



Assessment of Invasion Extent of Asian Swamp Eels in Ponds and Backwater Marshes Adjacent to the Chattahoochee River

With Consideration of Management and Control Strategies

Natural Resource Report NPS/CHAT/NRR—2022/2357



**ON THIS PAGE**

Leaf litter traps deployed to detect juvenile Asian Swamp Eels (*Monopterus albus*) within marshes of the Chattahoochee River
Photograph by Jeffery Johnson, Oklahoma State University

ON THE COVER

Juvenile Asian Swamp Eels (*Monopterus albus*) sampled with leaf litter traps from the Chattahoochee River marshes
Photograph by Jeffery Johnson, Oklahoma State University

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Natural Resource Report NPS/CHAT/NRR—2022/2357

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Executive Summary

Asian Swamp Eels (*Monopterus albus*; ASEs) were first documented in the contiguous U.S. in 1994 within several ponds on the Chattahoochee Nature Center (CNC), a private nature preserve in Roswell, Georgia. Subsequent surveys from 2000 to 2005 detected adult and juvenile eels within adjacent marsh habitats along the Chattahoochee River, within the boundaries of the Chattahoochee River National Recreation Area. However, previous studies were somewhat limited by low capture rates of eels within the marshes. Herein, we expand upon these previous studies to provide updated estimates of invasion extent (spatial distribution and adult abundance) and to assess potential management and control strategies for ASEs within the Chattahoochee River marshes.

Leaf litter trapping of juvenile ASEs allowed for the estimation of detection and occupancy probabilities within the marshes immediately adjacent to the CNC, as well as surrounding marsh habitats. In 2015, a juvenile eel was detected 1.5 km downstream from the CNC ponds, and in 2016 juveniles were detected in a marsh situated across the mainstem Chattahoochee River. Occupancy probabilities increased with vegetation cover and amount of silt substrate, and maps of occupancy probability suggested eels could occupy several marsh areas where they had not been previously detected, including portions of the Willeo Creek marsh and an unnamed tributary to the North Marsh. Subsequent canoe-based backpack electrofishing captured six adult eels from the unnamed tributary to the North Marsh in 2017, confirming eels have expanded their range within the marshes and interconnected habitats. Because capture rates of adult eels from the marshes were low, we used next-generation sequencing of 60 juvenile eels to estimate an approximate minimum bounds on the number of reproducing adults in the marshes in 2015. The pedigree reconstruction of maximum likelihood indicated that 86 adults contributed to our sample of juveniles, and point estimates of the effective number of breeders ranged from 57 to 154.

Canoe-based electrofishing routes produced five adult eels from the marshes adjacent to the CNC in May through July 2016, but electrofishing circuits failed to detect eels in the Middle, North, or South marshes in July 2017. We assessed the potential use of canoe-based electrofishing to deplete eels within Beaver and Kingfisher ponds on the CNC property, both of which were known to produce high catch rates of eels. Catch rates fell with subsequent depletion passes within a day (likely trap-shyness), but catch rates were approximately equal (~ 0.20 eels/minute) across four consecutive days of effort with no noticeable depletion trend in either pond (despite the removal of a total of 142 eels). Baited stationary traps and baited eel pots failed to capture eels within the marshes or CNC ponds.

Finally, we integrate our results with existing literature to examine management and control options for ASEs within the Chattahoochee River marshes. Given the reproductive strategies of the species (e.g., protogynous sequential hermaphroditism and polygamy wherein females visit multiple males within a breeding season) and low catchability of adult eels from the marshes, removal and control efforts will be costly and eradication will be virtually impossible. Moving forward, the costs of management actions should be balanced with known and other, perhaps unforeseen, ecological risks of population persistence or expansion.

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Multi-agency sampling effort for Asian Swamp Eels conducted in July 2017.

Introduction

Asian Swamp Eels (*Monopterus albus*; ASEs) are native to southeastern Asia and Indonesia where they inhabit a variety of different habitats, including swamps, rice fields, ponds, and streams (Liem 1967). Asian Swamp Eels feature a number of adaptations that allow them to persist in rice fields and swamps that are subject to seasonal habitat desiccation and hypoxic conditions. Many of these same adaptations and life history strategies make ASEs a particularly interesting and problematic invasive species.

Asian Swamp Eels are obligate air breathers and may travel short distances over land (Liem 1967, 1987). A thick mucus layer covers their scale-less body, which presumably retains moisture and allows ASEs to more easily burrow into muddy substrates to avoid desiccation as habitats dry (Liem 1967, 1987). The reproductive strategies of ASEs are thought to allow populations to recover following prolonged droughts or other harsh conditions (Liem 1963). In general, ASEs are regarded as sequential protogynous hermaphrodites, wherein all individuals begin life as females, with some portion of older adults within the population entering a natural intersex phase and transitioning to male (Liem 1963). Age-1 eels are capable of being sexually mature and are consistently female, and sex reversal can begin at approximately 2.5 to 3 years of age (Liem 1963; Yang and Xiong 2010). Previous studies have reported female-to-male ratios from 2:1 to 3:1 (Liem 1963; Mei et al. 1993; Yang and Xiong 2010). Population density and food availability are thought to influence sex ratios and sex reversal timing, however, these drivers are poorly understood (Liem 1963; Shi 2005). In their native range, spawning begins as water temperatures approach 28°C, and males construct three-chambered breeding burrows and defend this territory from competing males (Matsumoto et al. 2011). Females visit and breed with multiple males within a single breeding season, forming a male-territory-visiting polygamy (Matsumoto et al. 2011). Males then maintain clutches of eggs within bubble nests constructed on the water's surface near their burrows, and following hatch, males mouth-brood the larvae until the juvenile eels reach approximately 32–37 mm TL (Matsumoto and Iwata 1997).

Large markets exist for ASEs as a food fish (Guan et al. 1996), and since the 1990s or earlier ASEs were imported into the U.S. and sold at ethnic live food markets – the most likely source for wild introductions of ASEs in the U.S. (Nico et al. 2011). Although ASEs were purposefully introduced into Oahu, Hawaii, prior to 1900 (Mundy 2005), the first established population of ASEs in the contiguous U.S. was documented in private ponds in Roswell, Georgia, in 1994 (Freeman and Burgess 2000). Since, established populations of ASEs have been discovered in several distinct regions of southern Florida in the late 1990s and in New Jersey in 2008 (Fuller et al. 2019). Asian Swamp Eels or close relatives (*Monopterus* spp.) have also been detected in Maryland (2014), Texas (2014), a disjunct stream system in Georgia (2016), Michigan (2017), and New York (2017); however, none of these recent detections are currently known to represent established populations (Fuller et al. 2019; Nielsen and Fuller 2019).

Asian Swamp Eels were first detected in 1994 within several ponds (Beaver, Kingfisher, and Frog ponds) on the Chattahoochee Nature Center (CNC) property, a private environmental education

facility (Starnes et al. 2000; Freeman et al. 2005; Figure 1). The CNC is adjacent to the Chattahoochee River wherein the headwaters of Bull Sluice Lake create marsh habitats along the river's margins – this area is administered by the National Park Service as a portion of the Chattahoochee River National Recreation Area. Initial electrofishing surveys in 1996 failed to detect ASEs in the marshes or nearby tributaries of the Chattahoochee River (Starnes et al. 2000), but a single eel was collected from the marshes near the outflow from the CNC ponds in May 2000 (Freeman and Burgess 2000). Freeman and Burgess (2000) noted that the lack of individuals encountered within the marshes was problematic because it could indicate a population exists in the marshes that is difficult to detect, or it could represent an occasional migrant from the CNC ponds. Subsequent surveys from 2004 to 2005 captured juvenile and adult ASEs in the marshes near the outflow from the CNC ponds, but failed to detect ASEs in other nearby marshes (Freeman et al. 2005). Freeman et al. (2005) interpreted these results as indication of a “potentially breeding population within the adjacent marsh,” and the authors noted that temperatures within the marshes occasionally reached their hypothesized minimum of 25°C for spawning. The thermally-depressed temperature regime of the mainstem Chattahoochee River was also assumed to pose a barrier to reproduction and movement of the species (Freeman et al. 2005).



Figure 1. Study area in Roswell, Georgia, illustrating ponds within the Chattahoochee Nature Center and adjacent marsh habitats along the Chattahoochee River. Star indicates pond outflow to marshes.

Much of what is known about the ecology of this ASE population stems from studies of adults captured from the CNC ponds because of low captures of adult ASEs from the marshes. Examination of gut contents indicates that ASEs primarily feed upon aquatic invertebrates, primarily consisting of Odonata, Trichoptera, Mollusca, and Diptera (Freeman and Burgess 2000; Freeman et al. 2005). Fish were found to represent a small percentage of diet items (less than approximately 10%; Freeman and Burgess 2000; Freeman et al. 2005). A stable isotope study of 26 adult eels from Beaver Pond (2 to 49 cm total length) revealed that ASEs occupy a similar trophic position as insectivorous fish like *Gambusia* sp.; thus, the expected primary impact of ASEs within the food web would be reducing available food for other insectivorous fishes in the system (Straight et al. 2005). Estimated ages of eels from the marshes ranged from 1 to 4 years old, whereas individuals from the ponds ranged from 1 to 7 years old (Freeman and Burgess 2000; Freeman et al. 2005). Examination of gonads revealed that females have the potential for multiple spawns within a single breeding season, and the overall female-to-male ratio was approximately 5:1 (Freeman et al. 2005; Long and LaFleur 2011).

Low capture rates have limited previous studies of ASE in the area, particularly in the marshes of the Chattahoochee River. A number of active and passive sampling gears have been assessed, including backpack electrofishing, boat-mounted electrofishing, benthic-grid electrofishing, barrel traps, and leaf litter traps (Freeman and Burgess 2000; Freeman et al. 2005). Much of these efforts proved unfruitful, either failing to capture a single ASE or requiring an insurmountable amount of effort to capture one or a few ASE. Canoe-mounted backpack electrofishing had moderate success at capturing adult eels; however, mark-recapture models estimated individual capture and recapture probabilities < 1% (Freeman et al. 2005). Leaf litter traps proved useful for capturing juvenile eels in pond and marsh habitats (Freeman et al. 2005).

The present study builds upon previous studies to update the invasion extent of ASE within the Chattahoochee River marshes and to evaluate several potential control strategies. We conclude by integrating our results with existing literature in order to examine potential management and control options for ASE within the study system.

Objectives

With the goal of informing management and control efforts for Asian Swamp Eel within the ponds and backwater marshes adjacent to the Chattahoochee River, this study addressed the following objectives:

1. Determine extent of Asian Swamp eel invasion
 - a. Model detection and occupancy of juvenile Asian Swamp Eels to delineate current invasion extent in the Chattahoochee River
 - b. Model detection and occupancy of adult Asian Swamp Eels for future invasion extent research
 - c. Evaluate the efficacy of environmental DNA (eDNA) for Asian Swamp Eel detection for future invasion extent monitoring
2. Determine the size of the breeding population in the marshes using genetic analyses
3. Evaluate management and control strategies

Methods

Objective 1a – Detection and Occupancy of Juvenile Eels

From June through August in both 2015 and 2016, leaf litter traps were deployed along the Chattahoochee River and its backwater marsh habitats within a 2-km radius of the CNC pond outflow. Detections and associated sampling and habitat data were used to estimate detection and occupancy probabilities for juvenile ASE. For detailed methods regarding field sampling and model construction, see Master's thesis by Johnson (2017).

Objective 1b – Detection and Occupancy of Adults Eels

Canoe-based backpack electrofishing was used to sample 10 x 2 m shoreline transects in Beaver and Kingfisher ponds, as well as in the marsh habitats immediately adjacent to the CNC from May 27 to June 2, 2016. For detailed methods regarding field sampling and model construction, see master's thesis by Johnson (2017).

On July 17, 2017, we conducted exploratory sampling for adult ASEs in an unnamed tributary stream to the North Marsh of the Chattahoochee River (Figure 1). Although ASEs of different life stages have been variously reported from backwater marsh habitats (Freeman and Burgess 2000; Freeman et al. 2005; Johnson 2017), to our knowledge, tributary streams to these marshes have received no directed sampling for ASEs. We employed canoe-based backpack electrofishing methods that have proven effective at capturing adult ASEs within the study area (Freeman et al. 2005; Johnson 2017). We recorded the number of ASEs caught, the number of ASEs missed (i.e., seen but not captured), and electrofishing effort (min; i.e., on-time for shocking). All captured eels were immediately placed in an anesthetizing solution of MS-222 (270 mg/L) and preserved in 70% ethanol.

