MOLECULAR ECOLOGY WILEY

Comparative population genomics of manta rays has global implications for management

Emily Humble[1,2] ◉ | Jane Hosegood[2,3] | Gary Carvalho[3] | Mark de Bruyn[3,4] ◉ |
Simon Creer[3] | Guy M. W. Stevens[2] | Amelia Armstrong[5] | Ramon Bonfil[6,7,8] |
Mark Deakos[9] | Daniel Fernando[2,10] | Niv Froman[2] | Lauren R. Peel[2,11,12] |
Stephen Pollett[2] | Alessandro Ponzo[13] | Joshua D. Stewart[2,14] | Sabine Wintner[15,16] |
Rob Ogden[1]

Correspondence
Emily Humble, Royal (Dick) School of
Veterinary Studies and the Roslin Institute,
University of Edinburgh, Edinburgh EH25
9RG, UK.
Email: emily.humble@ed.ac.uk

Handling Editor: Alana Alexander

Abstract

Understanding population connectivity and genetic diversity is of fundamental importance to conservation. However, in globally threatened marine megafauna, challenges remain due to their elusive nature and wide-ranging distributions. As overexploitation continues to threaten biodiversity across the globe, such knowledge gaps compromise both the suitability and effectiveness of management actions. Here, we use a comparative framework to investigate genetic differentiation and diversity of manta rays, one of the most iconic yet vulnerable groups of elasmobranchs on the planet. Despite their recent divergence, we show how oceanic manta rays (*Mobula birostris*) display significantly higher heterozygosity than reef manta rays (*Mobula alfredi*) and that *M. birostris* populations display higher connectivity worldwide. Through inferring modes of colonization, we reveal how both contemporary and historical forces have likely influenced these patterns, with important implications for population management. Our findings highlight the potential for fisheries to disrupt population dynamics at both local and global scales and therefore have direct relevance for international conservation of marine species.

KEYWORDS
conservation genomics, fisheries management, genetic diversity, population structure, ddRAD

1 | INTRODUCTION

Understanding the extent to which populations are connected is key to exploring population dynamics, predicting extinction risk and informing conservation management (Hanski & Gilpin, 1991; Lowe & Allendorf, 2010; Mills & Allendorf, 1996). In species with isolated populations characterized by limited dispersal, the risk of extirpation from local depletion is high (Reed, 2004). In such cases,

---

Emily Humble and Jane Hosegood: Joint first authors.

For Affiliation refer page on 9

local and regional scale management will be most appropriate for preventing and reversing population declines (Palumbi, 2003). In contrast, species with high rates of gene flow are potentially demographically and genetically more resilient to extrinsic factors (Lowe & Allendorf, 2010; Pascual et al., 2017). However, in order to maintain connectivity and mitigate genetic diversity loss in these taxa, management measures must be coordinated and encompass migratory corridors. As overexploitation and habitat destruction threaten to disrupt population dynamics at a global scale, characterizing genetic variation and connectivity has become more important than ever before (Funk et al., 2012; Kardos et al., 2021; Palsbøll et al., 2007).

In widely distributed marine species with high dispersal potential, genetic differentiation is often found to be subtle or non-existent (Palumbi, 2003; Waples, 1998; Ward et al., 1994). Such patterns can arise from a range of mechanisms – from high contemporary gene flow to recent divergence of historically large populations (Palumbi, 2003; Waples et al., 2008; Waples & Gaggiotti, 2006) – and can therefore be difficult to interpret. The latter scenario reflects a disconnect between demographic and genetic connectivity and has important implications for species resilience (Bailleul et al., 2018; Lowe & Allendorf, 2010; Waples, 1998). This is because populations that appear genetically connected may not operate as a single demographic unit, making them more vulnerable to overexploitation. High-resolution SNP datasets go some way to addressing this problem by providing greater power to detect subtle differences at both neutral and adaptive loci (Gagnaire et al., 2015; Hauser & Carvalho, 2008). However, since population genetic differentiation can be affected by past, as well as contemporary patterns, parallel inference of historical relationships and genetic variation can allow the relative contribution of historical processes to be explicitly evaluated (Foote & Morin, 2016; Liu et al., 2022; Louis et al., 2021). Furthermore, when carried out within a comparative framework, such an approach can provide powerful insights into the drivers of population divergence and therefore improve recommendations for conservation management (Gagnaire, 2020).

Manta rays are large, mobile elasmobranchs inhabiting tropical and sub-tropical oceans (Couturier et al., 2012) (Figure 1a,c) and provide an excellent opportunity to evaluate the genomic consequences of historical and contemporary population processes within a comparative framework. They comprise two described species estimated to have diverged less than 0.5 Mya as a result of distinct habitat preferences (Kashiwagi et al., 2012). The reef manta ray (*Mobula alfredi*) frequents near-shore tropical reef environments, such as coral atolls and barrier reefs (Kashiwagi et al., 2011), with a high degree of residency (Braun et al., 2015; Deakos et al., 2011; Germanov et al., 2022; Jaine et al., 2014; Knochel, Hussey, et al., 2022; Peel et al., 2019; Setyawan et al., 2018). In contrast, while the oceanic manta ray (*Mobula birostris*) also inhabits near-shore environments, it is often found ranging into sub-tropical habitats along continental coastlines and at oceanic islands, usually adjacent to productive deep-water

upwellings (Andrzejaczek et al., 2021; Kashiwagi et al., 2011). As a result of these differences in habitat use, *M. alfredi* and *M. birostris* have long been considered to display marked differences in their migratory abilities and levels of gene flow. Yet, only a handful of long-distance movements have ever been recorded in *M. birostris* (Andrzejaczek et al., 2021; Knochel, Cochran, et al., 2022) alongside observations of site fidelity (Cabral et al., 2023; Garzon et al., 2023; Gordon & Vierus, 2022), raising questions about the extent to which population structure and genetic variation may differ across species. To date, assessments of genetic differentiation in *M. alfredi* have focussed on local and regional patterns (Lassauce et al., 2022; Venables et al., 2021; Whitney et al., 2023), and we have little understanding of how genetic variation is distributed across the species' range. In, *M. birostris*, the situation is even less clear, with studies reporting both widespread connectivity and population differentiation (Hosegood et al., 2020; López et al., 2022; Stewart et al., 2016). Critically, these differences and uncertainties exist against a background of ongoing global exploitation and uncertain implications for management.

Targeted and incidental fisheries, driven in part by increasing demand for mobulid gill plates (Couturier et al., 2012; O'Malley et al., 2017), have led to widespread population declines in manta rays (Carpenter et al., 2023; Croll et al., 2016; Moazzam, 2018; Rohner et al., 2017; Ward-Paige et al., 2013). Currently, both species are managed through a patchwork of local, regional and international measures with varying levels of implementation and enforcement (Fernando & Stewart, 2021; Lawson et al., 2017; Lawson & Fordham, 2018). To determine the appropriateness of management measures and assess population vulnerability, a global assessment of management units is urgently required (Lawson et al., 2017; Stewart, Jaine, et al., 2018). Here, we undertake a comparative genomic analysis of manta ray populations from across their global distribution to investigate connectivity, genetic variation and historical relationships with an aim to guide effective fisheries management and emphasize the value of genomic research for advancing knowledge of understudied elasmobranchs.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Tissue samples were opportunistically collected from 12 geographic locations to represent the global distribution of each species (Figure 1a,c). For *M. alfredi* (total $n = 120$), these originated from the Chagos Archipelago ($n = 5$), the Maldives ($n = 48$), Seychelles ($n = 24$), Australia Pacific ($n = 4$), Fiji ($n = 9$) and Hawaii ($n = 30$). For *M. birostris* (total $n = 112$), these originated from Sri Lanka ($n = 43$), the Philippines ($n = 37$), South Africa ($n = 3$), Mexico Caribbean ($n = 4$), Mexico Pacific ($n = 13$) and Peru ($n = 12$). Samples from Mexico Caribbean, where a third putative manta ray species occurs in sympatry (*Mobula* cf. *birostris*, Hinojosa-Alvarez et al., 2016; Hosegood et al., 2020), were visually and genetically
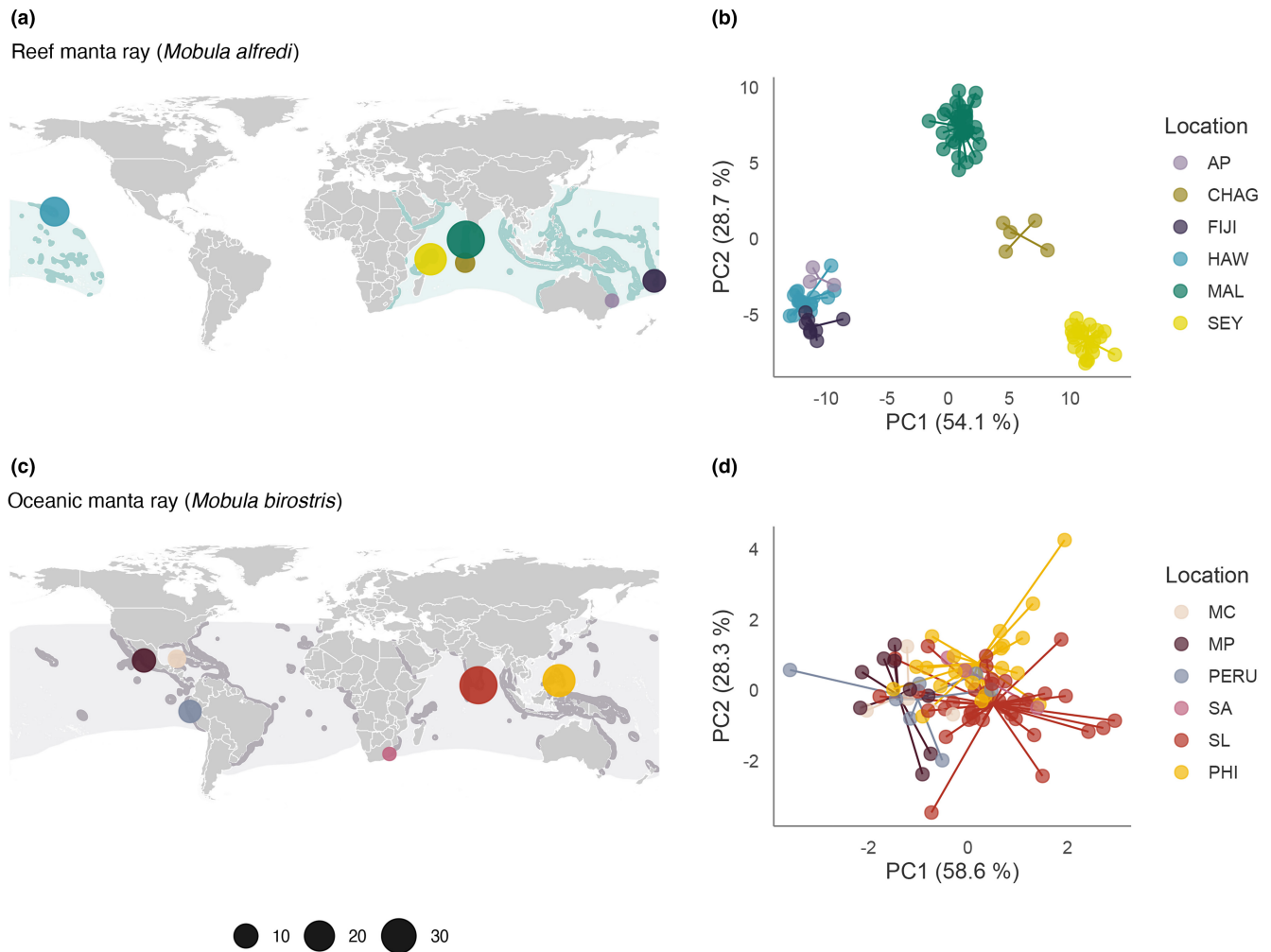
(a)

