ABSTRACT

Title of Document: ANGLER PERCEPTION AND POPULATION DYNAMICS OF THE NORTHERN SNAKEHEAD (*CHANNA ARGUS*) IN THE POTOMAC RIVER & TRIBUTARIES

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Our research sought to address the extent to which the northern snakehead (*Channa argus*), an invasive fish species, represents a threat to the Potomac River ecosystem. The first goal of our research was to survey the perceptions and opinions of recreational anglers on the effects of the snakehead population in the Potomac River ecosystem. To determine angler perceptions, we created and administered 113 surveys from June – September 2014 at recreational boat ramps along the Potomac River. Our surveys were designed to expand information collected during previous surveys conducted by the U.S. Fish and Wildlife Service. Our results indicated recreational anglers perceive that abundances and catch rates of target species, specifically largemouth bass, have declined since snakehead became established in the river.

The second goal of our research was to determine the genetic diversity and potential of the snakehead population to expand in the Potomac River. We hypothesized that the effective genetic population size would be much less than the census size of the snakehead population in the Potomac River. We collected tissue samples (fin clippings) from 79 snakehead collected in a recreational tournament held between Fort Washington and Wilson's Landing, MD on the Potomac River and from electrofishing sampling conducted by the Maryland Department of Natural Resources in Pomonkey Creek, a tributary of the Potomac River. DNA was extracted from the tissue samples and scored for 12 microsatellite markers, which had previously been identified for Potomac River snakehead. Microsatellite allele frequency data were recorded and analyzed in the software programs GenAlEx and NeEstimator to estimate heterozygosity and effective genetic population size. Resampling simulations indicated that the number of microsatellites and the number of fish analyzed provided sufficient precision. Simulations indicated that the effective population size estimate would expect to stabilize for samples > 70 individual snakehead. Based on a sample of 79 fish scored for 12 microsatellites, we calculated an N_e of 15.3 individuals. This is substantially smaller than both the sample size and estimated population size. We conclude that genetic diversity in the snakehead population in the Potomac River is low because the population has yet to recover from a genetic bottleneck associated with a founder effect due to their recent introduction into the system.

Angler Perception and Population Dynamics of the Invasive Northern Snakehead (*Channa argus*) in the Potomac River & Tributaries

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Chapter 1

An Introduction to Northern Snakehead in the Potomac River

1.1 Defining Invasive Species

An invasive species, once introduced, may establish itself within an ecosystem, often causing populations of native plants and animals to decline. For example, the mute swan (*Cygnus olor*), native to Europe and Asia, was introduced to the Chesapeake Bay area when five captive birds escaped in 1962. The population of mute swans remained under 500 until 1986, when it underwent a rapid increase until it reached almost 4,000 birds in 1999. Mute swans forage on and among aquatic plants, endangering the populations of submerged aquatic vegetation by uprooting plants and reducing their ability to reproduce. They also displace existing waterfowl populations by taking over their resources and habitat and even attacking and killing native species. The Maryland Department of Natural Resources (DNR) responded by creating a Mute Swan Task Force to control the population (MD DNR, 2011).

Invasive species also have economic and ecological consequences for cultivated, protected and restored landscapes. Invasive species can exert particular important effects in strongly size-structured aquatic systems. For example, the invasion of zebra mussel (*Dreissena polymorpha*) into aquatic ecosystems throughout the Mississippi and Atlantic drainages of North America increased the presence of toxic algal blooms and decreased zooplankton populations, causing a trophic chain reaction that reduced populations of many native species, even driving some to local extirpation. Zebra mussel populations also affected water-dependent infrastructure such as nuclear power plants, dam structures, and water treatment plants (O’Neil, 1997). Three hundred thirty-nine facilities made efforts to reduce and ameliorate the negative impacts of this invasive species. The cost of these efforts increased over time as the zebra mussel population expanded--in 1995, facilities spent over $17 million, compared to just $200,000 in 1989 (O'Neil, 1997). State and federal governments have been taking measures to control the zebra mussel, targeting it in the National Invasive Species Act of 1996. However, it continues to cost businesses money and time, and to fundamentally alter the aquatic ecosystems it invades.

In addition to invasive species, we will define the terms "native" and "naturalized." A native species is a species that is present in a given ecosystem before a given time--in the United States, a species is considered naturalized if it was introduced during European settlement. White oaks are considered native to Maryland, but English boxwoods are considered naturalized because English colonists brought them to North America to be planted ornamentally (Invasive and Exotic, n.d.).

1.2 Background: the Northern Snakehead

Discovered by an angler fishing in a pond in Crofton, Maryland in 2002, and then introduced in the Potomac River in 2004, the northern snakehead (*Channa argus*) became an invasive species in the Potomac River watershed. Characterized by a prominent dorsal fin and mottled appearance (Figure 1), the snakehead quickly spread throughout the pond. The Department of Natural Resources poisoned the pond with rotenone, a commonly-used piscicide, later that year in an effort to eliminate snakehead from the system (Courtenay & Williams, 2004). However, other related introductions (e.g., Dogue Creek, Virginia) resulted in the spread of snakehead throughout the Potomac River and its freshwater drainages (Orrell et al., 2005; Dolin, 2003).

Figure 1
Northern snakehead (U.S. Geological Survey Archive).

The native range of the northern snakehead is in river drainages of China, Thailand, Korea, and southeast Russia (Courtenay & Williams, 2004). When the species was introduced into the Potomac River, the media sensationalized many of the northern snakehead's traits. Media outlets publicized that northern snakehead could walk on land. Even though many of these concerns are exaggerations, the northern snakehead is a resilient, highly adaptable species. It is a member of the Channidae (snakehead) family. A suite of unique characteristics define the snakehead family of fishes, including the ability to breathe air. Snakehead have supra-branchial chambers that function as lungs supporting aerial respiration that are particularly active during the juvenile stage (Courtenay & Williams, 2004). The northern snakehead specifically is an obligate air breather (Courtenay & Williams, 2004). Although some species of snakehead are able to travel overland as adults, the northern snakehead's ability to do so is very limited, and only if some water is present (i.e. muddy, flooded conditions) (Courtenay & Williams, 2004).

1.3 The Northern Snakehead in the Ecosystem

The northern snakehead (hereafter snakehead) can reach up to 85cm in length, with males generally growing larger than females (Courtenay & Williams, 2004). The maximum length found in the Potomac River is 89.2cm (Newhard, 2015), and the largest snakehead caught in Maryland weighed in at 17.49lbs (Welsh, 2016). The species prefers stagnant ponds, swamps, and slow-moving streams, but also occurs in lakes, rivers, and canals--virtually any temperate freshwater body is appropriate habitat for snakehead (Courtenay & Williams, 2004). Snakehead can survive at temperatures between 0^{o}C to 30^{o}C, and have an upper salinity tolerance level of 18ppt. However, their distribution within the Chesapeake Bay suggests that this may not be a lethal limit. As an obligate air breather, the snakehead can live in waterbodies with low oxygen levels (Courtenay & Williams, 2004). Overall, the snakehead is capable of tolerating a wide range of environmental conditions (Odenkirk & Owens, 2007).

The snakehead is an apex predator that consumes fish and other organisms. Snakehead feed on plankton in the post-larval stage and begin to feed on crustaceans and fish larvae as juveniles. Adult snakehead consume almost any small aquatic organism. Based on analysis of gut contents, adult snakehead in the Potomac River consume a diet composed of ninety seven percent fish (Saylor, Lapointe, & Angermeier, 2012). Snakehead mainly prey on smaller fish but are opportunistic omnivores (Saylor et al., 2012). They can feed on frogs, crustaceans and insects (Courtenay & Williams, 2004). When diets of the snakehead, yellow perch (*Perca flavescens*), American eel (*Angulla rostrata*), and largemouth bass (*Mircropterus salmoides*) in the Potomac River were compared, a significant overlap was found only between snakehead and largemouth bass

(Saylor et al., 2012). This is an indicator of potential competition between species: a non-native predator can force a native or naturalized predator to change trophic position (Saylor et al., 2012).

Snakehead were sensationalized by the media. One myth that was perpetuated was that snakehead have no natural predators in the Potomac area; however, snakehead fry (juveniles) are eaten by other piscivores, and raptors such as the osprey (*Pandion haliaetus)* are known to prey on snakehead (Odenkirk, 2015).

Studies of snakehead in the Potomac indicate that the species demonstrates high growth rates. Odenkirk et al. (2013) studied growth via both release and recapture of tagged fish and from analyzing otoliths. These authors report growth rates of snakehead juveniles of up to 0.89 mm/d. Although studies have not been completed, this suggests that snakehead may mature at a young age. Mature female snakehead have high fertility and reproductive rates, and are highly adaptable to changing environments (DelViscio, 2004). Mature females produce an average of 40,000 eggs per spawning (Odenkirk & Owens, 2007). Jiao et al. (2009) used a stochastic, stage-based model to examine the likely dynamics of the snakehead population in the Potomac River. Jiao et al. estimated a positive net population growth rate (λ) for snakehead of 1.13 (where $\lambda>1$ indicates an increasing population). Data on the abundance of snakehead in the river support this estimate. Electrofishing was used to monitor the snakehead population in the Potomac River from 2004 to 2006. In 2004, mean catch per unit effort was 0.2 fish/hr, but had increased thirtyfold to 6.1 fish/hr by 2006 (Herborg, Mandrak, Cudmore, & MacIsaac, 2007). Data on recreational catches support this population increase. Reported angler catches in 2006 equaled the combined total of reported catches in 2004 and 2005, and the maximum reported fish size increased each year (Herborg et al., 2007).

Due to their high individual growth rates and opportunistic diet, snakehead have the potential to outcompete with native and naturalized fish, especially those with slower individual growth rates and more specialized diet, for the resources the fish need in order to survive. This could cause the populations of these native and naturalized fish to decline. By combining this risk with high reproductive rates and high population growth rate, the snakehead have the potential to quickly expand and outcompete fish throughout their expanded range, negatively impacting a greater number of fish individuals and species, which may include the largemouth bass (Odenkirk et al., 2013).

1.4 Regulation and Stakeholders

As snakehead populations continue to expand, state and federal governments have taken action to regulate these populations. In October 2002, the U.S. Fish & Wildlife Service added the snakehead family to the list of injurious wildlife under the Lacey Act. The implications of this listing are that it is now illegal both to import snakehead into the U.S. and to transport it alive across state borders without a permit. The state of Maryland banned the possession of live snakehead in July 2004, following the ban implemented by 15 other states in 2002 (Courtenay & Williams, 2004). Since then, tournaments to catch snakehead have become popular in Maryland.

The Potomac River Snakehead Tournament, an annual snakehead fishing competition to remove as many snakehead as possible from the Potomac River watershed, removed more than half a ton of snakehead biomass from the Potomac in

2012 (Fears, 2012). The tournament is hosted by Potomac Snakehead, an organization that collaborates with government agencies. Despite these efforts, the species appears to be spreading widely from its initial introduction into the Potomac watershed, as it can now be found in many widespread areas, including the upper Potomac (via the C&O canal), the Patuxent River, and the Wicomico and Marshyhope Creeks on the Eastern Shore (Knauss, 2015).

Stakeholders such as anglers, wholesalers and restauranteurs that sell and serve fish, and conservation groups can potentially play roles in controlling and managing the snakehead population. Stakeholder based programs have been successful in controlling invasive species in other locations. For example, the Minnesota Department of Natural Resources engaged stakeholders in that state to control aquatic invasive species. The Minnesota DNR began an aquatic invasive species program focusing on recreational anglers and boaters. This program educated participants on aquatic invasive species. A 1994 survey found that respondents were more educated on invasive species than during previous surveys and that many anglers and boaters changed their behavior towards invasive species (Larson et al., 2011). Minnesota’s experience indicated that anglers can be potential mediators of impacts of invasive species such as snakehead, and that government agencies can use recreational anglers to their advantage in order to manage a species.

A main incentive for reducing the snakehead population is to protect native and naturalized species that are targeted by anglers, like the largemouth bass. Population models have indicated that largemouth bass populations decline in the presence of uncontrolled snakehead populations, with one model predicting a 35.5% reduction in the largemouth bass population (Love & Newhard, 2012). Therefore, harvesting snakehead to reduce its abundance may help protect the largemouth bass population.

1.5 Understanding Snakehead Populations

Before management goals can be identified, it is crucial to understand the size, extent, and potential of the snakehead population to expand. Determining whether an invasive species will become established sometimes requires assessment of its abundance. Love et al. (2015) used a habitat suitability approach to estimate the area of habitat available to snakehead in the Potomac River. They then scaled this area by an average abundance to yield an estimated population size of the snakehead population in the 44 tidal freshwater tributaries of the Potomac River drainage of 21,179 individuals (Love et al., 2015). However, the size of a population (N) does not fully capture the extent of naturalization. Populations of newly introduced or newly established species have several unique characteristics, including limited genetic diversity. This restriction, or bottleneck, on genetic diversity in invasive species is called the "founder effect" (Hamilton, 2009). As a population overcomes the founder effect, it is crucial to measure its genetic diversity, which can be done by quantifying its effective population size. N_e may be defined as the size of the randomly breeding, ideal population that maintains as much genetic variation as the target population, regardless of its census size (Hamilton, 2009). This is what Ne represents when the sample does not include overlapping generations. However, the population of snakehead in the Potomac River does have overlapping generations. Therefore, for the purpose of this paper, we define N_e as the number of effective breeding pairs within a population. This estimate informs us how many individuals are contributing the genetic diversity of the population. N_e is the effective population size, where the total number of individuals in a population is N. A similar N and N_e would suggest that the population is mostly free of genetic drift and is likely in Hardy-Weinberg equilibrium. Therefore, N_e can be defined by the rate of loss of genetic diversity in a population, as such:

$$H_t = H_0[1 - \frac{1}{2N_e}]$$

where t = number of generations and H_t and H_0 are random-mating frequencies of heterozygous genotypes at generation t (Doyle et al., 2001).