Objective 1c – Efficacy of eDNA Detection

Triplicate 1L water samples were collected at 10 transects where ASEs had previously been detected. Each transect was sampled on five separate occasions for a total of 150 water samples. Additionally, triplicate water samples of a negative control were collected from a local water source at the testing laboratory in Warm Springs, Georgia, that had no possibility of ASE contamination (15 water samples). Water samples for eDNA analysis were preserved with 30ml of 95% ethanol and 1.5ml of 3M sodium acetate (Ficetola et al. 2008). The eDNA analysis was in collaboration with G. Moyer, formerly with the USFWS, Warm Springs Conservation Genetics Laboratory, now at Mansfield University of Pennsylvania. Water samples were filtered (0.45 µm) for eDNA at the Oklahoma Cooperative Fish and Wildlife Research Unit lab at Oklahoma State University. Post-filtration, DNA extraction procedures were conducted with the RapidWater™ DNA isolation Kit (MO BIO Laboratories, Inc), and analyzed for a genetic marker specific to ASEs. For detailed methods, see report by G. Moyer, Mansfield University (Appendix A).

Objective 1d – Breeding Population Size

Previous research documented adult ASE in the marsh habitats of the Chattahoochee River adjacent to the Chattahoochee Nature Center (CNC; Freeman et al. 2005; Johnson 2017). Capture and recapture probabilities of adult ASE have been extremely low and variable (Freeman et al. 2005; Johnson 2017), which has hindered ability to produce meaningful estimates of the size and scope of

the ASE population that could inform management and control in the marshes. Juvenile ASE are more-readily captured with leaf litter traps in marsh habitats, suggesting an adult ASE population that is reproducing independent from ASE in the CNC ponds (Johnson 2017). Despite the cryptic nature of adult ASE in the marsh habitats, next-generation sequencing of captured juvenile ASE could provide inference into the size of the breeding adult population in the marshes.

Juvenile ASE belonging to the 2015 cohort were collected using leaf litter traps between June and August, 2015, primarily from the Middle Marsh, but also from a few locations in the North and South marshes. Specimens were stored in 70% ethanol at room temperature. In 2018, genomic DNA was extracted using the DNeasy® Tissue Kit (Qiagen Inc., Valencia CA) following manufacturer's protocols, and stored in DNA hydrating solution. Samples were sent to the University of Georgia Genomics and Bioinformatics Core Facility (GCBC, Athens, Georgia) for genotyping-by-sequencing (GBS) following Elshire et al. (2011) using the *ApeKI* restriction enzyme for sample digestion. The resulting library was quantified using qPCR and sequenced on an Illumina NextSeq for 150 cycles at mid output for single end 150bp sequencing. To obtain a subset of informative single-nucleotide polymorphism (SNP) loci, reads were filtered, clustered, and aligned within the STACKS pipeline (Catchen et al. 2013) with the following parameters specified: 1 population, minimum depth of coverage (m in *ustacks*) of 7, and a locus-specific missing data threshold (r) of 0.2 (i.e., a locus could have up to 80% missing data across individuals). We then used the *pegas* package (Paradis 2010) in Program R (R Core Development Team 2019) to perform Hardy-Weinberg exact tests at each locus with 1,000 MCMC reps. Significant deviations were identified at $p < 0.05$ and a Bonferroni adjustment was made to adjust for multiple comparisons (Rice 1989).

We then used the pedigree reconstruction of maximum likelihood in Program COLONY v 2.0.6.5 (Jones and Wang 2010) to estimate full- and half-sib relationships of juvenile ASE (designated as offspring in models). Because the ASE population likely originated from a few founder individuals, we accounted for inbreeding in the model parameterization. We estimated the contemporary number of effective breeders (N_b) of ASE that produced our sample of juveniles based on the frequencies of full- and half-sibs inferred from the estimated sibship assignments (Wang 2009). Two sets of N_b estimates were produced: one based on the assumption of random mating in the population, and another that accounts for non-random mating by accounting for estimated deviation from Hardy-Weinberg equilibrium. The effective number of breeders represents the number of reproducing individuals in an idealized population that would lose genetic variation at the same rate as those in the study population. In other words, N_b would theoretically equal the census size (N) of reproductively mature eels contributing to our sample of juvenile ASEs if the adult population conformed to the conditions of an "ideal population" that features an unlimited number of breeders, equal sex ratio among breeders, random variation in family size, non-overlapping generations, and absence of migration, mutation, or selection (Hallerman 2003).

Objective 2 – Management and Control

To better inform management and control strategies for ASE in the CNC ponds and in marshes of the Chattahoochee River, we examined the efficacy of several different sampling and removal strategies using information in published and unpublished literature and through *in-situ* experimentation (see

below). *In-situ* studies included using canoe-based backpack electrofishing to deplete adult ASE numbers in the marshes and CNC ponds as well as testing two stationary trapping methods (baited eel-pots and baited PVC traps). Finally, we examined the ecology of invasive ASE by recording a variety of ecological data on captured adults, including sex, age estimates, and stomach contents.

Depletion Sampling

Depletion methods are commonly used in fisheries science to obtain estimates of initial population size when mark-recapture techniques are impractical. The underlying principle of depletion sampling is that the rate at which catches fall off with subsequent sampling passes is directly related to the unknown initial population size and the known number of fish removed (Cowx 1983). Depletion methods assume closed populations, constant catchability across all removals, substantial proportions of the population removed by sampling efforts, catchability is equal across all fish (i.e., vulnerability to capture), and units of effort are independent (i.e., sampling passes) (Cowx 1983; Ogle 2016). Catchability (q) is the probability of any single fish being captured during a sampling pass, and violations to catchability-related assumptions are typically the most problematic with depletion methods (Cowx 1983). Catchability commonly declines with successive sampling passes, as fish can develop trap-shyness or other physiological responses that can inherently change vulnerability to capture (Cowx 1983; Mesa and Schrek 1989; Rosenberger and Dunham 2005). For example, ASE may grow increasingly wary and avoid electrofishing gear, thus decreasing q . Similarly, ASE may change their vulnerability of capture during or between passes by moving in and out of burrows within the substrate.

We used canoe-based backpack electrofishing methods that have proven effective at capturing adult ASE within the study area (Freeman et al. 2005; Johnson 2017). We assessed two different depletion sampling schemes. One scheme involved the completion of multiple full sampling circuits (all shoreline at a given site) within one day. A second scheme involved the completion of only one full sampling circuit per day, but continued consecutively over multiple days. For each depletion circuit, we recorded the number of ASE caught, the number of ASE missed (i.e., seen but not captured), and electrofishing effort (min; i.e., on-time for shocking). All ASE were immediately anesthetized in MS-222 (270 mg/L) and preserved in 70% ethanol.

To estimate initial population size of adult ASE in each waterbody from the depletion sampling data, we used the DeLury (1947) model to relate catch-per-unit-effort (CPUE; ASE/min) and cumulative effort (min). The DeLury model is

$$\log\left(\frac{C_i}{f_i}\right) = \log(qN_0) - qE_{i-1}$$

where C_i is the catch for sample i ; f_i is the fishing effort (min) for sample i ; q is the catchability coefficient; N_0 is initial population size; and $E_{(i-1)}$ is the cumulative fishing effort prior to sample i . We followed Ogle (2016) to build DeLury models and to calculate confidence intervals for estimated N_0 using the *FSA* package in Program R.

Stationary Trapping

We tested two stationary trapping methods, baited eel-pots and baited PVC traps, that had not previously been evaluated as a means of detecting and removing ASE from the study area. First, eel pots (Gee's Minnow Trap/Eel Pot Trap #G40EP) were fished overnight in two ways, either deployed as floating or submerged traps. All eel pots were baited with canned tuna. Although a variety of passive gear types have been previously tested for sampling adult ASE (Freeman and Burgess 2000; Freeman et al. 2005), recent literature has mentioned the *Tukil* – a traditional cylindrical tool (previously made of hollowed bamboo, modern version made of PVC pipe) that is used by locals to catch ASE in rice paddy habitats (Yin et al. 2012; Yin et al. 2018). A quick web search revealed numerous modern adaptations of the *Tukil* catching ASE within their native range. If such methods are effective at sampling ASE from marsh habitats, they may be conducive to a volunteer-based program that National Park Service biologists could leverage to remove adult ASE and possibly suppress population growth. To that end, we created two PVC interpretations of the *Tukil* to fish in the marsh habitats: a “large” version (based off of this video: <https://www.youtube.com/watch?v=kSWeBvCKguA>) and a “small” version of similar design. These stationary traps were deployed with different combinations of the following baits: mesh bags containing chopped earthworms or mesh bags of MAGIC© brand preserved bait fish (either “Baby Shad” or “Emerald Shiner” varieties). Traps were positioned in areas of the marshes that generally retained water despite fluctuating river levels, and were fished for variable amounts of time between checking and re-baiting.

Ecological Data

In a laboratory setting, we examined all ASE that were stored in 70% ethanol and recorded total length (TL; mm) and weight (g). We extracted sagittal otoliths for age estimation (*see* Freeman et al. 2005) and sectioned otoliths along a transverse plane with a low-speed saw following Secor et al. (1992). Age was independently estimated by two readers by interpreting annuli from one otolith belonging to each specimen. Where discrepancies occurred among readers, a concert read was performed to obtain a consensus age estimate for each specimen. We identified sex (male or female) based on gross internal anatomy of the gonads, and where neither eggs nor milt were apparent, we recorded sex as unknown. Stomach contents were removed and identified to taxonomic order (when possible), similar to Freeman and Burgess (2000) and Freeman et al. (2005). Diet items were summed across all individuals in a sample to calculate an overall percentage of diet items belonging to each order.

Results

Objective 1a – Detection and Occupancy of Juvenile Eels

In 2015, 10 sampling occasions were conducted and 31 juvenile ASEs were captured at 14 unique transects. Juvenile ASEs were detected up to 1.5 km away from the CNC pond outflow (presumed invasion point) during 2015. In 2016, 36 juveniles were collected at 10 unique transects, including 3 transects within a marsh situated directly across the mainstem river from the CNC. For detailed maps of capture locations, see Johnson (2017).

The top model of detection probability included sampling year, temperature, and depth at time of sampling, with detection probability increasing with sampling depth and temperature. The top model of occupancy probability contained both proportion of vegetated area and proportion of silt substrate area, with occupancy positively associated with both variables. In general, higher occupancy probabilities were estimated in backwater marsh habitats, including several marshes where ASE were not detected. These areas included the Willeo Creek marsh and an unnamed tributary to the North Marsh. Occupancy probabilities were generally much lower along the banks of the mainstem Chattahoochee River. For detailed model results, see Johnson (2017).

Objective 1b – Detection and Occupancy of Adults Eels

Over 10 sampling occasions, 76 adult ASEs were captured from all 10 transects in Beaver Pond and 19 adult ASEs capture from 9 unique transects were captured from Kingfisher Pond. Three adult ASEs were captured from three unique transects within the marsh, with catch per sampling occasion in the marsh varying from zero to two eels. An inadequate number of detections were made in the marsh to allow for modeling of detection and occupancy of adult ASE in the marshes. Data from Beaver and Kingfisher ponds allowed for modeling detection probabilities, but the high proportion of occupied transects precluded modeling of occupancy probabilities in either pond. Point estimates of adult ASE detection probability were 0.45 in Beaver Pond and 0.19 in Kingfisher Pond. The top model of detection probability in Beaver Pond included temperature (inversely related to detection probability), whereas the top model in Kingfisher Pond was the null model. For detailed capture and model results, see Johnson (2017).

Based on results from juvenile eel sampling, a multi-agency team from U.S. Geological Survey, Oklahoma State University, and the National Park Service conducted exploratory sampling efforts on July 17, 2017, within the apparently suitable unnamed tributary to the North Marsh. A total of six adult ASE were collected, and an additional seven ASE were observed but evaded capture, during 48 min of electrofishing effort distributed across two sampling passes. Each sampling pass covered approximately 125 m of both stream banks upstream of the Azalea Dr. bridge (Table 1; Figure 2). The collection of adult ASE in the North marsh tributary represented an expansion of the known invaded range of ASE.

Table 1. Sample data for adult Asian Swamp Eels during exploratory sampling of an unnamed tributary to the North Marsh. Catch-per-unit-effort (CPUE) is measured in fish/minute.

Date	Catch	Missed	Effort (min)	CPUE
7/17/2017	5	3	25	0.20
7/17/2017	1	4	23	0.04



Figure 2. The unnamed tributary to the North Marsh, looking upstream from near the Azalea Drive bridge. St. Francis School is located on the upstream-right bank. Upstream extent of sample reach was located near the coordinates: 34.007669, -84.374018.

Objective 1c – Efficacy of eDNA Detection

All positive controls and internal standards amplified, suggesting the eDNA assay worked as intended and there were no PCR inhibitors limiting reactions. However, the assay failed to detect ASE in all but one sample, suggesting this method was ineffective at detecting ASE within the study area. One proposed limitation of an eDNA detection is that ASE may not slough off their DNA into their aquatic environment as many other Teleost fishes do, perhaps because of their increased mucus layer that contains glycoproteins but is void of tissue and DNA (see Appendix A for more details).

Objective 1d – Breeding Population Size

A total of $n = 88$ individuals were submitted for GBS analysis, 82 of which were juvenile ASE trapped from the marshes in 2015. Due to the low number of reads associated with some specimens ($< 10\%$ of SNP loci scored), 60 juvenile eels were retained for analysis. The STACKS pipeline produced genotypes consisting of 1,003 SNP loci. Following tests for conformance to Hardy-Weinberg, 976 SNP loci remained for data analysis. Because juvenile ASE specimens had been stored for over two years in 70% ethanol at room temperature, DNA degradation led to elevated percentages of missing SNP scores (38% – 95% of loci missing per individual genotype). Preserved specimens commonly produce SNP datasets with high levels of missing data, but this does not bias results if missing data are properly accounted for in population genetic analyses (Graham et al. 2015).