Reef manta ray (*Mobula alfredi*)

(b)

(c)

Oceanic manta ray (*Mobula birostris*)

(d)

FIGURE 1 Contrasting patterns of population structure in manta rays. (a, c) Geographic distributions of (a) *Mobula alfredi* and (c) *Mobula birostris* visualized together with the locations of samples used in this study. Dark-shaded distributions denote the confirmed species range, and light-shaded distributions denote the expected species range. Sampling location points are distinguished by colour and scaled by the number of samples. Further details are provided in the Appendix S1. (b, d) Scatterplots showing individual variation in principal components (PC) one and two derived from discriminant analysis of principal components analysis for (b) *M. alfredi* and (d) *M. birostris* individuals. The amount of variance explained by each PC is shown in parentheses. Population abbreviations: AP, Australia Pacific; CHAG, Chagos; FIJI, Fiji; HAW, Hawaii; MAL, Maldives; MC, Mexico Caribbean; MP, Mexico Pacific; PERU, Peru; PHI, the Philippines; SA, South Africa; SEY, Seychelles; SL, Sri Lanka.

confirmed as *M. birostris*. For both species, samples were collected from a combination of live animals and fisheries specimens (see Appendix S1 for further information).

2.2 | DNA extraction and ddRAD sequencing

Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit and quantified using a Qubit 3.0 Broad Range Assay. Double digest restriction-site associated DNA (ddRAD) libraries were prepared following the Peterson et al. (2012) protocol with modifications described in Palaiokostas et al. (2015) and were 125 bp paired-end sequenced on an Illumina HiSeq. Sequencing reads from both species were assessed for quality using FastQC and processed together using the Stacks v2.54 de novo assembly

pipeline (Catchen et al., 2013). The three main assembly parameters were chosen following the optimization procedure outlined in Rochette and Catchen (2017) (Figures S1 and S2). Initial quality filters were applied to the resulting genotypes before generating three high-quality datasets for use in downstream analysis: two species-specific datasets, and one dataset comprising both species. For the species-specific datasets, we extracted either *M. birostris* or *M. alfredi* individuals, removed individuals with high relatedness coefficients (Korneliussen & Moltke, 2015; Waples et al., 2019, Figure S3) and filtered out SNPs with a minor allele count of less than 3, a genotyping rate less than 90% and that were in linkage disequilibrium using PLINK. For the dataset comprising both species, we first removed closely related individuals and then filtered out SNPs with a minor allele count of less than 3 and a genotyping rate less than 90%. Population genetic summary statistics were calculated using the R

package diversity (Keenan et al., 2013, Table S1). See Appendix S1 for further information on library preparation, read processing and SNP and individual filtering.

2.3 | Population structure

To investigate population structure, we used the species-specific datasets and three complementary approaches. First, we carried out a discriminant analysis of principal components (DAPC) using the R package adegenet (Jombart, 2008). This approach initially transforms the SNP data using a principal components analysis (PCA) and then performs a discriminant analysis on the retained PCs. This serves to maximize discrimination of individuals between groups while minimizing variation within (Jombart et al., 2010). Following the recommendations outlined in Thia (2023), the number of PCs retained as predictors was determined based on the K–1 criterion, where K is equal to the number of effective populations. For *M. alfredi*, this was set to 5, under the assumption that each sample site reflects a separate population. For *M. birostris*, this was set to 4 under the assumption that Mexico Pacific and Peru may represent a single population given their close geographic proximity. Second, we estimated admixture proportions for the individuals in each dataset using ADMIXTURE. Admixture runs were performed for ancestry clusters ranging from $K=1$–8, with 10 runs for each K. The optimal K was identified based on the lowest cross-validation error. The runs with the highest likelihood were visualized. Third, we estimated pairwise genetic differentiation between populations within each species using the Weir and Cockerham F_{ST} value (Weir & Cockerham, 1984) calculated in the R package dartR (Gruber et al., 2018). Confidence intervals and *p*-values were estimated based on bootstrap resampling of individuals within each population 1000 times. *Mobula alfredi* samples from Australia Pacific and *M. birostris* samples from South Africa were excluded from this analysis due to low sample sizes.

2.4 | Isolation by distance

To investigate patterns of isolation by distance, we examined the relationship between genetic and geographic distance between all pairs of populations in each species. Genetic distances were based on the pairwise F_{ST} estimates calculated above. Geographic distances were determined based on a least-cost path analysis implemented using the R package marmap (Pante & Simon-Bouhet, 2013) with a minimum depth constraint of −10 m in order to prevent paths overland. The significance of associations between genetic and geographic distance matrices was inferred using distance-based Moran's eigenvector maps (dbMEM) by redundancy analysis (RDA, Legendre et al., 2015). For this, geographical distances were transformed into dbMEMs using the R package adespatial, and genetic distances were decomposed into principal components using the R function prcomp. RDA was then

performed using the R package vegan, with significance tested using 1000 permutations.

2.5 | Contemporary gene flow

To infer the strength and directionality of contemporary gene flow between populations, we used the programme BA3-SNPs BayesAss v1.1 (Mussmann et al., 2019) which estimates the proportion of immigrants in a given population using Bayesian inference. This analysis was restricted to *M. alfredi* as it assumes low levels of connectivity and imposes an upper bound on the proportion of non-migrants in a population. We first performed initial runs of BayesAss to determine optimal mixing parameters (dM = migration rate, dA = allele frequency and dF = inbreeding coefficient) using the autotune function in BA3-SNPs. We then ran BayesAss-3 with 10,000,000 iterations, a burn-in of 1,000,000 and a sampling interval of 1000. Mixing parameters were set to dM = 0.21, dA = 0.44 and dF = 0.08. Results were averaged across five replicate runs, and migration rates were considered significant if 95% credible sets (mean migration rate ± 1.96 × mean standard deviation) did not overlap zero. Chain convergence was assessed, and migration rates were visualized using R (Figure S4).

2.6 | Historical relationships among populations

To explore historical relationships among populations of *M. alfredi* and *M. birostris*, we used the programme TreeMix (Pritchard et al., 2000). TreeMix uses population allele frequencies to estimate a bifurcating maximum likelihood tree with which to infer historical population splits, admixture events and the degree of genetic drift. We first supplemented the *M. alfredi* dataset with one randomly selected *M. birostris* individual and the *M. birostris* dataset with one randomly selected *M. alfredi* to act as outgroups when rooting the trees. Both datasets were then filtered for linkage, a minor allele count of less than 3, a genotyping rate less than 90% and related individuals using PLINK v1.9 (Purcell et al., 2007). Allele frequencies for each population were then calculated using the –freq and –within arguments in PLINK. For both the *M. birostris* and *M. alfredi* datasets, we then performed 10 initial runs of TreeMix for each migration event (M) ranging from 0 to 10. The number of migration edges that explained 99.8% of the variance was selected as the best model for each species (*M. birostris*: M = 0; *M. alfredi*: M = 2, Figure S5). We then re-ran TreeMix 100 times using the optimal number of migration edges. Consensus trees and bootstrap values were estimated and visualized using code modified from the BITE R package (Milanesi et al., 2017).

2.7 | Genome-wide heterozygosity

To assess levels of genetic variation both within and between species, we used the high-quality SNP dataset comprising both species.

We then calculated multi-locus heterozygosity for each individual using the R package inbreedR (Stoffel et al., 2016).

3 | RESULTS

High-quality SNPs were genotyped in 173 individuals from 12 locations representing the global distribution of each species (Figure 1a,c). The species-specific datasets contained a total of 1553 SNPs in 91 *M. alfredi* individuals, and 6278 SNPs in 82 *M. birostris* individuals, while the full dataset contained a total of 15,312 SNPs called across both species. See Materials and Methods and Appendix S1 for details.

