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Using eDNA to Supplement Population Genetic Analyses for Cryptic Marine Species: Identifying Population Boundaries for Alaska Harbour Porpoises

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ABSTRACT

Isolation by distance and biogeographical boundaries define patterns of population genetic structure for harbour porpoise along the Pacific coast from California to British Columbia. Until recently, inadequate sample sizes in many regions constrained efforts to characterise population genetic structure throughout the coastal waters of Alaska. Here, tissue samples from beachcast strandings and fisheries bycatch were supplemented with targeted environmental DNA (eDNA) samples in key regions of Alaska coastal and inland waters. Using a geographically explicit, hierarchical approach, we examined the genetic structure of Alaska harbour porpoises, using both mitochondrial DNA (mtDNA) sequence data and multilocus SNP genotypes. Despite a lack of evidence of genetic differentiation from nuclear SNP loci, patterns of relatedness and genetic differentiation from mtDNA suggest natal philopatry at multiple geographic scales, with limited gene flow among sites possibly mediated by male dispersal. A priori clustering of sampled areas at an intermediate scale (eastern and western Bering Sea, Gulf of Alaska and Southeast Alaska) best explained the genetic variance (12.37%) among regions. In addition, mtDNA differentiation between the Gulf of Alaska and eastern Bering Sea, and among regions within the Gulf of Alaska, indicated significant genetic structuring of harbour porpoise populations in Southeast Alaska. The targeted collection of eDNA samples from strata within Southeast Alaska was key for elevating the statistical power of our mtDNA dataset, and findings indicate limited dispersal between neighbouring strata within coastal and inland waters. These results provide evidence supporting a population boundary within the currently recognised Southeast

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Alaska Stock. Together, these findings will prove useful for ongoing management efforts to reduce fisheries conflict and conserve genetic diversity in this iconic coastal species.

1 | Introduction

Population genetic data are widely recognised as an important line of evidence for delineating stock boundaries and, as such, are of particular value for small coastal cetaceans that are often vulnerable to nearshore fisheries and other anthropogenic impacts. Furthermore, knowledge of population genetic structure is vital to assess trends in abundance, to understand population-level effects of anthropogenic and environmental impacts, and to estimate levels of potential biological removal (PBR) for marine mammal stock assessments. Distributed throughout coastal temperate and sub-Arctic waters in the Northern Hemisphere, the harbour porpoise (*Phocoena phocoena*) is one of the smallest cetaceans in the eastern North Pacific, typically found in coastal areas along the continental shelf in waters < 200 m deep (Fontaine 2016; Read 1999). As a result of this nearshore distribution, harbour porpoises are particularly vulnerable to a wide range of anthropogenic activities including coastal development, offshore marine resource infrastructure (Tougaard, Henriksen, and Miller 2009; Vallejo et al. 2017) and commercial and subsistence fisheries (e.g., Bjørge, Skern-Mauritzen, and Rossman 2013; Quintela et al. 2020; Read, Drinker, and Northridge 2006).

While understanding patterns of genetic differentiation is critical for directing management and conservation actions, characterising the structure and connectivity of harbour porpoise populations is challenging as the species' small size and elusive behaviour makes traditional tissue sampling methods impractical (Parsons et al. 2018). To date, several studies have explored harbour porpoise population structure leveraging samples collected from beachcast carcasses and fisheries bycatch. Genetic structure was detected on relatively small geographical scales (i.e., 100s of kilometres; Morin et al. 2021) in several regions of the eastern Pacific and in the eastern and western Atlantic (Fontaine 2016; Fontaine et al. 2007, 2017; Lah et al. 2016; Rosel, Dizon, and Haygood 1995; Rosel et al. 1999). In the eastern North Pacific, significant genetic differentiation among harbour porpoises was initially described by Rosel, Dizon, and Haygood (1995) using mitochondrial control region sequence data. On a broad geographical scale, significant genetic differentiation was detected among geographically defined populations, suggesting genetic divergence between northern (AK and BC) and southern (WA and CA) coastal regions. Using both mitochondrial and nuclear (microsatellite) loci, Chivers et al. (2002) re-examined intraspecific genetic structure in the eastern North Pacific on a finer geographic scale, revealing significant genetic subdivision among a priori geographical strata and identifying a number of relatively small genetic units. Additional support for these initial findings of genetic structure among harbour porpoise populations in the North Pacific indicated a break in genetic variation between northern British Columbia (Taguchi et al. 2010) and coastal Washington (Morin et al. 2021). Previous analysis of 198 harbour porpoise from coastal British Columbia suggested a lack of genetic subdivision among porpoises in southern coastal British Columbia (Crossman, Barrett-Lennard, and Taylor 2014). However, recent efforts to characterise the population genetic structure of continuously distributed harbour porpoise along the Pacific coast using a larger set of nuclear SNP markers revealed significant genetic differentiation of porpoises in the Strait of Georgia, BC from the nearby San Juan Islands, WA (Morin et al. 2021). In addition to population genetic structure, studies of harbour porpoises in the inland waters of Washington State and British Columbia also detected genetic introgression with Dall's porpoises (*Phocoenoides dalli*) with evidence of F1 hybrids among samples collected from BC and WA (Crossman, Barrett-Lennard, and Taylor 2014; Morin et al. 2021). These studies all highlight varying degrees of geographical subdivision within *Phocoena phocoena* in the North Pacific, with several instances of genetic divergence on relatively small geographical scales (< 200 km; Morin et al. 2021).

Recent efforts have highlighted the need to better understand the effects of fisheries bycatch on harbour porpoise in Alaska inland waters (Dahlheim et al. 2015; Zerbini, Goetz, et al. 2022). These effects would depend on whether harbour porpoises in the area were panmictic or consisted of multiple populations in small geographical areas, as consistent with other areas along the west coast. In the United States, the Marine Mammal Protection Act (MMPA) manages marine mammals at the scale of demographically independent populations (DIPs). By definition, DIPs specify management units at the scale of demographic independence but evolutionary independence is not expected (Martien and Taylor 2003; Morin et al. 2021). Management units at the scale of DIPs are expected to exhibit differences in allele or mitochondrial DNA (mtDNA) haplotype frequencies consistent with restricted gene flow among geographically adjacent areas (Palsbøll, Bérubé, and Allendorf 2007; Taylor and Dizon 1999). Prior to this study, prohibitively small sample sizes (often ≤ 5 samples) within some Alaska coastal regions limited robust evaluation of genetic structure (Chivers et al. 2002), emphasising the need for further examination of genetic structuring of harbour porpoises in under-represented Alaska regions.

Recent years have seen a growing interest in the use of microhaplotypes: Loci containing multiple SNPs per amplicon whose individual alleles can be combined into haplotypes, substantially increasing power compared to singleton SNP loci (McKinney, Seeb, and Seeb 2017). The use of microhaplotypes may serve to increase power to detect population structure and ameliorate some of the limitations associated with small sample sizes. While power to detect population structure depends on divergent alleles, power to detect related individuals requires alleles with high minor allele frequencies and is less constrained by sample size (May et al. 2020). Thus, a given panel of SNP loci may be more powerful for one or the other type of analysis. As such, coupling traditional population

structure analyses (i.e., F_{ST}, AMOVA and STRUCTURE) with relatedness-based analyses can aid in describing broader evolutionary processes across interconnected populations (May et al. 2020). Furthermore, combining data from multiple marker types (e.g., nuclear and mitochondrial markers) can be highly informative for characterising contemporary population genetic structure, particularly where sex biases in dispersal or philopatry exist (Prugnolle and de Meeus 2002). Thus, combining analyses using sequence data from both nuclear and mitochondrial genomes may aid in resolving population dynamic processes or detecting signatures of historical population structure (Morin et al. 2021).

Here, we characterise the population genetic structure of harbour porpoises in Alaska using both mtDNA sequence data and nuclear SNP microhaplotypes. To maximise sample sizes from key geographical strata, we use a targeted eDNA sampling approach to supplement tissue specimens collected from beachcast strandings and fisheries bycatch. Using mitochondrial sequence data generated from both tissues and eDNA as well as a multiplexed sequencing approach (GTseq; Campbell, Harmon, and Narum 2014) to genotype tissue samples using a species-specific SNP panel (Morin et al. 2021), we characterise the genetic diversity among Alaska harbour porpoise. Specifically, we use an a priori geographically explicit hierarchical approach to examine genetic structuring among geographical strata, with particular emphasis on the inland waters of Southeast Alaska. Supplementing population genomic data with targeted amplicon sequences from eDNA samples highlights the value of indirect genetic for addressing sample limitations when examining population structure in difficult to sample marine species.