From the 60 juvenile ASE retained for analysis from 2015, Program COLONY pedigree of maximum likelihood revealed that 25 individuals had no sibship to other juveniles in our sample. A total of 12 family groups were identified: 5 full-sib only groups (the largest containing 4 individuals), 4 half-sib only groups, and 3 groups containing both full- and half-sibs. Among all groups, there were 15 full-sib relationships and 16 half-sib relationships, and there was an overall inbreeding coefficient of 0.5947 (i.e., 59% of alleles are identical by descent). The pedigree reconstruction also revealed that 86 hypothetical adults contributed to our sample of the 2015 cohort. Family reconstruction supported polygamy among hypothetical adults of both sexes within the breeding season, with a maximum of 3 partners observed for any one hypothetical adult. At least one juvenile from the North Marsh and from the South Marsh were identified as half-sibs with juveniles from the Middle Marsh, indicating adults moved at least 0.5 km during the breeding season and mated in multiple marshes. When assuming random mating $N_b = 154$ (95% CI's: 107 – 226), whereas $N_b = 57$ (95% CI's: 36 – 96) when assuming non-random mating.

The results of the sibship reconstruction used to estimate N_b should be interpreted carefully because the methodology is sensitive to the assumption that offspring are sampled in a random fashion across all available families (Wang 2009). First, we cannot completely rule out that some of the juveniles from the Middle Marsh were spawned in the CNC ponds, which would upwardly bias estimates of breeding adults in the marshes. However, we believe the likelihood of including juvenile eels that originated from the ponds is low. In their native range, adult male ASEs have been observed mouth brooding juveniles until about 37 mm TL (Matsumoto and Iawata 1997), and juveniles included in this analysis had an average TL of 65 mm (range: 20–96 mm). Considering the estimated growth rate of 2 mm per day for juvenile ASEs in this population (Long and LaFleur 2011), these juveniles had approximately 14 days to disperse from a breeding territory, providing further support that juveniles included were likely sampled from the immediate vicinity of where they were brooded. Second, even if some of the juveniles in our sample did originate from the ponds instead of the marshes, our estimates of breeding adults within the marshes would still provide a conservative estimate of the number of breeding adults within the marshes because some juveniles were documented in marshes farther away from the marshes included in this study. For example, Johnson (2017) captured a juvenile ASE 1584 m away (38 mm TL) from the outflow pipe and captured of juvenile ASEs on the opposite side of the river (38 mm TL to 43 mm TL). If juveniles from these locations had been

included, one would expect, at the very least, a slight increase in N_b estimates. All considered, the reported estimates likely represent a conservative baseline for the breeding population in the marshes.

Objective 2 – Management and Control

Depletion Sampling

A multi-agency team from U.S. Geological Survey, Oklahoma State University, the National Park Service, the U.S. Fish and Wildlife Service, Georgia Power Company, and Georgia Department of Natural Resources conducted depletion sampling passes in July 2017. On July 18, 2017, one complete circuit of shoreline sampling was conducted in the Middle, North, and South marshes, with no adult ASE captured (Table 2). One crew reported missing a single adult ASE in the South Marsh. At the time of sampling, the marshes were shallow and riddled with dense emergent and submergent vegetation (Figure 3) that likely hindered catchability of adult ASE. Because an assessment of depletion sampling schemes necessitated capture and removal of ASE, we discontinued sampling of the marshes in favor of Beaver and Kingfisher ponds, which have consistently had the highest catch rates of adult ASE in the study area (Freeman and Burgess 2000; Freeman et al. 2005; Johnson 2017).



Figure 3. Thick emergent and submergent vegetation in the Middle Marsh of Chattahoochee River in July, 2017.

Table 2. Sample data for adult Asian Swamp Eels during depletion sampling of two Chattahoochee Nature Center ponds and adjacent Chattahoochee River marsh habitats. Catch-per-unit-effort (CPUE) is measured in fish/minute.

Waterbody	Date	Pass	Catch	Missed	Effort (min)	CPUE
North Marsh	7/18/2017	1	0	0	– ^a	0.00
Middle Marsh	7/18/2017	1	0	0	– ^a	0.00
South Marsh	7/18/2017	1	0	1	81	0.00
Beaver Pond	7/18/2017	1	8	2	49	0.16
Beaver Pond	7/18/2017	2	7	2	67	0.10
Kingfisher Pond	7/18/2017	1	15	7	150	0.10
Kingfisher Pond	7/18/2017	2	8	4	63	0.13
Beaver Pond	7/19/2017	1	15	7	107	0.14
Beaver Pond	7/19/2017	2	11	3	103	0.11
Beaver Pond	7/19/2017	3	6	1	56	0.11
Beaver Pond	7/19/2017	4	5	1	78	0.06
Kingfisher Pond	7/19/2017	1	9	6	97	0.09
Kingfisher Pond	7/19/2017	2	9	6	74	0.12
Kingfisher Pond	7/19/2017	3	7	4	66	0.11
Kingfisher Pond	7/19/2017	4	2	4	75	0.03

^a The backpack electrofishing units deployed in the North and Middle marshes did not record electrofishing time, but it would have been approximately equivalent to the effort spent in the South Marsh.

On July 18 and 19, 2017, we assessed the depletion scheme involving the completion of multiple sampling circuits within a single day. When multiple sampling teams (i.e., canoes) combined to complete one full sampling circuit of a pond, the team's collective efforts were totaled per sampling pass. Two sampling passes were completed on July 18, 2017 in Beaver and Kingfisher ponds, yielding 15 and 23 adult ASE, respectively. However, CNC camp activities delayed morning depletion passes in both ponds, making additional depletion passes difficult to complete that day. On July 19, 2017, four depletion passes were completed in each pond, and captures of adult ASE totaled 37 in Beaver Pond and 27 in Kingfisher Pond (Table 2). In both ponds, CPUE was relatively consistent at approximately 0.10 fish/min until the fourth pass, during which CPUE was 0.06 fish/min in Beaver Pond and 0.03 fish/min in Kingfisher Pond. DeLury models estimated $N_0 = 59$ (95% CI's: 0 – 128) with $q = 0.002$ (95% CI's: 0.000 – 0.007) in Beaver Pond and $N_0 = 30$ (95% CI's: 0 – 90) with $q = 0.004$ (95% CI's: 0.000 – 0.020) in Kingfisher Pond (Figure 4). Given that previous research has estimated incredibly low individual-level capture probabilities (0.0001%) of ASE (Freeman et al. 2005) and relatively low average species-level detection estimates (0.19 – 0.45; Johnson 2017), the resulting N_0 and q estimates better reflect individuals vulnerable to capture on a

given day and not actual population size. Repeated sampling with electrofishing methods can result in behavioral avoidance and physiological changes that alter catchability (Mesa and Schrek 1989), leading to decreased sampling efficiency over time that downwardly bias population size estimates (Rosenberger and Dunham 2005). The daily depletions (see next paragraph) further supported the notion that the depletion trends obtained were likely caused by a behavioral avoidance response to multiple sampling passes within a day, rather than actual population depletion (also supported by Freeman et al. 2005).

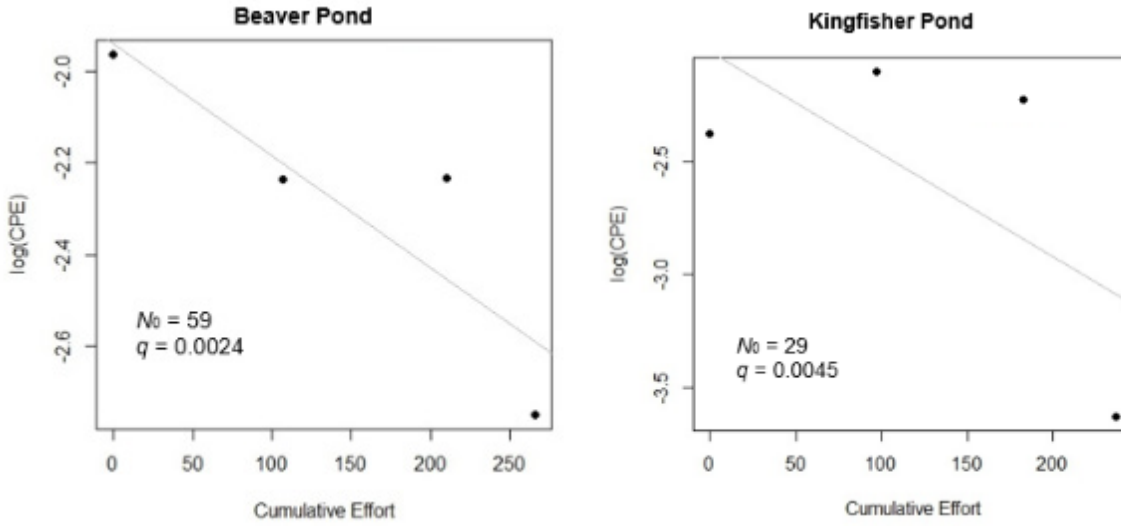


Figure 4. DeLury depletion models for July 19, 2017, sampling of CNC ponds, including estimates of initial population size (N_0) and a catchability coefficient (q).

From April 30th to May 3rd, 2018, we examined the daily depletion sampling scheme in Beaver and Kingfisher ponds. With an experienced two-person team, we completed one full circuit of the shoreline of Beaver and Kingfisher ponds each day while attempting to maintain approximately equal effort across days. We obtained rather consistent catches of ASE from each pond over the four consecutive sampling days. In Beaver Pond, a complete sampling circuit consisted of an average of 85 min of sampling effort, and ASE catch averaged 22 (range: 15 – 26; Table 3). In Kingfisher Pond, a depletion circuit averaged 54 min of sampling effort, and ASE catch averaged 13 (range: 11 – 16). No clear pattern of depletion was obtained from either pond over the four days of sampling. In fact, DeLury estimates of N_0 and q were invalid because positive slopes between CPUE and cumulative effort resulted in negative estimates (Figure 5). Over the four-day sampling period, a total of 89 ASEs were removed from Beaver Pond and 53 from Kingfisher pond.

Table 3. Sample data for adult Asian Swamp Eels during daily depletion electrofishing circuits of two Chattahoochee Nature Center ponds. Catch-per-unit-effort (CPUE) is measured in fish/minute.

Waterbody	Date	Pass	Catch	Missed	Effort (min)	CPUE
Beaver Pond	4/30/2018	1	26	9	115	0.23
Beaver Pond	5/1/2018	2	21	7	81	0.26
Beaver Pond	5/2/2018	3	27	7	66	0.41
Beaver Pond	5/3/2018	4	15	4	79	0.19
Kingfisher Pond	4/30/2018	1	11	3	45	0.24
Kingfisher Pond	5/1/2018	2	14	5	53	0.26
Kingfisher Pond	5/2/2018	3	12	5	57	0.21
Kingfisher Pond	5/3/2018	4	16	4	59	0.27

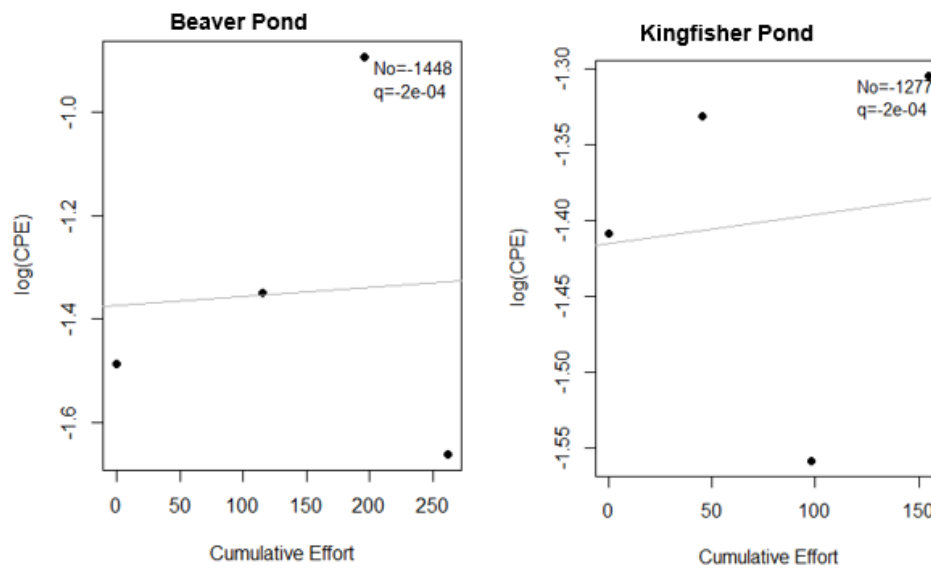


Figure 5. DeLury depletion models for sampling of Chattahoochee Nature Center ponds each day from April 30th to May 3rd, 2018. Estimates include initial population size (N_0) and a catchability coefficient (q).

