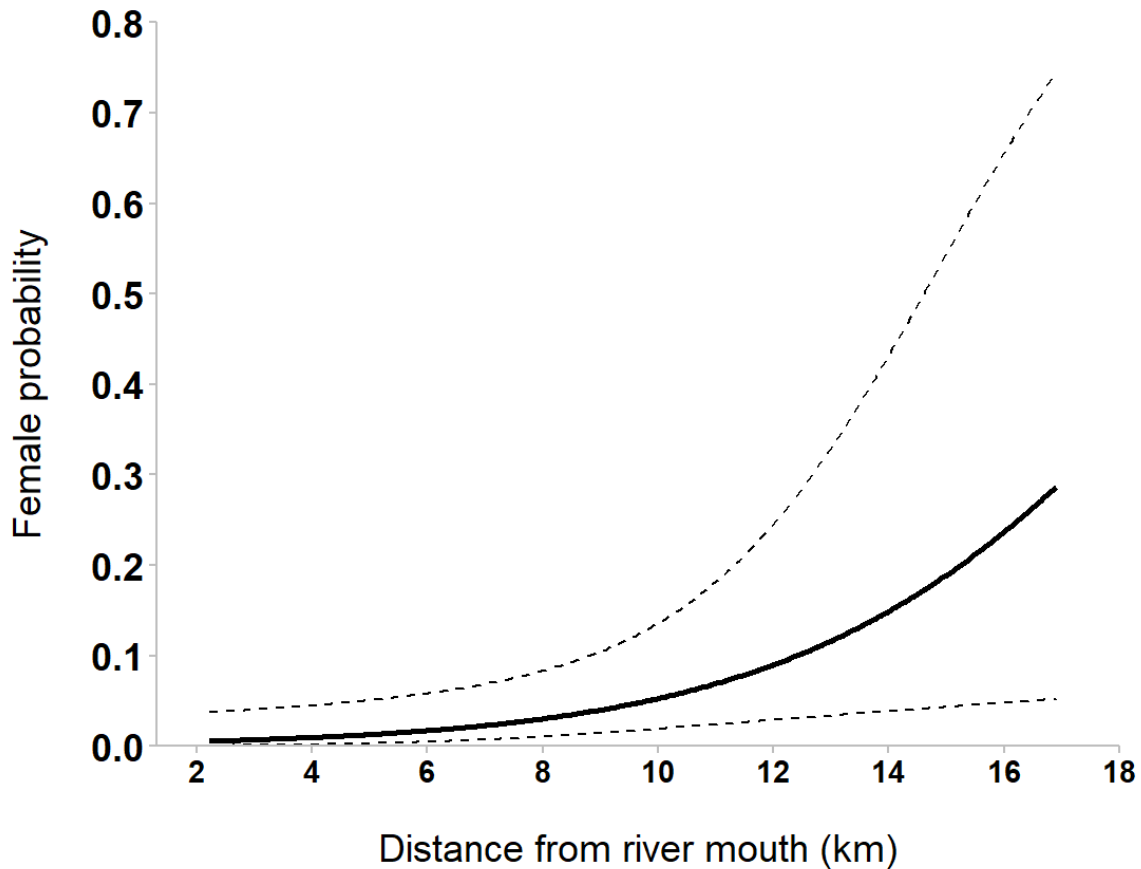


Figure SI 1. — Probability of female eel presence given the distance from the river mouth (solid line) and 95% confidence intervals (dashed lines) respectively. These values were calculated by fitting the top logistic regression model with predictors: distance from the river mouth, length, and an interaction between the 2 predictors to 287 observations of American Eel length and scaled distance from river mouth values from 23 sampling sites (dry season) of which 14 were resampled (wet season). Scaled distance to mouth values displayed on the x-axis were back transformed to their original scale in kilometers.

