FINAL TECHNICAL REPORT

Assessing abundance, sex ratio, and space use by suckermouth armored catfish to enhance control efforts

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Funding for this work was provided by the Texas Parks and Wildlife Department Aquatic Invasive Species Research Grant Program through Contract Number CA-0002259A.

EXECUTIVE SUMMARY

This study focused on advancing management of non-native populations of suckermouth armored catfish (family Loracariidae). Fish from this family, particularly the genera *Hypostomus* and *Pterygoplichthys*, have been introduced to freshwater ecosystems on a global scale and threaten native biota and ecosystems through competition, potential consumption of eggs, and modifications to banks caused by burrowing activity. In Texas, suckermouth armored catfish occur in thermally stable ecosystems such as power plant reservoirs, bayous near Houston, irrigation canals and oxbow lakes (resacas) in south Texas, and several groundwater-fed spring systems such as the Comal, San Antonio, and San Marcos rivers in central Texas and San Felipe Creek in West Texas. In the San Marcos River, the population of *Hypostomus* is managed through spearfishing, a form of control that successfully provides short-term suppression of armored catfish biomass and survival. The current study sought to advance this management effort by providing supporting information on movement, population size, sex ratios and sex determination methods, and the potential for exclusionary devices to block armored catfish from entering artificial refuges (e.g., concrete crevices). Four objectives were developed to achieve this goal with a focus on the genus *Hypostomus*, including (1) providing estimates of the total number of fish in the river, (2) estimation of the sex ratio in the population, (3) estimates of site fidelity and short-term movement based on tagged fish, and (4) experimental exclusion of tagged fish.

Estimates of total abundance were derived using a floating raft fitted with underwater lights and cameras to collect fish count data for the area between Spring Lake Dam and Purgatory Creek (1 km of stream) using three passes during three seasons (spring 2022, fall 2022, spring 2023). These data were combined with geospatial data characterizing water depth, water velocity, the distance to artificial refuges in the river, and aquatic vegetation coverage estimated from seasonal drone imagery to fit a zero-inflated Poisson N-mixture model to assess abundance and detection probability. Sex ratio was estimated using dissections of 385 specimens collected during a purposeful dewatering of the Rio Vista Falls during December 2021. A subset of 212 of these specimens were used to develop a model to predict sex based on external morphology. Site fidelity and movement were assessed using 20 fish fitted with ultrasonic transmitters during 2022 (n = 10) and 2023 (n = 10), and a subset of the tagged fish from 2023 were used to assess the efficacy of an exclusionary device made of hardware cloth that prevented fish from entering a crevice they were known to habitually use.

Estimates of total fish in the 1-km reach of the upper San Marcos River were 4,585 (95% confidence interval = 3,025-7,089) for spring 2022, 4,695 (2,122-10,704) for fall 2022, and 2,942 (1,567-5,996) for spring 2023. Armored Catfish abundance declined over the period of

study as water levels dropped, but was consistently highest near to artificial structures, in shallower depths, and in faster velocities. The detection of armored catfish through visual survey was negatively correlated with vegetation density. The sex ratio in the population was skewed towards females (60% female) based on the snapshot of individuals collected from the dewatering event. The sexual dimorphism model showed that elongated dorsal and anal fins of males were diagnostic of sex for specimens >240 mm total length, the same size at which we estimated reproductive maturity occurs. Tagged catfish showed high site fidelity and strong diel activity patterns characterized by greater activity away from crevices at night and greater time spent within specific crevices during the day. Exclusion of one tagged catfish using hardware cloth caused the fish to move to an adjacent artificial crevice, and there was some evidence that this movement displaced another fish from the secondary crevice.

Collectively, these findings advance management of the population by providing the first fishery-independent estimate of abundance at a riverscape scale, that can be used in future management strategies requiring information on the total number of fish. For example, genetic biocontrol methods that seek to bias sex ratios towards males require knowledge of total population size. Our results further inform such management actions by providing evidence that the sex ratio is currently biased towards females, perhaps because males are larger and more readily removed during ongoing spearfishing suppression. Our results also support previous conclusions that armored catfish in the San Marcos River are largely restricted in their movement, and therefore local management and suppression is likely to be successful in reducing the number of fish, but unlikely to result in total local eradication because of recolonization by a small number of dispersing individuals. Exclusion devices may be a useful complementary control strategy if implemented on a broad scale at the same locations where spatial aggregation of armored catfish occurs, and our work provides spatially explicit heatmaps of armored catfish across a 1-km segment of river. A portion of this study, focusing on sex ratios and sexual dimorphism, is now published in the North American Journal of Fisheries Management and additional publications are anticipated.

INTRODUCTION

Loricariidae is the most speciose family of catfishes (Siluriformes), with over 1,040 valid species across 115 genera (Fricke et al. 2023). Colloquially known as suckermouth armored catfishes (SAC), these fishes are native to Central and South America (Reis et al. 2016). However, introductions of SAC have occurred globally via the aquarium trade due to intentional releases or escape into natural ecosystems (Orfinger and Goodding 2018; Blanton et al. 2020). Impacts of introduced SAC include competition with or exclusion of native species (Pound et al. 2011; Owsley et al. 2017), altered nutrient cycling (Scott et al. 2012; Datri et al. 2015), disturbance and consumption of fish eggs (Cook-Hildreth 2009), and increased erosion and bank instability due to burrowing activity (Hoover et al. 2014). As a result, interest in managing, or potentially eradicating, non-native SAC populations is increasing (Blanton et al. 2020; Hussan et al. 2021). Spearfishing removal programs in high clarity, spring-fed systems have shown success in either (1) eradication when SAC populations are detected early (Hill and Sowards 2015) or (2) suppression after populations are well-established (Blanton et al. 2020). However, development of additional tools for effective management of SAC will require additional natural history information, such as the identification of sexual dimorphism (Hay et al. 2022). For example, the YY Male or Trojan Y Chromosome technique is used to bias the sex ratio within a population strongly, or completely towards males (Teem and Gutiérrez 2010; Schill et al. 2017; Kennedy et al. 2018). Applying this biocontrol method to SAC populations will first require a greater understanding of background sex ratios in invasive populations (Hay et al. 2022), sex determination (Rocha-Reis et al. 2018), and the extent to which existing control methods might bias sex ratios (Blanton et al. 2020).

Despite the high diversity of SAC, and variation of sexual dimorphism among other loricariid genera (Rapp Py-Daniel and Fernandes 2005), there is limited research on secondary sexual characteristics (i.e., dimorphic morphological traits that do not directly contribute to reproduction; Purdom 1993) that could be used to externally determine sex within the genus *Hypostomus*, enabling data collection during spearfishing events to support research and management efforts. Rapp Py-Daniel and Fernandes (2005) reviewed sexual dimorphism among siluriform fishes from the Amazon and noted differences existed for body size, fin size, urogenital papilla shape, odontode number and size, and buccal teeth arrangement. More recently, obvious sexual dimorphism was noted among a newly described genus and species of SAC, *Hirtella carinata*, involving differences in total length, head and mouth morphology, odontode size, and fin length and width (Pereira et al. 2014; Reis et al. 2022). However, limited evidence for sexual dimorphism exists within the globally invasive genus *Hypostomus* (Hoover et al. 2014; Blanton et al. 2020). Gubiani et al. (2009) reported differences in length-weight relationships between males and females, as well as larger body size among male *H. derbyi* in Brazilian reservoirs, while Rocha-Reis et al. (2020) referenced sexual dimorphism among *Hypostomus* without providing detailed examples. Tencatt et al. (2014) reported pelvic-fin spine enlargement in male *Hypostomus basilisko*. Given the uncertainty of the presence of sexual dimorphism among *Hypostomus* spp., additional research is needed to explore the utility of external morphological characters for identification of sex, and ultimately advance the control of invasive SAC populations.

The San Marcos River in Texas, USA is inhabited by non-native SAC representative of two genera, *Hypostomus* and *Pterygoplichthys*. *Hypostomus plecostomus* was historically used to denote the species inhabiting the river, with introduction likely occurring during the 1980s (Perkin and Bonner 2011). However, the exact species identification remains unknown, and work is ongoing to determine the species of *Hypostomus* within the river (N. Lujan, University of Toronto, *personal communication*). *Pterygoplichthys* sp. are occasionally collected from the San Marcos River, but the genus is relatively rare. Populations of SACs within the San Marcos River are currently managed with spearfishing to remove both genera (Blanton et al. 2020; Hay et al. 2022). Blanton et al. (2020) found that of 6,046 SAC removed during spearfishing tournaments in the upper San Marcos River during 2014–2018, only 19 of those fish were recorded as *Pterygoplichthys* sp. while *Hypostomus* sp. persists in the river despite evidence that spearfishing has reduced the population (Blanton et al. 2020; Hay et al. 2022).

The main goal of our study was to advance management of the SAC population in the genus *Hypostomus* in the upper San Marcos River. Our first objective was to estimate total abundance within a 1-km long stretch of the river using N-mixture modeling (Kellner et al. 2023). Our second objective was to characterize the reproductive biology of *Hypostomus* sp. in the San Marcos River as a means of advancing management. We leveraged a planned dewatering of the river for the purpose of repairing collapsing artificial chutes to collect SACs from Rio Vista Park, the same location where Hay et al. (2022) documented high densities of *Hypostomus* sp. Our first subobjective was to estimate the sex ratio for the population as it currently exists within the river. Our second subobjective was to test for sexual dimorphism based on a suite of external morphological characters. We hypothesized that sexual dimorphism would be present and identifiable based on differences in head size and fin length between males and females as identified in previous work by Rapp Py-Daniels and Fernandes (2005) and Reis et al. (2022) for other species of SACs, and that these differences would be positively related to fish age and growth metrics. Our third subobjective was to investigate reproductive investment across a size gradient of *Hypostomus* sp. to estimate the size at which reproduction might begin and how this relates to sexual dimorphism. We hypothesized *Hypostomus* sp. would begin to reach sexual maturation at SL classes ranging 144–160 mm SL (equivalent to TL 198–217 mm). Our fourth subobjective was to assess what proportion of fish removed during spearfishing could be used to track sex ratios or test for gear-specific sex bias during removal. Our third objective was to assess fine scale movement of fish using ultrasonic transmitters and submersible ultrasonic receivers to log time spent within and outside of crevices across day and night. Our fourth and final objective was to use a subset of tagged fish to measure the responses of fish that were excluded from their typical habitats to test for change in movement and habitat use relative to home range.

METHODS

Study area. – The upper San Marcos River is a spring-fed, groundwater-dependent system that arises from a series of artesian wells within Spring Lake in San Marcos, Texas. The water temperature is a nearly constant 22 °C in the upper 2.2 km of the river, where the water is clear and aquatic vegetation is abundant (Lemke 1989; Groeger et al. 1997). The upper San Marcos River flows through urbanized areas where the channel has been stabilized by concrete bulkheads, particularly within parks managed by the City of San Marcos or Texas State University (Hay et al. 2022). The fish used to study sex ratio and reproductive biology were obtained from the San Marcos River at Rio Vista Park (29.878570 North, -97.932742 West), where a series of artificial chutes were constructed for recreational activities in the river. The chutes were originally installed during 2006 but required repair during 2021 and 2022 because of erosion and collapse. Abundance of *Hypostomus* sp. was assessed along a 1-km reach of the San Marcos River between Spring Lake Dam and the confluence with Purgatory Creek. Movement of tagged fish occurred in Sewell Park and near the outflow of Spring Lake Dam, both of which have sufficient crevices and overhangs in the failing infrastructure.

Raft floats for abundance estimates. – We used video footage to count the number of *Hypostomus* sp. present in the 1-km reach by fitting a small raft with underwater cameras and lights. The raft had two waterproof LED lights mounted to an underwater frame and two 4K high-definition cameras, with a 170° field of view similar to that of an individual with a dive mask, were mounted in the center of the frame, with one camera facing downward and one camera facing forward (Figure 1). Transect width, or recorded width, varied with depth, vegetation, and distance from structures, but viewable distance stayed consistently greater than that which disturbed the SAC, even for the deepest areas surveyed. The raft had an onboard GPS unit that tracked the path of the raft during each deployment. We began floats at the entry to the river near the Spring Lake Dam and continuously monitored abundances of fish as the raft passed overhead at the rate of approximately 0.5 m per second. A diver with a mask and snorkel swam with the raft to guide it down the river to the takeout just downstream of the Purgatory Creek confluence. Care was taken to follow the same path during each float occasion, primarily following the deepest part of the channel, while including areas of artificial structures of known SAC use. These single transect raft floats were conducted at night, at approximately weekly intervals, on three occasions during spring 2022 (April 22, April 29, May 6), fall 2022 (October 7, October 17, October 26), and spring 2023 (April 3, April 11, and April 18). Videos from each camera were synchronized and stitched together for each float. A set of two observers reviewed the footage from the cameras and enumerated fish within 10-second intervals. Two observers were used as high-density areas would have too many fish within a 10-second interval to accurately count for a single observer. The time stamps from the GPS and the cameras were then aligned so that the location of each 10-second interval could be georeferenced along the river and were matched to the aerial imagery on ArcGIS Pro creating a spatial representation of the path and all observed fish.

Fish collection for reproductive biology, sex ratio, and sexual dimorphism. – The upper San Marcos River flowing through Rio Vista Park was diverted from the river channel during a scheduled dewatering beginning in December 2021. Streamflow was routed around the series of artificial chutes with pipes to bypass the channel for 130 m before being returned to the channel, with the use of gas-powered pumps to dewater the remaining pools in the riverbed. As the water receded on December 15, 2021, crews were onsite to relocate any native species downstream of the dewatered section, while non-native species were removed. We randomly collected 385 (excluding 21 small individuals from the grand total of 406) SACs consisting entirely of *Hypostomus* sp. from the dewatered portion of the river. All SACs were transported to Texas A&M University (College Station, Texas) with a fish hauling unit. Fish were transported in river water and provided oxygen from a 7,080 L oxygen tank connected to two air stone diffusers. In the laboratory, SACs were euthanized through immersion in a lethal dose of clove oil following Texas A&M University Institutional Animal Care and Use Committee approved protocols. Following euthanasia, 213 specimens were selected at random from the fish hauling unit and preserved in 10% formalin solution and later transferred to 70% ethanol, while the remaining 172 specimens were frozen without formalin preservation. The 172 specimens were frozen due to a lack of space and volume of formalin required to adequately preserve all 385 collected SAC. A subset of 30 of the formalin-fixed specimens used in this study were deposited within the Collection of Fishes at the Biodiversity Research and Teaching Collections at Texas A&M University under TCWC 20634.01.

