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Preservation of genetic diversity and selection over a century in a coral reef fish
(*Taeniamia zosterophora*) in the Philippines

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Abstract

Evaluating the evolutionary impacts of anthropogenic activity on populations is key to understanding species resiliency and to designing effective conservation strategies. Sequencing DNA from historical specimens provides the opportunity to establish a historical baseline and empirically assess changes in genetic diversity, changes in effective population size, and selection over time. Here, we sequenced historical and contemporary samples of the cardinalfish (*Taeniamia zosterophora*) collected in 1908 and in 2021-2022 across two sites with differing human impact in the Philippines. At both sites, genetic diversity increased over time, with contemporary samples having significantly higher Watterson's θ than historical samples. This diversity increase was primarily attributable to positive selection on low-frequency alleles such that they increased towards intermediate frequencies through time. For the putatively neutral fraction of the genome, in contrast, there was a slight but significant decline in Watterson's θ at both low and high human impact sites, suggesting that drift strengthened and effective population sizes declined through time. There was more evidence for selection and greater loss of neutral diversity at the site with higher human impact. Our results provide empirical evidence for the surprising preservation of genetic diversity through the action of natural selection in the face of anthropogenic impacts.

Introduction

Understanding how ecosystems respond to anthropogenic activities is a core interest in the field of global change biology. Overall, there has been a decrease in biodiversity and biomass in many ecosystems over the last century (Christensen et al. 2014; Hooper et al. 2012; Lotze et al. 2019; Romero-Muñoz et al. 2021), which has led to changes to community structure, species

interactions, and ecosystem functions and services (Grimm et al. 2013; Scheffers et al. 2016; Scholes 2016). For example, over-exploitation can reshape trophic structure and community interactions when predators are eliminated from systems (Baden et al. 2012; Heithaus et al. 2008; Ripple et al. 2024), and habitat degradation can lead to local extirpations and changes in community dynamics (Martínez-Ramos et al. 2016; Peterson and Lowe 2009; Wilson et al. 2010). Evolutionary impacts such changes in genetic diversity, effective population size, and natural selection are also expected and may be even more impactful and difficult to reverse than ecological impacts (Bradshaw and Holzapfel 2006; Law 2007). However, studying and understanding evolutionary changes has been substantially more difficult, in part because phenotypic plasticity and evolution can produce equivalent morphological changes (Merilä and Hendry 2014). In this context, whole genome sequencing from historical and contemporary samples can provide a powerful approach to “functional resurveys” that illuminates otherwise invisible evolutionary processes and impacts.

Evolutionary Consequences of Anthropogenic Activities

Anthropogenic activities are likely to have substantial evolutionary consequences for ecosystems, but these are substantially less well understood than ecological impacts. Genetic diversity is considered a critical aspect of biodiversity and system resilience, not least because genetic diversity forms the basis for a species' ability to adapt to changing environments and to maintain resilience in the face of such change (Ellegren and Galtier 2016; Messer and Petrov 2013). Preserving genetic diversity contributes to reducing species extinction risk, reducing inbreeding depression, enhancing species diversity, bolstering ecosystem resilience, and facilitating ecosystem function and services (Forester et al. 2022; Hoban et al. 2020; Hughes et

al. 2008). Genetic diversity is expected to decline in response to overexploitation and habitat degradation, as both factors contribute to population declines, with fewer individuals contributing genomic variation to the next generation and stronger genetic drift (Ellegren and Galtier 2016). It is possible for genetic diversity to be preserved despite these stressors if effective population size is large or increases, if gene flow introduces new alleles to the population, or if selection or other factors increase allele frequencies towards intermediate values in many loci (Gum et al. 2014; Pinsky et al. 2021; Setzke et al. 2021; Therkildsen et al. 2010). Despite biodiversity being the greatest in tropical systems, most studies have focused on temperate species, leaving large gaps in our understanding of how genetic diversity has changed in tropical populations over time (R. E. Shaw et al. 2025). In addition, many studies of genetic diversity change even in temperate systems have been limited by low numbers of loci that diminished their statistical power (Hutchinson et al. 2003a; Pinsky and Palumbi 2014; Therkildsen et al. 2010; Waples et al. 2018).

Adaptation in response to altered selective pressures is another evolutionary consequence of anthropogenic activities. In the most severe circumstances, adaptation via natural selection can save a population from a severe decline or even extirpation, a process called evolutionary rescue (Bell 2017). Adaptation in response to overexploitation has been studied with fishing (fisheries-induced evolution) and hunting. A greater presence of tusklessness in elephants and smaller horns in big-horned sheep has been documented after hunting (Campbell-Staton et al. 2021; Douhard et al. 2016). Earlier maturation has been documented in wild populations of Atlantic cod (Olsen et al. 2004; Swain et al. 2007). Evolution towards smaller average lengths in fish populations has been found in experimental and simulation studies (Conover and Munch 2002;

van Wijk et al. 2013). Recent studies employing genome scans have found evidence of selection over time, with two studies identifying genomic regions under selection in overfished freshwater or lab-reared populations (Chebib et al. 2016; Therkildsen et al. 2019) and studies on wild marine populations finding evidence of a highly polygenic response to fisheries-induced selection (Pinsky et al. 2021; B. N. Reid et al. 2023). Pollution has also been shown to induce rapid adaptation in wild fish populations, including the identification of selective sweeps of toxin-tolerant genomic variants in brackish-water killifish (Oziolor et al. 2019; N. M. Reid et al. 2016) and freshwater guppies (Hamilton et al. 2017). Studies assessing adaptation in response to habitat degradation are lacking, but theory suggests species in patchy or fragmented habitats may evolve shorter dispersal distances to maximize chances of finding suitable habitat (Baskett et al. 2007; A. K. Shaw et al. 2019). Recent advances in genome scan methods and historical DNA extraction methods (Sproul and Maddison 2017) can be particularly important for addressing this knowledge gap because comparisons between historical and contemporary specimens can establish clear historical baselines and provide direct observations of genomic changes (Clark et al. 2023).

Another key consideration is that anthropogenic influence is not uniform across space. Instead, gradients of human impact create some areas with heavy anthropogenic influence and others are less strongly influenced (Halpern et al. 2008). The type or types of anthropogenic influence can vary across a region as well. We might, for example, expect areas with higher human influence to show a larger decrease in genetic diversity and stronger selection than areas with lower human influence. The genomic regions responding to selection may also differ among geographic regions with more and less human influence. Testing these hypotheses is key for understanding

the evolutionary trajectory of marine ecosystems and for developing effective conservation strategies, but until now, they have not been evaluated rigorously. Understanding which types of areas have preserved genetic diversity, for example, could help designate target areas for marine reserves.

Temporal Genomics to Evaluate Evolutionary Dynamics

Sequencing historical and contemporary samples from the same location has emerged as an increasingly powerful approach to monitor populations and evaluate evolutionary dynamics over time (Clark et al. 2023; Jensen and Leigh 2022). Specimens stored in museums present an excellent opportunity to establish an empirical historical baseline, which can otherwise be obscured or lost entirely by gene flow, drift, selection, and other events in the intervening generations (Raxworthy and Smith 2021). DNA degradation is a challenge when working with historical samples, especially older samples or samples not stored with DNA preservation in mind. In recent years, however, advances in DNA extraction and library preparation protocols along with bioinformatic methods to address biases from degradation in DNA have made sequencing historical DNA more feasible, accurate, and cost-effective (Hofreiter et al. 2015; Link et al. 2017). Studies have successfully evaluated changes in genetic diversity and effective population size over time using historical DNA (Dures et al. 2019; Gauthier et al. 2020; Pacioni et al. 2015; Therkildsen et al. 2010) and tested for evidence of selection across the genome to understand how anthropogenic actions have affected the evolutionary trajectory of populations (Dehasque et al. 2020; Gignoux-Wolfsohn et al. 2021; Lilley et al. 2020; Therkildsen et al. 2013) across a wide range of taxa.

Temporal Genomic Research from the R/V Albatross Expedition to the Philippines

To evaluate the effects of anthropogenic influence on genetic diversity and adaptation, here we sequenced the genomes of historical and contemporary samples of coral reef fishes in the Philippines. The Smithsonian National Museum of Natural History (NMNH) houses a large collection of tropical fishes from the U.S. Research Vessel *Albatross*'s expedition from 1907 to 1909 throughout the Philippines (Smith and Williams 1999). This collection of more than 79,000 specimens from more than 120 geographic locations provides a unique opportunity to empirically investigate long-term genomic changes in the tropics across a gradient of human influence. Of particular value, these specimens were fixed and stored in ethanol, which preserves DNA, rather than formalin, a common fixation agent for fishes that crosslinks DNA and complicates sequencing (Chakraborty et al. 2006).

The Philippines is an ideal study site for evaluating evolutionary changes in tropical ecosystems. Coral reefs in the Philippines are considered marine biodiversity hotspots (Allen 2008; Sanciangco et al. 2013) and have the highest concentration of fish species per unit area in the world (Carpenter and Springer 2005). Philippine coral reef fishes have experienced a decline in biodiversity and abundance over the last century that coincides with increased anthropogenic activities such as fishing and coastal development (Lavides et al. 2016; Nañola et al. 2011). Habitat degradation has also increased, with the latest Philippine National Assessment of Coral Reefs concluding that no sites had “excellent” live coral cover and that 90% of sites had “poor” or “fair” coral cover (Licuanan et al. 2019).

In this study, we used low-coverage whole genome sequencing of historical and contemporary individuals at sites with differing human population densities to evaluate if and how anthropogenic pressures have impacted genetic diversity and selection over the last century in the reef-associated cardinalfish, *Taeniamia zosterophora*. With this data, we evaluated two questions: **1) Does the change in genetic diversity over the last century differ between fish from areas with lower human impact and areas with higher human impact?**, and **2) Does evidence for selection differ between areas with lower human impact and areas with higher human impact?** For Q1, we hypothesized that because of population declines associated with habitat degradation or fish collection, there would be an overall decline in genetic diversity between 1908 and 2022, with greater declines in areas with higher human impact. For Q2, we hypothesized that there would be regions of the genome under selection in fish from both sampling sites, and we expected more loci under selection in areas with higher human impact. Our findings revealed recent evolutionary changes in tropical marine fishes associated with intensified anthropogenic activities, which have not been extensively studied in wild populations to date.

Methods

Sample Collection

The Smithsonian NMNH *Albatross* collection contains samples collected from across the Philippines between 1907 and 1909 (Smith and Williams 1999). During the voyage, whole fish specimens were stored in high proof rum distillate obtained in the Philippines before being transferred to 75% ethanol for storage at the Smithsonian Institution, Washington, D.C. As a proxy for human impact, we identified areas with low (under 200 people/km²) and high (over

800 people/km²) human population density according to 2020 data (WorldPop 2020). Human population density is not an exact representation of human influence on coastal areas, though density provides a consistent proxy for coastal development and human activity in a region (Gallardo, Zieritz, and Aldridge 2015), and the data are available for all coastlines of the Philippines (WorldPop 2020).