2 | Methods

Nuclear SNP genotypes and mitochondrial control region sequence data were generated to evaluate population genetic structure and characterise patterns of gene flow among Alaska harbour porpoises. We employed a GTseq multiplex sequencing protocol (Campbell, Harmon, and Narum 2014) to generate individual SNP genotypes targeting 292 microhaplotype loci containing one or more SNPs (Morin et al. 2021). The harbour porpoise SNP panel was designed from next generation sequencing data from an indexed pool comprising genomic DNA from 12 North Pacific and 12 North Atlantic harbour porpoises (see Morin et al. 2021 for details). This multiplexed amplicon sequencing approach was applied to genomic DNA extracted from harbour porpoise skin tissue samples collected from fisheries bycatch or beach-cast carcasses throughout Alaska (1988–2016). Identification of SNP loci, microhaplotype genotype calling, quality assessment and error rate estimates were previously described in Morin et al. (2021). Targeted amplicon sequencing of the hypervariable 5′ region (396 bp) of the mitochondrial genome control region was performed for all tissue and eDNA samples using previously described protocols (Morin et al. 2021; Parsons et al. 2018); these methods are briefly described below. Data and scripts used in this study are publicly available at github.com/SMay1/AK_Harbor_Porpoise_PopGen.

2.1 | Identifying Inter-Generic Hybrid Porpoises

Inter-generic hybrids between parapatric harbour and Dall's porpoises have been previously identified among samples from the coasts of California, Washington and British Columbia based on both phenotype and genotype (Baird et al. 1998; Crossman, Barrett-Lennard, and Taylor 2014; Morin et al. 2021; Willis et al. 2004). Since introgression of hybrid alleles may confound species-specific analyses of population structure (Davies, Villablanca, and Roderick 1999; Miller, Adams, and Waits 2003), nuclear SNP data were explored to detect hybrid porpoises or putative introgressed alleles from Dall's porpoises. Data generated for the current Alaska study were expanded coastwide to include previously published data generated for porpoises sampled throughout the California Current system (Morin et al. 2021). Singleton SNP and microhaplotype SNP genotypes were generated for 388 porpoise samples collected from the eastern North Pacific from southern California to Barrow, Alaska. Samples identified to species based on morphological examination at the time of collection included 367 harbour porpoises, 11 Dall's porpoises and 5 putative inter-generic hybrid individuals from British Columbia (Crossman, Barrett-Lennard, and Taylor 2014).

To validate species identification of carcasses based on morphology, a discriminant analysis of principal components (DAPC) was applied to the SNP dataset using the package *adegenet* v.2.1.2 (Jombart 2008) in R (Team 2013). The optimal number of retained principal components (PC's) was determined using the function *optim.a.score*, with 1000 simulations per number of possible PC's. The function *loadingplot* was used to identify loci contributing significantly to species differentiation. Mitochondrial control region haplotypes (see below) in putative hybrids were used to identify the maternal contribution for intergeneric matings. Full details on inter-generic analyses are provided in the supplemental materials.

2.2 | Population Genetic Structure

A hierarchical spatial approach was adopted to investigate the magnitude and patterns of spatial population genetic structure among Alaska harbour porpoises. Geographical regions represented by porpoise samples were stratified into three hierarchical levels of a priori Strata (Figure 1). Strata were defined as geographically disjunct regions. At the highest level, harbour porpoise samples collected from Alaska waters were assigned to one of four broad geographical Basins (Strata-1): Western Bering Sea (W_BERING), Eastern Bering Sea (E_BERING), Barrow (BARROW) or Gulf of Alaska (GOA). The intermediate geographical strata (Strata-2) further divides the GOA stratum into three discrete sampling Regions (Cook Inlet [COOK], Copper River [COPPER], and Southeast AK [SEAK]), each consisting of one or more Locations (Strata-3; Figure 1). At the finest scale (Strata-3), Southeast AK is further divided into N_SEAK and S_SEAK, where N_SEAK includes the inland waters of Frederick Sound and waters to the North and West, including lower Chatham Strait, and S_SEAK includes waters South of Frederick Sound and East of Chatham Strait (Figure 1). Geographical groups within this stratification scheme are more

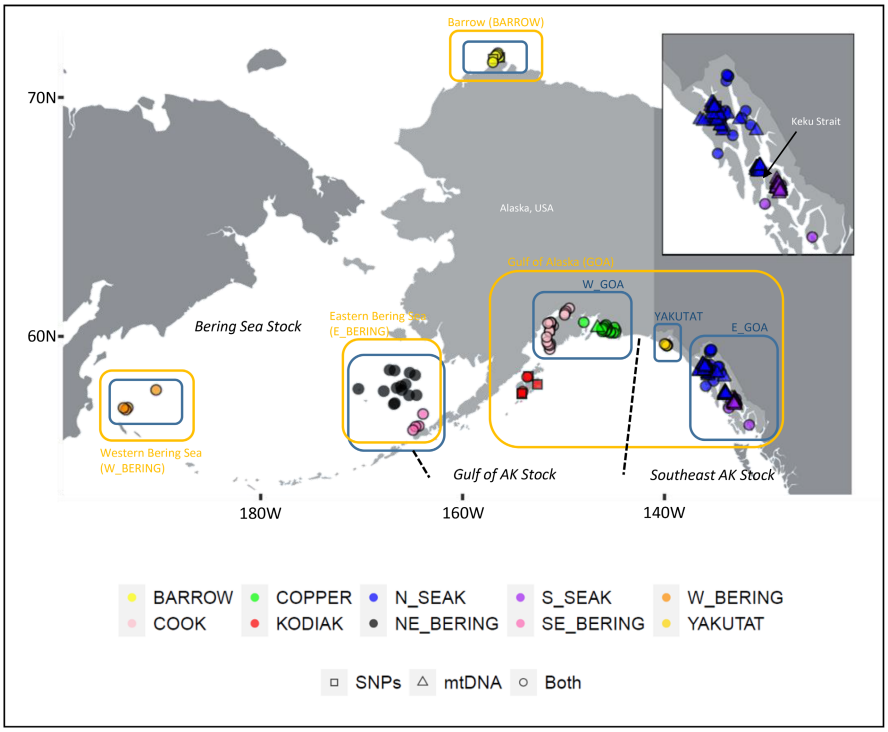

FIGURE 1 | Map of locations (points jittered) for AK harbour porpoise samples and current US stock boundaries (broken lines). The Bering Sea Stock is inclusive of coastal waters up to and including Barrow, AK. Colour text and rectangles represent a priori geographic stratifications: Strata-1 ('Basins')—yellow rectangles, Strata-2 ('Regions')—blue rectangles. Strata-3 ('Locations') are indicated by the coloured sampling locations. The shape of each point indicates genetic data generated for each sample and included in population genetic analyses: SNP loci (square), mtDNA control region (triangle), or both (circle). Sample locations in Southeast Alaska are magnified in the inset map. Note that for samples in the Eastern Bering (E_BERING) and Beaufort Seas (BARROW), Strata-1 and Strata-2 are synonymous.

generally referred to as 'strata' and the specific hierarchies are referred to as 'Basins', 'Regions' and 'Locations' for Stratas 1, 2 and 3, respectively.

2.3 | Genetic Clusters and Relatedness From Nuclear SNPs

Population genetic analyses were applied to multilocus SNP data generated from harbour porpoise tissue samples from throughout Alaska and the Western Bering Sea (Figure 1). We removed monomorphic loci and samples or loci with >25% missing data. Data from two geographically isolated Basins where sample sizes were ≥15 (Gulf of Alaska [GOA]; $N=54$) and Eastern Bering Sea (E_BERING, $N=15$) were used to test for linkage disequilibrium and deviations from Hardy–Weinberg Equilibrium (HWE). The package *StrataG* (v.2.4.905, Archer, Adams, and Schneiders 2017) in R (version 4.0.2, R Core Team 2020) was used to evaluate the significance of linkage disequilibrium (LD) between all pairs of loci and a sequential correction for multiple testing was applied (Holm 1979). One locus from each pair was excluded if in significant LD in the two Basins. Subsequently, loci were evaluated for deviations from Hardy–Weinberg Equilibrium (HWE) using *Pegas* (v.0.13, Paradis 2010) and similarly were excluded if they deviated significantly from HWE in both GOA and E_BERING after applying Holm's multiple testing correction. To remove potentially non-neutral loci and loci exhibiting non-Mendelian inheritance, we excluded loci with minor allele frequencies (MAF) < 0.05, and loci with a difference between observed and expected heterozygosity > 0.2 in both Basins. Lastly, we checked for duplicated individuals (identity > 80%) using *CKMRsim* (Anderson 2020).

