Evaluating the Effects of Reintroduction on Population Viability and Genetic Diversity of the Orinoco Crocodile (*Crocodylus intermedius*) in Venezuela

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Abstract

Captive breeding and translocation have been central components of Orinoco crocodile conservation in Venezuela for more than three decades. This report integrates ecological monitoring and population genomic analyses to evaluate demographic and genetic consequences of reintroductions. Ecological surveys included spotlight counts, population structure assessments, nest monitoring, and reproductive population estimates in the Cojedes, Capanaparo, and Manapire river systems. Genomic analyses used 3RAD sequencing and approximately 12,200 SNPs from wild and captive populations. Contrasting demographic trajectories among river systems. Capanaparo retained the highest abundance and genomic diversity, Cojedes showed evidence of recruitment and persistence of adults, whereas Manapire exhibited lower genetic diversity and smaller effective population size. Genomic analyses revealed extensive admixture between captive and wild populations and strong kinship links consistent with successful survival and reproduction of released individuals. Evidence suggests that translocations have contributed both demographic reinforcement and genetic augmentation. However, population recovery remains uneven and constrained by habitat degradation, nest loss, poaching, and fragmentation. Conservation success will require integration of demographic monitoring, adaptive release strategies, genomic management of captive stocks, and long-term protection of nesting habitats. The combined evidence supports reintroduction as a valuable conservation tool, while emphasizing the need for genetically informed management to secure long-term viability of *Crocodylus intermedius*.

Introduction

The Orinoco Crocodile (*Crocodylus intermedius*) is classified as Critically Endangered and remains one of the most threatened crocodilians globally. Historical overexploitation, habitat alteration, nest loss, and illegal hunting caused severe declines and fragmentation (Seijas, 2010; Rodríguez et al, 2015; Balaguera-Reina et al., 2018). Despite legal protection dating back to the 1970s and the establishment of protected areas (Ayarzagüena, 1987; Godshalk, 1978; González-Fernández, 1995; Seijas, 2008), along with efforts in population promotion, restoration (Babarro, 2014), cessation of hunting and collection, and artificial incubation of eggs (Espinosa-Blanco et al., 2013), Venezuelan populations of C. intermedius have not exhibited the anticipated recovery (Mendoza & Seijas, 2007). Presently, the primary threats impeding their recovery are believed to be egg loss due to flash floods, human collection and nest plundering, habitat loss, and illicit hunting (Morales-Betancourt et al., 2013). Moreover, there are concerns of ongoing decline in various population remnants (Espinosa-Blanco & Seijas, 2012; Moreno et al., 2017; Seijas, 2010).

Translocations and population reinforcement have been successful in recovering crocodilian populations threatened by overexploitation in various parts of the world (Elsey & Woodward, 2010; Fergusson, 2010; Verdade et al., 2010; Webb et al., 2010). Population assessment and conservation genomics now provides tools to evaluate whether released individuals survive, reproduce, and contribute genetically to population recovery (Weeks et al., 2011; Angeloni et al., 2012). The objectives were to evaluate demographic recovery, reproductive performance, population structure, genomic diversity, and the contribution of translocated individuals to wild populations.

Methods

Study areas included the Cojedes River System, Capanaparo River, Manapire River, and additional captive and wild populations. Population monitoring relied on nocturnal spotlight surveys, abundance indices (individuals/km), size-class analyses, and nest surveys. Reproductive ecology assessments quantified nests, active females, recruitment, and reproductive population size. Genomic analyses used DNA extracted from tissue samples, 3RAD sequencing, SNP discovery, population structure analyses, admixture inference, diversity metrics, and kinship analyses (Figure 1).

A total of 147 tissue samples were collected from 14 *C. intermedius* populations in Venezuela, including four wild populations and ten ex situ populations. Wild populations comprised the Capanaparo River, Cojedes River System, Manapire River, and the reintroduced population at El Frío Biological Station. Ex situ samples were obtained from major captive-breeding facilities, including UNELLEZ, La Patrona, Agropecuaria Puerto Miranda, Masaguaral, and the El Frío breeding center. Additional samples were collected from zoological institutions participating in the Venezuelan Orinoco Crocodile Conservation Program, which contribute to captive breeding, population management, and the release of captive-bred individuals into the wild.

