

Population genomics of Baltic Harbour Porpoises: SNP panel development and WGS



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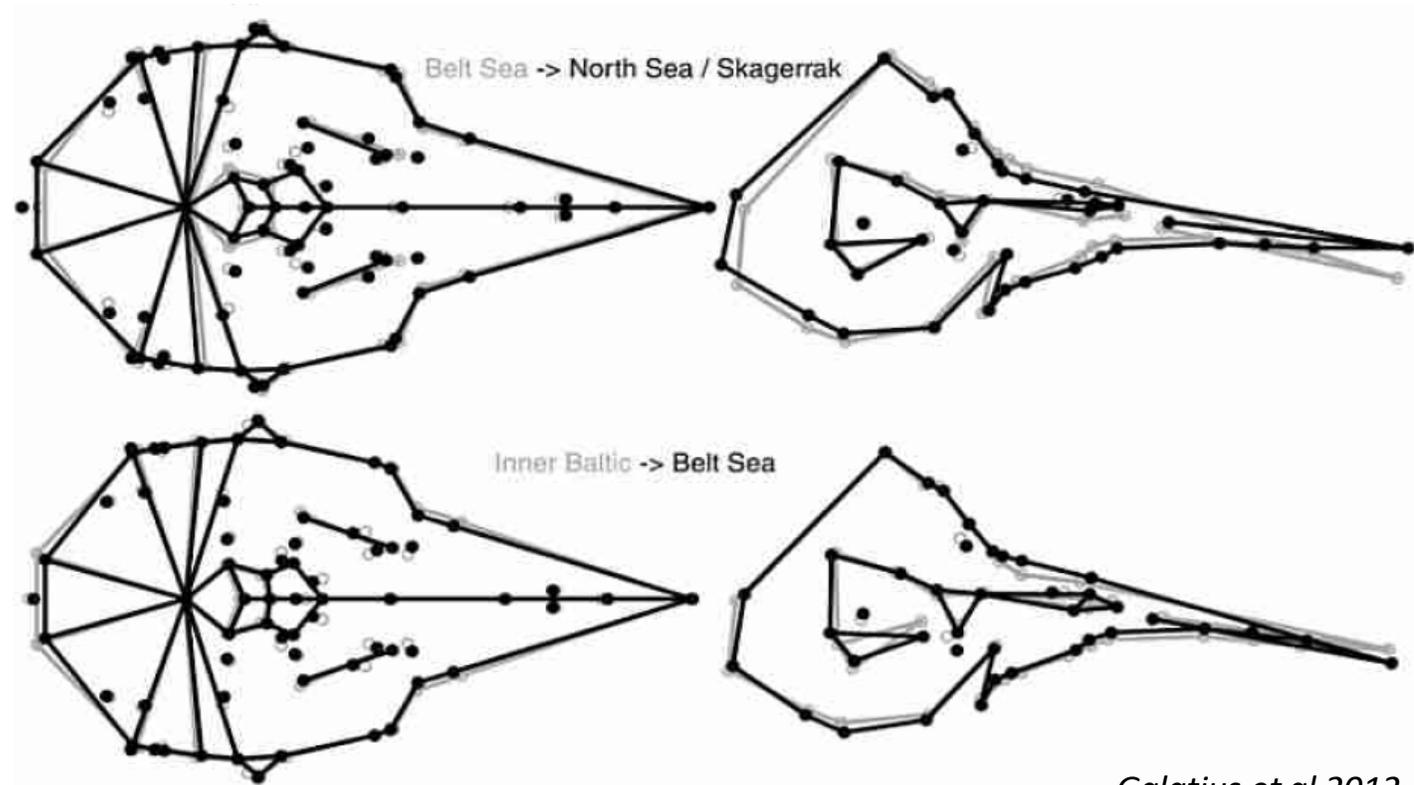
Harbour porpoise in the Baltic region

- *Phocoenaphocoena*
- Only cetacean breeding and occurring regularly
- 3 putative populations
- NOS and IBS Least Concerned (IUCN Red List, Braulik et al. 2020)
- IBS population “Critically Endangered”, 71–1105 inds. (IUCN Red List, Hammond et al. 2016, Amundin et al. 2021)



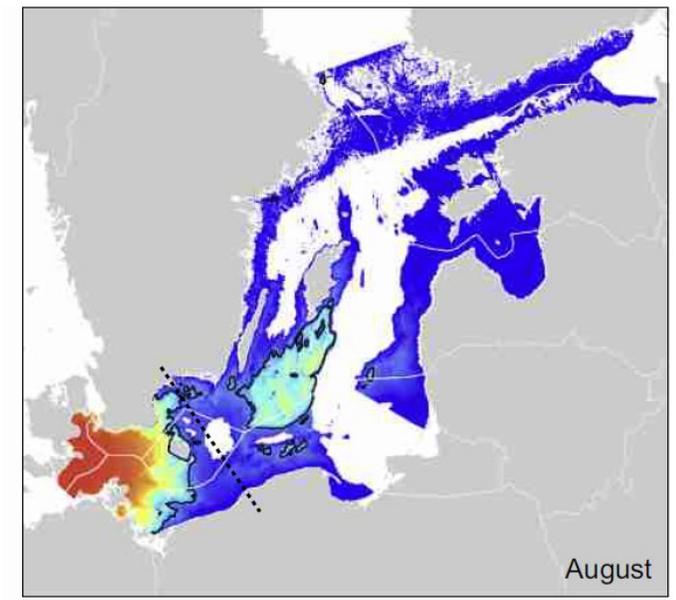
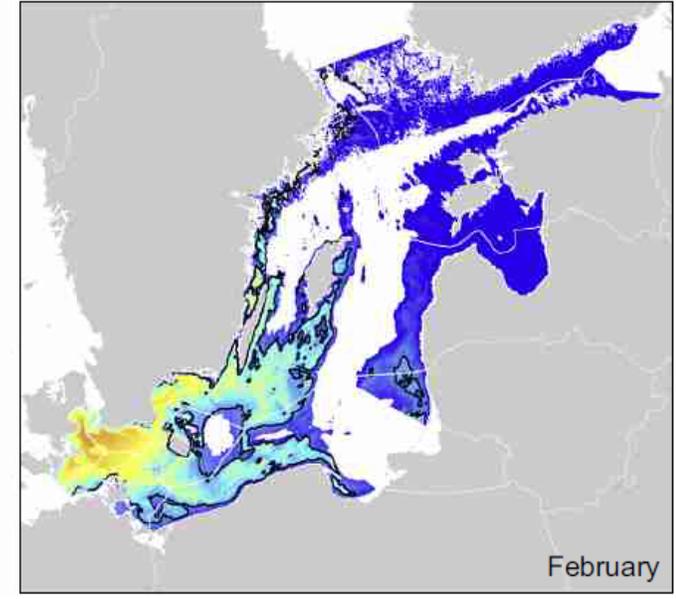
Non genetic evidences