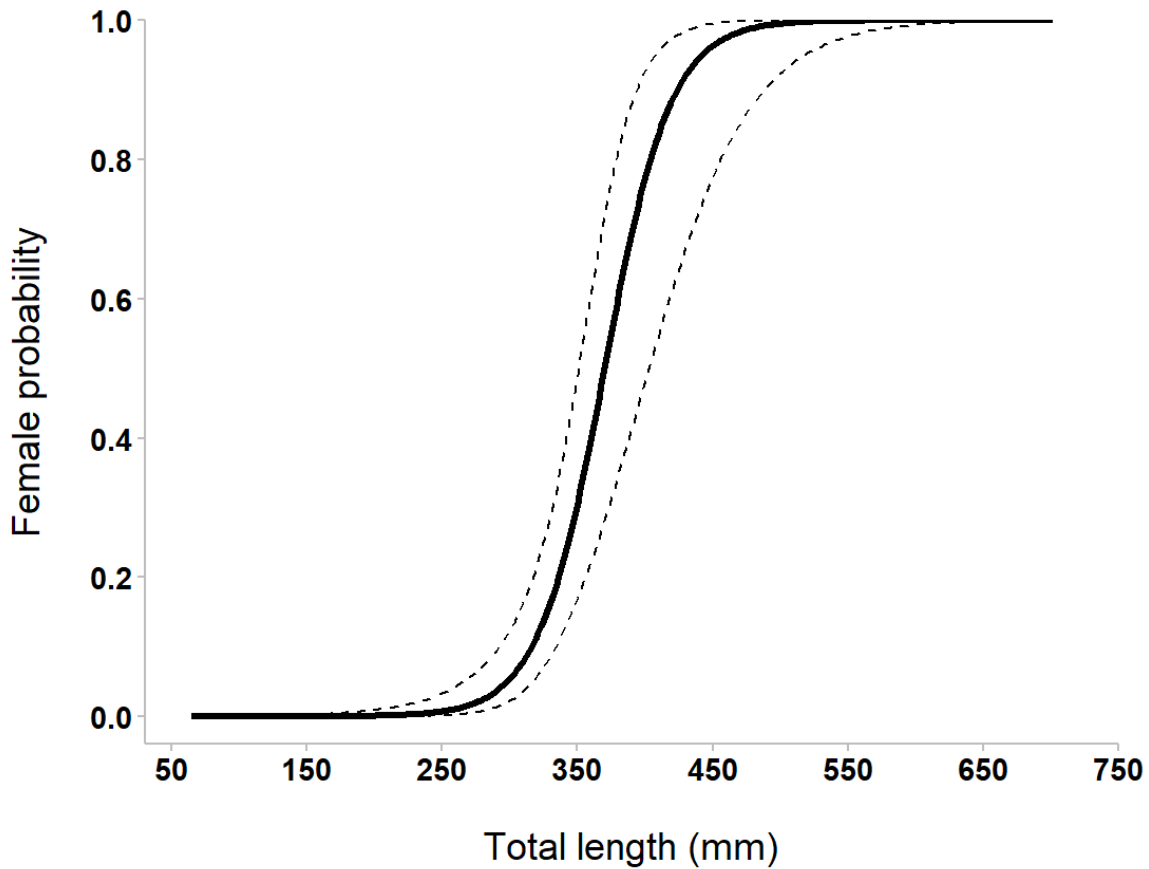


Figure SI 2. — Probability of female eel presence given length (solid line) and 95% confidence intervals (dashed lines) respectively. These values were calculated by fitting the logistic regression model with predictors: distance from the river mouth, length, and an interaction between the 2 predictors to 287 observations of American Eel length and scaled distance from river mouth values from 23 sampling sites (dry season) of which 14 were resampled (wet season). Scaled length values displayed on the x-axis were back transformed to their original scale in mm.

Appendix B

Table SI 1. Coefficients ($\hat{\beta}$) from the top multiple linear regression model for American Eel condition as a function of distance from the river mouth, abbreviated as 'dtm'. The numerical predictor was scaled. P-values were calculated at $\alpha=0.05$.

Predictor variable	$\hat{\beta}$	SE	P-value
intercept	1.07	0.02	<2.0e-16***
distance from the river mouth 'dtm'	-0.0448	0.02	0.023*

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Table SI 2. Coefficients ($\hat{\beta}$) from the competitive model for American Eel condition as a function of the numerical predictor distance from the river mouth, abbreviated as ‘dtm’, and the categorical predictor ‘river’. The numerical predictor was scaled. P-values were calculated at $\alpha=0.05$.

Predictor variable	$\hat{\beta}$	SE	P-value
Intercept	1.05	0.02	<2.0e-16***
distance from the river mouth ‘dtm’	-0.0683	0.02	0.0072**
Río Matilde	-0.00728	0.07	0.91
Río Piedras	0.121	0.07	0.08 .

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Table SI 3. Coefficients ($\hat{\beta}$) from the competitive model for American Eel condition as a function of the numerical predictors distance from the river mouth, abbreviated as ‘dtm’, Monogenean parasite count, abbreviated as ‘mpcount’, and the categorical predictor ‘river’. The numerical predictors were scaled. P-values were calculated at $\alpha=0.05$.

Predictor variable	$\hat{\beta}$	SE	P-value
intercept	1.06	0.02	<2.0e-16***
distance from the river mouth ‘dtm’	-0.0738	0.02	0.0041**
Monogenean counts ‘mpcount’	0.0364	0.03	0.18
Río Matilde	-0.0817	0.08	0.34
Río Piedras	0.121	0.07	0.07 .

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .

Table SI 4. Coefficients ($\hat{\beta}$) from the competitive model for American Eel condition as a function of the numerical predictors distance from the river mouth, abbreviated as ‘dtm’, and Monogenean parasite count, abbreviated as ‘mpcount’. The numerical predictors were scaled. P-values were calculated at $\alpha=0.05$.

Predictor variable	$\hat{\beta}$	SE	P-value
intercept	1.07	0.02	<2.0e-16***
distance from the river mouth ‘dtm’	-0.0478	0.02	0.021*
Monogenean counts ‘mpcount’	0.00975	0.02	0.63

Significance level: 0 ***, 0.001 **, 0.01 *, 0.05 .