Stationary Trapping

Eel pots were fished within Beaver and Kingfisher ponds at the CNC, as well as in the marsh habitats immediately adjacent to the CNC from May 27, 2016 to June 06, 2016 for a total of 175 pot nights, but no ASEs were captured (Johnson, 2017).

Between September 13 and 14, 2018, a total of 13 separate locations were fished with the stationary PVC traps, including locations in the North Marsh tributary, the North Marsh, Middle Marsh, and Kingfisher Pond in the CNC (Table 4; Figure 6). Total elapsed trapping time was 191 hours, during

which no ASEs were caught or detected. Traps had very little bycatch, except for one crayfish caught in a large-sized PVC trap deployed in the Middle Marsh (trap #5; Table 4).

Table 4. Summary of stationary PVC trapping data for Asian Swamp Eels conducted on September 13 and 14, 2018.

Trap #	Type	Latitude	Longitude	Elapsed time (hr:min)	Bait type(s)	Eel catch
1	Small	34.006581	-84.374110	22:54	shad	0
2	Large	34.005685	-84.373736	22:47	shad + worms	0
3	Large	34.005497	-84.373725	22:42	shiner + worms	0
4	Small	34.003117	-84.377283	22:24	shad	0
5	Large	34.002933	-84.377469	22:30	shad + worms	0
6	Large	34.003117	-84.377317	22:03	shiner + worms	0
7	Large	34.004967	-84.375767	16:22	shiner + worms	0
8	Large	34.004483	-84.376150	22:04	shad + worms	0
9	Large	34.004333	-84.373633	4:23	worms	0
10	Large	34.002786	-84.380631	3:24	fish + worms	0
11	Large	34.002779	-84.380658	3:24	fish + worms	0
12	Large	34.002835	-84.381421	3:03	fish + worms	0
13	Large	34.002870	-84.381483	3:00	fish + worms	0



Figure 6. Large (left) and small (right) baited PVC traps deployed in the Middle Marsh.

Ecological Data

In 2017, a total of 108 ASEs were captured, averaging 327 mm TL (SD: 110 mm; range: 118 – 537 mm). Weight of preserved eels averaged 37.2 g (SD: 34.6 g; range: 1.0 – 131.8 g). A total of 81 (75%) individuals were assigned to sex based on gross internal anatomy; of those, only 4 (5%) were male. Sex composition appeared similar in each waterbody (female:male:unknown): 3:0:3 in the North marsh tributary, 37:2:13 in Beaver Pond, and 37:2:11 in Kingfisher pond. These results were more skewed towards females than previously documented. Freeman et al. 2005 documented a female:male:intersex ratio of 28:6:7; however, direct comparisons are difficult between the studies because some of the unknown individuals in our dataset likely represented intersex or male eels. Total length differed slightly with sex, wherein females averaged 308 mm TL and males averaged 439 mm TL. Smaller individuals were sometimes classified as unknown (i.e., possibly reproductively immature), but an average of 362 mm TL for eels of unknown sex demonstrates that sex was not discernible in some adult fish (i.e., a lack of visible gonads). Regarding the diet of the ASEs sampled in 2017, 22% of eels had empty stomachs, 28% of eels ate fish, and 67% had detritus or plant material in their stomach. The most common diet items recovered were Amphipoda (37%), followed by Mollusca (14%) and fish (13%; Figure 7).

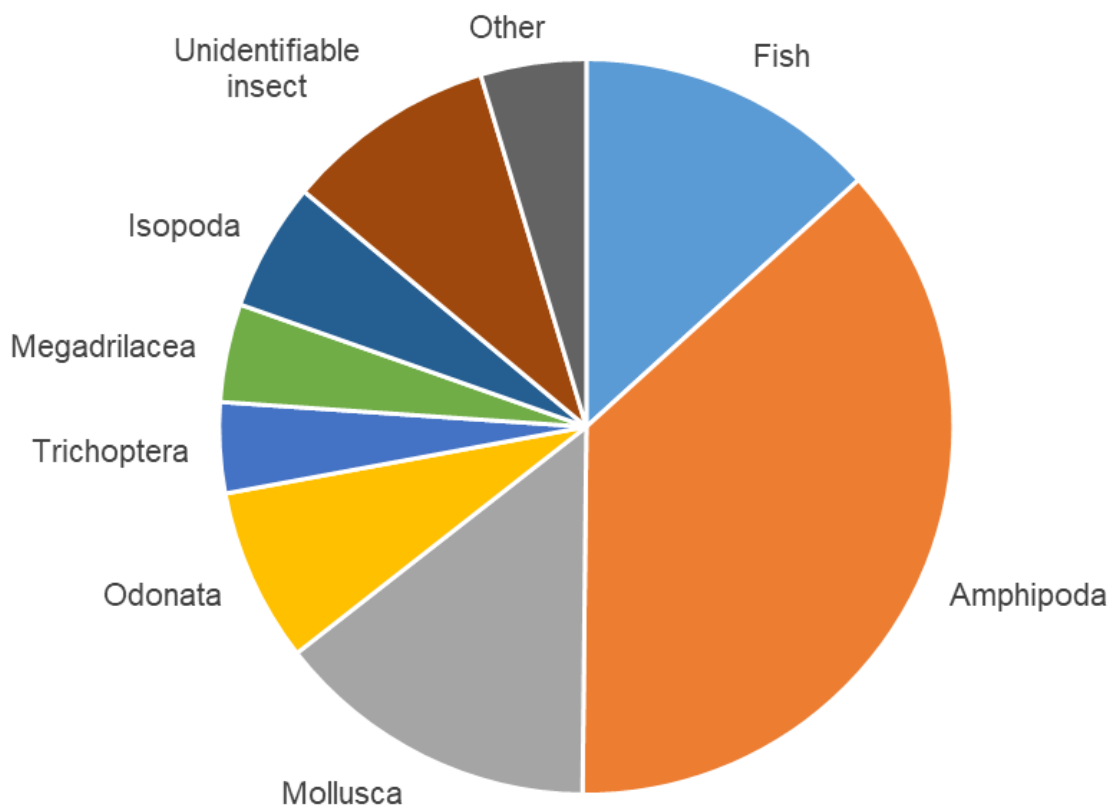


Figure 7. Overall percent diet items recovered from 107 Asian Swamp Eels sampled from the North Marsh tributary, Beaver Pond, and Kingfisher Pond in 2017.

In 2018, a total of 142 ASE were collected from Beaver and Kingfisher ponds during depletion sampling. In general, ASE captured during May 2018 were larger than those captured in July 2017. For example, 2018 ASE had an average TL of 406 mm (SD: 104 mm; range: 165 – 649 mm) and an average preserved weight of 66.7 g (SD: 50.0 g; range: 2.9 – 263.1 g). To increase our sample coverage of diet and sex of larger eels, we examined $n = 52$ specimens from 2018 collections that had a TL ≥ 450 mm. Sex composition appeared slightly less skewed towards females with a ratio of 25:7:20 (female:male:unknown), suggesting that adult ASEs may have been more vulnerable to capture in late April and early May of 2018 than in July of 2017. Because males occupy breeding burrows during the spawning season (Matsumoto et al. 2011), our data suggest that the breeding season may begin sometime around May within the CNC ponds. However, the timing of spawning activity is likely variable, because Freeman et al. (2005) documented the first juvenile ASE captures in March and April of 2005, suggesting that first spawning activity occurred much earlier than May in that year. Empty stomachs were only found from 6% of individuals, 42% had consumed fish, and 67% had detritus or plant material in their diet. Overall percentage of gut content items was dominated by Odonata (47%), followed by Mollusca (17%) and fish (13%; Figure 8).

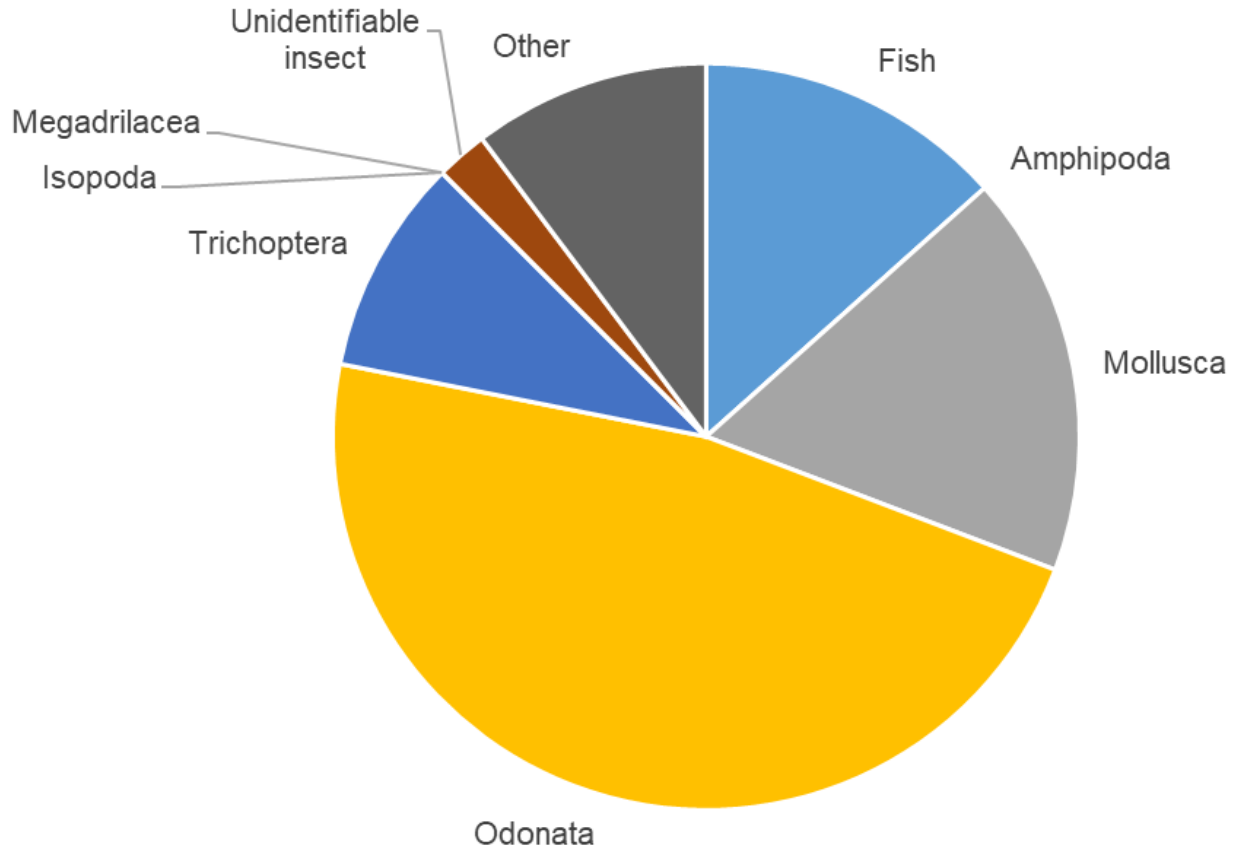


Figure 8. Overall percent diet items recovered from 53 Asian Swamp Eels sampled from Beaver Pond and Kingfisher Pond in 2018.

We estimated age from a total of 244 ASE ($n = 108$ from 2017; $n = 136$ from 2018), which ranged from 1 to 19 yrs (Figure 9). The longevity observed is in stark contrast to the maximum estimated age of 7 to 9 yrs from previous studies (Freeman and Burgess 2000; Freeman et al. 2005); however, ASE in captivity have lived more than 15 years (Matsumoto et al. 2011), lending credence to our estimates. The majority of eels examined were estimated to be between 2 and 8 years of age; only 31 individuals were aged ≥ 10 years (Figure 10). Males ($n = 11$) were estimated between 6 and 19 years old, whereas female ages ($n = 101$) ranged from 1 to 16 years. The majority of females ($n = 66$), however, ranged from 2 to 6 years of age. We estimated age for 6 adult eels captured in the North Marsh tributary, of wherein one individual was aged at 7 years and all others were aged at either 2 or 3 years, lending support to a breeding adult population within the marshes and interconnected habitats.