Fish collection for tagging. – We collected fish for tagging during March 2022 and April 2023 using hand nets carried by self-contained underwater breathing apparatus (SCUBA) divers or by swimming along the walls of the river and quickly grabbing fish as they remained motionless and suctioned to the wall. We tagged 10 fish in 2022 and an additional 10 fish in 2023 using ultrasonic transmitters (Sonotronics model PT-4) surgically inserted into the body cavity. We anesthetized fish with clove oil and made a 2-cm incision on the ventral side just off the midline and inserted the transmitter. We closed the incision with two interrupted sutures and medical grade adhesive (VetBond Surgical Glue). Fish were held for 2 hours post-surgery to monitor short term survival and then were released back into the location from which they were captured.

Morphometrics. – We measured nine external morphological traits based on characters identified by Rapp Py-Daniel and Fernandes (2005) and Reis et al. (2022) as being related to sexual dimorphism in other species of SAC. We focused on measurements that could be easily collected from fish in the field without dissection (Figure 2). Cryptic, subjective, or otherwise difficult to analyze characteristics identified as being related to sexual dimorphism in SAC, such as abdomen or odontode shape, or results of histological analysis of gonads, were excluded from consideration. We measured total length (TL; tip of snout to posterior point of caudal fin) and standard length (SL; tip of snout to posterior end of hypural plate) to the nearest millimeter with a measuring board. Measurements of all median and paired fins were recorded to 0.1 mm with digital calipers, except caudal-fin length (CFL), which was calculated by subtracting SL from TL. Fin measurements included depressed dorsal-fin length (DFL; anterior origin of dorsal-fin spine to furthest posterior tip of fin when depressed against the body), pectoral-fin spine length (PSL; base of pectoral-fin spine to tip of spine), pectoral-fin spine width (PSW; widest point of pectoral-fin spine), pelvic-fin length (PFL; base of outermost pelvic-fin ray to tip), and anal-fin length (AFL; anterior origin of first anal-fin ray to furthest posterior tip of fin when depressed against the body). We also measured head width (HW) at the widest anterior point of the cheek plates. Each fish was then incised on the ventral surface from the urogenital opening to the base of the pectoral girdle. We removed, weighed (± 0.0001 g) and identified gonads as ovaries or testes to determine sex, then retained the gonads in 70% ethanol. Eviscerated fish were weighed to the nearest 0.001 kg and returned to 70% ethanol for storage. Histological analysis of the gonads was not performed as it fell outside the scope of this study. The 172 frozen fish were measured for TL and dissected to determine sex based on the same methods as for the preserved fish, but additional morphometrics were not measured.

Tracking methods. – We deployed submersible ultrasonic receivers (SURs) to monitor site fidelity of *Hypostomus* sp. During 2022, we deployed two SURs in Sewell Park at the upstream and downstream ends of a large wall on the west side of the park in the deepest part of the river located between the two walking bridges (Figure 3 Inset B). These two SURs were 15 m apart, which was far enough apart to not overlap in their listening range, but close enough together to assess movement of fish between SURs. We monitored movements of fish between March and August of 2022 to develop a baseline assessment of site fidelity and diel activity patterns. Based on high site fidelity and strong nightly activity patterns in 2022, we then deployed four SURs farther upstream near the Spring Lake Dam during 2023 (Figure 3 Inset B and Figure 4). We placed SURs in crevices from which divers pulled fish for tagging. SURs were removed every 2-3 weeks to download data and change batteries and were then immediately redeployed.

Exclusion methods. – Using data obtained from SURs from May 8, 2023 to June 21, 2023, a pattern was assessed for an individual fish (ID 20233) that had consistent usage of a single crevice, showing when the fish could be expected to leave the crevice for the night. On June 23, 2023, we inserted a mesh hardware cloth barrier at night while the fish was away from the crevice, but the SUR was still inside the crevice. We then monitored fish activity for the next three weeks to assess behavioral responses. Additional SURs were placed in the next available crevices both upstream and downstream of the exclusionary mesh crevice.

Statistical analyses. – We estimated abundance of *Hypostomus* sp. using N-mixture modeling. This modeling framework allows for development of abundance estimates while accounting for incomplete detection, or the condition in which fish are present but not observed. We fit a zero-inflated Poisson error distribution because there were many zeros in the dataset. We created a 3m by 3 m grid across the river and assigned fish abundance estimates to each grid cell along the raft float path. We then used a geographic information system to derive grid-cell-specific estimates for water velocity (m/s) and depth (m) based on a Multidimensional Surface Water Model that was created by the Meadows Center for Water and the Environment (MCWE). We then used aerial imagery to create polygons of aquatic vegetation based on seasonal drone flights conducted by MCWE, which were then used to assign the proportion of each grid cell that was covered by aquatic vegetation. Finally, we calculated the distance between each grid cell and anthropogenic structures (e.g., concrete walls, riprap, bridge pillars) for the entire length of the 1-km segment. We used these data to fit the N-mixture model of Royle (2004) using the zero-inflated Poisson mixture, three abundance covariates (depth, velocity, distance to structures), and a single detection covariate (proportional coverage of aquatic vegetation). We fit separate models to each season and used Akaike Information Criterion selection methods to identify top-ranked models in which subsets of abundance covariates and a null model (no covariates) were compared.

We assessed sex ratios and morphological differences among female and male SACs based on univariate and multivariate statistics. We tested for differences in length frequency distributions between sexes with a Kolmogorov-Smirnov test (KS test) implemented with the 'ks.test' function and tested for deviation from a 1:1 sex ratio based on a test of equal proportions implemented with the 'prop.test' function from the 'stats' package in R version 4.2.0 (R Core Team 2022). These tests were conducted on all dissected fish (preserved and frozen). We then selected seven of the nine morphological measurements, CFL, DFL, PSL, PSW, PFL, AFL, and HW, to be standardized to TL to create the following ratios that were used in our model: caudal-fin ratio (CFR), dorsal-fin ratio (DFR), pectoral-length ratio (PLR), pectoral spine-width ratio (PWR), pelvic-fin ratio (PFR), anal-fin ratio (AFR), and head-width ratio (HWR). Morphological measurements were standardized to TL as opposed to SL because length data from SACs returned during spearfishing tournaments have historically been measured to TL (Blanton et al. 2020), and because preliminary models fit with SL produced similar results. This ensured consistency and transferability of data among stakeholders involved in management of the population and meant that historically collected TL data could be compared with results generated from this study. We performed t-tests to assess differences in the distributions of morphological characters between sexes with the 't.test' function from the 'stats' package in R. We then used a generalized boosted regression model (GBM) to assess classification strength for sex based on the seven external morphological ratios (Table 1). The GBM is a machine learning method that employs construction of decision trees based on random subsets of the total dataset and builds trees sequentially so that new trees correct the errors associated with the previous tree (Elith et al. 2008). We used binomial sex assignment (female = 0, male = 1) as the response variable and all morphological ratios as predictor variables to build the model based on the 'gbm' function from the 'gbm' package in R (Greenwell et al. 2022). We used 500 trees, set the minimum number of observations in terminal nodes to 10, used a shrinkage rate of 0.01, and used 10-fold cross validation for model assessment. We assessed model performance with the 'confusionMatrix' from the 'caret' package in R (Kuhn 2022) to estimate mean accuracy (i.e., proportion of correct assignments) across cross-validation subsets, Kappa (i.e., ratio of observed to expected accuracy), sensitivity (i.e., proportion of males correctly classified) and specificity (i.e., proportion of females correctly classified). We first fit a global model with all seven ratios as predictor variables, and then based on the relative contribution of variables to model explanatory power, fit a reduced model with only the two most influential variables.

We tested the hypothesis that sex classification from the GBM model was correlated with fish size and reproductive investment based on generalized linear and additive models. We tested whether classification accuracy from the GBM was related to fish size via logistic regression with the ‘glm’ function and binomial error distribution from the ‘stats’ package in R (R Core Team 2022) with a binary response variable coded as incorrect (0) or correct (1) classification of known sex by the reduced GBM. Next, we calculated the gonadosomatic index (GSI) based on the equation:

$$GSI = \left(\frac{Gonad\ weight}{Eviscerated\ weight}\right) * 100$$

where gonad weight and eviscerated weight are measured to the nearest 0.0001 g (Nikolsky 1963). The GSI ranges from 0 to a theoretical maximum of 100, with values close to zero representing minimal reproductive investment (Nikolsky 1963). We tested for a relationship between GSI (dependent variable) and total length (independent variable) for females and males separately with generalized additive models (GAMs). The GAM approach was necessary because the response variable was bound by zero and relationships were non-linear (Wood 2004). We fit GAMs with the ‘gam’ function from the ‘mgcv’ (Mixed GAM Computation Vehicle) package version 1.8–40 in R (Wood 2017) and used a “quasipoisson” error distribution to account for the zero-truncated, non-integer response variable. Separate GAMs were fit for females and males because of large differences in GSI values between the sexes. We assessed the TL at which classification accuracy transitioned from below to above 75%. The selection of 75% accuracy was arbitrary and was selected based on the shape of the slope of the logistic regression model fit to the relationship between TL and classification accuracy. This threshold was then compared to changes in reproductive investment (measured as GSI) for both sexes.

Dimorphism validation exercises. – We validated the utility of the GBM developed here for advancing management of *Hypostomus* sp. in the San Marcos River based on two approaches. First, we used a retrospective approach to determine the proportion of fish removed during past spearfishing for which the GBM might have been used to estimate sex. We obtained TL data for all fish spearfished during 2014–2018 from Blanton et al. (2020) and calculated the proportion ≥240 mm TL, the size at which predictive accuracy of the model was >0.75. Second, we developed predictions from the reduced GBM across all values of AFR and DFR included in the training dataset and generated a model output map of probabilities that fish were classified as female versus male. We solicited seven volunteer raters to measure the AFR and DFR of 30 *Hypostomus* sp. speared by Atlas Environmental on the morning of February 21, 2023. Each fish was measured for TL to the nearest mm with a measuring board before each volunteer rater independently measured AFR and DFR from the same 30 fish. Four raters used digital calipers and three used a metric ruler to conduct measurements. Values from each volunteer rater were used in conjunction with the model output map to assign sex to each fish. An analysis of inter-rater reliability was conducted across all raters as well as pairwise among raters based on Fleiss' Kappa (Fleiss 1971) implemented with the ‘kappum.fleiss’ function from the ‘irr’ package in R (Gamer et al. 2019). Fleiss’ Kappa ranges <0–1, where negative values represent less agreement than expected by random chance, 0 represents no agreement among raters (other than that expected by random chance), and 1 represents complete agreement among raters. Landis and Koch (1977) suggested agreement among raters could be interpreted from Fleiss’ Kappa values as poor (<0), slight (0.01–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), and almost perfect (0.81–1.00). After all volunteer raters had completed their measurements, fish were dissected to assess sex based on gonads and the proportion of correct classifications for each rater was calculated as the number of correct sex assignments divided by the total number of fish assessed (i.e., 30). We then tested for a relationship between TL and the proportion of raters that assigned the correct sex to each of the 30 fish with a generalized linear model (GLM) with a quasibinomial error distribution to represent the continuous yet bound (by 0 and 1) nature of the response variable. A single rater with low reliability was removed from this regression analysis (see Results). Finally, on the afternoon of February 21, 2023, while the volunteer raters were conducting their work, an additional 29 *Hypostomus* sp. were speared and a single rater used the model output map to classify each of the fish as another form of validation for the model. The accuracy (i.e., proportion of correct sex classifications) of these fish was assessed and the relationship between probability of correct classification and TL was tested based on a GLM with a binomial error distribution with the 'glm' function from the 'stats' package in R (R Core Team 2022).

RESULTS

N-mixture model comparison revealed the model with the full complement of abundance covariates and vegetation as a detection covariate was well supported for each season. Across seasons, predicted abundance was greatest nearest to anthropogenic structures, in higher velocities, and in shallower depths (Figure 5). Detection declined with increasing vegetation density across all seasons (Figure 6). The resulting estimates (95% confidence intervals) for the entire 1-km segment were 4,585 (3,025-7,089) for spring 2022, 4,695 (2,122-10,704) for fall 2022, and 2,942 (1,567-5,996) for spring 2023 (Figure 7). Mapped abundance illustrated areas of high fish density near the headwaters and Sewell Park during spring and fall of 2022, then areas concentrated near the Aquarena Springs Drive crossing and East Hopkins Street crossing for spring 2023 (Figure 8). The decline in abundance of *Hypostomus* sp. across the riverscape correlated with declines in flow over the course of the study (Figure 9).

Length frequency distributions differed (D = 0.22, $P < 0.001$) for males (n = 154) versus females (n = 231) and illustrated greater abundance of females for fish 250–320 mm TL but greater abundance of males for fish $\geq$340 mm TL (Figure 10). The difference in proportions of females (0.60) and males (0.40) was 0.20 (95% CI = 0.13–0.27) and was significantly different from zero (df = 1, $\chi^2 = 30.01$, $P < 0.001$). Of the 213 preserved SAC used in the GBM, 108 were females, 105 were males, and the proportion of females (0.50) and males (0.50) did not differ (df = 1, $\chi^2 = 0.04$, $P = 0.85$). All measurements were on average greater for males compared to females, and only standard length was non-significant (Table 1). Among the seven ratios calculated to control for total length, male ratios were on average larger for five (PLR, PWR, PFR, DFR, and AFR) but similar between sexes for two (HWR and CFR).

The global and reduced GBMs had similarly high predictive capability. The global model accuracy was 0.87 (0.82–0.91), Kappa was 0.74, sensitivity was 0.88, and specificity was 0.86. Based on the relative contributions of morphometrics to model classification accuracy in the global model (Table 2), we created a reduced model with only AFR and DFR as predictor variables. The reduced model accuracy was 0.83 (0.77–0.88), Kappa was 0.67, sensitivity was 0.84, and specificity was 0.83. In the reduced model, the relative contribution of AFR was 63% and DFR was 37% (Figure 11A). Probability of classification as male increased rapidly between AFR values of 0.105 and 0.111 (Figure 11B) and DFR values of 0.325 and 0.330 (Figure 11C). Males and females separated in multivariate space along axes of AFR and DFR, with model predictions aligning with the major boundary between the sexes (Figure 11D).

Model accuracy varied among fish of different sizes. The slope of the logistic regression model fit to probability of correct sex classification as a function of TL was significant (slope estimate = 0.013, $t = 3.22$, $P = 0.001$) and declined from a maximum fitted value of 0.95 at 385 mm TL to a minimum fitted value of 0.52 at 164 mm TL (Figure 12A). Classification accuracy fell below 0.75 at 240 mm TL. Reproductive investment measured as GSI for females increased with fish total length ($F_{1.1,1.3} = 6.07$, $P = 0.015$), though little variation was explained (adjusted $R^2 = 0.06$, deviance explained = 6.84%) because of high variation in reproductive investment among larger specimens (Figure 12B). Female GSI values were consistently <2% for individuals <240 mm TL, but individuals >240 mm TL had GSI values ranging up to 20%. Male reproductive investment increased with total length ($F_{2.5,3.2} = 8.49$, $P = 0.015$) with greater variation (relative to females) captured by the model (adjusted $R^2 = 0.20$, deviance explained = 21.9%). Male GSI increased with total length in an approximately linear fashion and the lowest male GSI values occurred among specimens <240 mm TL (Figure 12C).