- Morphological: Skull shape



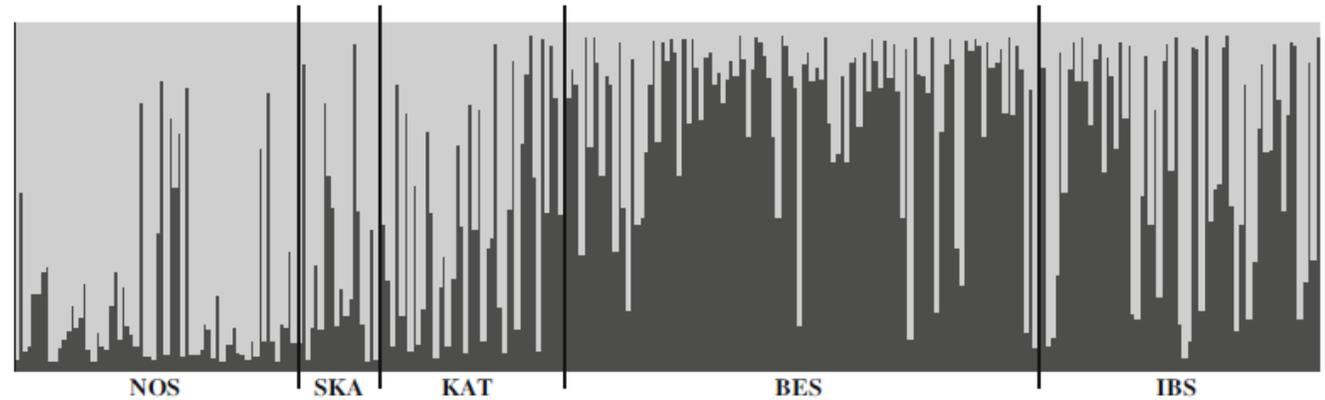
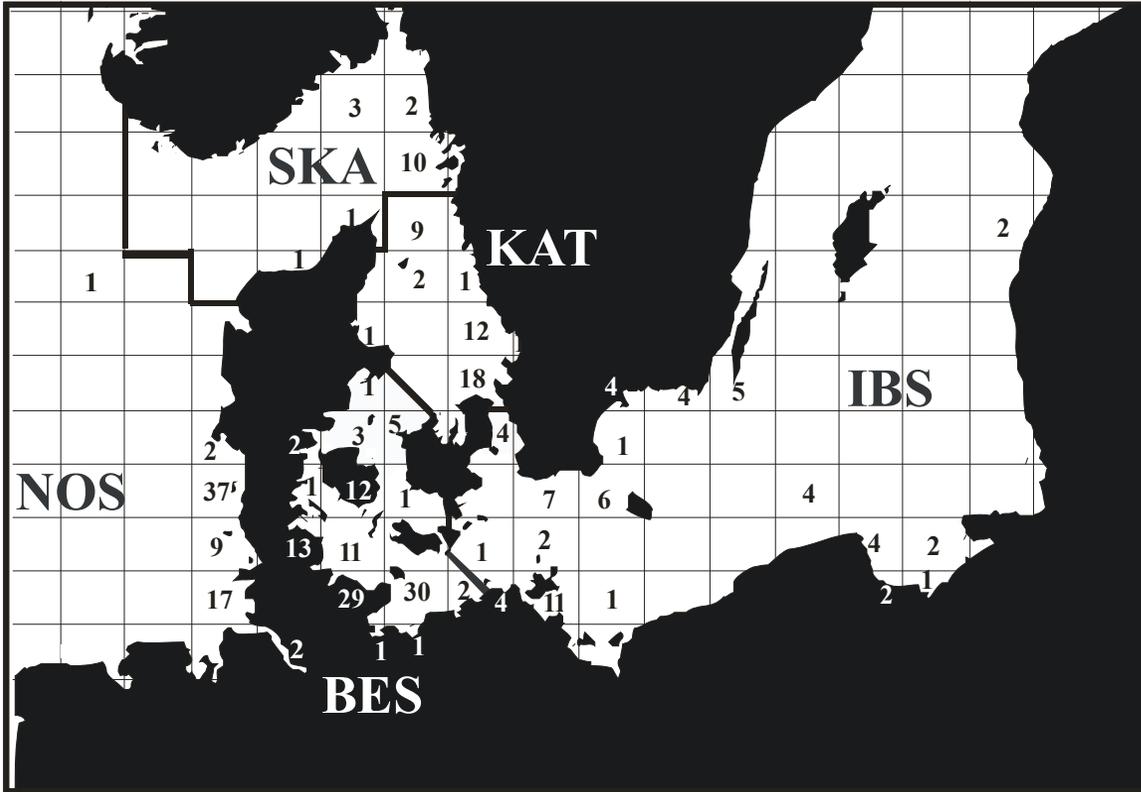
Galatius et al 2012

