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Invasion Genetics of the Blue Catfish (Ictalurus Furcatus) Range Expansion into Large River Ecosystems of the Chesapeake Bay Watershed

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INVASION GENETICS OF THE BLUE CATFISH (*ICTALURUS FURCATUS*) RANGE EXPANSION INTO LARGE RIVER ECOSYSTEMS OF THE CHESAPEAKE BAY WATERSHED

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University

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Abstract

INVASION GENETICS OF THE BLUE CATFISH (*ICTALURUS FURCATUS*) RANGE EXPANSION INTO LARGE RIVER ECOSYSTEMS OF THE CHESAPEAKE BAY WATERSHED

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The blue catfish, *Ictalurus furcatus* (Ictaluridae), is ranked among the most invasive, non-native species of concern in the Chesapeake Bay watershed. This species, intentionally introduced to three major tributaries and a number of impoundments between 1974 and 1989 for sport fishing, has spread into three additional tributaries. Using samples from the introduced tributary populations as a baseline, we evaluated microsatellite genetic variation in light of demographic and ecological data to elucidate the potential sources of the invasive *I. furcatus* populations. In general, the populations surveyed in the Chesapeake Bay watershed were considerably more inbred (F ranged from 0.03 – 0.27) than four native populations (all $F = 0.03$) and they exhibited 12% lower allelic diversity

than native populations, showing evidence consistent with a founder effect. Lack of evidence for significant bottlenecks combined with high effective migration rates suggested that there may be a great deal more movement of this species within the Bay than was previously thought. Two proposed scenarios for expansion (dispersal from introduced populations and intentional surreptitious introductions) were evaluated. Although not inconceivable, genetic evidence did not support the Bubba mechanism as the primary mode of expansion and dispersal was found to be the most probable mode underlying the recent range expansion. However, a number of characteristics of the population genetic and mixed stock analyses indicate that a separate scenario, escapement from impoundments, is worth investigating as a substantial source of the expansion. The study has important implications for ecosystem-based management because it is the first application of mixed stock analysis to an invasive species.

INTRODUCTION

The negative impact of invasive species on native species diversity, ecological communities and ecosystem functioning has been recognized as a significant component of current global change (Vitousek et al. 1996). It has been estimated that 42% of species listed as threatened or endangered in the U.S. under the Endangered Species Act are at risk due to the presence of nonindigenous species (Wilcove et al 1998). Aside from the threat to biodiversity around the world, it has become increasingly apparent that these losses have economic consequences as well. A recent study (Pimentel et al. 2000) estimates that the ecological and economic costs in the U.S. due to invasive species are approximately $137 billion per year. In response to executive order #13112, federal, state, and local agencies have been working to complete risk analyses and craft management plans that will control and minimize the impacts of invasive species. In the specific instance of aquatic species, controlling invasives is all the more challenging because of cross-purpose activities of native and nonnative sport fish management (Clarkson et al. 2005). Declines in native freshwater ichthyofauna in the southwestern U.S. over the past 20 years have been attributed to the presence of nonnative sport fish; effectively precluding or negating restoration efforts (Mueller 2005). Understanding the genetic architecture of a successful and expanding invasive species population offers insight into the role of genetic diversity in invasion success (Baker and Stebbins 1964) and more importantly can provide information about the sources of recent range expansions that can be used in turn to predict how they might continue to spread.

Blue catfish in the Chesapeake Bay watershed

Among the most invasive species in the Chesapeake Bay watershed is the blue catfish, *Ictalurus furcatus* (Ictaluridae). This species is ranked in the top five "species of concern" in Virginia, also as a high priority in Maryland, by the U.S. Environmental Protection Agency's Chesapeake Bay Program, and was identified as a species for which a risk assessment plan is needed (Moser 2002). As a sport fishing enhancement measure, the Virginia Department of Game and Inland Fisheries and the US Fish and Wildlife Service introduced *I. furcatus* into 70 impoundments and reservoirs in Virginia (>330,000 fingerlings between 1981 and 1989) and into the James, Rappahannock, and Mattaponi Rivers (>130,000 fingerlings between 1974 and 1989; Table 1). Until the early 1990s, *I. furcatus* were documented only in the river systems where they had been introduced. Recently, breeding populations of *I. furcatus* have been recorded in three additional rivers: Pamunkey, upper Potomac, and Piankatank (Edmonds 2003) effectively extending their range to all major tributaries in the Virginia portion of Chesapeake Bay (Figure 1).

Ictaluridae is the largest freshwater family of fishes endemic to North America. Its broad native distribution (Graham 1999) includes large rivers of the Mississippi, Missouri, and Ohio River basins and coastal drainages of the Gulf of Mexico from Alabama and into the Rio Grande extending south into Mexico, Belize and Northern Guatemala (Etnier and Starnes 1993). Unlike the introduced Chesapeake Bay populations, *I. furcatus* in their native range have experienced an overall decline in abundance and a contracting range due to the construction of impoundments, channelization and increases in siltation (Graham 1999). Although *I. furcatus* inhabit

primarily deep swift flowing areas of large rivers and lakes in their native ranges (Etnier and Starnes 1993), they have been observed in tributaries of the Bay to inhabit even shallow creeks (R. Greenlee, VDGIF, personal communication). *I. furcatus* have a wide salinity tolerance, and have been observed inhabiting waters ranging in salinity from 3.7 ppt to 15 ppt (Ross 2001). *I. furcatus* are the most migratory of the ictalurids, moving in response to water temperatures and have demonstrated the ability to move great distances in search of spawning habitat (Graham 1999). Nests are built in sheltered areas, protected by either the male or both sexes; no other North American freshwater fish is known to provide the same level of parental care. Life span is known to exceed 29 years (Graham 1999).

Although highly adaptable in their feeding habits, three general feeding stages have been determined for *I. furcatus* based on size and age classes. As young (<100 mm) they feed primarily on zooplankton, as juveniles (up to 240 mm) they feed on small benthic invertebrates, and as adults, they feed on larger and more mobile organisms becoming primarily nocturnal piscivores as adults (Ross et al. 2004). In the Bay, *I. furcatus* growth rates are dependent upon the amount of biomass consumed (Chandler 1998) and studies of native populations indicate that growth rates increase substantially after they reach a piscivorous state (Graham 1999). Known for the ability to grow impressively large and for their aggressive nature, *I. furcatus* is a desirable species for recreational and commercial fishing. These were the primary justifications for introducing *I. furcatus* to lakes and tributaries in the Chesapeake Bay watershed beginning in 1974.