If the snakehead were a naturalized species that had enjoyed multiple generations of constant demographic structure and population size, we would expect the effective population size to be within an order of magnitude of the census population size (Doyle et al., 2001). N_e can be used to determine the effect of inbreeding and genetic erosion on a population, and therefore a larger N_e/N ratio indicates a healthy population (Doyle et al., 2001). However, since snakehead is an introduced species, we expect to see a smaller N_e, reflecting the lower genetic diversity resulting from the founder effect. This would also imply a lower N_e/N ratio. Indeed past management practices have suggested the desire to maintain a smaller population (Snakehead Plan Development Committee, 2014). If the population is kept smaller, genetic diversity and therefore N_e will remain low due to the bottleneck effect.

With respect to control and management, there are several advantages to having an invasive species with a low or limited genetic diversity (the consequence of a genetic bottleneck as well as the founder effect). Firstly, a low genetic diversity limits the ability of an invasive species to adapt to environmental changes or disruptions. For example, if a large portion of the population of an invasive species is killed due to some event, then the species is much less likely to adapt and persist than it is to be extirpated.

1.6 Research Questions

We developed two questions to guide our research.

Research Question 1: How do anglers perceive the presence of the northern snakehead in the Potomac River?

To address this first question, we evaluated the public perception of northern snakehead in the Potomac River drainages. We sought to determine:

1. Whether anglers could identify snakehead?
2. How anglers perceive snakehead in relation to their fishing experience (positive or negative impact)?
3. Their perception of the potential for recreational control of the snakehead population
4. Whether they thought the snakehead population was increasing or decreasing?

We conducted short surveys throughout popular Potomac tributaries to determine angler perception of snakehead.

Research Question 2: What does the effective size of the snakehead population in the Potomac River tell us about the potential for snakehead population to continue to expand?

To address this question, we sought to estimate the minimum effective population size (N_e) of the snakehead population in the Potomac River. We addressed this objective by quantifying the diversity of microsatellite genetic markers in the population. We also calculated the heterozygosity, or allele diversity, of the population.

Chapter 2

A survey of angler attitudes towards Northern Snakehead in the Potomac River & Tributaries (MD)

2.1 Introduction

Recreational fishing has over the last 50 years become an increasingly important consideration in the management of coastal fisheries, particularly in terms of its economic impact, the number of participants, and the magnitude of catches (Ihde, Wilberg, Loewensteiner, Secor, & Miller, 2011). Recreational fisheries have become especially important in comparison to commercial fisheries, as recreational harvests have dominated the total marine harvests in the United States since the 1960s (Ihde et al., 2011).

Despite their increasing importance, it can be difficult to assess the impacts of recreational anglers on fisheries (Arlinghaus, Mehner, & Cowx, 2002). Recreational fisheries typically have low entry requirements, tackle is relatively cheap, and licenses are often not required (Arlinghaus et al., 2002). This can lead to high levels of public participation in recreational fisheries, making it difficult to evaluate the population of participating anglers.

Recreational fisheries have multiple access points, adding an additional challenge for gauging their users. Although commercial fisheries often land their catch in a few documented ports, recreational fisheries land their catches in numerous ports and private access points. This can make surveys of recreational anglers expensive and difficult to design, further affecting the ability of management organizations to measure the effects of recreational anglers on fisheries (Arlinghaus, Cooke, Schwab, & Cowx, 2007).

In order to address these challenges, researchers often utilize creel or intercept surveys to collect information from recreational anglers. These surveys can be used to collect information on specific fish species, angling pressure on fish populations, and angler attitudes. Intercept surveys typically “intercept” people while they are fishing, and administer the survey in person. This method originated in freshwater areas where anglers kept their catch in a basket, or creel. For example, McCormick et al. (2015) utilized creel surveys to investigate the reporting accuracy of steelhead (*Oncorhynchus mykiss*) harvests in Idaho, as well as angler attitudes toward steelhead. Similarly, McCormick et al. (2013) utilized creel surveys to investigate the angling effort of Chinook salmon (*Oncorhynchus tshawytscha*) in Washington and Oregon. These studies demonstrate the ability of creel surveys to effectively measure recreational anglers’ attitudes and effort, despite the difficulties and costs associated with measuring anglers’ impact on fisheries.

There is evidence that snakehead compete with other native and naturalized fishes in the Potomac. Regional management agencies are interested in limiting the negative impacts of this species on the ecosystem (Snakehead Plan Development Committee, 2014). One approach to meet this objective is to encourage catches and removal of snakehead from the system by recreational anglers. Participation by recreational anglers in this method of control presumes that they perceive snakehead as a threat to the integrity of the ecosystem and that they would be willing to participate.

Here we report the results of a creel intercept survey on recreational anglers in the Potomac River. The survey was designed to be consistent with surveys conducted by Maryland Department of Natural Resources (MD DNR) and the Maryland Fish & Wildlife Conservation Office (MFWCO) of the US Fish and Wildlife Service. The goals of our survey were: to determine whether or not recreational anglers could identify snakehead; to understand how recreational anglers perceive snakehead in relation to their recreational fishing experience; to assess the potential for recreational control of snakehead; to evaluate the perception of whether snakehead are increasing or decreasing in population size in the Potomac River; and to evaluate the success of past management actions that encouraged harvest, including raffles.

2.2 Methodology

When designing our survey, we asked questions that were strategically important to help achieve our previously stated goals and provide MD DNR and MFWCO with valuable information relevant to the management of snakehead and previously conducted creel surveys (Appendix A). A full copy of the survey is provided in Appendix B. We obtained a waiver from the University of Maryland, College Park Institutional Review Board because no personal identifiers were being retained in the survey.

Interviews were conducted at Maryland boat ramps on the Potomac River and Potomac River drainages between Washington D.C. and Charles County (Appendix C Table 1; Figure 1). Survey locations and frequencies were selected using a stratified sampling of boat docks in the target areas (Appendix C, Table 1). After determining the number of effort days, we decided to survey at the same boat ramps from the most recent MFWCO creel survey. The number of visits to each boat ramp were weighted based off the number of responses they generated in the past (Appendix C, Table 1). Sites with more responses in the MFWCO survey were weighted more heavily, and sites with fewer responses weighted less heavily. This allowed for highly trafficked sites to be sampled more frequently and less trafficked sites less frequently. Using the weights, each site was assigned a fraction of the total target number of visits. Visits were distributed throughout

June, July, August, and September so that each site's visits were equally distributed through each month. Within each month, sites were randomly assigned to a weekday morning, a weekday afternoon, a weekend morning, or a weekend afternoon (Appendix C, Table 2). Interviews were conducted an average of two times per week from June to September 2014, for a total of 113 interviews (Appendix D).

On each survey visit, teams of two to four researchers would visit the assigned boat ramp or fishing location and intercept anglers in person. One researcher would ask the angler survey questions, and a second would record responses on a paper and clipboard. After an angler's response was recorded, it was coded into a master data file using a coding key in Microsoft Excel (Appendix E). This coded data was analyzed in SPSS (v.32, Chicago, Illinois).

In addition to a few basic questions on fishing preferences (fishing style, length of trip, target species, etc.), anglers were asked several questions regarding their feelings and experiences with snakehead. The survey tested the anglers' ability to identify a snakehead. Anglers were also asked several questions regarding their feelings and awareness surrounding the snakehead's presence in Maryland waters.

Can anglers identify snakehead? Anglers were given a sheet with four color images of unidentified fish, and asked to identify each to the best of their ability (Appendix B). Research members recorded whether they correctly identified each image. This question was used to assess public knowledge of snakehead. This helped in evaluating the potential for recreational control, as recreational control can only be effective if the public can correctly identify the fish. Additionally, the question informs us on the effectiveness of past awareness campaigns by MD DNR and MFWCO. This question further helped to assess the internal validity of the survey results, as further questions about snakehead have limited value if the respondent cannot identify a snakehead.

How do anglers perceive snakehead in relation to their recreational fishing experience? Anglers were asked what their target species was, whether there had been a significant increase or decrease in the species over different time frames, if they enjoyed catching snakehead, if snakehead have a positive impact on the environment, and if snakehead have a positive impact on their fishing enjoyment.

Assess potential for recreational control. We used these questions to determine angler motivation for catching snakehead, and therefore the potential for anglers to catch snakehead as a population control measure. We also asked questions to determine the market potential of snakehead as a food fish. Anglers were asked how long their typical fishing trip lasts, if they would fish specifically for snakehead, if they would sell their catch commercially, if they enjoyed eating snakehead, if they would recommend it as a food product, and if they would consider work as a government contractor to catch snakehead.

Evaluate success of past incentives. Anglers were asked if they were aware of past or ongoing raffles involving snakehead. According to the Snakehead Taskforce Meeting 2015, anglers who submit a species survey including snakehead catches are entered into a raffle. The raffle is drawn annually and the winner receives a prize. Many anglers made additional comments concerning snakehead control incentives that were not included in the survey questions.

2.3 Results

We conducted and completed 113 interviews at 6 different boat ramps from June – September 2014. Of the 113 interviews, the majority of anglers (69%, n = 78) reported that they were targeting bass, mostly largemouth bass. The only other target species at a substantial level were catfish (11.5%, n = 13) and snakehead (8.8%, n = 10). Over fifty percent (55.8%, n = 63) of anglers reported having caught snakehead, and almost all of those anglers (98.4%, n = 62) could accurately identify a snakehead by picture. About eighty percent of all anglers interviewed (78.8%, n = 89), regardless of whether they had previously caught a snakehead, could accurately identify a snakehead by picture.

There was a general trend that anglers claimed to be finding fewer of their target species in the last five years, especially among those anglers who reported targeting largemouth bass (Appendix F, Table 1 & Table 2). In contrast, almost fifty percent of anglers interviewed (46.9%, n = 53) believed snakehead had a positive impact on their fishing enjoyment. Of the remaining interviewees, less than two percent (1.8%, n = 2) were neutral about the impact of snakehead, and around seven percent (7.1%, n = 8) believed the introduction of snakehead had had a negative impact on their fishing enjoyment. Of the anglers interviewed, over forty percent (44.2%, n = 50) did not answer the question. Upon prompting with the Likert-scale questions, some anglers would give non-verbal responses such as shrugging, express a desire not to answer the question, or otherwise respond in a way that the interviewer could not interpret as an appropriate response on the Likert scale.

In expressing their interest in being an agent of control of the increasing snakehead population, less than sixty percent of anglers (57.5%, n = 65) claimed that, if given the legal opportunity, they would sell their recreational snakehead catch commercially. Moreover, about fifty eight percent of anglers (57.5%, n = 65) stated that they would consider fishing exclusively for snakehead, and about fifty eight percent (58.4%, n = 66) of anglers expressed interest in a hypothetical government contracting position fishing for snakehead as a control measure.

Although less than forty percent (37.2%, n = 42) of the anglers had eaten snakehead, all (100%, n = 42) of those who had eaten snakehead would recommend trying it, and some anglers specifically mentioned having tasted it at the snakehead tournament in June 2014. Finally, about a third (32.7%, n = 37) of anglers were unaware of any past or ongoing raffles connected to the snakehead.

2.4 Discussion

We were able to successfully complete a survey of the attitudes of recreational anglers toward snakehead in the Potomac River. Our results indicate that most anglers can accurately identify a snakehead, especially when they have previously caught a snakehead. This information indicates that efforts by MD DNR and USFWS to raise awareness about the snakehead have been effective. However, is the ability of recreational anglers to identify a snakehead sufficient for population control? We can infer that if 81% of anglers can identify a snakehead, initial awareness programs have succeeded. However, it is important to continue offering educational materials, such as brochures with the purchase of a fishing license, to ensure continued capability of identification for future generations of anglers. The National Snakehead Control and Management Plan (2014), assembled by the Snakehead Plan Development Committee, suggests the use of outreach materials that include contact information of individuals involved with snakehead management. The plan also recommends an explanation of natural resources stewardship, environmental and health concerns in relation to snakehead, and actions that should be taken if a snakehead is caught. We agree that outreach materials that include information on why anglers should participate in snakehead population control should be included with the purchase of a fishing license. Anglers that understand the motivation behind snakehead population control may be more inclined to target snakehead.

Our results indicate that one third of anglers are unaware of any past or ongoing raffles concerning snakehead (33%, n = 37). Following the question about raffle awareness, we frequently received anecdotal responses (not included in the survey) from anglers who told us they were aware of the annual Potomac Snakehead Tournament. From our conversations with anglers, we believe that more anglers are aware of the snakehead tournament than of the snakehead raffle contests promoted by MD DNR. Therefore, we suggest that MD DNR consider more effective ways to market their efforts to anglers if they are to continue with this program.