We selected *T. zosterophora* as our study species, as the *Albatross* voyage sampled it in both low and high human population density areas. *Taeniamia zosterophora* is a small, mouth-brooding cardinalfish (Thresher 1984), and dispersal during the larval stage is likely limited based on findings of high genetic differentiation over small spatial scales in other cardinalfish species (Gerlach et al. 2016; Hoffman et al. 2005; Rueger et al. 2020; Vagelli et al. 2008). The pelagic larval duration for species in the closely-related *Apogon* genus ranges from 14-25 days (Leis et al. 2015). We expected that gene flow and effective population size would be low relative to many other fishes as only the larvae of mouth brooders can be dispersed via ocean currents, whereas many fishes can be dispersed by ocean currents at both the egg and larval stage (Selkoe and Toonen 2011). A member of the family Apogonidae, *T. zosterophora* was previously classified as *Archamia zosterophora* and was re-classified into the genus *Taeniamia* in 2013 (Fraser 2013; Mabuchi et al. 2014). Cardinalfishes are nocturnal and demonstrate high fidelity to diurnal refugia locations in branching corals or caves (Fukumori et al. 2010; Gardiner and Jones 2005; Marnane and Bellwood 2002). *Taeniamia zosterophora* has high site fidelity and relatively specific habitat preferences, often found only on reef patches that include branching *Acropora*, *Pavona*, and *Porites* corals and with a preference for *Porites cylindrica* (Gardiner and Jones 2005 2010 2016). These habitat preferences may make the species more vulnerable to population

decline, because coral bleaching is accelerating globally, especially in the Coral Triangle, and suitable habitat for the species continues to be lost (McManus et al. 2020; Sully et al. 2019).

Taeniamia zosterophora was collected by the *Albatross* voyage in the west Philippines (Malampaya, Palawan), an area with lower human population density (<200 people/km²), and in the central Philippines (Mantatao Island, Bohol) at a site with higher human population density (>800 people/km²) (fig. 1; table S1). A 1903 census estimated approximately 5 people/km² in Palawan and 70 people/km² in Bohol (Sanger et al. 1905). Contemporary samples were collected by local fishers at locations as close as logistically feasible to the historical collection locations and were stored in DESS, a solution composed of EDTA, DMSO, and NaCl (Yoder et al. 2006). Historical samples at Malampaya were collected at 10.923 °N, 119.249 °E in December 1908, while contemporary samples were collected at 10.858 °N, 119.354 °E in December 2022 (~13 km between historical and contemporary sampling locations). Historical individuals at Mantatao Island were collected at the coordinates 9.940 °N, 123.869 °E in April 1908, and contemporary individuals were collected at 9.941 °N, 123.853 °E in November 2021 (~2 km between historical and contemporary sampling locations).

Other environmental data for both sampling sites is difficult to obtain, but there is nearby coral cover data available. Two coral monitoring stations near our high human impact site of Mantatao Island received an “Excellent” hard coral cover rating in 2017 (Licuanan et al. 2017). The monitoring station closest to our low human impact site of Malampaya, Palawan was also rated as “Excellent”, but a second station slightly farther away was rated as “Poor” (Licuanan et al. 2017). There are also land use differences between the two sampling sites, with Mantatao

historically having more cultivated agricultural land and Malampaya more forested land. Bohol (where Mantatao is located) has a lengthy history of agriculture, with records showing the cultivation of sugar from the 1800's and land use maps showing the island as mainly cultivated land in 1942 and 1973 (*Philippine Islands Land Utilization* 1942; *Philippines - Land Utilization* 1973; Sanger et al. 1905). In contrast, land near Malampaya was historically classified as “Forest Lands” or “Commercial Forest” (*Philippine Islands Land Utilization* 1942; *Philippines - Land Utilization* 1973). These land use trends have continued into the present day with land cover classified in 2020 as “Open Forest” in Malampaya and “Perennial Crop” in Mantatao (Dag-uman 2022). Mantatao likely has more runoff from agriculture than Malampaya. However, logging in Malampaya can also cause sedimentation.

Sequencing, Bioinformatics, and Population Genomics

DNA was extracted at Old Dominion University from 223 total specimens (95 historical and 128 contemporary) using 45-50 mg of muscle tissue per individual and Qiagen DNeasy 96-well Blood and Tissue kits. The amounts of Proteinase K, Buffer ATL and RNase A were double the standard Qiagen protocol. Samples were then prepared for low-coverage whole genome sequencing by the Texas AandM University - Corpus Christi Genomics Core Laboratory using KAPA Hyperplus library kits and Adapterama stubby adapters and indexed primers (Glenn et al. 2019) following Roberts (Roberts 2023). The individuals were sequenced across two-thirds of 6 lanes of an Illumina NovaSeq X Plus 10B flowcell. We targeted 2x coverage of each individual. Sequence data were initially processed with the *pire_fq_gz_processing* pipeline (Bird, Garcia, Brendan N. Reid, Rene D. Clark, et al. 2024), which includes quality checks, trimming, deduplication, and the removal of exogenous DNA. Subsequently, a *de novo* genome was

generated from a single contemporary individual (~125x coverage, sequenced via shotgun sequencing on an Illumina HiSeq lane using the same extraction and library preparation kits as the low-coverage sequencing) following the *pire_ssl_sequencing* pipeline (Garcia, Roberts, Reid, Labrador, et al. 2024). In short, this latter pipeline first utilizes Jellyfish v.2.3.0 (Marçais and Kingsford 2011) and GenomeScope v.2.0 (Vurture et al. 2017) to estimate the heterozygosity and genome size of the species before using SPAdes v.3.15.3 (Bankevich et al. 2012; Prjibelski et al. 2014 2020; Vasilinetc et al. 2015) to build an assembly to the scaffold level using short-read data. Assembly quality was subsequently assessed with QUAST v.5.1.0 (Gurevich et al. 2013) and BUSCO v.5.1.2 (Seppey et al. 2019; Simão et al. 2015; Waterhouse et al. 2018). For other resequencing data, we ran mapping, rescaling, and indel realignment using the GenErode pipeline (Kutschera et al. 2022). The GenErode pipeline uses BWA (Li 2013; Li and Durbin 2009) for mapping (BWA aln for historical samples, BWA mem for contemporary samples), MapDamage2.0 (Jónsson et al. 2013) for rescaling to account for DNA damage patterns, and GATK v.3.7 (Van der Auwera et al. 2013) indel realigner tool for indel realignment.

We performed SNP calling and allele frequency calculations using ANGSD v.0.940 (Korneliussen, Albrechtsen, and Nielsen 2014). We first generated a list of SNPs for further analysis in ANGSD using the rescaled and realigned bam files generated with GenErode as inputs. We set minimum depth filters that corresponded to 1x per individual, maximum depth filters corresponding to 15x per individual, a minimum individual filter of 50% of the total number of individuals (111), a mapping quality filter of 30, a minimum allele frequency filter of 0.001, and a SNP p-value of 10^{-6} . Unless otherwise noted, all subsequent analyses in ANGSD were run on this list of SNPs to ensure that comparisons among populations were made on the

same parts of the genome. We excluded transitions from all analyses in ANGSD to remove potential sequencing errors due to DNA degradation (e.g., cytosine deamination towards fragment ends that result in a C→T transition on the affected strand) that may not have been fully accounted for by MapDamage2.0. We used the list of SNPs along with the SamTools model (-GL 1 option) to estimate genotype likelihoods and to generate a BEAGLE file for all individuals.

Population structure analysis (PCA and Admixture) was performed in PCAngsd, using the MAP test to select the most supported value for K (Meisner and Albrechtsen 2018). We also generated minimum allele frequencies and site allele frequencies for each population using the list of SNPs generated. We defined a population as the group of individuals at the same location during the same time period. Our study therefore had four populations: Malampaya-Historical, Malampaya-Contemporary, Mantatao Island-Historical, and Mantatao Island-Contemporary. We calculated minimum allele frequencies by inferring major and minor alleles from genotype likelihoods (-doMajorMinor 1 option) and the EM algorithm (-doMaf 1 option) in ANGSD. Allele frequency estimation averages over the uncertainty in the individual genotype calls and produces substantially more accurate allele frequency estimates than do hard genotype calls for low coverage data and does equivalently well as hard genotypes calls with moderate-coverage data (Lou et al. 2021; Maruki and Lynch 2017).

Selection Analysis

We identified SNPs under selection over time using the adapted Cochran-Mantel-Haenszel (CMH) test and the adapted Chi-squared test (Spitzer et al. 2020). The adapted CMH test

requires replicate populations per time-period, and we ran the test with our two sampling sites per time period to detect selection present across both sampling sites. We also ran the adapted Chi-squared test individually for each sampling site to detect SNPs under selection that might be missed by the adapted CMH test because they are only under selection at one sampling site. For both tests, we utilized our N_e estimate calculated from the Jorde and Ryman estimator and a generation time of one year (Kume et al. 2003). We utilized the `p.adjust()` function in R to perform an FDR correction on p -values. We considered SNPs with a q -value < 0.05 to be under selection. We report the allele that increased in frequency on average across both sites through time.

Genetic Diversity and Differentiation

Genetic diversity and F_{ST} were calculated by generating site allele frequency files and a site frequency spectrum for each population. We calculated a folded site allele frequency spectrum based on individual genotype likelihoods (`-doSaf 1` option in ANGSD). To calculate genetic diversity metrics (Watterson's θ , nucleotide diversity, Tajima's D), we used the folded site frequency spectra to generate per-site thetas from each scaffold. In our case, site refers to the list of SNPs we first generated and therefore does not include sites monomorphic in all sampled populations. This analysis identified that genetic diversity was somewhat sensitive to sequencing depth below 3x or above 6x (figs. S1-S2); we therefore restricted analyses on genetic diversity metrics to the 2,291 scaffolds with 3-6x depth. We used this range out of an abundance of caution given that depth-based correlations can sometimes be an issue in studies using low-coverage data (Kardos and Waples 2024). If base calling error rates are miscalibrated, it is possible that the uncertainty incorporated into genotype likelihoods could influence a genetic

diversity versus depth trend, but we would need additional higher coverage data (15-20x or greater) to fully evaluate this. With the available data, we do not see indications that this is an issue for our dataset. We also used the folded site frequency spectra to calculate average weighted pairwise F_{ST} estimates among populations. We would not expect the use of the folded site frequency spectra to influence our results, as we only used the genetic diversity metrics that are reliable with folded spectra (Korneliussen et al. 2014). To test for how selection may have been affecting genetic diversity and population structure results, we also ran the above analyses on a list of SNPs that excluded loci detected as under selection (see Selection Analysis section above for more details).

Statistical Analysis

Statistical analysis was conducted in R v.4.4.1 (R Core Team 2024) unless otherwise noted. To test whether there was a change in genetic diversity over time (Q1), we used the Watterson's theta and nucleotide diversity estimates for each population. We ran paired t-tests on these theta and pi values between the historical and contemporary populations at each sampling site, which produced one paired t-test of Malampaya-Historical vs. Malampaya-Contemporary for each diversity metric and a second paired t-test of Mantatao Island-Historical vs. Mantatao Island-Contemporary. We used a significance threshold of $p < 0.05$ to reject the null hypothesis in all of our statistical tests unless otherwise noted. We utilized the boot package in R to generate 95% confidence intervals for our theta estimates by bootstrapping the mean across scaffolds using 1,000 replicates for each population.

Effective Population Size

We calculated effective population size estimates for each sampling site using the change in allele frequencies at the putatively neutral SNPs through time. We utilized Jorde and Ryman's estimator (2007) and NeEstimator v2.1 (Do et al. 2014) to perform the calculations. We used a generation time of one year in our calculations based on estimates of sexual maturity for cardinalfish (Kume et al. 2003). We generated 95% confidence intervals using the boot package in R and bootstrapping Jorde and Ryman's estimator across SNPs with 1,000 replicates for each sampling site.