3.1 | Contrasting patterns of population structure at a global scale

To investigate population differentiation within each species, we used four complementary approaches: discriminant analysis of principal components (DAPC), admixture, pairwise F_{ST} and isolation by distance analysis. In *M. alfredi*, all methods supported the presence of strong population structure at both global and regional scales. Populations inhabiting different ocean basins displayed the highest degree of differentiation in the DAPC, with Pacific and Indian

Ocean populations forming distinct clusters along PC1 (Figure 1b). Regional differentiation was also detected, with Seychelles, Chagos and the Maldives clustering apart along PC2, and Hawaii separating from Australia Pacific and Fiji along PC3 (Figure 1b and Figure S6A). These patterns were reinforced in the admixture analysis which highlighted two major ancestral source populations, inferred an optimal value of $K=4$ and resolved hierarchical structure up to $K=7$ (Figures S7 and S8A). Interestingly, only weak separation was observed between Australia Pacific and Fiji; however, this pattern may be confounded by the small sample size of the former, which can lead to spurious merging of distinct populations (Puechmaille, 2016). Pairwise F_{ST} estimates between ocean basins were on average over two times higher than those within (mean pairwise F_{ST} between ocean basins = 0.30, mean pairwise F_{ST} within ocean basins = 0.13, Figure 2a); yet all population comparisons were found to be significant (Figure S9A, mean = 0.23, min = 0.08, max = 0.43). Finally, we detected a significant relationship between pairwise F_{ST} and geographic distance (adjusted $R^2 = .65$, $p = .03$) indicating an effect of isolation by distance (Figure 2b).

In stark contrast, *M. birostris* displayed little evidence of strong population structure across all methods. Individuals from different ocean basins clustered closely together along each axis in the DAPC (Figure 1d and Figure S6B). Admixture identified $K=1$ as the optimal number of clusters, with increasing values of K merely introducing additional mixing (Figures S7 and S8B). Pairwise F_{ST} estimates
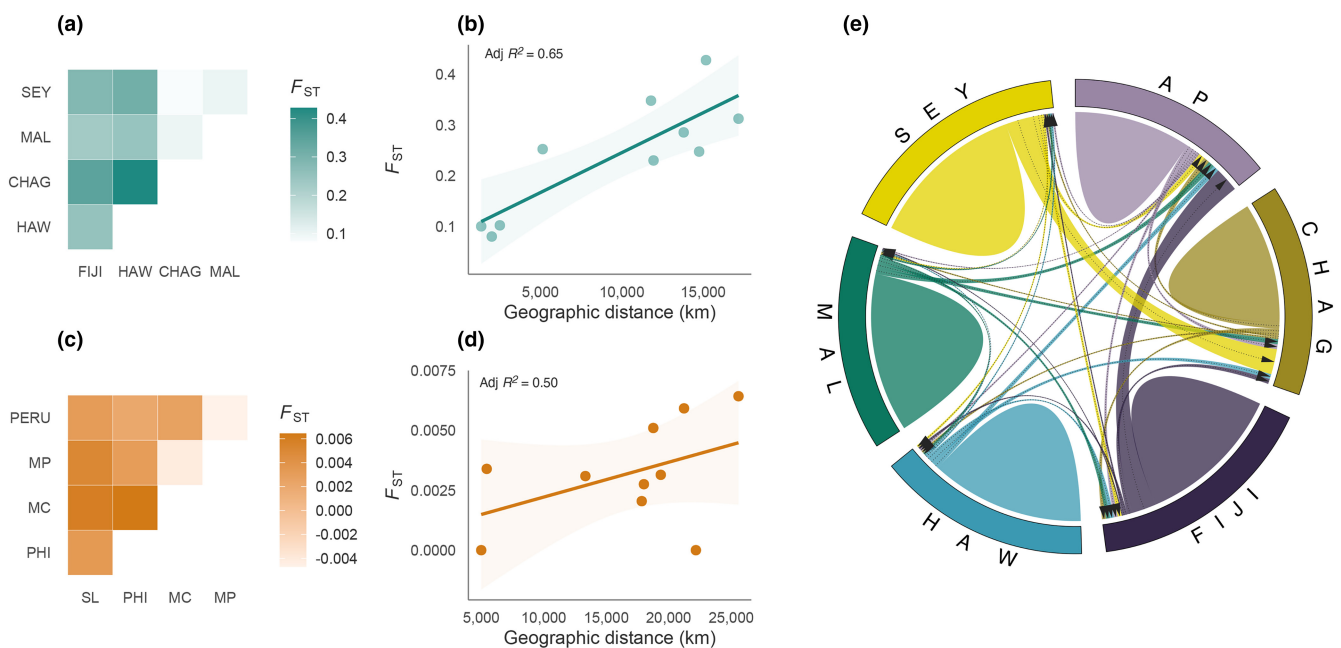

FIGURE 2 Population genetic differentiation, isolation by distance and contemporary migration in manta rays. (a, c) Pairwise F_{ST} estimates between sampling locations for (a) *M. alfredi* and (c) *M. birostris*. Samples from Australia Pacific and South Africa were excluded from this analysis due to low sample sizes. (b, d) Relationship between genetic (F_{ST}) and geographic distance as calculated by least-cost path analysis for all pairwise population comparisons in (b) *M. alfredi* and (d) *M. birostris*. Solid lines and shaded areas reflect the regression slopes and standard errors, respectively, based on a linear model. Samples from Australia Pacific and South Africa were excluded from this analysis due to low sample sizes. Note that F_{ST} scales differ between (a) and (c), and axis scales differ between (b) and (d). (e) Contemporary gene flow estimates between populations of *M. alfredi*. The direction of each arrow represents the direction of gene flow, and the width of each ribbon reflects the relative amount of gene flow. Population abbreviations: AP, Australia Pacific; CHAG, Chagos; FIJI, Fiji; HAW, Hawaii; MAL, Maldives; MC, Mexico Caribbean; MP, Mexico Pacific; PERU, Peru; PHI, the Philippines; SEY, Seychelles; SL, Sri Lanka.

were two-fold lower than in *M. alfredi*, with no pairwise comparison falling above 0.007 (mean = 0.002, min = −0.005, max = 0.006, Figure 2c). Nevertheless, despite these broad patterns, several lines of evidence indicate the presence of subtle geographic differentiation in this species. First, individuals from Mexico Pacific, Peru and Mexico Caribbean clustered separately from those sampled in South Africa, Sri Lanka and the Philippines along PC1 (Figure 1d). Second, despite pairwise F_{ST} estimates being low, comparisons between Eastern-Pacific and Indo-Pacific populations, and between Sri Lanka and the Philippines were statistically significant (Figure S9B). Small F_{ST} values are expected when minor allele frequencies are low and therefore do not necessarily reflect an absence of differentiation (Jakobsson et al., 2013). Furthermore, it is possible that overall levels of population structure were underestimated due to the small sample size of two of our six populations (Puechmaille, 2016). Finally, while no significant relationship was observed between pairwise F_{ST} and geographic distance (adjusted $R^2 = .50$, $p = .10$), there was a tendency for populations separated by greater distances to display higher differentiation (Figure 2d).

3.2 | Contemporary gene flow

To characterize the strength and direction of gene flow between populations, we used the programme BA3-SNPs (Mussmann et al., 2019) to estimate recent migration. As this method assumes low levels of connectivity and imposes an upper bound on the proportion of non-migrants in a population, this analysis was restricted to *M. alfredi*. As expected, contemporary gene flow was low (Figure 2e); the average migration rate between populations, measured as the estimated number of migrants per generation, was 0.029 (min = 0.008, max = 0.15), with this figure falling to 0.018 (min = 0.008, max = 0.03) when considering gene flow between populations in different ocean basins. Migration into both Hawaii and the Maldives was lowest, indicating these populations are the most isolated of those sampled (Table S2). Migration rates were only deemed significant between Seychelles and Chagos (0.15) and between Fiji and Australia Pacific (0.15), in line with these populations being last to separate in the admixture analysis. These patterns highlight that while *M. alfredi* may have the propensity to travel over large distances, restricted movement likely dominates.

3.3 | Historical relationships among populations

To place patterns of genetic differentiation into a historical context, we investigated population origins and colonization patterns using TreeMix (Pritchard et al., 2000). This programme uses allele frequency data to infer patterns of population splits and admixture events through the construction of a maximum likelihood tree. In *M. alfredi*, internal branch lengths were relatively long, with an initial split clearly separating populations in the Indian and Pacific Oceans (Figure 3a). The Maldives and Australia Pacific were the first to separate within

each locality and displayed the lowest levels of genetic drift overall. Hawaii was among the last populations to split and displayed the highest amount of drift, in line with its geographic isolation. The best supported model inferred two migration events (Figure S5A): one from the *M. alfredi* population in Seychelles into *M. birostris* and one from Chagos into Hawaii. However, because not all geographic regions are represented in our dataset, the true sources and sinks of these admixture events may originate from related ghost populations. In contrast to *M. alfredi*, the addition of migration events led to no substantial improvement in the model for *M. birostris* (Figure S5B), and therefore the tree without migration is presented here. Interestingly, internal branch lengths were considerably shorter in *M. birostris*, indicating rapid radiation from a shared ancestral source population (Figure 3b). External branch lengths were also short, consistent with larger populations displaying marginal drift and low divergence. Nevertheless, despite these patterns, some geographic signal was detected in the *M. birostris* tree, with individuals originating from the Eastern Pacific and the Caribbean (Peru, Mexico Caribbean and Mexico Pacific) grouping separately from those originating from the Atlantic and Indo-Pacific (South Africa, the Philippines and Sri Lanka), although bootstrap support was overall low.