There is a gap between recognition of snakehead and snakehead fishing effort: 81% of anglers could identify a snakehead, but only 57% have actually caught a snakehead. Creel surveys completed in 2009 by the Maryland DNR indicated that 95% of anglers knew what a snakehead was, but only 28% had caught at least one snakehead. Firstly, it is important to note that the Maryland DNR and our team measured different variables when measuring snakehead recognition. Where the Maryland DNR measures whether or not an angler had heard of the fish, our team measured whether or not an angler could identify the fish. In other words, the drop in recognition percentage (from 95% to 81% in 5 years) can be explained by the fact that USFW creel surveys asked if anglers knew what a snakehead was, while we asked anglers to identify snakehead among pictures of other species of fish. However, the gap between anglers who can recognize snakehead and anglers who have caught snakehead was substantially smaller in 2014 than in 2009. The rise in the amount of anglers who have caught snakehead (28% to 57% in 5 years) can be attributed to several factors. The most likely reasons are either that the snakehead population increased significantly over those five years, or that anglers are more inclined to catch snakehead. The only census population estimate calculated a population size of about 21,000 snakehead (Love et al., 2015).

A minority of anglers disagreed that snakehead positively affected their fishing enjoyment (45.1%) and almost all perceived a lower abundance of their target species in the Potomac tributaries than in the past. However, a majority (54.9%) of anglers are either neutral about snakehead or perceive them to be positively affecting their fishing enjoyment, indicating that there are numerous different perceptions of the fish among recreational anglers. This latter figure indicates that further education of recreational anglers by regional management agencies on the objectives of environmental stewardship may be needed.

Over half of the anglers we surveyed claimed that, if given the legal opportunity, they would sell their snakehead catch commercially; 57% of anglers said that they would consider fishing exclusively for snakehead, regardless of whether the fishing was commercial or not, and 58% of anglers expressed interest in a hypothetical government contracted position fishing for snakehead. Under appropriate conditions, anglers could be amenable to assisting agencies with snakehead control measures. In fact, Maryland House Bill 1387, passed in 2016, allows bowfishers to purchase a fifteen dollar license allowing them to sell their snakehead catch legally.

Although only a third of the anglers have eaten snakehead, every angler who had eaten snakehead would recommend trying it. This means the snakehead may have the potential to become a popular food fish in the national capital region. Indeed, the snakehead is a highly-sought-after food fish in its native range (Snakehead Plan Development Committee, 2014). Although it should not and cannot be available in live food fish markets, snakehead may easily be caught and prepared as food in the region. Consumption of snakehead can also be encouraged as snakehead typically contains fewer contaminants than other fish in the DC area making it safer to consume (DOEE, 2016).As many as 40 restaurants in the area already serve snakehead (Rogers, 2013). In 2014, 2,400 pounds of snakehead were commercially harvested in the Potomac tributary (Snakehead Task Force Meeting Summary, 2015). ProFish, a large commercial seafood wholesaler, offers northern snakehead to commercial clients (Rogers, 2013). However, the number of snakehead caught per year by commercial fishers who supply ProFish is miniscule in comparison to common food fish such as tilapia (Rogers, 2013). It is a young fishery, and ideal methods have not been developed for catching snakehead (Rogers, 2013). Therefore, snakehead is currently extremely expensive to obtain for restaurateurs. However, we believe that with time, commercial snakehead harvest and focus on the snakehead as a food fish can become a viable control measure, as it has been for past species.

We encountered many limitations during our survey process. We only surveyed in Maryland, could only access public areas, and only in known snakehead range. We did not investigate change in fishing effort as a result of snakehead introduction, since we were only able to survey for one summer. For future surveys, we recommend a long-term project definitively measuring snakehead fishing effort over a period of time--this could perhaps be included in state creel surveys, perhaps alongside questions asking if anglers are aware of laws pertaining to snakehead and what to do if snakehead is caught. Our survey also focused on late morning and afternoon anglers. This stratification overlooked bowfishers as they typically fish during the night and return in the early morning, before our designated survey times. Bowfishers, some of whom may be considered “snakehead specialists”, contribute significantly to snakehead fishing efforts and their opinions should be taken into account in future surveys,

We recommend the formulation of a long-term snakehead-focused survey by government agencies, and that it be repeated every summer. If agencies were to use our survey as a baseline, we would recommend keeping the identification question. Identifying the snakehead from a group of other fish species is an effective way to ensure that anglers can actually recognize snakehead on sight. We recommend that the fishing effort question be reformatted; blocks of time are difficult to quantify, and if we were to survey again we would ask anglers to estimate the number of hours their fishing trip would last. In a new survey, we would narrow the Likert scale responses to simply “agree,” “neutral” and “disagree.” Including “strongly agree” and “strongly disagree” made responses difficult to quantify because some anglers would simply say “yes” or “no,” and when asked to choose an answer from the scale, would waver between options. Narrowing down the scale would allow for an easier process and more reliable answers.

We would include a question about the snakehead tournament asking anglers if they have heard of it and if they have participated before. Additionally, we would like to see how anglers who fish for bass like fishing for snakehead, so we would include a question reflecting this. Finally, we recommend forming a pilot survey, testing it for a summer, and after analyzing data formulating a survey to be conducted over a longer period. Since our survey was only done once, we were unable to refocus the questions to reflect our goals. Therefore, we recommend a pilot survey with a following long-term annual survey lasting 10-15 years. Surveying for over a decade ensures that several generations of snakehead are covered during the time period.

This survey was designed to answer the overarching question: how do anglers perceive the presence of snakehead in the Potomac River? In order to answer this question, we evaluated a series of research questions. First, can anglers identify snakehead? Our survey indicates that anglers are successful in identifying snakehead, especially anglers who have caught a snakehead. Second, how does the snakehead affect anglers’ fishing experience? In future surveys, we recommend that surveys be vetted for their ability to pinpoint angler perception, as many anglers would shrug or give an answer outside the designated scale. Third, what is the potential for recreational angler control? We found that a large percentage of anglers are willing to fish specifically for snakehead, especially given the opportunity for monetary incentive. We also found that anglers enjoy eating snakehead, indicating its potential as a food fish. Finally, how successful have snakehead control incentives been? Although we only asked about the raffle, our results demonstrate that the Potomac Snakehead Tournament reaches a larger audience than the raffle. We recommend that government agencies conduct research on improving awareness of a variety of control methods. Overall, although anglers have caught more snakehead in past years, perception of snakehead is not clearly defined by the angler community.

Chapter 3

Estimation of the effective population size of the Northern Snakehead population in the Potomac River, MD

3.1 Introduction

Knowledge of fundamental aspects of biology is important for management and control of invasive species. An estimated $1 billion in economic losses due to non-indigenous fish species occurs in the United States every year (Pimentel, Zuniga, & Morrison, 2005). Management and control of introduced species is therefore important in reducing environmental and economic damages. Studying the population biology of an introduced species can also give insight into the genetic diversity and the potential for rapid adaptation and establishment as an invasive species. The probability of a species becoming invasive is dependent on genetic and environmental factors (Allendorf & Lundquist, 2003).

When a non-indigenous species is introduced, typically a small number of individuals establish the population in the new habitat. This can cause a “founder effect” in which the new population is characterized by a reduced genetic diversity compared to the larger population from which it was derived. This genetic bottleneck, where a few individuals remain from a larger population, implies that only a limited number of alleles will contribute to future generations (Hamilton, 2009). As the newly founded population grows in size, the genetic variation in allelic frequency may increase. This increase is due to random mutation as well as genetic drift. For invasive species, this results in a paradox. Genetic bottlenecks tend to be harmful and limit population growth of a newly established population, through inbreeding and/or the limited ability to evolve and adapt to new environments. However, invasive species can overcome the founder effect and the genetic bottleneck to out-compete and take over native species’ niches because they are typically species with fast population growth due to the higher rate of potentially mutated alleles in offspring (Allendorf & Lundquist, 2003). Invasive species like snakehead may not experience negative effects of homozygosity and low genetic diversity. Genetic studies and population biology research help to elucidate this paradox.

The snakehead has the potential to damage native fish and wildlife populations within the Potomac River and its tributaries. For example, Iwanowicz et al. (2013) identified that some snakehead in Virginia waters carry the largemouth bass virus (LMBV), and acknowledged that little is known about other pathogens snakehead may be carrying. In addition, the snakehead overlaps with largemouth bass in habitat use and diet, and many anglers fishing for bass will catch snakehead instead (Love & Newhard, 2012). Our survey of angler attitudes indicated that recreational anglers in the Potomac River believe that their catch rate of largemouth bass has declined since snakehead became established (Chapter 1). Furthermore, a modeling study also indicated that if the snakehead continue to expand without control measures in place, the largemouth bass population could decrease by 35.5% (Love & Newhard, 2012).

The abundance of snakehead in the river has been monitored since 2004. Previous electrofishing and collection studies have shown that the snakehead population has increased since its introduction in 2004. Between 2004 and 2006, the population increased dramatically, with the mean catch in a standardized electrofishing survey increasing 30.5-fold, from 0.2 fish per hour to 6.1 fish per hour in 2004 and 2006, respectively (Odenkirk & Owens, 2007). Further, studies found that adults can disperse over large distances, suggesting that the snakehead population can rapidly increase in abundance and/or change distribution (Lapointe, Odenkirk, & Angermeier, 2013). As a recent introduction, the snakehead population likely also experiences low genetic diversity as a result of a founder effect.

Several genetic studies of snakehead have already been conducted that assess the genetic diversity of different snakehead populations in several areas. Zhu, Li, Xie, Zhu, Wang, & Yue (2014) analyzed the genetic diversity of a snakehead population in China using ten microsatellite loci. These authors found that compared to other freshwater fishes, snakehead in its native range had a high allelic diversity (Zhu et al., 2014). King and Johnson (2011) collected fin clippings of snakehead from the lower Potomac River in Virginia and developed microsatellite loci to map likely patterns of introduction of the species in the mid-Atlantic area. They identified 19 individual tetra-nucleotide markers that were tested on a collection of snakehead from New York. Orrell et al. (2005) assessed the population viability in the Potomac River using mitochondrial DNA sequencing to determine the number of haplotypes, a set of DNA variations or polymorphisms that are usually inherited together, in the populations sampled. Orrell et al. (2005) determined that in the mid-Atlantic, and in Maryland, there was more than one independent introduction, and that no two introductions came from the same maternal source. As more snakehead were caught and identified and more genetic analyses were completed for the population, it became clear that there were in fact multiple introductions. However, additional DNA evidence is still required to create a more thorough picture of the snakehead population and its impact in the area (Orrell et al., 2005). Therefore, we aim to measure effective population size to predict genetic diversity.

Estimates of the effective genetic population size incorporate how a population reproduces and how, subsequently, the genetic diversity changes. It can also indicate a presence of past or ongoing selective pressure. For example, populations of snapper (*Pagrus auratus*) in New Zealand were sampled from 1950 to 1986, and in 1998, and their effective population size, N_e, was measured (Hauser, Adcock, Smith, Ramírez, & Carvalho, 2002). The estimated N_e of snapper showed temporal fluctuations in the allele frequencies and decreases in heterozygosity, and a low effective population size in an otherwise large snapper population, implying low genetic variation (Hauser et al., 2002). However, the snapper is a marine fish. In marine organisms, factors such as high, size-dependent fecundity and a strong bias in reproductive success may significantly reduce the N_e (Hauser et al., 2002). The effective population size can demonstrate the actual breeding population and help make predictions about the potential for populations to increase or decrease in size.

N_e can be estimated using microsatellites. Microsatellites are short segments of DNA composed of two to four nucleotides in a tandem repeat, for a total of up to a few hundred base pairs. They make for suitable genetic markers because they are extremely abundant and dispersed throughout the eukaryotic genome and have high levels of allelic variation. Microsatellites vary in length, as they are highly susceptible to length mutations at each locus. These mutations can be due to slipped-strand mispairing or slippage during DNA replication (Wright & Bentzen, 1994). The diversity and distribution of these mutations in microsatellites in a population is a direct measure of heterozygosity (Hamilton, 2009). We can use microsatellites associated with snakehead to determine the effective population size and other values of genetic diversity. These can help us determine how the population's genetics are evolving and whether the population is losing any genetic diversity (Luikart, Allendorf, Cornuet, & Sherwin, 1998). There are several factors that can influence effective population size in a species, perhaps the most important of which is admixture disequilibrium. Admixture disequilibrium occurs when more than one source population is introduced. Individuals from these separate populations interbreed, mixing the gene pools. Admixture can be beneficial to invasive species, increasing genetic variation and even causing novel genotypes with new trait combinations (Verhoeven, Macel, Wolfe & Biere, 2010). However, admixture disequilibrium can skew N_e results, causing the output to be lower than the actual N_e.

Since the introduction of snakehead to the Potomac River was a recent event, and the number of successful introductions is small, we believe that the effective population size will be significantly smaller than the actual population size. According to Frankham (1995), the "standard" N_e/N ratio for vertebrates is 0.1. We hypothesized that the Potomac River snakehead N_e/N ratio would be much lower. If no substantial difference exists between the effective population size and actual population size, this would suggest that either there were a much higher number of introductions or that the mutation rate in the snakehead is much higher than previously hypothesized.