Retrospective length frequency analysis for *Hypostomus* sp. spearfished from the San Marcos River during 2014–2018 revealed 73% of fish were ≥240 mm and could have been included in the GBM to assign sex with high accuracy (Figure 13). The multiple rater validation exercise that used 30 *Hypostomus* sp. (range 207–365 mm TL) indicated there was fair agreement among all raters (Fleiss' Kappa = 0.33) and the level of agreement was greater than would be expected by random chance ($Z = 8.21$; $P < 0.001$). Pairwise Fleiss' Kappa values revealed rater #4 had low agreement (i.e., all Kappa values negative) with all other raters, and Rater #1 had the second lowest level of agreement (Table 3). Remaining raters had substantial agreement in pairwise comparisons (Fleiss' Kappa values >0.61). There was a non-significant increase (slope estimate = 0.011, $t = 1.24$, $P = 0.23$) in the proportion of the six volunteer raters (i.e., Rater #4 excluded) that correctly classified the sex of fish as the TL of fish increased (Figure 15A). Classification accuracy of the 29 *Hypostomus* sp. (range 182–284 mm TL) speared in the afternoon of the validation exercise and rated by a single individual was 0.56. There was a significant increase in the probability of correct classification as TL increased (slope estimate = 0.042, $Z = 2.17$, $P = 0.03$), including a high probability (>0.75) of correct classification of fish ≥ 240 mm TL (Figure 15B).

Movement by tagged *Hypostomus* sp. indicated strong site fidelity. During 2022, there was evidence of diel movement and activity behaviors as some fish moved into the range of a SUR only during nighttime hours, while others spent most of the day within a crevice (Figure 16). Notably, fish 20225 and 20228 appeared to spend daytime hours near the upstream SUR, but then would move into detection range of the downstream SUR primarily at night. During 2023, fish 20233 spent most of the day and occasionally nights in the vertical crevice, with increased occurrence just prior to the June 21, 2023 download, until the exclusion device was installed on June 23, 2023 (Figure 17A). An exit time of approximately 9:00pm was identified from detection patterns which allowed for the successful installation of an exclusion mesh while the crevice was vacant. Following installation of the exclusion device, fish 20233 moved to the corner crevice, the next available crevice downstream of the vertical crevice, where it spent most of the day and occasionally nights (Figure 17B). Meanwhile, Fish 20235 remained within the Sessoms Creek outflow for the duration of the time a SUR was present to monitor detections (Figure 17C). Fish 20233 would occasionally use the horizontal crevice (Figure 17D), but seemingly only during periods when fish 20239 was not present or only occasionally present (Figure 17E). Fish 20238 occasionally used the horizontal crevice but was killed by spearfishing just prior to the exclusion experiment on June 22, 2023, and therefore was not tracked any longer. There was a strong negative relationship between the number of detections for fish 20239 versus fish 20233 in the horizontal crevice, and this persisted for both day and night (Figure 18), suggesting fish 20233 was potentially capable of displacing fish 20239. After both the removal of fish 20238 from spearing and the displacement of fish 20233 to a downstream crevice, following the installation of the exclusionary mesh, the frequency of detections for fish 20239 in the horizontal crevice increased (Figure 17E). The exclusion device successfully caused a reduction of reads for fish 20233 in the vertical crevice (Figure 19).

DISCUSSION

Our work demonstrates the presence of sexual dimorphism within the invasive population of *Hypostomus* sp. inhabiting the San Marcos River, Texas, USA and suggests sex classification is possible with the tool developed in this study. We found that the largest fish captured during the dewatering event were male, though females were more abundant overall (60% of specimens) and within intermediate size ranges (250–320 mm TL). Detailed morphometric analysis revealed that males tended to have larger characters, even after adjusting by TL to account for overall body size differences between sexes. The most striking differences between sexes were larger dorsal- and anal-fin lengths for males as compared to females. We leveraged these differences in fin size to develop a model that discriminated between males and females based on external morphology, particularly for specimens ≥240 mm TL. This length-based threshold in classification accuracy was meaningful because it corresponded with similar increases in reproductive investment, supporting the idea that secondary sexual characteristics are most useful for discriminating among sexes after the onset of reproductive maturity. Our results have potential to advance management of the invasive population given that most fish collected during spearfishing tournaments might be used to monitor sex ratios (Blanton et al. 2020), and spearfishing control experiments and movement studies might use our methodology to easily assess and include sex as a covariate for modeling behavior (Hay et al. 2022). Our discovery of a bias in sex ratios towards females also indicates that management actions aimed at creating bias towards males (i.e., the goal of genetic biocontrol through YY males; Teem and Gutiérrez 2010) might have to overcome an already-present bias towards females.

Enlargement of fins may be a common secondary sexual characteristic among armored catfishes. Rapp Py-Daniel and Fernandes (2005) reviewed sexual dimorphism among catfishes native to the Amazon River basin and found that some of the most common forms of dimorphism among loricariid catfishes included enlargement of dorsal fins, pectoral spines, and anal fins in males. Though the diversity of Loricariidae is high and some genera express multiple sexually dimorphic characteristics simultaneously (e.g., Pereira et al. 2014), Rapp Py-Daniel and Fernandes (2005) included only one example of sexual dimorphism for *Hypostomus*. This example was that of larger body sizes among males for *H. hermanni* as documented by Nomura and Mueller (1980). Dimorphism within the genus *Hypostomus* is apparently uncommon given that studies have concluded a lack of sexual dimorphism for *H. commersonii* (Goulart and Verani 1992), *H. affins* and *H. luetkeni* (Mazzoni and Caramaschi 1995), *H. velhochico* (Zawadzki et al. 2017), *H. subcarinatus* (Zawadzki et al. 2019), and *H. froehlichi* (Zawadzki et al. 2021). Our work supports the pattern of larger body size in males for congeneric *H. hermanni* documented by Nomura and Mueller (1980) as well as the >100-year-old report by Miranda Riberio (1918) of enlarged fins in males of *H. albopunctatus* (Zawadzki et al. 2020). Differences in total body size may cause other morphometrics to increase in size due solely to correlation with total size, thus it is common to calculate ratios of morphometric measures to total or standard length as we did in our study (Reis et al. 2022). When we adjusted measurements to account for differences in total length, we found evidence of proportionally larger dorsal-, anal-, and pectoral-fin lengths for males in the population of *Hypostomus* sp. in the San Marcos River and this pattern might exist for other SAC populations.

Reproductive maturity typically correlates with secondary sexual characteristics and the emergence of sexual dimorphism presents information that can be integrated into management objectives. The reduced GBM we developed most accurately discriminated between sexes for individuals ≥240 mm TL, the same size at which we noted increases in reproductive investment. This suggests that sexual dimorphism is most apparent and determinable among larger, sexually mature individuals. Though the size at which *Hypostomus* sp. in the San Marcos River reach sexual maturity is not currently known (Cook-Hildreth et al. 2016), we predicted that sexual maturity might begin to be reached by individuals at total lengths ranging 198–217 mm TL (equivalent to 144–160 mm SL) based on previous research on *H. affinis* and *H. leutkeni* (Mazzoni and Caramaschi 1995). However, the correlation between sexual discrimination and GSIs for females and males collectively suggest *Hypostomus* sp. in the San Marcos River might reach maturity somewhere around 240 mm TL. The relatively minor difference between the expected and observed length at maturity (i.e., only 2 cm) lends support to the hypothesis that the onset of reproductive maturity correlates with detectable sexual dimorphism. This is the case for other highly managed fish species, such as Alligator Gar *Atractosteus spatula* (McDonald et al. 2013, 2018) and Walleye *Sander vitreus* (Wszola et al. 2022). Validation of the GBM model by multiple volunteer raters as well as the 29 fish assessed by a single rater further supported the notion that classification of sex was more accurate for larger individuals. The relationship between fish size and the proportion of raters that correctly classified sex was positive, though not significant because of an outlier female 345 mm TL classified as male by all but one rater. This, combined with the general low reliability of Rater #4, suggests that individual rater characteristics, such as level of training or expertise, is an important consideration for use of the tool developed in this paper. Still, inter-rater reliability was generally higher for larger fish with mature gonads. The 29 fish classified by a single rater were relatively small (e.g., 66% had TL < 240 mm), yet classification accuracy significantly increased as fish size increased to ≥240 mm TL. This independent validation suggests the model fit to the training dataset obtained during the dewatering event is directly applicable to fish speared from other parts of the river and supports the use of fish ≥240 mm TL for sex determination.

Differences between sexes are often overlooked in fisheries management, in part because it can be challenging to distinguish between sexes based on commonly collected data (Hanson et al. 2008). However, this does not negate the potential importance of sex information in management. For example, Wszola et al. (2022) found that differences in growth rate and size between male and female Walleye *Sander vitreus* resulted in males spending more of their lifetime within the range of lengths at which harvest was greatest, resulting in disproportional harvest of males. We found that female *Hypostomus* sp. were much more abundant than males within the range of 250–320 mm TL based on a fisheries independent capture method (i.e., dewatering). This is the same size range from which the greatest number of fish were harvested by spearfishing during 2014–2018 (Blanton et al. 2020). Though greater information on sex-specific growth rates and sex ratios of harvested fish are needed, it is possible that targeting fish in the 250–320 mm TL size range could help to continue disproportional harvest of females. Stock depletion through overfishing generally operates through the systematic loss of larger fish (Froese et al. 2018). In the case of *Hypostomus* sp. in the San Marcos River, our data suggest males might be disproportionally removed if only the largest members of the population were targeted. However, an important consideration of spearfishing is the potential to target a broader range of sizes relative to traditional capture techniques, including smaller individuals (Welch et al. 2010). The high proportion of fish collected from spearfishing for which sex can be accurately predicted by our model suggests that consideration of differences between sexes could be further integrated into management in the future (Hanson et al. 2008).

A limitation of our model is that the entire training sample originated from one location within the San Marcos River. This was a consequence of the opportunistic nature of the dewatering event that affected only one portion of the river, albeit a portion known to be inhabited by high densities of *Hypostomus* sp. (Hay et al. 2022). Future habitat restoration and bank stabilization projects that involve the temporary drying of sections of river might present further opportunities to obtain fishery independent data on *Hypostomus* sp. abundance and sex ratios. Fish included in the validation exercises were speared from other locations in the river, suggesting a general applicability of the model results along the segment of river inhabited by SAC (Hay et al. 2022). A second limitation is that our discriminant model is only applicable to the sizes of fish included in the training dataset (165–396 mm TL) and is most accurate for individuals ≥240 mm TL. This leaves uncertainty regarding sex ratios for smaller and immature specimens. Although these smaller individuals are not traditionally harvested during spearfishing, monitoring the sex ratio within the broader population will require capture of smaller individuals. Based on our knowledge of this river system, small individuals are most abundant in upstream portions of the river, where shallower riffle habitats might be conducive to sampling with seines. A third limitation is that although we detected differences in size between sexes and apparent reproductive maturation at 240 mm TL, we cannot assign age to fish sizes. Future research should focus on application of methods that could allow for estimation of age structure, including studying growth rates, assessment of hard part aging methods, or perhaps captive raising of fish in temperature and photoperiod regimes consistent with the river that would allow for validating length-based changes in age. A fourth limitation is that we focused only on morphometrics that might be easily implemented in the field from a fisheries management perspective; other forms of dimorphism may be present but undetected in this work. Future research could focus on identifying cryptic dimorphic characteristics that could potentially exist within SAC (Reis et al. 2022).

Results from this study have the potential to advance management of SAC invasions both in the San Marcos River and potentially across a broad geographic scale. Evidence from past research suggests the spearfishing control method is effective at reducing biomass by increasing short-term mortality (Blanton et al. 2020; Hay et al. 2022). Reduction of biomass below the level at which deleterious effects are caused might be possible through increasing spearfishing effort (Hay et al. 2022). Such a reduction in biomass would be equivalent to what Green and Grosholz (2021) define as functional eradication, in which the invasive species persists but at relatively small abundances. A far greater challenge would come with attempting total eradication, which is typically most achievable in aquatic ecosystems that can be completely dewatered or chemically treated. When these eradication methods are not feasible, biological control methods, such as YY male technology, might provide an alternative approach to reach total eradication (Teem et al. 2020). Critical first steps in moving towards biological control methods include greater understanding of standing sex ratios, development of methods to easily monitor sex ratios, and evaluation of whether existing control methods can aid in biasing the sex ratio towards males. Our assessment of the current sex ratio and development of a model to assign sex based on external characteristics provides multiple avenues to advance management. First, our work provides a fisheries independent baseline against which sex ratios of speared fish might be compared to assess sex bias in spearfishing. If no such bias exists, then speared fish could be used to track the sex ratio of the population. Our work also provides insight into sexually dimorphic characters that inform aquaculture of *Hypostomus* sp. during the future development of trojan genes as a form of biological control (Reis et al. 2022). Analysis of existing data and field validation of the GBM model suggest sex classification of *Hypostomus* sp. in the San Marcos River is possible with the tool developed from this study.

MANAGEMENT IMPLICATIONS

Our work has implications for the management of *Hypostomus* sp. in multiple ways. First, we provide an estimate of total fish abundance in the upstream-most segment of river and demonstrate that abundances fluctuate through time. Knowledge of total abundance is necessary for application of biocontrol methods such as trojan genes because the fraction of the total population removed or replaced by feminized supermales must be considered. Second, our work also provides critical sex ratio information for control methods that seek to bias sex ratios towards males. We also provide a field-based method for estimating the sex of fish that are >240 mm TL that can be used to collect data from spearfishing tournaments and other removal efforts. Third, we demonstrate that fish abundance is highest in areas near artificial structures, as previously suggested by Hay et al. (2022). Our work produced spatially explicit maps of abundance that can be used to direct allocation of ongoing removal efforts (Blanton et al. 2020). Fourth, our work demonstrates that *Hypostomus* sp. have high site fidelity and strong diel movement and behavioral tendencies, suggesting control and removal efforts that target nighttime hours when fish are more active might be more effective. Fifth, we show that installation of a small-scale exclusion device caused the excluded fish to move to another nearby crevice, and that potential exists for competitive exclusion by some individuals.

