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Integrating genetic data and population viability analyses for the identification of harbour seal (*Phoca vitulina*) populations and management units

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Abstract

Identification of populations and management units is an essential step in the study of natural systems. Still, there is limited consensus regarding how to define populations and management units, and whether genetic methods allow for inference at the relevant spatial and temporal scale. Here, we present a novel approach, integrating genetic, life history and demographic data to identify populations and management units in southern Scandinavian harbour seals. First, 15 microsatellite markers and model- and distance-based genetic clustering methods were used to determine the population genetic structure in harbour seals. Second, we used harbour seal demographic and life history data to conduct population viability analyses (PVAs) in the VORTEX simulation model in order to determine whether the inferred genetic units could be classified as management units according to Lowe and Allendorf's (Molecular Ecology, 19, 2010, 3038) 'population viability criterion' for demographic independence. The genetic analyses revealed fine-scale population structuring in southern Scandinavian harbour seals and pointed to the existence of several genetic units. The PVAs indicated that the census population size of each of these genetic units was sufficiently large for long-term population viability, and hence that the units could be classified as demographically independent management units. Our study suggests that population genetic inference can offer the same degree of temporal and spatial resolution as 'nongenetic' methods and that the combined use of genetic data and PVAs constitutes a promising approach for delineating populations and management units.

Keywords: demographic independence, management units, microsatellites, minimum viable population size

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Introduction

Identification of populations is a fundamental aspect in the study of biological systems. From an evolutionary perspective, understanding population structure is essential for the inference of a species' evolutionary history and investigating how selective forces may drive local adaptation. In management and conservation, the identification of populations is paramount for delineating management units and assessing the effects of harvesting, pathogens, interspecific competition, habitat fragmentation and climate change.

In marine organisms, assessment of population structuring and delineation of management units is challenging because these organisms typically have a large

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potential for dispersal and wide, seemingly continuous distributions (Bérubé *et al.* 1998; Iacchei *et al.* 2013; Wallace *et al.* 2010; White *et al.* 2011). This is particularly true for many marine mammals (e.g. whales and seals) where assessments may be further hampered by their large body sizes, low densities and/or complex life histories, as well as ethical issues. Valuable insights can be obtained from tagging and tracking (Block *et al.* 2011), photo-ID (Katona & Whitehead 1981; Smith *et al.* 1999), trace element analyses (Kelly 2000), morphometrics (Galatius *et al.* 2012) and acoustics (Bjorgesaeter *et al.* 2004; Delarue *et al.* 2009), but such approaches to population identification are typically time- and resource-consuming and may be logistically and technically complex.

For these reasons, identification of populations and delineation of management units in marine mammals – and many other organisms – has relied heavily on estimates of genetic variation and differentiation (Andersen *et al.* 1998; Baker *et al.* 1993; Davis *et al.* 2008; Foote *et al.* 2010; Nielsen *et al.* 2012). However, genetic approaches to identify populations and delineate management units are not without their limitations (Hedrick 1999; Kool *et al.* 2013; Lowe & Allendorf 2010; Palsbøll *et al.* 2007, 2010; Waples 1998; Waples & Gaggiotti 2006). For example, traditional population genetic analyses typically apply to evolutionary timescales and may not inform about contemporary processes of relevance to management (Palsbøll *et al.* 2013; Pearse & Crandall 2004). Thus, several authors have advocated a shift in the use and interpretation of genetic data for the identification of populations – particularly when using these to define management units (Hedrick 1999; Lowe & Allendorf 2010; Palsbøll *et al.* 2007; Waples 1998; Waples & Gaggiotti 2006). Specifically, they suggest that populations and management units should be defined by demographics rather than by genetics. That is, they should reflect demographically independent units where population dynamics are determined by local birth and mortality rates rather than immigration from neighbouring populations.

However, despite the great appeal and gradual adoption of this definition, the most appropriate path for delineating such demographically independent units remains unclear. In particular, two questions warrant consideration: (i) can current genetic methods detect population structuring at the temporal and spatial scale relevant for management? and (ii) what is the most appropriate approach for evaluating whether these genetically identified populations should be treated as separate management units or not?

The harbour seal population of southern Scandinavian comprises a well-suited model system for addressing these questions. Several decades of research have resulted in a large amount of 'nongenetic' data on movement patterns (Dietz *et al.* 2012b, 2003; Härkönen & Harding 2001; Teilmann *et al.* 2010; R. Dietz unpublished), disease epidemiology (Härkönen *et al.* 2006), population dynamics (Heide-Jørgensen & Härkönen 1988; Olsen *et al.* 2010), contaminant loads (Dietz *et al.* 2012a) and habitat characteristics (Heide-Jørgensen & Härkönen 1988; Jepsen 2005; Olsen *et al.* 2010), allowing for a solid comparison with results based on genetic methods. Moreover, while the harbour seal is one of the best-studied marine mammals, central questions regarding population structuring and delineation of management units remain unanswered. As would be expected for a marine mammal with few physical barriers to dispersal, genetic studies of harbour seals typically report about genetic differentiation over scales of several hundred kilometres (Andersen *et al.* 2011; Burg *et al.* 1999; Goodman 1998; Lamont *et al.* 1996; Stanley *et al.* 1996; Westlake & O'Corry-Crowe 2002), suggesting frequent gene flow among haul-out sites and the existence of relatively large populations. In contrast, tagging studies have revealed that, although harbour seals may undertake long-distance foraging trips, the range and duration of movements decrease substantially during the mating season (Bjørge *et al.* 2002a,b; Dietz *et al.* 2012b; Härkönen & Harding 2001; Small *et al.* 2005; Teilmann *et al.* 2010; Thompson *et al.* 1996; Tollit *et al.* 1998), indicative of population structuring at much finer scales than has been reported in genetic studies. Resolving this discrepancy is not only of interest for understanding harbour seal biology, but also for other species in which genetic and nongenetic methods have yielded contrasting results.

The purpose of our study was threefold: (i) to apply model- and distance-based genetic methods to identify genetic units within the harbour seal study population, (ii) to discuss and compare these units with information from nongenetic studies to assess whether genetic methods can provide inference at the spatial and temporal scale relevant for management and (iii) to examine the utility of two approaches for determining whether the inferred harbour seal units can be classified as demographically independent and thus may be appointed the status of management units: first, a novel approach using population viability analysis (PVA) and the 'population viability criterion' of Lowe and Allendorf (2010), and then, a previously described approach based on genetic migrant detection and the '10% migration criterion' discussed by Waples and Gaggiotti (2006).

Materials and methods

Sampling and experimental conditions

Tissue samples from 259 harbour seals were gathered from 12 well-defined and closely spaced haul-out sites

Fig. 1 The study area and 12 sampling localities (filled circles) used for the present study, as well as other unsampled haul-out sites within the region (open circles). Abbreviations refer to haul-out sites listed in Table 1.

in southern Scandinavia (Fig. 1; Table 1). Total genomic DNA was extracted using a modified CTAB method with Proteinase K (Andersen *et al.* 2004) and each individual genotyped at fifteen previously developed and published polymorphic microsatellite markers (Table S1, Supporting Information). PCRs were performed in a total volume of 10 μL, including 1–2 μL genomic DNA, 0.5 μM of each primer, 5 μM dNTPs, 0.5 U Taq Polymerase, 1 mM $MgCl_2$ and 10x DNA polymerase buffer with 10 mM Tris-HCl, 10 mM KCl and 0.1% Triton X-100. PCR amplifications were initialized with 3-min of denaturation at 95 °C followed by 35 cycles with 45-s denaturation at 94 °C, 45-s annealing at 47–55°C depending on primers and 20-s extension at 72 °C (Table S1, Supporting Information). Finally, 10-min extension at 72 °C ended each reaction. PCR products were electrophoresed on a polyacrylamide gel in an ABI PRISM 377 DNA Sequencer. Presence of small allele dominance, stuttering and null alleles were tested using the program MICROCHECKER v. 2.2.3 (van Oosterhout *et al.* 2004). This program was also used to estimate the frequency of null alleles.