3.4 | Heterozygosity landscape across species and populations

To explore how patterns of population structure and colonization are associated with genome-wide variation, we compared individual multi-locus heterozygosity between species and among populations. Strikingly, heterozygosity was on average over three times higher in *M. birostris* (mean = 0.10, min = 0.053, max = 0.12) than in *M. alfredi* (mean = 0.03, min = 0.01, max = 0.051), with every individual displaying a higher value than any *M. alfredi* ($\beta = .07$, 95% CI = 0.06–0.07, $p = <2.2 \times 10^{-16}$, Figure 3c). This finding is in line with the patterns of population structure and historical splits we observed in each species. Variation in heterozygosity was also observed at a population level (Figure 3d,e). In *M. alfredi*, the Maldives and Australia Pacific had the highest levels of heterozygosity within each ocean basin, in line with these populations being first to split in the TreeMix analysis. Indian Ocean populations displayed higher overall heterozygosity than Pacific populations and had mean values that were overall similar. Interestingly, heterozygosity within Pacific populations declined steeply from west to east, with heterozygosity in Hawaiian individuals being around half that of the Australian animals (Figure 3d), in line with this population being last to split in the TreeMix analysis and displaying the highest amount of drift. In contrast, *M. birostris* populations displayed less extreme variation in heterozygosity, with mean values differing by less than 0.02 and having no clear geographical pattern (Figure 3e). Furthermore, variance within populations was an order of magnitude greater in *M. birostris* than in *M. alfredi*, and was particularly large in Sri Lanka, the Philippines and Mexico Pacific populations.
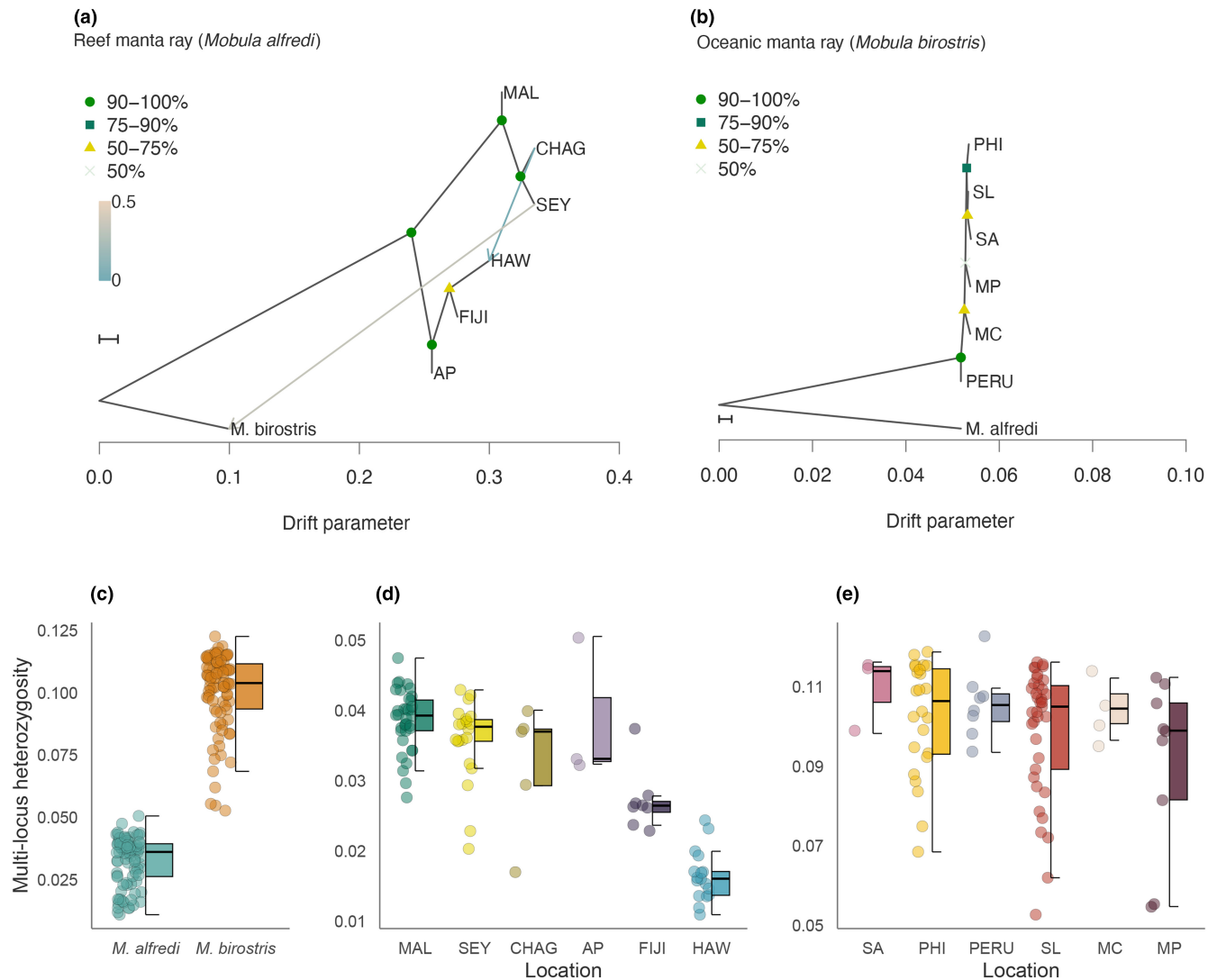
FIGURE 3 Historical relationships and heterozygosity in manta rays. (a, b) TreeMix maximum likelihood consensus tree displaying the historical relationships among (a) *M. alfredi* and (b) *M. birostris* populations. Horizontal branch lengths reflect the amount of genetic drift that has occurred along each branch. Bootstrap support values for each node are indicated. Migration edges inferred using TreeMix are represented as arrows and coloured according to their migration weight. The scale bar reflects 10 times the average standard error of the entries in the sample covariance matrix. (c–e) Variation in individual multi-locus heterozygosity between (c) species and among populations of (d) *M. alfredi* and (e) *M. birostris*. Note that *y*-axis scales differ for (d) and (e). Centre lines of boxplots reflect the median, bounds of the boxes extend from the first to the third quartiles, and upper and lower whiskers reflect the largest and smallest values but no further than 1.5 × the interquartile range from the hinge. Population abbreviations: AP, Australia Pacific; CHAG, Chagos; FIJI, Fiji; HAW, Hawaii; MAL, Maldives; MC, Mexico Caribbean; MP, Mexico Pacific; PERU, Peru; PHI, the Philippines; SA, South Africa; SEY, Seychelles; SL, Sri Lanka.

4 | DISCUSSION

Manta rays are iconic megafauna with cultural, socio-economic and ecological significance. Due to targeted and bycatch fisheries operating across their broad-ranging distributions, populations are declining worldwide. Elucidating levels of connectivity and genetic variation remain a crucial priority for conservation management. We use reduced-representation sequencing on a global set of samples and reveal striking differences in the population genetic landscape of two recently diverged manta ray species. By considering the influence of both contemporary and historical processes, our study provides a precautionary framework for assessing conservation units in widely distributed marine species.

We first demonstrate the presence of strong genetic differentiation in *M. alfredi* at a global and regional scale. From a total of six sampling locations, we found evidence for at least five genetically distinct, and by extension, demographically independent populations. Two of these were separated by a distance of ~1200 km, which is close to the maximum recorded movement in the species (Armstrong et al., 2019; Jaine et al., 2014), indicating that long-distance migrations are likely rare. Indeed, contemporary gene flow was low – especially between geographically

distant locations – with only a small proportion of individuals in any population being identified as first- or second-generation migrants. Furthermore, when gene flow was observed, it tended to be unidirectional. These results are in line with recent studies demonstrating population differentiation between Western Australia and Mozambique (Venables et al., 2021) and between Eastern Australia and New Caledonia (Lassauce et al., 2022), together highlighting how large ocean basins form significant barriers to dispersal in coastal elasmobranchs (Hirschfeld et al., 2021). High site fidelity has been widely reported in *M. alfredi* based on tagging and mark-recapture studies (Braun et al., 2015; Deakos et al., 2011; Germanov et al., 2022; Jaine et al., 2014; Knochel, Hussey, et al., 2022; Peel et al., 2019; Setyawan et al., 2018). However, the degree of residency has been shown to vary, with movements rarely exceeding a few hundred kilometres in some locations (Braun et al., 2015; Deakos et al., 2011; Kessel et al., 2017; Knochel, Hussey, et al., 2022; Setyawan et al., 2018) yet reaching over 1000 km in others (Armstrong et al., 2019; Germanov & Marshall, 2014). Our study presents a comparatively broad-scale analysis relevant to regional and global management planning. Further work on local patterns of population structure will shed light on the nuances and drivers of fine-scale movement patterns in this species (Whitney et al., 2023).

To explore the mechanism by which manta rays colonized their distribution, we reconstructed historical relationships and assessed levels of heterozygosity. In *M. alfredi*, we found strong evidence for an initial split between the Indian and Pacific Oceans followed by further separation within each locality. Furthermore, populations in the Pacific displayed a signal of declining heterozygosity from west to east, together suggesting that *M. alfredi* underwent a stepping-stone pattern of range expansion from an Indo-Pacific Ocean origin, involving opportunistic long-range movements and associated founder events. This is consistent with a recent observation of a pregnant *M. alfredi* individual at Cocos Island, Costa Rica (Arauz et al., 2019), almost 6000 km east of the nearest confirmed sighting, and the first record of *M. alfredi* in the Eastern Pacific. Range expansion inherently impacts genetic variation, with a stepping-stone model of colonization predicted to result in the strongest cumulative effect of founder events (Le Corre & Kremer, 1998). Among our sampled populations, Hawaii is the most geographically isolated, situated at the edge of the *M. alfredi* distribution. Interestingly, not only was Hawaii the most genetically differentiated from all populations in our study, but it displayed the longest external branch lengths in the TreeMix analysis and the lowest levels of heterozygosity. This could be suggestive of a single founder event by a small population. Genetic variation is fundamental for enabling populations to adapt in response to selection (Bonnet et al., 2022; Kardos et al., 2021; Lai et al., 2019). Our findings therefore expose how isolated *M. alfredi* populations at the periphery of their distribution may be intrinsically more vulnerable to changing environmental conditions and the genetic impacts of population decline.