ACKNOWLEDGMENTS

This project was funded by the Texas Parks and Wildlife Department through the Statewide Aquatic Vegetation and Invasive Species Management program (Grant CA-0002259A) and by the United States National Institute of Food and Agriculture (HATCH Project 1017538). This study represents a publication of the TAMU Biodiversity Research and Teaching Collections and publication 12 of the TAMU Aquarium Research Facility. Assistance with fish collection was provided by B. Littrell and Bio-West, Inc., M. Howard and the City of San Marcos, and K. Smith and the Edwards Aquifer Authority. Assistance with model validation was provided by K. Byckovski, K. Chappel, M. Conley, S. Fisher, S. Julian, K. Lee, M. Martinez, A. Panther, C. Piel, A. Puzon, and S. Schultz. Assistance with museum preparation of fish was provided by S. Parker, and H. Prestridge. This work benefited from correspondence with J. Armbruster and N. Lujan. Fish handling methods during the dewatering event were approved by the Texas A&M University Institutional Animal Care and Use Committee (Animal Use Protocol 2021-0054).

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Table 1. List of morphological measurements (see Figure 1) and ratios (i.e., measurement divided by total length) taken from 213 (108 female, 105 male) preserved *Hypostomus* sp. collected from the San Marcos River, Texas, USA. Variable descriptions and codes as well as range, mean, and standard deviation for female and male fish are given. The univariate test statistic (*t*) and P-value (*P*) illustrate the results of a test for differences between female and male fish.

Variable	Code	Female		Male		Univariate test	
		Range (mm)	Mean ± SD (mm)	Range (mm)	Mean ± SD (mm)	*t*	*P*
Standard Length	SL	119-296	215.1 (36.9)	143-316	225.6 (44.0)	1.89	0.059
Total Length	TL	164-375	280.6 (42.8)	195-392	295.7 (52.3)	2.31	0.022
Head Width	HW	32.55-85.72	56.8 (9.7)	35.49-87.82	60.47 (11.6)	2.49	0.013
Pectoral-Fin Spine Length	PSL	35.88-77.63	59.2 (7.7)	41.33-94.47	65.69 (11.0)	4.98	<0.001
Pectoral-Fin Spine Width	PSW	3.39-14.87	7.3 (1.9)	3.50-15.43	8.53 (2.8)	3.62	<0.001
Pelvic-Fin Length	PFL	29.57-68.70	52.9 (7.64)	36.68-81.54	57.35 (9.7)	3.68	<0.001
Depressed Dorsal-Fin Length	DFL	48.43-123.66	87.8 (14.3)	58.57-165.65	98.08 (20.4)	4.23	<0.001
Depressed Anal-Fin Length	AFL	15.36-41.06	28.2 (4.8)	18.18-56.03	33.30 (7.7)	5.78	<0.001
Caudal-Fin Length (calculated as TL-SL)	CFL	45-93	66.1 (8.1)	49-96	70.05 (9.5)	3.25	0.001
Head Width Ratio	HWR	0.174-0.229	0.2 (0.012)	0.171-0.230	0.2 (0.01)	0.72	0.470
Pectoral Length Ratio	PLR	0.185-0.236	0.2 (0.01)	0.180-0.243	0.2 (0.01)	7.02	<0.001
Pectoral Width Ratio	PWR	0.017-0.040	0.03 (0.003)	0.018-0.040	0.03 (0.004)	3.89	<0.001
Pelvic-Fin Ratio	PFR	0.161-0.205	0.2 (0.008)	0.166-0.212	0.2 (0.009)	4.36	<0.001
Dorsal-Fin Ratio	DFR	0.284-0.351	0.3 (0.01)	0.295-0.362	0.3 (0.01)	9.85	<0.001
Anal-Fin Ratio	AFR	0.085-0.129	0.1 (0.006)	0.092-0.131	0.1 (0.007)	11.28	<0.001
Caudal-Fin Ratio	CFR	0.184-0.301	0.2 (0.02)	0.191-0.284	0.2 (0.02)	0.70	0.487

Table 2. Results from two generalized boosted classification models fit to 210 *Hypostomus* sp. collected from the San Marcos River, Texas, USA and used to predict sex (codes as female = 0, male = 1) based on external morphometrics (see Table 1 for code definitions). Accuracy denotes the proportion of fish in the model that were correctly classified, the no information rate is the proportion of male fish in the training dataset, Kappa is the ratio of observed accuracy versus expected accuracy, sensitivity is the proportion of males correctly classified, and specificity is the proportion of females correctly classified. Percent contribution of individual morphometrics to overall explained variation is given for seven ratios in the global model (all data) and the only two ratios included in the reduced model.

Parameter	Global Model	Reduced Model
Accuracy (95% CI)	0.87 (0.82 – 0.91)	0.83 (0.77 – 0.88)
No Information Rate (NIR)	0.50	0.51
Significance of accuracy > NIR	<0.001	<0.001
Kappa	0.74	0.67
Sensitivity	0.88	0.84
Specificity	0.86	0.83
AFR	44%	63%
DFR	26%	37%
PLR	19%	-
PWR	6%	-
CFR	3%	-
PFR	2%	-
HWR	<1%	-

Table 3. Results of model validation based on seven independent raters that measured depressed dorsal-fin length and depressed anal-fin length from 30 *Hypostomus* sp., and then assigned sex based on the chart shown in Figure 6. Fleiss' Kappa (upper diagonal of table) and associated Z-value (lower diagonal of table) was used to assess inter rater reliability (bolded values represent significant pairwise correlations). Raters used either a metric ruler or digital calipers to measure depressed fin lengths and exhibited varying accuracy (i.e., proportion of 30 fish with sex correctly predicted).

Rater	1	2	3	4	5	6	7	Tool	Accuracy
1	-	0.28	0.15	-0.25	0.31	**0.52**	**0.37**	Ruler	0.67
2	1.55	-	**0.46**	-0.25	**0.62**	**0.68**	**0.68**	Calipers	0.80
3	0.84	**2.52**	-	**-0.39**	**0.83**	**0.56**	**0.56**	Calipers	0.77
4	-1.34	-1.34	**-2.13**	-	**-0.39**	**-0.44**	**-0.44**	Calipers	0.43
5	1.68	**3.37**	**4.54**	**-2.13**	-	**0.73**	**0.73**	Calipers	0.83
6	**2.87**	**3.74**	**3.04**	**-2.41**	**4.02**	-	**0.81**	Ruler	0.73
7	**2.00**	**3.74**	**3.04**	**-2.41**	**4.02**	**4.46**	-	Ruler	0.80

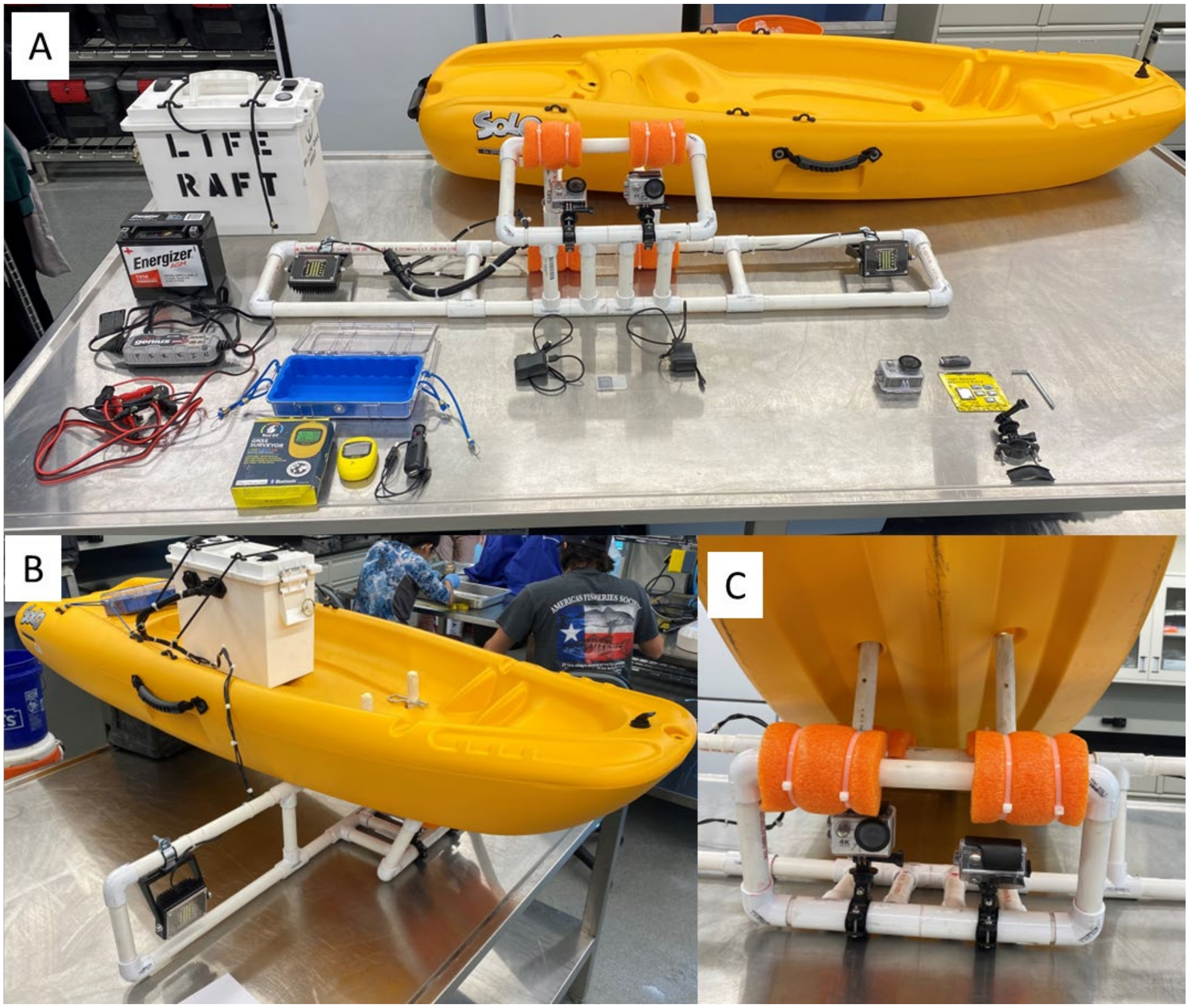


Figure 1. Raft fitted with underwater lights and cameras used to monitor *Hypostomus* sp. abundances in the San Marcos River. (A) Shows the components used in the raft, including cameras, lights, a GPS unit, and onboard battery. (B) Shows the raft fully assembled and (C) is a close up of the two cameras with differing angles.

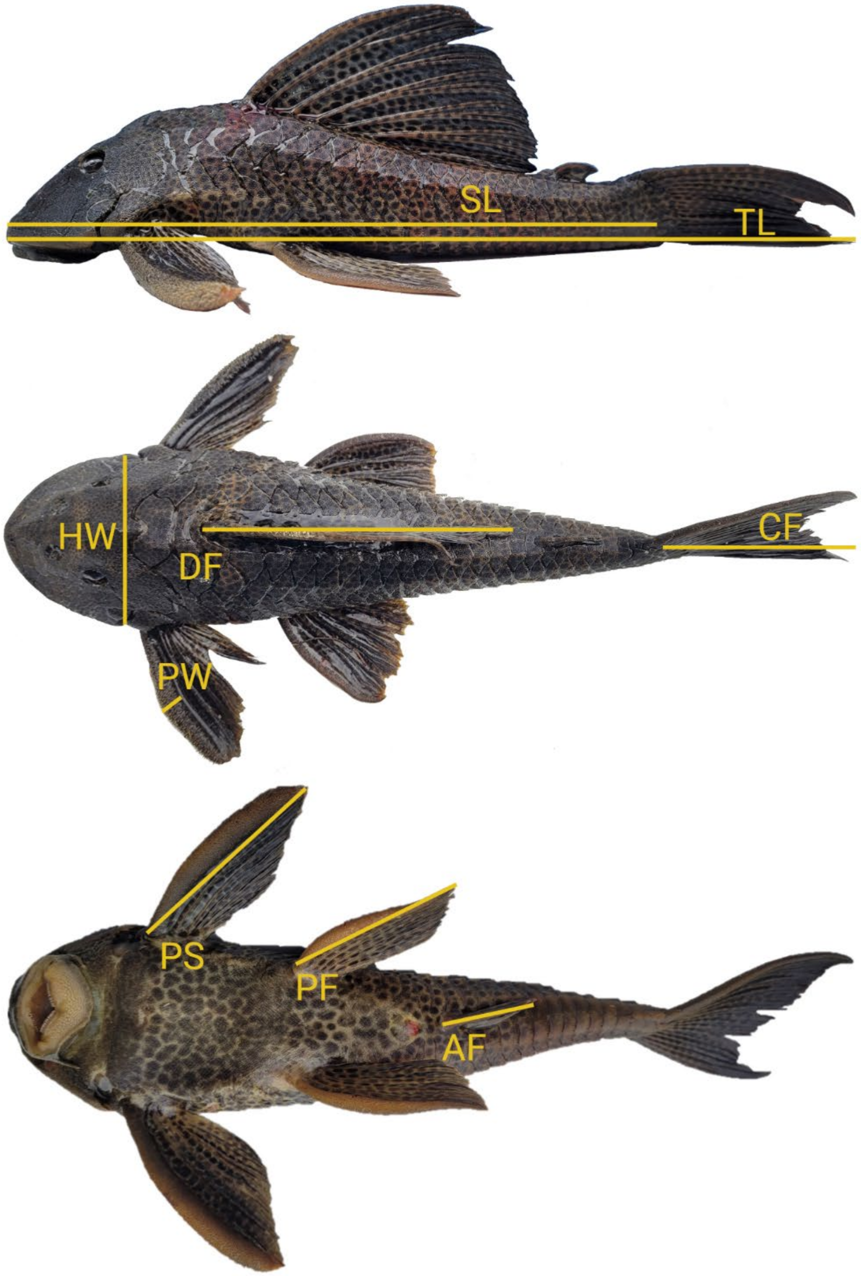
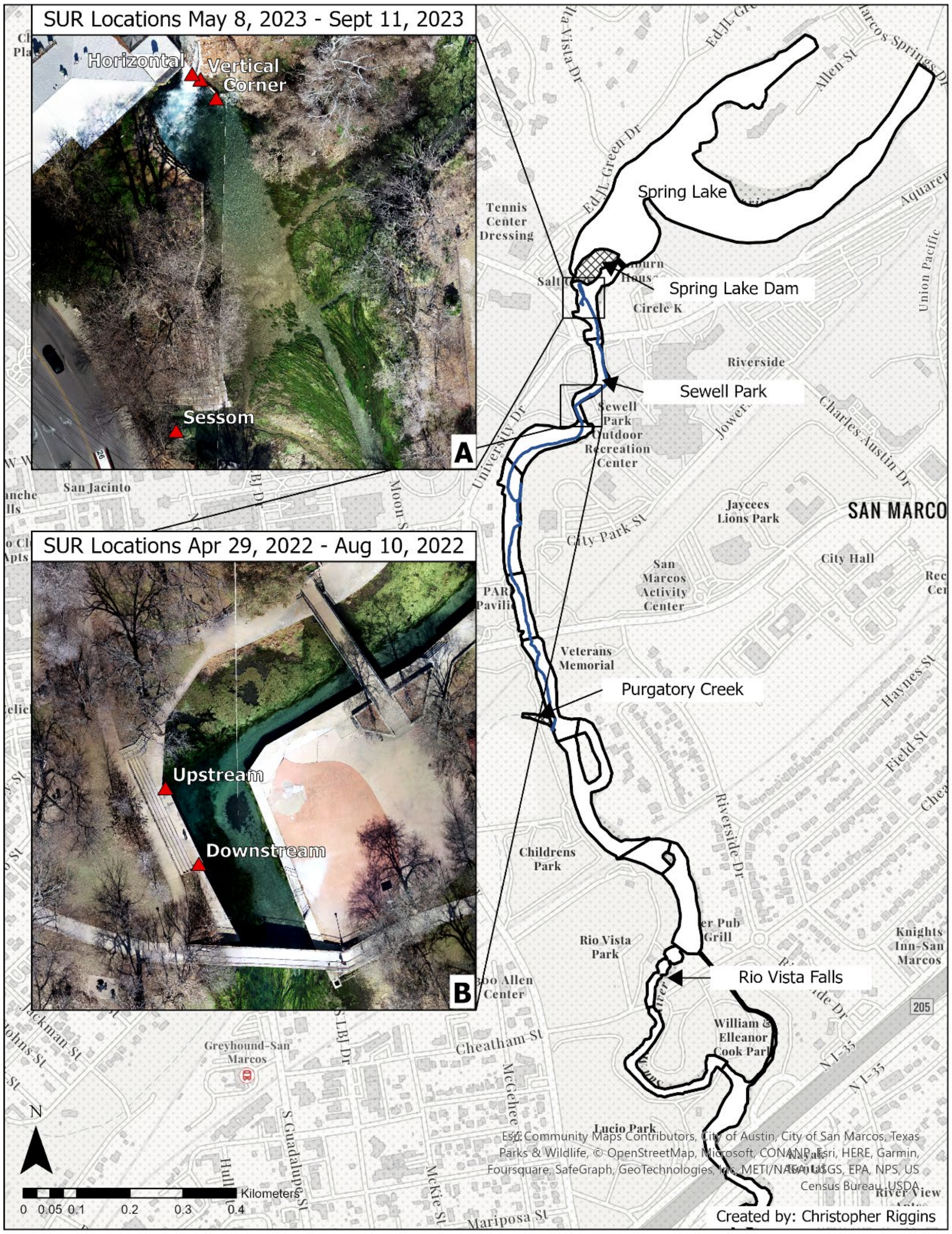