Genomic DNA was extracted from tissue samples using the DNeasy Blood and Tissue Kit (Qiagen), and DNA quality was assessed with Qubit fluorometry. Genomic libraries were prepared following the 3RAD sequencing protocol, which combines triple-enzyme restriction digestion, adapter ligation, PCR amplification, and size selection, and were sequenced on an Illumina NextSeq 500 platform. Sequence reads were processed using Stacks and pyRAD, including quality filtering, demultiplexing, and both de novo and reference-based locus assembly using the *C. porosus* genome. SNP datasets were subjected to stringent filtering to remove low-quality loci, excessive missing data, rare alleles, deviations from Hardy–Weinberg equilibrium, and putative paralogs. Population genomic analyses included Bayesian clustering (STRUCTURE), Principal Coordinates Analysis (PCoA), Discriminant Analysis of Principal Components (DAPC), phylogenetic clustering, and estimates of heterozygosity, inbreeding, genetic differentiation, and relatedness. Genomic patterns were subsequently compared with historical records of captive-bred crocodile releases to evaluate the genetic contribution of reintroduction programs to the recovery of wild *C. intermedius* populations.

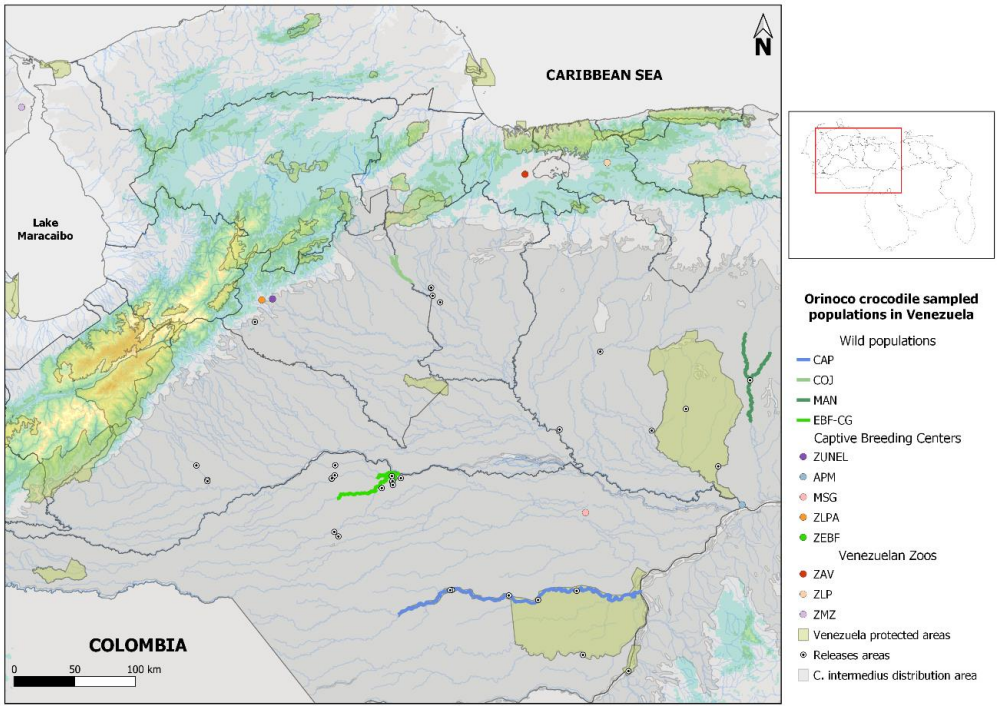


Figure 1. Historical geographic distribution and sampling localities of the Orinoco crocodile across the Orinoco Basin in Venezuela. Circular symbols represent ex situ populations (captive breeding centers and zoos), while shaded areas indicate wild populations of the species in Venezuela.