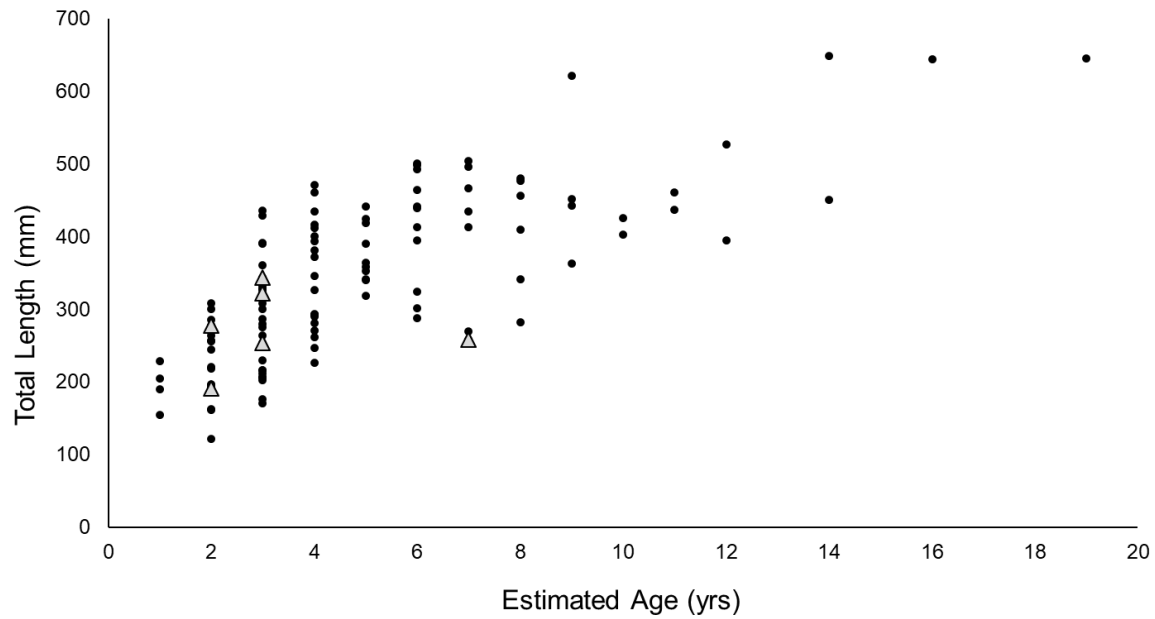


Figure 9. Length-at-age of Asian Swamp Eels collected from Beaver and Kingfisher ponds (black circles) and from the North Marsh tributary (grey triangles) in 2017 and 2018.

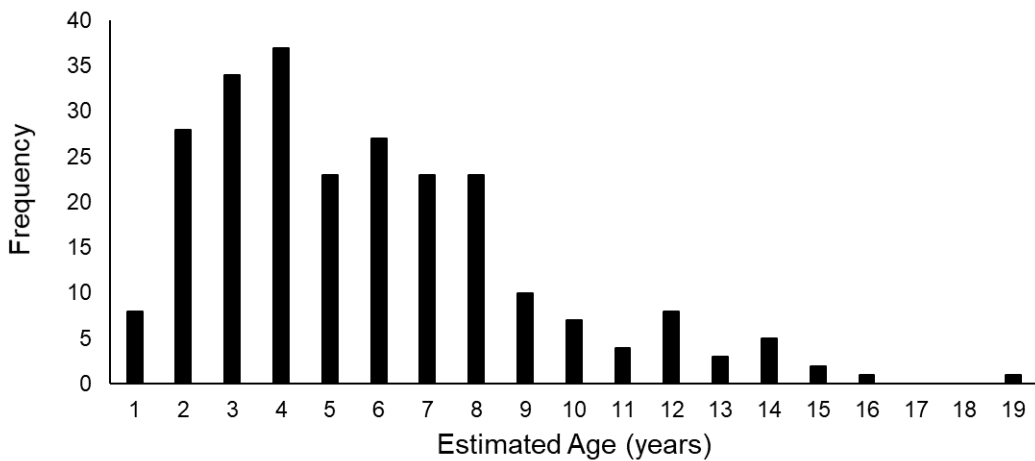


Figure 10. Age-frequency histogram of Asian Swamp Eels collected from Beaver Pond, Kingfisher Pond, and the North Marsh tributary in 2017 and 2018.

We assigned individuals to year class by considering estimated age and year of capture (Figure 11). In a population with consistent recruitment, these plots would feature a unimodal curve with a descending limb among older cohorts (representative of mortality) and an ascending limb representative of younger cohorts that are not fully recruited to the sampling gear. In Beaver Pond, there was a disruption in the expected unimodal curve that was most noticeable in 2012. Interestingly, Beaver Pond was drawn down by approximately 4 ft. during May 2012 to complete repairs to its overflow pipe (H. von Schmeling, Chattahoochee Nature Center, personal communication), which possibly dewatered breeding chambers and interrupted spawning and rearing. However, we cannot exclude alternative explanations. For example, perhaps recruitment was much higher in Beaver Pond in 2010 and 2011 than in years immediately before and after. Severe droughts occurred in the Upper Chattahoochee River Basin in 2008, 2009, and 2012, suggesting that drought conditions may also suppress recruitment relative to wet years.

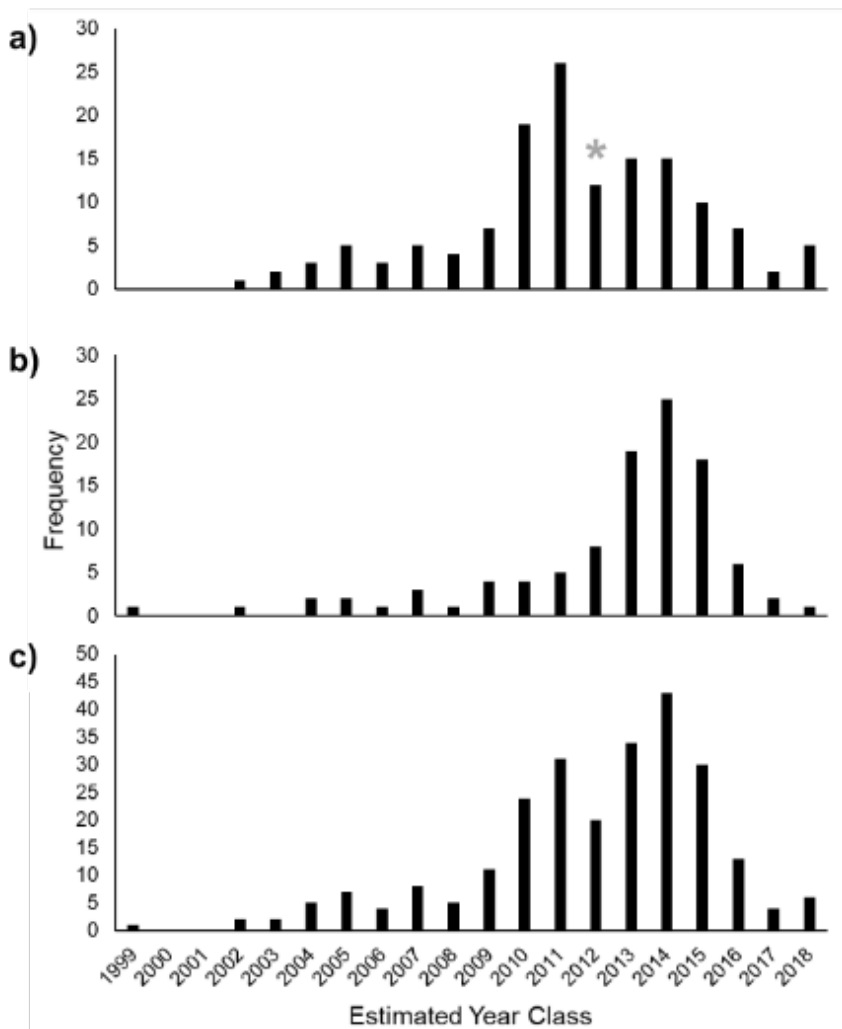


Figure 11. Catch from Beaver Pond (a); Kingfisher Pond (b); and combined catch from Beaver Pond, Kingfisher Pond, and the North Marsh Tributary (c) assigned to estimated year class. Asterisk in indicates potential effects of a lake drawdown in Beaver Pond in May 2012.

Discussion

Overall, results of this study provide strong evidence that ASE are not only reproducing within the marshes of the Chattahoochee River, but that their invasion extent has expanded. Juvenile eels were detected in a marsh across the mainstem Chattahoochee River (previously hypothesized to represent a thermal barrier to movement and reproduction; Freeman et al. 2005), as well as up to 1.5 km downstream of the CNC pond outflow. Estimated occupancy probabilities were high in some areas that had been sampled and where juvenile ASE had not yet been detected. An electrofishing survey in one such area lacking detection of juvenile ASE (unnamed tributary stream to the North Marsh) produced adult ASE. Next-generation genotyping of juvenile eels revealed that, at a very minimum, approximately 100 reproducing adults occupied the marshes adjacent to the CNC in 2015. Furthermore, genetic results supported polygamy among both sexes and adult movements of at least 0.5 km between marshes.

We evaluated eDNA detection, two stationary trapping methods, and active depletion with canoe-based backpack electrofishing. Unfortunately, eDNA trials proved ineffective at reliably detecting ASE in our system. Sampling trials with baited eel pots and baited PVC traps failed to capture ASE. Although PVC traps appear effective within the ASE's native range, differences in bait type, environmental conditions (e.g., water level, temperature, or clarity), and density of adult ASE in the area could all have influenced our results. Canoe-based depletion trials in the CNC ponds suggested that repeated sampling passes within a day invoked an avoidance behavior (i.e., trap-shyness), limiting the effectiveness of multiple sampling passes within a day. However, when depletion passes were conducted once per day for four consecutive days, no noticeable depletion occurred, suggesting high adult abundance in both Beaver and Kingfisher ponds. Efforts to detect or remove adult ASE from the marshes with canoe-based electrofishing produced low capture rates (approximately 0.02 eels/min; Johnson 2017). In contrast to previous studies, our results suggest that low capture rates of adult ASEs in the marshes is likely attributed to low sampling efficiency, not low abundance. For example, when considering only five adults were captured by Johnson (2017) in the marshes out of an approximate minimum of 100 reproducing adults in the area, sampling efficiency is at best 5%, but perhaps much lower. Sampling efficiency is likely hindered by both habitat (shallow, heavily-vegetated marshes) and ASE behavior (burrowed ASE may be unavailable for capture for prolonged periods; Freeman et al. 2005).

Ecological data from adult ASE captured in Beaver and Kingfisher ponds revealed that longevity was markedly higher (19 yrs) than found in previous studies of the same populations (7 yrs; Freeman et al. 2005). Despite the prevalence of older individuals, sex ratio was more skewed towards females than observed previously. This was an unexpected result that contradicted common observations; for example, in China female ASE were 1 to 5 yrs old (180 to 580 mm TL) and male ASE were 3 to 9 yrs old (380 to 860 mm TL; Yang and Xiong 2010). A possible mechanism for our results is a high density of adult ASE in the CNC ponds. High densities could limit space available for males to establish breeding territories, making sex reversal from female to male less advantageous (and therefore, delayed) because of increased competition among males for breeding territories (see Matsumoto et al. 2011). ASE diets were similar to those reported by Freeman and Burgess (2000)

and Freeman et al. (2005), dominated by Odonata, Trichoptera, and Amphipoda. Elevated percentages of mollusks and fishes were observed compared to previous studies, which could be explained by the increased representation of older and larger ASE within our sample.

Management and Control Strategies

Management or control efforts directed at the ASE population in the CNC ponds and Chattahoochee River marshes would likely be most impactful within an integrated pest management approach. Such approaches rely on the best available information on life history, invasion status, and effectiveness of control methods to manage an invasive species in an economical fashion, while also considering possible hazards associated with management actions. An adaptive management framework that monitors the relative effectiveness of control actions towards meeting management goals would help refine such a strategy over time. We integrate our findings with available literature to review potential management and control strategies for ASE within the study system, and conclude with a few options that represent a reasonable balance of ecological risks versus costs of management actions.

Control Strategies

Control of invasive species generally involves efforts to suppress abundance and to contain the spatial extent of the invasion. Suppression strategies for invasive fishes generally fall into three categories: chemical, mechanical, and biological (Wydoski and Wiley 1999).

Suppression

Chemical

Chemical suppression is one of the most popular options for control of unwanted fishes because of the ease of application, ability to achieve lasting results, and lower costs compared to mechanical and biological suppression options (Wydoski and Wiley 1999). However, chemical suppression is seldom specific to the target species and oftentimes poses hazards to non-target organisms (Wydoski and Wiley 1999; Bettoli and Maceina 1996). Two of the most popular chemical piscicides, rotenone and Antimycin-A, have been tested on ASE in laboratory settings. Trials with rotenone reported 100% mortality at 8 mg/L, but this is a much higher concentration than is typically used for freshwater pond treatments (3–5 mg/L; Schofield and Nico 2007). Antimycin-A concentrations of 2, 5, 10, and 100 nL/L were tested on juvenile ASEs, but no concentration was effective at killing eels despite onset of death of Golden Shiners (*Notemigonus crysoleucas*) at 10 nL/L (Reinert et al. 2006). Furthermore, ASEs may be able to avoid chemical-induced mortality via escaping onto a bank or occupying burrows in the substrate (Reinert et al. 2006; Schofield and Nico 2007). Chemical methods alone are not promising for ASE suppression, because the concentrations necessary to elicit mortality in ASE would cause widespread mortality of non-target fishes, but probably not 100% mortality of the target species.