In stark contrast to the patterns observed in *M. alfredi*, *M. birostris* displayed markedly higher levels of heterozygosity and only

subtle genetic differentiation across ocean basins. Weak population structure is common in highly mobile marine species (Leslie & Morin, 2018; Nikolic et al., 2023; Vignaud et al., 2014), and yet warrants careful interpretation, particularly considering management recommendations (Younger et al., 2017). On the one hand, these findings may be an indication of high contemporary gene flow and low natal philopatry, in line with the species' occurrence at remote oceanic islands, tendency to range into sub-tropical habitats and lower overall re-sight rates than *M. alfredi* (Couturier et al., 2014; Harty et al., 2022; Rambahiniarison et al., 2023). To date, our understanding of the movement behaviour in *M. birostris* has largely been based on coastal aggregations of adult individuals over relatively short timeframes (Beale et al., 2019; Harty et al., 2022; Rohner et al., 2013; Stewart et al., 2016). Such studies have a tendency to capture seasonal migrations as opposed to dispersal events and may explain why only a handful of long-distance (~1000 km) movements have been recorded in the species (Andrzejaczek et al., 2021; Knochel, Cochran, et al., 2022). In an infinite island model, only a few migrants per generation are required to obscure strong population structure when N_e is large (Wright, 1931), and therefore it is possible the patterns we observe translate to infrequent dispersal events. Furthermore, dispersal could be segregated by age and/or sex (McClain et al., 2022; Phillips et al., 2021) and may vary among individuals (Papastamatiou et al., 2013; Perryman et al., 2022; Thorburn et al., 2019). While challenging, there is benefit in extending future tagging efforts to transient individuals away from known aggregation sites (Garzon et al., 2023), as well as previously underrepresented age classes – such as juveniles – to capture what may be infrequent yet evolutionarily relevant movements.

An alternative explanation for the patterns we observe in *M. birostris* is that insufficient time has elapsed to reliably identify recent genetic divergence among localities. In contrast to *M. alfredi*, our TreeMix analysis indicated that *M. birostris* rapidly radiated from a large ancestral source, with only marginal genetic drift occurring between regions. This was further evidenced by substantially higher levels of genetic variation that differed little across sampling locations. In addition, little differentiation was observed between Mexico Pacific and Mexico Caribbean, two regions that have been geographically separated since the emergence of the Isthmus of Panama. These findings are consistent with a recent mark-re-sight analysis that estimated the population of *M. birostris* in coastal Ecuador to number at least 22,000 individuals (Harty et al., 2022). Large effective population sizes and high genetic variation increase the time taken for populations to diverge due to genetic drift (Bailleul et al., 2018; Taylor & Dizon, 1996; Wright, 1931). This is further compounded in species with long and overlapping generations (Hoffman et al., 2017) as is the case for manta rays (Dulvy et al., 2014). Taken together, genetic similarities among *M. birostris* localities may be partially confounded by recent shared ancestry and large effective population size.

On the basis of these considerations, we propose that a combination of large historical population size and contemporary gene flow has contributed to the comparatively high levels of heterozygosity

and genetic homogeneity in *M. birostris*. The subtle population differentiation we observe between the Indian Ocean, South-East Asia and the Eastern Pacific is likely best explained by the geographic limits of dispersal as opposed to complete geographic isolation. Yet, unlike in *M. alfredi* where genetic clusters almost certainly reflect discrete demographic units relevant for conservation management, the extent to which genetic connectivity in *M. birostris* reflects demographic connectivity is less clear. For example, in extreme cases, the number of migrants required to eliminate signals of population structure will not be enough to demographically link populations, and more importantly, replenish those that have been depleted (Waples, 1998). Interestingly, while re-sight rates are typically lower in *M. birostris* than *M. alfredi*, demographic independence has been implicated in several mark-recapture studies where re-sightings follow predictable patterns (Beale et al., 2019; Cabral et al., 2023). Furthermore, a population genetic analysis based on F_{ST} outliers uncovered allele frequency differences between two Mexican locations and Sri Lanka (Stewart et al., 2016), suggesting recent divergence against a background of ongoing gene flow. Taken together, we highlight the potential for further work investigating adaptive divergence between *M. birostris* populations and emphasize the need to combine molecular measures of connectivity with empirical demographic data in this species (Cayuela et al., 2018; Lowe & Allendorf, 2010; Younger et al., 2017).

4.1 | Conservation implications

The remarkable differences we observe in the population genetics of manta rays directly inform likely response to continued exploitation and respective conservation measures. At present, *M. alfredi* is among the most protected mobulid species worldwide, with some management frameworks in place at local, national and international levels (Lawson et al., 2017; Stevens et al., 2018). Our findings of global population structure underline how local initiatives recognizing populations as distinct management units will be most appropriate for this species. However, we also demonstrate the consequence of geographic isolation on genetic variation and reveal how *M. alfredi* likely faces a greater risk from local depletion. This is especially true for populations at the edge of the species range and in regions with high coastal fishing pressure. Prioritizing these populations in conservation action plans and maintaining local connectivity will therefore be crucial for boosting resilience and preventing local extinction in this vulnerable species.

The implications of our findings for *M. birostris* are more nuanced. Despite detecting only subtle population genetic differentiation, we cannot rule out the possibility that historical processes and large effective population size are obscuring a higher degree of contemporary demographic separation. Together with studies reporting high site fidelity and restricted movement patterns, our findings strongly suggest that local and national management actions should be considered essential for protecting resident aggregations of *M. birostris*. Nevertheless, we expect that weak population structure and high

genetic variation are simultaneously being driven by some degree of contemporary dispersal. Consequently, any fishing activity taking place along migratory corridors threatens to disrupt a mode of gene flow that may be fundamental for long-term resilience of the species. Similarly, although we have limited understanding of the number and distribution of breeding and nursery grounds (Knochel, Cochran, et al., 2022; Pate & Marshall, 2020; Stewart, Nuttall, et al., 2018), significant reduction in local stocks may impact long-term recruitment at oceanic and even global scales. We therefore emphasize the escalating need to improve the implementation of regional and international measures that seek to protect taxa in the high seas. Together with local scale management, appropriate evidence-based actions will contribute to maintaining large, connected and genetically diverse populations of manta rays into the future.

AFFILIATIONS
[1]Royal (Dick) School of Veterinary Studies and the Roslin Institute, University of Edinburgh, Edinburgh, UK
[2]The Manta Trust, Catemwood House, Dorset, UK
[3]Molecular Ecology and Evolution Group, Bangor University, Bangor, UK
[4]Australian Research Centre for Human Evolution, Griffith University, Nathan, Queensland, Australia
[5]School of Biomedical Sciences, The University of Queensland, St. Lucia, Queensland, Australia
[6]Océanos Vivientes AC, Mexico City, Mexico
[7]Consejo Nacional de Humanidades Ciencia y Tecnología (CONAHCyT), Mexico City, Mexico
[8]El Colegio de la Frontera Sur, Unidad Chetumal, Chetumal, Mexico
[9]Hawai'i Association for Marine Education and Research, Lahaina, USA
[10]Blue Resources Trust, Colombo, Sri Lanka
[11]Save Our Seas Foundation – D'Arros Research Centre, Geneva, Switzerland
[12]School of Biological Sciences, Oceans Institute and Oceans Graduate School, The University of Western Australia, Crawley, Western Australia, Australia
[13]Large Marine Vertebrates Research Institute Philippines, Jagna, Philippines
[14]Ocean Ecology Lab, Marine Mammal Institute, Department of Fisheries, Wildlife & Conservation Sciences, Oregon State University, Newport, Oregon, USA
[15]KwaZulu-Natal Sharks Board, Umhlanga Rocks, South Africa
[16]School of Life Sciences, University of KwaZulu-Natal, Durban, South Africa

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

FUNDING INFORMATION

BENEFIT-SHARING STATEMENT

A research collaboration was developed with scientists from regions providing genetic samples to advance the conservation of manta rays and their relatives through evidence-based research, international collaboration and institutional capacity building. Data and analysis pipelines associated with this work have been shared on public databases for the benefit of this wider cause.

DATA AVAILABILITY STATEMENT

Sequencing data have been deposited to the European Nucleotide Archive under study accession number PRJEB66437. Analysis code is available at https://github.com/elhumble/manta_pop_gen_2022.