The introduction of piscivorous *I. furcatus* in Virginia has been associated with declines in anadromous clupeid populations of American shad (*Alsoa sapidissima*) and blueback herring (*A. aestivalis*), possibly compromising major restoration programs, and adding to the documented negative economic and ecological effects of invasive species range expansion (Ashley and Buff 1987, MacAvoy et al. 2000). Among the deleterious impacts on native aquatic communities is the alteration of habitats, especially by nest building species such as *I. furcatus* (Courtenay and Stauffer 1984). Alteration of Chesapeake Bay tributaries from historically bottom-up biomass controlled processes to one that is 'top heavy' with predators has been suggested to be a serious consequence of the introduction and spread of *I. furcatus* (Garman et al. 1991).

Assessing modes of invasion

In 1974-1977, James and Rappahannock Rivers were stocked with assemblages of *I. furcatus* collected from a number of hatcheries outside the state (121,950 fish, Table1). During the period 1981-1985, stocking efforts concentrated on impoundments, nine of them located in the Potomac River basin. Of more than 79,000 *I. furcatus* stocked into impoundments in the Potomac basin, the majority (78%) were introduced in 1985 and consisted of the same group of fish that were also stocked into the Mattaponi and James Rivers in that year (1,850 and 13,764 fish, respectively; Table 1). Two years later, 1987, *I. furcatus* were noted in upper Potomac River (Nammack and Fulton 1987), likely a result of escapement from the stocked impoundments. Similarly, the Pamunkey *I. furcatus* population (first collected in 1994) is presumed to have arisen from the Mattaponi via dispersal. However, the Piankatank River invasion, believed to have

occurred within the last five years (G. Garman, personal communication), is a novel system in which the source of colonization is more equivocal. A number of scenarios have been suggested to account for the appearance of *I. furcatus* populations found outside of their introduced range including intentional transplantation of live fish harvested from nearby tributaries, dispersal from one tributary to another via the Chesapeake Bay, and escapement from impoundments.

This study was designed to make use of inherent genetic variation to evaluate the potential sources of the secondary *I. furcatus* populations in the Bay and to investigate the role of genetic diversity during the invasion. The utility of genetic mixed stock analysis for elaborating the source(s) of an invasive species range expansion is also investigated. In this study we posed two primary invasion scenarios regarding the sources of the secondary populations: (1) *Dispersal:* recruits moved from a nearby stocked river through the Bay during periods of significant freshwater influx, and (2) *Bubba*: the *I. furcatus* range expansion was intentionally facilitated by anglers or commercial fisherman. A third possibility, e*scapement* from nearby impoundments resulting in development of secondary *I. furcatus* populations could not be tested because samples could not be obtained from the impoundments. Hypotheses concerning the origin of the 'secondary' *I. furcatus* populations (Pamunkey, Potomac, and Piankatank Rivers) in the Chesapeake Bay watershed were tested by comparing population genetic variation at six polymorphic microsatellite loci to the 'introduced' populations (James, Rappahannock, and Mattaponi Rivers), as well as four native populations (Alabama, Mississippi, Ohio, and Tennessee Rivers). Genetic architecture of the Bay populations was then brought into focus using population genetic analyses and genetic Mixed Stock

Analysis (MSA). These comparisons were used to search for evidence of relatedness, founder effects, and genetic drift. Ultimately, this study was intended to provide information regarding to two issues relevant to invasive species management: will *I furcatus* remain in the tributaries they currently occupy or continue to expand, and is MSA an effective tool for determining the source(s) of the secondary populations and therefore useful in assessing risk for invasive species management?

A number of assumptions were embedded within the analyses. That *I. furcatus* in Chesapeake Bay represent one large panmictic population was the null hypothesis being tested by population genetic analyses. However, it was expected that populations of *I. furcatus* in the original introduced populations (James, Rappahannock, Mattaponi) would differ significantly because it is believed that they have not interbred over the past 20-30 years since their introduction. The Pamunkey River was expected under the dispersal scenario to have a high degree of genetic similarity to the Mattaponi population and to have less genetic diversity as compared to the Mattaponi. Conversely, the other secondarily colonized populations (Potomac and Piankatank Rivers) were expected to show various degrees of relatedness to source populations depending on geographic distance and length of time since colonization. It was expected that genetic diversity would be greater in the "ancestral" introduced populations as compared to the secondary populations at the furthest reaches of the expanded range. As observed for a number of other species (Marsden et al. 1996, Pollux et al. 2003, Elderkin et al. 2001, Lewis et al. 2000, Marsden et al. 1995), loss of genetic diversity due to founder effects was expected in each of the secondary populations. Loss of heterozygosity, shifts in allele and genotype frequencies, genetic drift, and allele fixation were expected to be observed in

the comparison to both the native and introduced populations, especially in the most recent populations of the Potomac and Piankatank Rivers.

Using MSA to elaborate the sources of range expansion

Originally developed for application in fisheries management, MSA provides statistical estimates of the presence and relative proportions of specific contributing populations in mixture samples. Mixture proportion estimates are determined using all known source populations to produce baseline allele frequencies against which the mixture populations in question are compared. For such an analysis, large sample sizes and multiple independent polymorphic loci are necessary for calculations of baseline data and mixture estimates. The application of mtDNA and microsatellite variation in MSA has been successfully performed to address mixed stock harvesting of several anadromous fish species, American Shad (*Alosa sapidissima*, Epifanio et al. 1995, Brown et al. 1996, Brown et al. 1999), sockeye salmon (*Oncorhynchus nerka*, Beacham and Wood 1999), and Atlantic cod (*Gadus morhua*) (Ruzzante et al. 2000). Genetic MSA also has been applied in conservation studies of migratory species such as harbour porpoises (*Phocoena phocoena*, Anderson et al. 2001) in the north Atlantic and in Loggerhead sea turtles (*Caretta caretta*, Witzell et al. 2002) in Florida. Although it is a novel application, use of MSA to evaluate the current range expansion of *I. furcatus* is the best currently available tool to elucidate the phenomenon as it is unfolding in the Chesapeake Bay watershed and constitutes an informative case study in the investigation of invasive species.

Ecological genetic patterns expected in a range expansion

The relative roles that genetic drift plays in determining variation patterns in allele frequencies of native versus introduced populations are inconclusive (Antonovics 1976, Crawley 1986, Lindholm et al. 2005). More important may be life history, founder population size, the number and frequency of introductions, and the spatial distribution of the invasion are important factors to consider when comparing the genetic diversity of the source and colonizing populations (Gray 1986). Nevertheless, the two scenarios have been hypothesized to account for secondary *I. furcatus* populations have predictable characteristics that are testable with population genetic and mixed stock analyses:

1) Dispersal scenario

Prior studies have demonstrated high gene flow accompanied by an initial loss of genetic diversity in introduced populations and further losses as an introduced species expands its range (Marsden et al. 1995, Lewis et al. 2000, Pollux et al. 2003). A colonized population, such as the one in Pamunkey River, is therefore expected to be less genetically diverse than the source population from which it originated (a population bottleneck; Sakai et al. 2001). Novel populations would be characterized by high estimates of gene flow with the founding population(s), higher levels of inbreeding (F_{IS}) than the founder(s), and percent composition would be heavily weighted for one major source.