Hardy–Weinberg proportions, genotypic linkage disequilibrium and genetic diversity

The degree of heterozygosity was assessed across loci and sampling localities by F_{IS} (Weir & Cockerham 1984) and deviations from Hardy–Weinberg proportions tested using 10 000 randomizations. Tests for genotypic linkage disequilibrium were performed with 1 000 000 iterations, and significance of *P*-values was assessed by applying the sequential Bonferroni correction to adjust for the effect of multiple tests (Rice 1989). Further, we estimated expected heterozygosity H_E, the number of sampled alleles N_A, the number of unique alleles (N_U) and allele frequencies. All estimates and tests were performed in FSTAT ver. 2.9.3.2 (Goudet 1995) or in the R package HIERFSTAT ver. 0.04-10 (Goudet 2005).

Table 1 Characteristics of the 12 sampling sites in southern Scandinavia

Name	Locality	*n*	N_{2000}	N_A	N_A/n	A_R	N_U	H_O	H_E	F_{IS}
Skagerrak-Kattegat-western Baltic										
VAE	Väderöarna	25	1698	54	2.16	3.35	1	0.512	0.517	0.009
TIS	Tistlarna	16	2293	47	2.94	3.10	1	0.488	0.491	0.007
LAE	Læsø	14	1453	46	3.29	3.07	0	0.505	0.486	−0.039
ANH	Anholt	17	1488	49	2.88	3.15	1	0.451	0.450	−0.003
HES	Hesselø	14	1330	49	3.50	3.27	0	0.424	0.453	0.065
HAL	Hallands Väderö	18	1190	56	3.11	3.55	1	0.515	0.496	−0.037
SAM	Samsø	31	1488	54	1.74	3.21	1	0.438	0.435	−0.006
MAA	Måkläppen	26	158	50	1.92	3.15	0	0.480	0.451	−0.063
ROE	Rødsand	15	280	54	3.60	3.54	2	0.484	0.501	0.034
Limfjorden-Wadden Sea										
LIE	Eastern Limfjorden	28	1278	43	1.54	2.70	1	0.470	0.454	−0.035
LIW	Western Limfjorden	27	210	46	1.70	2.79	0	0.474	0.455	−0.042
WAD	Wadden Sea	28	3754	45	1.61	2.71	2	0.381	0.407	0.064

n, number of samples; N_{2000}, abundance in year 2000; N_A, number of alleles; N_A/n, the ratio between alleles and samples; A_R, allelic richness; N_U, number of unique (private) alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, Weir and Cockerham's (1984) measure of correlation of genes within individuals within populations (inbreeding coefficient) in a sample site. None of the F_{IS} estimates were significant at $P < 0.05$.

Model-based genetic clustering approaches

Inference from model-based clustering approaches may be biased in data sets characterized by isolation by distance (IBD) (Frantz *et al.* 2009; Guillot *et al.* 2005). Thus, prior to the cluster analyses, we explored the potential relationship between genetic and geographical distance by regressing the pairwise values of $\theta/(1-\theta)$ (Rousset 1997) estimated in FSTAT v. 2.9.3.2 (Goudet 1995) against the shortest waterway distance between haul-out sites calculated in GoogleMaps (©2013 Google). The strength and significance of the correlation between geographical and genetic distance were estimated using a simple Mantel test with 10 000 iterations (Mantel 1967) as implemented in the R package *ncf* version 1.1-4 (Bjornstad 2012).

Following the IBD analysis, the number of genetic units in southern Scandinavian harbour seals was inferred using STRUCTURE ver. 2.3.1 (Hubisz *et al.* 2009; Pritchard *et al.* 2000) and GENELAND ver. 3.1.4 (Guillot *et al.* 2005). Analyses in STRUCTURE were performed under the admixture model, using the model of correlated allele frequencies between clusters and locations as priors. For each value of K from 1–10, ten runs were performed, each with 100 000 initial steps of burn-in followed by 1 000 000 iterations. As our data set was characterized by some degree of IBD (see results), analyses were conducted for the entire data set and for the two geographically defined regions: the Limfjorden–Wadden Sea and the Skagerrak–Kattegat–western Baltic region separately. Output data were processed in STRUCTURE HARVESTER (Earl 2009) and CLUMPP (Jakobsson & Rosenberg 2007) and graphically displayed using DISTRUCT (Rosenberg 2004). As inference of the number of clusters K can be difficult under scenarios of extensive admixture and IBD (Falush *et al.* 2003; Pritchard *et al.* 2000), we applied Evanno's ΔK as an additional predictor of K (Evanno *et al.* 2005).

Analyses in GENELAND were performed under the spatial model assuming correlated frequencies. Inference of population structuring was based on 10 independent runs, each allowing the number of populations to vary between 1 and 10. Each run consisted of 1 000 000 MCMC iterations with a thinning interval of 100. The maximum rate of Poisson processes and maximum number of nuclei were set at 100 and 300, respectively. The spatial coordinates of each sampling site were obtained for each sampling site using GoogleMaps (©2013 Google) and expressed in degrees latitude and longitude. Data were postprocessed with a burn-in of 1000 and the number of pixels in the spatial domain set at 300 times 500. The analyses in GENELAND were performed on the entire data set, as well as on the two subsets as described above.

Distance-based genetic clustering approaches

The genetic distance among individuals and sampling localities was inferred by estimation of the Cavalli-Sforza and Edwards' (1967) genetic chord distance. Unrooted trees were estimated using the neighbour-joining algorithm, and support for tree nodes was assessed by bootstrapping across loci (10 000 iterations). Calculations and bootstrapping were performed in POPULATIONS ver. 1.2.30 (Langella 1999), and the resulting trees were graphically displayed in FIGTREE ver. 1.4.0 (Rambaut 2012).

In addition, the summary statistic θ, Weir & Cockerham's (1984) unbiased estimator of F_{ST}, was estimated for comparing levels of genetic differentiation within southern Scandinavia with previous genetic studies on harbour seals and pinnipeds in general. As there is some debate with regard to the applicability of F_{ST} (Gerlach *et al.* 2010; Heller & Siegismund 2009; Jost 2009; Ryman & Leimar 2009; Whitlock 2011), we included Jost's (2008) D_{est} as an additional estimate of genetic differentiation. Calculations were performed in the programs FSTAT v. 2.9.3.2 (Goudet 1995) and DEMETICS (Gerlach *et al.* 2010), and significance was determined by 95% bootstrap confidence intervals (1000 iterations).

Demographic independence

Given our focus on conservation and management, a genetic unit was defined as demographically independent when elimination of dispersal did not result in decreased population viability and local extinction (Lowe & Allendorf 2010). To assess whether the identified units satisfied this 'population viability criterion', we first used demographic simulations to determine the threshold carrying capacity of a population (minimum viable population size, MVP_K) with no immigration and emigration where the probability of persistence (PP) is >95% or >99% (Reed *et al.* 2003). Next, this MVP_K was compared to the census population size estimates in year 2000 (N_{2000}) for the inferred genetic units. Our rationale was that if the census estimates N_{2000} were above the estimated MVP_K, then the units were likely to be viable and long-term persistent, satisfied our criterion for demographic independence and thus could be classified as separate management units.