Results

Genome Assembly

The *T. zosterophora* genome assembly yielded a total length of 835 million base pairs with 63,527 scaffolds, an N50 of 19,682 base pairs, and an L50 of 11,491 scaffolds. This falls within the Jellyfish kmer-based genome length prediction of 817-884 million base pairs. Genome completeness based on BUSCO for the Actinopterygii lineage was 71.1% for single copy genes, and the GC percentage was 39.21%. Genomes from other cardinalfish in the Apogonidae family have GC contents of 38% (*Sphaeramia orbicularis*) and 37.71% (*Sphiamia tubifer*) and have lengths of 1.3 Gb (*S. orbicularis*) and 1.2 Gb (*S. tubifer*) (Gould et al. 2022; Venkatesh 2018). We filtered the genome to include only scaffolds with 50,000 base pairs or longer. After this filtering, the genome had a length of 412 million base pairs and 11,150 scaffolds.

Sequencing

We sequenced a total of 95 historical (64 at Malampaya, 31 at Mantatao Island) and 128 contemporary individuals (64 at Malampaya, 64 at Mantatao Island). After filtering, ANGSD

returned likelihoods for 1,784,878 SNPs from a mean depth of 2.59x per individual. After filtering for an average depth per scaffold between 3 and 6x, we retained 2,291 scaffolds with 452,496 SNPs for calculating genetic diversity metrics (Watterson's theta, nucleotide diversity, and Tajima's D). These latter scaffolds represent 72 million base pairs, approximately 18% of the filtered genome that we mapped to and had a mean depth of 3.97x per individual. There were no notable systematic differences in patterns of missingness, with similar fractions of individuals having data at a SNP position regardless of the population they were from. The median percentage of individuals having data at a SNP position was around 60% for all populations (56% Malampaya-Historical, 64% Malampaya-Contemporary, 67% Mantatao Island-Historical, 65% Mantatao Island-Contemporary).

Selection Analysis

We found evidence of selection over time across the genome. Using the adapted CMH test, 69,940 SNPs (3.9% of all 1.8M SNPs) were identified as potentially under selection at both sampling sites (fig. 2). Of these SNPs under selection, 63,694 (91%) showed a greater change in frequency at Mantatao Island, the site with higher human impact. In addition, 30,318 of the SNPs under selection showed an increase in allele frequency over time at both sites, and 27,336 SNPs (90.16%) had a greater increase in frequency for the Mantatao Island site. The mean allele frequency for these 69,940 SNPs putatively under selection in Malampaya remained relatively stable over time, increasing only slightly from a historical mean of 0.2554 ± 0.0016 [standard error] in 1908 to a contemporary mean of 0.2564 ± 0.0016 (fig. 2A). In Mantatao Island, mean allele frequency increased more substantially from 0.2490 ± 0.0015 to 0.2960 ± 0.0015 over time (fig. 2B).

An adapted Chi-squared test to detect the presence of selection over time at each sampling site individually identified 231,450 SNPs at Malampaya and 275,831 SNPs at Mantatao Island (13.0% and 15.5% of all 1.8 million SNPs respectively) as potentially under selection, despite larger sample sizes and greater power to detect selection at Malampaya. The difference in numbers of SNPs was statistically significant (chi-squared test, $\chi^2 = 2,301,351$, $df = 3$, $p\text{-value} < 2.2 \times 10^{-16}$). In Malampaya, mean allele frequency for the 231,450 SNPs putatively under selection increased over time from 0.0186 ± 0.0001 [standard error] to 0.1121 ± 0.0003 (fig. 2C). Similarly, in Mantatao Island, mean allele frequency for the 275,831 SNPs putatively under selection increased from 0.0138 ± 0.0001 to 0.1098 ± 0.003 (fig. 2D).

Population Structure

Both PCA and Admixture analyses identified population structure between historical and contemporary individuals (fig. 3). The PCA and Admixture analyses mostly grouped individuals together according to their time period, suggesting some genetic differentiation over time. Individuals appeared to be genetically quite similar between sites within the same era. A MAP test yielded $K=2$ as the most supported number of source populations. Supporting this grouping, pairwise F_{ST} values between sites within time periods were lower than pairwise F_{ST} between time periods, though we also note that all F_{ST} values were below 0.04 (table 1). Population structure analyses run on the putatively neutral SNPs showed less differentiation than when run on all SNPs (see figs. 3B and D). However, the overall pattern of population structure between historical and contemporary individuals remained consistent. Pairwise F_{ST} values were lower for the putatively neutral SNPs than for all SNPs (table 1).

Genetic Diversity

Across all sites, Watterson's theta increased significantly over time in both the low and high human impact sites. At our low human impact site (Malampaya, Palawan), the mean Watterson's theta was 0.0791 ± 0.00020 [standard error] for historical individuals, while the mean Watterson's theta was significantly higher at 0.0804 ± 0.00020 for contemporary individuals (figs. 4 and S1; paired t-test: $t = -9.6916$, $df = 2290$, $p\text{-value} < 2.2 \times 10^{-16}$). For our high human impact site (Mantatao Island), the mean Watterson's theta was 0.0744 ± 0.00024 for historical individuals and also significantly higher for contemporary individuals (0.0885 ± 0.00017 , paired t-test: $t = -77.89$, $df = 2290$, $p\text{-value} < 2.2 \times 10^{-16}$). Nucleotide diversity also increased significantly over time. In Malampaya, nucleotide diversity significantly increased from 0.0659 ± 0.0035 historically to 0.0735 ± 0.00041 in contemporary individuals (figs. 4 and S1; $t = -42.344$, $df = 2290$, $p\text{-value} < 2.2 \times 10^{-16}$). At Mantatao Island, there was also a significant increase in nucleotide diversity, from 0.0644 ± 0.00034 in historical individuals to 0.0724 ± 0.00038 in contemporary individuals ($t = -47.459$, $df = 2290$, $p\text{-value} < 2.2 \times 10^{-16}$).

To test how changes in selection over time may have impacted the changes in genetic diversity we observed, we also calculated Watterson's theta and nucleotide diversity on SNPs that were identified as putatively neutral. In Malampaya, the mean Watterson's theta was 0.0934 ± 0.00022 for historical individuals but was significantly lower in contemporary individuals with a mean of 0.0880 ± 0.00024 (figs. 4 and S2, paired t-test: $t = 36.172$, $df = 1914$, $p\text{-value} < 2.2 \times 10^{-16}$). In Mantatao Island, there was a slight but significant drop in theta from a mean theta in historical individuals of 0.0906 ± 0.00027 to 0.0895 ± 0.00022 in contemporary individuals

(paired t-test $t = 6.3556$, $df = 1914$, $p\text{-value} = 2.6 \times 10^{-10}$). Mean nucleotide diversity in Malampaya was not significantly different over time with a historical mean of 0.0858 ± 0.00047 and a contemporary mean of 0.0858 ± 0.00051 ($t = -0.073435$, $df = 1914$, $p\text{-value} = 0.94$). In Mantatao Island, mean nucleotide diversity significantly decreased from 0.08488 ± 0.00048 in the historical individuals to 0.0819 ± 0.00049 in contemporary individuals ($t = 17.001$, $df = 1914$, $p\text{-value} < 2.2 \times 10^{-16}$).

Effective Population Size

We obtained a contemporary effective population size point estimate of 2311 (95% CI: 2294-2331) from the temporal changes in allele frequencies for our low human impact site at Malampaya, Palawan, and an estimate of 2230 (95% CI: 2207-2253) at our high human impact site on Mantatao Island. Tajima's D distributions peaked at less than one for both the historical and contemporary samples, suggesting there has been a long-term increase in effective population size over time (figs. 5 and S3).

Discussion

Our findings reveal evidence for the intensification of both neutral drift and adaptive evolution in coral reef fish populations in the face of anthropogenic impacts. Using low-coverage whole genome sequencing data from *T. zosterophora* collected across more than a century, we unexpectedly found significant increases in genome-wide genetic diversity that could be largely attributed to positive selection. In contrast, the putatively neutral fraction of the genome lost diversity, with somewhat stronger signals of loss in the site with larger human influence. Selection scans revealed that approximately 4% of SNPs showed evidence of selection over the

last century at both of our sampling sites, with stronger signals of selection at the site with larger human influence. Our results provide empirical evidence for the interplay of drift and selection in the face of rapid environmental change in a tropical marine fish.

Population Structure

Our population structure analyses revealed substantial genetic differentiation through time that could be explained by a combination of drift and selection. Patterns of differentiation across time have been observed in songbirds (Turbek et al. 2023), while studies in fishes have found temporal stability across time and effective population sizes above 10,000 in some species (Pinsky et al. 2021; Therkildsen et al. 2010) and strong drift and low effective population sizes in others (Hauser et al. 2002; Hutchinson et al. 2003b). Our effective population size estimates of just over 2,000 individuals suggest that *T. zosterophora* has medium-sized populations that may experience stronger genetic drift than would pelagic species with larger population sizes (Allendorf et al. 2013; Poulsen et al. 2006; Whitlock 2000). Further, we found that loci under temporal selection explained roughly half of the differentiation observed, suggesting a strong role for selection in creating population structure.

There were indications that *T. zosterophora* was more dispersive than anticipated based on their mouth brooding and the limited pelagic larval duration of Apogonids. Other cardinalfishes exhibit higher levels of genetic differentiation among populations (Gerlach et al. 2016; Hoffman et al. 2005; Rueger et al. 2020; Vagelli et al. 2008). However, the cardinalfish *Siphamia tubifer* in Okinawa exhibited mostly insignificant structure (Gould and Dunlap 2017), which was attributed to the Kuroshio Current. In the Philippines, the Sulu Sea Throughflow may similarly

connect the populations sampled here by transporting larvae in a stepping stone manner from western Palawan into the Sulu Sea and other inland seas (including the Bohol Sea) (Gordon et al. 2011; Pata and Yñiguez 2019 2021; Raynal et al. 2014).

Genetic Diversity

Contrary to our hypotheses that genetic drift would be a dominant force that eroded genome-wide genetic diversity through time, we found a surprising and significant increase in genome-wide genetic diversity in *T. zosterophora* over the last century at both our low and high human impact sites. Despite extensive documentation of diversity loss across mammals, birds, fishes, and other animals (Leigh et al. 2019; R. E. Shaw et al. 2025), studies sequencing historical and contemporary samples of Atlantic cod (*Gadus morhua*) (Pinsky et al. 2021; Therkildsen et al. 2010) and kokanee salmon (*Oncorhynchus nerka*) (Setzke et al. 2021) found that genetic diversity did not significantly decline over time, with large and/or increasing effective population size, gene flow, and small allele frequency changes in many loci presented as possible explanations for the preservation of genetic diversity. In Lake Constance, the whitefish *Coregonus wartmanni* also maintained genetic diversity over time (Gum et al. 2014), with evidence of new alleles potentially introduced to the population from anthropogenic stocking. Conversely, multiple studies have found empirical evidence for a decline in genetic diversity in marine fishes, including *Gadus morhua* in the North Sea (Hutchinson et al. 2003a), Chinook salmon (*Oncorhynchus tshawytscha*) (Johnson et al. 2018), and New Zealand snapper (*Pagrus auratus*) (Hauser et al. 2002). Periods of prolonged genetic drift, declining effective population size following overexploitation, and selection that brings alleles closer to fixation (including

examples of fisheries-induced selection) are cited as possible reasons for the declines in genetic diversity in the studies above.