2) Bubba scenario

Given the extremely low number of individuals likely to be involved in a small-scale intentional introduction, such as has been suggested to be the sole source of the Potomac and Piankatank River populations, a severe genetic bottleneck is expected. This scenario

would be characterized by a sharp reduction in allelic diversity. The secondary population would show greatest similarity to a nearby population, would have a percent composition estimate that would be heavily weighted for a single major source, and would exhibit high levels of inbreeding (F_{IS}).

MATERIALS AND METHODS

Sample collection

Samples were obtained during the summer of 2004, from six *I. furcatus* populations in Chesapeake Bay tributaries using a combination of high and low frequency electrofishing and from four native populations using gill net and electrofishing (Table 2). Samples were stored in 70% isopopanol at the site of collection.

Microsatellite identification and optimization

Twenty-two published microsatellite sequences for *I. punctatus* were surveyed to determine levels of polymorphism in *I. furcatus*. In addition, a microsatellite-enriched library was prepared from a mixture of 5 μg of total nucleic acid pooled from several *I. furcatus* specimens that was then digested with *Sau*3AI, ligated to linkers, and hybridized to a cocktail of biotinylated tandem repeat oligonucleotides [$(AAC)_{11}$, $(GAAT)_{10}$, $(ACAT)_{11}$, $(AAAG)_{11}$, $(GTA)_{15}$ and $(AAT)_{15}$]. Coupled molecules were separated from non-repeat sequences using avidin, PCR repaired, and TA-cloned with the TOPO™ vector (Invitrogen). Approximately 100 colonies with inserts were picked and subjected to PCR using M13 primers. Appropriately sized amplicons (500-1200 bp) were sequenced in both directions resulting in a suite of 20 repeat-containing sequences.

DNA preparation and genotyping

For each specimen, DNA was extracted from 50 mg of tissue using the PureGene™ method. Three primer sets were directly labeled with FAM, TET, or HEX, and three others were modified as described by Boutin et al. (2001) with the addition of a unique sequence to the 5′ end of one of each pair (referred to hereafter as modified primer) as shown in Table 3. Each 6 μL PCR reaction contained 1 μL of template, 0.6 μL of 0.5 μM primer mix, 1 μL H_2O, 0.2 μL 4mM spermidine, and 3 μL of JumpStart Red Taq (Sigma–Aldrich). PCR was performed using MJ Research PTC100 thermal cyclers to cycle through the following steps: 2 min denaturation at 95°C, followed by 30 sec at 94°C, 30 sec annealing at the appropriate temperature (Table 3), and 50 sec extension at 72°C. These three steps, repeated 40 times. The 5′-modified primers allowed use of the third fluorescently labeled primer in PCR, which facilitated pooling of PCR reactions and automated detection and genotyping using a BaseStation 51™ DNA fragment analyzer (MJ Research). Each lane of each ultra thin gel contained a 70-400 base pair ROX-labeled molecular marker (BioVentures). All genotypes were scored individually with the use of automated Cartographer® genotyping software.

Statistical tests

To calculate allele frequencies and genotypic proportions, GENEPOP Version 3.4 (Raymond and Rousset 1995) was used. Linkage disequilibrium was tested with the probability test using a Markov chain method (Guo and Thompson 1992) and global tests were performed across all populations with Fisher's method. The significance of deviation from Hardy-Weinberg expectations was examined with exact *P*-values that

were estimated using a Markov chain method and tests for heterozygote excess and heterozygote deficiency for each locus were conducted. All Markov chain runs consisted of 1000 dememorization steps, 100 batches, and 1000 iterations. In each instance where multiple independent tests were performed, significance levels (α) were revised by Bonferroni correction (Rice 1989).

Population genetic structure was examined using Arlequin version 2.00 (Schneider et al. 2000) in terms of Φ_{ST} calculated by AMOVA (Excoffier et al. 1992), pairwise genetic differentiation among populations, and *F*-statistics (Wright 1946). As a further indication of how genetic variation was distributed among populations, a population topology was determined using GENO (Dyer 2005). Multilocus inbreeding estimates, originally described by Ayres and Balding (2001) and subsequently illustrated by Dyer (2005) to be useful in consideration of inbreeding in wild populations, were examined in each of the ten *I. furcatus* populations. The distribution of inbreeding coefficients, *F*, generated by GENO was plotted to compare estimated levels of inbreeding. Nei's standard genetic distance (D_S; Nei 1987) was calculated for each population pair using MICROSAT Version 1.5d (Minch 1997) and PHYLIP phylogenetic software (Felsenstein 1993) was used to obtain a neighbor-joining tree (Saitou and Nei 1987) based on D_S-values. The extent of gene flow was evaluated by calculating the effective migration rate (N_em) using the standard relationship of N_em to F_{ST} (Wright 1946) and with GENEPOP using private allele frequencies (Barton and Slatkin 1986; Slatkin 1985).

The possibility of recent effective population size reductions was examined using BOTTLENECK (Ver 1.2; Cornuet and Luikart 19976). The Wilcoxon sign-rank statistic

tested for heterozygosity deficiency or excess, and the allele frequency distribution mode shift analyses (Luikart and Cornuet 1998) were performed using the heterozygosity data results to detect recent population bottlenecks under the two-phased model (TPM). The TPM was selected because it accepts lower numbers of loci and smaller sample sizes than the other two models implemented by BOTTLENECK (Luikart and Cornuet 1998).

Unconditional genetic mixed stock analysis was used to identify the sources of the three secondary populations: Pamunkey, Piankatank, and Potomac Rivers, using the Statistics Program for Analyzing Mixtures (SPAM version 3.7, Pella and Masuda 2001). SPAM estimated the relative contributions of discrete populations (in this case the original rivers into which *I. furcatus* were introduced) to each of the three mixture samples. Settings for each run of SPAM included activation of the IRLS algorithm and use of the Pella-Masuda model for determining the baseline posterior allele frequency distributions. All models were run with 95% confidence intervals and 100 resamplings of the baseline populations.

RESULTS

Of more than 2,000 *I. furcatus* collected, we obtained genotype data for 1,376. Genetic sample sizes for the Bay populations ranged from n = 119 to n = 265, and for the four comparative native populations genetic sample size ranged form n = 38 to n = 96.