3.2 Methodology

We used DNA microsatellite techniques to estimate the effective population size of northern snakehead in the Potomac River. King and Johnson identified 19 microsatellites from snakehead in Virginia that they applied to snakehead in New York. While there were multiple introductions of snakehead into the Potomac River system, the microsatellites were identified from fish in close proximity to the fish we analyzed and were used effectively for snakehead in New York, indicating that the microsatellites would be sufficient for our use (Orrell et al., 2005; King & Johnson, 2011). All loci used nuclear DNA. We did not use all 19 microsatellites in our analysis as a smaller number of loci were sufficient for an effective genetic diversity analysis (Lane, Symonds, & Ritchie, 2016; Zhu et al., 2014). We selected 12 microsatellite markers to ensure that we would obtain at least 9 workable loci.

Samples were obtained from the annual Potomac Snakehead Tournament and the MD DNR. Tournament samples were collected from the Potomac River and tributaries between Fort Washington and Wilson's Landing from May 31, 2014 to June 1, 2014. Samples from the MD DNR were collected from Pomonkey River from May to August 2014 (Appendix C, Figure 1). We obtained a total of 79 fin clips, which were stored in ethanol at 4^{o}C. Our team obtained an exemption from the University of Maryland, College Park IACUC as no team members were directly involved in snakehead harvest.

DNA Extraction

Twenty-five (25)-milligram sub samples were taken from the 79 preserved samples of snakehead collected in the Potomac River. Each small 25 mg sub sample was ground for efficient DNA extraction. DNA was extracted using the DNEasy Blood and Tissue kit from Qiagen (Hilden, Germany). Based on the kit protocol, we incubated the fin clips for at least 1 hour for proper lysing at 56^{o}C, while vortexing each sample every 30 to 45 minutes. We checked the quality of DNA extracted using a spectrophotometer. Our samples had an absorbance range between 1.77 and 2.08 µg/µl of DNA and the lowest concentration of our extractions was 97.3 ng/µl.

Microsatellite Analysis

Using the microsatellites identified from King and Johnson, we selected 12 markers to analyze based on the number of alleles they found per microsatellite. King and Johnson also identified the primers for each microsatellite and using those results, we ordered primers from Integrated DNA Technologies (Coralville, Iowa) with 6-FAM fluorescent tag on the 5' end primers of each pair. Each primer was diluted to a 5 µM working solution.

Microsatellites in the population were identified based on PCR of DNA fragments extracted from the 25 mg sub samples. PCR reagents (dNTPs, 10x Buffer, and Taq polymerase) were obtained from ClonTech (Mountain View, CA). Each reaction included 8.2 µl of ddH_2O, 1.2 µl dNTPs, 1.5 µl of 10x Buffer, 1.5 µl of the forward primer and reverse primer, 0.1 µl of Taq polymerase, and 1 µl of template DNA. The thermal cycle was programmed for 2 minutes at 94°C for the initial denaturation, followed by 30 cycles of 30 seconds of 94°C for denaturation, 1 minute at 55°C for annealing, 1 minute at 72°C for extension, with a final extension for 9 minutes at 72°C. Once the PCR was complete, 1 µl of each PCR reaction was transferred to 8.5 µl of HiDi (ThermoFisher Scientific, Waltham, MA) and 0.5 µl of ROX-350 ladder (ThermoFisher Scientific). The PCR products were sequenced in a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA).

3.3 Data Analysis

Results from the ABI 3730 xl were quantified using a custom MATLAB script that produced graphs which plotted the standards versus the sample data, and could be read to describe the hetero or homozygosity of each microsatellite in a sample (Appendix G). In order to compare samples and standards, standard ladders were rescaled from logarithmic to linear scaling using a Fourier transform to match the scaling of the sample data. The standard ladder was used as a method of reference for allelic size. The ladder determined whether or not samples were in the range required by a certain microsatellite. Each allele had to be within a specific size range in order to be analyzed.

Final graphs created by the code displayed the sample data, in blue, overlaid with the standard ladder, in red. The graphs were scored individually to determine heterozygosity versus homozygosity of genotypes. In order to score the data, each graph was analyzed and alleles were marked as heterozygous or homozygous based on frequency of allele presence. Each graph was printed and uniformly measured to the nearest 0.5mm in order to determine the alleles present.

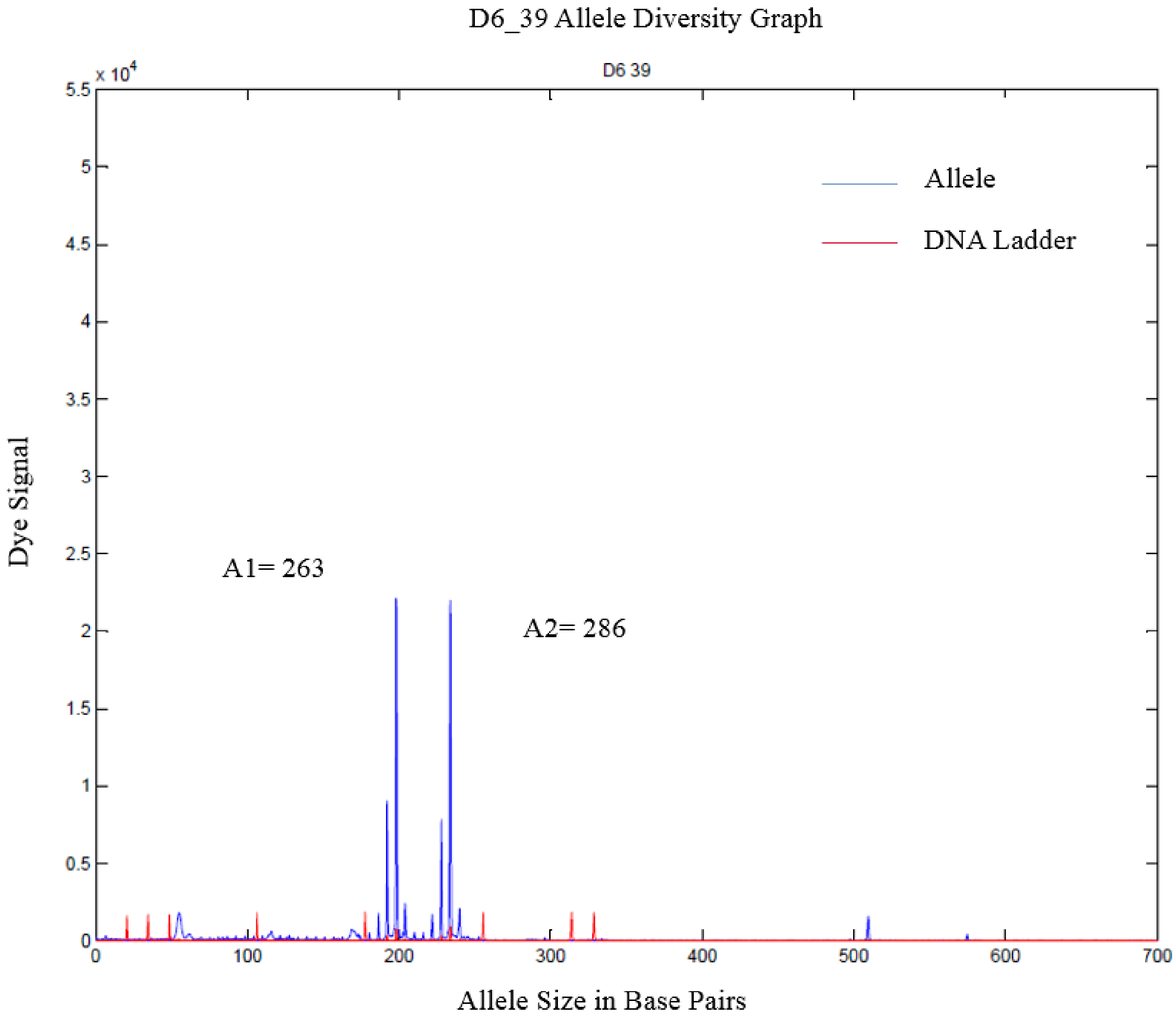

Figure 2
Graph depicting analysis of microsatellite D6 of fish 39. The blue peaks in Figure 2 represent the alleles present whereas the smaller red peaks represent the size standards from the DNA ladder.

Figure 2 presents a heterozygous fish with alleles A1 and A2 for the D6 microsatellite. (Figure 2; Appendix H, Figure 1). Each allele of each microsatellite was scored and consistently numbered based on its size. These numbers only serve to consistently identify and differentiate among the alleles for each microsatellite.

To calculate heterozygosity, we used package GenAlEx (v.6.5) in Microsoft Excel. We conducted an Fstat test under the codominant method. The effective population size (N_e) was estimated from the microsatellite allelic frequencies using the

N_e Estimator program (Molecular Fisheries Laboratory, V2.01, Queensland, Australia). There are three main single sample techniques for estimating N_e in NeEstimator: the linkage disequilibrium method, the heterozygote excess method, and the molecular co-ancestry method. The linkage disequilibrium with random mating method measures linkage disequilibrium between two loci by maximum likelihood from the diploid genotype frequency in a random mating population. This method is based on the fact that genetic drift can cause non-random association by chance among alleles in different loci. Haploid genotypes can be identified either from a sample of chromosomes from the population which are made homozygous or by test crossing. The estimate of D, the degree of linkage disequilibrium, has the same variance from haploids and diploids if both loci are codominant. The equation to calculate variance is

$$Var(D) = \frac{p(1-p)q(1-q)}{N}$$

where p and q are gene frequencies at the two loci being considered**.** With a dominance at either locus, the Var(D) is lower for haploid than diploid samples of the same size. This method is useful because N_{eb} can be estimated from a single cohort sample; however, one critical drawback to this method is the assumption that the population is an isolated equilibrium population with a constant effective size, which may not be always be tenable in the wild (Hill, 1981). An additional disadvantage of this method is that when a small N is used, the N_e may falsely suggest the presence of linkage disequilibrium.

The heterozygote excess method utilizes an expected excess of heterozygosity, caused by a binomial sampling error, in a population with a limited number of males and females to indirectly calculate an estimate of effective number of breeders. The small population's binomial sampling error produces male and female breeder allele frequency differences. The expected heterozygosity excess is estimated using the following equation:

$$H' = 2pq + \frac{pq}{n} = 2pz(1 + \frac{1}{2n})$$

This equation was manipulated to ascertain the effective number of breeding pairs. Again, this method requires only a single cohort and is easily computed, however the method has low precision, so there are fewer applications for this method (Pudovkin, Zaykin, & Hedgecock, 1996). This method is more accurate for populations with a very small N_e.

The molecular co-ancestry method utilizes two simulated population models, a non-inbred population which consists of non-inbred and non-related parents, and an inbred population that is composed of inbred and related parents. Both yield practically unbiased estimates of N_{eb} when applied to the non-inbred population. However, in the inbred population, this method gives a downward biased estimate, with a narrowed confidence interval compared with that in the non-inbred population. The estimate from the heterozygote-excess method is nearly unbiased in the inbred population, but has a larger confidence interval. When the estimates from both methods are combined as a harmonic mean, the reliability improves (Dadi, Tibbo, Takahashi, Nomura, Hanada, & Amano, 2008).

Microsatellite allele frequency data were assembled into a standard Fstat format for use in the software. Each allele was named using a two digit naming convention maintained from King and Johnson. The input file was identified, containing one population, eleven microsatellites, and five maximum alleles. We scored the graphs and used a plot of the relative frequency of sizes of observed microsatellites to determine the numbers and locations of alleles (Figure 3). As an example, for microsatellite D108, we determined allele A1 ranged from 113.7 to 116 and A2 ranged from 122.2 to 125. We then used these scores to estimate A1 to be 115 and A2 to be 123.

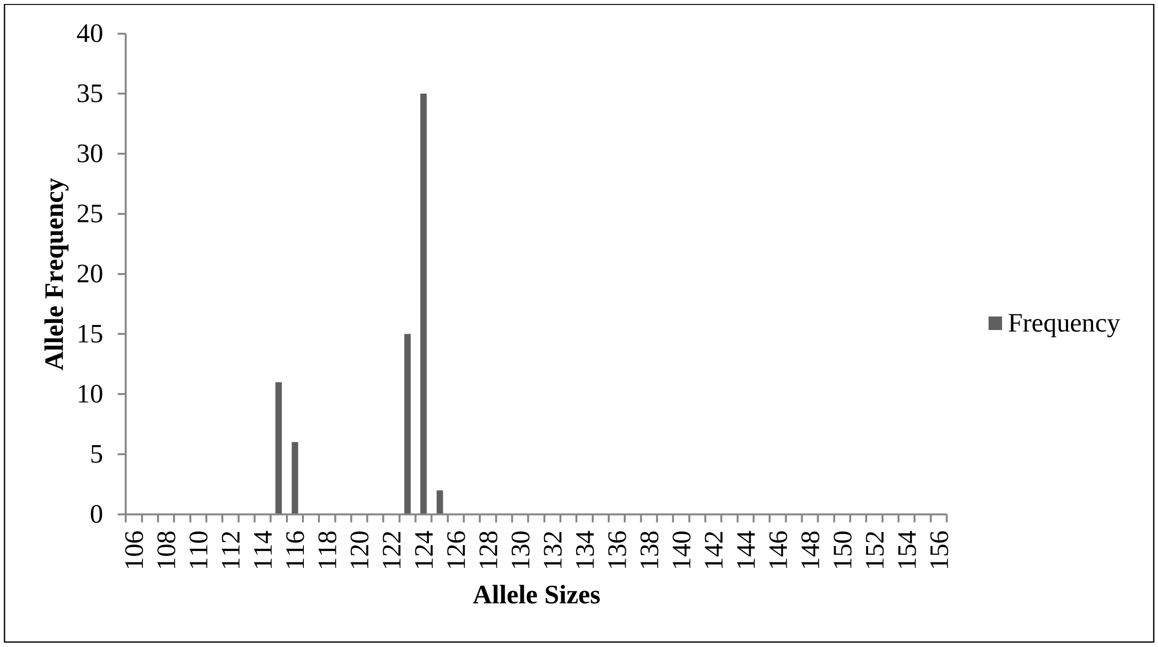

Figure 3
D108 allele size frequency.