To examine the extent of spatial genetic structuring and patterns of isolation by distance (IBD), we estimated pairwise least-cost distances over water between individual sample sites using *marmap* (Pante and Simon-Bouhet 2013) with a resolution of 1 min, and we plotted pairwise genetic distance (Smouse and Peakall 1999) against pairwise geographical distance for each pair of samples. Pairwise Mantel tests were executed in *ade4* (Dray and Dufour 2007). Additionally, a genetic spatial autocorrelation analysis was performed in *GenAlEx*, which can be more sensitive to spatial genetic structure than Mantel tests (Peakall and Smouse 2006).

The optimal number of a posteriori genetic clusters among Alaska harbour porpoises was estimated using a Bayesian clustering approach implemented in *STRUCTURE* (v.2.3.4, Falush, Stephens, and Pritchard 2003; Hubisz et al. 2009; Pritchard, Stephens, and Donnelly 2000). To minimise the effects of sample size variance among regions (Toyama, Crochet, and Leblois 2020; Wang 2017), this analysis included only the largest sampling Locations, represented by sample sizes similar in magnitude (N_BERING $n=12$, COOK $n=17$, COPPER $n=14$, and N_SEAK $n=15$). Within *STRUCTURE*, the LOCPRIOR model was implemented with a burn-in length of 50,000 iterations and 100,000 repetitions for values of K from 1 to 10. Optimum K was

estimated following Evanno et al. 2005. *STRUCTURE* barplots were generated from the mean of five repetitions per *K* value using *CLUMPAK* (Kopelman et al. 2015). A genetic principal component analysis (PCA) was performed in R package *adegenet* v.2.1.2 (Jombart 2008) including samples from all Locations for each of the three geographical stratifications.

Many population genetic analyses assume a representative sample to accurately estimate population allele frequencies, and best practices for estimating the power of a dataset for population assignment suggest splitting the data into 'training' and 'holdout' datasets (Anderson 2008). Unfortunately, this is frequently not possible due to the small sample sizes often representative of cetacean studies and can introduce the possibility of a high-grading bias. Morin, Martien, and Taylor (2009) found that at least 80 SNP loci and large sample sizes were needed to detect genetic structure among populations with low F_{ST} values (i.e., $F_{ST} < 0.005$), a characteristic common to many cetacean species. To examine the power of the GTseq SNP panel (Morin et al. 2021) to assign individuals to their population of origin at the fine-scale Strata-3 level, we estimated the probability of assignment of 1000 simulated individuals per Location ($n \geq 5$) using the leave-one-out approach of Anderson, Waples, and Kalinowski (2008), executed in *rubias* (Anderson 2018) with 1000 MCMC repetitions.

To test the power of SNP loci for inferring pairwise relatedness, we simulated multilocus genotypes for pairs of related individuals in *CKMRsim*. We estimated false negative and false positive rates for full-sibling, half-sibling and parent–offspring pairs compared to unrelated pairs. To estimate geographical patterns of relatedness among Alaska harbour porpoises and estimate whether individuals within geographical strata were more related than expected by chance, we used the relatedness metric of Wang (2002) implemented in the R package, *related* (Pew et al. 2015). The range of dispersal was estimated by testing for the presence of close-kin pairs in our dataset, using *CKMRsim* to calculate log-likelihood ratios between all pairs of individuals. We used an estimated census population size of 80,000 harbour porpoises for all of Alaska (Muto et al. 2020) to set a conservative log-likelihood threshold for false negative rates equal to 10% of the number of possible pairwise comparisons. Relationships are difficult to distinguish without generational information; therefore, we refer to relationships in generalised terms as first- or second-order kin for parent-offspring/full-sibling pairs and half-siblings, respectively.

2.4 | MtDNA Haplotype Diversity

2.4.1 | Marine eDNA Samples

Surface seawater eDNA samples (2–3 L) were collected during July and September 2016 (published in Parsons et al. 2018), and September 2018 through October 2019 in the inshore waters of Southeast Alaska and Western Gulf of Alaska. Sampling efforts were conducted opportunistically and during line-transect surveys in areas of harbour porpoise aggregations. All eDNA samples were collected in the fluke print of an individual harbour porpoise in groups of one or more animals. This targeted sampling approach was used to generate spatially 'tagged' mtDNA sequence data that can be incorporated into traditional population genetic analyses (Adams et al. 2019; Parsons et al. 2018).

Environmental DNA samples may contain genetic material from one or several individuals and estimating the number of genetic contributors is not possible using mtDNA sequence data alone. Therefore, a conservative approach to estimating haplotype frequencies was adopted by counting each unique mtDNA haplotype as a single occurrence for each collection event. eDNA sample processing, DNA extraction and amplicon sequencing protocols follow Parsons et al. (2018). Briefly, eDNA filters (0.45 μm MCE filter membranes) were stored in Longmire's lysis buffer (Longmire, Maltbie, and Baker 1997) and extracted using a modified phenol:chloroform:isopropanol extraction method. Primers Ppho_Con1F-Illumina (TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG TAC TCC TTG AAA AAG CCC ATT GTA) and Ppho_Con7R-Illumina (GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GAT GGT CCT GAA GTA AGA ACC AGA TG; Parsons et al. 2018) were used to amplify 379 bp of the mitochondrial control region from each eDNA sample, followed by a second PCR to tag amplicons from each eDNA sample with unique barcodes using Illumina indexes. Negative controls were included for each set ($n = 24$) of eDNA samples and were carried through the first PCR amplification. If no amplicon was detected, the PCR was not indexed/sequenced. Technical replicates (independent, duplicate PCR replicates) were included for > 10% of samples that successfully amplified. All eDNA amplicons were sequenced on an Illumina MiSeq sequencing platform. All sequence data generated from marine eDNA samples (2016, 2018 and 2019) were quality filtered and processed using the *Anacapa Toolkit Sequence QC and ASV Parsing* module with a quality score of 30 (Curd et al. 2019). ASVs were filtered to retain sequences present in > 2% of read depth per sample as detailed in Parsons et al. (2018). A custom reference database was constructed using all previously generated relevant mitochondrial control region sequences including tissue samples from harbour porpoise, Dall's porpoise and the haplotypes derived from eDNA in Parsons et al. 2018 (Sequence alignment available at github.com/SMay1/AK_Harbor_Porpoise_PopGen). Haplotypes were assigned using *blast* with 98% identity and 90% query coverage following Parsons et al. (2018).

2.4.2 | mtDNA Sequence Diversity and Phylogeography

MtDNA sequences were generated for the control region from tissue samples using primer pair L15926 and H16498 (Rosel, Dizon, and Heyning 1994) according to previously published methods (Chivers et al. 2002; Chivers et al. 2007; Morin et al. 2021) and sequenced on ABI 3500 and 3730 genetic analyzers (Applied Biosystems).

MtDNA haplotypes generated from the 2018/2019 eDNA samples were integrated into the larger dataset comprising haplotype frequencies generated from tissue samples and previously published eDNA data from samples collected in Southeast Alaska in 2016 (Parsons et al. 2018). Control region sequences generated from eDNA samples were assigned to geographical strata (Figure 1) based on collection locations. Prior to combining eDNA sequence data from the two time periods (2016 vs. 2018/2019), genetic differentiation metrics (F_{ST} and Φ_{ST}) were

estimated and compared within and among collection periods. Samples collected in 2019 in Keku Strait, AK ($n = 15$; Figure S1) were geographically intermediate to a priori strata N_SEAK and S_SEAK. Thus, N_SEAK, S_SEAK, and this small subset of SEAK samples were further examined for genetic differentiation using pairwise F_{ST} and Φ_{ST} estimates to evaluate patterns of genetic differentiation among these neighbouring geographical locations (Figure 1, Figure S1). Additionally, data simulations were performed based on observed frequencies of mtDNA haplotypes generated from eDNA and tissue samples to evaluate whether the frequency of control region haplotypes generated from eDNA samples were correlated with those generated from tissue samples. Confidence intervals were generated from 1000 nonparametric bootstraps of each dataset.

Haplotype (h) and nucleotide (π) diversity were estimated for each sampled a priori region (Strata-3) in *pegas*. We assessed the power of mtDNA haplotypes to assign individuals to their population of origin using the leave-one-out approach of Anderson, Waples, and Kalinowski (2008). This simulation was executed in *rubias* using the same methods as with the GTseq SNP data above, except in this case markers were coded as haploid.