- Abundance: Passive acoustic detection



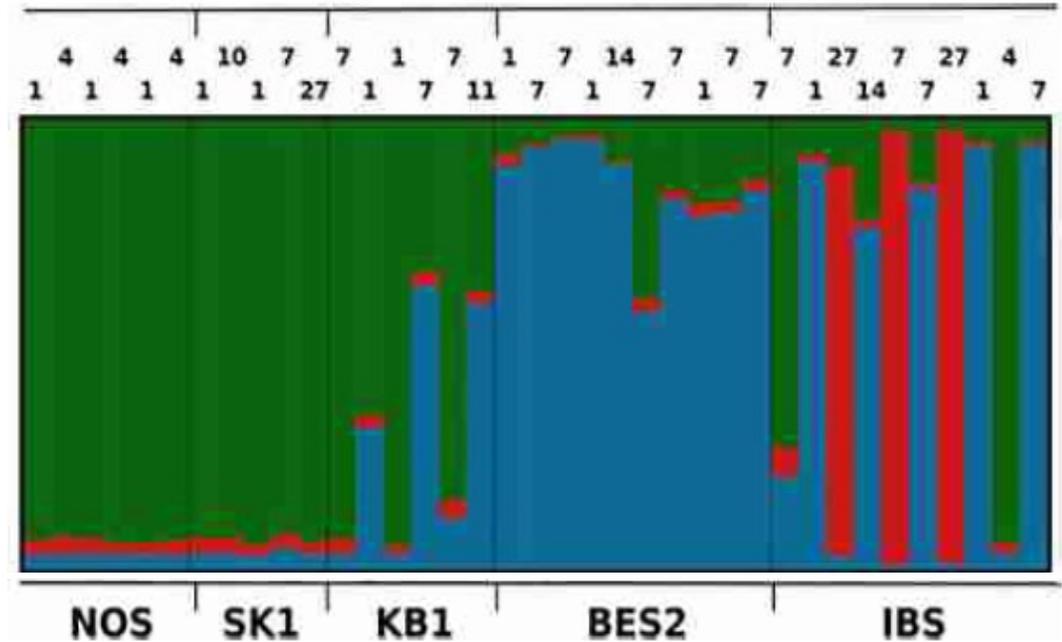
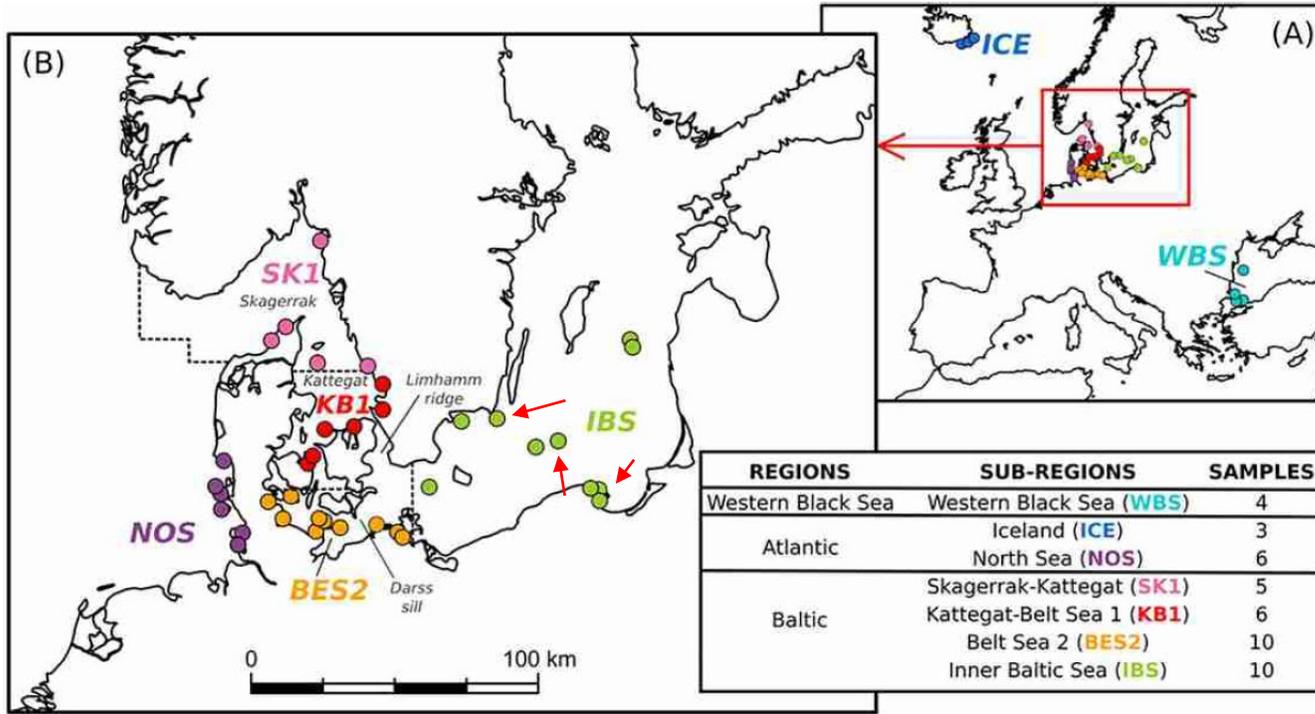
Carlén et al 2018

Wiemann et al 2010 – 15 Microsatellites



- IBS Darss Sill border
- NOS-BES genetic differentiation

Lah et al 2016 – 1st ddRAD (1,874 SNPs)

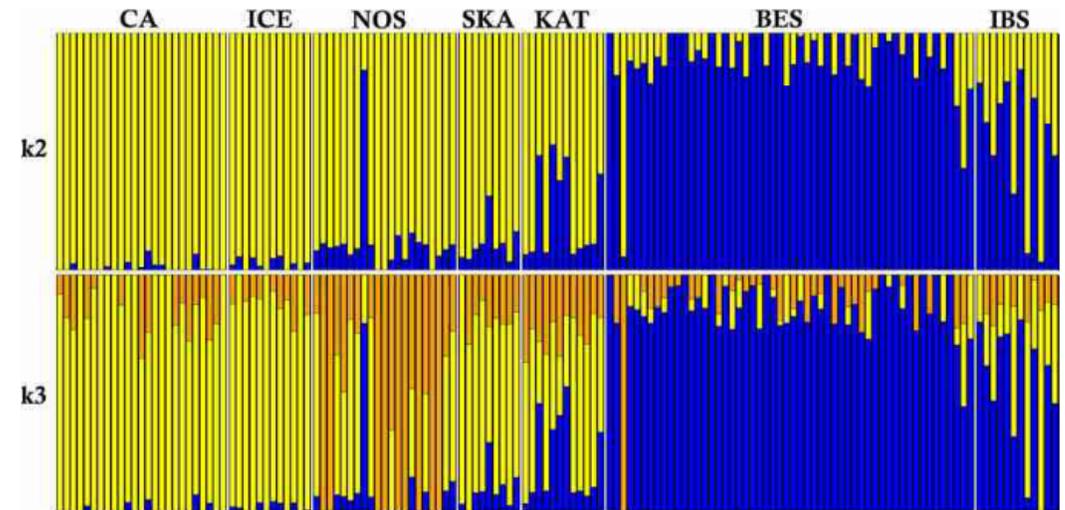
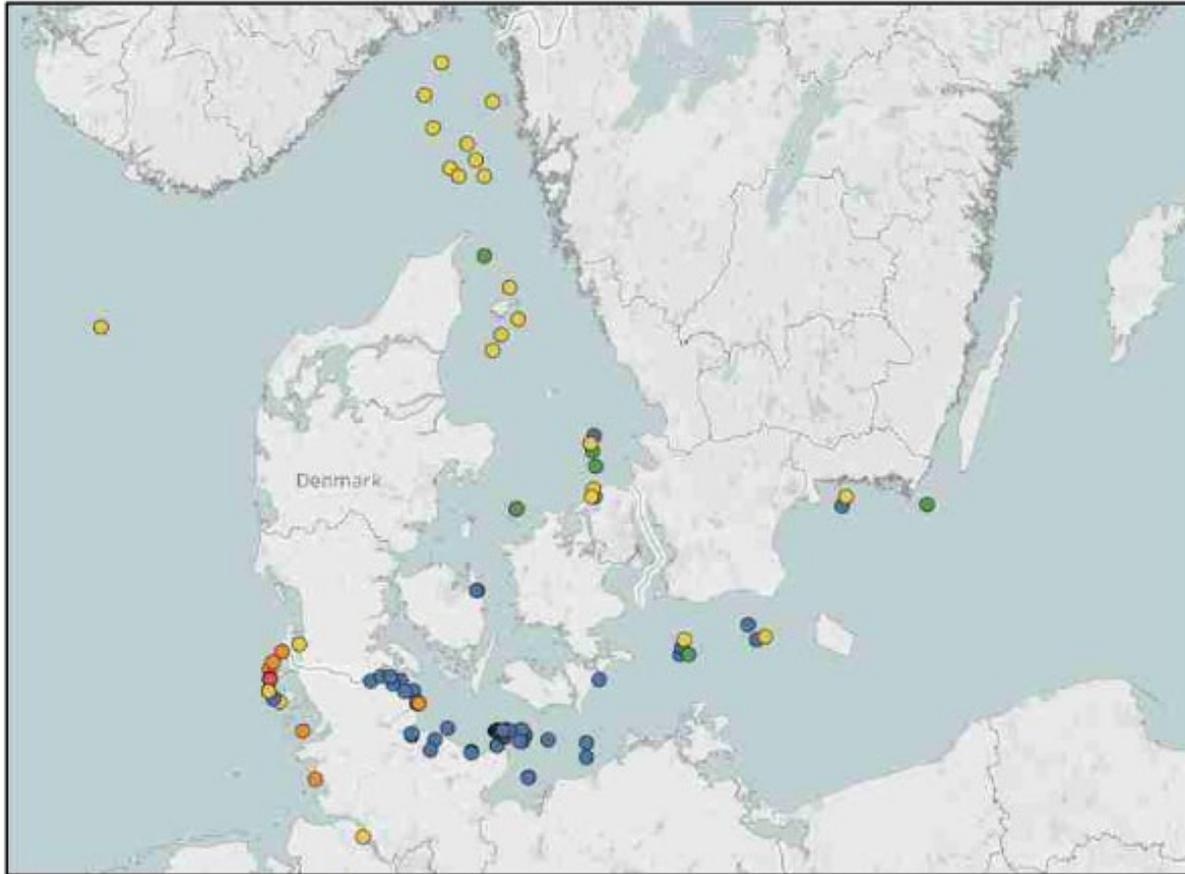