Census population size estimates (N_{2000}) were obtained from aerial surveys carried out 2 years prior to genetic sampling. Seals were counted on the haul-out sites during the breeding and moulting period, and subsequent adjustments were made for animals not hauling during the time of survey (Heide-Jørgensen & Härkönen 1988; Härkönen & Harding 2001; Olsen *et al.* 2010). Demographic simulations were performed in the

individual-based, age-structured population simulation model VORTEX ver. 9.99b (Lacy *et al.* 2009) using input parameters from a long list of harbour seal studies (Coltman *et al.* 1998; Hayes *et al.* 2006; Härkönen *et al.* 2006, 2007, 2005, 2002; Härkönen & Heide-Jørgensen 1990) (Table S2, Supporting Information). VORTEX can include carrying capacity, density dependence, demographic and environmental stochasticity, catastrophes and inbreeding depression. Populations were modelled for 50 or 200 years, with 1000 iterations, 10% environmental variation in carrying capacity N_K, and an initial population size N_0 equal to N_K. The populations were defined as extinct when their size was 10% of N_K. Further, we assumed a stable age distribution (Härkönen & Heide-Jørgensen 1990), a conservative scenario with catastrophic declines caused by phocine distemper virus (PDV) epidemics at 14-year intervals (Härkönen *et al.* 2006), and no inbreeding depression, because this appears to have relatively small effects on extinction probability relative to demographic and environmental stochasticity (Lande 1988).

In addition, we assessed the utility of the '10% migration criterion' proposed by Waples and Gaggiotti (2006) as a preliminary criterion for demographic independence. Contemporary migration rates among putative genetic units were estimated as the number of migrant individuals in the sample using the 'detection of first-generation migrants' option in GENECLASS2 (Piry *et al.* 2004). The model uses the Bayesian computation criteria of Rannala and Mountain (1997) to estimate the likelihood of each individual genotype within the unit where the individual was sampled $L = L_{HOME}/L_{MAX}$ (Paetkau *et al.* 2004), and classify individuals as migrants if they have a higher likelihood of belonging to another unit than the one they were sampled in. Significance was assessed by a Monte Carlo resampling algorithm simulating 10 000 individuals (Paetkau *et al.* 2004).

Results

Sampling and experimental conditions

Complete genotypes were obtained for all but two samples, corresponding to a genotype scoring success above 99.9%. As previously reported by Gemmell *et al.* (1997), the BG microsatellite amplified two loci: one with allele size range 253–255 bp and one with range 278–308 bp (Table S1, Supporting Information). The former of these two loci proved difficult to score consistently and was omitted from further analysis. The test for null alleles (van Oosterhout *et al.* 2004) indicated the presence of null alleles in 12 of 180 locus–sampling sites combinations (i.e. 12 sites × 15 microsatellites). By chance alone, nine loci would be expected to contain null alleles (i.e. 5% of 180 locus–locality combinations), indicating that the probability of null alleles biasing the analyses in the present sample was low.

Hardy–Weinberg proportions, genotypic linkage disequilibrium and genetic diversity

Three loci (Lc26, SGPV11 and TBPV2) had larger-than-expected F_{IS} values across sampling sites indicative of null allele, allelic dropout, selection against heterozygotes, inbreeding and/or Wahlund effect (Table S1, Supporting Information). Further testing of these three loci revealed that deviations only occurred within the Kattegat region, suggesting that the observed heterozygote deficiency may be due to a Wahlund effect. Indeed, several of our other analyses indicated substructuring within this region (see below). No significant deviations from genotypic linkage equilibrium were detected, indicating that the loci can be considered statistically independent (data not shown). A total of 77 alleles were observed of which ten were unique to one of the 12 haul-out sites (Table 1). In particular, the Rødsand (ROE) haul-out site had relatively high frequencies of unique alleles at loci SGPV2 (allele 161; 10%) and Hg8.10 (allele 189; 13%). Overall observed heterozygosity was moderate $H_O = 0.47$, with estimates ranging between $H_O = 0.38$ in the Wadden Sea (WAD) and $H_O = 0.52$ at Väderöarna (VAE) in Skagerrak and Hallands Väderö (HAL) in Kattegat, pointing towards lower genetic diversity in Wadden Sea–Limfjorden than in Skagerrak–Kattegat–western Baltic.

Model-based genetic clustering approaches

We observed a significant correlation between geographical distance and genetic differentiation (θ), indicating an IBD effect across haul-out sites in southern Scandinavia (Mantel's $Z = 0.689$, $P = 0.0009$) (Fig. 2A). However, this trend was mainly driven by the genetic differentiation among sites in the Skagerrak–Kattegat–western Baltic and Limfjorden–Wadden Sea region, respectively. When these regions were analysed separately, the correlation was weaker and nonsignificant for Skagerrak–Kattegat–western Baltic (Mantel's $Z = 0.316$, $P = 0.0570$) (Fig. 2B). It did not make sense to assess IBD for the three sites in the Limfjorden–Wadden Sea region.

The spatial models implemented in STRUCTURE version 2.3.1 (Hubisz *et al.* 2009; Pritchard *et al.* 2000) and GENELAND (Guillot *et al.* 2005) suggested the existence of several genetic units within each region, but the two approaches converged on slightly different clustering solutions. In the Skagerrak–Kattegat–western Baltic

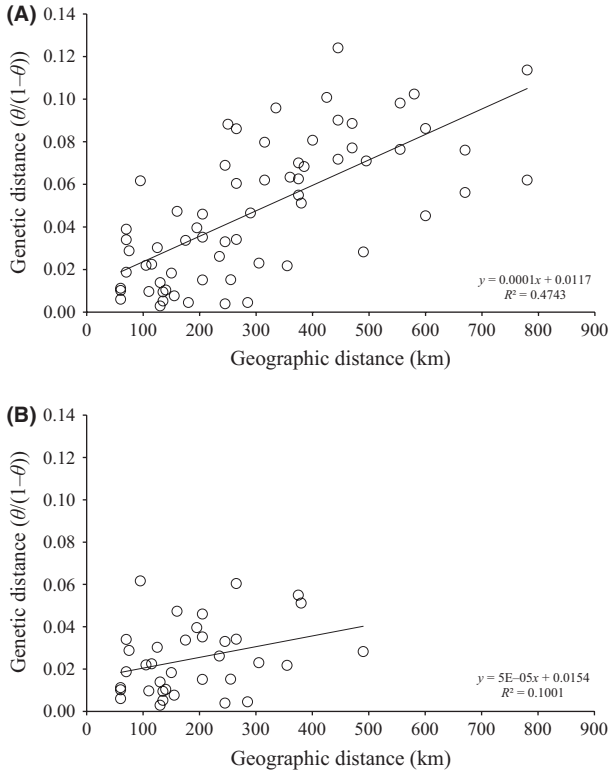

Fig. 2 Isolation by distance (IBD) in southern Scandinavian harbour seals. Plot of genetic distance $\theta/(1-\theta)$ among pairs of sampling localities against their geographical distance measured in kilometres (Rousset 1997). (A) IBD plot for the entire data set. (B) IBD plot for the Skagerrak–Kattegat–western Baltic region.

region, GENELAND pointed to the existence of five genetic units within the region, which corresponded to the northern Skagerrak (VAE), southern Skagerrak (TIS), northern Kattegat (LAE), southern Kattegat (HES, HAL, SAM) and the western Baltic (MAA and ROE) (Fig. 3A). Animals at the Anholt (ANH) haul-out site clustered to both the northern and southern Kattegat units. In contrast, although some clustering could be detected at higher *K*s, STRUCTURE mainly supported the existence of two genetic units separating Skagerrak (VAE, TIS) from the western Baltic (MAA, ROE), with the intermediate Kattegat localities (LAE, ANH, HES, HAL, SAM) having varying degrees of genetic affiliation to either of these (Fig. 4A). In the Limfjorden–Wadden Sea region, GENELAND indicated separate eastern Limfjorden (LIE), western Limfjorden (LIW) and Wadden Sea (WAD) genetic units (Fig. 3B), whereas STRUCTURE suggested that animals from the LIW locality are of mixed ancestry (Fig. 4B).