The spatial distribution of mtDNA haplotypes and their phylogenetic relationships were visualised using a median joining network constructed in *pegas* (Paradis 2010). To investigate the evolutionary relationships of mtDNA haplotypes, an unweighted pair group method with arithmetic mean (UPGMA) tree and consensus network from 1000 bootstraps were generated using the JC69 model implemented in the package *phangorn* (Schliep 2011). The sequences generated for Alaska harbour porpoises from both tissue and eDNA samples (current study) were examined within the context of previously published harbour porpoise control region haplotypes by expanding the dataset used to generate the UPGMA to include published data (Chivers et al. 2002; Chivers et al. 2007; Crossman, Barrett-Lennard, and Taylor 2014; Morin et al. 2021; Parsons et al. 2018), using the most recently published sequence data for a given sample where haplotypes were updated following re-sequencing (Morin et al. 2021).

2.5 | Hierarchical Spatial Genetic Structure in Alaska Harbour Porpoises

The geographically explicit hierarchical stratification of samples allowed direct evaluation of population differentiation among the represented sampling Locations (Figure 1). Pairwise measures of genetic differentiation for both nuclear SNPs (F_{ST}) and mtDNA sequence data (F_{ST} and Φ_{ST}) were estimated among all a priori strata, at all three levels. Pairwise F_{ST} values were estimated from SNP data using *diveRsity* (Keenan et al. 2013), and the R package *StrataG* (Archer, Adams, and Schneiders 2017) was used to calculate pairwise divergence metrics from control regions sequences. Statistical significance was assessed with 1000 random re-samplings of the data for both datasets. Population structure was further assessed using a hierarchical analysis of molecular variance (AMOVA) to calculate the degree of genetic variation attributed to the spatial clustering of samples within and among a priori strata (Figure 1). The AMOVA was executed in *Arlequin (v.3.5.2.2*, Excoffier and Lischer 2010) for both datasets, using F_{ST} as genetic distance for SNP data and Kimura's (1980) estimate of genetic distance for mtDNA data with 20,000 permutations to assess significance.

3 | Results

3.1 | Natal Philopatry, Intergeneric Hybrids and Population of Origin From GTseq SNP Data

Of the 383 coastwide porpoise samples (including 367 harbour porpoise samples), all individual samples with high quality genotypes clustered with a priori phenotypic species assignments. Evidence of intergeneric hybridisation was detected in the same five individuals from Washington state previously identified by Morin et al. (2021), but no hybrids were identified among the porpoise samples collected in Alaska (see Supporting Information for additional details; Figures S2 and S3). A total of 85 *P. phocoena* samples were collected in Alaska and the Western Bering Sea. After filtering for >25% missing data, 80 individuals and 285 SNP loci were retained. No loci were in significant LD in both the GOA and E_BERING. Two loci deviated significantly from HWE in both of these strata and were removed. We removed 10 loci with minor allele frequencies <0.05, and 29 loci with a difference in observed and expected heterozygosity >0.2. No duplicated individuals were identified, and 243 loci were retained in the final dataset for population genetic analyses.

Pairwise geographic distances based on sample collection locations ranged from 0 to 4153 km. Geographical distance had a slight but significant negative effect on genetic distance in a simple linear regression ($\beta = -0.0015$; adj $R^2 = 0.0111$; $p = < 0.001$; Figure S4a), yet a corresponding Mantel test failed to reveal significant spatial genetic structure ($p = 0.98$; Figure S4b). A genetic spatial autocorrelation analysis detected weak but significant positive spatial autocorrelation on the scale of 1500 km (Figure S4c). The Bayesian *STRUCTURE* analysis indicated $K = 7$, based on the Evanno method, as the most likely number of independent genetic clusters (Figure S5); however, admixture coefficients were evenly distributed among samples, indicating a lack of detectable genetic structure based on SNP genotypes (Figure S5). A genetic PCA generally indicated a lack of detectable population genetic structure based on the existing SNP genotypes at the geographical scales represented herein (Figure S6).

Using the filtered SNP dataset, we established a log-likelihood threshold of 4.7 to obtain a 1.81e-5 false positive rate and 0.0022 false negative rate in the ability to distinguish first-order relatives (parent–offspring and full-sib pairs) from unrelated individuals, demonstrating the substantial power of our SNP panel for relationship inference (Figure S7). Mean pairwise relatedness within fine-scale geographical strata (Strata-3) was significantly higher than expected in seven out of nine sampling Locations ($p < 0.05$; Figure 2A). Within a Location, mean relatedness ($r = 0.151$) was significantly greater than would be expected by chance alone given the observed allele frequencies ($p < 0.001$; Figure 2B).

To test for the presence of close-kin in our dataset, we set a conservative false positive rate (FPR) threshold for pairwise log-likelihood values equal to 20 pairs for first-order kin. Simulations indicated a lack of power to distinguish second-order kin from unrelated pairs (Figure S7). We identified five putative

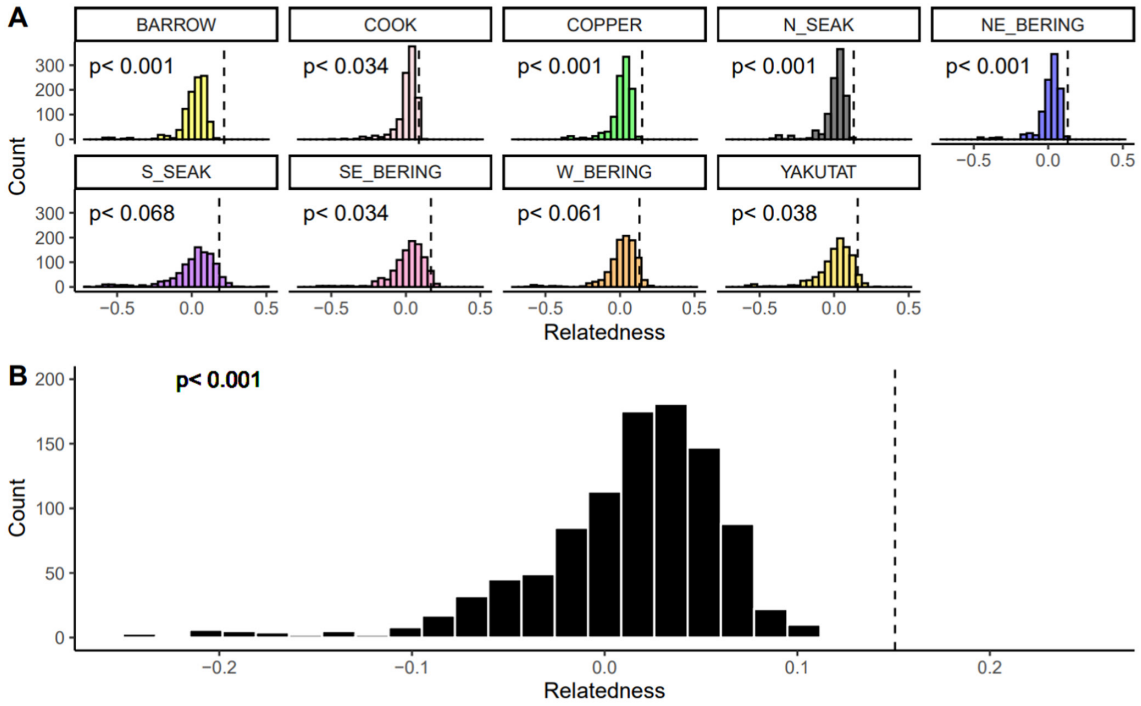

FIGURE 2 | Mean relatedness based on nuclear SNP genotypes (A) within sampling locations, and (B) across all Strata-3 Locations. Mean observed (empirical) relatedness indicated by dashed line, and mean expected relatedness for 1000 random permutations (histogram) represents the null hypothesis of random spatial structure.

first-order-related pairs in our sample of 80 individual harbour porpoises (3120 pairwise comparisons). Two of the close-kin pairs had sequential field ID numbers, indicating they likely stranded at the same time and were potentially mother–calf pairs, although size and age data were unavailable. Of the remaining three close-kin pairs, one pair was from the same sampled population and two pairs were from neighbouring populations, yielding potential estimates for dispersal distances of 175—440 km (Table 1).

3.2 | mtDNA Diversity and Distribution of Haplotypes Among a Priori Strata

3.2.1 | Generating Population Genetic Data From Marine eDNA Samples

Thirty-eight eDNA samples were collected during 2018/2019 field surveys, 37 of which were collected in harbour porpoise fluke prints. Collections were made in the wake of a single porpoise, with the number of porpoises in visual range of the collection location ranging from 1 to 12 animals. Amplicons were successfully generated, cleaned and sequenced from 31 eDNA samples using the Ppho_Con1F-Illumina and Ppho_Con7R-Illumina primers (Parsons et al. 2018), resulting in high-quality sequence data. Technical replicates were sequenced from six of the 31 samples.