Explanations for increasing genetic diversity through time in *T. zosterophora* include positive selection on rare alleles, novel gene flow, and an increase in effective population size. We find this first explanation most convincing for *T. zosterophora*. First, we found evidence that selection was acting on some rare alleles at both of our sampling sites, increasing their frequency towards 50%. This trend was especially apparent when using the adapted Chi-squared test, with a substantial fraction of alleles increasing towards intermediate frequencies over time in Malampaya and Mantatao Island. This increase in frequencies for initially rare alleles would increase genetic diversity, including for nucleotide diversity. For nucleotide diversity, there are more pairwise nucleotide differences between samples when both alleles are more similar in frequency. Our finding is similar in some ways to the negative frequency-dependent selection that was found to have increased genetic diversity over a five-year experiment in tropical palms (Browne and Karubian 2016). In that experiment, a survival advantage was found for rare genotypes, possibly related to lower pathogen or predator attack or reduced competition through niche partitioning. Other studies have found candidate SNPs under selection with low to intermediate frequencies (Gignoux-Wolfsohn et al. 2021) and evidence for selection across many loci of small effect (B. N. Reid et al. 2023) but without increases in genetic diversity.

In stark contrast to the genome-wide results, most measures of genetic diversity declined through time when calculated on only putatively neutral SNPs (i.e., those SNPs presumably affected most strongly by drift rather than selection). These results suggest that drift did, in fact, reduce genetic

diversity through time as we had hypothesized, but that this effect was offset at a genome-wide level by positive selection on rare alleles. Selection, however, can also reduce genetic diversity, particularly if selective pressures are strong enough to send multiple alleles to fixation or close to fixation (Ellegren and Galtier 2016).

Alternatively, gene flow between populations can maintain and even increase genetic diversity through the transfer of alleles (Allendorf et al. 2013; Turbek et al. 2023). However, our population structure analysis revealed substantial gene flow between our two sampling sites even in historical time periods, with no evidence for increasing gene flow through time. There may have been increased gene flow over time from populations not sampled by our study, but we would need additional sampling locations to evaluate this. As another alternative, an increase in effective population size over the last century would have weakened genetic drift and increased the drift-mutation equilibrium level of genetic diversity (Charlesworth 2009). *T. zosterofera* is highly site specific and adapted to low light conditions (Gardiner and Jones 2016; Luehrmann et al. 2020). It is possible that coastal development would have led to increased sedimentation, reduced light, and an opportunity for population sizes of this species to grow over the past century. However, we found reduced diversity across the putatively neutral fraction of the genome, suggesting a decline rather than an increase in effective population size. This evidence leaves selection as the more likely explanation for increasing genome-wide genetic diversity through time.

Although the genome-wide increase in Watterson's θ was statistically significant at both sites, there was a difference in the magnitude of change between sites with a 1.6% increase in

mean Watterson's θ at our low human impact site and a 19.0% increase at our high human impact site. This is the opposite of what we hypothesized in Q1, but these results are nonetheless consistent with the differences in drift and selection that we hypothesized and that we detected across sites. We found support for our Q1 hypothesis (greater diversity loss in the site with greater human impact) in the putatively neutral fraction of the genome, with more diversity loss in Mantatao Island than in Malampaya. In addition, we found greater evidence for selection at Mantatao Island and evidence that selection led to increases in genetic diversity by increasing the frequency of rare alleles towards intermediate values. Together, these findings reveal a complex interplay of drift and selection across the genome during strong environmental change.

Selection and Adaptation

We found evidence for adaptation over the last century in *T. zosterophora*, with approximately 4% of SNPs identified as under selection at both our sampling sites. This adaptation may have occurred in response to changing environmental pressures over the last century, including pollution, sedimentation, and the loss of one-third of Philippine coral reef habitat in the last decade (Licuanan et al. 2019). Our result concurs with a growing body of work that has identified selection over time using temporal sampling in taxa from fish to birds to mammals (Bi et al. 2019; Chebib et al. 2016; Gignoux-Wolfsohn et al. 2021; Therkildsen et al. 2019; Turbek et al. 2023).

We found differences in selection identified between our sampling sites that suggested stronger selective pressures over the last century at Mantatao Island than in Malampaya. When we tested each sampling site individually for the presence of selection at individual loci, we found a

somewhat higher fraction of SNPs under selection at our high human impact site than our low human impact site. We also found that the high human impact site of Mantatao Island had the majority of SNPs with a greater increase in frequency, suggesting that selection was stronger over the last century in Mantatao Island. We do not see evidence for issues of low statistical power contributing to the difference in results between the two sites. If low power were an issue with our Mantatao population, we would expect to see less selection detected in Mantatao than in Malampaya. We found the opposite. Stronger selection could be the result of higher levels of environmental change, including pollution, sedimentation, and habitat degradation, or overexploitation from aquarium trade (Oziolor et al. 2019; N. M. Reid et al. 2016; Rhyne et al. 2017). With Bohol's history of agriculture that persists to the present day, there is likely runoff and sedimentation entering waters from agricultural fields. This may have created stronger selective pressures for the Mantatao Island population, compared to Malampaya where there is little agriculture. Our results show similarities to sequencing on lab-reared populations of fish, where a greater number of significant allele frequency shifts was observed in populations with higher selective pressures (Therkildsen et al. 2019). Our results show that populations within the same species may respond differently based on local selective pressures, making sampling across a variety of environments crucial for understanding the evolutionary responses and future conservation outlook for a species.

Implications for Conservation

Our results both show that genetic diversity can be preserved in some coral reef fishes even in the face of anthropogenic impacts and suggest that such patterns are unlikely to persist into the future. Preserving genetic diversity is a key component of biodiversity conservation efforts

(Hoban et al. 2020). Genetic diversity that increases because positive selection shifted rare alleles towards intermediate frequencies, however, is subsequently highly sensitive to those same selective pressures reducing genetic diversity as frequencies increase further towards fixation. In addition, it remains unknown if any fitness tradeoffs accompanied adaptive evolution. The evolution of smaller body size in cod, for example, is thought to make them more vulnerable to predation (Hočevar and Kuparinen 2021).

Our effective population size estimates of just over 2,000 individuals for each sampling site are greater than the commonly used recommended effective population density of 500 to preserve long-term adaptive potential (Franklin 1980). However, more recent analyses show that effective population sizes should be in the thousands to be resilient to environmental changes and catastrophic events (Traill et al. 2010). Although the areas around Mantatao Island have presumably higher human impacts than does Malampaya in Palawan, there have been efforts to conserve coral reefs in both areas, such as through the creation of networks of No Take Marine Reserves (NTMRs) (Alcala and Russ 2006; Lowry et al. 2009). Previous work has shown that NTMRs in the region increase coral cover (Magdaong et al. 2014), increase predator fish biomass (Russ and Alcala 2010), increase herbivorous fish biomass and decrease macroalgal biomass (Stockwell et al. 2009), increase reef fish diversity (Muallil et al. 2015), and increase total reef fish biomass (Aurellado et al. 2021). NTMRs in the central Philippines are generally small in area ($<1 \text{ km}^2$) but are spaced closely enough together that larval dispersal in reef fishes, such as *Chaetodon vagabundus*, connect one reserve to another (Abesamis et al. 2017; Weeks et al. 2010). This close spacing of reserves may support ample habitat for larvae settlement, enabling population growth (Gaines et al. 2010; Harrison et al. 2012). Additional sampling

across space would help to identify geographic patterns of gene flow and connectivity for protected area design.

Conclusions

Our study empirically investigated evolutionary change over a time period with rapid changes in the selective landscape due to anthropogenic activities. We found that genetic diversity significantly increased over the last century of anthropogenic change in a tropical marine fish. Our results suggest that it is possible for populations to preserve genetic diversity in the face of anthropogenic-driven environmental changes, with selection on rare alleles as a mechanism for preserving and increasing diversity. These populations may be more resilient to subsequent disturbances, as they may have ample genetic variation for adaptation to future changes. Local marine conservation efforts including designating and enforcing marine reserves may support the preservation of genetic diversity even in the face of unprecedented global change. Our study shows the value of utilizing historical collections and contemporary resampling to illuminate patterns of evolution in response to global change.

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Statement of Authorship

K.E.C., C.E.B., and M.L.P. conceptualized the project and acquired funding. K.E.C., J.P.B., A.A.B., R.D.C., E.G., and I.R.L. collected and curated data. K.S.F., C.E.B., S.F.M., J.P.B., R.D.C., E.G., M.J.M., I.R.L., and B.R.R. developed methodology. K.S.F. analyzed the data with assistance from J.P.B., R.D.C., M.J.M., and B.R.R. R.N.M., L.R.P., R.A.A., and M.D.S. and M.L.P. provided resources and supervision. K.S.F. wrote the original draft, and all authors reviewed and edited the manuscript.

Data and Code Availability

Sequence data is archived to the NCBI SRA. The BioProject numbers and links to data are as follows: PRJNA1185294 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1185294>), PRJNA1185280 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1185280>), PRJNA1185271 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1185271>), PRJNA1185264 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1185264>). Scripts for bioinformatics and

population genomics analysis are available at <https://doi.org/10.5281/zenodo.15384658> (Fitz 2025).

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Tables

Table 1: Weighted pairwise F_{ST} values among populations calculated in ANGSD.

	Historical- Malampaya, Palawan (N=64)	Historical-Mantatao Island (N=31)	Contemporary- Malampaya, Palawan (N=64)
Historical-Mantatao Island (N=31)	0.010 (0.007)		
Contemporary- Malampaya, Palawan (N=64)	0.030 (0.016)	0.037 (0.021)	
Contemporary- Mantatao Island (N=64)	0.033 (0.020)	0.030 (0.016)	0.009 (0.008)

Note: The first value was calculated across all 1.78 million SNPs, and the value in parentheses was calculated across 1.3 million putatively neutral SNPs. Comparisons within the same time period are bolded.

Figure Legends

Figure 1: *Taeniamia zosterophora* was collected and sequenced at one lower human impact site (Malampaya, Palawan) and one higher human impact site (Mantatao Island, Bohol). The circle represents the lower human impact, and the triangle represents the higher human impact.

Alt Text: A map of the Philippines with study sites marked. Malampaya, Palawan is located in the western Philippines, and Mantatao, Bohol is located in the central Philippines.

Figure 2: Allele frequencies for the 69,940 candidate SNPs identified as under selection by the adapted CMH test for A) Malampaya and B) Mantatao Island. In plots C) and D), allele frequencies for the 231,450 SNPs at C) Malampaya and 275,831 SNPs at D) Mantatao Island identified as under selection by the adapted Chi-squared test.

Alt Text: Four histograms that display the change in allele frequencies over time at both study sites. The histograms show a slight increase in allele frequencies over time.

Figure 3: PCA and Admixture with genomic data from 223 individuals. A) and C) were run across all 1.78 million SNPs and B) and D) across 1.3 million putatively neutral SNPs. For A) and B), Malampaya (lower human impact) data is represented by a circle and Mantatao Island (higher human impact) is represented by a triangle. In C) and D), the white dashed line separates each sampling site/era combination. From left to right, the order is Malampaya-Historical, Mantatao Island-Historical, Malampaya-Contemporary, Mantatao-Island Contemporary.

Alt Text: The PCA plots display historical individuals clumped together on the left side of the plot, and contemporary individuals are clumped together on the right side of the plot. The

Admixture plots show historical individuals as mostly having the same ancestry, and contemporary individuals as also mostly having the same ancestry.

Figure 4: Mean A) Watterson's θ estimates and B) nucleotide diversity (π) estimates for each population calculated on the 452,496 SNPs on scaffolds between 3-6x depth with 95% confidence intervals calculated from bootstrapping. Mean C) Watterson's θ estimates and D) nucleotide diversity estimates calculated on the neutral SNPs on scaffolds between 3-6x depth with 95% confidence intervals calculated from bootstrapping.