Subsampling simulations of the allelic frequencies for the 12 microsatellites for 79 fish were used to determine whether we had sampled microsatellites and individual fish with sufficient intensity to obtain reliable estimates of N_e. First, N_e was estimated for varying numbers of alleles for all 79 individuals. Then, N_e was measured by varying the number of individuals against all 11 alleles. Each sampling group consisted of 25 random combinations, from which the average N_e and standard deviation were calculated and plotted.

3.4 Results

We tested 12 microsatellite loci and 11 of them were used in the analysis. One microsatellite - D129 - was excluded. D129 did not give us consistent results in our initial sampling. We were not able to discern individual alleles for this loci, and this loci was subsequently removed from the analysis.

We estimated a grand mean for observed heterozygosity (H_o) of 0.494) and 0.537 for expected heterozygosity (H_e). We also calculated N_{eb}, the heterozygosity excess. We found an observed N_{eb} of 0.494 ±0.069 (grand mean over all loci). However, N_{eb} is most useful when a population is at equilibrium. Since our population is likely not at Hardy-Weinberg equilibrium due to founder effects, we cannot tell from the N_{eb} if the alleles are associated because of disequilibrium and genetic drift or just due to a lack of genetic diversity.

Some of our loci had a higher heterozygosity than others. Some alleles were not in the range defined by King and Johnson (2011). Initial microsatellite sizes were based on the fish that King and Johnson characterized. We found alleles that had more tandem repeats than was described by King and Johnson (Table 1). This could be due to the fact that King & Johnson (2011) used only ten fish to characterize their microsatellites. It is also possible, but less likely, that there have been mutations in the snakehead population which have created new alleles in six of the microsatellites since King & Johnson performed their study.

Table 1
This table depicts the number of alleles, the number of expected alleles, the observed heterozygosity, and the expected heterozygosity for each loci.

	D6	**D119**	**D126**	**D138**	**D139**	**C6**	**C7**	**D108**	**D116**
Range	**257-327**	**273-345***	**216-288***	**326-433***	**235-281***	**213-244***	**253-305**	**106-156**	**244-300***

**	257-327	273-329	216-273	326-418	253-281	216-244	253-305	106-156	244-292
Na	2	5	4	2	2	2	5	2	3
Ne	1.938	3.667	3.641	1.893	1.914	1.440	3.317	1.947	1.841
Ho	0.359	0.772	0.603	0.338	0.338	0.325	0.848	0.329	0.539
He	0.484	0.727	0.725	0.472	0.477	0.306	0.699	0.486	0.457

*Ranges used for these loci are different than the ranges that King and Johnson have published
**This row denotes the ranges of allele size that King and Johnson found for the same microsatellites

We calculated minimum effective population size at 95% confidence intervals given the number of alleles sampled (Figure 4, Table 2). We also calculated minimum effective population size at 95% confidence intervals given the number of individuals sampled (Figure 5, Table 3).

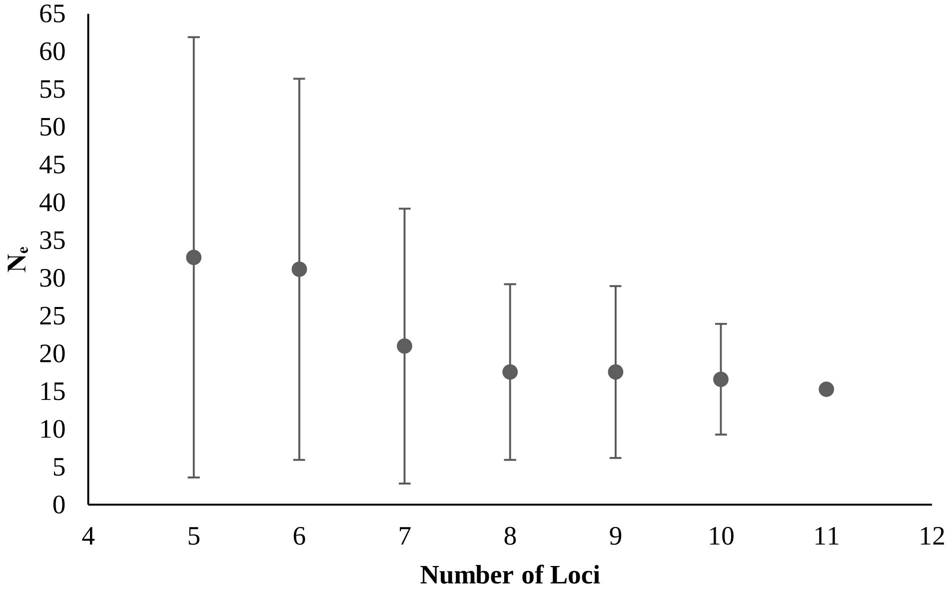

Figure 4
Number of loci vs. N_e using the linkage disequilibrium method at 95% confidence. Error bars at each point signify the standard deviation of N_e values found for each subsample.

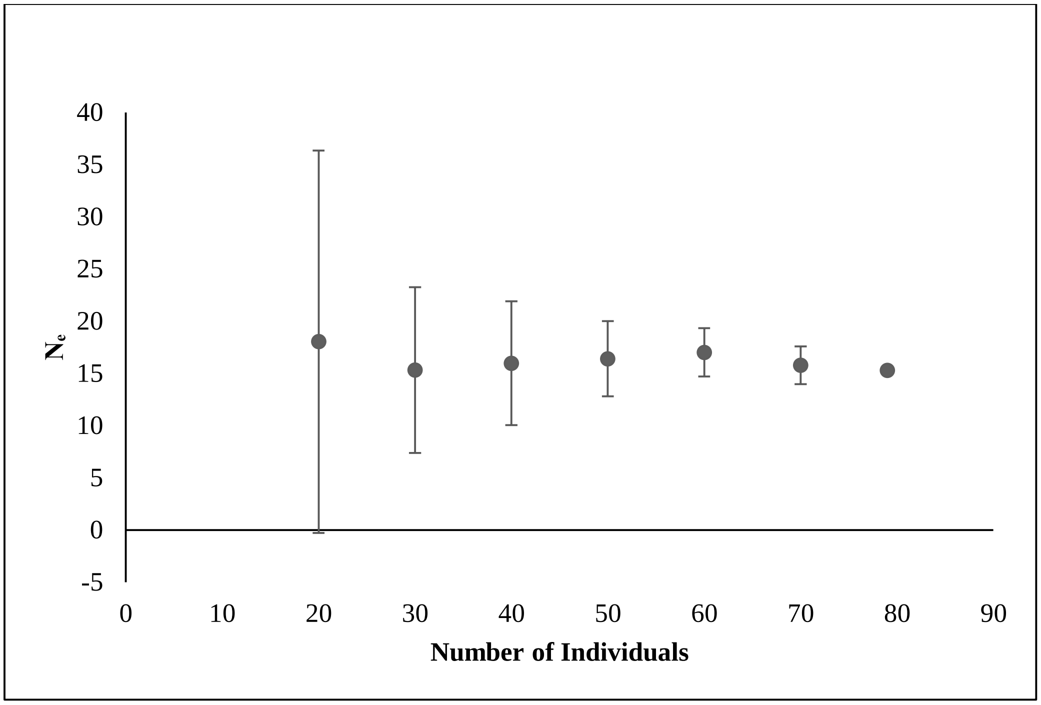

Figure 5
Number of individuals vs. N_e using the linkage disequilibrium method at 95% confidence. Error bars at each point signify the standard deviation of N_e values found for each subsample.

Table 2
Average N_e and standard deviations calculated from loci groups using all 79 individuals.

Number of Microsatellites	**LDM (.05)**		**LDM (.01)**		**MCM**	
	AVG	**STDEV**	**AVG**	**STDEV**	**AVG**	**STDEV**
5	32.756	29.14554	34.808	35.31443	16.54545	21.32802
6	31.192	25.2232	32.22	26.52094	13.12105	13.56407
7	21.012	18.18029	21.984	18.85574	10.52083	14.19498
8	17.592	11.62486	18.34	12.44836	7.55	8.663044
9	17.576	11.36754	18.348	12.14476	6.413043	5.356925
10	16.61818	7.321724	17.09091	7.682246	6.018182	5.252393
11	15.3	0	15.7	0	4	0

Table 3
Average N_e and standard deviations calculated from groups of individuals using all 11 loci.

Number of Individuals	**LDM (.05)**		**LDM (.01)**		**MCM**	
	AVG	**STDEV**	**AVG**	**STDEV**	**AVG**	**STDEV**
20	18.044	18.31145	20.408	19.77378	5.348	3.175311
30	15.328	7.933501	16.8	8.733365	5.512	4.261193
40	15.984	5.933779	18.148	7.203363	3.92	0.715891
50	16.412	3.60212	18.076	3.9027	4.26	0.857321
60	17.024	2.310534	17.36	2.028135	4.052	0.862709
70	15.7812	1.808453	16.116	1.699431	3.82	0.51316
79	15.3	0	15.7	0	4	0

Any population has a finite number of effective breeding pairs. We sought to determine if our sample size and number of microsatellites was appropriate for the chosen population. We used a sample of 79 fish with 11 microsatellites. The N_e estimation (Figure 4) begins to level off around 8 alleles; therefore, 11 alleles was a sufficient number of alleles to accurately estimate the minimum effective population size. Tables 2 and 3 demonstrate the same trend for each model tested: the linkage disequilibrium at 95% and 99% confidence, and the molecular coancestry method. We do not report results from the heterozygote excess method because the results were inconclusive. Increasing the number of individuals only served to reduce the standard deviation of the N_e estimates. Since the average N_e within each sampling group remained relatively constant, 79 individuals was a sufficient sample size (Figure 5).

Initially, we only used 47 fish in our analysis to determine N_e. Allelic frequency data from this small sample of fish yielded an estimate of N_e of 21.0 (95% CI: 10.8 - 46.6) from the linkage disequilibrium method. The jackknife interval had a lower bound of 6.0 and an upper bound of 127.8, which gives us a more robust confidence interval by averaging variances. This N_e appeared to be low based on the expansion of the snakehead previously reported by other researchers (Odenkirk & Owens, 2007; Love & Newhard, 2012). Consequently, we decided to use the remaining the 32 samples. Using all 79 samples, we obtained an N_e of 15.3 (95% CI, 3.8-44.1).

3.5 Discussion

Based on 79 tissue samples and 11 microsatellites, we estimated the effective size of the snakehead population in the Potomac River to be 15.3. Within our representative population of 79 fish, there are only 15 effective breeding pairs, suggesting that the genetic diversity of the snakehead population is low. If Orrell et al. (2005) are correct that there have been four introductions of snakehead at random from a large genepool, the maximum N_e possible at the time of introduction was 8, assuming each founding pair was heterozygous and did not share microsatellite alleles. The low value of N_e we estimate reflects the severe genetic bottleneck expected in an introduced species. Less than 15 years have passed since the first introduction of the snakehead into the Potomac system, and snakeheads mature at about 3 years old (Courtenay & Williams, 2004). Only 4 or 5 generations have matured since the original snakehead introduction, so there has been limited opportunity for genetic diversity to occur within the population. The snakehead census population size was estimated to be about 21,000 individuals in 2013 (Love et al., 2015). Together these estimates indicate a ratio of N_e/N of 0.00072, suggesting N_e is four orders of magnitude lower than N. King and Johnson (2011) calculated an N_e of 9.1 for a sample size of 22 fish in Meadow Lake, New York City, New York. It is important to consider that their N_e estimate, while small, is from a newer and more contained population of snakehead than that of the Potomac. King and Johnson tested 19 microsatellites, but their N_e reflects a sample of 22 fish. The difference in N_e estimates between these two locations suggests that the Potomac population is more diverse than the Meadow Lake population, likely due to a larger census size with more introductions. It is important to note that while a low N_e is cause for concern for endangered species, there is little evidence this study or in the literature that a low N_e is an indicator of limited longevity in invasive species.

We calculated a grand mean for observed heterozygosity (H_o) of 0.494 and an expected heterozygosity (H_e) of 0.537. Since these values are close, we can assume that no one allele is dominant across the microsatellites. H_o and H_e for each locus in the population is shown in Table 1. Zhu et al. (2014) measured genetic diversity for several wild populations of snakehead in its native range (China) found an average of 0.70-0.85. King and Johnson (2011) observed an expected average heterozygosity of 0.742.

Future studies should estimate N_e as well as heterozygosity. To enhance the understanding of the expansion and diversity of the snakehead population, we suggest continued microsatellite marker analysis across generations. These calculations should be done every few years to minimize generational overlap confusion (Hauser et al., 2002). As the snakehead population becomes more established, a series of N_e calculations over time will inform an increase, decrease, or stabilization in snakehead genetic diversity.