Genetic variation among populations of native and Chesapeake Bay I. furcatus

Of 22 *Ictalurus punctatus* loci examined, four were polymorphic in *I. furcatus*. Of 20 microsatellite sequences isolated for *I. furcatus*, primers were designed for seven, and of those only two loci produced at least two different alleles. In combination with one previously published locus for channel catfish (*I. punctatus*; Liu et al. 1999) and three primer sets designed from published sequences for *I. punctatus* (Table 3), a total of six polymorphic loci yielded sufficient data for discrimination among the Chesapeake Bay as well as native populations. Across the ten populations examined, a total of 72 alleles were detected. The total number of alleles per locus, ranged from a low of 3 for *Ifu* F43B to a high of 23 alleles for *Ifu* F42A. Mean allelic diversity, *A*, observed for Chesapeake Bay populations averaged 3.5, 12% lower than observed for the native populations (*A* =4.1). For the six Bay populations, *A* ranged from a median of 3.0 to 4.2 with the Mattaponi / Pamunkey populations both having the lowest and James / Rappahannock populations having the highest, whereas for the native populations *A* ranged from a median of 3.7 to 4.7. The secondary Potomac and Piankatank populations both had higher allelic diversity than the introduced Mattaponi population, but less than the James

and Rappahannock (Table 4). Of particular note, five alleles for *Ifu* F42A were unique to the Potomac population. Five instances of significant linkage were observed (of 15 comparisons) all of which involved *Ipu*13 or *Ipu*15, indicating a possibility of null alleles at these two loci. Gene diversity did not reveal a clear trend in terms of native, introduced, and secondary populations. Although a number of individual loci were in Hardy-Weinburg Equilibrium (HWE) in various population samples, none of the six Bay populations conformed to HWE overall (Table 4). BOTTLENECK analyses indicated that severe reductions in population size resulting in genetic bottlenecks were not a likely factor for non-conformance to HWE in any populations including the native samples. For the six Bay populations, theta ($4N_e\mu$) ranged from a low of 6.72 for Pamunkey to a high of 36.47 for Rapahannock. By comparison, the native *I. furcatus* populations sampled had much lower values of theta (3.00 – 13.12).

Analysis of molecular variance resulted in 18% of genetic variation detected among the native and introduced groups, and 6% of the diversity was due to differences among Chesapeake populations (Table 5). Exact tests of population differentiation using only 3 loci (*Ipu*13, *Ipu*15, and *Ipu*270) among all ten native and introduced population samples, revealed that the native populations of Mississippi and Ohio did not differ significantly ($P = 0.79$), nor did the samples collected from Tennessee and Rappahannock Rivers ($P = 0.14$). Considering all six loci for all six populations in the Bay, the microsatellite allele frequency distributions differed significantly ($\chi^2 = \infty$, $P = 0.000$) among each of the Chesapeake Bay population pairs, thus each of the six populations were genetically distinct and therefore considered separately in all subsequent analyses.

Pairwise F_{ST} estimates, ranging from 0.042 to 0.183 ($P = 0.000$), provided evidence of moderate population substructure sufficient to perform MSA (Table 6). Estimates of D_S between population pairs ranged from a low of 0.019 between Rappahannock and Piankatank to a high of 0.194 between Rappahannock and Pamunkey (Table 7). The neighbor-joining tree based on D_S resulted in strong association between Rappahannock and Piankatank and a weaker cluster of Mattaponi, Pamunkey and Potomac (Figure 3). The overall effective migration rate (N_em) for the six Bay populations was very high 37.88, and gene flow was observed among all populations ranging from a low between Rapahannock and Pamunkey (0.58) to very high between Piankatank and Rapahannock (13.75; Table 7). The N_em values between the James and Piankatank (5.77) and between Mattaponi and Potomac (12.44) were also high.

Considering the multilocus inbreeding F analysis, Piankatank had the lowest observed level of inbreeding of all Bay populations, not significantly different from the native populations ($P > 0.50$) and significantly less than the Bay populations ($P < 0.05$), with a median value $F = 0.03$. The least genetically diverse Pamunkey population had the highest level of inbreeding, 0.27. The James and Potomac populations had a median $F = 0.22$ and were not significantly different (P>0.05). The Rappahannock and the Mattaponi were the least inbred of the introduced populations having similar median F of 0.15 and 0.17, respectively (Figure 2).

Maximum likelihood estimates of secondary populations

Three separate sets of admixture analysis were conducted. The first employed a baseline consisting of only the three original introduced populations and examined Pamunkey,

Potomac and Piankatank sets as mixtures. Because the Pamunkey population has been self-sustaining since the early-1990s, a second analysis employed the three original introduced populations plus the Pamunkey in the baseline to determine the percent compositions of Potomac and Piankatank only. Because Potomac, itself a mixture, could conceivable be contributing to the Piankatank population, a third baseline containing Potomac was employed to analyze the Piankatank mixture. In each of the three instances (Table 8), there was a single major contributing population and a relatively large component that was unknown (9.5 – 16.5%). The Pamunkey River *I. furcatus* population was estimated to be 83% derived form Mattaponi, 16.5% unknown and <0.5% each of Rappahannock and James. Using the three-source baseline, the Potomac population was derived primarily of Mattaponi (74%), followed by Rapahannock (16%) and 10% unknown. Including Pamunkey in the baseline group, dropped the Mattaponi percentage to 52%, complemented by 21% Pamunkey, and the Rapahannock and unknown portions of composition remained approximately the same as in the prior analysis. The James population was not observed to contribute in either instance to the Potomac population. Analysis of the newest population, Piankatank, revealed a more complex mixture consistent across the three- and four-population baseline analyses where Rappahannock was the major contributing population (71%), followed by an unknown group (14%), James (10-11%) and Mattaponi (4-5%). When Potomac was added to the baseline, all of the Mattaponi and 5% of Rappahannock's contribution to Piankatank were replaced by Potomac (10%).

DISCUSSION

Each of the six Chesapeake Bay *I. furcatus* populations was genetically distinct from the others and moderate population substructure was observed within the Bay (Figure 4). In general, the Bay populations were considerably more inbred than the native populations and they exhibited lower allelic diversity, showing evidence typical of the founder effect. However, the high N_em rates suggest that there may be a great deal more movement of this species within the Bay than was previously thought. The known predilection to seasonal migration combined with the wide range of salinity tolerance provides ample support that the observed levels of effective migration are contemporary estimates, as opposed to reflecting historical stocking activities. Long range movement is further supported by significant high flow storm-related events that could facilitate far range movement over short time periods. However, without physical tagging, there is little recourse to verify the absolute extent of movement and effective migration.

