

Rapid Communication**First record of occurrence and genetic characterization of a population of northern snakehead *Channa argus* (Cantor, 1842) in Georgia, USA**Hunter J. Roop¹, Nathan V. Whelan^{2,3}, Ashantye' S. Williams² and Jim Page^{1,*}¹Georgia Department of Natural Resources, Wildlife Resources Division, 2150 Dawsonville Hwy, Gainesville, GA 30501, USA²U.S. Fish and Wildlife Service, Warm Springs Fish Technology Center, 203 Swingle Hall, Auburn, AL 36849, USA³Auburn University, School of Fisheries, Aquaculture and Aquatic Sciences, 203 Swingle Hall, Auburn, AL 36849, USAAuthor e-mails: Hunter.Roop@dnr.ga.gov (HJR), Nathan_Whelan@fws.gov (NVW), Ashantye_Williams@fws.gov (ASW), Jim.Page@dnr.ga.gov (JP)

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OPEN ACCESS**Abstract**

On October 4th, 2019, an angler caught and released a single northern snakehead (*Channa argus*) in a private pond in Gwinnett County, Georgia, USA. Pictures of the specimen were reported to the Georgia Department of Natural Resources, Wildlife Resources Division, Fisheries Management Section (DNR), and subsequent investigations by the DNR including electrofishing and rotenone surveys resulted in the capture and removal of 34 individuals from the area. Genetic analyses of fin clips from 33 specimens indicated the population consisted of a combination of juveniles from a breeding pair of captured adults and other unsampled adults. This discovery constitutes the first record of this non-native species in Georgia, and the results demonstrate how genetic analyses can facilitate effective understanding of invasion dynamics during rapid response operations.

Key words: Channidae, introduction, invasive, non-native, vector**Introduction***Species background*

The northern snakehead *Channa argus* (Cantor, 1842) (Perciformes: Channidae) is a popular food and sportfish native to eastern Asia that has been infamized in the United States following discoveries of illegally introduced populations since 2002 (Courtenay and Williams 2004). The native range of the northern snakehead includes the Amur River basin (Asia): Songhua River, Manchuria; Tunguska River, Russia; Ussuri River basin; Lake Khanka; Korea (excluding the northeastern region); and several reservoirs and rivers in China (Courtenay and Williams 2004). The species has been introduced to numerous locations throughout the world, including central and southern Japan, Czechoslovakia, Kazakhstan, Uzbekistan, Turkmenistan, and the United States (Courtenay and Williams 2004). Historical accounts of isolated encounters (i.e., not reproducing populations) of northern snakehead in the United States have occurred

since 1997 (Silverwood Lake, California) and include the St. Johns River in Florida; Newton and Massapoag Ponds in Massachusetts; Wheaton, Maryland; Lake Wylie and the South Fork Catawba River, North Carolina; Lake Michigan (Illinois); a park pond in the District of Columbia; and waters adjacent to established populations in eastern Arkansas including Mississippi (Lake Whittington) and Missouri (St. Francis River levee system; SPDC 2014; Benson 2019). Established populations of northern snakehead in the United States have been discovered in various waterbodies in Maryland, Pennsylvania, Virginia, New York, the District of Columbia, Arkansas, New Jersey, and Delaware (Love and Newhard 2012; SPDC 2014; Benson 2019).

Georgia discovery

On October 4th, 2019, a northern snakehead was captured by an angler while fishing a privately owned pond in Gwinnett County, Georgia, USA. The pond is within the Yellow River watershed (Altamaha River basin), and is located 31 km NE of Atlanta, Georgia. The angler landed the specimen, photographed the fish, and returned it to the pond alive. The angler then contacted the Georgia Department of Natural Resources, Wildlife Resources Division (DNR) to report the catch and provide photos of the specimen to DNR staff. Examination of the photos by DNR staff led to positive identification of the specimen as a northern snakehead. This paper reports the discovery of an established northern snakehead population in Gwinnett County, Georgia, USA—the first record of northern snakehead in the state. Results of length-frequency histogram data and genetic parentage analyses provided insight regarding the timing of the invasion and possible recruitment dynamics. The potential ecological effects of this newly discovered population are currently unknown but could be significant should the population expand its range within and outside of the watershed.