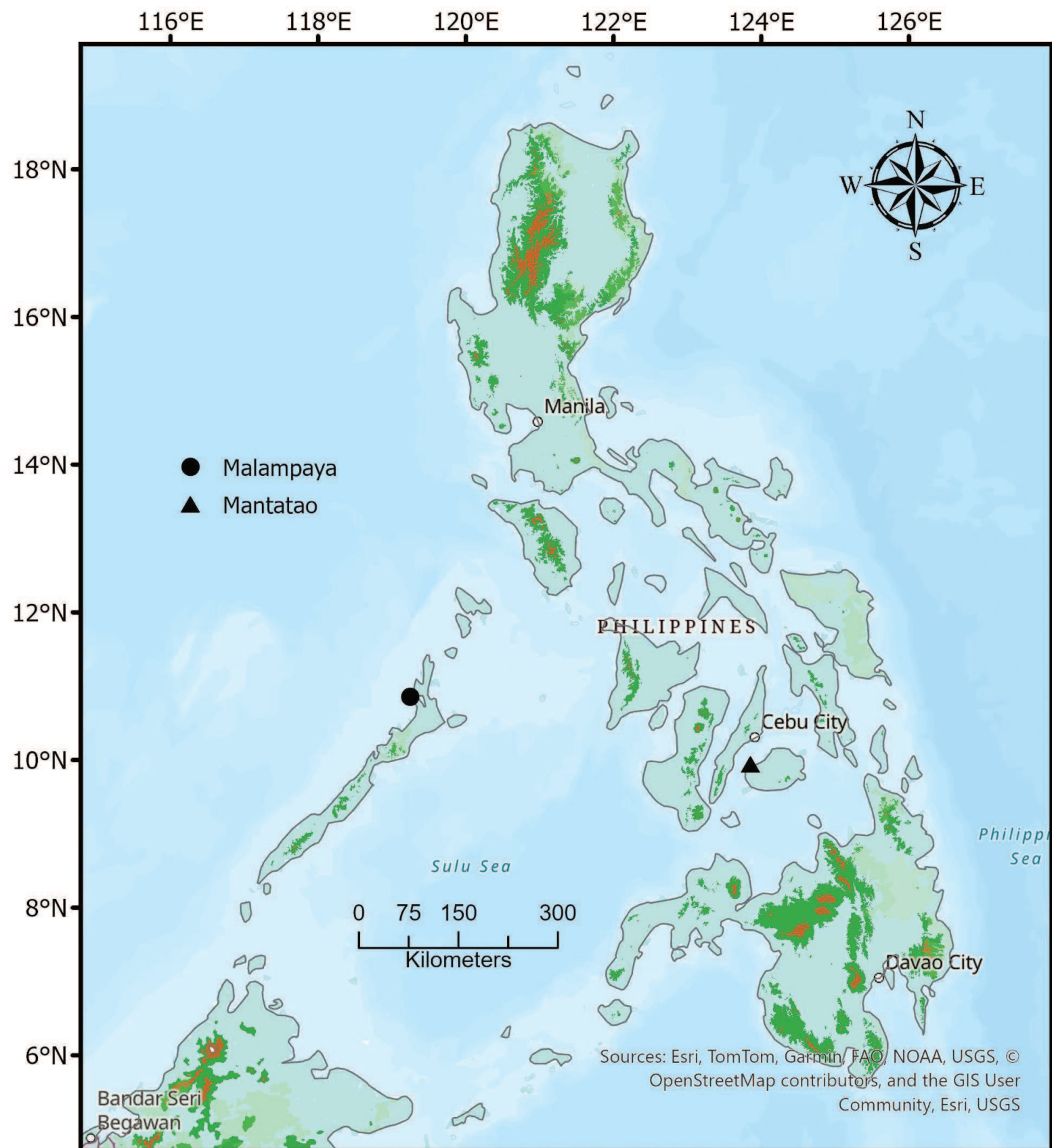
Alt Text: Four plots with a circle or triangle representing genetic diversity in the historical fish and a line connecting that to the genetic diversity value in the contemporary fish. The plots show an increase in genetic diversity over time when calculated over all SNPs but show a decrease or no change in genetic diversity over time when calculated over only the putatively neutral SNPs.

Figure 5: Density plots showing the distribution of Tajima's D calculated using all SNPs on scaffolds between 3-6x depth in A) Malampaya (lower human impact) and B) Mantatao Island (higher human impact).

Alt Text: Two density plots showing density on the y-axis and Tajima's D on the x-axis. Density peaks between 0 and -1 for historical and contemporary data.