Pamunkey expansion

Pamunkey was the most inbred of all *I. furcatus* populations examined. The Mattaponi and Pamunkey populations had the lowest allelic diversity of all populations studied, 28% less than Rappahannock or James, reflecting the stocking history in which only 1,850 fingerlings were introduced into Mattaponi River in 1985. The MSA procedure worked well for analyzing *I. furcatus* in Chesapeake Bay as shown by the result that the Mattaponi population was the primary contributing source for the

Pamunkey population, 82%, as expected from its geographic proximity. Unexpectedly, no loss of genetic diversity was observed between the source (Mattaponi) and the secondarily colonized (Pamunkey) populations in this expansion event. A note of caution is that a major contribution of an unknown source to Pamunkey (16%), likely indicates that the baseline could have been better characterized by addition of more loci. This proportion of unknown in the mixture estimate was consistent with other MSA performed for the other two secondary populations (Potomac and Piankatank). A subsequent simulation using the program WHICHLOCI indicated that the 6-locus data set for the Bay baseline populations provided 86% accuracy in population assignment and no misassignments. Overall, population genetic and MSA analyses indicate that the Pamunkey expansion conforms to the dispersal scenario.

Potomac expansion

The Potomac population, observed to date only in the upper reaches of Potomac River near Occoquan Bay, exhibited a 22% higher diversity than the primary contributor identified by MSA (Mattaponi/Pamunkey) accompanied by a 12% drop in allelic diversity as compared to the second highest contributor (Rappahannock). In the case of range expansion via dispersal, although lower diversity than Rappahannock is expected, it is an apparent contradiction for this novel population to have higher allelic diversity than a major source located two drainages away (Mattaponi). Based on the MSA results alone, these conflicting data are difficult to explain. However, considering the fact that the northern Virginia impoundments were stocked at the same time with ~70,000 of the same hatchery stock of fingerlings as were stocked into Mattaponi and James Rivers

(~2000 and ~13,000, respectively), it is possible that the contribution attributed to Mattaponi and James may actually be a genetic signal of shared ancestry with fish stocked in impoundments in northern Virginia. The fact that five *Ifu* 42A alleles were found exclusively in Potomac, constitutes additional evidence in support of the possibility that escapees from lakes are the more likely source(s) of the secondary Potomac population. Finally, lack of evidence for a genetic bottleneck effectively rules out the possibility that this population was founded solely by one or more intentional introductions. Taking into account the stocking history, population genetics, and MSA analyses, the Potomac expansion conforms best to a scenario involving escapement from impoundments. However, although evidence points to such a scenario, this conclusion cannot be supported without genetic samples from such impoundment populations.

Piankatank expansion

Never observed prior to 2002, the Piankatank population appeared after an extended season of high flow, the highest annual discharge since 1981 (USGS Dragon Swamp station #01669520). Collections from this population exhibited a substantial reduction in allelic diversity compared to its primary contributors, the Rappahannock, Potomac, and James populations (16%, 18%, and 28% less, respectively). The observations of reduced allelic diversity and high genetic similarity to the source are consistent with the Piankatank population being founded by either dispersal or many intentional introductions, assumedly from the geographically proximal Rapahannock. Because Piankatank had the most diverse maximum likelihood estimate of composition, the second highest theta value, and the lowest observed level of inbreeding of all Bay

populations, intentional introductions alone are not a likely source of the recent Piankatank population. Furthermore, the lack of evidence for a genetic bottleneck effectively discounts the possibility that this population was founded solely by one or a few small-scale intentional introductions. Therefore, based on quantitative and qualitative considerations, the sudden Piankatank expansion conforms best to the dispersal scenario.

CONCLUSION

This analysis of the *I. furcatus* range expansion among Chesapeake Bay tributaries provides practical information that is relevant to a watershed-wide risk assessment. The ecological and genetic data provide quantitative measures of the potential for migration among tributaries and indicate that dispersal and escapement are the primary modes for the recent range expansion and that intentional introductions are not an effective explanation for the sudden appearance of Potomac and Piankatank secondary populations. Because one interpretation of the MSA results indicates that escapees from impoundments may be important components of the *I. furcatus* range expansion, this implies that such ecosystems may be more connected to watershed biology than previously recognized. This, in turn, may provide important information as these results imply that impoundments may be much more intimately connected to watershed ecology than previously recognized, and therefore may be an important component of river ecosystem management. This study also has proven to be an informative system for exploring the utility of a MSA in the study of invasive species. By combining MSA with other more typical population genetic analyses and ecological information, it was possible to select the most likely scenario to account for three separate expansion events. In each of the three cases, had we used only population genetics analyses and ecological data, we would have detected only decreased genetic diversity and the major contributing populations. By including MSA in the total analysis, we obtained more complete information on the sources of the range expansion and acquired higher degree of

confidence in the ability to estimate sources (roughly 86%) providing information that will be useful in determining future risk.

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Table 1. History of stocking and range expansion of *I. furcatus* in Chesapeake Bay tributaries.

	Record of stocking date or date of first observation	
Population	**Years**	**Numbers**
Introduced populations:		
James	1975	64,100
	1985	13,764
Rappahannock	1974	37,750
	1977	20,100
Mattaponi	1985	1,850
Impoundments	1983	10,149
	1985	59,982
Secondary populations:		
Pamunkey	1988	
Potomac	2001	
Piankatank	2003	--

Table 2. Sites sampled for blue catfish, *Ictalurus furcatus*, during 2003-2005 from tributaries of the Chesapeake Bay and from four native range tributaries of the Mississippi River. Abbreviations in parentheses are used in subsequent tables and figures.

Location	Site	Latitude	Longitude	N
CHESAPEAKE BAY, Virginia*				
James River	Jordan Point	37.31506	77.22561	10
	Turkey Cut	37.35283	77.27533	81
	Jordan Point	37.31506	77.22561	75
	Sandy Point	37.23673	76.94156	26
Rappahannock River	Stony Creek VA	37.30540	77.25944	78
	Horse Head Point VA	38.16223	77.06153	52
	Skinker's	38.22512	77.27812	38
	Highway 360 Bridge	37.93231	76.8483	30
	Fowners	38.06674	76.92224	38
Mattaponi River	Clifton	37.60632	76.82020	29
	Melrose	37.63773	76.85596	32
	Muddy Point	37.58265	76.79433	30
	Powerline	37.54675	76.77821	29
	RT 30 Bridge	37.53851	76.78919	30
	Walkerton	37.72390	77.01260	42
Pamunkey River	Indian Resevoir	37.65350	76.90605	19
	Brickhouse	37.55712	76.94315	23
	Cohoke Creek	37.57481	76.95834	25
	Cumberland	37.54622	76.97657	15
	Grimes Landing	37.64694	77.11376	21
	Hill Marsh	37.57985	76.85693	16
Potomac River	Ft. Washington	38.70137	77.05574	236
Pinakatank River	RM 15 – 16 Station	37.56062	76.55084	178
NATIVE**				
Mississippi River	Herculoneum, MO	38.2527	90.3674	96
Ohio River	Cincinnati, OH	39.0925	84.5164	46
Tennessee River	Muscle Shoals, AL	34.7444	87.6503	45
Alabama River	Miller's Ferry Power House, AL	32°05.27	87°24.00	28
	Hwy 84 Bridge, AL	31°36.33	87°33.02	66
	Chastain's Hole, AL	31°35.48	87°32.47	4

* Samples obtained through VDGIF and VCU.