ORCID

Emily Humble https://orcid.org/0000-0001-6841-1269
Mark de Bruyn https://orcid.org/0000-0003-1528-9604

REFERENCES

Andrzejaczek, S., Schallert, R. J., Forsberg, K., Arnoldi, N. S., Cabanillas-Torpoco, M., Purizaca, W., & Block, B. A. (2021). Reverse diel vertical movements of oceanic manta rays off the northern coast of Peru and implications for conservation. *Ecological Solutions and Evidence*, 2(1), e12051. https://doi.org/10.1002/2688-8319.12051

Arauz, R., Chávez, E. J., Hoyos-Padilla, E. M., & Marshall, A. D. (2019). First record of the reef manta ray, *Mobula alfredi*, from the eastern Pacific. *Marine Biodiversity Records*, 12(1), 3. https://doi.org/10.1186/s41200-019-0162-9

Armstrong, A. O., Armstrong, A. J., Bennett, M. B., Richardson, A. J., Townsend, K. A., & Dudgeon, C. L. (2019). Photographic identification and citizen science combine to reveal long distance movements of individual reef manta rays *Mobula alfredi* along Australia's east coast. *Marine Biodiversity Records*, 12(1), 14. https://doi.org/10.1186/s41200-019-0173-6

Bailleul, D., Mackenzie, A., Sacchi, O., Poisson, F., Bierne, N., & Arnaud-Haond, S. (2018). Large-scale genetic panmixia in the blue shark (*Prionace glauca*): A single worldwide population, or a genetic lag-time effect of the "grey zone" of differentiation? *Evolutionary Applications*, 11(5), 614–630. https://doi.org/10.1111/eva.12591

Beale, C. S., Stewart, J. D., Setyawan, E., Sianipar, A. B., & Erdmann, M. V. (2019). Population dynamics of oceanic manta rays (*Mobula birostris*) in the Raja Ampat Archipelago, West Papua, Indonesia, and the impacts of the El Niño–Southern Oscillation on their movement ecology. *Diversity and Distributions*, 25(9), 1472–1487. https://doi.org/10.1111/ddi.12962

Bonnet, T., Morrissey, M. B., de Villemereuil, P., Alberts, S. C., Arcese, P., Bailey, L. D., Boutin, S., Brekke, P., Brent, L. J. N., Camenisch, G., Charmantier, A., Clutton-Brock, T. H., Cockburn, A., Coltman, D. W., Courtiol, A., Davidian, E., Evans, S. R., Ewen, J. G., Festa-Bianchet, M., … Kruuk, L. E. B. (2022). Genetic variance in fitness indicates rapid contemporary adaptive evolution in wild animals. *Science*, 376(6596), 1012–1016. https://doi.org/10.1126/science.abk0853

Braun, C. D., Skomal, G. B., Thorrold, S. R., & Berumen, M. L. (2015). Movements of the reef manta ray (*Manta alfredi*) in the Red Sea using satellite and acoustic telemetry. *Marine Biology*, 162(12), 2351–2362. https://doi.org/10.1007/s00227-015-2760-3

Cabral, M. M. P., Stewart, J. D., Marques, T. A., Ketchum, J. T., Ayala-Bocos, A., Hoyos-Padilla, E. M., & Reyes-Bonilla, H. (2023). The influence of El Niño Southern Oscillation on the population dynamics of oceanic manta rays in the Mexican Pacific. *Hydrobiologia*, 850(2), 257–267. https://doi.org/10.1007/s10750-022-05047-9

Carpenter, M., Parker, D., Dicken, M. L., & Griffiths, C. L. (2023). Multi-decade catches of manta rays (*Mobula alfredi*, *M. birostris*) from South Africa reveal significant decline. *Frontiers in Marine Science*, 10, 1128819. https://doi.org/10.3389/fmars.2023.1128819

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, 22(11), 3124–3140.

Cayuela, H., Rougemont, Q., Prunier, J. G., Moore, J.-S., Clobert, J., Besnard, A., & Bernatchez, L. (2018). Demographic and genetic approaches to study dispersal in wild animal populations: A methodological review. *Molecular Ecology*, 27(20), 3976–4010. https://doi.org/10.1111/mec.14848

Couturier, L. I. E., Dudgeon, C. L., Pollock, K. H., Jaine, F. R. A., Bennett, M. B., Townsend, K. A., Weeks, S. J., & Richardson, A. J. (2014). Population dynamics of the reef manta ray *Manta alfredi* in eastern Australia. *Coral Reefs*, 33(2), 329–342. https://doi.org/10.1007/s00338-014-1126-5

Couturier, L. I. E., Marshall, A. D., Jaine, F. R. A., Kashiwagi, T., Pierce, S. J., Townsend, K. A., Weeks, S. J., Bennett, M. B., & Richardson, A. J. (2012). Biology, ecology and conservation of the Mobulidae. *Journal of Fish Biology*, 80(5), 1075–1119. https://doi.org/10.1111/j.1095-8649.2012.03264.x

Croll, D. A., Dewar, H., Dulvy, N. K., Fernando, D., Francis, M. P., Galván-Magaña, F., Hall, M., Heinrichs, S., Marshall, A., Mccauley, D., Newton, K. M., Notarbartolo di Sciara, G., O'Malley, M., O'Sullivan, J., Poortvliet, M., Roman, M., Stevens, G., Tershy, B. R., & White, W. T. (2016). Vulnerabilities and fisheries impacts: The uncertain future of manta and devil rays. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 26(3), 562–575. https://doi.org/10.1002/aqc.2591

Deakos, M. H., Baker, J. D., & Bejder, L. (2011). Characteristics of a manta ray *Manta alfredi* population off Maui, Hawaii, and implications for management. *Marine Ecology Progress Series*, 429, 245–260. https://doi.org/10.3354/meps09085

Dulvy, N. K., Pardo, S. A., Simpfendorfer, C. A., & Carlson, J. K. (2014). Diagnosing the dangerous demography of manta rays using life history theory. *PeerJ*, 2, e400. https://doi.org/10.7717/peerj.400

Fernando, D., & Stewart, J. D. (2021). High bycatch rates of manta and devil rays in the "small-scale" artisanal fisheries of Sri Lanka. *PeerJ*, 9, e11994. https://doi.org/10.7717/peerj.11994

Foote, A. D., & Morin, P. A. (2016). Genome-wide SNP data suggest complex ancestry of sympatric North Pacific killer whale ecotypes. *Heredity*, 117(5), 316–325. https://doi.org/10.1038/hdy.2016.54

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution*, 27(9), 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Gagnaire, P.-A. (2020). Comparative genomics approach to evolutionary process connectivity. *Evolutionary Applications*, 13(6), 1320–1334. https://doi.org/10.1111/eva.12978

Gagnaire, P.-A., Broquet, T., Aurelle, D., Viard, F., Souissi, A., Bonhomme, F., Arnaud-Haond, S., & Bierne, N. (2015). Using neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the genomic era. *Evolutionary Applications*, 8(8), 769–786. https://doi.org/10.1111/eva.12288

Garzon, F., Seymour, Z. T. A., Monteiro, Z. L., & Graham, R. T. (2023). Spatial ecology of a newly described oceanic manta ray population in the Atlantic Ocean. *Marine Biology*, 170(6), 68. https://doi.org/10.1007/s00227-023-04219-y

Germanov, E. S., & Marshall, A. D. (2014). Running the gauntlet: Regional movement patterns of *Manta alfredi* through a complex of parks and fisheries. *PLoS One*, 9(10), e110071. https://doi.org/10.1371/journal.pone.0110071

Germanov, E. S., Pierce, S. J., Marshall, A. D., Hendrawan, I. G., Kefi, A., Bejder, L., & Loneragan, N. (2022). Residency, movement patterns, behavior and demographics of reef manta rays in Komodo National Park. *PeerJ*, 10, e13302. https://doi.org/10.7717/peerj.13302

Gordon, L., & Vierus, T. (2022). First photographic evidence of oceanic manta rays (*Mobula birostris*) at two locations in the Fiji Islands. *PeerJ*, 10, e13883. https://doi.org/10.7717/peerj.13883

Gruber, B., Unmack, P. J., Berry, O. F., & Georges, A. (2018). dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources*, 18(3), 691–699. https://doi.org/10.1111/1755-0998.12745

Hanski, I., & Gilpin, M. (1991). Metapopulation dynamics: Brief history and conceptual domain. *Biological Journal of the Linnean Society*, 42(1–2), 3–16. https://doi.org/10.1111/j.1095-8312.1991.tb00548.x

Harty, K., Guerrero, M., Knochel, A. M., Stevens, G. M. W., Marshall, A., Burgess, K., & Stewart, J. D. (2022). Demographics and dynamics of the world's largest known population of oceanic manta rays *Mobula birostris* in coastal Ecuador. *Marine Ecology Progress Series*, 700, 145–159. https://doi.org/10.3354/meps14189

Hauser, L., & Carvalho, G. R. (2008). Paradigm shifts in marine fisheries genetics: Ugly hypotheses slain by beautiful facts. *Fish and Fisheries*, 9(4), 333–362. https://doi.org/10.1111/j.1467-2979.2008.00299.x

Hinojosa-Alvarez, S., Walter, R. P., Diaz-Jaimes, P., Galván-Magaña, F., & Paig-Tran, E. M. (2016). A potential third Manta Ray species near the Yucatán Peninsula? Evidence for a recently diverged and novel genetic Manta group from the Gulf of Mexico. *PeerJ*, 4, e2586. https://doi.org/10.7717/peerj.2586

Hirschfeld, M., Dudgeon, C., Sheaves, M., & Barnett, A. (2021). Barriers in a sea of elasmobranchs: From fishing for populations to testing hypotheses in population genetics. *Global Ecology and Biogeography*, 30(11), 2147–2163. https://doi.org/10.1111/geb.13379

Hoffman, J. R., Willoughby, J. R., Swanson, B. J., Pangle, K. L., & Zanatta, D. T. (2017). Detection of barriers to dispersal is masked by long lifespans and large population sizes. *Ecology and Evolution*, 7(22), 9613–9623. https://doi.org/10.1002/ece3.3470

Hosegood, J., Humble, E., Ogden, R., de Bruyn, M., Creer, S., Stevens, G. M. W., Abudaya, M., Bassos-Hull, K., Bonfil, R., Fernando, D., Foote, A. D., Hipperson, H., Jabado, R. W., Kaden, J., Moazzam, M., Peel, L. R., Pollett, S., Ponzo, A., Poortvliet, M., ... Carvalho, G. (2020). Phylogenomics and species delimitation for effective conservation of manta and devil rays. *Molecular Ecology*, 29(24), 4783–4796. https://doi.org/10.1111/mec.15683

Jaine, F. R. A., Rohner, C. A., Weeks, S. J., Couturier, L. I. E., Bennett, M. B., Townsend, K. A., & Richardson, A. J. (2014). Movements and habitat use of reef manta rays off eastern Australia: Offshore excursions, deep diving and eddy affinity revealed by satellite telemetry. *Marine Ecology Progress Series*, 510, 73–86. https://doi.org/10.3354/meps10910

Jakobsson, M., Edge, M. D., & Rosenberg, N. A. (2013). The relationship between *Fst* and the frequency of the most frequent allele. *Genetics*, 193(2), 515–528. https://doi.org/10.1534/genetics.112.144758

Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24(11), 1403–1405.