Figure 2: Morphometric features measured for suckermouth armored catfish *Hypostomus* sp. collected from the San Marcos River, Texas, USA. All measurements were taken to the nearest mm and included total length (TL), standard length (SL), head width (HW), dorsal-fin length (DF), caudal-fin length (CF; inferred from the difference between TL and SL), pectoral-fin spine width (PW), pectoral-fin spine length (PS), pelvic-fin length (PF), and anal-fin length (AF). Fin length measurements (DF, PF, AF) were all measured with the fin depressed against the body. Note that TL and SL use the same anterior-most point on the body but are shown as non-overlapping lines for illustration.



SUR Locations May 8, 2023 - Sept 11, 2023

SUR Locations Apr 29, 2022 - Aug 10, 2022

Esri Community Maps Contributors, City of Austin, City of San Marcos, Texas Parks & Wildlife, © OpenStreetMap, Microsoft, CONANP, Esri, HERE, Garmin, Foursquare, SafeGraph, GeoTechnologies, Inc, METI/NASA, USGS, EPA, NPS, US Census Bureau, USDA

Created by: Christopher Riggins

Figure 3. Map showing the locations of the submersible ultrasonic receivers (SURs) deployed below Spring Lake Dam during 2023 (Inset A) and the upstream and downstream SURS in Sewell Park during 2022 (Inset B). The video raft survey path (Blue Line) began the western spillway of Spring Lake Dam down to the Purgatory Creek outflow. The dewatering event and resulting suckermouth armored catfish collection occurred at Rio Vista Falls.

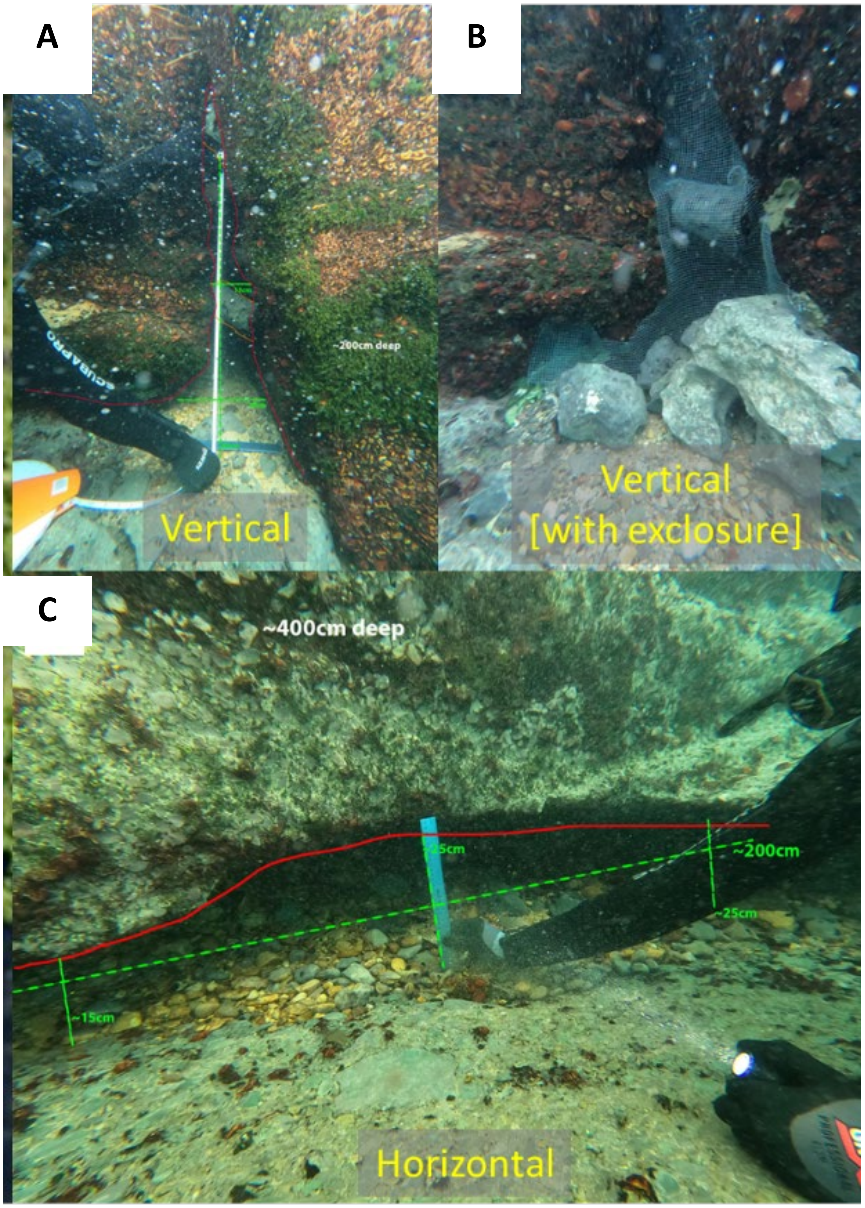


Figure 4. Map showing locations of submersible ultrasonic receivers (SURs) and underwater crevices near Spring Lake Dam used to monitor fish movement and exclusion during 2023. Referenced from the locations in Figure 3 map, (A) shows the vertical crevice without the exclusion device, (B) shows the vertical crevice with the exclusion device, and (C) shows the horizontal crevice that remained open during the experiment.

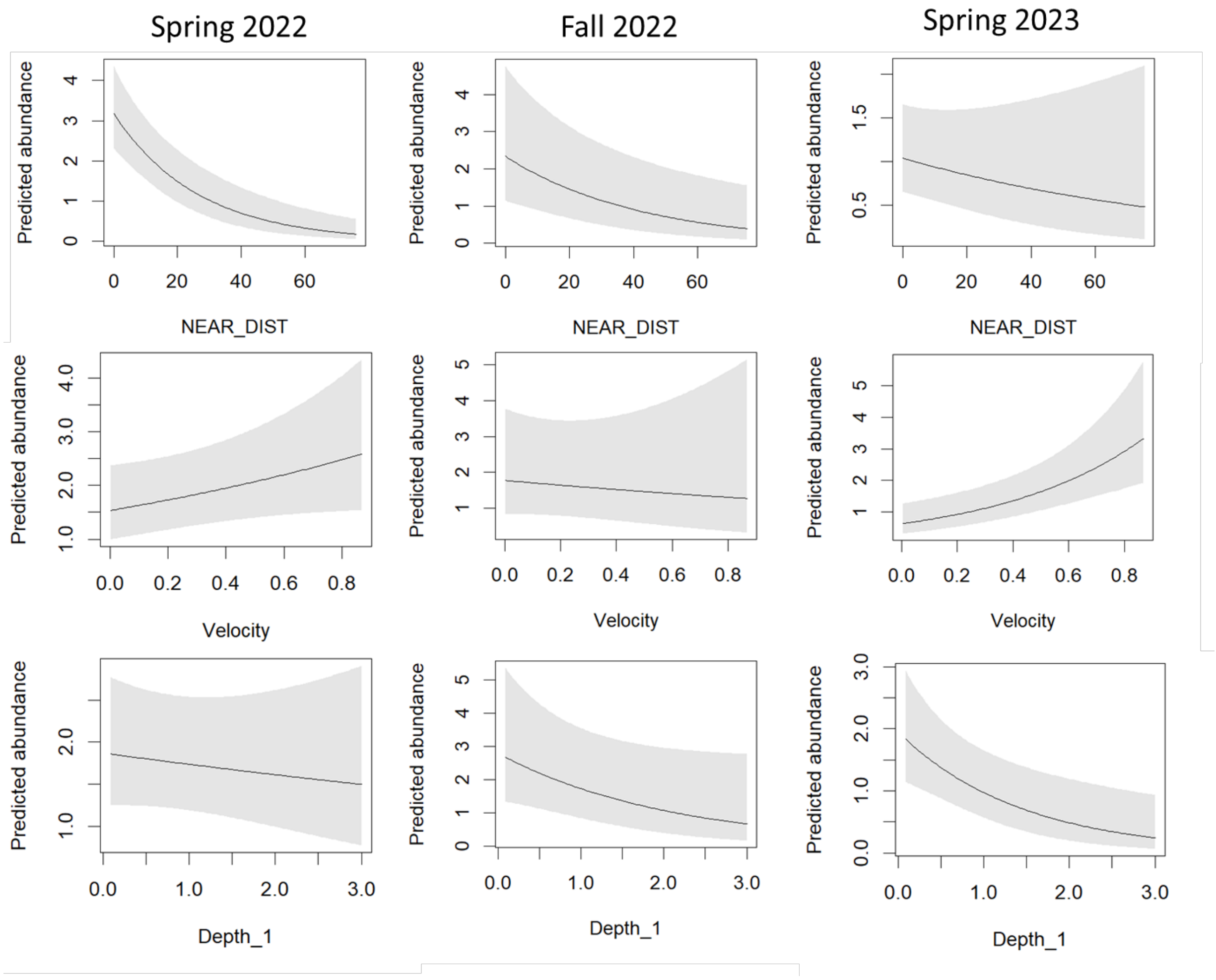


Figure 5. Partial dependent plots showing the relationship between *Hypostomus* sp. abundance and distance to the nearest anthropogenic structure (NEAR_DIST), water velocity (Velocity), and water depth (Depth_1) for the three study seasons.

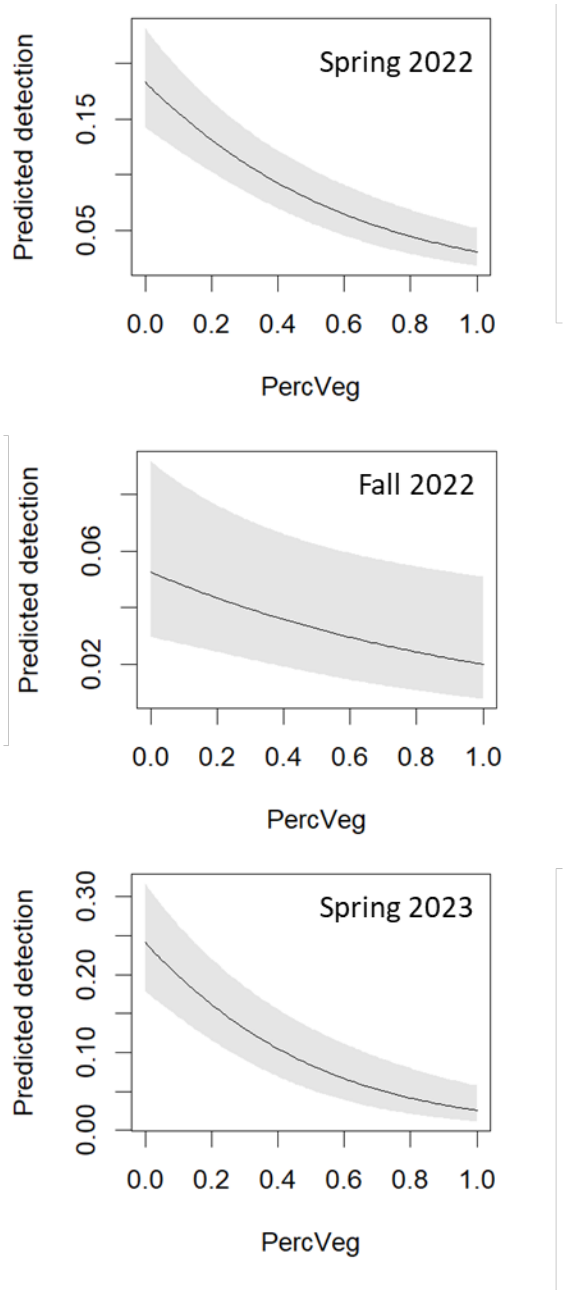


Figure 6. Relationship between detection probability for *Hypostomus* sp. and vegetation density (PercVeg) for the three seasons of the study.