In the analyses of the entire data set, GENELAND suggested the existence of six clusters with LIW being equally assigned to eastern Limfjorden and the Wadden Sea (Fig. 3C). Curiously, two of the ten replicate runs performed in GENELAND suggested a split between the western Baltic sites MAA and ROE into separate genetic units. The STRUCTURE model converged at two different clustering solutions (Fig. 4C, Table S3, Supporting Information), where the one with the highest support separated the Skagerrak–Kattegat–western Baltic and the Limfjorden–Wadden Sea region and the other divided these further into the same six groupings as suggested by GENELAND.

Distance-based genetic clustering approaches

The Cavalli-Sforza & Edwards' (1967) chord distance provided support for a separation of the harbour seals in the Skagerrak–Kattegat–western Baltic and the Limfjorden–Wadden Sea region (Fig. 5). Within these regions, Limfjorden haul-out sites LIE and LIW were separated from WAD, and the haul-outs VAE, TIS and ROE appeared to be distinct from the other haul-outs in the Skagerrak–Kattegat–western Baltic region. The individual-based tree suggested some clustering of Skagerrak, Kattegat, western Baltic, Limfjorden and Wadden Sea animals, but bootstrap supports for all internal branches were very low (Fig. S1, Supporting Information).

Estimates of genetic differentiation within southern Scandinavia were moderate $\theta = 0.050$ (95% CI: 0.028–0.081) and appeared higher within the Limfjorden–Wadden Sea region $\theta = 0.042$ (95% CI: 0.020–0.069) than within the Skagerrak–Kattegat–western Baltic $\theta = 0.026$ (95% CI: 0.020–0.031). The pairwise estimates of genetic differentiation among haul-out sites were near identical for the two summary statistics θ and D_{est} and pointed to overall similar patterns of genetic clustering as the STRUCTURE and GENELAND models (Table 2).

To sum up, the genetic approaches appear to support the existence of two genetically differentiated regions which each are partitioned into smaller genetic units with some haul-out sites predominantly being inhabited by seals with a uniform genetic background while others mainly support animals with mixed genetic backgrounds. The inferred number of genetic units ranged from four to eight, depending on model and data set. The Skagerrak (VAE, TIS), the western Baltic (MAA, ROE), eastern Limfjorden (LIE) and the Wadden Sea (WAD) were consistently identified as separate genetic units, whereas there was some inconsistency with regard to the clustering of western Limfjorden and the sites in the Kattegat region. Specifically, while the overall results from GENELAND and STRUCTURE were similar, the two models suggested different degrees of genetic structuring, with the former indicating more fine-scale structure than the latter.

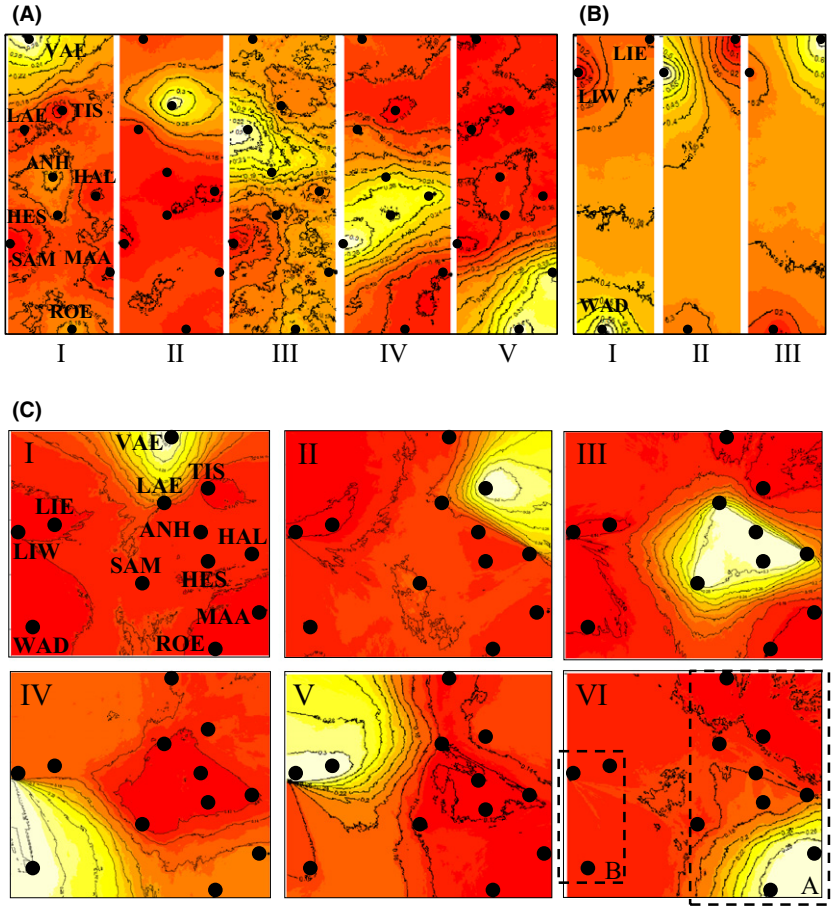

Fig. 3 Estimation of the number of harbour seal populations represented in southern Scandinavia using the program GENELAND ver. 3.1.4 (Guillot *et al.* 2005). The sampling sites are imposed on a probability landscape with lighter yellow colours denoting a high probability of belonging to a specific cluster and darker red values denoting low probability. (A) The results from the analysis of the Skagerrak–Kattegat–western Baltic region. A-I: northern Skagerrak cluster (VAE). A-II: southern Skagerrak cluster (TIS). A-III: northern Kattegat cluster (LAE and partly ANH). A-IV: southern Kattegat cluster (HES, HAL, SAM and partly ANH). A-V: western Baltic cluster (MAA and ROE). (B) The results from the analysis of the Limfjorden–Wadden Sea region. B-I: Wadden Sea cluster (WAD). B-II: western Limfjorden cluster (LIW). B-III: eastern Limfjorden cluster (LIE). (C) The six clusters detected when analysing the entire sample. C–I: northern Skagerrak cluster (VAE). C-II: southern Skagerrak cluster (TIS). C-III: Kattegat cluster (LAE, ANH, HES, HAL and SAM). C-IV: Wadden Sea cluster (WAD). C-V: eastern Limfjorden cluster (LIE). C-VI: western Baltic cluster (MAA and ROE). For interpretation of the references to color in this figure legend, the reader is referred to the online version of this paper.

Demographic independence

The demographic simulations in VORTEX (Lacy *et al.* 2009) suggested that a MVP_K of approximately 75 seals was sufficient to ensure a *PP* greater than 95% at a time frame of 50 years, and a MVP_K of approximately 200 seals sufficed to ensure population persistence for 200 years (Fig. 6A). A population size in the excess of 1000 was required for a *PP* of more than 99%, regardless of the time frame (Fig. 6B). Comparing these numbers with the census population size estimates (N_{2000}) indicated that each of the genetic units identified in the four- and the six-cluster scenario can be considered viable at a 95% *PP* and hence demographically independent under the 'population viability criterion' (Lowe & Allendorf 2010) (Fig. 7A; Fig. 7B). However, at a 99% *PP*, the western Baltic genetic unit (MAA and ROE) cannot be considered viable and consequently fails our criterion for demographic independence. In the eight-cluster scenario, all genetic units passed the criterion for demographic independence at a 95% *PP*, but western Limfjorden only marginally so, and at a 99% *PP*, both the western Baltic and western Limfjorden genetic units failed the criterion (Fig. 7C).