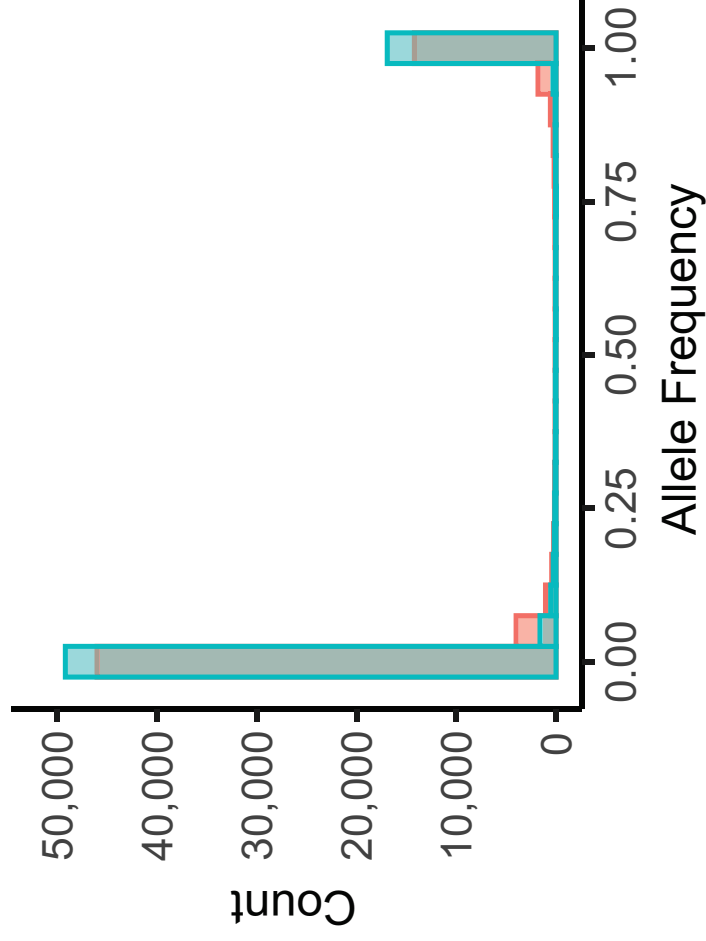
Figure 1

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A

Malampaya

**B**

Mantatao Island

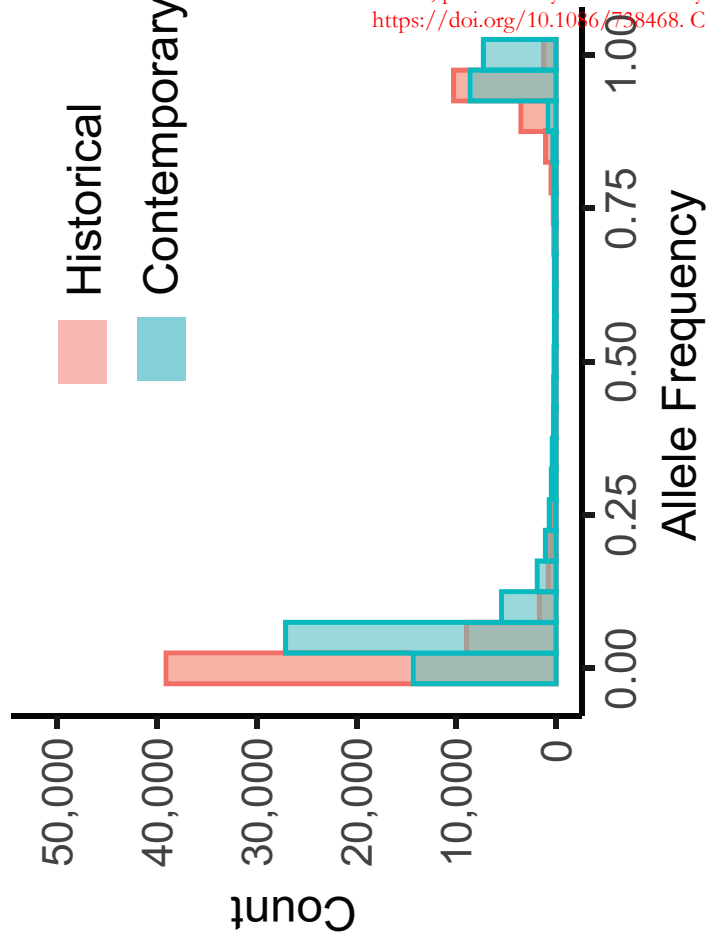
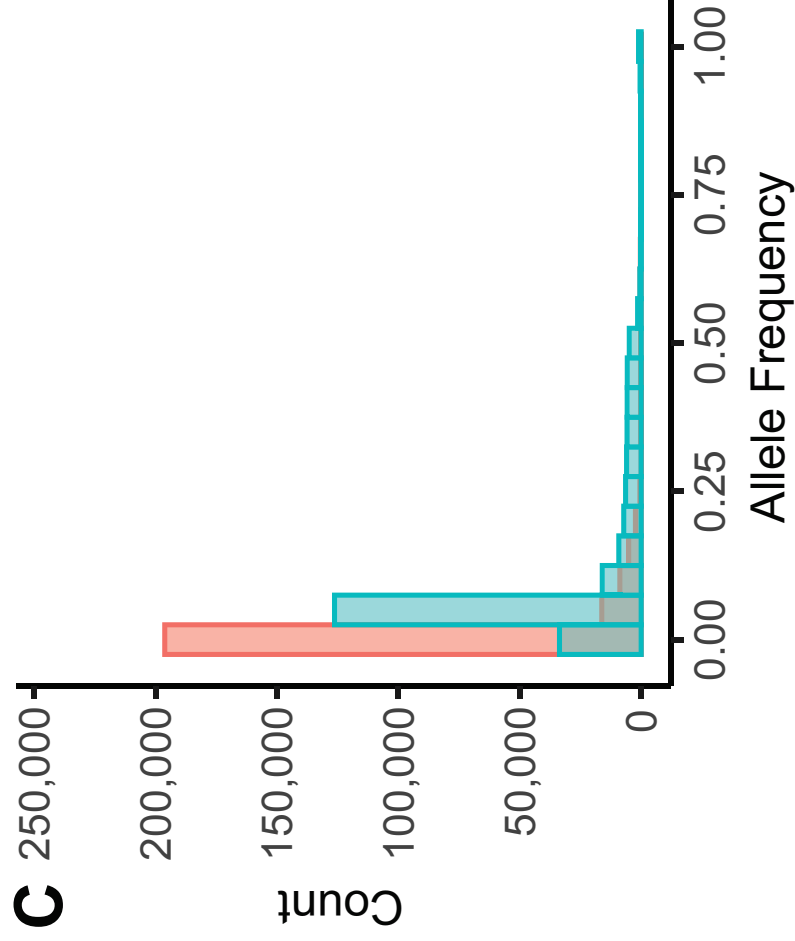
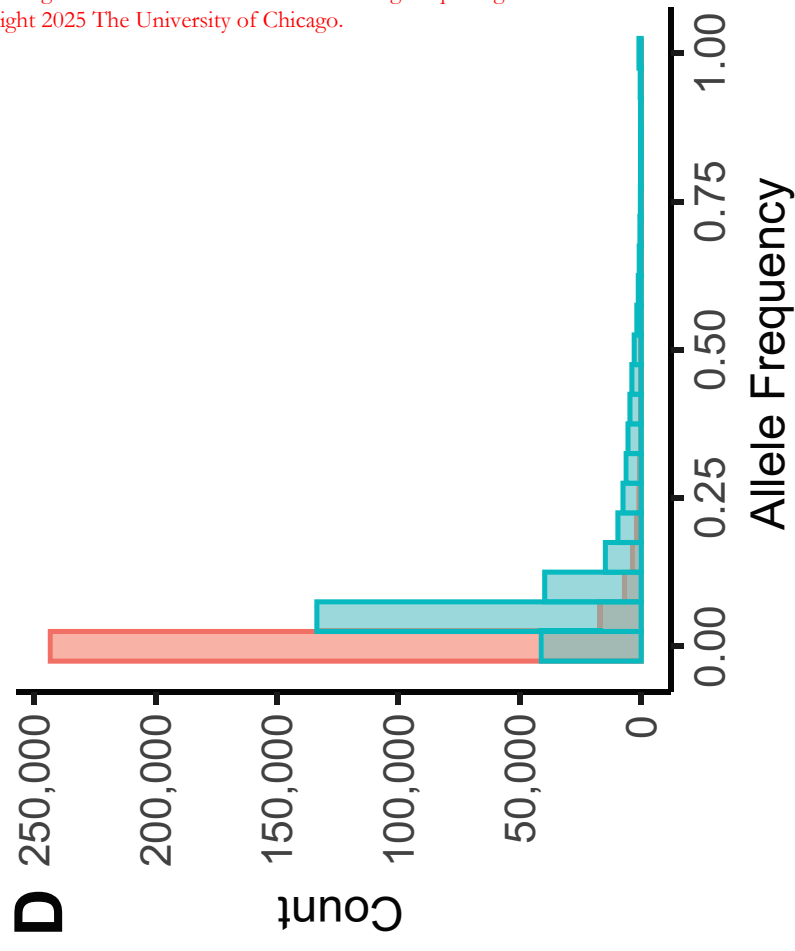
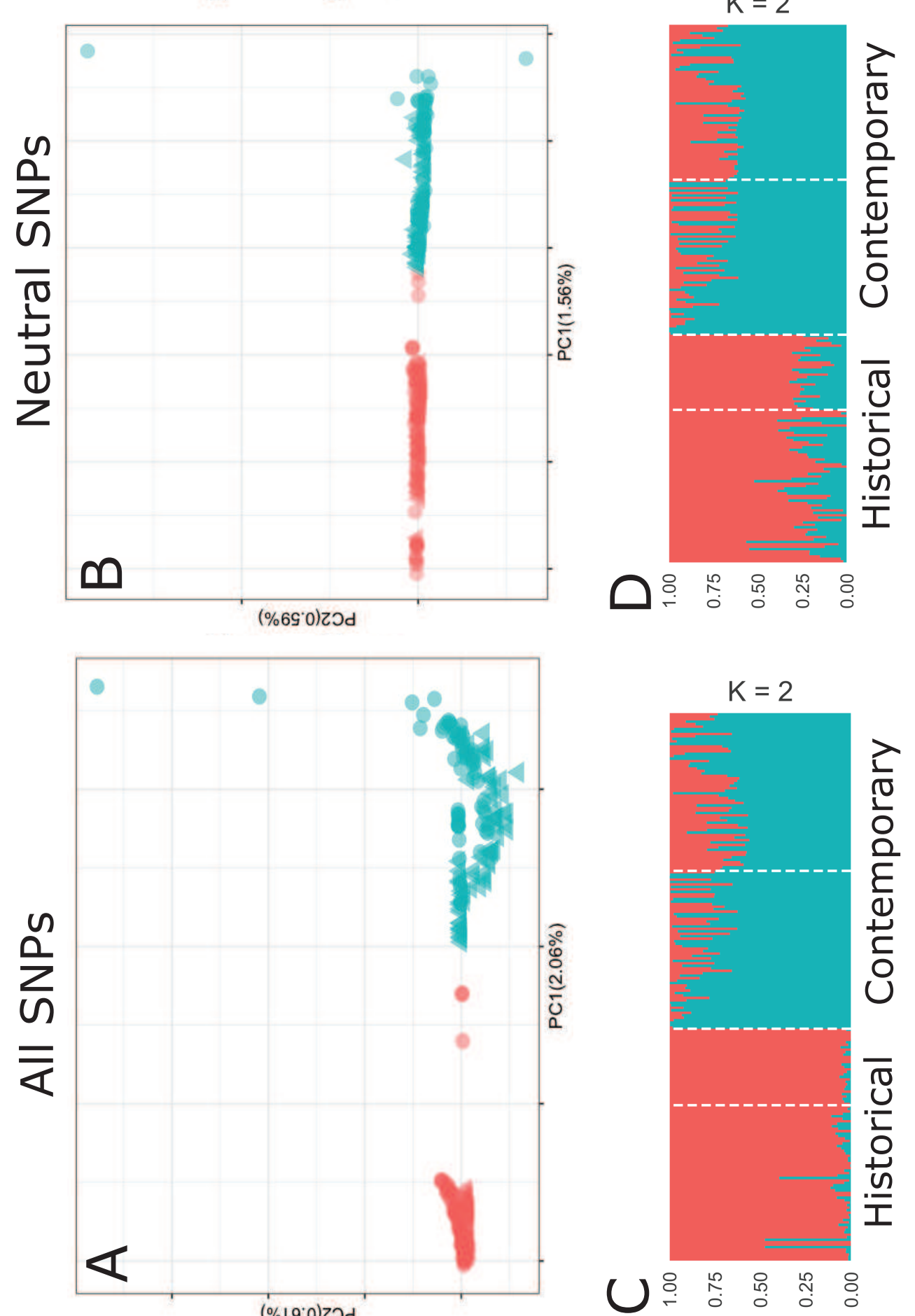
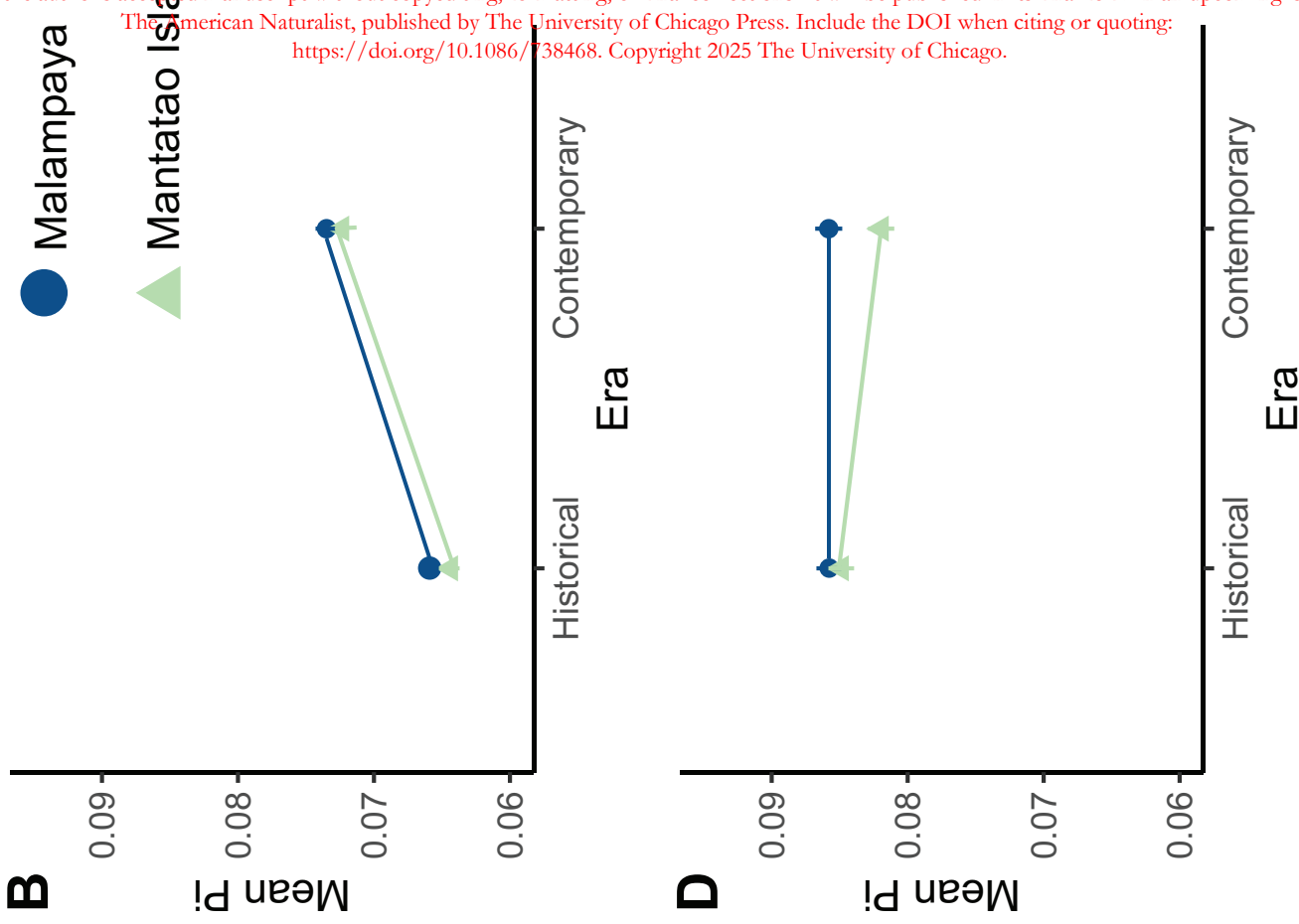
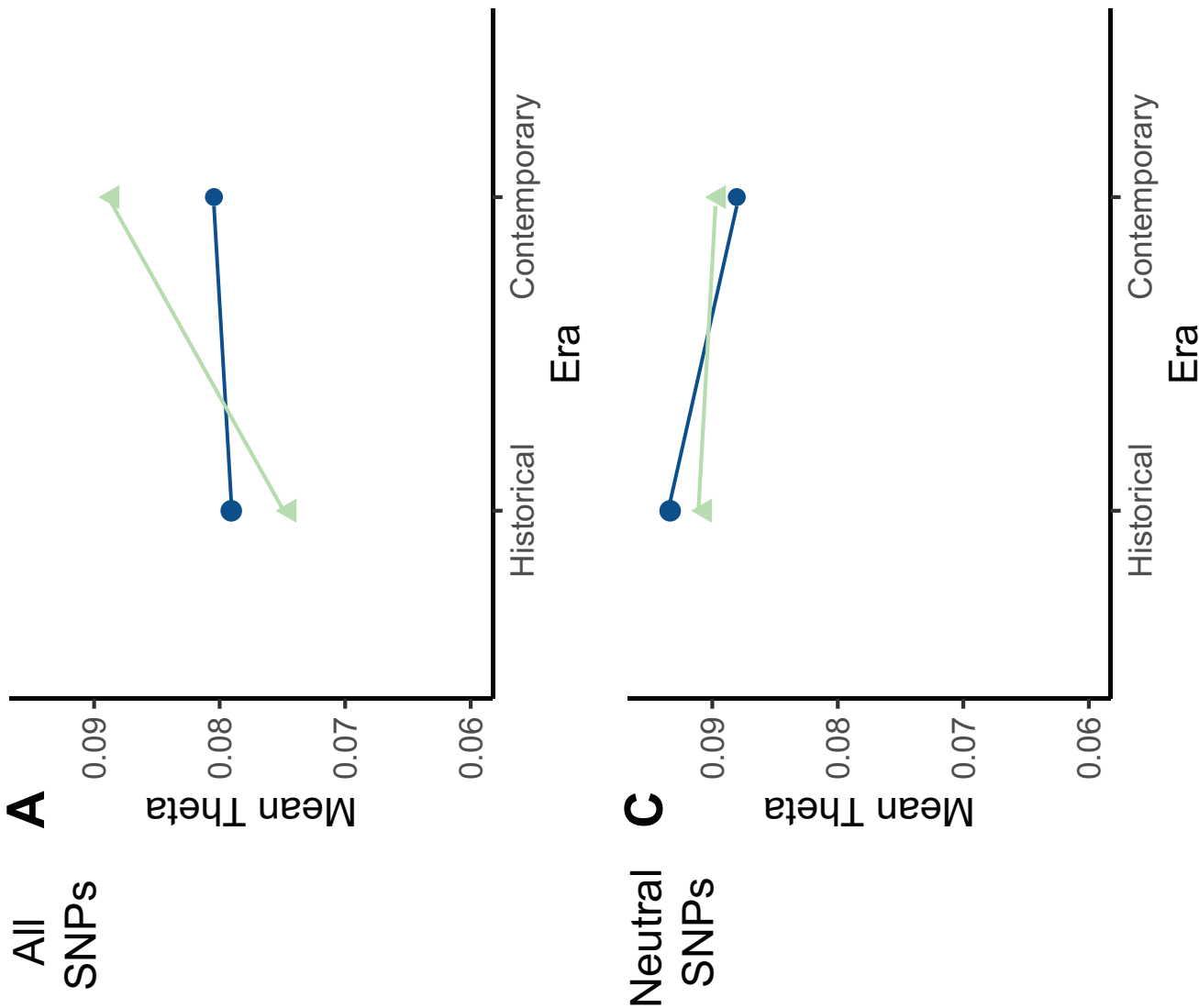
**C****D**