Results

Population monitoring conducted between February and May 2016 evaluated the demographic status, reproductive activity, and population structure of key *C. intermedius* populations in Venezuela. Surveys combining diurnal and nocturnal spotlight counts, population structure assessments, and nest monitoring revealed contrasting recovery trajectories among river systems. In the Cojedes River system, abundance was highest in the Sarare River and La Batea–Confluencia sectors, where suitable nesting habitat and reduced hunting pressure likely promoted population persistence. However, nest density remained low (0.49 nests/km), and the minimum reproductive population was estimated at only 19 adults, suggesting a long-term population decline despite the continued presence of breeding individuals.

A reintroduced population established through previous release efforts exhibited relatively high local densities, particularly in Laguna Larga (LL), and was dominated by adult and subadult individuals, indicating long-term survival of released crocodiles. Nevertheless, reproductive output was extremely limited, with only two nests recorded and an estimated reproductive population of four adults, suggesting that demographic persistence has not yet translated into substantial natural recruitment.

The Capanaparo River population remained the largest and most demographically robust population surveyed. Adult individuals predominated, although juveniles and subadults were well represented, indicating ongoing recruitment. Despite low nesting density (0.14 nests/km) and evidence of nest poaching, the population was estimated at 132 individuals, including a minimum of 39 breeding adults. Population abundance increased by approximately 29% in sectors receiving captive-bred juveniles, providing strong evidence that reintroduction efforts have contributed to demographic recovery.

Genomic analyses based on 3RAD sequencing generated approximately 12,200 high-quality SNPs from 147 sampled individuals representing both wild and captive populations. Following stringent quality filtering, 100 individuals genotyped at 477 loci were retained for downstream analyses. Clone detection identified two technical replicates and one highly similar individual pair, which were removed to maximize dataset reliability.

Population structure analyses consistently identified three major genetic clusters corresponding to the Capanaparo (CAP), Cojedes (COJ), and Manapire (MAN) river systems (Figure 2). Bayesian clustering analyses implemented in STRUCTURE supported K = 3 as the most likely number of populations, a result independently corroborated by DAPC ordination. Captive individuals were predominantly assigned to the Cojedes genetic cluster, indicating that this population has historically served as the principal source for ex situ breeding and reintroduction programs.

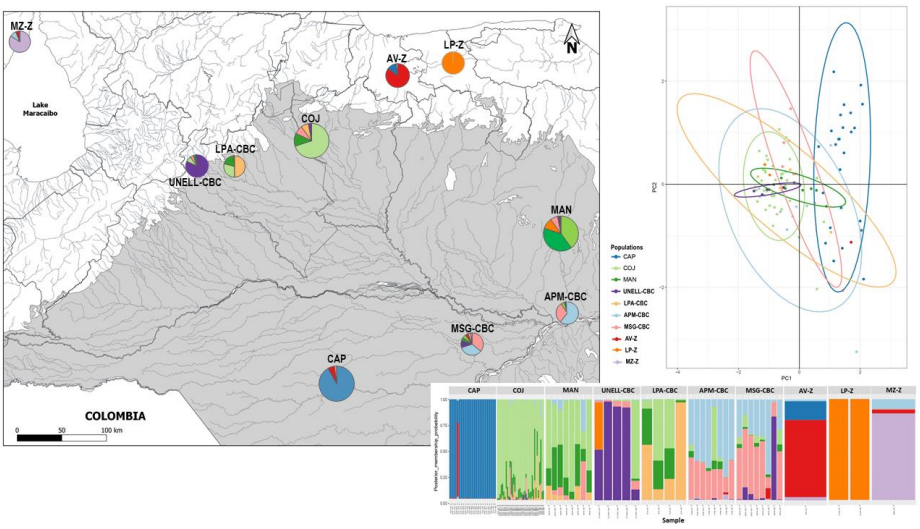


Figure 2. Geographic distribution, population structure, and genomic clustering of wild and captive populations of the Orinoco crocodile (*Crocodylus intermedius*) in Venezuela.