In the alternative approach based on the '10% migration criterion' (Waples & Gaggiotti 2006), the program GENECLASS2 (Piry *et al.* 2004) detected a total of 11, 20 and 23 animals with less than 5% probability of originating from the genetic unit where they were sampled, indicating that these seals are immigrants (Table 3). Thus, if we apply the '10% migration criterion' for demographic

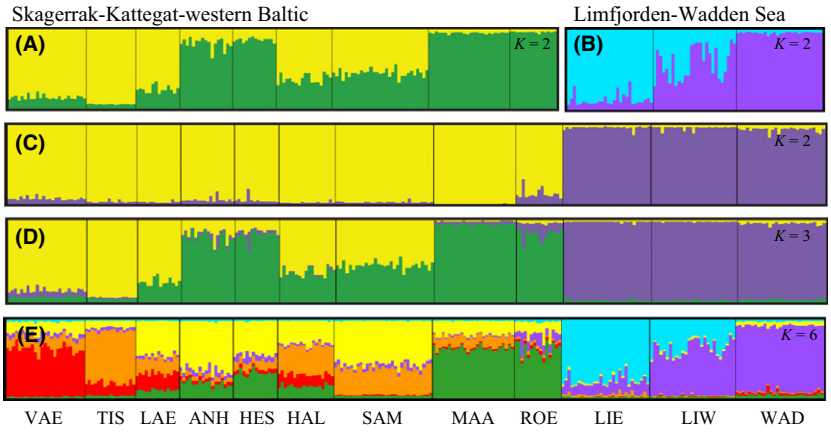

Fig. 4 Estimation of the number of harbour seal populations represented in southern Scandinavia using the program STRUCTURE ver. 2.3.1 (Hubisz *et al.* 2009; Pritchard *et al.* 2000). Each vertical bar represents a sampled individual and the colouring its proportion of membership to each of *K* clusters. (A) Plot for $K = 2$ in the analysis of the Skagerrak–Kattegat–western Baltic region. (B) Plot for $K = 2$ in the analysis of the Limfjorden–Wadden Sea region. (C–E) Plot for $K = 2$, $K = 3$ and $K = 6$ in the analysis of the entire data set. For interpretation of the references to color in this figure legend, the reader is referred to the online version of this paper.

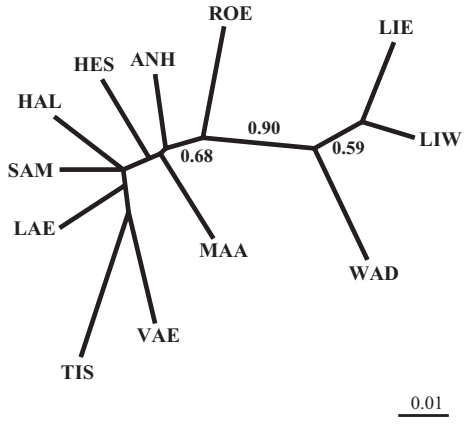

Fig. 5 Unrooted neighbour-joining tree for harbour seals in southern Scandinavia. The tree is based on Cavalli-Sforza and Edwards (1967) genetic distance d_{chord} showing the relationship between sampling localities. The scale bar is in units of d_{chord}. Bootstrap values below 0.50 are omitted.

independence, rather than the 'population viability criterion', three genetic units are classified as demographic independent and one unit is not in the four-cluster scenario. For the six-cluster scenario, three genetic units can be considered demographically independent and three cannot, and for the eight-cluster scenario, four units can and four cannot be considered demographically independent using the '10% migration criterion'.

Discussion

Harbour seal populations and management units

Our analyses revealed structuring at finer scale than previous genetic studies of harbour seals and pinnipeds in general (Andersen *et al.* 2011; Davis *et al.* 2008; Goodman 1998; Graves *et al.* 2009; Westlake & O'Corry-Crowe 2002), as well as a high degree of congruence with findings from nongenetic studies (Fig. 8A–H). We found that southern Scandinavian harbour seals are partitioned into two main genetic lineages represented by haul-out sites in the Skagerrak–Kattegat–western Baltic and the Limfjorden–Wadden Sea regions, respectively. These two regions are further divided into smaller genetic entities where some haul-out sites are dominated by seals with a uniform genetic background, while other sites primarily support animals with mixed genetic backgrounds.

The observed division between Skagerrak–Kattegat–western Baltic and Limfjorden–Wadden Sea haul-out sites has also been reported in previous genetic studies (Goodman 1998; Stanley *et al.* 1996), and it has been proposed that this genetic differentiation results from historic colonization patterns (Härkönen *et al.* 2005). The observation that Limfjord seals were infected from the Wadden Sea rather than from Kattegat during the 1988 and 2002 PDV epidemics (Härkönen *et al.* 2006), as well as regional differences in contaminant loads (Dietz *et al.* 2012a), suggests the genetic differentiation is maintained by contemporary barriers to dispersal at the Skagerrak–North Sea transition.

Within the Limfjorden–Wadden Sea region, our data suggested a genetic and demographic separation of the Wadden Sea (WAD) and eastern Limfjorden (LIE). Geological and historical records suggest that Limfjorden and the North Sea were separated by land until the 1820s (Olsen *et al.* 2010 and references therein), which may explain the observed genetic separation, and indicate that the demographic and evolutionary history of

Table 2 Pairwise genetic differentiation between southern Scandinavian harbour seal localities expressed in terms of θ (Weir & Cockerham 1984) above the diagonal and D_{est} (Jost 2008) below the diagonal. Significance was determined by 95% bootstrap confidence intervals (CI). The correlation between estimates of θ and D_{est} was strong and highly significant (Mantel's $Z = 0.977$, $P = 0.0002$).

	VAE	TIS	LAE	ANH	HES	HAL	SAM	MAA	ROE	LIE	LIW	WAD
VAE		0.022*	0.018	0.034**	0.033**	0.015*	0.023**	0.049**	0.028**	0.060**	0.045**	0.072**
TIS	0.027**		0.011	0.058**	0.045**	0.010	0.015	0.057**	0.052**	0.088**	0.079**	0.110**
LAE	0.022*	0.010		0.018	0.014	0.003	0.008	0.032**	0.021	0.074**	0.065**	0.092**
ANH	0.038**	0.060**	0.020*		0.010	0.033*	0.022*	0.033*	0.005	0.064**	0.058*	0.066
HES	0.038**	0.045**	0.009	0.006		0.006	0.028*	0.005	0.004	0.067**	0.059**	0.071*
HAL	0.022**	0.010	0.000	0.033**	0.004		0.009	0.029**	0.026	0.083**	0.066**	0.089**
SAM	0.023**	0.013*	0.003	0.021**	0.023*	0.008		0.044**	0.038**	0.081**	0.075**	0.093**
MAA	0.060**	0.057**	0.029**	0.030**	0.003	0.030**	0.043**		0.010	0.071**	0.079**	0.102**
ROE	0.041**	0.059**	0.027*	0.004	0.001	0.033**	0.039**	0.009		0.053**	0.043*	0.058**
LIE	0.071**	0.091**	0.078**	0.068**	0.071**	0.088**	0.081**	0.071**	0.062**		0.038**	0.081**
LIW	0.054**	0.086**	0.064**	0.054**	0.056**	0.066**	0.071**	0.074**	0.043**	0.034**		0.005
WAD	0.076**	0.107**	0.079**	0.057**	0.059**	0.081**	0.083**	0.086**	0.050**	0.076**	0.002	

*: $P < 0.05$; **: $P < 0.01$.