Our resampling simulations indicate that our sample size (n = 79) and number of microsatellites (11) were sufficient to provide accurate estimates of N_e with uncertainty. Future microsatellite studies should use at least 80 individuals. Although we are comfortable with our sample size of 79 due to minimal fluctuation of N_e results and an increasingly precise standard deviation, 80 samples is an appropriate benchmark. Studies should determine at least 8 usable loci, because N_e becomes more consistent. However, we would recommend using at least 11 or 12 microsatellites, to ensure that at least 8 loci are polymorphic. Future studies can also, as King and Johnson did, determine whether the population represented is at mutation-drift equilibrium. The Meadow Lake population was not at equilibrium at the time of King and Johnson's publication, due to introductory (founder) effects; because our population exhibits the same effects, we can infer that the Potomac population is not in equilibrium either. However, performing mutation-drift equilibrium tests as the population establishes itself will help determine if and when snakehead populations reach equilibrium after initial introduction.

Future snakehead genetic diversity research could be done by incorporating otoliths; a bone in fish which allows for the collection of information about the individual fish's age, as well as their life history patterns. Originally, our team hoped to collect data on the age of individuals from which we collected fin clippings for our genetic analysis. This data would have allowed us to create a life table and determine whether N_e had changed between younger fish (the most recent additions to the snakehead populations) and older fish (the original founders of the Potomac population). Sampling by age class would have better complied with the assumptions of our N_e estimation methods because we would have met the non-overlapping generation assumption and would have given us more accurate estimates. However, at the time of the initiation of our research, there had been no published literature on northern snakehead otoliths, and it would have been beyond the scope of our project to develop such a study.

It is worth noting that we considered separating our N_e analysis based on the fish samples from Pomonkey Creek and the samples attained from the Potomac River

Snakehead Tournament at Sweden Point. The reason we did not make such a separation was because we had less than 20 samples from Pomonkey Creek and from Figure 5, it was apparent that a randomly subsampled group of less than 20 fish would have had a variance that was too high for us to view the N_e results as reliable. Pomonkey Creek is about 7 miles from Sweden Point and we had no geographical locations for where the fish at the tournament were caught. Therefore we could not assume that the tournament fish were not caught in Pomonkey Creek.

In addition, our estimate of N_e does not take into account certain factors related to linkage disequilibrium. Linkage disequilibrium is influenced by multiple phenomena including genetic drift, mating systems, admixture, and null alleles among others. The mating systems of snakehead might lead to limited genetic exchange within the population. This may be the result of mating pairs persisting throughout multiple spawning seasons. However, since the mating systems of snakehead are still largely unknown, we could not effectively address this bias in our estimate. Furthermore, admixture in the population could have pulled down our N_e since two or more introductions of genetically distinct populations leads to linkage disequilibrium and therefore a skewed N_e. Null alleles occur when certain alleles do not amplify well during PCR or are very close to each other and individuals are scored as homozygous as a result. This decreases the genetic diversity in our sample and also acts to lowers our estimate of N_e. We were unable to address these factors in our assessment of N_e due to the software we used.

Our study should be repeated in other known snakehead regions, as well as across the entire snakehead range in the Potomac. We were only able to obtain samples from one region. Calculating the N_e across the Potomac will allow us to determine whether the population is homogeneous, or made up of subpopulations that rarely interact. The study can be repeated every generation using a single age class. This would imply estimates every three years. If the N_e continues to grow and the population diversifies, naturalization can be expected. Conversely, if snakehead population diversity stagnates or declines, it could suggest several outcomes. The snakehead may be unable to adapt to the Potomac River over an extended period; fishing or predatory pressures could increase, causing a high mortality rate that prevents further establishment. Continued research will inform snakehead management and, over time, define the status of the snakehead population in the Potomac.

Chapter 4

Conclusions and management recommendations on the Northern Snakehead in the Potomac River

According to the Snakehead Plan Development Committee, eradication is impossible where snakehead have already been established, especially in river systems such as the Potomac. As a result, it is more feasible to attempt to manage the snakehead population as opposed to attempting to eradicate it completely. In the future, given the spatial heterogeneity of the ecosystem, it is likely that the population size of the snakehead will continue to increase. To mitigate these effects, it is possible to use recreational anglers to aid in managing the snakehead population.

Since it is unlikely that snakehead can be eradicated from where they have already been established, it is important to increase the amount of recreational anglers fishing for them. Snakehead have been portrayed as a “bad” fish in Maryland and a case could be made to change that perception. To achieve a perception change, it might be beneficial to determine if it is appropriate to add snakehead to the Tidal and Non-Tidal Seasons, Minimum Sizes, Daily Creel & Possession Limits section of the Maryland Fishing Guide. There is already a size limit and creel limit set at “none,” and the season is set for year round, similar to the white perch. However, this information is in the invasive species section of the guide. The remarks column could express the fact that it is invasive and must be kept if caught, or mention that the fish is a good fighting fish with an appealing taste. Moving the snakehead to this section has the potential to influence anglers to view snakehead as a common fish to catch rather than only seeing it in the invasive species section. Giving the fish legitimacy could increase its popularity and increase the fishing pressure on the fish.

Since government resources are limited, it might be more effective to focus government control methods on areas where bass tournaments are held and encourage recreational fishing for snakehead in other areas. Focusing control methods such as electrofishing in tournament areas should reduce the population of snakehead in those areas. It is true that snakeheads from other areas may move in, but continued pressure should help keep their numbers low in the tournament areas. While there is no direct evidence that the snakehead population negatively impacts the bass population, because of the overlap in diet, there is a potential for harm to bass (Love & Newhard, 2012). These recommendations can be helpful if it is determined that snakehead do negatively impact bass populations and bass fishing.

We would recommend an increase in the efforts to remove invasive sub aquatic vegetation (SAVs) from the river system. Snakehead use invasive SAVs such as *Hydrilla verticillata* to make their floating nests as well as to hide their young. We suggest not only increasing the government's man hours devoted to removing the SAVs, but also increasing awareness of the problems with invasive SAVs. Possible events similar to the snakehead tournaments but focused on removing the SAVs from the river could be helpful in removing habitat that is needed for snakehead reproduction. Furthermore, removing invasive SAVs would promote growth of native SAVs in the Potomac, a crucial resource in the river ecosystem.

Our survey results indicate a correlation between snakehead population growth and largemouth bass yield as reported by anglers. As the population of snakehead has spread through the Potomac River system over the past few years, our survey reports that anglers have almost universally reported a decline in largemouth bass fishing yield. Thirty-one anglers who indicated largemouth bass as their target species reported a perceived decrease in largemouth bass availability, while two anglers reported a perceived increase.

This does not necessarily imply there is a definite causation-link between the two, but the considerable overlap in diet combined with the former data is something that should be considered when pursuing future areas of research.

Based on our genetic analysis, we found that our calculated N_e was very low with respect to the census size found in 2013. However, these populations and the total area inhabited by the snakehead have increased, and as such the snakehead spread does not appear to have been limited by low genetic diversity. Due to the low N_e/N ratio we measured for the Potomac River, we hypothesize that the snakehead may be limited in its ability to adapt to new pressures quickly.

Areas of future research should focus on recalculating N_e and heterozygosity over generations of snakehead. This is to determine stability in the snakehead's genetic diversity over time as the population becomes more established within the Potomac. Creation of a life table would also assist in understanding population dynamics over time. In areas where snakehead have recently been sighted, such as the Choptank River, agencies should determine if these populations are disjunct from previously established populations (Northern Snakehead Taskforce, 2015). Based on genetic analysis, we can determine if the Choptank population originated from a new introduction, if it is from the same population that is in the Potomac, or both. Resolution of this question is important because if the Choptank River population was seeded from the Potomac River, it may suggest that snakehead are able to tolerate higher levels of salinity than previously believed.

The suggested improvements to our genetic analysis and surveying techniques in conjunction with each other can improve the quality and scope of our research by further understanding the extent and range of snakehead within our local ecosystem. Having a life table developed in combination with knowledge of snakehead presence (partially determined by snakehead yield of anglers) and places of new introduction, as determined by our genetic analysis, can be used to focus on mitigating the emerging presence in those locations. In summation, the combination of future improvements to genetic analysis and surveying techniques as well as strategic fishing methods can contribute to the management of snakehead in affected river systems in this region.

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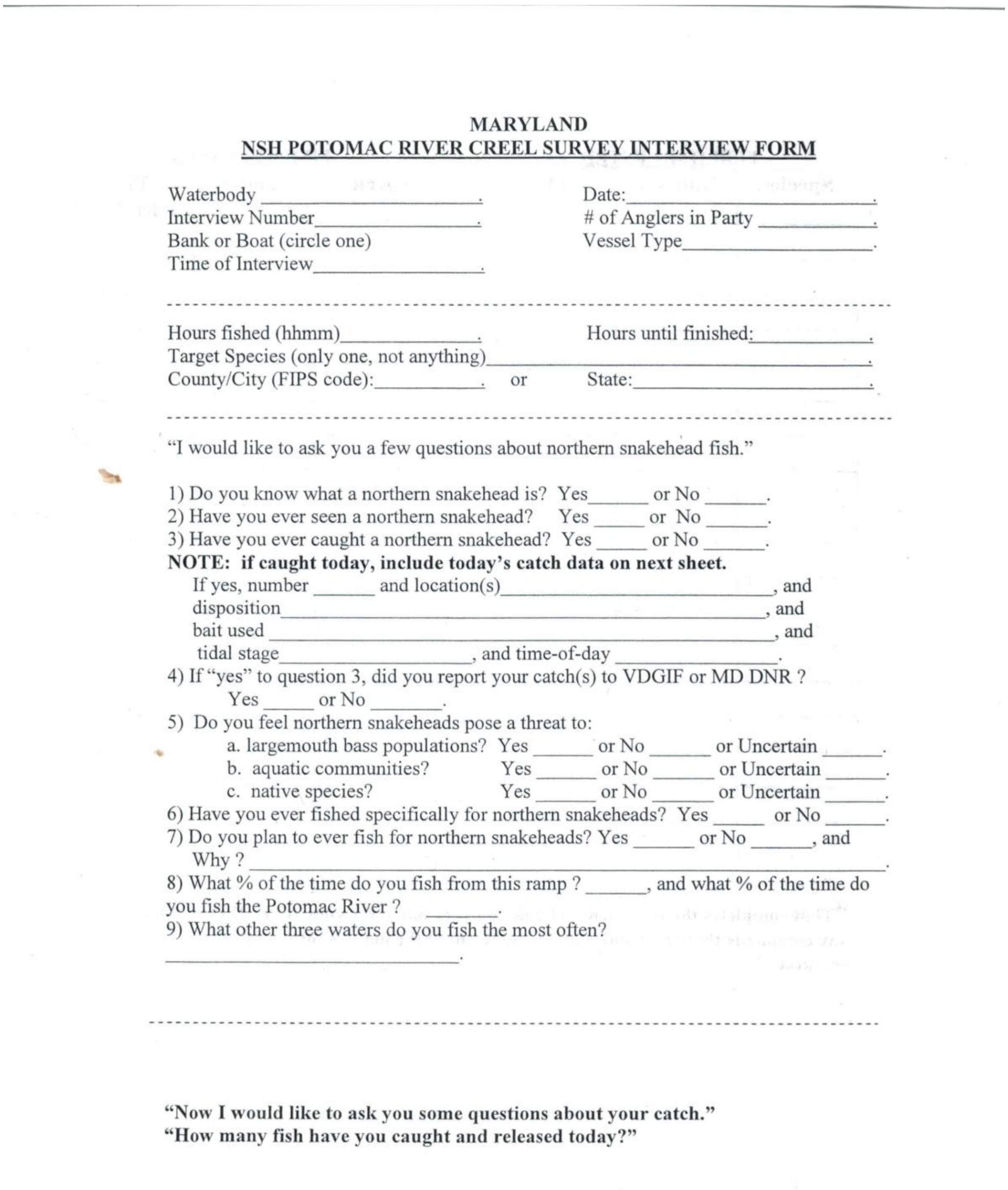
Appendix A
MFRO Potomac River Creel Survey



MARYLAND

NSH POTOMAC RIVER CREEL SURVEY INTERVIEW FORM

Waterbody ______________________.
Date:________________________.
Interview Number________________.
# of Anglers in Party ___________.
Bank or Boat (circle one)
Vessel Type__________________.
Time of Interview_________________.

Hours fished (hhmm)_____________.
Hours until finished:___________.
Target Species (only one, not anything)______________________________________.
County/City (FIPS code):__________. or State:_______________________.

"I would like to ask you a few questions about northern snakehead fish."

1) Do you know what a northern snakehead is? Yes______ or No ______.
2) Have you ever seen a northern snakehead? Yes _____ or No ______.
3) Have you ever caught a northern snakehead? Yes _____ or No ______.

NOTE: if caught today, include today's catch data on next sheet.

If yes, number ______ and location(s)__________________________, and
disposition____, and
bait used ____, and
tidal stage___________________, and time-of-day ________________.

4) If "yes" to question 3, did you report your catch(s) to VDGIF or MD DNR ?
Yes _____ or No _______.

5) Do you feel northern snakeheads pose a threat to:
a. largemouth bass populations? Yes ______ or No ______ or Uncertain ______.
b. aquatic communities? Yes ______ or No ______ or Uncertain ______.
c. native species? Yes ______ or No ______ or Uncertain ______.