Genetic diversity differed markedly among populations. Capanaparo exhibited the highest diversity (H' = 3.638), followed by Cojedes (H' = 3.219), whereas Manapire showed substantially lower diversity (H' = 2.079), suggesting increased vulnerability to genetic drift and inbreeding. Admixture and kinship analyses revealed extensive genetic exchange between captive and wild populations and identified close genetic relationships between released and wild-born individuals. Together, the ecological and genomic evidence demonstrates that reintroduction programs have generated both demographic reinforcement and measurable genetic contributions to wild populations, highlighting their importance for the long-term recovery and viability of the Orinoco crocodile.

The DAPC revealed clear genetic differentiation among major *C. intermedius* Venezuelan populations, with three principal clusters corresponding to the Capanaparo, Cojedes, and Manapire river systems. Captive populations largely overlapped with the Cojedes cluster, reflecting their historical origin. Moderate overlap among some groups suggests admixture and gene flow associated with long-term translocation and reintroduction efforts, while maintaining detectable population structure (Figure 3).

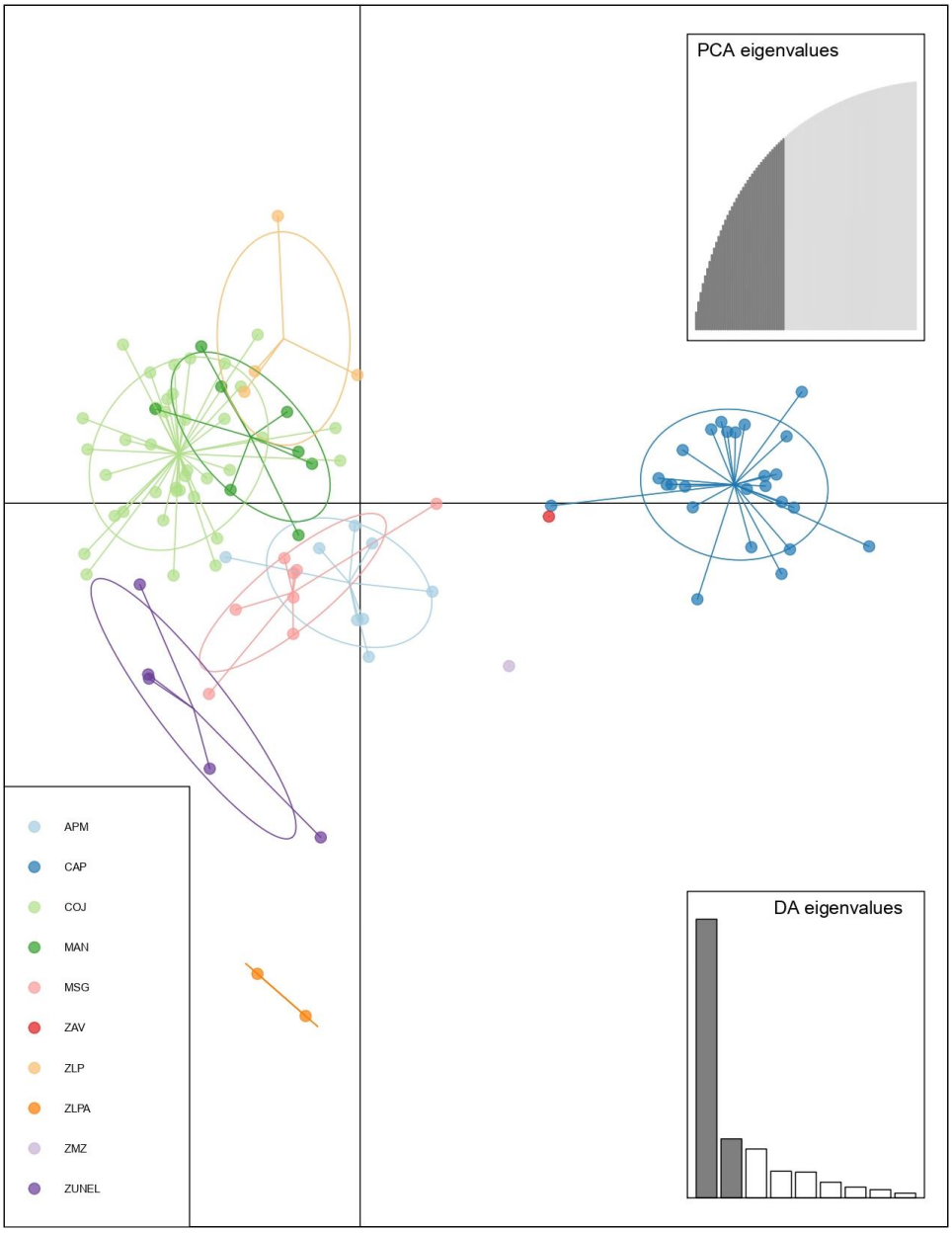


Figure 3. Genetic relationships among Venezuelan populations of *Crocodylus intermedius* inferred

Discussion

The demographic patterns observed among the three river systems reveal contrasting recovery trajectories for *C. intermedius* in Venezuela and underscore the importance of long-term population monitoring in evaluating conservation interventions. The Capanaparo River currently represents the most viable wild population of the species, supporting the largest abundance, the highest number of breeding adults, and evidence of sustained recruitment through the presence of juveniles and subadults. The observed increase in abundance in sectors receiving captive-bred juveniles suggests that reintroduction efforts have contributed substantially to demographic reinforcement. Similar outcomes have been documented in recovery programs for other crocodilians, including *Alligator mississippiensis*, *C. porosus*, and *Caiman latirostris*, where sustained releases and habitat protection promoted population recovery and increased long-term persistence (Elsey & Woodward 2010; Webb et al. 2010; Verdade et al. 2010).

In contrast, the Cojedes and reintroduced populations exhibited adult-dominated population structures and low nesting activity, suggesting that demographic recovery remains incomplete. Although breeding individuals persist, reduced nest densities may indicate recruitment bottlenecks associated with habitat degradation, nest poaching, hydrological alterations, and low juvenile survival. Similar patterns have been reported for declining populations of *C. intermedius* in Venezuela, where anthropogenic pressures continue to limit natural population growth despite decades of conservation efforts (Espinosa-Blanco & Seijas 2012; Balaguera-Reina et al. 2017). Consequently, the persistence of these populations may still depend on continued management interventions and reinforcement programs.