Figure 2

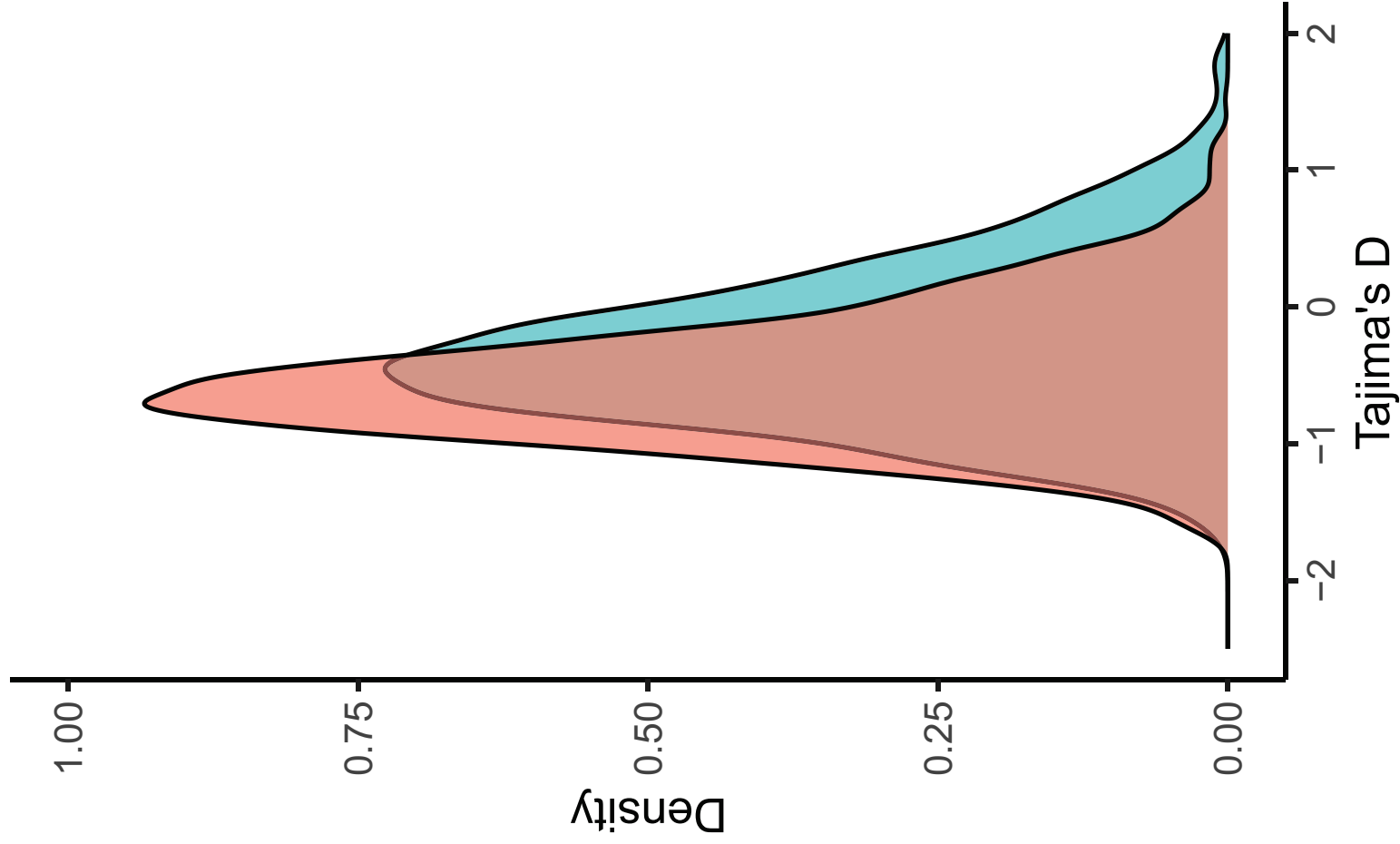
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A

Malampaya

**B**

Mantatao Island

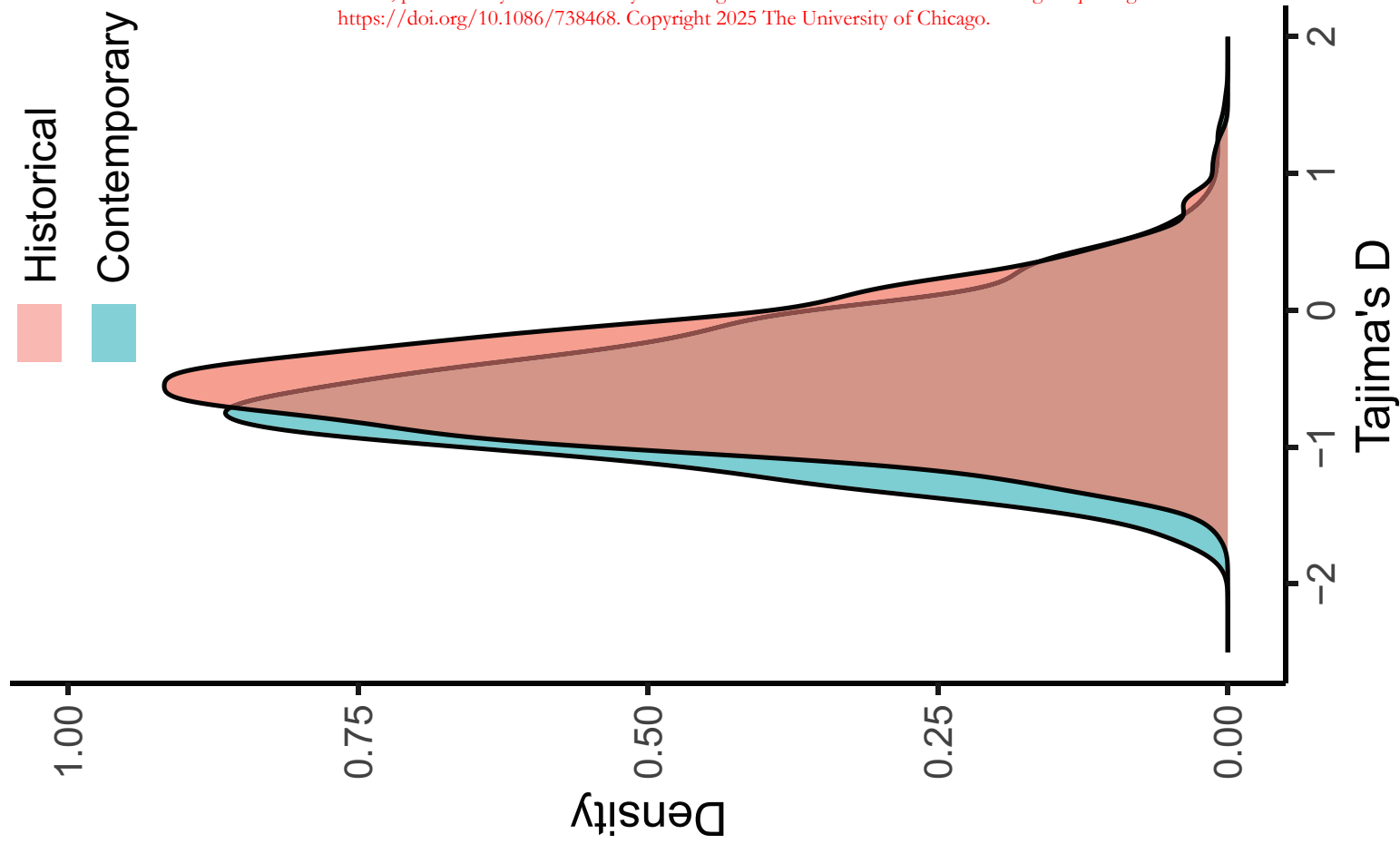


Figure 5

This is the author's accepted manuscript without copyediting, formatting, or final corrections. It will be published in its final form in an upcoming issue of The American Naturalist, published by The University of Chicago Press. Include the DOI when citing or quoting: <https://doi.org/10.1086/738468>. Copyright 2025 The University of Chicago.

Supplement:

Preservation of genetic diversity and selection over a century in a coral reef fish

(*Taeniamia zosterophora*) in the Philippines

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Contents: Table S1, Figures S1-S3

Supplementary Table

Table S1. Collection details. Collection locations, dates, coordinates, lot numbers, and number of individuals sequenced. Lot numbers for historical samples can be used to look up the specimens in the USNM database.