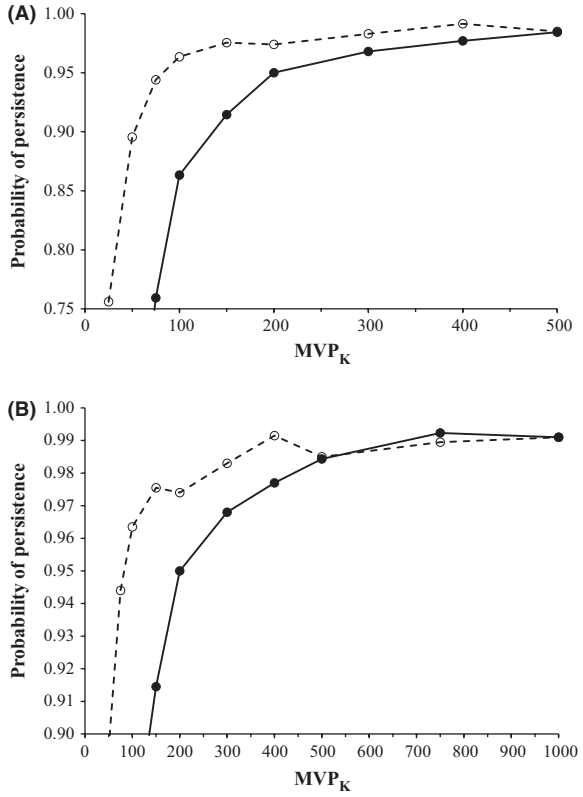

Fig. 6 Estimation of persistence probability (*PP*) for different minimum viable population sizes (MVP_K) of harbour seals over a 50-year (stippled line) or 200-year (solid line) time frame, based on VORTEX simulations (Lacy *et al.* 2009). (A) Simulations ranging from a *PP* of 75–100% and up to $MVP_K = 500$. (B) The range of *PP* from 90–100% and MVP_K up to 1000.

Limfjorden harbour seals may be very different from other populations in the North Sea. Although the region-specific GENELAND analysis did indicate that western Limfjorden (LIW) haul-out site constituted a separate genetic unit, both STRUCTURE and the distance-based methods suggested that animals at this site are of mixed genetic background. This pattern is supported by tagging data and the observed spread of the PDV (Härkönen *et al.* 2006; Teilmann *et al.* 2010). Thus, most evidence suggests that western Limfjorden is a satellite of the Wadden Sea and eastern Limfjorden, and it is likely that these two management units will become genetically and demographically connected in future. Also, it is likely that Danish Wadden Sea harbour seals are connected to the German and Dutch Wadden Sea harbour seals, but this awaits further analyses.

The Skagerrak–Kattegat–western Baltic region appears to be divided into several distinct genetic units, which may serve as management units. The existence of such fine-scale genetic and demographic structuring within Skagerrak and Kattegat is strongly supported by nongenetic data. In a tagging and monitoring study of 163 harbour seal pups over a 14-year period, sexually mature animals were not observed more than 32 km away from the site of tagging at any time (Härkönen & Harding 2001). Site fidelity was also observed for 27 animals tagged at the Anholt haul-out site in central Kattegat (Dietz *et al.* 2012b), and harbour seals in the region appear to have different contaminant loads (Dietz *et al.* 2012a). The near-continuous distribution of haul-out sites in Skagerrak prevents a clear definition of management unit boundaries in Skagerrak and Kattegat, but for now we suggest a delineation between the northern Skagerrak (VAE), the southern Skagerrak (TIS) and the Kattegat (LAE, ANH, HES, HAL, SAM), respectively. The distance-based estimates of genetic differentiation, the region-specific analyses performed in GENELAND and existing tagging data indicate that

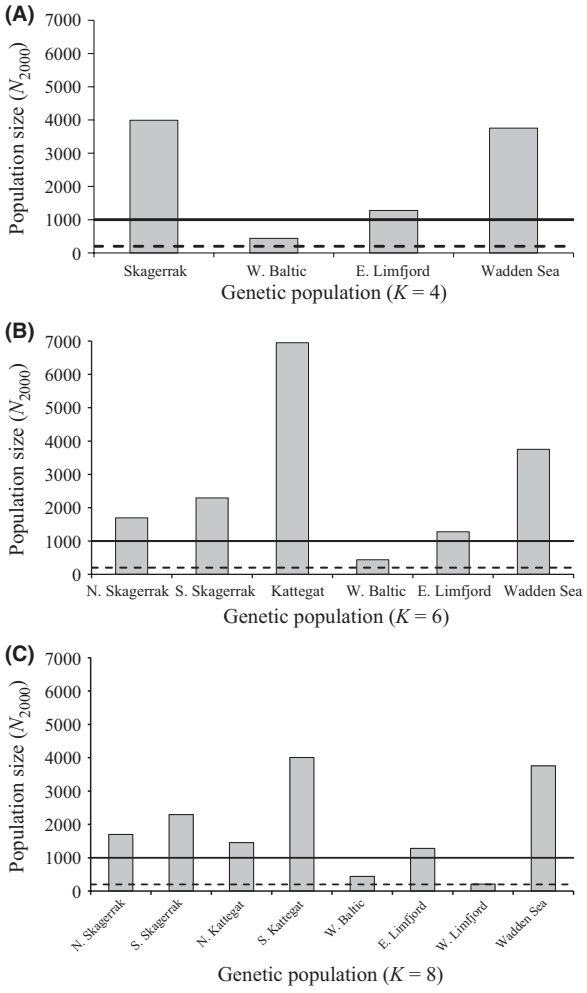

Fig. 7 Defining management units according to the 'population viability criterion' by comparison of census population size in year 2000 (N_{2000}) and the minimum viable population size (MVP_K) estimated for each of the identified genetic units. Units for which the N_{2000} is above MVP_K = 200 (full line) have a *PP* above 95% over a 200-year time frame and are consequently considered demographically independent. For comparison, the stippled line marks the MVP_K for a *PP* of 99%, regardless of time frame. (A–C) classification for each of the genetic units identified under the four-, six- and eight-cluster scenarios, respectively, obtained for different models and data subsets.

haul-out sites in Kattegat may be further divided. Finally, harbour seals in the western Baltic were genetically and demographically independent of the Kattegat, but the results were unclear with regard to the degree of differentiation between localities Måkläppen (MAA) and Rødsand (ROE) within the western Baltic. This could be due to gene flow from now extinct populations in the western Baltic region and/or the small extant population in the eastern Baltic (Goodman 1998; Härkönen *et al.* 2005).

The above management unit delineations may aid in pointing out marine-protected areas for harbour seals,

Table 3 Defining management units according to the '10% migration criterion' by detection of first-generation migrants in the genetic units identified by STRUCTURE and GENELAND. The four-cluster scenario was suggested by STRUCTURE analysis on data subsets. The six-cluster scenario was suggested by STRUCTURE and GENELAND analyses on the entire data set. The eight-cluster scenario was suggested by GENELAND analyses on data subsets.

Genetic unit	*N*	*M*n	*M* (%)	*DI*
Four-cluster scenario (K = 4)				
Skagerrak	41	2	4.9	Yes
W. Baltic	41	4	9.8	Yes
E. Limfjord	28	4	14.3	No
Wadden Sea	28	1	3.6	Yes
Six-cluster scenario (K = 6)				
N. Skagerrak	25	2	8.0	Yes
S. Skagerrak	16	2	12.5	No
Kattegat	94	5	5.3	Yes
W. Baltic	41	4	9.8	Yes
E. Limfjord	28	4	14.3	No
Wadden Sea	28	3	10.7	No
Eight-cluster scenario (K = 8)				
N. Skagerrak	25	2	8.0	Yes
S. Skagerrak	16	2	12.5	No
N. Kattegat	14	1	7.1	Yes
S. Kattegat	63	4	6.3	Yes
W. Baltic	41	5	12.2	No
E. Limfjord	28	4	14.3	No
W. Limfjord	27	2	7.4	Yes
Wadden Sea	28	3	10.7	No

N, number of animals sampled in each unit; *M*n, number of animals sampled in each unit which was detected as first-generation migrant by GENECLASS2; *M* (%), the proportion of animals sampled in each unit that were migrants; *DI*, classified as demographic independent using the '10% migration criteria'.