Mechanical

Mechanical suppression techniques are generally more selective than chemical suppression, but also more costly in the amount of effort and time required to sustain effectiveness (Wydoski and Wiley 1999). Numerous passive sampling strategies such as barrel traps, baited PVC traps, and baited eel pots have failed to reliably capture ASE from the marshes (Freeman et al. 2005; Johnson 2017). Leaf litter traps provide a potential means of removing juvenile ASE and dampening recruitment, but intensive effort would be required during peak conditions to obtain population-level effects (Freeman

et al. 2005; Johnson 2017). Active canoe-based backpack electrofishing has proven successful at capturing adult ASE (Freeman et al. 2005; Johnson 2017), but with very low catchability (0.002–0.004 probability of catching any single fish/minute of electrofishing). Based on our results and previous studies, canoe-based backpack electrofishing has an even lower sampling efficiency in the marshes compared to the ponds, but adjusting effort and periodicity prior-to and during the spawning season might allow for such efforts to suppress adult ASE abundance. However, multiple electrofishing passes within a day appeared to elicit trap-shyness (lowering q), a behavioral response (plausibly burrowing) that reduces exposure to capture, thus limiting cumulative catches within a day. Alternatively, conducting single passes for each day over a four-day period failed to appreciably reduce the number of eels captured in the CNC ponds, suggesting that many days of sampling would be needed to achieve any discernible depletion of the population.

Other mechanical means of suppression include manipulations to ASE habitat: water depth, shoreline habitats, or even water quality parameters (e.g., pH, salinity, or temperature). Freeman et al. (2005) suggested that increasing the slope and depth along the CNC pond shorelines could limit burrowing habitats. Marshes with sandy substrates had lower occupancy probabilities for juvenile ASEs than did silty substrates (Johnson 2017), suggesting that sandy substrates may also limit burrowing and reproduction. Freeman et al. (2005) also suggested increasing salinities near shoreline breeding habitats, but Schofield and Nico (2009) demonstrated a broad range of salinity tolerance in ASEs. One could also envision disrupting recruitment by targeting changes to the flow and temperature regimes within the Chattahoochee River and its adjoining marshes via manipulation of the release schedule at Buford Dam (upstream) and Morgan Falls Dam (downstream); however, little data presently exist to inform such endeavors and the ASE population in the marshes has already demonstrated its ability to adapt to the dynamic water regime at present. Year class estimates from Beaver Pond suggest that lowering of the water level in Beaver Pond during May 2012 may have depressed recruitment, but further research is needed to directly quantify such effects. Despite the promise that these mechanical suppression techniques hold, ASE are highly adaptable to a variety of habitat conditions and have evolved reproductive strategies to maintain their reproductive capacity following extreme events (e.g., extreme droughts within their native range; Liem 1963, 1967). As such, habitat manipulations may prove cost-prohibitive in the long-term because sustained efforts would be necessary to offset any compensatory responses by the remaining portion of the population.

Biological

Traditional biological suppression techniques in fisheries management include the introduction of predatory species or the stocking of sterile hybrids to limit the availability of viable mates (Wydoski and Wiley 1999). Certainly, wading birds act as a predator on ASE (Taylor et al. 2018), but to what degree is unknown. Also unknown is the degree of predation pressure exerted by piscivorous fish such as Largemouth Bass (*Micropterus salmoides*), although it has been noted to occur (Shafland et al. 2010). Juvenile ASEs have even been observed to be eaten by mosquitofish (*Gambusia* spp.; J. Johnson, Oklahoma State University, personal observation), but, again, the degree to which this predation affects ASE survivorship is unknown. Regardless, the likelihood of increasing predation pressure on ASE in marshes that experience daily water level fluctuations through some sort of predator-stocking program appears unlikely.

Targeted bio-control methods that integrate species-specific physiology and life history strategies (Lennox et al. 2015) may have promise for suppressing ASE invasion, but little research has been conducted in this area. Based on early-life history, larval ASE within bubble nests are highly susceptible to mortality because they rely on access to highly oxygenated water at or near the surface to survive (Liem 1981). The froth found in the bubble nests of ASE appears to be very important for egg hatching, with hatching rates from 64% to 97%, depending on temperature, for eggs in froth compared to hatching rates from 15% to 34% with no froth (Yin and Liu 2010). Moreover, eggs of ASE removed from bubble nests only hatched when directly exposed to aeration (Matsumoto and Iwata 1997). Therefore, a targeted spraying of surfactant or similar compound that would rupture bubble nests during the spawning season could induce low recruitment, but would have to be repeated approximately every five days from March through October to be effective. In our literature research, we have failed to identify any such compound that has been used for this purpose, but additional research would help determine this potential, which could benefit not just ASE control but potentially other invasive bubble-nest builders in other parts of the world (e.g., Three-spot Gourami *Trichopodus trichopterus* in India; Knight 2010).

Another potential biological suppression tactic would be to interrupt the natural sex reversal of ASE females to males, limiting the potential number of breeding males available in the population. A recent study discovered that sex reversal in ASE is related to differential gene expression in gonadal tissues (Chi et al. 2017), and aquaculture studies have shown that sex reversal in ASE can be induced with exposure to gonadotropin releasing hormone agonists (Tao et al. 1993) and inhibited with exposure to estradiol (Chu et al. 2011). Although promising in theory, all biological suppression possibilities would require further directed study to evaluate the efficacy of each method and effects to non-target organisms.

Containment

Containment of ASEs within the study area represents a logistically challenging control option. Perhaps the most realistic containment option was presented by Freeman et al. (2005): modifying the outflow of the CNC ponds to make them less appealing invasion vectors for ASEs. In fact, our current ecological data from the CNC ponds (e.g., high longevity, skewed female sex ratio) suggests that these populations may be overcrowded, creating a propagule source for invasion into the marshes via the outflow of the CNC ponds. ASEs within the marshes of the Chattahoochee River would be practically impossible to contain given the openness of the system and the dramatic fluctuations in river level. Whether or not the thermally-depressed mainstem of the Chattahoochee River will serve as a partial barrier to further invasion (both upstream and downstream) is unknown, but the detection of juvenile ASEs in a marsh directly across the mainstem from the CNC suggests that, at the very least, ASEs make occasional forays into the mainstem river.

Additionally, a separate population of ASE in a small creek further downstream in the Chattahoochee River basin was discovered in 2016 (Fuller et al. 2019). At least 9 specimens were collected, ranging in size from 102 mm to 275 mm TL, suggesting a viable population. Should the ASE population in the vicinity of the CNC be contained, this other population represents an additional source of propagules that could also invade the system.

Monitoring

To best inform future management or control strategies within an adaptive framework, monitoring efforts are warranted. Monitoring would ideally include updated estimates of the spatial extent of invasion as well as population abundance. Leaf litter trapping of juvenile ASEs is an effective tool for detecting the species from which occupancy probabilities can be used to estimate factors affecting their spatial distribution (Johnson 2017). Furthermore, leaf litter traps could be deployed strategically to update invasion extent upstream and downstream of the current study area. For detecting adult ASEs, canoe-based backpack electrofishing is currently the best available option in the backwater marshes of the Chattahoochee River and ponds of the CNC despite low capture efficiency. Nature photographers along the extensive board walk system that traverses the margins of the marshes may also contribute detections of adult ASE that can update the spatial extent of the invasion. For example, on December 19, 2017, Linda Marchetti photographed an adult ASE being depredated by a Great Blue Heron in the North Marsh – an area that had been sampled several times prior with canoe-based electrofishing without detecting an adult ASE (Taylor et al. 2018). In 2018, another record of an adult ASE in the Middle Marsh was submitted on iNaturalist (<https://www.inaturalist.org/observations/14387782>). A directed citizen-science effort to crowdsource photographs and locations of ASEs could help track the upstream and downstream invasion extent of ASEs in this system (Taylor et al. 2018). Estimating abundance of ASE has proved challenging because of sampling limitations. Previous mark-recapture efforts by Freeman et al. (2005) failed because of extremely low capture and recapture rates. However, through next-generation sequencing of juvenile ASEs, we estimated a minimum bound on the number of reproducing adults contributing to a cohort of juvenile eels, demonstrating a breeding population far larger than suggested by adult captures alone would indicate.

Balancing risks and costs

“Risk management of non-native fishes should ensure that actions taken are commensurate with the level of risk posed by that species in the environment.” – Britton et al. (2011)

From a management perspective, the best possible actions taken to control ASEs would represent a reasonable balance in the ecological risks posed by the species and the costs associated with those actions. Ecological risks associated with ASE invasion primarily surround the diet of the species. Sensational reports of the ASEs as voracious piscivores circulated early (e.g., Starnes et al. 1998), but several diet studies within our study area (Freeman and Burgess 2000; Freeman et al. 2005; and the present study) as well as in invaded regions of Florida (Hill and Watson 2007; Shafland et al. 2010) have since revealed that ASEs within their invaded range primarily feed on aquatic invertebrates, and rarely, small-bodied fishes. Additionally, a stable isotope study indicated that ASEs from the CNC ponds occupied a trophic level similar to insectivorous fishes (e.g., *Gambusia* sp.) and that native sunfishes remained top predators within the system (Straight et al. 2005). In total, these studies indicate that ASEs primarily alter the food web by consuming particular groups of aquatic invertebrates (e.g., Odonata, Trichoptera, and Mollusca) and thus reducing the availability of these food resources for other insectivorous fishes. Given this information, one may conclude that ASEs represent minimal ecological risk, at least within the study area, which already features an altered fish community as a result of hydroelectric dam operations upstream and downstream (Long

and Martin 2008). Therefore, the primary perceived risk would be the invasion of ASEs into upstream and downstream reaches of the Chattahoochee River and its tributaries, particularly in areas that harbor intact assemblages of native aquatic species. A precautionary approach, however, acknowledges scientific uncertainties surrounding the potential ecological impacts of ASEs as an invasive species. For example, the role of ASEs within the food web may change over time or ASEs may interact with other ecological factors to drive native species declines in unexpected ways (Leprieur et al. 2009).

Management actions to offset the perceived risks posed by ASEs must also account for the associated costs of the actions and the finite resources available for implementing control efforts. Given the minimal ecological risks associated with ASEs within the immediate study area, one reasonable option is a “do-nothing” strategy (Britton et al. 2011). A “do-nothing” strategy is best justified when costs of control exceed costs of invasive species impacts (Britton et al. 2010). A precautionary approach may include a program of sustained containment and suppression towards minimizing the risk of ASE spreading into new areas (Britton et al. 2010). Concerning containment, modifying the CNC pond outflow to inhibit ASE emigration represents a one-time fixed cost that would likely reduce number of propagules entering the marshes from the dense populations within the CNC ponds. Combining this containment strategy with sustained suppression efforts within the marshes (e.g., leaf litter trapping of juvenile ASEs in the summer months) could reduce opportunities for the spread of ASEs within the Chattahoochee River system.

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Appendix A: Monitoring for Asian swamp eels: an environmental DNA approach

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Overview

Traditionally, detection and surveillance of Asian swamp eel (*Monopterus albus*) has been conducted either by direct observation or collection of the species. The utility of environmental DNA (eDNA) raises the possibility to detect and monitor trace levels of aquatic invasive species. The goal of this project was to test for the presence/absence of the species from collected environmental samples.

Methods

DNA extraction

Filtered water samples were frozen and sent to Mansfield University via overnight courier. Filters were assigned a DNA extraction number (1–150), and DNA was extracted from each filter using the MoBio PowerWater DNA Isolation Kit following the manufacturer's instructions.

Detection of *M. albus* eDNA

Presence of swamp eel DNA in each water sample was determined using the quantitative (q) PCR Taqman assay protocol developed by Diaz-Ferguson and Moyer (2013). We targeted a 78 base pair fragment of the mitochondrially encoded 16S rRNA gene using primers FqSEGA (5'-AACTGTCTCCTCATTAAGT-3') and R16SSEq (5'-GGGTCTTCTCGTCTTATGGTGTTATC-3'). We confirmed the presence of swamp eel by visualizing this fragment on an Applied Biosystems 7300 Real-Time PCR System using a Taqman TAMRA labelled probe P16SSE (5'-Fam-AATAAAATTGATCTCCCCGTGCAGAAGCG-3').

Quantitative PCR reactions were optimized for 24uL reactions using the following final concentration of reagents: 4uL of DNA, 2.0uL of 5x Taq reaction buffer (Promega, Inc), 2.5uL MgCl₂ (25mM, Promega, Inc), 0.5uL dNTPs (10mM, Applied Biosystems, Inc), 1 uL of each primer (10uM each), 0.25uL probe (10uM), 0.4uL ROX size standard (50x Applied Biosystems, Inc), and 0.20uL Taq DNA polymerase (5U/uL, Promega, Inc). All qPCR Taqman assays were run using the following thermal profile: 60°C (1min), initial denaturation at 95°C for 10min., followed by 40 cycles of 95°C (15 s) and 60°C (1 min.).

A pGEM vector was used as an internal reference standard (Coyne et al. 2005) to monitor for PCR inhibition due to the potentially high humic content of the samples. PCR reactions were as above, except that instead of adding FqSEGA / R16SSEq primers and swamp eel specific Taqman probe to the PCR cocktail, pGEM DNA (1.44 X 10⁻⁶ ng/uL), primers (1.25uL, 10uM, Coyne et al. 2005), and MGB probe (0.5uL, 10uM, Coyne et al. 2005) were added.