- NOS, BES and indication of IBS
- 13,5°E IBS border
- Great potential in Polish samples?

A1993/05175	S Öland	M	August
Pol_67	Ustka	F	November
Pol_72	Wladyslawowo	F	February

Genome assembly (Autenrieth et al 2018) and 2nd ddRAD (11,978 SNPs)

- Whole genome assembled Kattegat porpoise: 2.39 Gb in 13,498 scaffolds
- Genome annotated: 22,154 predicted protein coding genes



- NOS more distinct than IBS?
- 13,5°E IBS border
- Very few (only 3) samples from Baltic Proper

SNP Panel Development

- **SNP Discovery:** ddRAD 145 individuals, 7 locations
 - 11,978 filtered SNPs (MAF<5%, QUAL<20, XDP<5)
- **SNP selection:** Fst (fixation index) outliers (NOS-BES-IBS)
 - Biallelic SNPs
 - High Fst
 - Low heterozygosity
 - Fst ranged 0,62 to 0,24

- **SNP genotyping:** Fluidigm



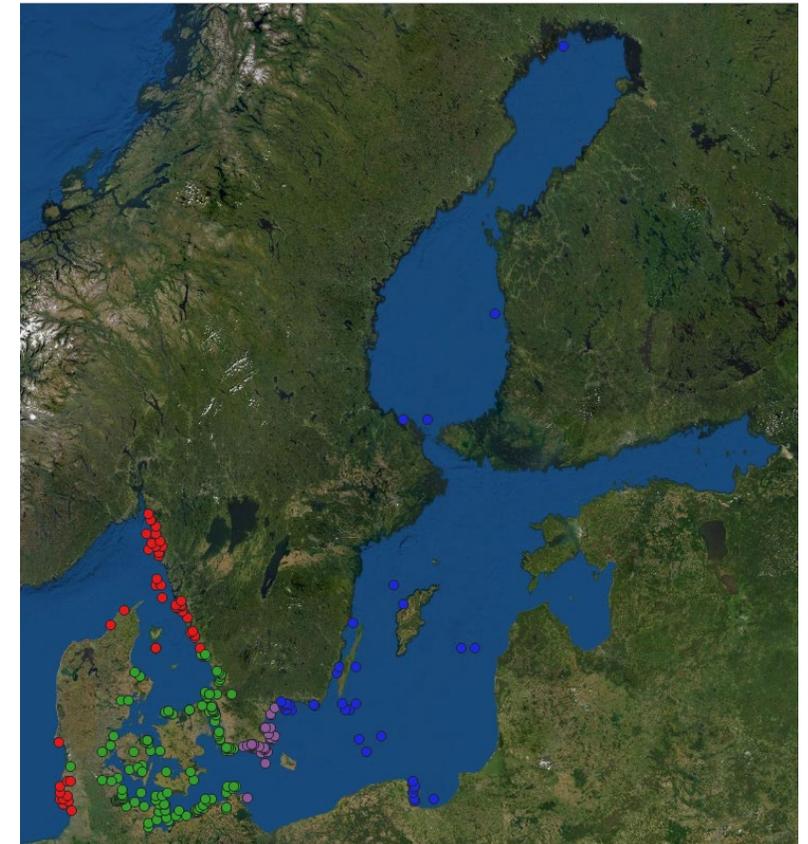
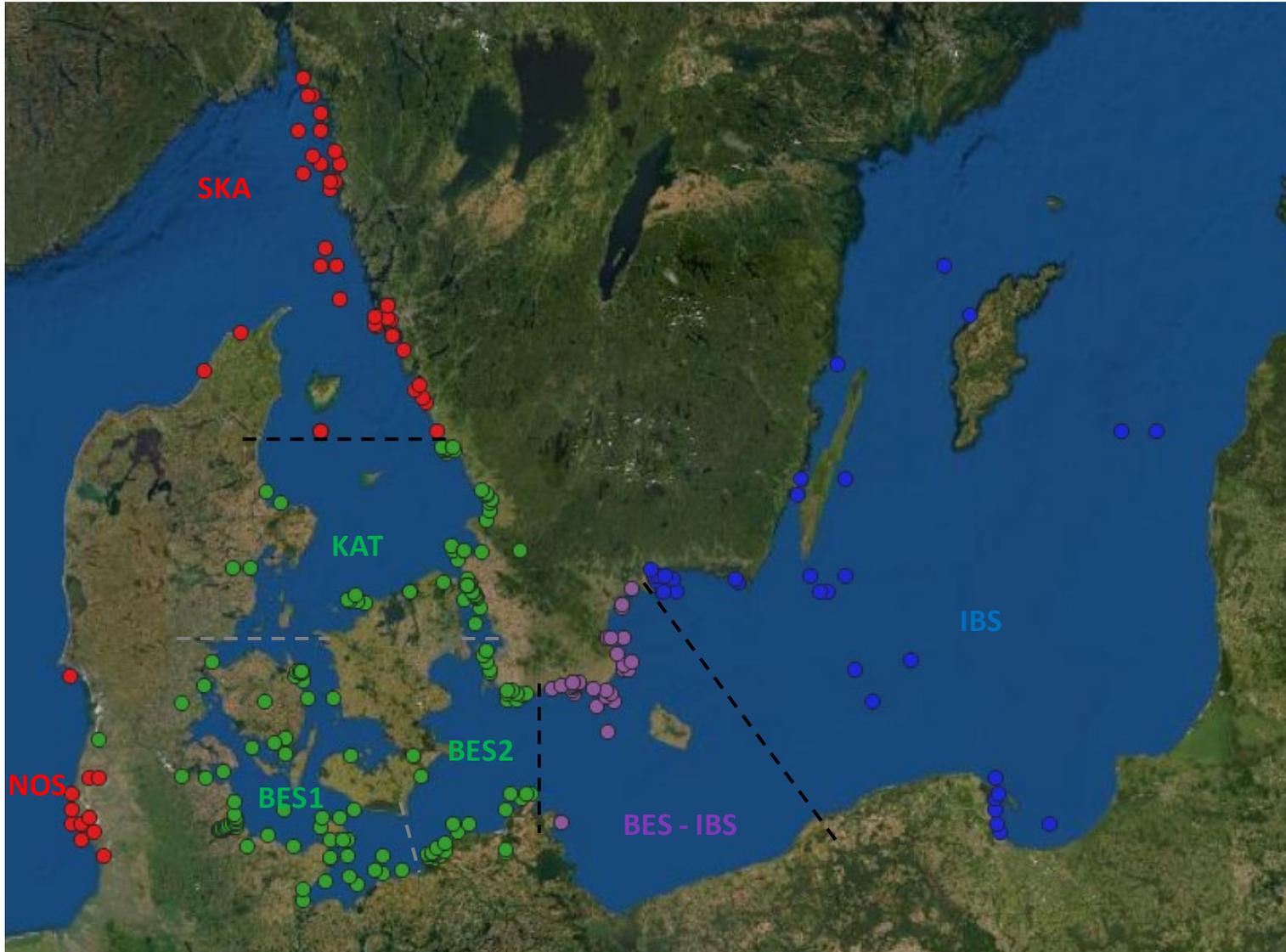
Juno