MtDNA haplotypes generated from the 2018/2019 eDNA samples were integrated into the larger dataset comprising haplotype frequencies generated from tissue samples (below) and previously published data (Parsons et al. 2018) from eDNA samples collected in Southeast Alaska in 2016 ($n = 154$ total samples, 28 unique haplotypes). Targeted amplicon sequencing of eDNA samples resulted in the detection of eight haplotypes from the 2016 samples and seven haplotypes from the 2018/2019 samples. Three haplotypes (CR01, CR09 and CR22) were common across all years, two of which (CR01 and CR09) were the most frequently occurring and abundant (sequence depth) across all eDNA samples.

The number of mtDNA haplotypes generated from an eDNA sample ranged from one to four haplotypes and was not correlated ($R^2 = 0.0034$, $p = 0.6354$) with the number of porpoises visually detected at the time of sampling (Figure S8). We observed multiple haplotypes in nine (5.49%) eDNA samples. Seven eDNA samples each generated two mtDNA haplotypes. One eDNA sample, processed in duplicate, generated the same three haplotypes in each technical replicate. All but two technical replicates generated the same mtDNA haplotypes in both independent replicates. Amplicon sequence variants from one eDNA sample revealed an additional two haplotypes in the technical replicate. In another eDNA sample, one technical replicate detected only two of the four haplotypes detected in the paired replicate, suggesting that despite positive amplifications, some degree of PCR inhibition may be present in some samples. Additionally, the stochastic nature of amplifying low template copy numbers may result in diminished detections of secondary and tertiary haplotypes represented by very low read count resulting from rare template copy numbers. Simulations comparing the frequency of mtDNA haplotypes generated from eDNA samples to those represented by tissue samples in the same geographic region failed to reveal any bias in the frequency of haplotypes generated from eDNA samples (Figure S9) supporting the integration of sequence data from eDNA and tissue samples into a single unified dataset for population genetic structure analyses (see below).

The 15 eDNA samples collected in Keku Strait in August 2019 exhibited a lack of genetic divergence from other N_SEAK samples

TABLE 1 | Five putative first-order relationships (parent–offspring or full-sibling) within the dataset, with log likelihood ratios estimated using *CKMRsim* on SNP genotypes.

Population of Individual 1	Population of Individual 2	Approximate distance (km)	LL ratio
Cook	Cook	0	32.1
Barrow	Barrow	0	26.6
Copper	Copper	0	22.3
Kodiak	Cook	175	22.3
Cook	Copper	450	20.3

TABLE 2 | Pairwise genetic divergence between eDNA samples collected in 2016 and 2018/2019 (collectively identified in the table as 2019) in both a priori strata within Southeast Alaska. $F_{ST}|\Phi_{ST}$ calculated from mtDNA sequence data. Significance was assessed via 1000 bootstraps per comparison across loci in *StrataG* (mtDNA haplotypes, (Archer, Adams, and Schneiders 2017); entries in bold typeface are significant ($p<0.05$). Sample sizes indicated for each stratum (left).

SEAK	2016_N_SEAK	2016_S_SEAK	2019_N_SEAK
2016_N_SEAK-16			
2016_S_SEAK-16	**0.1710|0.3305**		
2019_N_SEAK-30	0.0019|−0.0461	**0.1612|0.252**	
2019_S_SEAK-4	0.1021|0.2005	−0.1019|−0.1341	0.0398|0.0784

($F_{ST}=0.0452$, $p=0.0920$ and $\Phi_{ST}=0.0528$, $p=0.0920$) and significant differentiation from S_SEAK samples ($F_{ST}=0.0923$, $p=0.0480$ and $\Phi_{ST}=0.1654$, $p=0.0130$) and were subsequently assigned to the northern Southeast Alaska strata (N_SEAK) for population genetic analyses. A comparison of genetic divergence between the two eDNA sampling time periods (2016 vs. 2018/2019) revealed a lack of significant genetic differentiation between mtDNA sequence data generated in each region across the two time periods and, as such, eDNA data from both time periods were integrated into the larger dataset for population genetic analyses (Table 2).

3.2.2 | mtDNA Sequence Diversity and Phylogeography

We identified 31 unique control region haplotypes (389 bp) from all available genetic samples (156 harbour porpoise tissue and eDNA samples) collected in Alaska between 1988 and 2019. Two samples from Kodiak were only included in Strata-1 within the GOA; they were not included as their own group within Stratas-2 or 3, so analyses of these strata comprised 154 samples. Frequency of occurrence of each haplotype across the entire dataset ranged from 1 (0.0064) to 62 (0.3974) samples (Table S1), and the number of unique control region haplotypes per stratum ranged from 1 to 13 (Figure 3, Table S2). Nucleotide diversity within sampling sites varied from 0.000 to 0.008, with greatest haplotype diversity ($h=0.94853$) observed among samples from Cook Inlet (COOK) compared to other strata represented by greater than five samples (Table S2).

A median-joining network created from the mitochondrial control region haplotypes suggests a single group with a star-like phylogeny, with geographical strata represented by multiple haplotypes distributed evenly throughout the phylogenetic network (Figure 3). The most common mtDNA haplotype (CR01) occupied a central position in the network and was identified in all but one (Yakutat) of the sampled geographical locations. A consensus phylogenetic (UPGMA) tree including all eDNA samples and previously sequenced haplotypes from eastern North Pacific harbour porpoises revealed two clades among haplotypes represented by harbour porpoises from both Alaska and California Current ecosystems (Figure S10). All eDNA-derived haplotypes were placed within the larger of the two clades, which included all but two AK haplotypes. The two AK haplotypes in the smaller clade distributed among California Current haplotypes were represented by single occurrences in Locations COOK (CR02) and COPPER (CR52). From the Southeast Alaska inland waters, we found three shared haplotypes between N_SEAK and S_SEAK (CR01, CR09 and CR22). N_SEAK was represented by a greater number of unique haplotypes ($n=10$) than S_SEAK ($n=5$).

3.3 | Spatially Hierarchical Analysis of Genetic Differentiation

Pairwise genetic differentiation among a priori geographical strata for both nuclear SNPs and mtDNA data (Table 3) showed contrasting results. Based on SNP data generated using the GTseq approach, genetic differentiation was low and non-significant (Table S3) for most pairwise comparisons. Significant genetic differentiation ($p<0.05$) was estimated between BARROW and S_SEAK ($F_{ST}=0.0298$), BARROW and NE_BERING ($F_{ST}=0.0095$) and YAKUTAT and SE_BERING ($F_{ST}=0.0312$) despite small sample sizes ($n\leq12$). In contrast, mtDNA sequence data revealed significant (Table S3) genetic

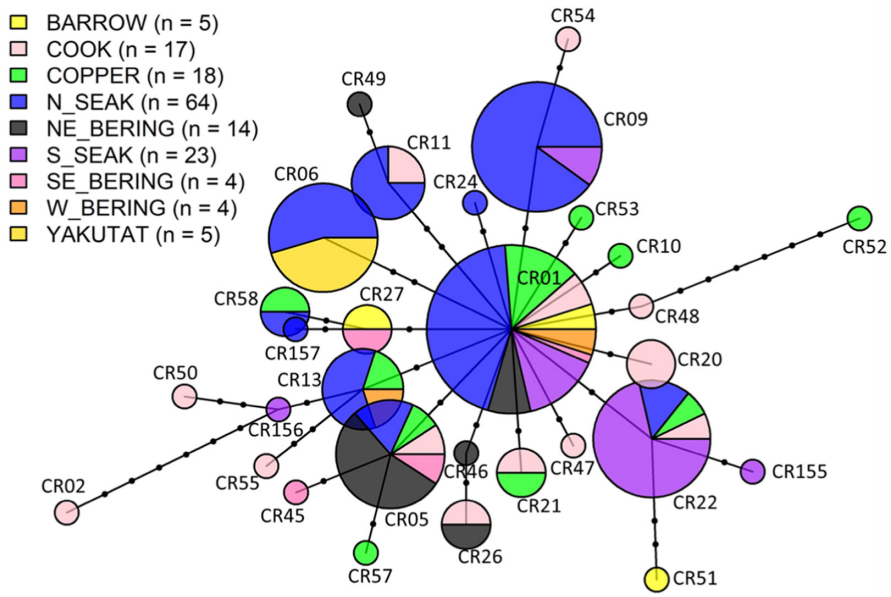

FIGURE 3 | Median-joining network of Alaska harbour porpoise mtDNA control region haplotypes. Node size is proportional to the log of haplotype frequency and colours represent the nine fine-scale a priori geographical strata or locations. Legend numbers indicate the number of samples in each stratum. Note that haplotypes generated from eDNA samples include: CR01, CR05, CR06, CR09, CR11, CR13, CR22, CR24, CR58, CR155, CR156 and CR157 (see also Figure S10).

differentiation between many pairs of geographical strata, including neighbouring strata GOA and E_BERING using both metrics, F_{ST} (0.0809, $p < 0.05$) and Φ_{ST} (0.0723, $p < 0.05$; Table 3).