Location	Date Collected	Coordinates	USNM Lot Number or Contemporary Lot Number	Number of Individuals Sequenced
Malampaya, Palawan	December 21, 1908	10.923 °N, 119.249 °E	171321	64
Malampaya, Palawan	December 14, 2022	10.858 °N, 119.354 °E	PAL- 2022_06_04	64
Mantatao Island	April 8, 1908	9.940 °N, 123.869 °E	171348	31
Mantatao Island	November 12, 2021	9.941 °N, 123.853 °E	Boh- 2021_09_01	64

Supplementary Figures

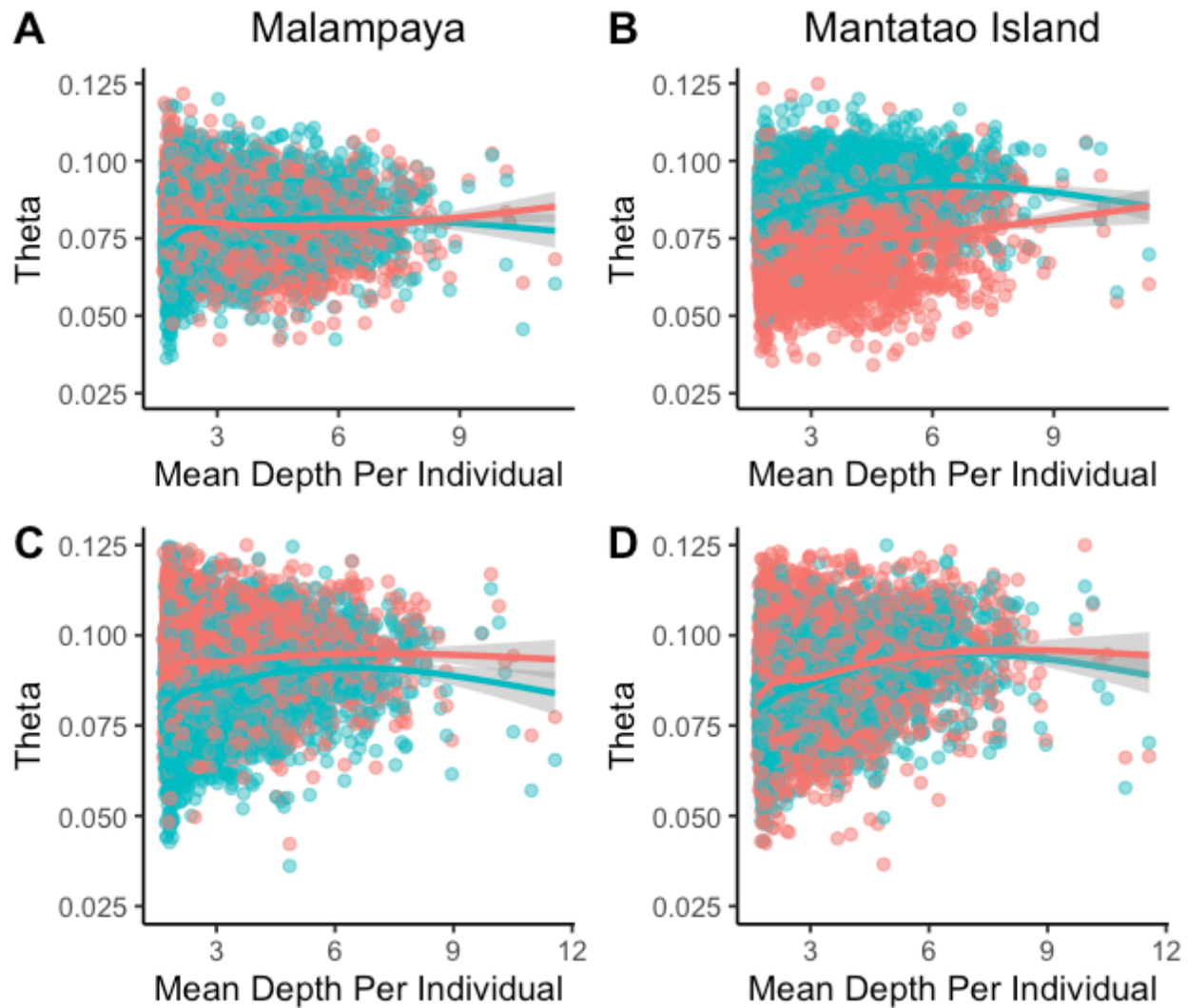


Figure S1. Watterson's theta calculated on all 1.8 million SNPs in A) and B) and on 1.3 million putatively neutral SNPs in C) and D) across each scaffold for each population versus the mean sequencing depth per individual. A) and C) show the lower impact site at Malampaya, Palawan and B) and D) show the higher impact site at Mantatao Island. Historical scaffolds are represented in red, and contemporary scaffolds are represented in blue.

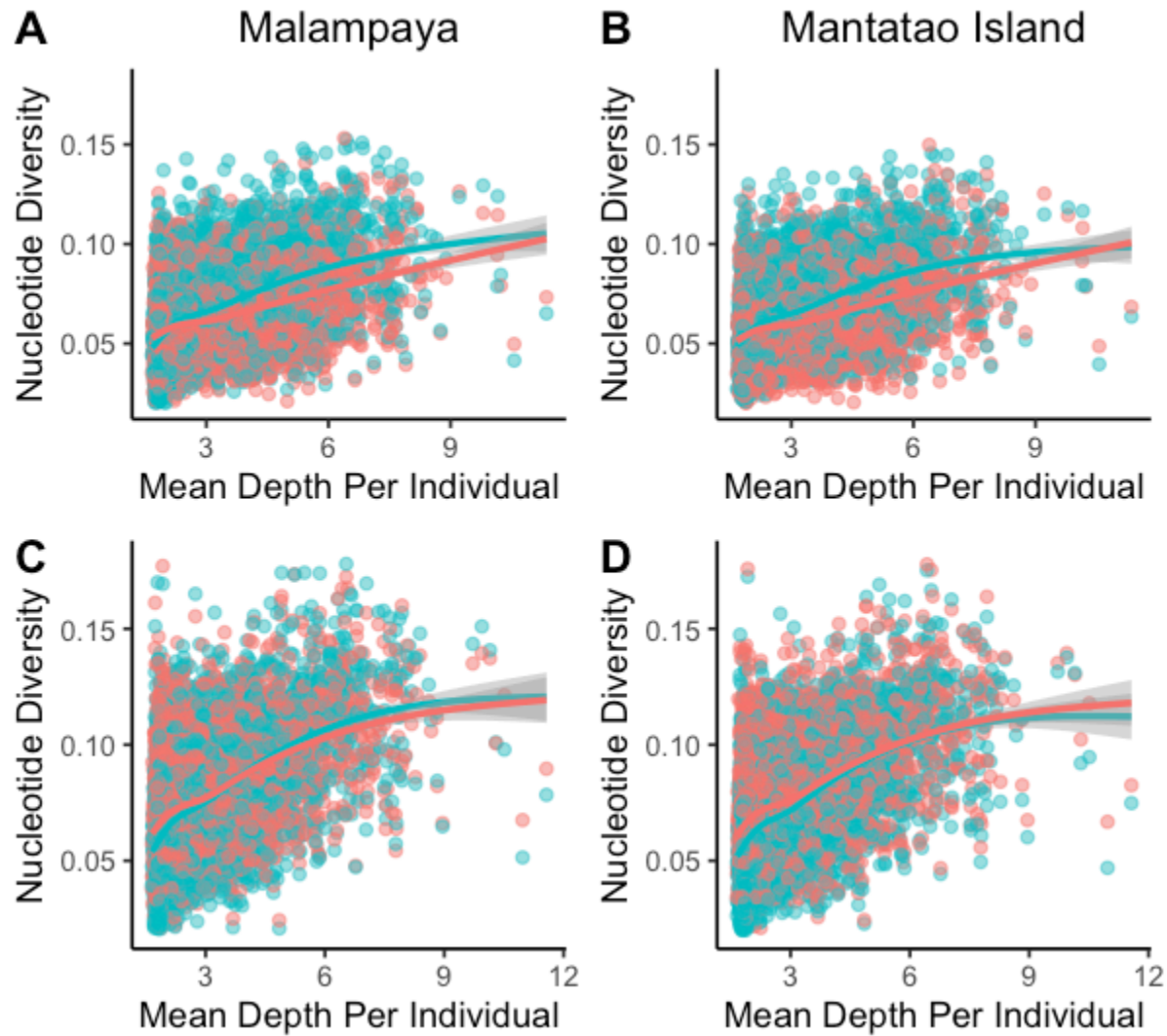


Figure S2. Nucleotide diversity calculated on all 1.8 million SNPs in A) and B) and on 1.3 million neutral SNPs in C) and D) across each scaffold for each population versus the mean sequencing depth per individual. A) and C) show the lower impact site at Malampaya, Palawan, and B) and D) show the higher impact site at Mantatao Island. Historical scaffolds are represented in red, and contemporary scaffolds are represented in blue.

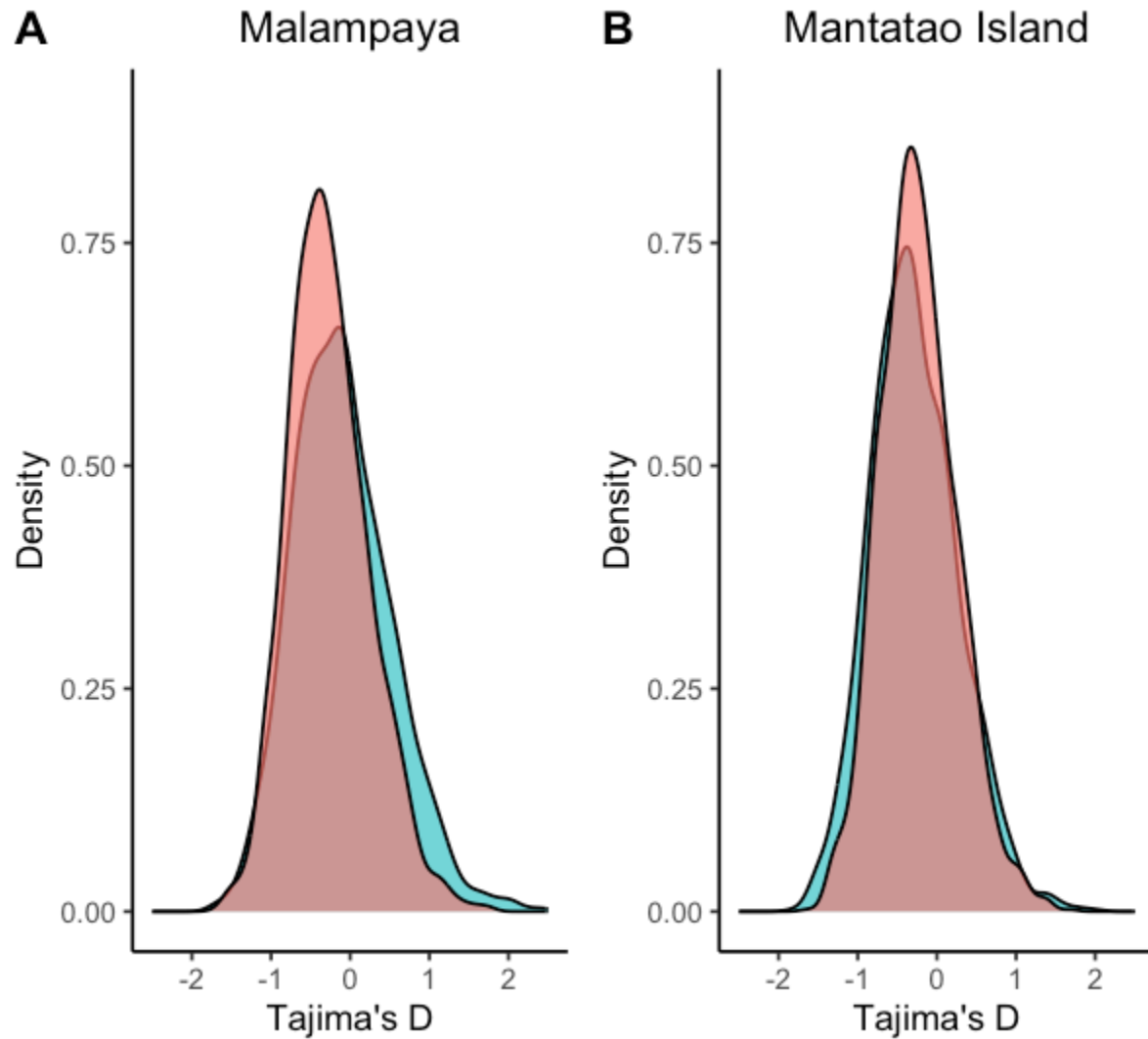


Figure S3. Density plots showing the distribution of Tajima's D calculated using neutral SNPs (on scaffolds between 3-6x depth) in A) Malampaya (lower human impact) and B) Mantatao Island (higher human impact). Historical data is in red, and contemporary data is in blue.