EP1

- **Population genetic analysis:** Structure, pairwise Fst, DAPC...

Fluidigm – 282 porpoises

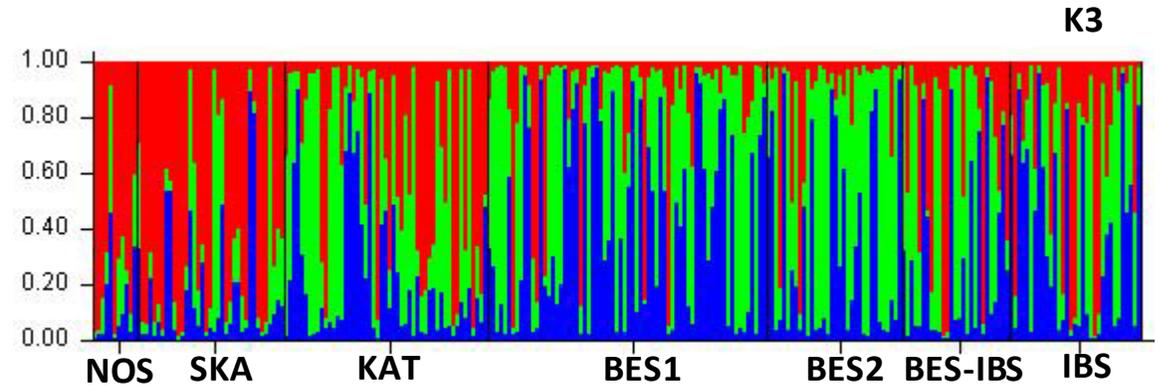
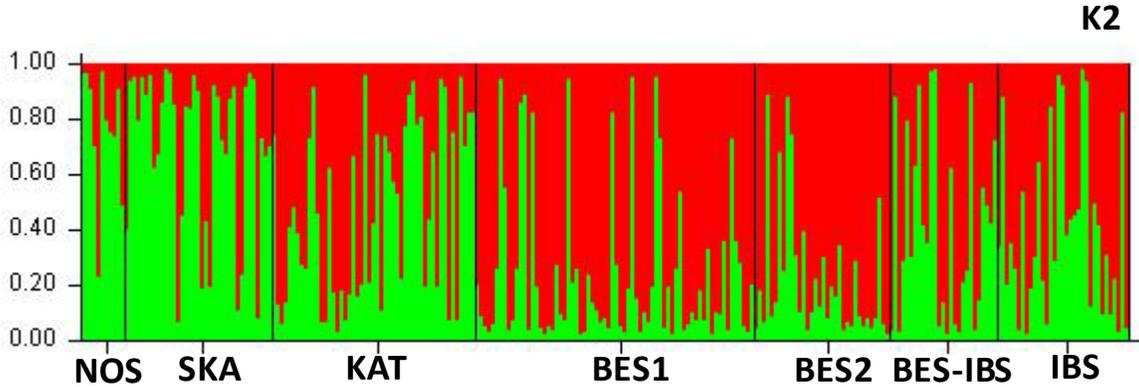


- **NOS/SKA:** 63 porpoises (37)
 - **NOS:** 19 porpoises (10)
 - **SKA:** 44 porpoises (27)
- **KAT/BES:** 150 porpoises (107 mating season)
 - **KAT:** 44 porpoises (26)
 - **BES1:** 66 porpoises (54)
 - **BES2:** 40 porpoises (27)
- **BES-IBS:** 31 porpoises (15)
- **IBS:** 37 porpoises (15)

(x) Porpoise “collected” in May-October

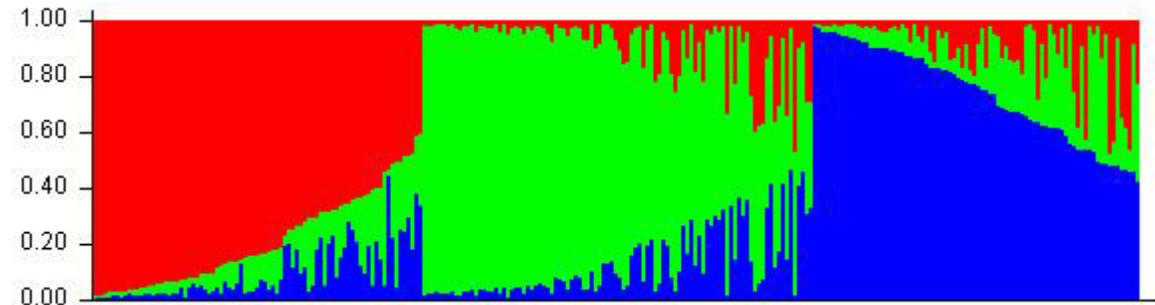
Fluidigm preliminary results – STRUCTURE