6) Have you ever fished specifically for northern snakeheads? Yes _____ or No ______.
7) Do you plan to ever fish for northern snakeheads? Yes ______ or No ______, and
Why ? ____.
8) What % of the time do you fish from this ramp ? ______, and what % of the time do you fish the Potomac River ? _______.
9) What other three waters do you fish the most often?
____________________________.

"Now I would like to ask you some questions about your catch."
"How many fish have you caught and released today?"

FISH HARVESTED

Species	Number	TL

FISH RELEASED

Species	Number	TL GROUP

"That completes the interview. Thank you very much for your time. Do you have any comments that you would like to make about the management of this resource?"

Appendix B
Team SAVIOR Creel Survey

Potomac River Creel Intercept Survey

Waterbody____________________
Date_________________________
Interview Number______________
Returning___ or Going Out___
# of Anglers in Party____________
Time of Interview ______________

"I am an undergraduate student at the University of Maryland doing research on angler perceptions. I would like to ask you a few questions about your experience with fishing."

1) Are you 18 or older? Yes___ or No___

If yes to question 1, then continue survey. If no, then terminate survey.

2) Target Species (two, one, or not anything)
   ____
3) Have you noticed a significant difference in finding these fish this year as opposed to in the past 5 years? Yes______ or No______

If fish listed for question 1, if "not anything" skip to question 5.

4) Have you been catching more or less of [fish 1] in the past 5 years? More___ or Less___
5) Have you been catching more or less of [fish 2] in the past 5 years? More___ or Less___
6) Do you prefer catch & release or catch & keep policy? Keep___ or Release___
7) Which of the following styles do you fish with most often?
   a. Rod and Reel___
   b. Fly Fishing___
   c. Bowfishing___
   d. Other___
8) How long do you normally end up fishing on your average fishing trip? 1-2 hours___ 2-4 hours___ 4-6 hours___ or 6+___

"Now I would like to show you a few pictures and ask you to name the fish. You are not being scored on your correctness, we merely want to get your opinion." (Pictures at end of survey)

9) Correctly identified the snakehead? Yes___ or No___
10) Correctly identified the striped bass? Yes___ or No___
11) Correctly identified the smallmouth bass? Yes___ or No___
12) Correctly identified the bowfin? Yes___ or No___
13) Have you ever caught a snakehead? Yes___ or No___

If "no" for question 13, skip to question 18.

14) Did you catch it from this site? Yes___ or No___

If "yes," skip to question 16.

15) What area or body of water did you catch it from?

____

16) Have you caught a snakehead in the last…
    a. 6 months? Yes___ or No___
    b. Year? Yes___ or No___
    c. 2 years? Yes___ or No___
    d. 5 or more years? Yes___ or No___

17) Did you enjoy catching the snakehead?
Strongly agree___ Agree___ Neutral___ Disagree___ or Strongly Disagree___

18) Have you eaten a snakehead? Yes___ or No___

If "no," skip to question 20.

19) Would you recommend it? Yes___ or No___

20) Are you aware of any past or ongoing raffles involving snakehead?
Yes___ or No___

21) Would you ever consider fishing specifically for snakehead?
Yes___ or No___

22) If you could sell your snakehead catch commercially through legal channels, would you?
Yes___ or No___

23) Would you consider being hired as an independent government contractor to fish specifically for snakehead?
Yes___ or No___

"For the following questions, please respond with the statements with of the following responses: strongly agree, agree, neutral, disagree, or strongly disagree. Again, you are not being scored for correctness, we are merely recording your opinions."

24) The snakehead have a positive impact on the environment.
Strongly Agree___ Agree___ Neutral___ Disagree___ Strongly Disagree___

25) The snakehead have a positive impact on your fishing enjoyment.
Strongly Agree___ Agree___ Neutral___ Disagree___ Strongly Disagree___

26) The depiction of snakehead by the media is negative.
Strongly Agree___ Agree___ Neutral___ Disagree___ Strongly Disagree___

27) The depiction of snakehead by government agencies is positive.
Strongly Agree___ Agree___ Neutral___ Disagree___ Strongly Disagree___

28) Recreational anglers can have an impact on the control or management of a fish species.
Strongly Agree___ Agree___ Neutral___ Disagree___ Strongly Disagree___

29) A role of government agencies is to manage exotic species.
Strongly Agree___ Agree___ Neutral___ Disagree___ Strongly Disagree___

30) Exotic species should be allowed to naturalize in our local ecosystems.
Strongly Agree___ Agree___ Neutral___ Disagree___ Strongly Disagree___

31) The management of exotic species should be decided by the public. Strongly Agree___
Agree___ Neutral___ Disagree___ Strongly Disagree___

“That’s all the questions I have for you today. Thank you for participating in our survey and have a good day, [sir or ma’am].”

A.

B.

C.

D.

Appendix C
Survey Stratification & Locations

Table 1
Stratification of ramp locations and frequency of visits.

Ramp Location	Days Surveyed	Responses at site	Average response number	Ratio of responses	Converted ratio	Site ID	Distance (miles)
Sweden Point	12	148	12.334	0.341	34	1	43
Marshall Hall	14	71	5.071	0.140	14	2	26.4
Fort Washington	4	23	5.75	0.159	16	3	36.4
Slavens Ramp	5	15	3	0.083	8	4	38
Friendship Landing	1	7	7	0.194	20	5	48.2
Wilson's Landing	0	0	3	0.083	8	6	50.5
Total			36.156		100		

Table 2
Stratification of survey times.

Time of Day	Time of Day Probability	Day Type	Day Type Probability
Afternoon	0.8	Weekend	0.8
Morning	0.2	Weekday	0.2

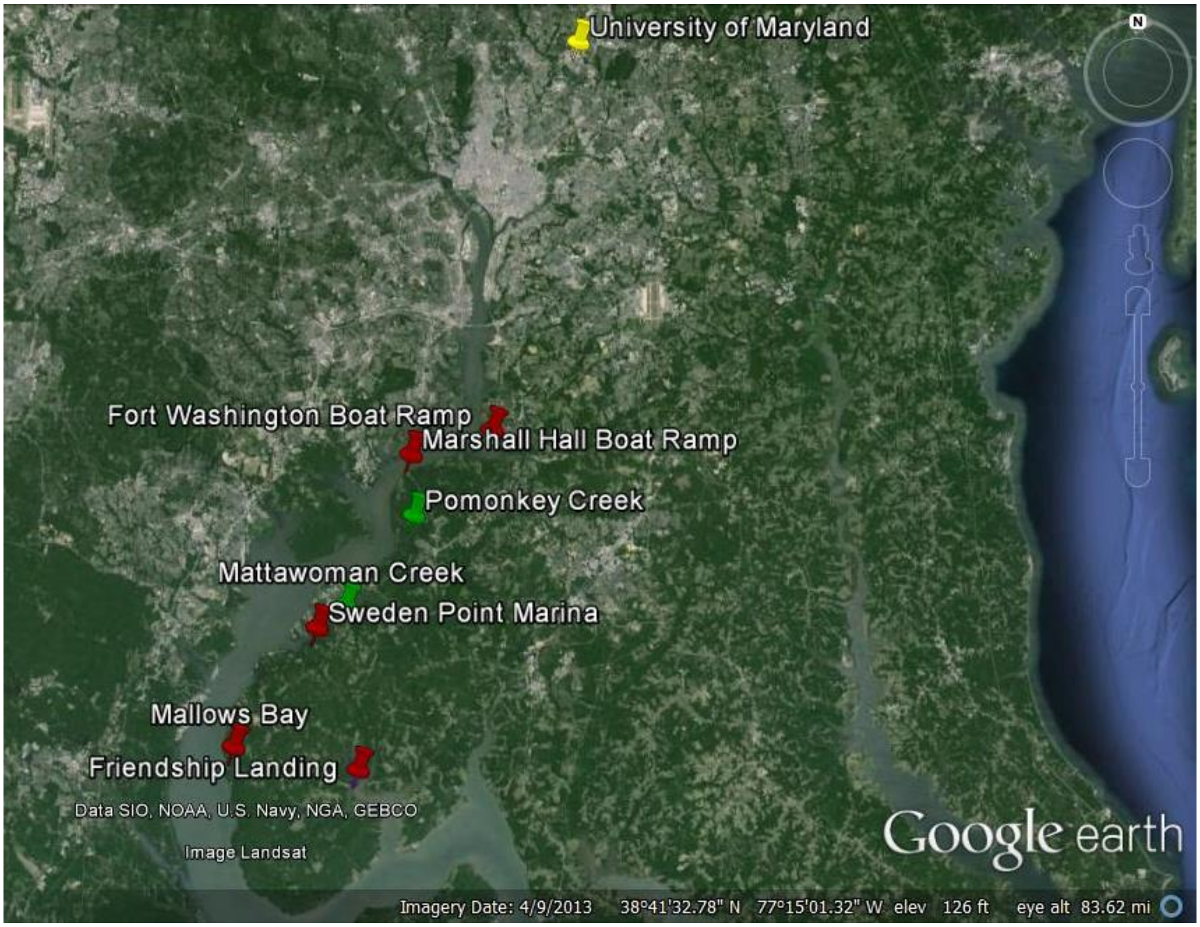


Figure 1

Map of Central Maryland delineating survey and sample sites along the Potomac River.
Red = survey site, green = fin clip sample site

Appendix D
Survey Schedule

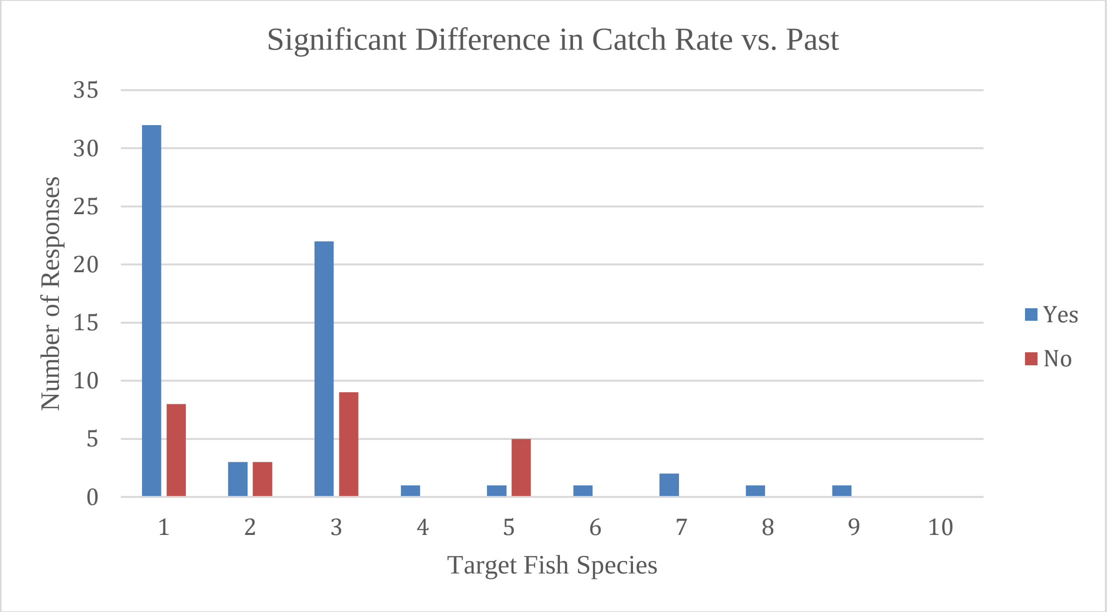
Table 1

Date	Time	Location	People
7-Jun-14	Late afternoon (3-6 pm)	Sweden Point	Trevor, Yasmine, Yvette
8-Jun-14	Early afternoon (12-3 pm)	Slavens Ramp	Brian, Isha, Zeke
10-Jun-14	Late afternoon	Friendship Landing	Greg, Trevor, Zeke
14-Jun-14	Late afternoon	Wilson Landing Road	Brian, Isha, Yvette
15-Jun-14	Late afternoon	Marshall Hall	Lauren, Trevor, Zeke
19-Jun-14	Late afternoon	Marshall Hall	Bobby, Greg, Zeke
21-Jun-14	Late afternoon	Sweden Point	Trevor, Yvette, Zeke
22-Jun-14	Late afternoon	Sweden Point	Bobby, Greg, Lauren
27-Jun-14	Late afternoon	Slavens Ramp	Bobby, Greg, Isha
28-Jun-14	Late afternoon	Fort Washington	Greg, Skyler, Zeke
29-Jun-14	Early afternoon	Sweden Point	Isha, Trevor, Yasmine
2-Jul-14	Late afternoon	Wilson Landing Road	Brian, Trevor, Yvette
5-Jul-14	Early afternoon	Sweden Point	Brian, Greg, Yvette
6-Jul-14	Late afternoon	Fort Washington	Brian, Greg, Zeke
12-Jul-14	Late afternoon	Slavens Ramp	Brian, Trevor, Zeke
13-Jul-14	Early afternoon	Fort Washington	Isha, Trevor, Yasmine
19-Jul-14	Early afternoon	Friendship Landing	Isha, Lauren, Skyler
20-Jul-14	Late afternoon	Sweden Point	Brian, Isha, Zeke
21-Jul-14	Late afternoon	Marshall Hall	Bobby, Brian, Greg
26-Jul-14	Late afternoon	Fort Washington	Brian, Trevor, Yasmine
27-Jul-14	Late afternoon	Sweden Point	Skyler, Yvette, Zeke
2-Aug-14	Late afternoon	Sweden Point	Brian, Isha, Yasmine
3-Aug-14	Late afternoon	Sweden Point	Lauren, Yvette, Zeke
9-Aug-14	Late afternoon	Slavens Ramp	Bobby, Greg, Yasmine
10-Aug-14	Late afternoon	Sweden Point	Lauren, Skyler, Yvette
13-Aug-14	Late afternoon	Marshall Hall	Brian, Isha, Yvette
16-Aug-14	Late afternoon	Friendship Landing	Isha, Trevor, Zeke
16-Aug-14	Late afternoon	Marshall Hall	Brian, Greg, Skyler
17-Aug-14	Early afternoon	Fort Washington	Lauren, Yasmine, Yvette
22-Aug-14	Late afternoon	Slavens Ramp	Bobby, Brian, Lauren, Zeke
23-Aug-14	Late afternoon	Friendship Landing	Isha, Yasmine, Yvette
24-Aug-14	Late afternoon	Wilson Landing Road	Bobby, Skyler, Zeke
6-Sep-14	Late afternoon	Sweden Point	Brian, Greg, Yvette
7-Sep-14	Late afternoon	Sweden Point	Bobby, Skyler, Natalie
7-Sep-14	Late afternoon	Friendship Landing	Lauren, Trevor, Zeke