Materials and methods

Field methods

On October 7th, in response to the angler's report and photos of the angled northern snakehead, DNR conducted boat electrofishing surveys in the pond, but recovered no northern snakehead. While conducting backpack electrofishing surveys in the stream channel flowing out of the pond, DNR staff captured and removed two juvenile (total length [TL] = 227 mm, 220 mm) fish identified as northern snakehead. DNR staff subsequently conducted a series of field surveys within the pond and waterbodies adjacent to the pond from October 9th–28th, 2019. These waterbodies included two ponds (0.61 ha), 5.5 km of first and second order tributaries connected to the pond, 36 ha of wetlands dispersed throughout the watershed

downstream of the pond, and a 3.7 km section of the Yellow River upstream of Lake Jackson. Survey methods included boat electrofishing in the pond and river, and backpack electrofishing in the pond, tributaries, and wetlands. Seine hauls were performed in the wetland immediately adjacent to the private pond, and experimental pyramidal crayfish traps were placed in the pond and its adjacent wetland to assess passive capture efficacy for northern snakehead. Crayfish traps were baited with sardines, cat food, and hotdogs to attract live fishes. All northern snakehead collected were removed from the pond. Total lengths (mm) and weight (g) were collected for each fish. A fin clip was removed from each fish, preserved in ACROS Organics™ 99.5% ethanol, and shipped to the United States Fish and Wildlife Service (USFWS) conservation genetics lab at Auburn University for genetic analysis (see genetics methods below). Physical specimens were transported to a DNR district office and frozen for later examination.

After collecting and removing sixteen individuals within and near the pond, DNR staff identified the pond, the wetland immediately southwest of the pond, and the channels connecting those waterbodies as the snakehead population's epicenter. In field surveys conducted prior to rotenone application, no threatened or endangered aquatic fish species (e.g., Altamaha shiner *Cyprinella xaenura*) were identified within the pond or local watershed. DNR determined that, given no occurrences of threatened or endangered species, combined with survey data indicating a potentially confined population within the sample area, rotenone (Prenfish™) could be used to remove northern snakehead in the apparent epicenter. Before rotenone application, fifteen largemouth bass > 305 mm were captured, removed, and held in an aerated stocking tank to be reintroduced to the pond as possible biological control after the rotenone was neutralized. A depth profile of the pond was obtained by recording depths on a roughly 6.1 m × 6.1 m grid throughout the pond. Average water depths in the wetland and channels connecting to the pond were estimated by random samples. Satellite imagery was used to estimate surface area of all treated waters. Using existing irrigation infrastructure at the site, DNR staff lowered the pond's elevation to reduce fish access to vegetative cover. The natural water supply to the pond was cut off by a temporary dam constructed at the pond's intake, and water levels in the wetland and interconnecting channels were also reduced as the pond was lowered. The total volume of water post-drawdown was estimated to be 0.31 ha-m. A total of 17 L rotenone was applied to the pond, wetlands, and their interconnecting channels. Potassium permanganate (KMNO₄; 22.7 kg) was used to neutralize the rotenone three days after application. Fourteen largemouth bass were returned to the pond after cage testing confirmed the rotenone was effectively neutralized. One thousand fingerling bluegill *Lepomis macrochirus* (Rafinesque, 1819) and 100 fingerling channel catfish *Ictalurus punctatus*

Table 1. Genetic diversity estimates for sampled population of northern snakehead (*Channa argus*) in Georgia.

| Age class | Locus | N | N _a | H _{obs} | H _{exp} | A _r |
|-----------|---------|----|----------------|------------------|------------------|----------------|
| Adults | CarC104 | 2 | 2.000 | 0.500 | 0.375 | 2.000 |
| | CarD1 | 2 | 1.000 | 0.000 | 0.000 | 1.000 |
| | CarD116 | 2 | 2.000 | 0.500 | 0.375 | 2.000 |
| | CarD126 | 2 | 1.000 | 0.000 | 0.000 | 1.000 |
| | CarD133 | 2 | 2.000 | 0.500 | 0.375 | 2.000 |
| | CarC113 | 2 | 2.000 | 1.000 | 0.500 | 2.000 |
| | CarC6 | 2 | 1.000 | 0.000 | 0.000 | 1.000 |
| | Average | | 1.571 | 0.357 | 0.232 | 1.570 |
| Juveniles | CarC104 | 30 | 3.000 | 0.800 | 0.551 | 2.145 |
| | CarD1 | 30 | 2.000 | 0.300 | 0.255 | 1.487 |
| | CarD116 | 30 | 4.000 | 0.767 | 0.550 | 2.170 |
| | CarD126 | 30 | 2.000 | 0.567 | 0.406 | 1.742 |
| | CarD133 | 30 | 2.000 | 0.533 | 0.391 | 1.718 |
| | CarC113 | 30 | 2.000 | 0.733 | 0.464 | 1.834 |
| | CarC6 | 30 | 2.000 | 0.600 | 0.420 | 1.764 |
| | Average | | 2.429 | 0.614 | 0.434 | 1.840 |