- 263 out 282 samples successfully genotyped at 93 SNPs



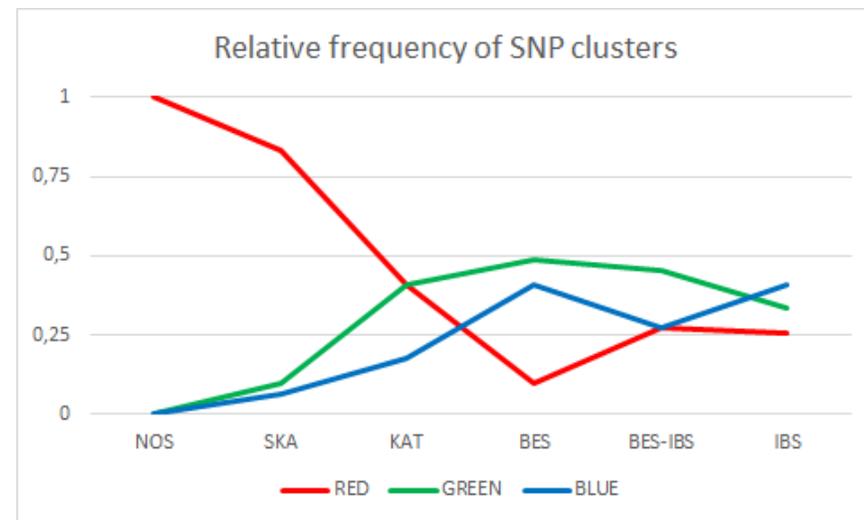
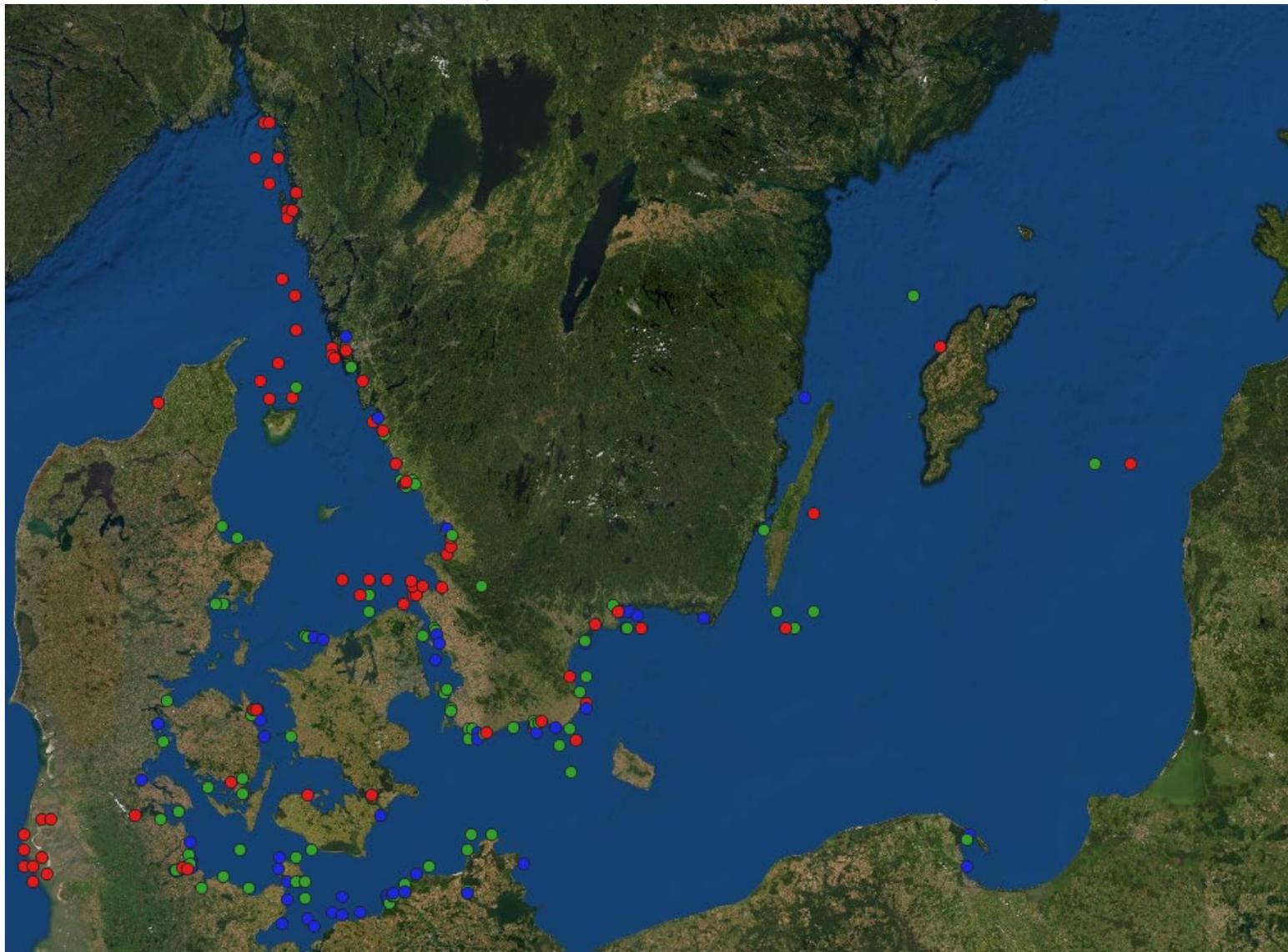
Proportion of membership of each pre-defined population in each of the 3 clusters

%ANCESTRY	RED	GREEN	BLUE	INDs
NOS	0.718	0.150	0.132	11
SKA	0.642	0.184	0.174	37
KAT	0.354	0.419	0.227	51
BES1	0.149	0.417	0.434	70
BES2	0.128	0.539	0.333	34
BES-IBS	0.302	0.413	0.285	27
IBS	0.294	0.334	0.372	33