** Native samples obtained through The Illinois Natural History Survey, Ohio River Valley Water Sanitation Commission, Auburn University, and the Alabama Department of Conservation and Natural Resources, respectively.

Table 3: Details for six microsatellite loci used in genetic analyses of *I. furcatus* populations.

Locus Name (Repeat)	GenBank Access. No.	Primer Sequences (5′ – 3′)	Anneal (°C)
Ifu F42-A	Pending	CAGTCGGGCGTCATCAATAAGGGCTAACTGGGATGT	53
$(CT)_9$		CTGCAAAGAGTAGAGGAAGAGT	
Ifu F43-B	Pending	GGTGCATACAGAGAATAAGGAACA	54
$(CA)_6$		CAGTCGGGCGTCATCAGAAAAGGGCATGCCAGGATAA	
Ipu 270	N/A	ACTCAATAAATCAAATCATGCG	58
$(ATTT)_{10}(TC)_{18}(CA)_{18}$	Liu et al. 1999	ATTTGTGAAACAAAATGAGTGG	
Ipu 13	BV078113	CACTCCGGTCACACTCTACG	57
$(AC)_{18}$		GTGGCTTTCTTATTTTTGTTTTTG	
Ipu 15	BV078115	GACGCTTTGTGGTTTCTCG	56
$(TTTG)_7$		TCAGTCGCGCCCTCATC	
Ipu 41	AF321241	CTTTGCTGGTTGAAATGGGATTA	57
$(GAA)_{10}$		TTGAGATAAAGAGCAATTCAGTCG	

Table 4: Diversity indices characterizing introduced and secondary populations of *Ictalurus furcatus* in the Chesapeake Bay watershed and four populations collected from the native range of the species. Maximum sample size (*N*), conformation to Hardy-Weinberg Equilibrium predictions (HWE), average allelic diversity (*A*), gene diversity (expected heterozygosity), inbreeding (*F*), and $Theta_{(hom)}$ which is = 4 $N_e\mu$.

Population	*N*	HWE	*A*	Gene Diver.	Inbreeding *F*	$Theta_{(hom)}$
JAM	192	No	4.17	0.94	0.22	15.70
POT	236	No	3.67	0.93	0.22	11.58
PIA	178	No	3.50	0.96	0.03	21.04
PAM	119	No	3.00	0.89	0.27	6.72
MAT	192	No	3.00	0.94	0.17	13.78
RAP	264	No	4.17	0.97	0.15	36.47
AL	38	No	3.67	0.79	0.03	3.0
MS	96	No	4.33	0.91	0.03	9.24
OH	47	No	3.67	0.88	0.03	5.98
TN	45	No	4.67	0.94	0.03	13.12

Table 5: Analysis of molecular variance for six *I. furcatus* populations in Chesapeake Bay and four native range samples.

Source of variation	d.f.	SS	Variance components	% variation
Native *vs.* Chesapeake	4	205.41	0.21 V_a	18.24
Among populations within Chesapeake Bay	5	129.41	0.07 V_b	6.10
Within populations	2620	2280.19	0.87 V_c	75.67
Total	2629	2615.01	1.15	

Table 6: Microsatellite genetic variation at three microsatellite loci in *I. furcatus* from six Chesapeake Bay and four native populations categorized above the diagonal as Φ_{ST} and below the diagonal as F_{ST} (P-values shown in parentheses).

	JAM	POT	PIA	PAM	MAT	RAP	AL	MS	OH	TN
JAM		0.065	0.048	0.175	-0.016	0.024	0.101	0.299	0.304	0.106
POT	0.104 (0.000)		0.087	0.034	0.053	0.091	0.240	0.055	0.305	0.292
PIA	0.091 (0.000)	0.056 (0.000)		0.189	0.113	0.010	0.285	0.206	0.237	0.280
PAM	0.183 (0.000)	0.051 (0.000)	0.146 (0.000)		0.062	0.150	0.366	0.025	0.027	0.436
MAT	0.042 (0.000)	0.044 (0.000)	0.077 (0.000)	0.079 (0.000)		0.113	0.122	0.154	0.157	0.169
RAP	0.092 (0.000)	0.052 (0.000)	0.010 (0.000)	0.126 (0.000)	0.071 (0.000)		0.273	0.166	0.188	0.220
AL	0.063 (0.000)	0.160 (0.000)	0.169 (0.000)	0.254 (0.000)	0.074 (0.000)	0.176 (0.000)		0.490	0.516	0.297
MS	0.342 (0.000)	0.274 (0.000)	0.273 (0.000)	0.290 (0.000)	0.282 (0.000)	0.249 (0.000)	0.435 (0.000)		0.007	0.533
OH	0.357 (0.000)	0.288 (0.000)	0.292 (0.000)	0.312 (0.000)	0.292 (0.000)	0.265 (0.000)	0.468 (0.000)	0.002 (0.082)		0.544
TN	0.069 (0.000)	0.202 (0.000)	0.167 (0.000)	0.314 (0.000)	0.107 (0.000)	0.140 (0.000)	0.176 (0.000)	0.393 (0.000)	0.423 (0.000)	

Table 7: Microsatellite genetic variation at six microsatellite loci in *I. furcatus* from six Chesapeake Bay populations categorized using pairwise estimates of genetic distance, D_S (above the diagonal), and effective migration rate, N_em (below the diagonal) using the private alleles method (overall $N_em = 37.88$).

	JAM	MAT	POT	PIA	PAM	RAP
JAM		0.046	0.094	0.098	0.166	0.124
MAT	1.25		0.031	0.087	0.058	0.131
POT	2.23	12.44		0.087	0.041	0.112
PIA	5.77	1.11	0.80		0.174	0.019
PAM	2.45	2.97	2.78	0.73		0.194
RAP	5.72	1.02	1.13	13.75	0.58	

Table 8. Estimated mixture proportions of secondary *I. furcatus* populations in Chesapeake Bay tributaries. Three models were used: JMR indicates baseline included James, Mattaponi and Rappahannock only; JPMR indicates baseline included introduced populations plus the secondary Pamunkey population; JPMRP indicates baseline included introduced populations plus the secondary Pamunkey and Potomac populations. SE and CV refer to the standard error and coefficient of variation of the estimates, respectively. N_em: effective migration between source and mixture. Relative percent change in *A* for secondary versus source. Population names in bold are the purported mixtures, whereas other populations are potential sources.