Western Bering Sea and Barrow strata were not significantly divergent from any other group at the broadest geographical stratification (Strata-1) based on either nuclear or mtDNA—potentially due to small sample sizes in these two strata ($n \leq 7$). Significant mtDNA genetic differentiation was estimated between YAKUTAT and all other a priori geographical strata at both intermediate (Strata-2) and fine-scale (Strata-3) stratification of samples. Among fine-scale geographical strata (Strata-3), patterns of genetic differentiation suggested significant divergence between N_SEAK and all other strata (COOK, COPPER, YAKUTAT and S_SEAK) in the Gulf of Alaska and Southeast Alaska, and S_SEAK was significantly differentiated from all other a priori strata represented by > 7 samples.

The power of our dataset to assign population of origin was generally low (i.e., probability of assignment < 0.95). However, some useful information about population structure may still be inferred from the probability of assignment scores, which varied substantially by geographical stratum and marker type (Figure S11). For example, SNP analyses demonstrated substantial power to assign population of origin in COOK, COPPER and N_SEAK (respective mean, median and SD assignment probabilities = COOK: 0.581, 0.806 and 0.433; COPPER: 0.625, 0.936 and 0.435; N_SEAK: 0.552, 0.717 and 0.437) with large variance in each stratum. In contrast, the power to assign population of origin using mtDNA haplotypes was low or near-zero in COOK and COPPER (mean, median and SD assignment probabilities = COOK: 0.045, 0.009 and 0.115; COPPER: 0.006, 0.002 and 0.013) likely due to the large number of shared haplotypes across strata and absence of unique haplotypes in these strata. The large variance around assignment probabilities reflects the low estimates of genetic differentiation for these strata based on nuclear SNP data (Table 3, Strata-3). However, in BARROW and NE_BERING, assignment based on mtDNA haplotypes was higher than with SNP data, possibly due to the presence of unique haplotypes in these locations. Two strata (YAKUTAT and S_SEAK) were excluded from assignment tests based on SNP data due to small sample sizes. Yet, mtDNA samples helped offset these data gaps, as the power to assign population of origin based on mtDNA for YAKUTAT was high (mean, median and SD assignment probability = 0.918, 0.936 and 0.068) and moderate for BARROW, NE_BERING, N_SEAK and S_SEAK (respective mean, median and SD assignment probability = BARROW: 0.402, 0.409 and 0.214; NE_BERING: 0.494, 0.331 and 0.347; N_SEAK: 0.441, 0.407, 0.288; S_SEAK: 0.529; 0.441;0.338). With the inclusion of eDNA samples in the mtDNA dataset, sample sizes from S_SEAK and N_SEAK were increased by greater than fourfold, substantially improving our ability to assign population of origin based on mtDNA and detect genetic differentiation between S_SEAK, N_SEAK and other sampled Alaska strata.

A hierarchical AMOVA was used to quantify the proportion of genetic variation that can be attributed to each a priori level of population substructure for both the mitochondrial control region sequences and multilocus nuclear SNP genotypes. Patterns of genetic differentiation contrasted greatly between the two marker types. A significant proportion of the genetic variance was explained by the geographic assignment of samples at the Strata-1 and Strata-2 levels (Figure 1) based on nuclear SNPs, but the estimated genetic differentiation was near-zero suggesting significant but subtle population structure (Table 4). In contrast,

TABLE 3 | Pairwise genetic divergence among a priori strata for both F_{ST} (SNP data—lower triangle) and $F_{ST}|\Phi_{ST}$ (mtDNA haplotypes—upper triangle). Sample size indicated for each stratum for nuclear SNPs (left) and mtDNA haplotypes (top) in each sub-table. Significance was assessed via 1000 bootstraps per comparison across loci in *diveRsity* (SNP data, (Keenan et al. 2013) and *StrataG* (mtDNA haplotypes, (Archer, Adams, and Schneiders 2017)); bolded entries are significant ($p < 0.05$).

Strata-1	BARROW-5	E_BERING-18	GOA-129	W_BERING-4
BARROW-7		0.0642|**0.2020**	0.0034|-0.0277	−0.0997|−0.0062
E_BERING-15	0.0090		**0.0809|0.0723**	0.1111|0.0019
GOA-54	0.0036	−0.0008		0.0120|−0.1900
W_BERING-4	0.0071	0.0087	−0.0010	

Strata-2	BARROW-5	E_BERING-18	E_GOA-87	W_BERING-4	W_GOA-35	YAKUTAT-5
BARROW-7		0.0642|**0.2020**	0.0228|0.0652	−0.0997|−0.0062	−0.0220|−0.0339	**0.6500|0.6153**
E_BERING-15	0.0090		**0.1085|0.1871**	0.1111|0.0019	**0.0433**|0.0304	**0.4749|0.7599**
E_GOA-17	0.0052	0.0025		0.0277|−0.0811	**0.0320|0.052**	**0.3989|0.7093**
W_BERING-4	0.0071	0.0087	−0.0016		−0.0097|−0.2323	**0.7810|0.9593**
W_GOA-31	0.0029	−0.0027	0.0027	0.0000		**0.3899|0.3785**
YAKUTAT-3	**0.0227**	0.0135	0.0037	0.0031	0.0037	

Strata-3	BARROW-5	COOK-17	COPPER-18	N_SEAK-64	NE_BERING-14	S_SEAK-23	SE_BERING-4	W_BERING-4	YAKUTAT-5
BARROW-7		0.0176|−0.051	−0.0521|−0.0369	0.0315|0.1412	0.0902|0.1797	0.1044|−0.0198	−0.0526|0.1195	−0.0997|−0.0062	**0.6500|0.6153**
COOK-17	0.0006		0.0187|−0.0581	**0.0662**|0.014	0.0538|0.0032	**0.0838**|0.0044	−0.0466|−0.1072	0.0581|−0.3186	**0.3751|0.3192**
COPPER-14	0.0099	0.0036		0.0400|**0.1201**	0.0654|0.0465	**0.0780|0.1276**	0.0035|−0.0556	−0.0713|−0.1573	**0.4749|0.5013**
N_SEAK-15	0.0034	0.0030	0.0036		**0.1234|0.137**	**0.1094|0.2031**	0.0607|0.1832	0.0322|−0.0623	**0.4025|0.6999**
NE_BERING-12	**0.0095**	−0.0005	−0.0020	0.0036		**0.1877|0.3377**	−0.0444|−0.0532	0.1256|−0.007	**0.5146|0.7515**
S_SEAK-2	**0.0298**	−0.0059	−0.0007	−0.0014	−0.0107		0.1217|**0.3836**	0.1321|0.139	**0.5149|0.8366**
SE_BERING-3	0.0171	−0.0088	0.0059	0.0052	0.0029	0.0106		0.0769|0.1514	**0.5522|0.8982**
W_BERING-4	0.0071	−0.0013	0.0045	−0.0009	0.0085	0.0085	0.0109		**0.7810|0.9593**
YAKUTAT-3	0.0227	0.0002	0.0119	0.0040	0.0099	0.0099	**0.0312**	0.0031	

TABLE 4 | Analyses of molecular variance (AMOVA) based on stratification of samples among nine geographical sampled regions in Alaska, grouped into a 3-level spatial hierarchical stratification (Figure 1). AMOVAs were performed in *Arlequin* using 20,000 permutations and the distance method of Kimura (1980) for the mtDNA control region (*F*, above dashed line) and F_{ST} for the SNP data (below dashed line).

Source of variation	Sum of squares	Variance	% of total variance	*F*	*p*
Among Strata-2	6.071	0.02685	**6.37**	**0.07122**	**<0.001**
Among Strata-3 within Strata-2	2.717	0.02811	**6.67**	**0.13039**	**<0.001**
Within Strata-3	53.153	0.36657	86.96	0.06370	0.153
Among Strata-1	2.362	−0.01405	**−3.39**	**0.11967**	**<0.001**
Among Strata-2 Within Strata-1	3.709	0.05132	**12.37**	**0.08985**	**<0.001**
Within Strata-2	55.870	0.37750	91.01	−0.03387	0.561
Among Strata-2	218.538	0.05726	0.13	−0.**00075**	**0.015**
Among Strata-3 within Strata 2	127.389	−0.09022	−0.21	−0.00206	0.161
Within Strata-3	6363.697	43.85461	100.08	0.00131	0.445
Among Strata-1	127.659	−0.09617	**−0.22**	**−0.00082**	**0.030**
Among Strata-2 Within Strata-1	90.878	0.06006	**0.14**	**0.00137**	**0.043**
Within Strata-2	6491.086	43.85869	100.08	−0.00219	0.703

Note: Bolded entries are significant ($p < 0.05$).

significant genetic differentiation was explained by all three hierarchical sample stratifications using the mtDNA sequence data (Table 4). Most of the genetic variance from mtDNA data was explained by the distribution of samples among Strata-2 regions within Strata-1 (12.37%), a geographically intermediate level of stratification that distinguishes among fine-scale geographical regions within the Gulf of Alaska Stock. Notably, a significant amount of genetic variance was explained by the fine scale stratification of samples among locations, including those within the currently defined Southeast Alaska Stock (YAKUTAT, N_SEAK and S_SEAK).