N: number of individuals, N_a: number of alleles, H_{obs}: Observed heterozygosity, H_{exp}: Expected heterozygosity, A_r: allelic richness.

(Rafinesque, 1818) were stocked in the pond on January 9th to re-establish the fishery. On November 19th, 2019 and April 1st, 2020 the pond and wetlands downstream of the pond were sampled with boat-mounted electrofishing equipment and backpack electrofishing equipment to determine presence/absence of northern snakehead.

Genetic data generation

DNA was extracted from fin clips using the Qiagen DNeasy Blood and Tissue kit. Fifteen microsatellite loci from King and Johnson (2011) were amplified, following their protocols. Microsatellite reactions were sent to Eton Bioscience and genotyped with an ABI 3730 genetic analyzer (Applied Biosystems, Inc.). Allele calls were made using GeneMapper® v4.0 (Applied Biosystems, Inc.) and checked by eye. Genotypes were screened for potential genotyping errors and the presence of null alleles with MICRO-CHECKER v2.2 (van Oosterhout et al. 2004), using a Bonferroni adjusted 95% confidence interval and 10,000 repetitions. Deviations from Hardy-Weinberg equilibrium (HWE) and for significant evidence of linkage disequilibrium of each locus were tested with GENEPOP v4.0.10 (Rousset 2008). Sequential Bonferroni correction was used to evaluate significance levels for simultaneous statistical tests of deviation from HWE and linkage disequilibrium (Rice 1989). See Table 1 for loci from King and Johnson (2011) used in downstream analyses.

Standard population genetic summary statistics (Table 1) were calculated with GenALEX (Peakall and Smouse 2012) and HP-RARE (Kalinowski 2005). Genetic clustering and admixture analyses were performed with the R (R Core Team 2019) package LEA (Frichot and François 2015). The best-fit number of clusters (K) was assessed by calculating an entropy criterion with



Figure 1. Northern snakehead specimen captured in a pond in Gwinnett County, Georgia, USA in October 2019. Photo courtesy of Brett Albanese (Georgia Department of Natural Resources, Wildlife Resources Division).

cross-validation using the *snmf* function, testing *K* values of 1–10 with fifty replicates. Lower cross-entropy values indicate better model fit (see Frichot and François 2015 for more details). The cross-entropy approach for assessing best-fit *K* was used because it can test fit for *K* = 1, unlike the Evanno et al. (2015) Delta*K* method. Individual admixture was assessed with the best scoring replicate of the best-fit *K* from the previous analysis with the LEA *snmf* function. Parentage analysis with CERVUS 3.0 (Kalinowski et al. 2007) was done to assess whether juveniles had the two sampled adults as parents. The CERVUS parent-pair analysis model designated all juveniles as potential offspring and both adults as potential parents. A simulation module was used to generate critical values of likelihood ratios so that parentage could be assigned with a known level of statistical confidence. Simulation parameters consisted of 10,000 offspring, two candidate parents, 85% proportion of loci typed, and a 1% error rate. Simulation results were used to assess whether juveniles were offspring of sampled adults. Assignments were made using the standard relaxed confidence interval of 80% (Kalinowski et al. 2007). The relaxed value was used because only a limited number of loci were genotyped, and a 95% confidence interval appeared too strict for our data to capture all parent-offspring relationships in our dataset.