Each assay (96 well plate) included the following: triplicate positive controls (1.44 X 10⁻⁶ ng/uL swamp eel DNA), triplicate negative controls (water instead of DNA), DNA sample, and internal pGEM (1.44 X 10⁻⁶ ng/uL) control (four random samples).

Results and Discussion

Based on a ten-fold serial dilution of a known concentration of DNA (20ng/uL), the lower limit of qPCR detection was 0.00002 ng/uL DNA with a cycle threshold value (Ct) of approximately 35.5 (Appendix A, Figures 1 and 2). Note that Ct is defined as the number of cycles required for the fluorescent signal to cross a threshold.

Levels are inversely proportional to the amount of target nucleic acid in the sample (i.e., the lower the Ct level the greater the amount of target nucleic acid in the sample). Values < 29 are typically considered strong positive reactions indicative of abundant target nucleic acid in the sample, values of 30–35.5 are positive reactions indicative of moderate amounts of target nucleic acid and values >35.5 could represent minimal amounts of target nucleic acid or environmental contamination, but are beyond the lower limit of detection for the qPCR assay.

All positive controls and internal standards amplified, indicating that the assay was working and inhibitors were not influencing the PCR reactions (Appendix A, Figure 3). A total of 150 samples were run on three 96-well plates (Appendix A, Figures 4, 5, and 6). No contamination was detected for each negative control and field blank. The assay failed to detect swamp eel DNA in all samples but one – sample 116 (site 153; date 8/4/2015; Appendix A, Figure 6). Note that numerous qPCR reactions showed amplification after the critical threshold value and is indicative of primers annealing with one another – termed primer dimers. Amplification artifacts such as primer–dimers can result in reduced yields of the desired PCR product; however, given that no strong qPCR signals of swamp eel DNA were detected and that the qPCR reactions were optimized to detect a ranged of swamp eel DNA, other extraneous factors may be limiting the ability to detect swamp eel DNA using this assay.

Limited detections have been observed in similar swamp eel studies (Moyer per sobs.), suggesting perhaps eDNA is being emitted in limited amounts by this organism. Key differences between Asian swamp eels and other aquatic organisms that emit eDNA, are lack of scales (sloughed scales are a potential form of eDNA) and increased mucus production (glycoproteins void of tissue and DNA). As such, controlled tank experiments using known quantities of eels may further benefit qPCR detection of swamp eel eDNA.

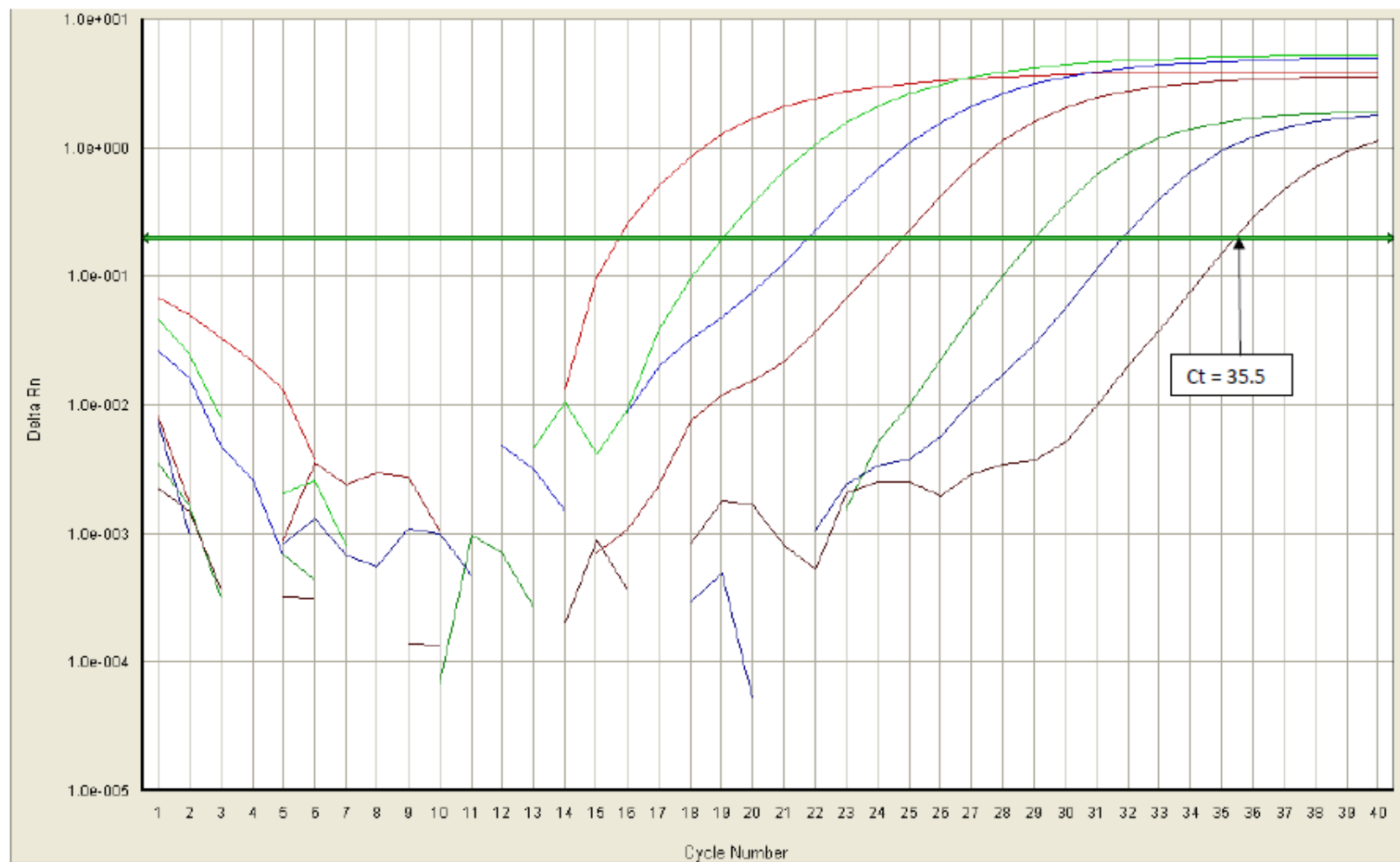


Figure A-1. Estimation of lower qPCR detection limits. Starting DNA concentration is 20 ng/uL of swamp eel DNA followed by ten-fold serial dilutions of this concentration.

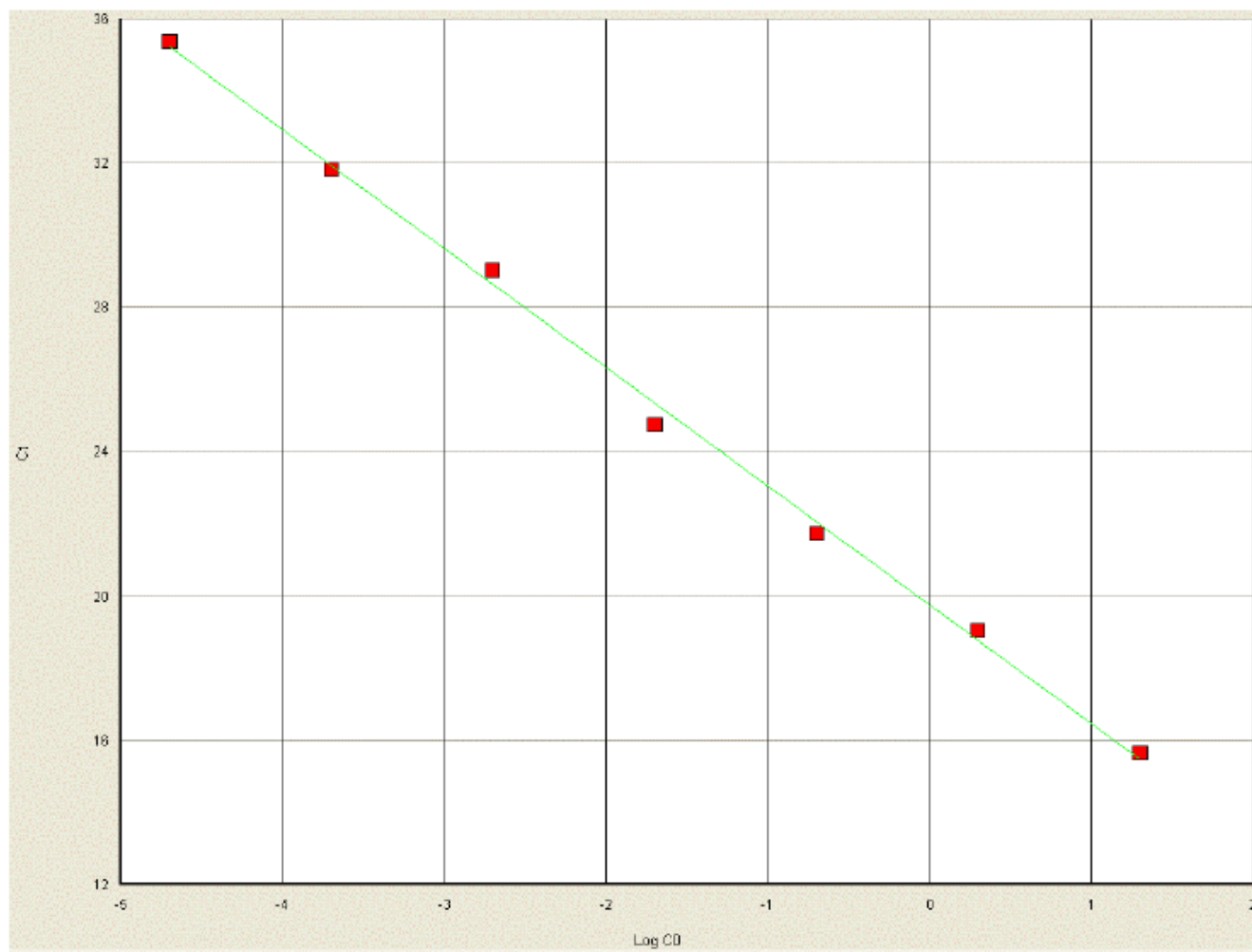


Figure A-2. Swamp eel standard curve using known concentrations of DNA extracted from tissue samples ($R^2=0.998$; lower limit $CT= 33$; minimum amount detected 0.00001ng/uL). Starting DNA concentration is 20 ng/uL followed by ten-fold serial dilutions of this concentration. The x-axis represents log DNA concentration.

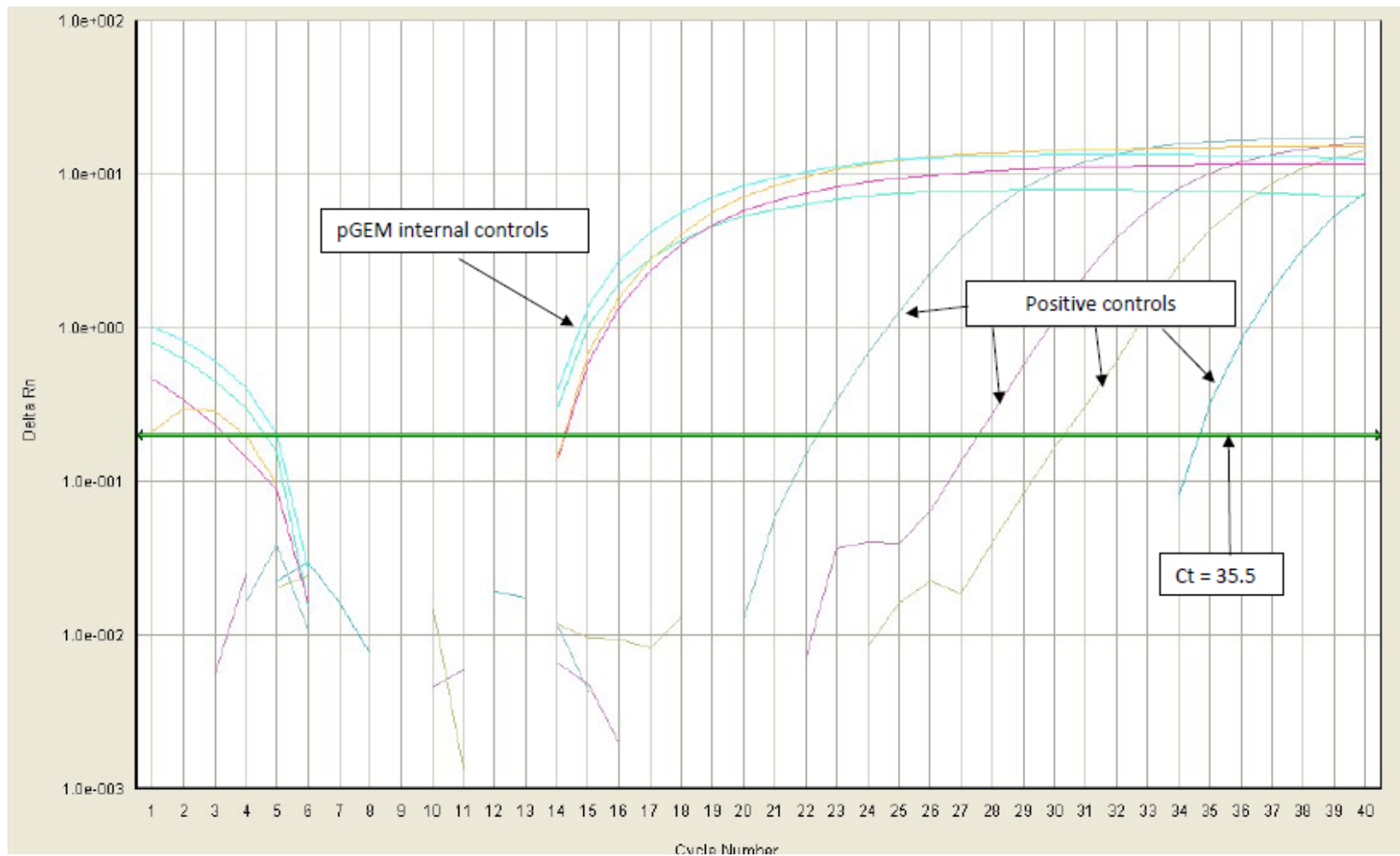


Figure A-3. Example of quantitative PCR reactions for pGEM internal controls and positive controls.