	MSA Estimates of %Composition									N_em	Rel. % change in *A*	Genetic Dist.
	JMR			JPMR			JPMRP					
	Estim. (%)	SE	CV	Estim. (%)	SE	CV	Estim. (%)	SE	CV			
Pamunkey												
JAM	0.4	0.88	2.20	--	--	--	--	--	--	2.8	-28	0.166
MAT	82.6	1.82	0.02	--	--	--	--	--	--	3.0	0	0.058
RAP	0.1	0.01	0.11	--	--	--	--	--	--	0.6	-28	0.194
Unknown	16.5	--	--	--	--	--	--	--	--			--
Potomac												
JAM	0.0	0.00	0.00	0.0	0.10	1.70	--	--	--	2.2	-12	0.094
PAM	--	--	--	20.8	--	--	--	--	--	2.8	22	0.041
MAT	74.1	3.40	0.04	51.9	4.80	0.09	--	--	--	12.4	22	0.031
RAP	16.3	3.31	0.20	17.8	3.30	0.19	--	--	--	1.1	-12	0.112
Unknown	9.6	--	--	9.5	--	--	--	--	--			
Piankatank												
JAM	10.0	3.97	0.39	11.3	4.13	0.36	11.3	3.64	0.32	5.8	-28	0.098
PAM	--	--	--	0.0	--	--	0.1	0.01	0.20	0.7	17	0.174
MAT	4.9	3.35	0.69	4.4	3.31	0.75	0.0	0.00	0.00	1.1	17	0.11
RAP	71.1	3.92	0.06	70.7	3.94	0.06	64.4	4.60	0.00	13.8	-16	0.019
POT	--	--	--	--	--	--	10.2	3.96	0.39	0.80	-18	0.087
Unknown	14.0	--	--	13.6	--	--	14.1	--	--			--

Figure 1: Map of the Virginia portion of Chesapeake Bay watershed denoting introduced (**bold**) and secondary (*italic*) populations of *I. furcatus*. See Table 1 for stocking years and numbers introduced.

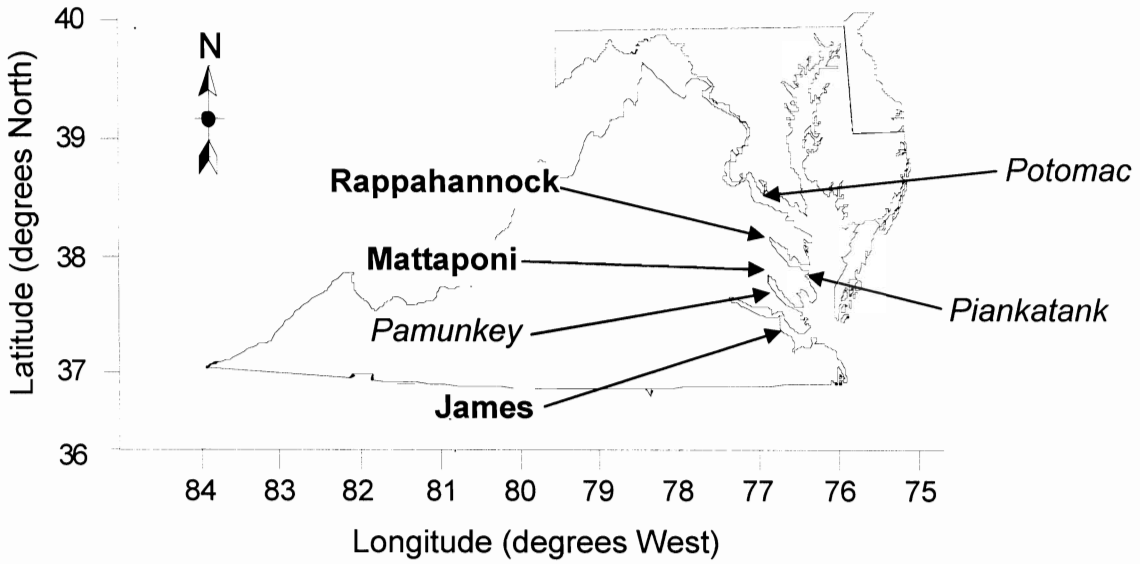


Figure 2: Distribution of inbreeding coefficients in six Chesapeake Bay and four native populations of blue catfish, *Ictalurus furcatus*. Frequencies appear on the y-axis and inbreeding coefficient values, *F*, along the x-axis.

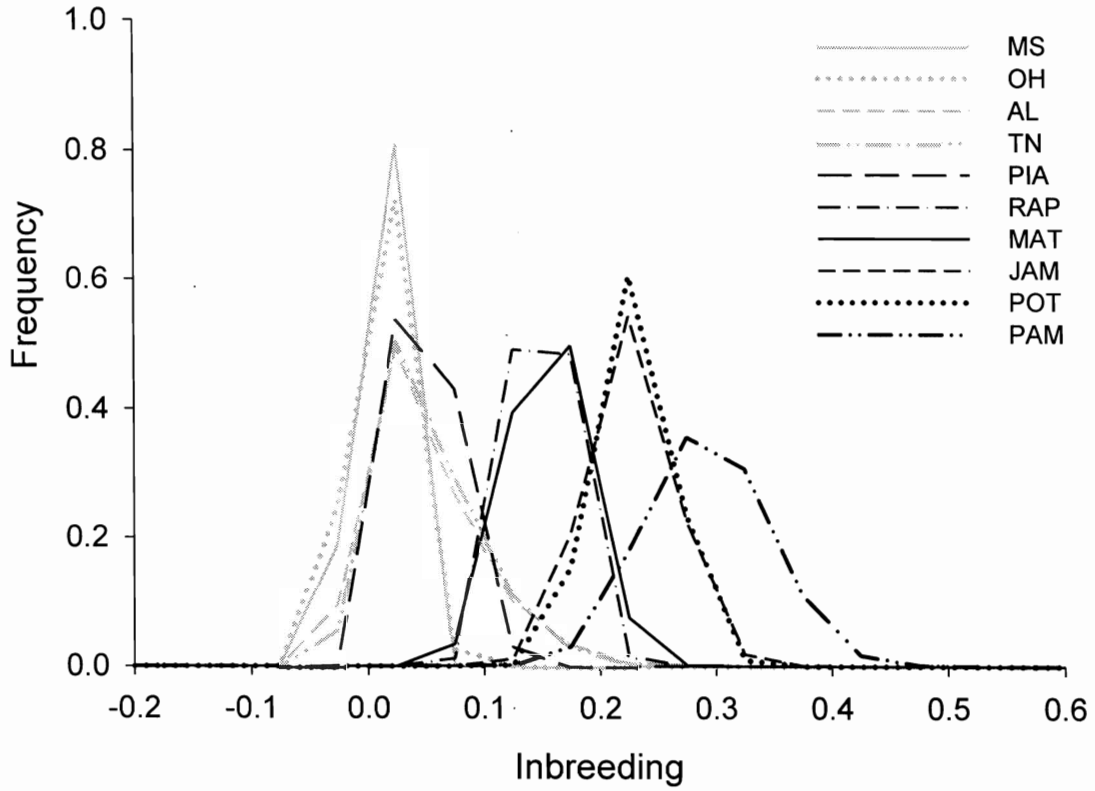


Figure 3: Neighbor joining tree constructed from Nei's standard genetic distance (DS) values among six populations (introduced and secondary colonization events) of *I. furcatus* in the Chesapeake Bay watershed. Bootstrap values at nodes indicate the percentage of unambiguous branches at that point.