4 | Discussion

Efforts to characterise harbour porpoise population structure in Alaska have been limited by sample availability, sample quality, genetic resolution and geographical coverage. This study aimed to overcome these limitations by leveraging (1) genetic samples collected over multiple decades; (2) a higher resolution species-specific SNP panel; and (3) mtDNA haplotypes from both tissue and eDNA samples to quantify patterns of genetic differentiation in harbour porpoises throughout Alaska coastal waters. Using a spatially hierarchical approach, we identified significant genetic divergence among geographically defined populations in the Gulf of Alaska and Bering Sea, despite low levels of genetic differentiation. Overall, we observed greater differentiation from mtDNA than nuclear SNP genotypes, suggesting male-mediated gene flow and evidence of natal philopatry based on estimates of relatedness within geographical strata. Importantly, integrating mtDNA haplotypes generated from both eDNA and tissue samples into a population genetic framework revealed significant genetic differentiation within the currently recognised Southeast Alaska management stock, highlighting the potential vulnerability of this small and elusive cetacean to anthropogenic threats.

Previous genetic studies of Alaska harbour porpoise were limited in power due to low sample sizes and limited numbers of genetic markers (e.g., Chivers et al. 2002). In contrast, the SNP panel used in the current study combined with increased numbers of samples maximised our ability to detect patterns of genetic structure. Nuclear SNP genotypes generated from tissue samples indicated weak, but significant positive spatial autocorrelation, albeit with a lack of detectable genetic structure using either a Bayesian clustering approach or genetic PCA. The lack of genetic differentiation observed between some distant geographic regions (e.g., Barrow vs. Gulf of Alaska) likely reflects the poor sample coverage from harbour porpoise around Barrow, AK. High mean pairwise relatedness observed within all but two strata emphasised the genetic divergence between geographic regions and indicated a lack of panmixia for this coastal species. Overall, the high degree of relatedness observed, despite few first-order relatives in the dataset, suggested a degree of natal philopatry among Alaska harbour porpoises. These results may reflect biogeographical barriers, including hiatuses in distribution due to complex seascapes and patterns of coastal productivity, where genetic divergence is higher than expected based on geographic distance alone, as previously described for harbour porpoise populations in the inland waters of southern British Columbia and Washington State (Morin et al. 2021).

Mitochondrial control region genetic diversity was relatively high and consistent with results previously described for this species in Alaska and other regions (Chivers et al. 2002; Morin et al. 2021; Rosel et al. 1999; Tolley and Rosel 2006). One haplotype (CR01) was identified in all but one (Yakutat) strata, and was central within the mtDNA haplotype network, indicating a highly common, ancestral maternal lineage across Alaska. Despite the widespread occurrence of this conserved haplotype, the geographical stratification of samples explained a large proportion of the genetic variance in the mtDNA sequence data, specifically between neighbouring strata in the eastern Bering

Sea and Gulf of Alaska, and within the Gulf of Alaska. Similar patterns of genetic diversity and haplotype occurrence were described by Morin et al. (2021) among West Coast porpoise populations between Washington State and southern California. Significant genetic divergence was evident from the mtDNA dataset across all three hierarchical levels of geographic stratification. These mtDNA results indicated genetic structuring within Alaska harbour porpoise despite the lack of genetic differentiation suggested by the nuclear SNP data. Together, we conclude that the maternally inherited mtDNA genetic variation of Alaska harbour porpoise are geographically structured on a finer scale than previously known (i.e., Chivers et al. 2002).

Despite using a SNP panel previously demonstrated to be adequate for detecting population structure among other harbour porpoise populations (Morin et al. 2021), genetic differentiation based on nuclear SNPs was generally low and lacked statistical significance among the a priori geographical strata. However, we found greater pairwise relatedness within sampled regions than expected by chance alone, and significant genetic divergence based on mtDNA sequence data. Together, both the nuclear SNP and mtDNA data indicate a lack of panmixia among Alaska harbour porpoises and the stronger mtDNA differentiation suggests sex differences in dispersal. Sex-biased dispersal (and consequently gene flow) are often reflected in contrasting patterns of genetic divergence between maternally inherited mtDNA and biparentally inherited nuclear loci (Prugnolle and de Meeus 2002). As a result of the maternal mode of inheritance, patterns of mitochondrial differentiation are particularly useful for assessing demographic independence. Female philopatry to either social groups or natal areas is often inferred where metrics of mtDNA genetic differentiation exceed those estimated from nuclear markers (e.g., Durante et al. 2022; Kerth, Mayer, and Petit 2002; Martien et al. 2014; Roycroft, Le Port, and Lavery 2019). Here, we see evidence suggesting that female natal philopatry may be driving population genetic structure among Alaska harbour porpoises based on contrasting patterns of genetic divergence from nuclear SNPs and mitochondrial control region sequences. Previous studies of harbour porpoise in the northwest Atlantic (Rosel et al. 1999), as well as the North Sea, Norwegian waters and Baltic Sea have described similar patterns of sex-biased genetic divergence (Tolley et al. 1999; Wiemann et al. 2010) supporting the hypothesis that female porpoises exhibit a higher degree of philopatry. Due to the limited sample sizes in many Alaska regions, stratifying analyses by sex was not possible. Although dispersal distances for this species were previously unknown, our genetic relatedness analyses identified putative pairs of first-order kin sampled between 170 and 450 km apart, providing some insight into possible dispersal distances. However, evidence of natal philopatry within this species as well as support for geographic genetic divergence provide evidence for genetic differentiation at a scale that warrants revisiting currently recognised management stock boundaries in Southeast Alaska.

Generating mitochondrial control region haplotypes from targeted eDNA samples in Southeast Alaska was key for examining population differentiation in a region where the number of genetic samples was previously prohibitively small. Both AMOVA and pairwise F_{ST} and Φ_{ST} indicated significant genetic differentiation within Southeast Alaska inland waters between N_SEAK and S_SEAK, indicating genetic structuring within a population that is currently managed as a single stock (Muto et al. 2021). The existence of two independent populations of harbour porpoise in this region was first suggested by Dahlheim et al. (2015) on the basis of markedly different demographic trends in abundance in the northern and southern inland waters of Southeast AK. This hypothesis was further supported by mtDNA haplotype data generated from eDNA samples by Parsons et al. (2018). Zerbini, Goetz, et al. (2022) and Zerbini, Parsons, et al. (2022) summarised harbour porpoise survey data dating back to the 1990s, identifying several regions in Southeast Alaska where the species does not regularly occur, suggesting distributional hiatuses and possible spatial boundaries in the inland waters. The patchy distribution of samples throughout geographically defined strata is unavoidable, and reflects the areas of high porpoise density encountered during 2016–2019 surveys. Additional survey efforts in future years are needed to work towards more even geographical sample coverage to further refine putative population genetic boundaries. However, despite the potential benefits that would be gained from additional samples in terms of power and geographical coverage, the genetic patterns described here in the context of divergent demographic trends and marked spatial distributions provide strong evidence for the presence of multiple demographically independent biological populations within a currently recognised Southeast Alaska management stock.

Importantly, we demonstrate that eDNA offers a valuable avenue for non-invasively generating data useful for some population genetic analyses, which historically relied on tissue samples. Collecting water in the fluke prints of porpoises offers a unique opportunity to supplement tissue sampling efforts which are severely restricted due to the species' small size and elusive nature. By using primers designed to amplify an informative section of the mitochondrial control region, eDNA-derived mtDNA haplotypes offered a valuable opportunity to supplement existing datasets, thus increasing overall sample sizes, strengthening estimates of genetic diversity and enhancing the power to detect population genetic structure (Dugal et al. 2022; Parsons et al. 2018; Sigsgaard et al. 2016). Furthermore, eDNA samples proved valuable for capturing additional genetic diversity (i.e., novel haplotypes) that were not previously detected among tissue samples despite many years of collection and dedicated resources. Recent discussions about the potential of eDNA as a population genetics tool have focused on both the scientific potential and limitations of these new approaches (Adams et al. 2019; Andres et al. 2023; Couton, Viard, and Altermatt 2023; Sigsgaard et al. 2020; Zanovello et al. 2023). Leveraging previously validated and optimised species-specific primers (Parsons et al. 2018), and published harbour porpoise control region reference haplotypes (Chivers et al. 2002; Morin et al. 2021; Parsons et al. 2018) provided prior knowledge of both the distribution of SNPs across the target amplicon and haplotype diversity, creating a valuable foundation for expanding datasets using eDNA samples.