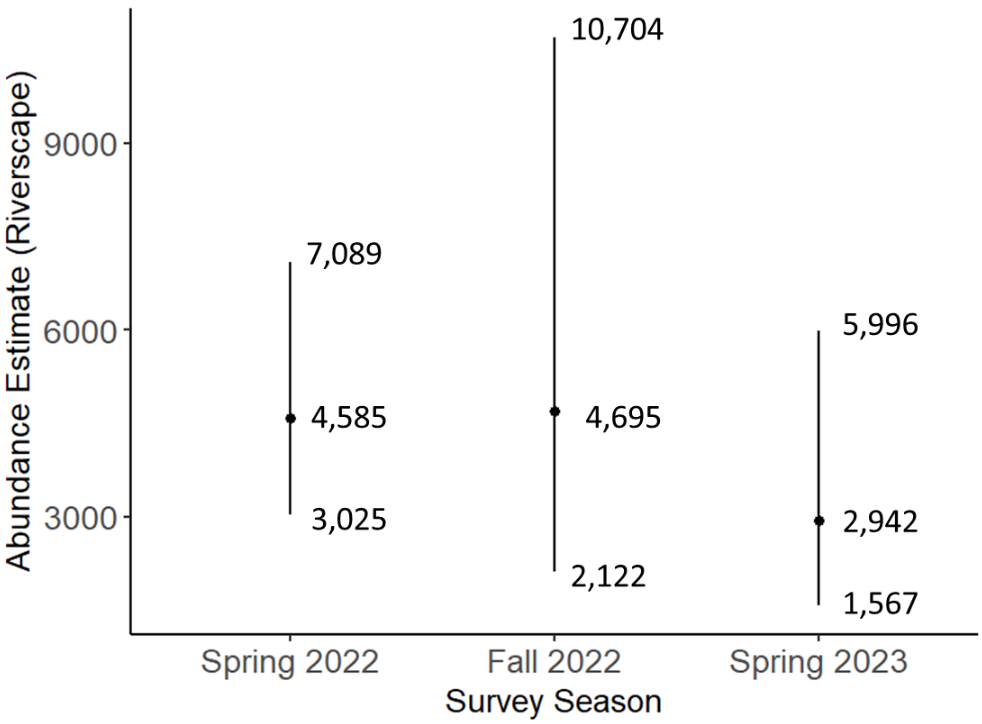


Figure 7. Estimates for the total number of *Hypostomus* sp. in the 1-km segment of the San Marcos River between Spring Lake Dam and the confluence with Purgatory Creek. The black circles and associated numbers are the estimates, and the bars and associated numbers represent 95% confidence intervals.

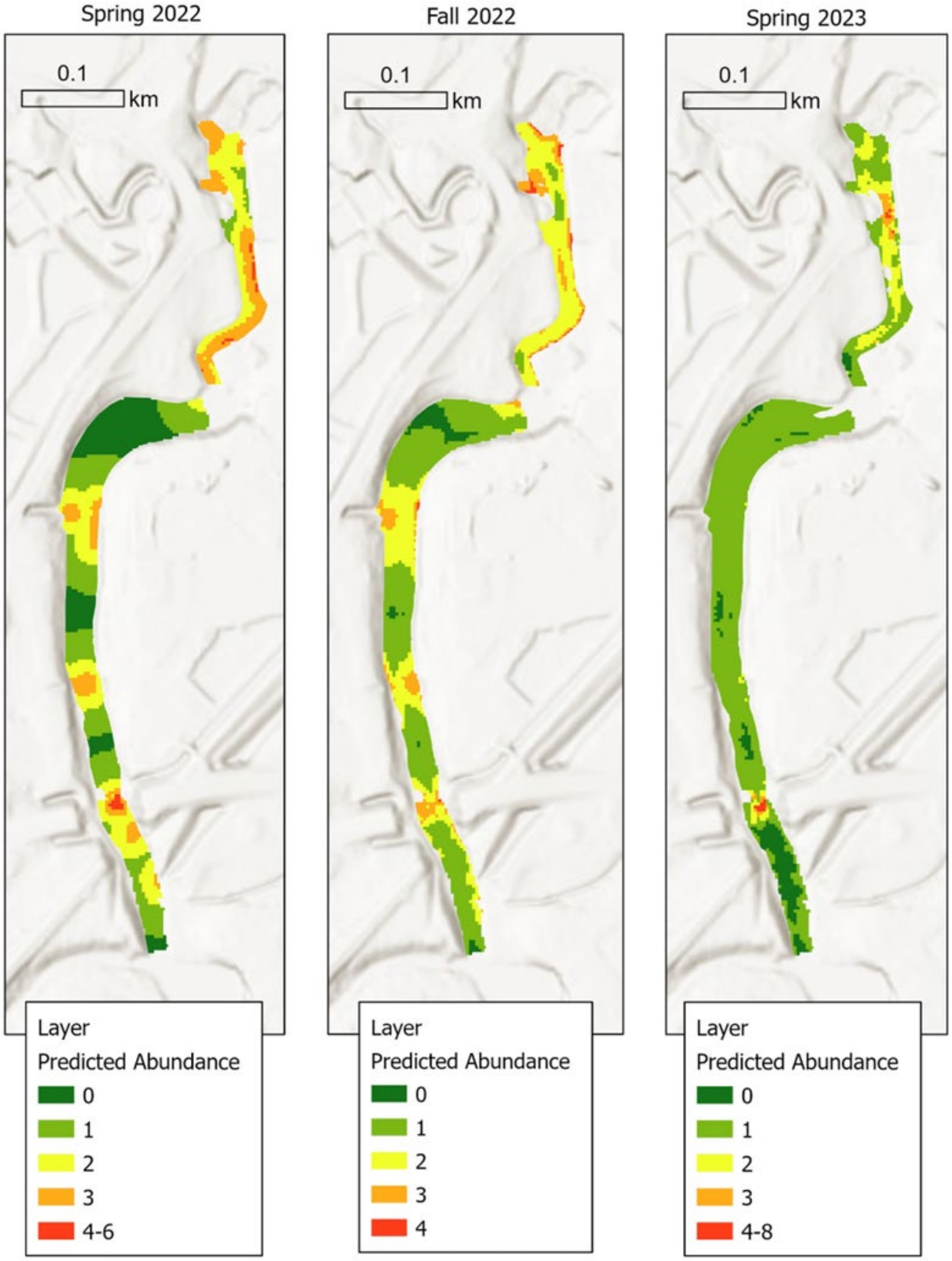


Figure 8. Mapped abundances of *Hypostomus* sp. in the upper San Marcos River across the three seasons in the study.

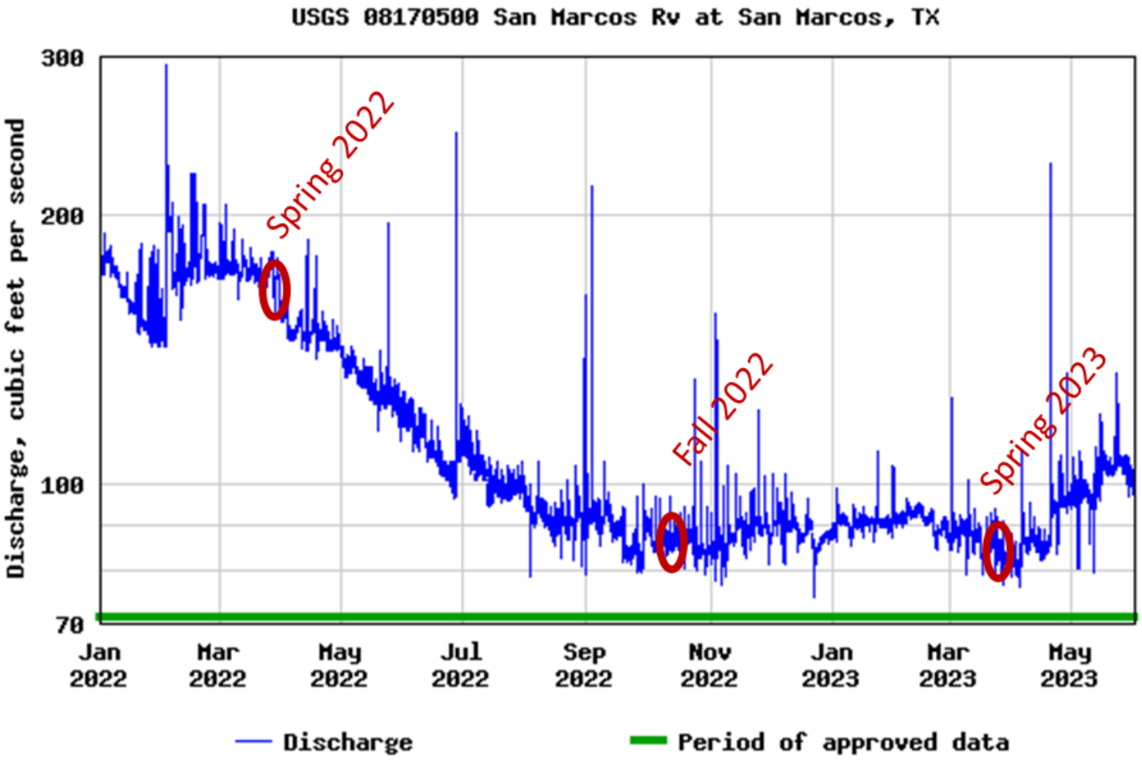


Figure 9. Hydrograph showing change in flow in the San Marcos River during the three seasons of *Hypostomus* sp. abundance monitoring.

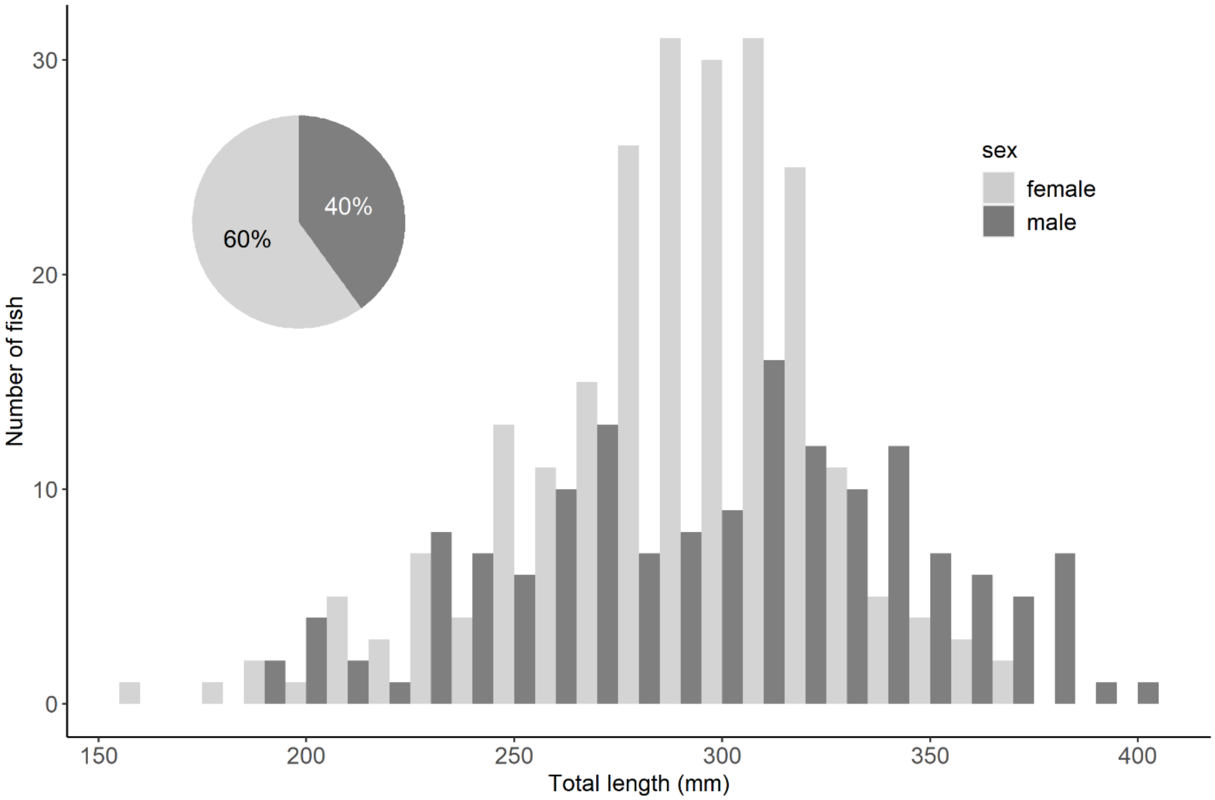


Figure 10. Length frequency distributions (bin width = 10 mm) for female (light gray; n = 231) and male (dark gray; n = 154) *Hypostomus* sp. collected from the San Marcos River, Texas, USA on December 15, 2021. The inset illustrates the proportion of specimens (n = 385) that were female versus male.