Samples assigned to K3

- 93 FLUIDIGM SNPs. Samples > 60% inferred ancestry to any K

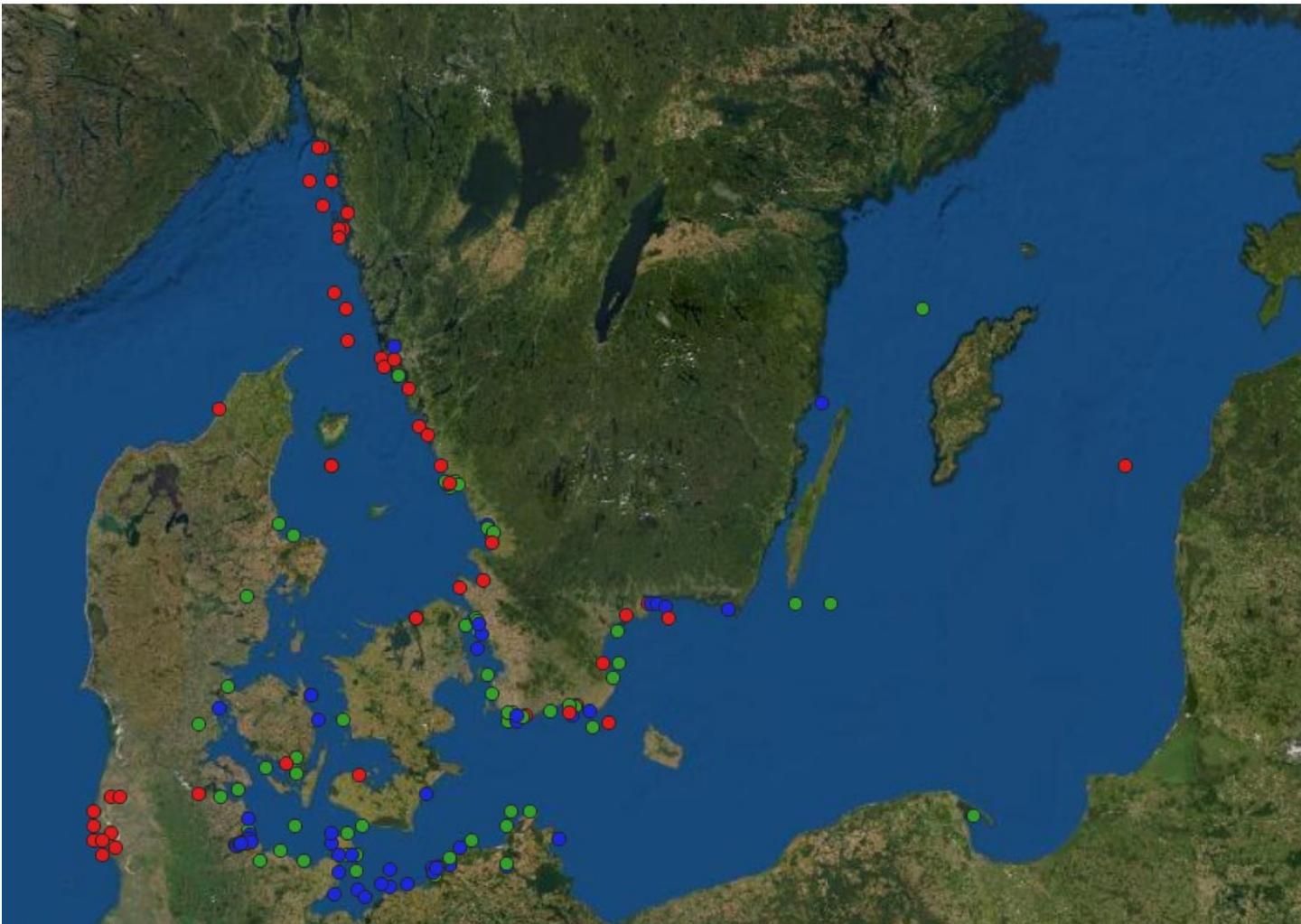


Number of samples assigned to each K (> 60% inferred ancestry)

	RED	GREEN	BLUE	% SAMPLES
NOS	9	-	-	100% RED
SKA	25	3	2	83,3% RED
KAT	16	16	7	41% RED / 41% GREEN
BES	9	44	37	48,8% GREEN / 41% BLUE
BES-IBS	6	10	6	45,5% GREEN / 27,25% BLUE
IBS	7	9	11	40% BLUE / 33% GREEN

Samples assigned to K3 – From May to October

- 93 FLUIDIGM SNPs. Samples > 60% inferred ancestry to any K



- Geographical pattern becomes slightly clearer:
- Red increases in SKA
- Red decreases and Green increase in KAT
- Blue increases and Green decreases in IBS

	RED	GREEN	BLUE	% SAMPLES
NOS	9	-	-	≈ 100% RED
SKA	24	2	1	↑ 92,3% RED
KAT	9	12	5	↓ 34,6% RED / 46,15% GREEN ↑
BES	7	39	35	≈ 48,1% GREEN / 43,2% BLUE ≈
BES-IBS	5	7	3	≈ 46,6% GREEN / 20% BLUE ↓
IBS	4	4	7	↑ 46,6% BLUE / 26,6% GREEN ↓

SNP Panel Interpretation

North Sea → Red very CLEAR!

Belt Sea → Green

Baltic Proper → Blue

- KAT is a transition zone between RED and GREEN
- Blue is very rare in NOS-SKA-KAT

• Promising preliminary results

- Pipeline to create SNP Panel is working
- Fluidigm works with porpoise samples
- Indication of 3 Baltic clusters, but not always exhibit geographic separation due to migration

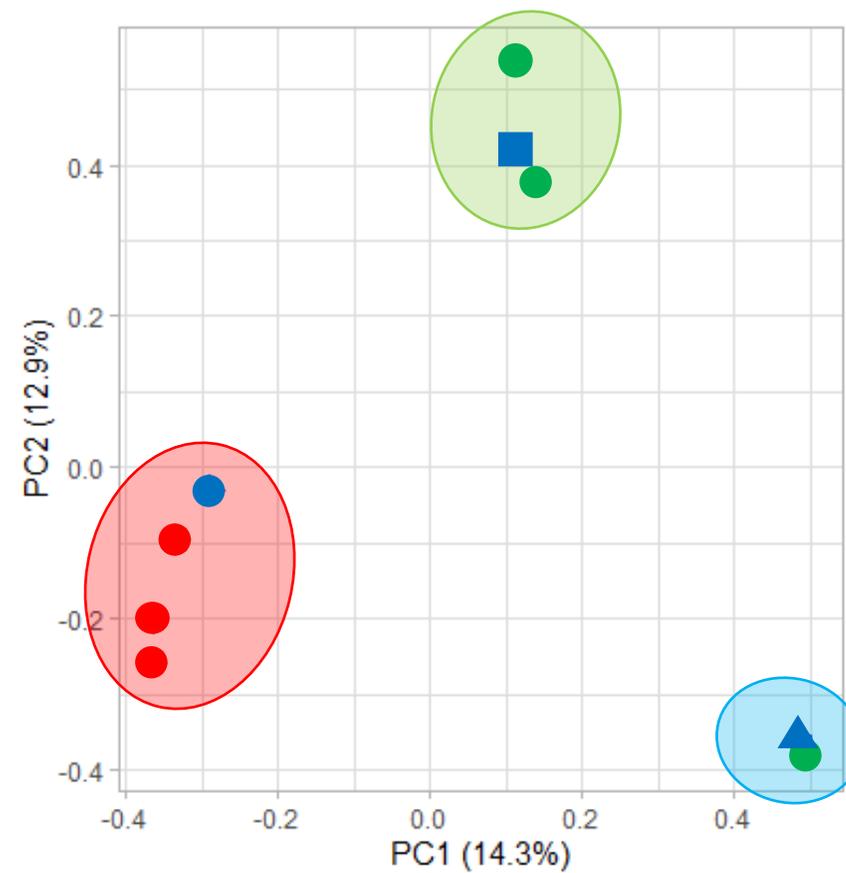
• Room for improvement

- Select SNPs per genetic cluster, rather than geographic location
- More samples from Baltic Proper in the SNP Discovery step
- 218/263 porpoise assigned (82%) → AIM >95%
- Bigger batch of samples