The use of eDNA for population genetics using mtDNA amplicon sequence data can be limited by the inability to estimate the number of genetic contributors in each sample. In the present study, eDNA samples were collected in the wake (or 'fluke print') of harbour porpoise(s) with the number of harbour porpoises ranging from single individuals to

dispersed groups of 15 porpoises within visual range at the time of sampling. Extrapolating from observed numbers of target individuals to the likely number of genetic contributors 'captured' in an eDNA sample is challenging due to the uncertainties around both biotic and abiotic elements affecting the shedding, transport and decay of eDNA in the marine environment (Andruszkiewicz et al. 2019; Harrison, Sunday, and Rogers 2019; Hinz et al. 2022; Lamb et al. 2022). Accurately estimating the number of individuals contributing genetic material to an eDNA or mixture sample is an area of active research that warrants additional studies to maximise the information gained from eDNA samples for population genetics. Further development validating the use of data generated from trace amounts of DNA using multiple, highly variable genetic markers (e.g., SNPs or STR loci) to estimate the number of individuals in each eDNA sample is needed (Andres et al. 2023; Shi et al. 2023).

Here, using data limited to mtDNA control region sequences, we adopted stringent quality controls to minimise potential effects of PCR and sequencing errors and a conservative approach to estimate genetic diversity from eDNA samples. We employed an approach similar to that in Parsons et al. (2018) including rigorous bioinformatic thresholds (e.g., sequencing depth, non-template controls) to ensure accurate haplotype assignments, an important consideration in species with high haplotypic diversity where haplotypes frequently differ by only a single nucleotide. Assigning a frequency of one to each haplotype detected in each eDNA sample addressed uncertainties around the number of unique porpoises represented in each eDNA sample. Nine eDNA samples collected in Southeast Alaska generated multiple unique haplotypes, highlighting the potential of capturing genetic material from multiple individuals in a single, 3 L water sample. However, the number of haplotypes resolved was not correlated with the number of porpoises observed at the time of sampling. Here, eDNA samples collected in the presence of multiple porpoises generated a single mtDNA haplotype and concomitantly, multiple haplotypes were generated from several eDNA samples where metadata indicated that only a single porpoise was visually detected. This lack of correlation is unsurprising considering the skewed haplotype frequencies typical for this species and our limited understanding of the fate and transport of eDNA in coastal marine systems (e.g., Andruszkiewicz et al. 2019; Harrison, Sunday, and Rogers 2019). These results suggest the likely persistence and/or transport of cellular debris from porpoises unobserved at the time of sampling and reinforces the need to better understand the myriad of factors contributing to the likelihood of capturing (and detecting) genetic material in marine eDNA samples. While true independence of eDNA samples cannot be confirmed in the absence of nuclear genotypes or allelic frequencies, the correlated mtDNA haplotype frequencies from eDNA and tissue samples suggest that the genetic data generated from eDNA samples was not heavily biased based on the approach adopted herein where each ASV is counted once regardless of the number of visually detected animals. In the absence of a validated approach for estimating the number of unique genetic contributors to each eDNA sample, we argue that assigning a frequency of one to each unique haplotype is the most conservative and unbiased approach for integrating eDNA haplotypes into a population genetic framework.

Without the ability to enumerate the number of porpoises contributing genetic material to each sample, duplicate samples from the same individual cannot be readily identified and excluded on the basis of genetic data alone. However, species behaviour likely plays an important role in determining the likelihood of genetic recaptures in eDNA samples. Unlike some cetaceans that are attracted to vessel presence, behavioural studies indicate that harbour porpoises are frequently displaced by vessel and anthropogenic activities, exhibiting interrupted foraging in some instances (Dyndo et al. 2015; Oakley, Williams, and Thomas 2017; Wisniewska et al. 2018). In general, harbour porpoises can be elusive and challenging to approach, thereby reducing the chance of resampling individuals. Additionally, satellite and VHF tagging studies have demonstrated that harbour porpoises are capable of long-distance movements, while also exhibiting a degree of site fidelity to regions within which short-term, large-scale movements may be associated with tidal fronts or seasonal prey movements (Hanson 2007; Read and Westgate 1997). The behavioural and ranging patterns exhibited by harbour porpoises likely reduce the probability of resampling the same individuals in sequential sampling events clustered in space and/or time, minimising genetic recaptures. Combined with relatively high haplotypic diversity and a method based on presence/absence of haplotypes in each sample, the rate of type 1 error is expected to be low, allowing us to integrate eDNA data into a population genetic framework and reliably detect significant genetic differentiation among harbour porpoises in Alaska.

4.1 | Management Implications

Both globally and locally, harbour porpoise populations are vulnerable to mortality in gillnet fisheries (Barlow and Hanan 1995; Helker et al. 2015; Manly 2015; Read et al. 1993; Reeves, McClellan, and Werner 2013; Tregenza et al. 1997; Trippel et al. 1996). Defining both population size and population structure are key requisites to accurately assess the impacts of anthropogenic activities on local harbour porpoise populations (e.g., Reeves, McClellan, and Werner 2013). Genetic and genomic data are frequently used to provide important lines of evidence for identifying demographically independent populations and delineating management stocks (Martien et al. 2019). Here, the observed patterns of genetic relatedness among porpoises within geographical strata suggest natal philopatry and limited gene flow among harbour porpoises throughout coastal Alaska, and genetic differentiation among geographical strata indicate patterns consistent with significant mtDNA genetic divergence indicating demographic independence despite inferred male-mediated gene flow.

A recent review (Zerbini, Goetz, et al. 2022; Zerbini, Parsons, et al. 2022) summarising multiple lines of evidence suggests that porpoises within N_SEAK and S_SEAK qualify as demographically independent populations (DIP; Martien et al. 2019) on the basis of a distributional hiatus, genetic divergence and contrasting trends in abundance. Survey data collected throughout the inland waters of Southeast Alaska (Dahlheim et al. 2015; Zerbini, Parsons, et al. 2022) indicate that harbour porpoises are not uniformly distributed throughout the region, suggesting a distributional hiatus between the two geographical strata (N_SEAK and S_SEAK). The

proposed geographical boundary between the two Locations is reflected in the a priori SEAK strata in the current study. Using data from multiple genetic markers and samples collected over multiple decades, the patterns of genetic divergence presented support a population boundary between the two DIPs. While additional sampling efforts, particularly in S_SEAK, may increase the power to resolve fine-scale boundaries between the two strata and more comprehensively capture the genetic diversity of this location, the existing data, together with demographic trends and distribution patterns, support delineating stock boundaries for harbour porpoise within Southeast Alaska. Estimates of incidental bycatch in salmon drift gillnet fisheries indicate a potential conservation concern for harbour porpoise in the S_SEAK population based on observations of mortality and serious injury (Helker et al. 2015; Manly 2015; Young et al. 2022). The collection of additional genetic data from harbour porpoises in Yakutat, AK may also help to resolve the limited samples available for that region and provide greater insight into the patterns of genetic distinctness suggested. Designating stocks to reflect demographically independent populations is critical for estimating minimum population size and the potential of biological removal (PBR), a key parameter for evaluating the impact of bycatch or other anthropogenic sources of mortality under the Marine Mammal Protection Act (Wade 1998). The patterns of genetic divergence presented here represent an important contribution to the refinement of stock boundaries and to effective long-term management of this sentinel, coastal cetacean in Alaska.

Author Contributions

K.M.P. and P.A.M. conceived of and designed the larger research project, and K.M.P., M.D. and L.P. designed and executed the eDNA study. K.M.P., S.A.M., Z.G. and P.A.M. performed analyses and wrote the manuscript. M.D., K.G. and A.N.Z. designed and conducted the vessel surveys in southeast Alaska. J.M.S., C.G. and J.R.M. lead efforts to collect eDNA samples opportunistically in southeast Alaska. All authors contributed to the preparation and review of this manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data and scripts used in this study are publicly available at github.com/SMay1/AK_Harbor_Porpoise_PopGen.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.