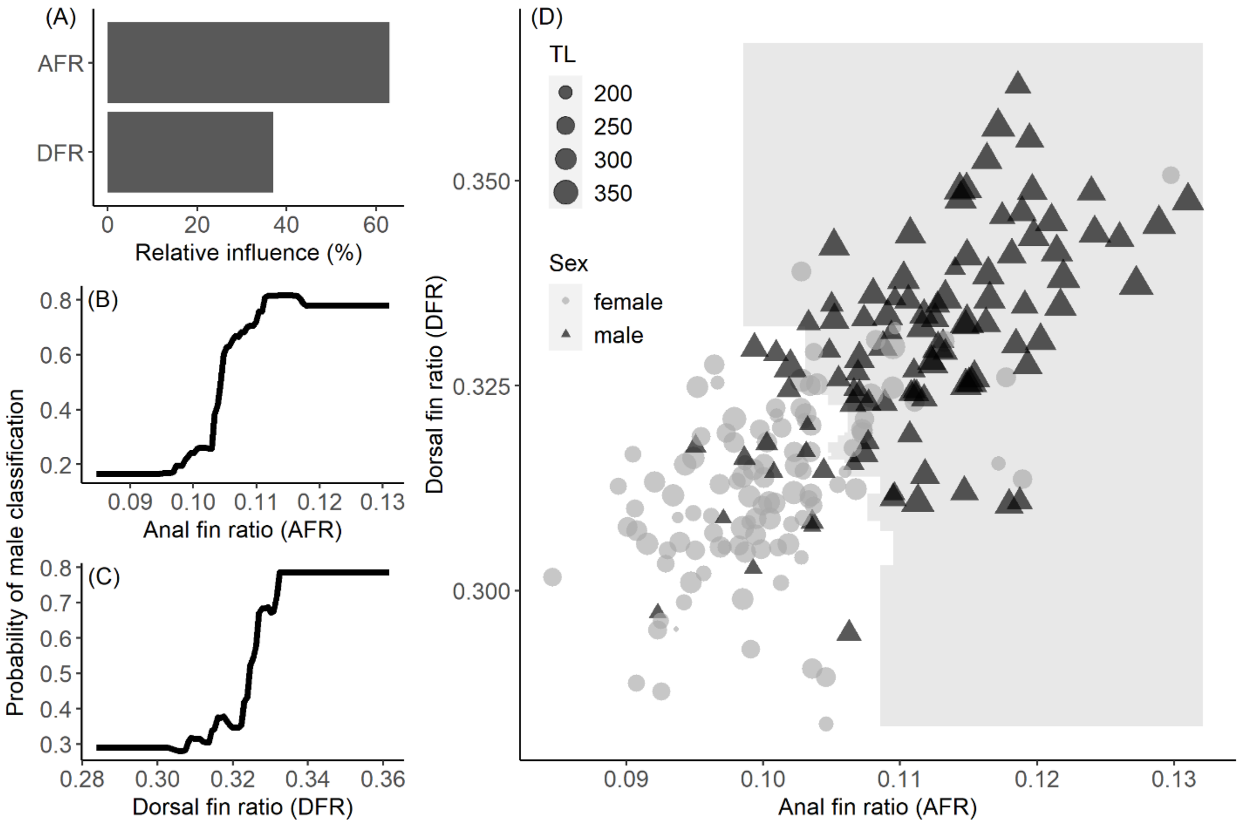


Figure 11. Results from the reduced generalized boosted classification model with only two variables (anal-fin ratio, AFR; dorsal-fin ratio, DFR) illustrating (A) the relative influence of AFR and DFR on classification predictions, (B) the partial dependence of classification on AFR (i.e., with DFR held at its mean), (C) the partial dependence of classification on DFR (i.e., with AFR held at its mean), and (D) a scatter plot of specimens included in the model (points) colored and shaped by male (black triangles) and female (gray circles) and sized by fish total length (TL, mm) across gradients of AFR and DFR for *Hypostomus* sp. collected from the San Marcos River Texas, USA. The gray shaded area in (D) represents the area where the probability of a fish being classified as a male is >0.5, where black triangles in the gray area represent correctly classified males and gray circles in the white area represent correctly classified females. Note that misclassified males (black triangles in white area in panel D) tend to have smaller total length.

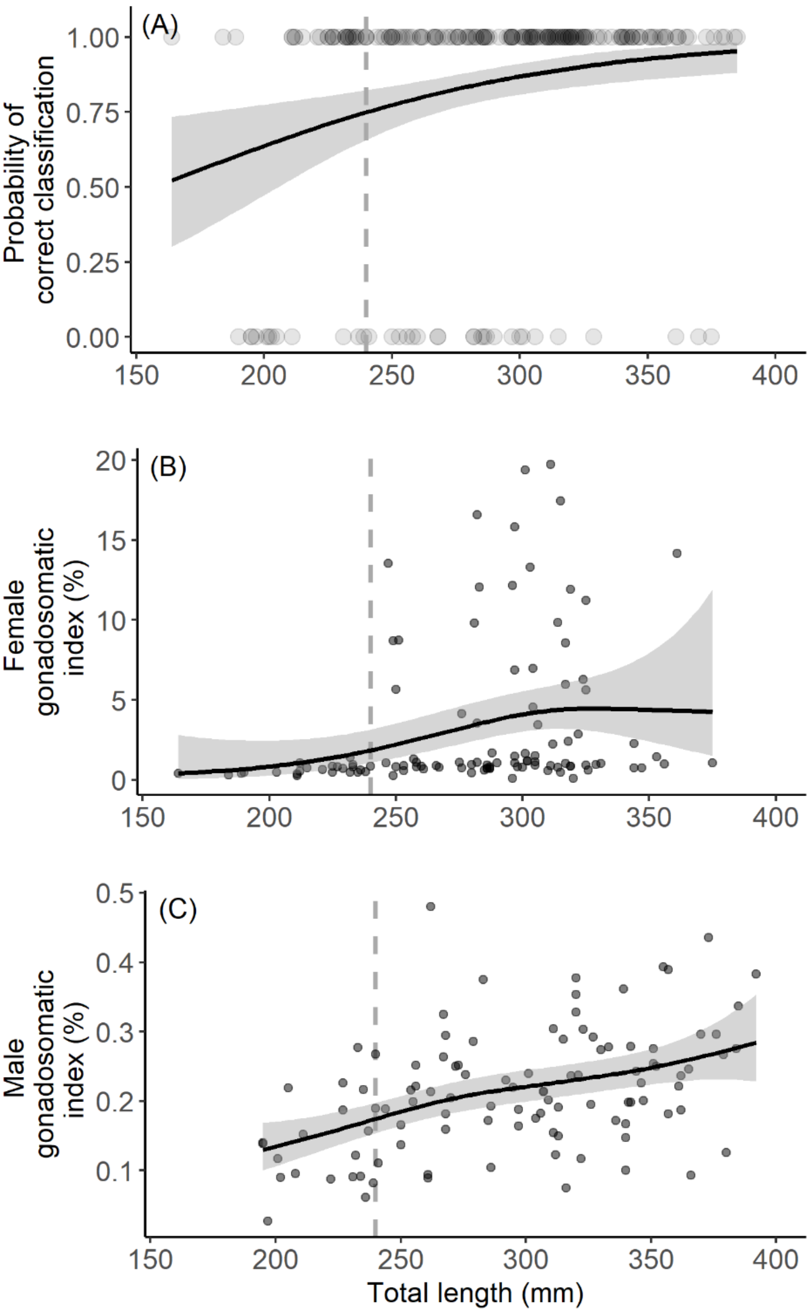


Figure 12. (A) Logistic regression model showing the relationship between total length (mm) and generalized boosted model sex classification accuracy of *Hypostomus* sp. collected from the San Marcos River, TX, USA. Points illustrate correct (1) or incorrect (0) classifications, the black line represents the fitted model prediction, and the gray area is the 95% confidence interval. (B) A generalized additive regression model (GAM) fit to the relationship between total length and female gonadosomatic index (GSI). (C) a GAM fit to the relationship between total length and male GSI. In (B) and (C) black lines represent model fits and gray areas are 95% confidence intervals. The vertical dashed line in each panel illustrates the corresponding change in classification accuracy (y-axis value = 0.75) and increase in reproductive activity at total length 240 mm.

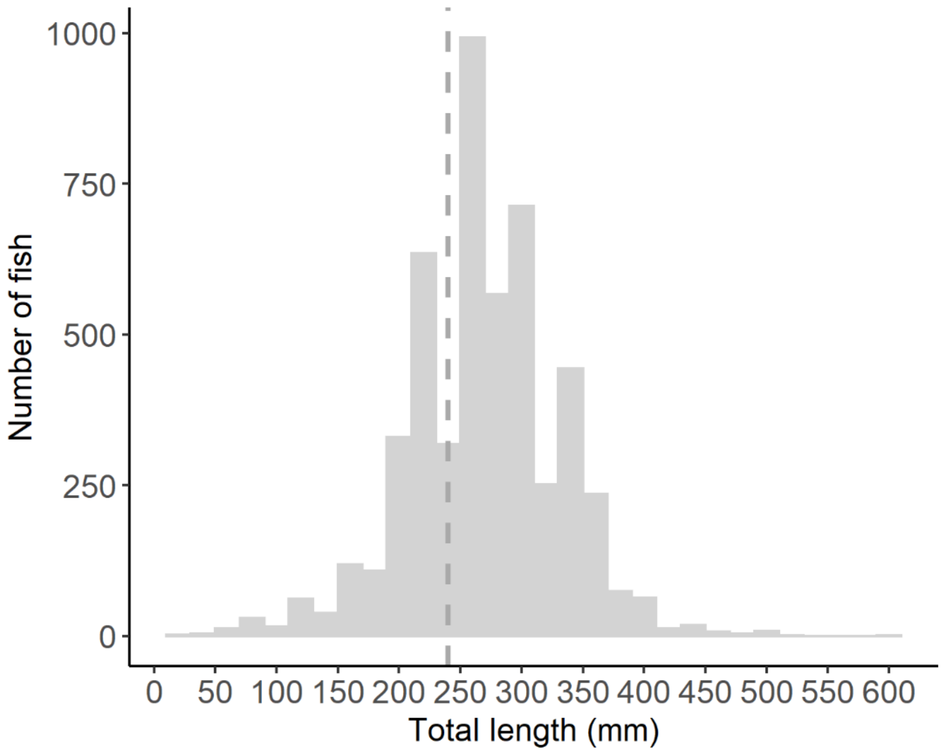


Figure 13. Length frequency histogram of *Hypostomus* sp. spearfished from the San Marcos River, TX, USA during 2014-2018 and reported by Blanton et al. (2020). The vertical dashed line indicates the length above which the probability of correct sex classification based on morphology exceeded 0.75 (i.e., 240 mm). The data distribution shows 5,067 fish, of which 3,710 (i.e., 73%) were ≥240 mm total length.

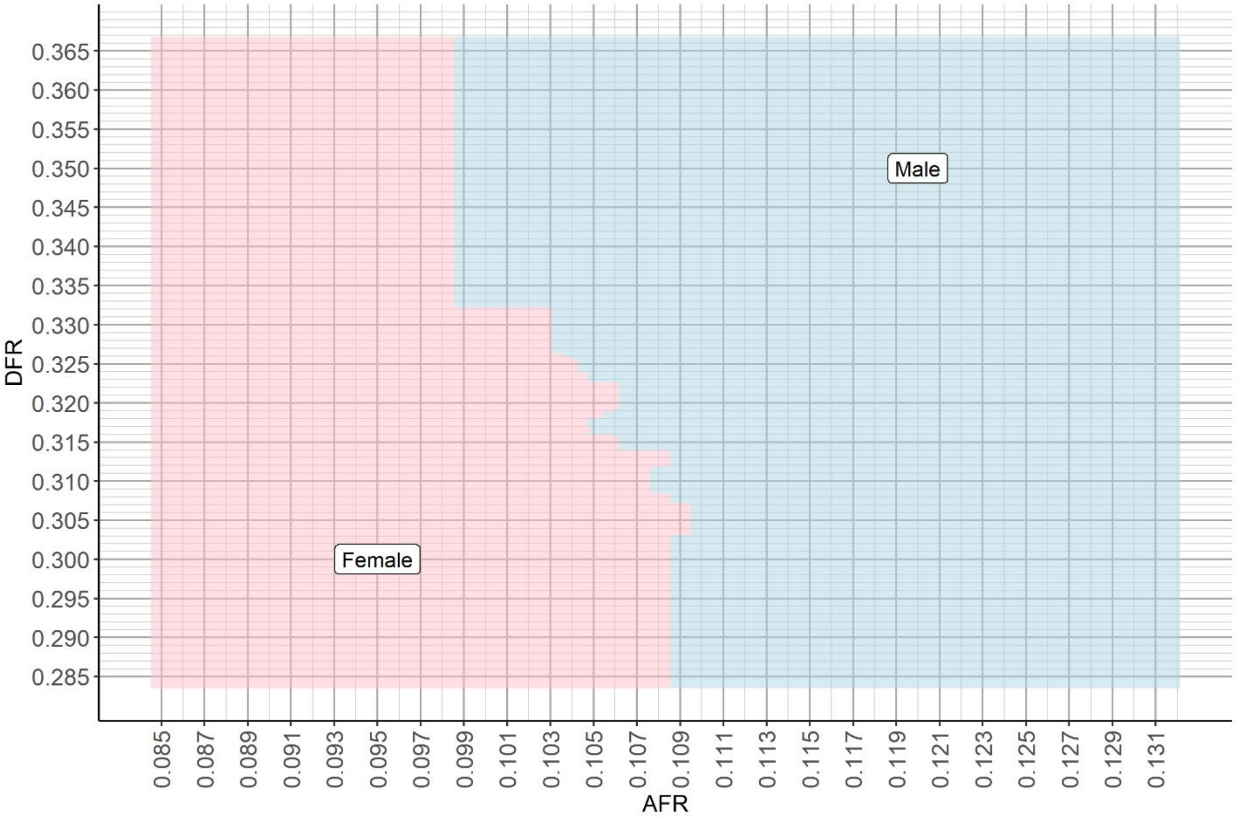


Figure 14. Two-dimensional predictions from the reduced generalized boosted classification model with only two variables (anal-fin ratio, AFR; dorsal-fin ratio, DFR) used to assign sex to *Hypostomus* sp. collected from the San Marcos River during model validation exercises. Ratios were rounded to three decimal places and are shown as gray lines, where line intersections occurring over the pink area represent females and line intersections occurring over the blue area represent males.

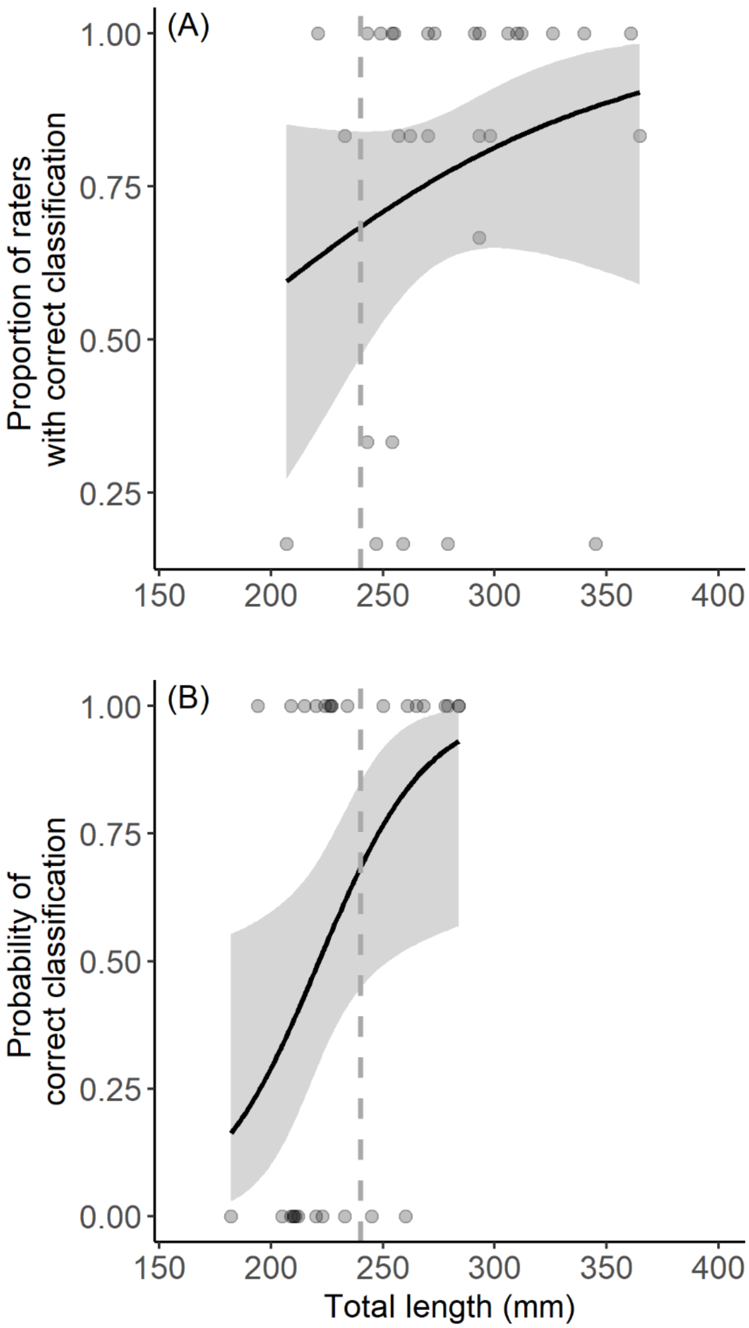


Figure 15. Results of classification model validation showing (A) proportion of six volunteer raters (i.e., excluding one unreliable rater; see Table 3) that correctly classified sex for 30 *Hypostomus* sp. collected from the San Marcos River and (B) the probability of correct classification for 29 *Hypostomus* sp. classified by a single rater. Each point represents an individual fish, black lines are fitted generalized linear models, and gray shaded areas are 95% confidence intervals. The vertical dashed line represents 240 mm total length, the size at which *Hypostomus* sp. began to exhibit mature gonads (see Figure 4).