Appendix E
Survey Response Coding Key

WTRBDY- Waterbody: Mattawoman Creek/Slavens Ramp/Mattingly Park (1), Sweden Point (2), Friendship Landing (3), Marshall Hall/Mallow's Bay (4), Wilson's Ramp (5), Fort Washington (6)
DATE- Date
INTVNMB- Interview Number
ANGSTAT- Returning (1) or Going Out (2)
ANGNUM- # of Anglers in Party
TIMINTV- Time of Interview
18OLD- Are you 18 or older? Yes (1) or No (2)
TARSPEC- Target Species: Largemouth Bass (1), Snakehead (2), Unspecified Bass (3), Smallmouth Bass (4), Unspecified Catfish (5), Rockfish/Striped Bass (6), Bluegill (7), Perch (8), Channel Catfish (9), Blue Catfish (10), Croaker (11), White Perch (12)
TARSPEC2- Target Species: Largemouth Bass (1), Snakehead (2). Bass (3), Smallmouth Bass (4), Catfish (5), Rockfish/Striped Bass (6), Bluegill (7), Perch (8), Channel Catfish (9), Blue Catfish (10), Croaker (11), White Perch (12)
SIGDIFF- Yes (1) or No (2)
MORE1- More (1) or Less (2)
MORE2- More (1) or Less (2)
CATCHSTY- Keep (1) or Release (2)
FISHSTY- Rod and Reel (1), Fly Fishing (2), Bowfishing (3), Other (4)
FISHTIME- 1-2 hours (1), 2-4 hours (2), 4-6 hours (3), or 6+ (4)
SNAKEID- Yes (1), No (2)
STRIPEID- Yes (1), No (2)
SMALLID- Yes (1), No (2)
BOWID- Yes (1), No (2)
CAUGHTS- Yes (1), No (2)
SITECATCH- Yes (1), No (2)
OTHSITE- Type response, we'll code once we see the responses
SCATCHT1- Yes (1), No (2)
SCATCHT2- Yes (1), No (2)
SCATCH3- Yes (1), No (2)
SCATCH4- Yes (1), No (2)
ENJCATCH- Strongly Agree (1), Agree (2), Neutral (3), Disagree (4), Strongly Disagree (5)
EATSNAKE- Yes (1), No (2)
ENJEATS- Yes (1), No (2)
SNAKERAF- Yes (1), No (2)
FISHSPEC- Yes (1), No (2)
SELLSNAKE- Yes (1), No (2)
HIREGOV- Yes (1), No (2)
SNAKEEnv- Strongly Agree (1), Agree (2), Neutral (3), Disagree (4), Strongly Disagree (5)
SNAKEEnj- Strongly Agree (1), Agree (2), Neutral (3), Disagree (4), Strongly Disagree (5)
SNAKEMed- Strongly Agree (1), Agree (2), Neutral (3), Disagree (4), Strongly Disagree (5)
SNAKEGov- Strongly Agree (1), Agree (2), Neutral (3), Disagree (4), Strongly Disagree (5)
SNAKECont- Strongly Agree (1), Agree (2), Neutral (3), Disagree (4), Strongly Disagree (5)
SNAKEMan- Strongly Agree (1), Agree (2), Neutral (3), Disagree (4), Strongly Disagree (5)
SNAKENature- Strongly Agree (1), Agree (2), Neutral (3), Disagree (4), Strongly Disagree (5)
SNAKEPub- Strongly Agree (1), Agree (2), Neutral (3), Disagree (4), Strongly Disagree (5)

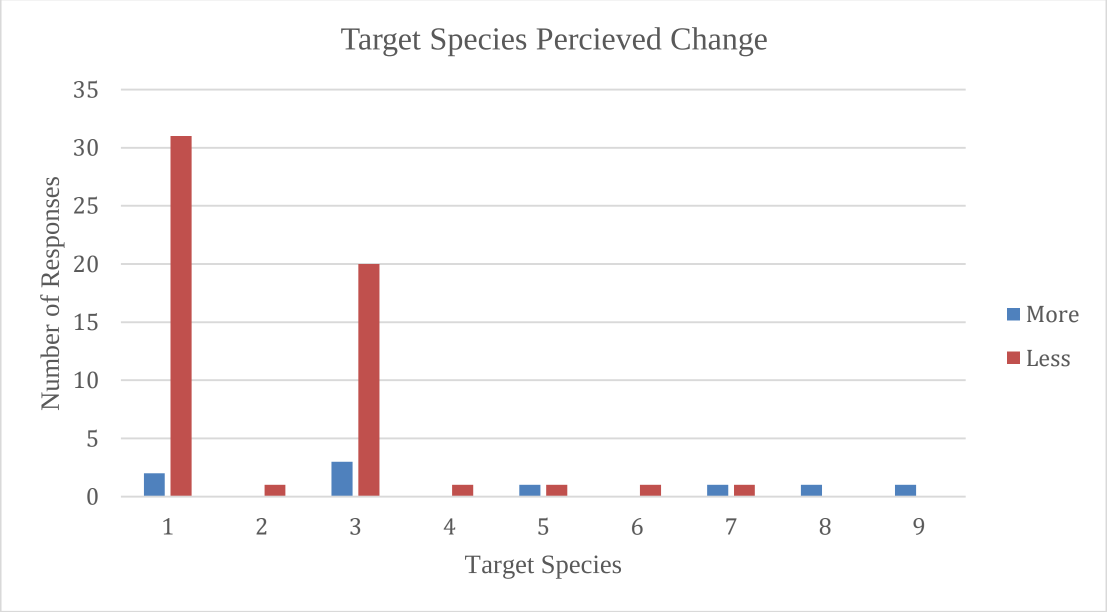
Appendix F
Angler Perceived Catch Changes



1=Largemouth Bass, 2= Snakehead, 3=Unspecified Bass, 4= Smallmouth Bass, 5=Unspecified Catfish, 6=Rockfish, 7=Bluegill, 8=White Perch, 9=Blue Catfish, 10=Croaker

Figure 1

Depicts if anglers perceived a change in target species catch rate.



1= Largemouth Bass, 2=Snakehead, 3=Unspecified Bass, 4=Smallmouth Bass, 5= Unspecified Catfish, 6=Rockfish, 7=White Perch, 8=Blue Catfish, 9=Croaker

Figure 2

Depicts if anglers perceived a higher or lower catch rate in their target species.

Appendix G
MATLAB Script

```
%% D126_92
% script file to extract data from AB 3730XL converted data file

%%
filename=’file location here’;

[s]=xml2struct(filename);

dye1txt=s.Children(6).Children(14).Children(4).Children.Data(:,:);
dye1dat=str2num(s.Children(6).Children(14).Children(12).Children.Data(:
,:))';
dye2txt=s.Children(6).Children(16).Children(4).Children.Data(:,:);
dye2dat=str2num(s.Children(6).Children(16).Children(12).Children.Data(:
,:))';
dye3txt=s.Children(6).Children(18).Children(4).Children.Data(:,:);
dye3dat=
str2num(s.Children(6).Children(18).Children(12).Children.Data(:,:))';
dye4txt=s.Children(6).Children(20).Children(4).Children.Data(:,:);
dye4dat=str2num(s.Children(6).Children(20).Children(12).Children.Data(:
,:))';

xdat=1:length(dye1dat);
xdat=xdat';
dyedat=horzcat(xdat,dye1dat,dye2dat,dye3dat,dye4dat);
%%
% now plot it
Init_fig_pos=[180 378];
Fig_size=[400 400];
Raw_fig=figure(1);
set(Raw_fig,'Position',[Init_fig_pos(1),Init_fig_pos(2),Fig_size(1),Fig
_size(2)]);
title('Raw Data');

plot(dyedat(:,1),dyedat(:,2),'-b')
title('D39 12');
hold on
plot(dyedat(:,1),dyedat(:,3),'-g')
plot(dyedat(:,1),dyedat(:,4),'c')
plot(dyedat(:,1),dyedat(:,5),'r')
legend('sample 1', 'sample 2', 'sample 3', 'standards')
hold off

%%
% now we have to rescale the x-axis based on the standard curve and
replot
% looking at the last dye that has the standards on it
 data=max(dyedat(:,5),0);

 % find the peaks
 [pks,locs]=findpeaks(data,'Minpeakheight',500);
```

```
 % plot the peaks to make sure they look ok
 Scale_fig=figure(2)

set(Scale_fig,'Position',[(Init_fig_pos(1)+Fig_size(1)),Init_fig_pos(2)
,Fig_size(1),Fig_size(2)]);

 plot(xdat,data,'r',locs,pks,'go')
 title('identified peaks');

 % now rescale the x axis based on the size standard used.
 % size standards from GS 350 are
 %  35   50 75 100 139  150  160  200  250  300  340  350
 % assume these are the largest 12 points found in locs

 gs=[35 50 75 100 139  150  160  200  250  300  340  350]';
 peak_locs=locs(length(locs)-11:length(locs));

 % now do the regression
 mdl=polyfit(peak_locs,gs,1);

 %rescale the x-axis
 scaled_x=mdl(2)+mdl(1).*dyedat(:,1);

 scaled_dyedat=dyedat;
 scaled_dyedat(:,1)=scaled_x;

 % only carry through that portion of data with >=0 x values
scaled_dyedat=scaled_dyedat(scaled_dyedat(:,1)>0,:);
% now delete all -ve values
scaled_dyedat=max(scaled_dyedat(:,:),0);

 % now replot
Rescaled_fig=figure(3)
set(Rescaled_fig,'Position',[Init_fig_pos(1)+2.*Fig_size(1),Init_fig_po
s(2),Fig_size(1),Fig_size(2)])

plot(scaled_dyedat(:,1),scaled_dyedat(:,2),'-b')
title('D126 92 RR');
axis([0 700 0 55000])
hold on
% plot(scaled_dyedat(:,1),scaled_dyedat(:,3),'-g')
% plot(scaled_dyedat(:,1),scaled_dyedat(:,4),'c')
plot(scaled_dyedat(:,1),scaled_dyedat(:,5),'r')
legend('sample 1','sample 3', 'standards');
hold off

%% Now extract the size information of the specific microsats on dye 3

microsat_data=scaled_dyedat(:,4);

[microsat_pks,microsat_locs]=findpeaks(microsat_data,'Minpeakheight',10
00);

Micro_bp=scaled_dyedat([microsat_locs],1)
```

Appendix H
Microsatellite Allele Scoring Methods

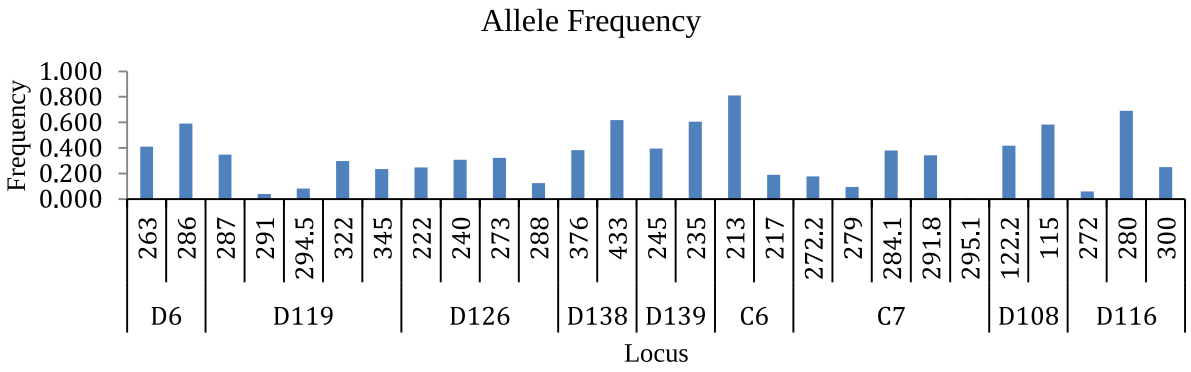


Figure 1
Allele frequencies are presented by locus, divided according to allelic sizes.