as well as for other marine mammals in the region such as the harbour porpoise (*Phocoena phocoena*) and the grey seal (*Halichoerus grypus*). Moreover, as our study system formed the epicentre of the 1988 and 2002 PDV epidemics, which caused the death of more than 50 000 European harbour seals (Dietz *et al.* 1989; Härkönen *et al.* 2006; Jensen *et al.* 2002), the inferred population structuring may provide a valuable piece of information for understanding the rise and spread of PDV and other marine mammal morbilliviruses. Finally, while the behaviour underlying the apparent strong site fidelity of harbour seals relative to other North Atlantic pinnipeds is unclear (Coltman *et al.* 2007; Davis *et al.* 2008; Folkow *et al.* 2004; Freitas *et al.* 2008; Gjertz *et al.* 2000; Kapel *et al.* 1998; Teilmann *et al.* 1999), this fidelity implies that local harbour seal populations may be susceptible to environmental change and shifts in the distribution of prey, predators, pathogens and interspecific competitors (Bolt *et al.* 2009; Bowen *et al.* 2003; Lonergan *et al.* 2007). For example, the abundance of

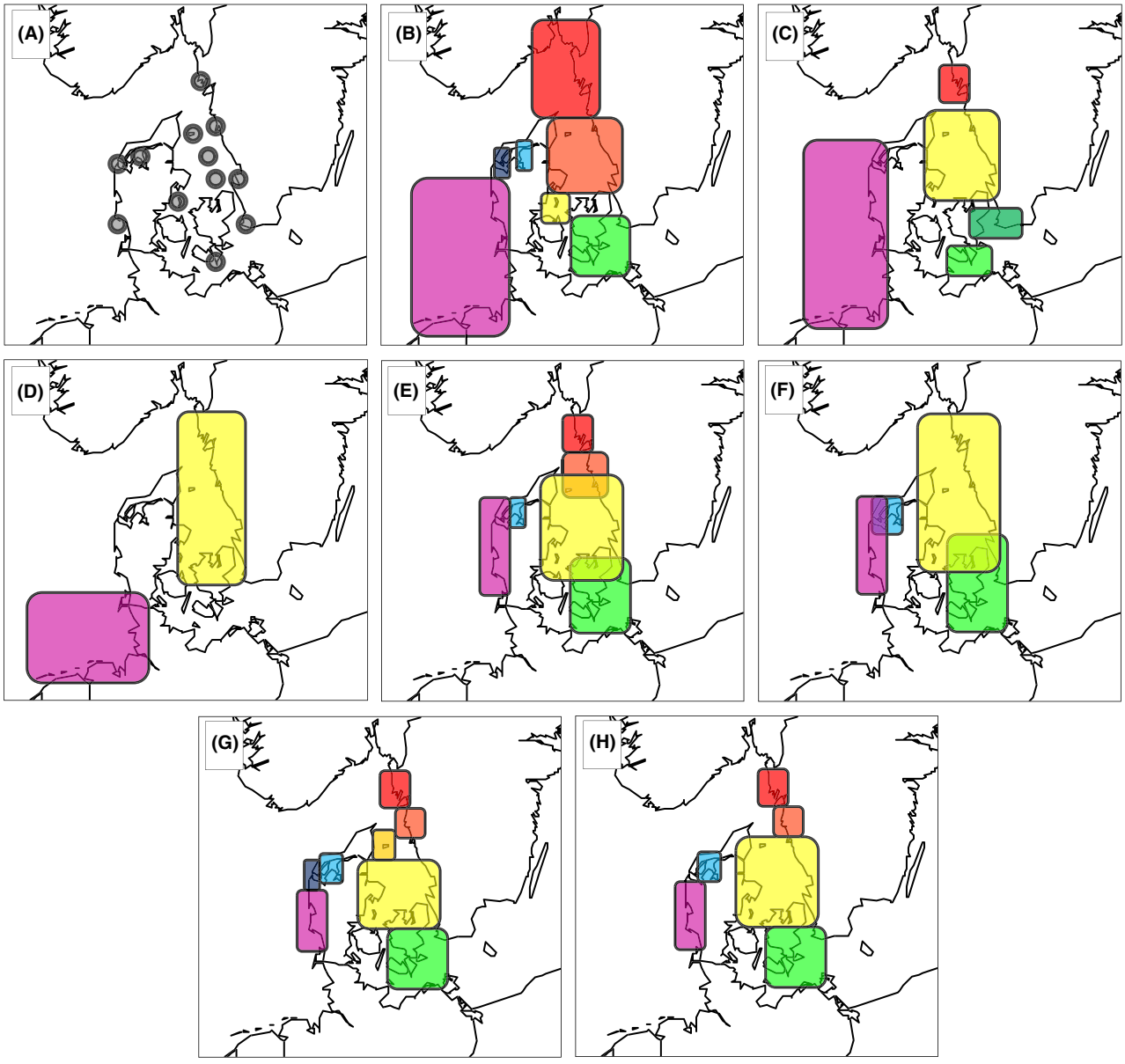

Fig. 8 Summary of southern Scandinavian harbour seal population structuring and management units inferred from this and previous studies. (A) sampling localities as described in Figure 1. (B) current delineation of management units based on geographical features and habitat characteristics (Heide-Jørgensen & Härkönen 1988; Jepsen 2005; Olsen *et al.* 2010). (C) harbour seal movement ranges detected by tagging studies (Dietz *et al.* 2012b; R. Dietz unpublished; Dietz *et al.* 2003; Härkönen & Harding 2001; Teilmann *et al.* 2010). (D) populations inferred from previous genetic studies (Goodman 1998; Stanley *et al.* 1996). (E–G) genetic units suggested in this study by pairwise estimates of θ and D_{est}, (E) STRUCTURE (F) and GENELAND (G). (H) The new management units suggested for harbour seals in southern Scandinavia. The units correspond to the northern Skagerrak (red), the southern Skagerrak (orange), the central and southwestern Kattegat (yellow), the western Baltic (green), eastern Limfjorden (blue) and the Wadden Sea (purple). The harbour seals in the Danish Wadden Sea are likely demographically connected to the German and Dutch Wadden Sea harbour seals. For interpretation of the references to color in this figure legend, the reader is referred to the online version of this paper.

grey seals has increased substantially in the study region in recent years and this species may become a competitor to harbour seals for local food resources (Olsen *et al.* 2010), as has been suggested for other regions where grey and harbour seals co-occur (Lonergan *et al.* 2007).

What is the spatial and temporal resolution of genetic methods?

An often criticized aspect of genetic methods for the identification of populations and management units is that they may fail to provide a baseline population

structure when gene flow is high or when populations have recently diverged (Palsbøll *et al.* 2010; Waples 1998; Waples & Gaggiotti 2006). That is, they may lack sufficient spatial and temporal resolution compared to nongenetic methods, such as satellite tracking, photo-ID, acoustics, stable isotopes and contaminant analyses.

Here, we combined fine-scale spatial sampling covering all major haul-out sites in the region, genotyping of 15 microsatellite markers and model-based genetic clustering analyses (Guillot *et al.* 2005; Hubisz *et al.* 2009; Pritchard *et al.* 2000) to identify harbour seal populations. In the analyses of the entire data set, both STRUCTURE and GENELAND suggested the existence of up to six genetic units. However, as this data set was characterized by IBD and it is well documented that inference of STRUCTURE and GENELAND can be affected by IBD (Blair *et al.* 2012; Frantz *et al.* 2009; Guillot *et al.* 2009, 2005; Meirmans 2012), we performed additional analyses on subsets of the data. In these analyses, the two approaches did not converge on the same solution with STRUCTURE generally detecting fewer genetic units (four, two in each region) and GENELAND detecting additional units (eight, three in one region and five in the other). So is STRUCTURE underestimating or is GENELAND overestimating the real number of genetic units? Empirical data suggest that the STRUCTURE model may suffer from low reproducibility when MCMC burn-in and run lengths are low (Gilbert *et al.* 2012), and both approaches may fail to detect clusters in data sets characterized by small sample sizes and low heterozygosity. Still, given the run settings used, our homogeneous sample distribution and F_{ST} values in the range of 0.010–0.050, both programs should perform reasonable well. The GENELAND model may perform slightly better than STRUCTURE at moderate to high levels of migration, possibly due GENELAND's use of geographical information in inferring population clusters (Blair *et al.* 2012; Frantz *et al.* 2009). Thus, given our sampling design and data, we suspect that the clustering identified by GENELAND provides a closer approximation to the real pattern.