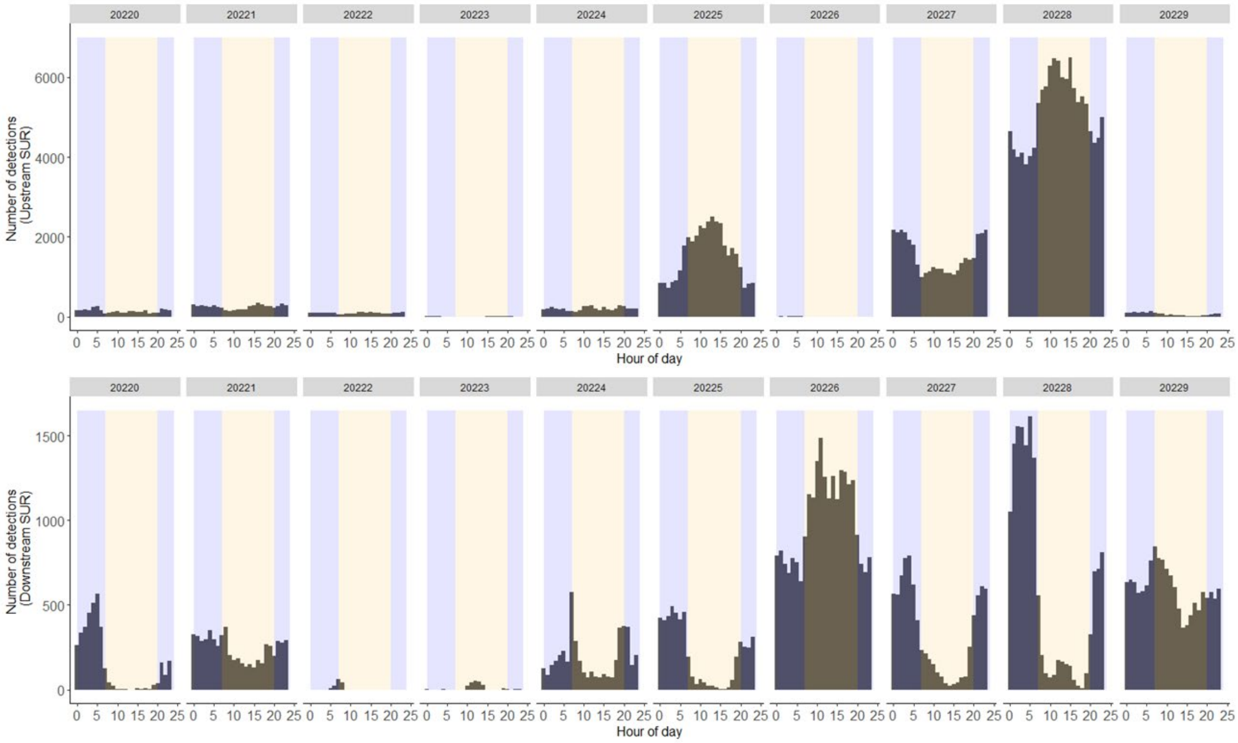


Figure 16. Plot showing diel (day = orange; night = blue) activity for 10 *Hypostomus* sp. tagged in the Sewell Park reach of the upper San Marcos River during March-August 2022. Black bars represent the number of detections per hour of the day at the upstream submersible ultrasonic receive (SUR; upper row) and the downstream SUR (lower row). Inference from these plots suggest fish 20220, 20221, and 20224 spend nights in the vicinity of the downstream SUR, fish 20225 and 20228 spent days near the upstream SUR but nights near the downstream SUR. The relatively higher bars in blue areas of the graphic illustrate high nighttime site fidelity.

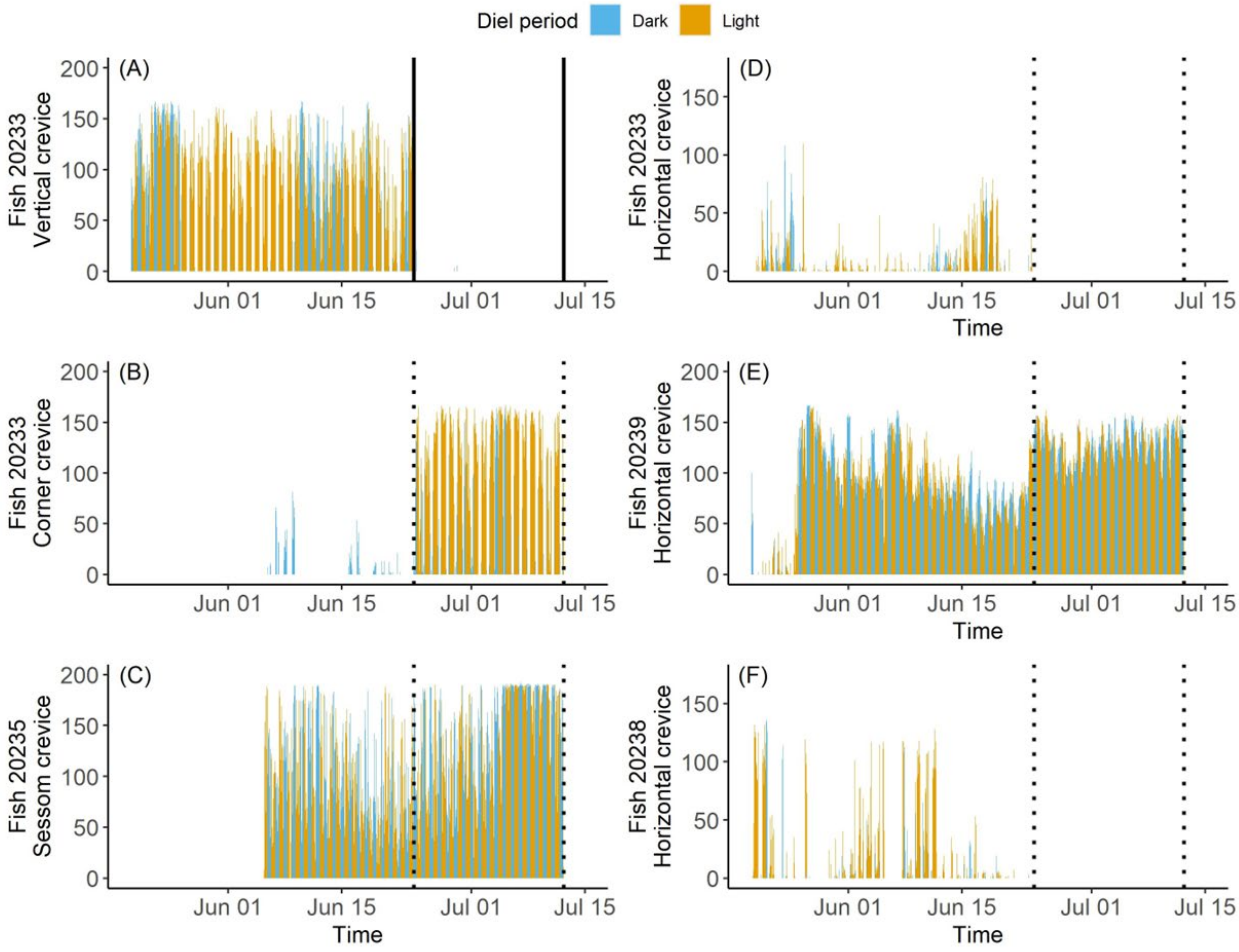


Figure 17. Hourly detections of three tagged suckermouth armored catfish among submersible ultrasonic receivers at four locations shown in Figure 4. (A) Fish 20233 inhabited the vertical crevice primarily during daytime (orange) though occasionally during nighttime (blue) periods until an exclusion device was installed on June 23 and removed July 12 (solid vertical bars). (B) Fish 20233 occasionally visited the corner crevice during nighttime hours prior to the exclusion from the vertical crevice but shifted to the corner crevice primarily during daytime after the exclusion device was in place. (C) Fish 20235 remained in the Sessom Creek outflow for the duration of tracking (SUR installed on June 6). (D) Fish 20233 occasionally used the horizontal crevice until the exclusion device was installed on the vertical crevice. (E) Fish 20239 constantly used the horizontal crevice, though reads increased after Fish 20233 moved to the corner crevice. (F) Fish 20238 occasionally used the horizontal crevice until it was speared just prior to the exclusion experiment.

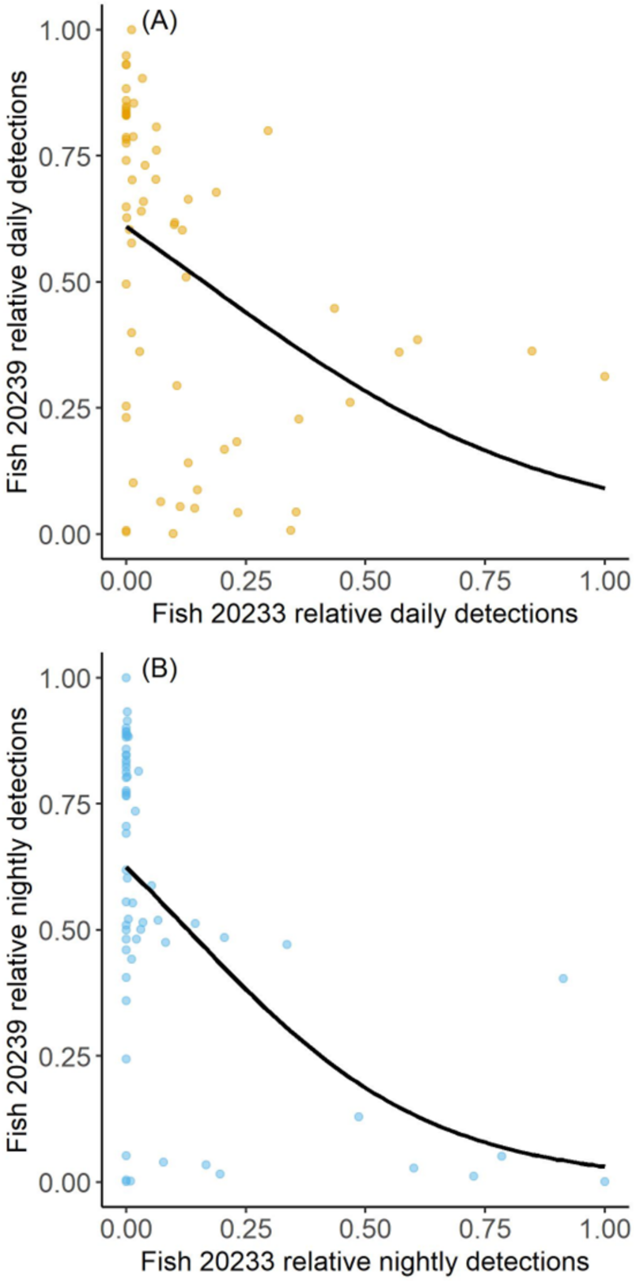


Figure 18. Regression models fit to the relationship between the number of detections for Fish 20239 as a function of detections for Fish 20233 in the horizontal crevice during (A) daytime and (B) nighttime hours. Note that number of detections are relativized to the maximum observed values by fish for daytime and nighttime hours.

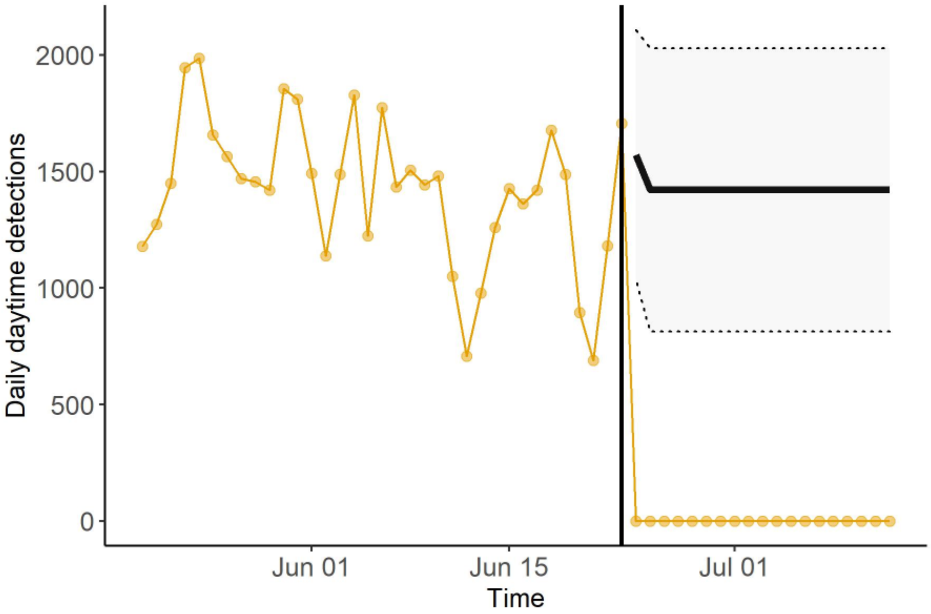


Figure 19. Observed daily daytime detections for Fish 20233 in the vertical crevice (orange points and lines) before and after installation of an exclusion device (vertical solid line) on June 23, 2023. The solid bold line and shaded area represents the fit and 95% confidence intervals from an autoregressive integrated moving average model used to project detections if the exclusion device had not been installed.