Results

A total of 34 northern snakehead, including the adult fish released by the angler (confirmed by a hook injury in the mouth), were captured and removed during eleven sampling days, resulting in approximately 23,000 sec of pedal time boat electrofishing, 137,554 sec of backpack electrofishing effort, 7,200 sec of seine hauls, and 13 trap nights. The only survey methods successful in capturing the target species were boat electrofishing (11 fish; catch-per-unit-effort [CPUE] = 1.72 fish/h), backpack electrofishing (5 fish; CPUE = 0.13 fish/h), and rotenone (18 fish). All specimens were identified as *Channa argus* based on the following characteristics (Figure 1): gular part

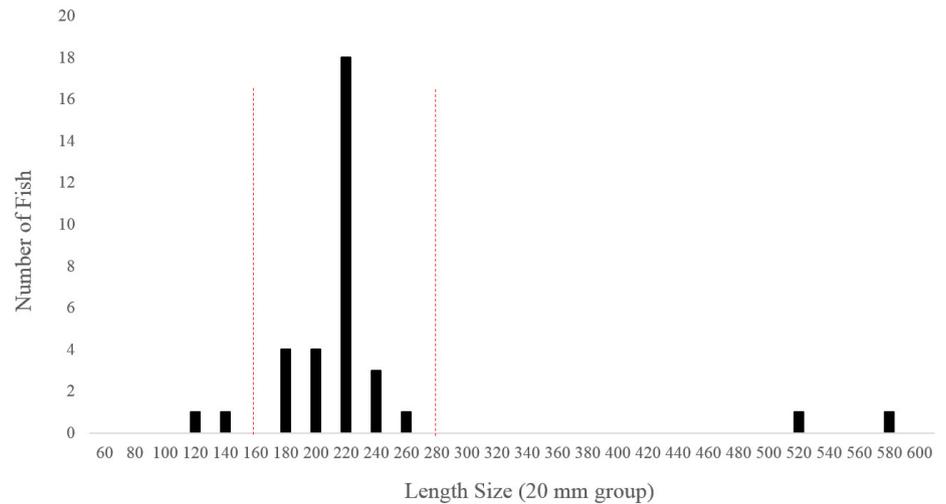


Figure 2. Length-frequency distribution of 34 northern snakehead collected from a pond in Gwinnett County (Georgia, USA) during October of 2019. Vertical dashed lines separate suspected cohorts ($n = 3$) based on visual assessment of the length-frequency chart.

of anteriorly depressed head without patch of scales; eye above middle of upper jaw; elongated dorsal fin with 49–50 rays; anal fin with 31–32 rays; and distinctive golden/brown background color on body with saddle-like blotches across the back (Courtenay and Williams 2004).

Collections of fish began within an hour of rotenone application and occurred daily for three days after application. It was determined that a complete kill had been achieved on day one of the rotenone survey, based on no visual signs of life within the pond or wetlands approximately three hours after the treatment. Over the course of three days, dead fish that floated to the surface were collected and identified to species. Non-target species found in the pond and wetlands were largemouth bass *Micropterus salmoides* (Lacepède, 1802), sunfish *Lepomis* spp., bullheads *Ictalurus* spp., golden shiners *Notemigonus crysoleucas* (Mitchill, 1814), mosquitofish *Gambusia affinis* (Baird & Girard, 1853), and black crappie *Pomoxis nigromaculatus* (Lesueur, 1829). Total lengths of northern snakehead removed from the site ranged from 117 mm to 575 mm. Two specimens were adults (one male and one female; confirmed via histological examination) while all others belonged to one of two distinct intra-annual cohorts of juveniles, based on length-frequency visualization, resulting in three size classes composing the entire sample (Figure 2).

Boat and backpack electrofishing efforts (2,537 sec) on November 19th, 2019 did not result in the capture or detection of any northern snakehead. Five largemouth bass > 305 mm were captured. There were visual observations of mosquitofish in the pond and wetlands. Similar electrofishing surveys conducted April 1st, 2020 (8,220 sec) did not result in the capture or detection of any northern snakehead. Largemouth bass, mosquitofish, and bluegill were observed.