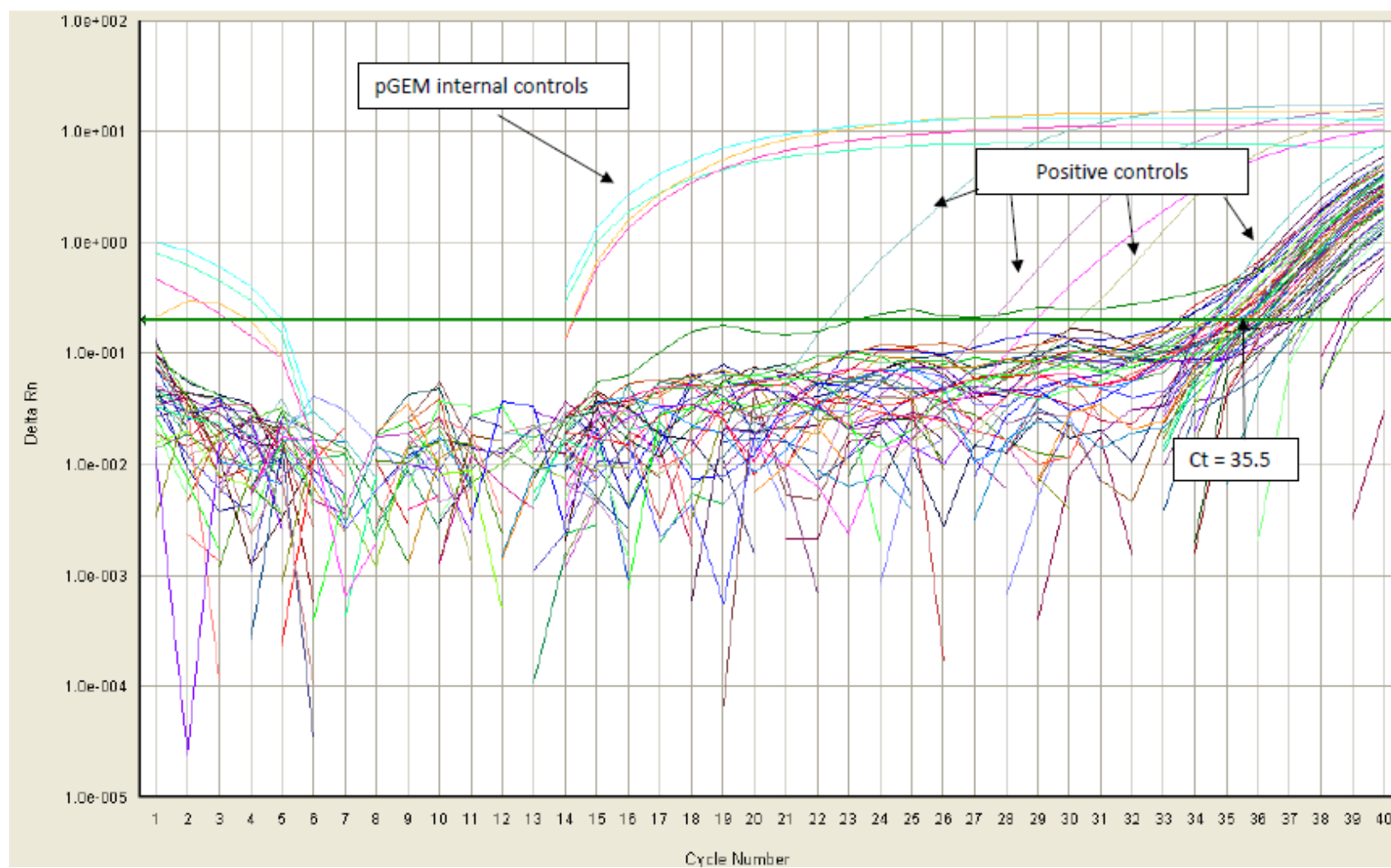


Figure A-4. Same reactions as above but including samples 41–100. Notice numerous samples amplified around Ct value indicating uncertainty in swamp eel detection because they appeared at the lower limit of detection for the assay.

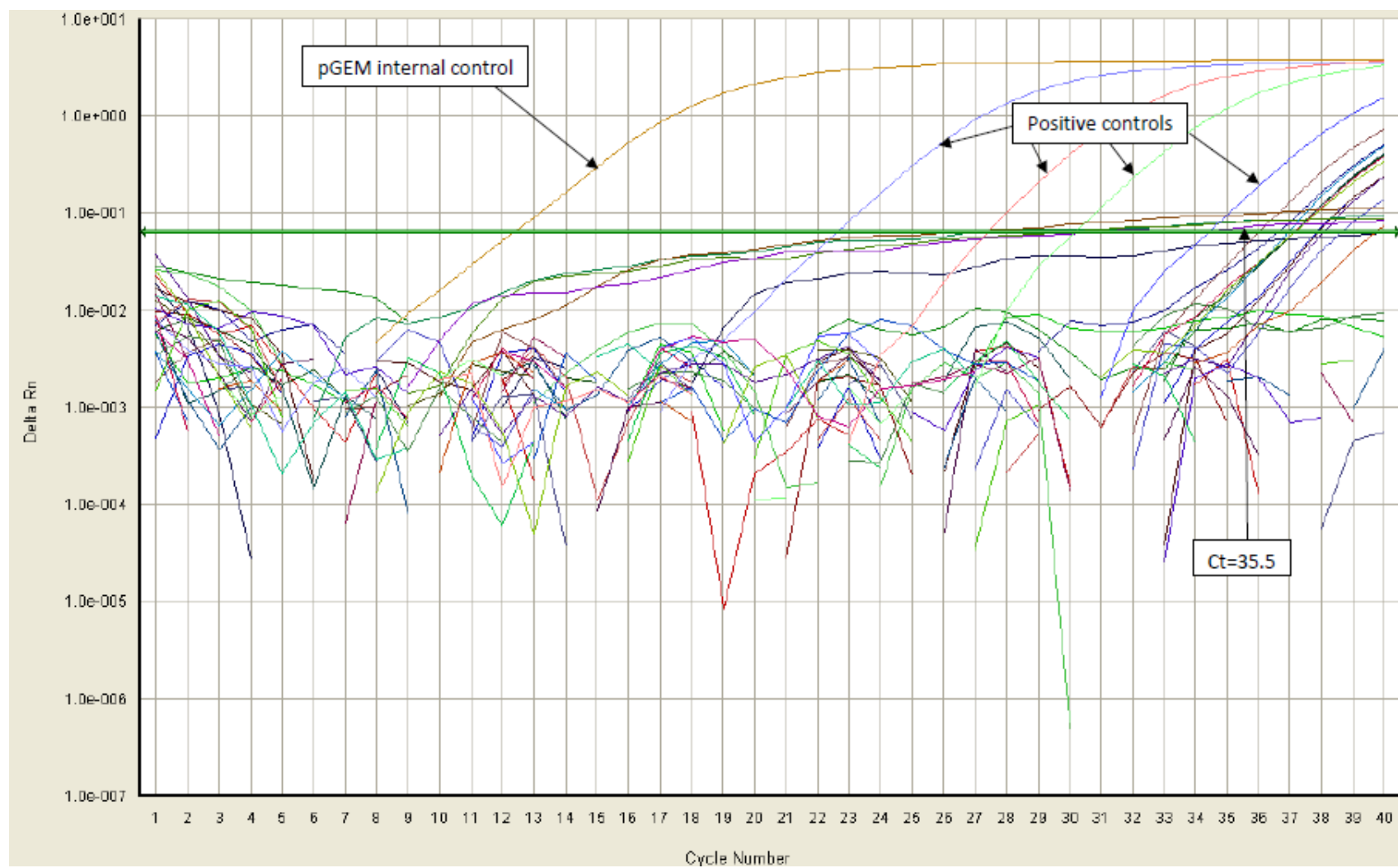


Figure A-5. Samples 1–40. Notice numerous samples amplified around Ct value indicating uncertainty in swamp eel detection because they appeared at the lower limit of detection for the assay.

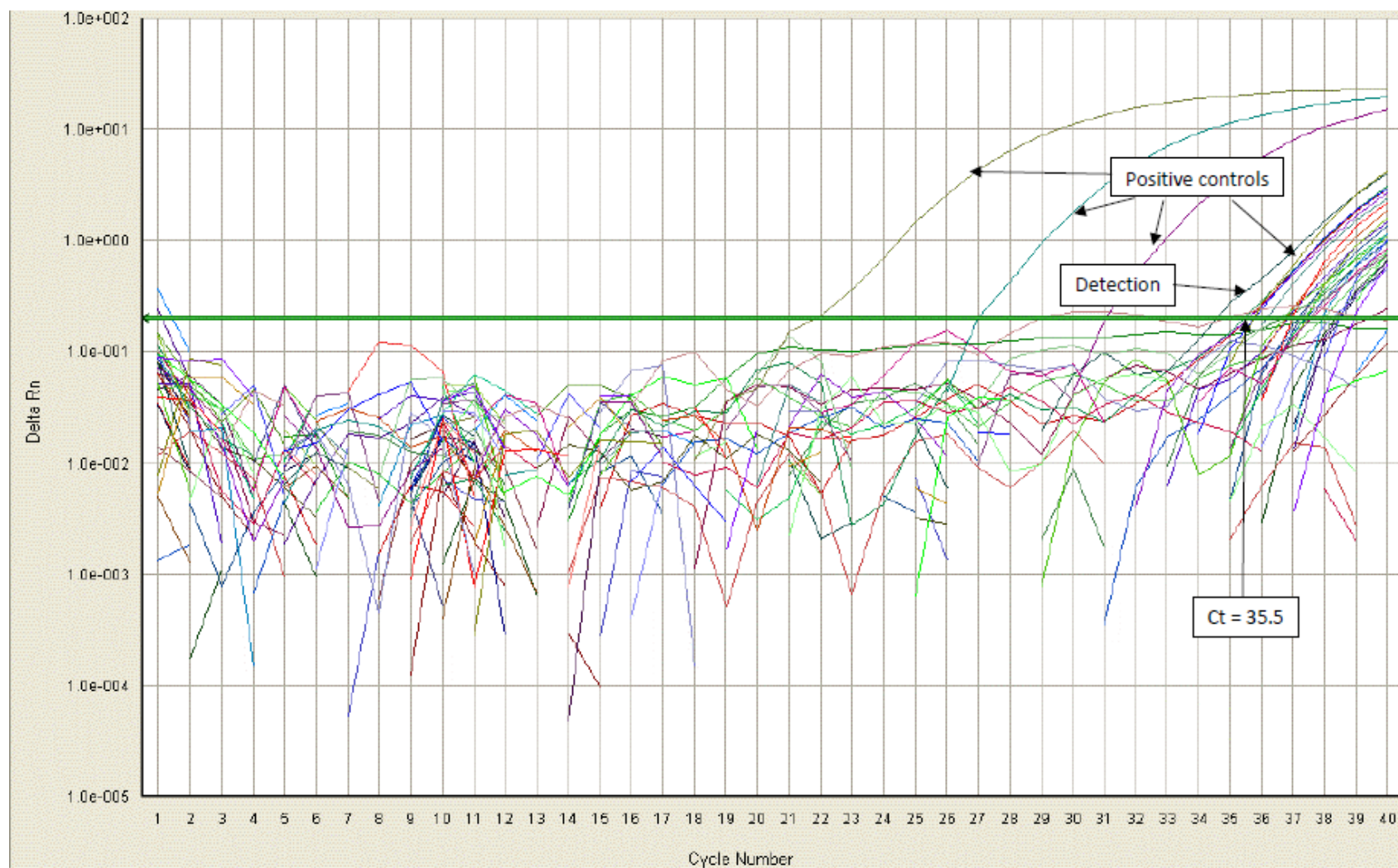


Figure A-6. Samples 101–150. Notice numerous samples amplified around Ct value indicating uncertainty in swamp eel detection because they appeared at the lower limit of detection for the assay. Potential detection was from sample 116.

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