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, 11(1), 94. https://doi.org/10.1186/1471-2156-11-94

Kardos, M., Armstrong, E. E., Fitzpatrick, S. W., Hauser, S., Hedrick, P. W., Miller, J. M., Tallmon, D. A., & Funk, W. C. (2021). The crucial role of genome-wide genetic variation in conservation. *Proceedings of the National Academy of Sciences of the United States of America*, 118(48), e2104642118. https://doi.org/10.1073/pnas.2104642118

Kashiwagi, T., Marshall, A. D., Bennett, M. B., Ovenden, J. R., (2011) Habitat segregation and mosaic sympatry of the two species of manta ray in the Indian and Pacific Oceans: Manta alfredi and M. birostris. *Marine Biodiversity Records 4*, 1–8.

Kashiwagi, T., Marshall, A. D., Bennett, M. B., & Ovenden, J. R. (2012). The genetic signature of recent speciation in manta rays (*Manta alfredi* and *M. birostris*). *Molecular Phylogenetics and Evolution*, 64(1), 212–218.

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, 4(8), 782–788. https://doi.org/10.1111/2041-210X.12067

Kessel, S. T., Elamin, N. A., Yurkowski, D. J., Chekchak, T., Walter, R. P., Klaus, R., Hill, G., & Hussey, N. E. (2017). Conservation of reef manta rays (*Manta alfredi*) in a UNESCO World Heritage Site: Large-scale Island development or sustainable tourism? *PLoS One*, 12(10), e0185419. https://doi.org/10.1371/journal.pone.0185419

Knochel, A. M., Cochran, J. E. M., Kattan, A., Stevens, G. M. W., Bojanowksi, E., & Berumen, M. L. (2022). Crowdsourced data reveal multinational connectivity, population demographics, and possible nursery ground of endangered oceanic manta rays in the Red Sea.

Aquatic Conservation: Marine and Freshwater Ecosystems, *32*(11), 1774–1786. https://doi.org/10.1002/aqc.3883

Knochel, A. M., Hussey, N. E., Kessel, S. T., Braun, C. D., Cochran, J. E. M., Hill, G., Klaus, R., Checkchak, T., Elamin El Hassen, N. M., Younnis, M., & Berumen, M. L. (2022). Home sweet home: Spatiotemporal distribution and site fidelity of the reef manta ray (*Mobula alfredi*) in Dungonab Bay, Sudan. *Movement Ecology*, *10*(1), 22. https://doi.org/10.1186/s40462-022-00314-9

Korneliussen, T., & Moltke, I. (2015). NgsRelate: A software tool for estimating pairwise relatedness from next-generation sequencing data. *Bioinformatics*, *31*, 4009–4011. https://doi.org/10.1093/bioinformatics/btv509

Lai, Y.-T., Yeung, C. K. L., Omland, K. E., Pang, E.-L., Hao, Y., Liao, B.-Y., Cao, H.-F., Zhang, B.-W., Yeh, C.-F., Hung, C.-M., Hung, H.-Y., Yang, M.-Y., Liang, W., Hsu, Y.-C., Yao, C.-T., Dong, L., Lin, K., & Li, S.-H. (2019). Standing genetic variation as the predominant source for adaptation of a songbird. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(6), 2152–2157. https://doi.org/10.1073/pnas.1813597116

Lassauce, H., Dudgeon, C. L., Armstrong, A. J., Wantiez, L., & Carroll, E. L. (2022). Evidence of fine-scale genetic structure for reef manta rays *Mobula alfredi* in New Caledonia. *Endangered Species Research*, *47*, 249–264. https://doi.org/10.3354/esr01178

Lawson, J. M., & Fordham, S. V. (2018). *Sharks ahead: Realizing the potential of the convention on migratory species to conserve elasmobranchs*. (Shark Advocates International, The Ocean Foundation, pp. 1–76) [Report]. https://www.bmis-bycatch.org/references/c8zv4pcf

Lawson, J. M., Fordham, S. V., O'Malley, M. P., Davidson, L. N. K., Walls, R. H. L., Heupel, M. R., Stevens, G., Fernando, D., Budziak, A., Simpfendorfer, C. A., Ender, I., Francis, M. P., di Sciara, G. N., & Dulvy, N. K. (2017). Sympathy for the devil: A conservation strategy for devil and manta rays. *PeerJ*, *5*, e3027. https://doi.org/10.7717/peerj.3027

Le Corre, V., & Kremer, A. (1998). Cumulative effects of founding events during colonisation on genetic diversity and differentiation in an Island and stepping-stone model. *Journal of Evolutionary Biology*, *11*(4), 495–512. https://doi.org/10.1046/j.1420-9101.1998.11040495.x

Legendre, P., Fortin, M.-J., & Borcard, D. (2015). Should the Mantel test be used in spatial analysis? *Methods in Ecology and Evolution*, *6*(11), 1239–1247. https://doi.org/10.1111/2041-210X.12425

Leslie, M. S., & Morin, P. A. (2018). Structure and phylogeography of two tropical predators, spinner (*Stenella longirostris*) and pantropical spotted (*S. attenuata*) dolphins, from SNP data. *Royal Society Open Science*, *5*(4), 171615. https://doi.org/10.1098/rsos.171615

Liu, X., Rønhøj Schjøtt, S., Granquist, S. M., Rosing-Asvid, A., Dietz, R., Teilmann, J., Galatius, A., Cammen, K., O'Corry-Crowe, G., Harding, K., Härkönen, T., Hall, A., Carroll, E. L., Kobayashi, Y., Hammill, M., Stenson, G., Kirstine Frie, A., Lydersen, C., Kovacs, K. M., ... Tange Olsen, M. (2022). Origin and expansion of the world's most widespread pinniped: Range-wide population genomics of the harbour seal (*Phoca vitulina*). *Molecular Ecology*, *31*(6), 1682–1699. https://doi.org/10.1111/mec.16365

López, K. E. R., Guadalupe, J. J., Gordillo-Romero, M., Montero-Oleas, A., Pazmiño, D. A., Guerrero, M., & de Torres, M. L. (2022). Exploring the genetic diversity and population structure of *Mobula birostris* in two key aggregation zones in the Eastern Tropical Pacific. *Marine Ecology Progress Series*, *699*, 75–89. https://doi.org/10.3354/meps14171

Louis, M., Galimberti, M., Archer, F., Berrow, S., Brownlow, A., Fallon, R., Nykänen, M., O'Brien, J., Roberston, K. M., Rosel, P. E., Simon-Bouhet, B., Wegmann, D., Fontaine, M. C., Foote, A. D., & Gaggiotti, O. E. (2021). Selection on ancestral genetic variation fuels repeated ecotype formation in bottlenose dolphins. *Science Advances*, *7*(44), eabg1245. https://doi.org/10.1126/sciadv.abg1245

Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular Ecology*, *19*(15), 3038–3051. https://doi.org/10.1111/j.1365-294X.2010.04688.x

McClain, M. A., Hammerschlag, N., Gallagher, A. J., Drymon, J. M., Grubbs, R. D., Guttridge, T. L., Smukall, M. J., Frazier, B. S., & Daly-Engel, T. S. (2022). Age-dependent dispersal and relatedness in tiger sharks (*Galeocerdo cuvier*). *Frontiers in Marine Science*, *9*, 900107. https://doi.org/10.3389/fmars.2022.900107

Milanesi, M., Capomaccio, S., Vajana, E., Bomba, L., Garcia, J. F., Ajmone-Marsan, P., & Colli, L. (2017). *BITE: An R package for biodiversity analyses*. (p. 181610). *bioRxiv*. https://doi.org/10.1101/181610

Mills, L. S., & Allendorf, F. W. (1996). The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, *10*(6), 1509–1518.

Moazzam, M. (2018). *Unprecedented decline in the catches of mobulids: An important component of tuna gillnet fisheries of the Northern Arabian Sea*. (IOTC – 14th Working Party on Ecosystems and Bycatch). IOTC-2018-WPEB14-30.

Mussmann, S. M., Douglas, M. R., Chafin, T. K., & Douglas, M. E. (2019). BA3-SNPs: Contemporary migration reconfigured in BayesAss for next-generation sequence data. *Methods in Ecology and Evolution*, *10*(10), 1808–1813. https://doi.org/10.1111/2041-210X.13252

Nikolic, N., Devloo-Delva, F., Bailleul, D., Noskova, E., Rougeux, C., Delord, C., Borsa, P., Liautard-Haag, C., Hassan, M., Marie, A. D., Feutry, P., Grewe, P., Davies, C., Farley, J., Fernando, D., Biton-Porsmoguer, S., Poisson, F., Parker, D., Leone, A., ... Arnaud-Haond, S. (2023). Stepping up to genome scan allows stock differentiation in the worldwide distributed blue shark *Prionace glauca*. *Molecular Ecology*, *32*(5), 1000–1019. https://doi.org/10.1111/mec.16822

O'Malley, M., Townsend, K. A., Hilton, P., Heinrichs, S., & Stewart, J. D. (2017). Characterization of the trade in manta and devil ray gill plates in China and South-East Asia through trader surveys. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*(2), 394–413. https://doi.org/10.1002/aqc.2670

Palaiokostas, C., Bekaert, M., Khan, M. G., Taggart, J. B., Gharbi, K., McAndrew, B. J., & Penman, D. J. (2015). A novel sex-determining QTL in Nile tilapia (*Oreochromis niloticus*). *BMC Genomics*, *16*(1), 171. https://doi.org/10.1186/s12864-015-1383-x

Palsbøll, P. J., Bérubé, M., & Allendorf, F. W. (2007). Identification of management units using population genetic data. *Trends in Ecology & Evolution*, *22*(1), 11–16. https://doi.org/10.1016/j.tree.2006.09.003