Notably, both model-based approaches yielded results, which were largely in accordance with those derived from distance-based genetic methods, as well as from nongenetic methods (Fig. 8A–H). This demonstrates that fine-scale spatial sampling, a relatively modest number of variable microsatellite markers and appropriate genetic analyses can allow for population structure inference at a temporal and spatial scale comparable to that of nongenetic methods, even at relatively low levels of genetic differentiation. Still, while model-based clustering approaches such as STRUCTURE and GENELAND indeed can provide inference at small spatial scales, their potential sensitivity to, for example, IBD, heterozygosity levels and sample size pinpoint that results from genetic analyses should always be interpreted with caution and integrated with ecological data if one is to understand whether populations are demographically independent.

The question of demographic independence

Although the idea that delineation of management units should be based on demographic independence is being gradually adopted, there is little consensus about the most appropriate path for assessing whether specific entities are demographically independent or not (Palsbøll *et al.* 2007).

A common approach is the use of genetic summary statistics. For example, in a study of North Pacific sperm whales (*Physeter macrocephalus*), Wright's (1943) island model $F_{ST} = 1/(4N_em + 1)$ was used to estimate F_{ST} from an inferred effective population size (N_e) and fixed migration rate, and populations were defined as demographically independent if pairwise estimates of F_{ST} were above zero (Mesnick *et al.* 2011). However, very few natural populations meet the assumptions of the island model (i.e. infinite number of subpopulations, N_e and m are constant and similar for each subpopulation, discrete generations), and if so, inference is on evolutionary timescales and typically does not reflect contemporary patterns (Palsbøll *et al.* 2013; Pearse & Crandall 2004).

Another approach is to estimate contemporary migration rates and compare these rates with a predefined migration threshold below which populations are considered to be demographically independent (Boessenkool *et al.* 2009; Hastings 1993; Waples & Gaggiotti 2006). Little is known about the dispersal rate at which populations move from demographic independence to dependence (Palsbøll *et al.* 2007; Waples & Gaggiotti 2006); however, given the theoretical results of Hastings (1993), Waples and Gaggiotti (2006) suggested that a 10% threshold could be a useful starting point when other information was lacking. We tested the utility of this '10% migration criterion' by estimating contemporary immigration rates in GENECLASS2 (Piry *et al.* 2004) for the inferred harbour seal genetic units and found that although the use of this criterion may be appealing, inference is highly affected by sampling design. For example, sampling a few animals more or less in western Baltic and Wadden Sea would likely change the demographic classification of these units. Thus, focussing on a specific threshold may result in units with only minor differences in immigration rates and sample size being classified differently. Clearly, such specific thresholds are not likely to apply to real systems (Lowe & Allendorf 2010).

In species and populations where the above approaches are unfeasible, inadequate or inappropriate, PVA models in programs such as VORTEX (Lacy *et al.* 2009) could provide an alternative path for delineating management units. In the present study, the inferred genetic units were defined to be demographically independent and separate management units when they, in the absence of immigration, were sufficiently large to ensure population persistence within the specified time frame. To obtain a probability of persistence greater than 95%, the MVP_K's were approximately 75 and 200 animals when considering a time frame of 50 and 200 years, respectively, while a probability of persistence above 99% required a population size in the excess of 1000 animals for persistence. How do these figures compare to observed harbour seal abundance and population dynamics? In southern Scandinavia, severe reductions in abundance due to PDV epidemics have been followed by annual growth rates of up to 12% and rapid increases in population size (Olsen *et al.* 2010). Hence, although the MVP_K's may seem too low for population viability and demographic independence, they are not biologically unrealistic for harbour seals.

The main attractiveness of using population viability as a criterion for demographic independence is that inference do not rely on a predefined migration threshold. The criteria are transparent and easily adjustable to specific management goals, such as persistence probability, time frame and population size. Furthermore, the data and criteria used for evaluation of demographic status are nongenetic, and classification is governed by actual population census size, rather than sample size. Importantly, both the delineation of genetic units and the PVA can be modified if new genetic or life-history data are obtained. Still, population viability simulations are not without their limitations (Beissinger & Westphal 1998; Coulson *et al.* 2001; Reed *et al.* 2003). All models are simplifications of real-world patterns and may not do well in capturing environmental and demographic stochasticity. Further, confidence in the simulations may be greatly reduced for less-studied species where reliable life history data are limited or unavailable.

In the present study, the VORTEX simulations were based on information from several decades of harbour seal research and monitoring (Coltman *et al.* 1998; Hayes *et al.* 2006; Härkönen *et al.* 2006, 2007, 2005, 2002; Härkönen & Heide-Jørgensen 1990), providing an unusual opportunity to model the extinction process. Moreover, our preliminary tests suggest that the simulations are relatively robust to variable life history parameters, and in a study of 102 species of vertebrates, Reed *et al.* (2003) found that MVP_K's did not correlate with major taxa, latitude or trophic level, but only with the simulated time frame and the population growth rate. Of these two parameters, the former is context specific and the latter can often be approximated from catch statistics or population surveys. Thus, despite the limitations of population viability simulations, reliable inference may be possible even for species with limited life history data – at least for a preliminary assessment.

Finally, it should be clear that our approach does not provide information about whether the populations in fact are demographically independent, but rather whether they can be managed as demographically independent. For example, the abundance of seals at both the SAM and HAL haul-out sites in Kattegat is well above the MVP_K suggested by the demographic simulations, indicating that these sites can support demographically independent populations. Still, because genetic and nongenetic results indicate some degree of connection to other Kattegat haul-out sites, SAM and HAL may not be classified as demographically independent in a conservation and management context. Conversely, although the western Baltic genetic unit (MAA and ROE) was found marginally too small for long-term persistence at a *PP* above 99% and hence may fail the criterion of demographic independence, this does not necessarily imply that it is demographically connected to other harbour seal localities. Rather, the western Baltic genetic unit may be at risk and need special attention.

The overall high degree of congruence between our genetic results, the demographic simulations and inference from a diverse body of nongenetic studies indicate that our results are not influenced by violations of method-specific assumptions. Thus, while further work is needed to thoroughly evaluate the utility of our approach, it may provide a good starting point for integrating genetic data and PVAs for the identification of populations and management units.

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M.T.O. designed and performed the research, analysed the data and wrote the manuscript. L.W.A. and H.R.S. provided input to laboratory analyses, genetic research design, commented and discussed data analyses and helped draft the manuscript. R.D., J.T.E. and T.H. aided in research design, provided all funding, contributed all samples and population survey data, commented on data analyses and helped draft the manuscript. All authors read and approved the final version of the manuscript.

Data accessibility

The microsatellite data in GENEPOP format (Table S4) and pairwise geographical distances between sites are in the online Supplementary Material.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Characteristics of the 15 analysed microsatellite loci.

Table S2 Parameter settings for the demographic simulations.

Table S3 Estimates of L(K), L′(K), |L′′(K)| and DeltaK for each cluster K inferred for the STRUCTURE analyses of the entire southern Scandinavia, the Skagerrak–Kattegat–Wadden Sea region and the Limfjorden–Wadden Sea region, respectively.

Table S4 Microsatellite data in GENEPOP format.

Fig. S1 Individual-based NJ tree made from pairwise estimates of Cavalli-Sforza and Edwards chord distance (1967).