Whole Genome Resequencing – Pilot Study



- 9 genomes, 3 per region
- Reads mapped to reference genome
- 3,873,977 filtered SNPS



Future plan – Further WGS

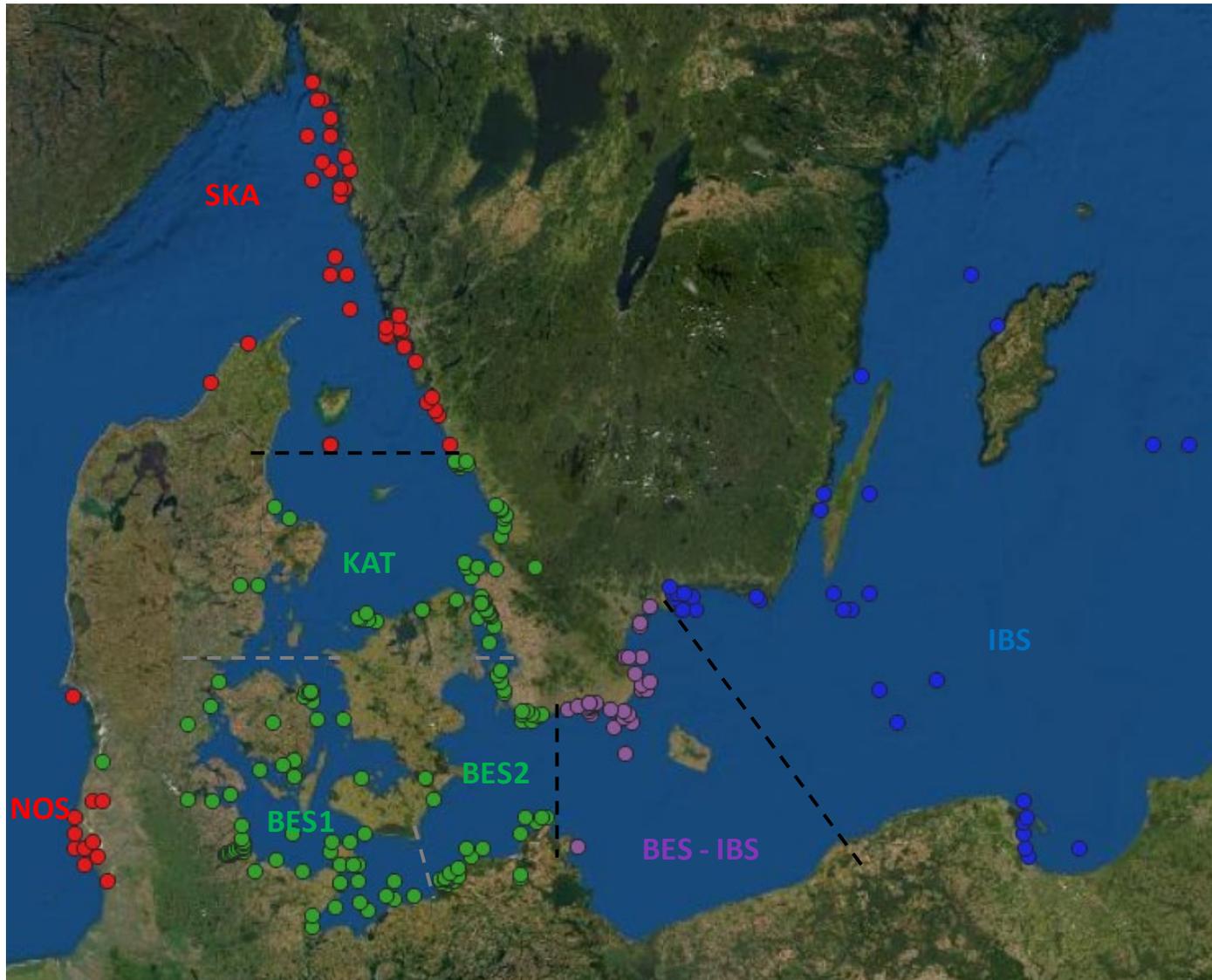


- 10 individuals high confidence (>80%) red cluster
- 20 individuals high confidence (>80%) green cluster
- 20 individuals high confidence (>80%) blue cluster
- Call SNPs and identify Baltic population structure
- Select SNPs with highest F_{ST} among three Baltic clusters
- Run Fluidigm for big batch of samples (>500)

Thanks for listening!
Muchas gracias! Danke schön! Tack så mycket!



Population differentiation – Fst Values



	NOS	SKA	KAT	BES1	BES2	BES-IBS	IBS
NOS	-						
SKA	0.02657	-					
KAT	0.05668 *	0.02501 *	-				
BES1	0.11451 *	0.06304 *	0.02447 *	-			
BES2	0.12237 *	0.07618 *	0.03173 *		-		
BES-IBS	0.06569 *	0.07618 *	0.01291	0.01630	0.02286	-	
IBS	0.08468 *	0.05077 *	0.02348	0.02733 *	0.04643 *	0.01183	-

	NOS/SKA	KAT/BES	BES-IBS	IBS
NOS/SKA	-			
KAT/BES	0.05273 *	-		
BES-IBS	0.04305 *	0.01099	-	
IBS	0.05699 *	0.02500 *	0.01183	-

Association between Fluidigm SNPs and Mt haplotypes

	RED	GREEN	BLUE
Phopho_1*	23	15	13
Phopho_4	2	-	-
Phopho_7*	13	29	26
Phopho_11*	-	-	3
Phopho_13	2	-	-
Phopho_14*	-	3	-
Phopho_19	1	-	-
Phopho_23	1	-	-
Phopho_24	2	-	-
Phopho_27	1	-	-
Phopho_30	-	-	1
Phopho_45	1	-	-
Phopho_70	1	-	-
Phopho_157	1	-	-

- Phopho_1 decreases when entering the Baltic
- Phopho_7 increases
- Phopho_14 restricted to Green
- Phopho_11 restricted to Blue
- No clear difference in Green-Blue clusters