Palumbi, S. R. (2003). Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, *13*(sp1), 146–158. https://doi.org/10.1890/1051-0761(2003)013[0146:PGDCAT]2.0.CO;2

Pante, E., & Simon-Bouhet, B. (2013). marmap: A package for importing, plotting and analyzing bathymetric and topographic data in R. *PLoS One*, *8*(9), e73051. https://doi.org/10.1371/journal.pone.0073051

Papastamatiou, Y. P., Meyer, C. G., Carvalho, F., Dale, J. J., Hutchinson, M. R., & Holland, K. N. (2013). Telemetry and random-walk models reveal complex patterns of partial migration in a large marine predator. *Ecology*, *94*(11), 2595–2606. https://doi.org/10.1890/12-2014.1

Pascual, M., Rives, B., Schunter, C., & Macpherson, E. (2017). Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. *PLoS One*, *12*(5), e0176419. https://doi.org/10.1371/journal.pone.0176419

Pate, J. H., & Marshall, A. D. (2020). Urban manta rays: Potential manta ray nursery habitat along a highly developed Florida coastline. *Endangered Species Research*, *43*, 51–64. https://doi.org/10.3354/esr01054

Peel, L. R., Stevens, G. M. W., Daly, R., Daly, C. A. K., Lea, J. S. E., Clarke, C. R., Collin, S. P., & Meekan, M. G. (2019). Movement and residency patterns of reef manta rays *Mobula alfredi* in the Amirante

Islands, Seychelles. *Marine Ecology Progress Series*, *621*, 169–184. https://doi.org/10.3354/meps12995

Perryman, R. J. Y., Mourier, J., Venables, S. K., Tapilatu, R. F., Setyawan, E., & Brown, C. (2022). Reef manta ray social dynamics depend on individual differences in behaviour. *Animal Behaviour*, *191*, 43–55. https://doi.org/10.1016/j.anbehav.2022.06.010

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double Digest RADseq: An inexpensive method for de novo snp discovery and genotyping in model and non-model species. *PLoS One*, *7*(5), e37135.

Phillips, N. M., Devloo-Delva, F., McCall, C., & Daly-Engel, T. S. (2021). Reviewing the genetic evidence for sex-biased dispersal in elasmobranchs. *Reviews in Fish Biology and Fisheries*, *31*(4), 821–841. https://doi.org/10.1007/s11160-021-09673-9

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.

Puechmaille, S. J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*(3), 608–627.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., de Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics*, *81*(3), 559–575.

Rambahiniarison, J., Agustines, A., Alexopoulos, K., Araujo, G., Armstrong, A. O., Arnold, S., Barruga, A., Cañete, T., Conales, S., Jr., Delijero, K., Enolva, N. P., Flam, A. L., Keane, E., Labaja, J., Legaspi, C. G., Murie, C., Murray, R., Oliver, S. P., Pierce, S. J., ... Barr, Y. (2023). Distribution of the reef manta ray *Mobula alfredi* and the oceanic manta ray *Mobula birostris* in The Philippines: A collaborative effort for conservation. *Journal of Fish Biology*, *102*(2), 492–503. https://doi.org/10.1111/jfb.15283

Reed, D. H. (2004). Extinction risk in fragmented habitats. *Animal Conservation Forum*, *7*(2), 181–191. https://doi.org/10.1017/S1367943004001313

Rochette, N. C., & Catchen, J. M. (2017). Deriving genotypes from RADseq short-read data using Stacks. *Nature Protocols*, *12*(12), 2640–2659. https://doi.org/10.1038/nprot.2017.123

Rohner, C. A., Flam, A. L., Pierce, S. J., & Marshall, A. D. (2017). Steep declines in sightings of manta rays and devilrays (Mobulidae) in southern Mozambique. *PeerJ Preprints*, *5*, e3051v1. https://doi.org/10.7287/peerj.preprints.3051v1

Rohner, C. A., Pierce, S. J., Marshall, A. D., Weeks, S. J., Bennett, M. B., & Richardson, A. J. (2013). Trends in sightings and environmental influences on a coastal aggregation of manta rays and whale sharks. *Marine Ecology Progress Series*, *482*, 153–168. https://doi.org/10.3354/meps10290

Setyawan, E., Sianipar, A. B., Erdmann, M. V., Fischer, A. M., Haddy, J. A., Beale, C. S., Lewis, S. A., & Mambrasar, R. (2018). Site fidelity and movement patterns of reef manta rays (*Mobula alfredi*: Mobulidae) using passive acoustic telemetry in northern Raja Ampat, Indonesia. *Nature Conservation Research*, *3*(4), 17–31. https://doi.org/10.24189/ncr.2018.043

Stevens, G., Fernando, D., & Sciara, G. N. D. (2018). *Guide to the manta and devil rays of the world*. Princeton University Press.

Stewart, J. D., Beale, C. S., Fernando, D., Sianipar, A. B., Burton, R. S., Semmens, B. X., & Aburto-Oropeza, O. (2016). Spatial ecology and conservation of *Manta birostris* in the Indo-Pacific. *Biological Conservation*, *200*, 178–183. https://doi.org/10.1016/j.biocon.2016.05.016

Stewart, J. D., Jaine, F. R. A., Armstrong, A. J., Armstrong, A. O., Bennett, M. B., Burgess, K. B., Couturier, L. I. E., Croll, D. A., Cronin, M. R., Deakos, M. H., Dudgeon, C. L., Fernando, D., Froman, N., Germanov, E. S., Hall, M. A., Hinojosa-Alvarez, S., Hosegood, J. E., Kashiwagi, T., Laglbauer, B. J. L., ... Stevens, G. M. W. (2018). Research priorities to support effective manta and devil ray conservation. *Frontiers in Marine Science*, *5*, 314. https://doi.org/10.3389/fmars.2018.00314

Stewart, J. D., Nuttall, M., Hickerson, E. L., & Johnston, M. A. (2018). Important juvenile manta ray habitat at Flower Garden Banks National Marine Sanctuary in the northwestern Gulf of Mexico. *Marine Biology*, *165*(7), 111. https://doi.org/10.1007/s00227-018-3364-5

Stoffel, M. A., Esser, M., Kardos, M., Humble, E., Nichols, H., David, P., & Hoffman, J. I. (2016). InbreedR: An R package for the analysis of inbreeding based on genetic markers. *Methods in Ecology and Evolution*, *7*(11), 1331–1339.

Taylor, B. L., & Dizon, A. E. (1996). The need to estimate power to link genetics and demography for conservation. *Conservation Biology*, *10*(2), 661–664.

Thia, J. A. (2023). Guidelines for standardizing the application of discriminant analysis of principal components to genotype data. *Molecular Ecology Resources*, *23*(3), 523–538. https://doi.org/10.1111/1755-0998.13706

Thorburn, J., Neat, F., Burrett, I., Henry, L.-A., Bailey, D. M., Jones, C. S., & Noble, L. R. (2019). Ontogenetic variation in movements and depth use, and evidence of partial migration in a benthopelagic elasmobranch. *Frontiers in Ecology and Evolution*, *7*, 353. https://doi.org/10.3389/fevo.2019.00353

Venables, S. K., Marshall, A. D., Armstrong, A. J., Tomkins, J. L., & Kennington, W. J. (2021). Genome-wide SNPs detect no evidence of genetic population structure for reef manta rays (*Mobula alfredi*) in southern Mozambique. *Heredity*, *126*(2), 308–319. https://doi.org/10.1038/s41437-020-00373-x

Vignaud, T. M., Maynard, J. A., Leblois, R., Meekan, M. G., Vázquez-Juárez, R., Ramírez-Macías, D., Pierce, S. J., Rowat, D., Berumen, M. L., Beeravolu, C., Baksay, S., & Planes, S. (2014). Genetic structure of populations of whale sharks among ocean basins and evidence for their historic rise and recent decline. *Molecular Ecology*, *23*(10), 2590–2601. https://doi.org/10.1111/mec.12754

Waples, R. (1998). Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, *89*(5), 438–450. https://doi.org/10.1093/jhered/89.5.438

Waples, R., & Gaggiotti, O. (2006). INVITED REVIEW: What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, *15*(6), 1419–1439. https://doi.org/10.1111/j.1365-294X.2006.02890.x

Waples, R., Punt, A. E., & Cope, J. M. (2008). Integrating genetic data into management of marine resources: How can we do it better? *Fish and Fisheries*, *9*(4), 423–449. https://doi.org/10.1111/j.1467-2979.2008.00303.x

Waples, R. K., Albrechtsen, A., & Moltke, I. (2019). Allele frequency-free inference of close familial relationships from genotypes or low-depth sequencing data. *Molecular Ecology*, *28*(1), 35–48. https://doi.org/10.1111/mec.14954

Ward, R. D., Woodwark, M., & Skibinski, D. O. F. (1994). A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology*, *44*(2), 213–232. https://doi.org/10.1111/j.1095-8649.1994.tb01200.x

Ward-Paige, C. A., Davis, B., & Worm, B. (2013). Global population trends and human use patterns of manta and mobula rays. *PLoS One*, *8*(9), e74835. https://doi.org/10.1371/journal.pone.0074835

Weir, B. S., & Cockerham, C. C. (1984). Estimating f-statistics for the analysis of population structure. *Evolution*, *38*(6), 1358–1370. https://doi.org/10.2307/2408641

Whitney, J. L., Coleman, R. R., & Deakos, M. H. (2023). Genomic evidence indicates small Island-resident populations and sex-biased behaviors of Hawaiian reef Manta Rays. *BMC Ecology and Evolution*, *23*(1), 31. https://doi.org/10.1186/s12862-023-02130-0

Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, *16*(2), 97–159.

Younger, J. L., Clucas, G. V., Kao, D., Rogers, A. D., Gharbi, K., Hart, T., & Miller, K. J. (2017). The challenges of detecting subtle population structure and its importance for the conservation of emperor penguins. *Molecular Ecology*, *26*(15), 3883–3897.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.