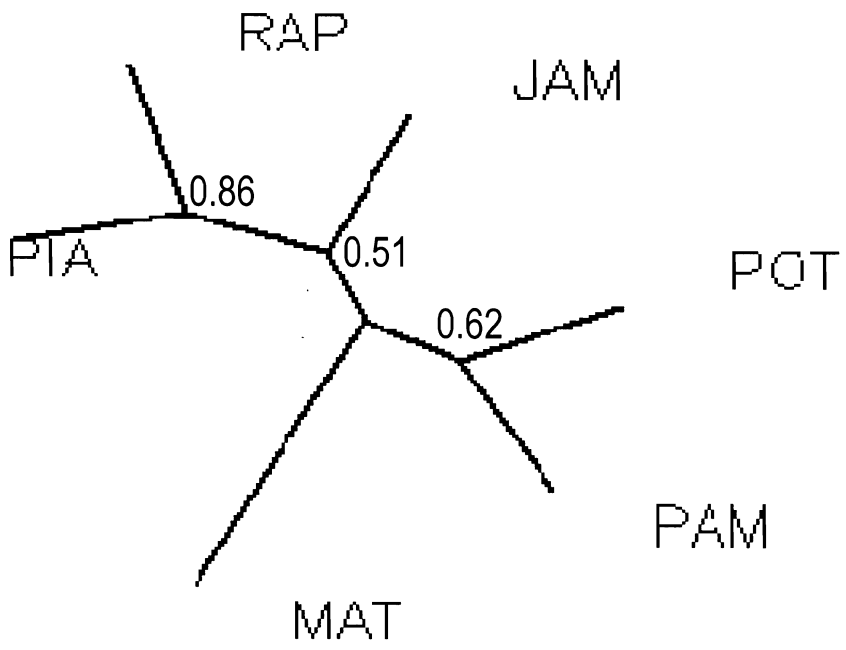
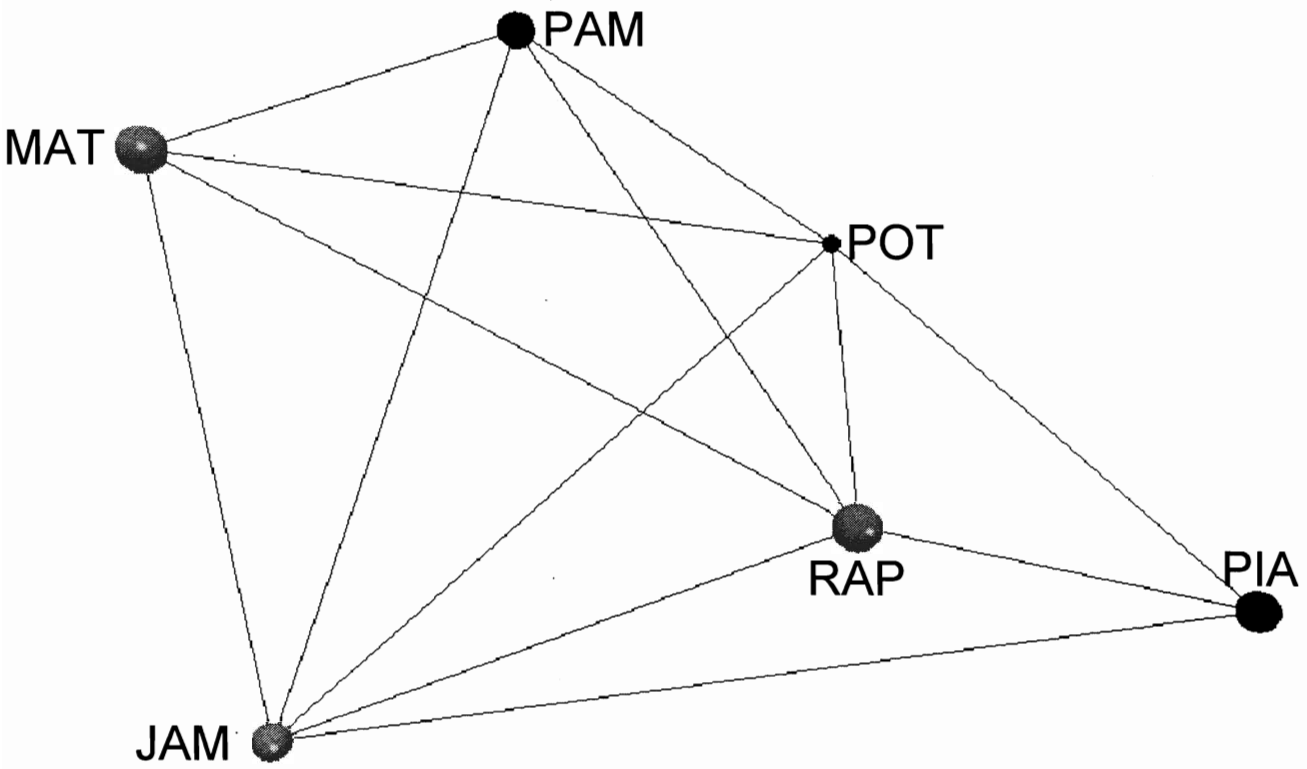


Figure 4: Population graph illustrating genetic relationships among Chesapeake Bay watershed introduced and secondary populations of *I. furcatus*. The variation among population samples is incorporated in the lengths of lines connecting nodes. Extent of within population genetic variability is illustrated by relative node size.



Vita

Colleen Beth Higgins was born on 14 March 1971, in Schenectady, New York, and is a patriotic American citizen who loves to fish and drive boats. She graduated from Plano Senior High School, Plano, Texas in 1989. She received her Bachelor of Arts in Middle East Area Studies with a minor in Religion from George Washington University, Washington, DC in 1994. She subsequently earned a Bachelor of Science in Biology from Virginia Commonwealth University, Richmond, Virginia in 2000. She has work experience in journalism, banking, analytical chemistry, wildlife education at The Maymont Foundation, and environmental sampling at Virginia Department of Environmental Quality in Richmond, Virginia.