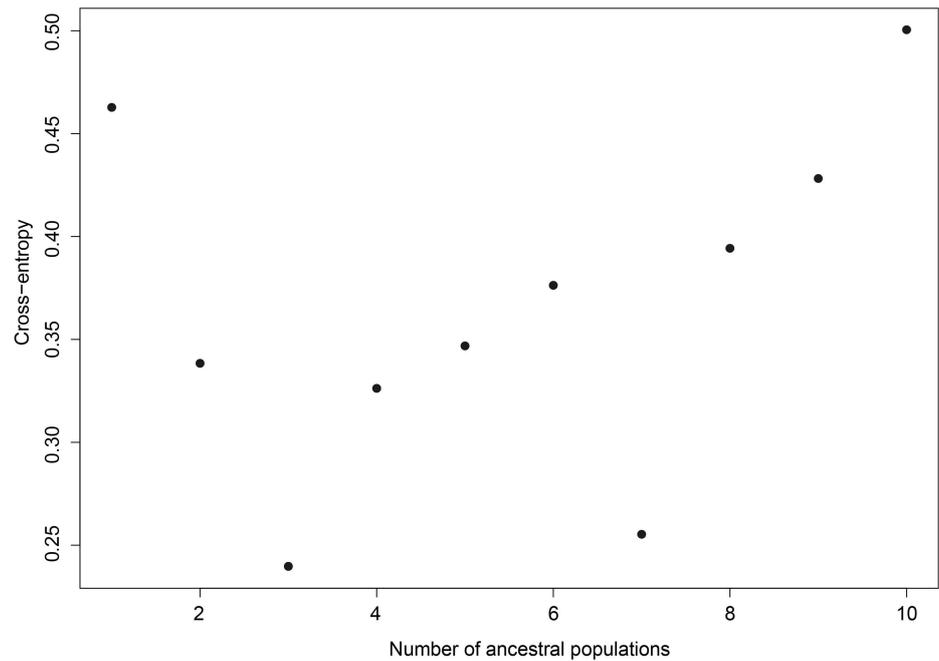


Figure 3. Cross-entropy values for each tested value of K.

Genetic results

We failed to generate genetic data from one individual, but both adults and 30 of 31 juveniles were successfully genotyped at 15 loci. We did not find evidence of scoring errors due to large allele dropout or stuttering. However, loci CarC110, CarC7, CarD6, CarD119, and CarD138 deviated from HWE ($p < 0.02$) and were subsequently removed from the study. We found no evidence that loci were under linkage disequilibrium ($p > 0.04$). Generally, genetic diversity was higher in the juveniles than in the two adults (Table 1), indicating that some individuals are offspring of unsampled fish. Three genetic clusters, or ancestral populations, were determined to best fit our data because $K = 3$ had the lowest cross-entropy score (Figure 3). LEA analyses indicated that most individuals showed no admixture (Figure 4). Notably, the two adults did not have identical admixture profiles (Figure 4). Parentage analysis indicated that five subadults had 1-to-1 matches with the two adults at an 80% confidence level in Cervus. Six fish matched to the sampled adult male and an unsampled female, and an additional six subadults matched to the sampled adult female and unknown male. Thirteen subadults did not appear to be offspring of either sampled adult.

Discussion

Introduction vectors

The discovery of a population of northern snakehead in Georgia raises questions regarding local vectors of introduction, timing of this introduction, and the possibility of other latent populations at large. The nearest known established population of northern snakehead is in the tidal