The genomic analyses provide strong evidence that reintroduction programs have generated not only demographic benefits but also measurable genetic contributions to wild populations. The identification of three genetically differentiated clusters corresponding to the Capanaparo, Cojedes, and Manapire river systems suggests that historical isolation and limited dispersal have shaped contemporary population structure. Despite more than three decades of translocations, both STRUCTURE and DAPC analyses recovered these clusters, indicating that population identity has been largely maintained.

The high genetic diversity observed in the Capanaparo population highlights its importance as a reservoir of evolutionary potential and a priority source population for future conservation actions. In contrast, the reduced diversity detected in Manapire may increase susceptibility to genetic drift, inbreeding, and loss of adaptive variation, factors known to reduce population viability in small and isolated populations (Frankham et al. 2010; Angeloni et al. 2012). Importantly, admixture and kinship analyses demonstrated that captive-bred individuals have successfully reproduced in the wild, contributing genetically to recipient populations. These findings are consistent with the principles of genetic rescue, whereby managed gene flow can enhance genetic diversity and reduce inbreeding depression while preserving adaptive potential (Weeks et al. 2011).

Overall, the combined ecological and genomic evidence indicates that the Venezuelan Orinoco Crocodile Conservation Program has contributed significantly to both demographic recovery and genetic reinforcement of *C. intermedius*. Future management should integrate genomic monitoring with demographic assessments to optimize source-population selection, maintain genetic diversity, and ensure the establishment of self-sustaining populations across the species' remaining range.

Future releases should prioritize genetically underrepresented populations, maximize effective population size, and minimize relatedness among breeders. Captive breeding centers should implement genomic screening to guide pairing decisions. Nesting habitats require enhanced protection and monitoring. Long-term monitoring should combine demographic surveys, mark-recapture studies, and genomic assessments. Adaptive management should evaluate survival, recruitment, and reproductive success of released cohorts.

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