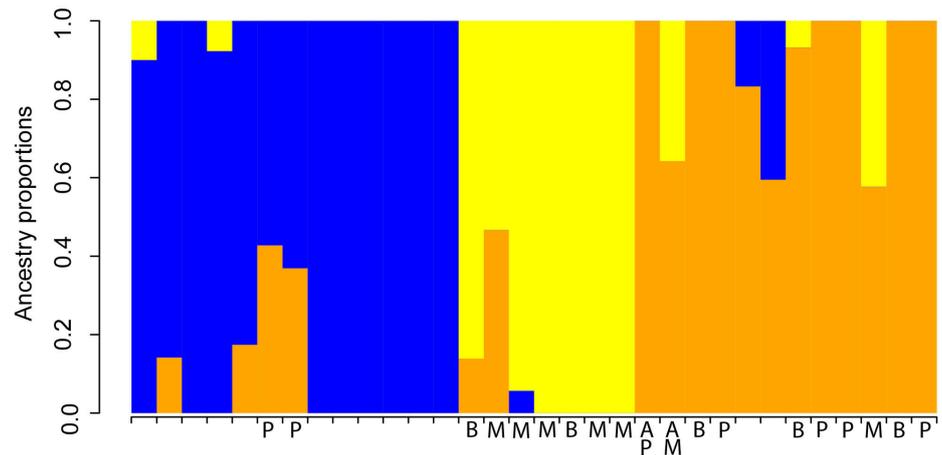


Figure 4. Admixture plot for genotyped *Channa argus*. Colors represent three ancestral populations. Each column is an individual. Columns are labelled to indicate results of genetic parentage analysis. “A” represent adults, “B” represents individuals with both maternal and paternal parentage, “M” represents individuals with maternal parentage, and “P” represents individuals with paternal parentage to the two adults.

and fresh waters of the Potomac River basin, thus eliminating natural dispersal as a mechanism for introduction and indicating the fish must have been illegally stocked through one of several potential scenarios. Because northern snakehead has historically been marketed online and via other illegal avenues for use in home aquariums, it is possible the fish were released by an aquarium owner who no longer wanted them. More likely, owing to the popularity of northern snakehead in the retail food industry and the continued importation of the species after its injurious listing under the Lacey act in 2002 (SPDC 2014), specimens purchased from live-food fish markets may have been intentionally released to establish a population for future harvest. Yet another plausible vector could be recreational angler(s) who wished to establish the species in Georgia and intentionally released the fish. The introduction of fish by anglers into a new area has been noted for many years throughout the USA, including in Georgia (Bonvechio et al. 2016). Considering the northern snakehead’s minimal requirements for transport, an angler could easily catch and transport northern snakehead from the Potomac River to Georgia. Still another possibility is that these fish were released as part of a religious observance (e.g., Shiu and Stokes 2008; Nico et al. 2019), though we acknowledge this vector is less likely than the aforementioned because intentional establishment of a non-native fish population does not align with the underlying motivations of religious release ceremonies. Identifying and stopping vectors of introduction are key steps to prevention and control of northern snakehead in the USA. An investigation into how and why northern snakehead were introduced to the pond is ongoing.

Genetic parentage analyses suggest that at least four non-sampled adults were the parents of some captured juveniles, and the higher genetic diversity in juveniles compared to the adults supports the presence of

unsampled parents. Genetic admixture analyses also corroborate this finding as some offspring have ancestry proportions that cannot be explained by reproduction from the two adults alone (Figure 4). The three genetic clusters present in our data represent genetic contribution from three ancestral populations to the sampled individuals, but this does not necessarily mean that the sampled fish were the result of multiple invasions. That is, a single release event could result in a genetically diverse group of invaded fish if the source population was genetically diverse. Furthermore, we cannot determine with our data whether reproduction occurred prior to release/invasion, and we cannot rule out the possibility that some adults present at the invasion site or outside of the rotenone treatment area were not sampled. Nevertheless, survey work after rotenone treatment did not recover live snakehead, possibly indicating that adults either died and were not collected after rotenone treatment or that a large number of juveniles were released into the pond without their parents. Even though LEA indicated a best-fit K of 3 for our data, survey work indicates that gene flow with fish outside the collection area is not ongoing, and we consider the reintroduced fish to be a single functional population. Future monitoring will help corroborate or reject these hypotheses, and additional genetic work with samples from putative source populations may improve our understanding of invasion source.

Effects on native fishes

Commensurate with the discovery of any new non-native species, questions regarding the ecological effects of northern snakehead, should they become established in Georgia, are inevitable. Such concerns have been expressed by other states where the species has been introduced (e.g., Delaware, Virginia, Maryland), though long-term trends have not been established regarding the effect of the species on native fishes in many of these environments, and most inquiries to date have focused on direct interactions between recreationally and economically important sportfish (e.g., largemouth bass) as opposed to assessing ecosystem-level impacts (Love and Newhard 2012; Saylor et al. 2012). While long-term effects may remain largely unknown, they are likely ecosystem specific and may vary based upon the presence of other native apex predators (Odenkirk 2018). Though we cannot conclusively state that a wild population of northern snakehead has established in Georgia based on this discovery, we also cannot conclude that additional specimens do not remain at large. Consequently, additional field surveys, public education efforts, and proactive management strategies to monitor the watershed and minimize the potential for future introductions are warranted.

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