# SNPs IMPROVE POPULATION RESOLUTION FOR THE HARBOUR PORPOISE (*Phocoena phocoena*)

Tiedemann, R., Autenrieth, M., Havenstein,K., Lah, L., Hartmann, S., Benke, H., Pawliczka, I., Roos, A., Siebert, U., Dennis, A.B.

THE HARBOUR PORPOISE IN THE BALTIC SEA

375,000
Atlantic Shelf

SKA

KAT

NOS

IBS

< 500

BES

40,000
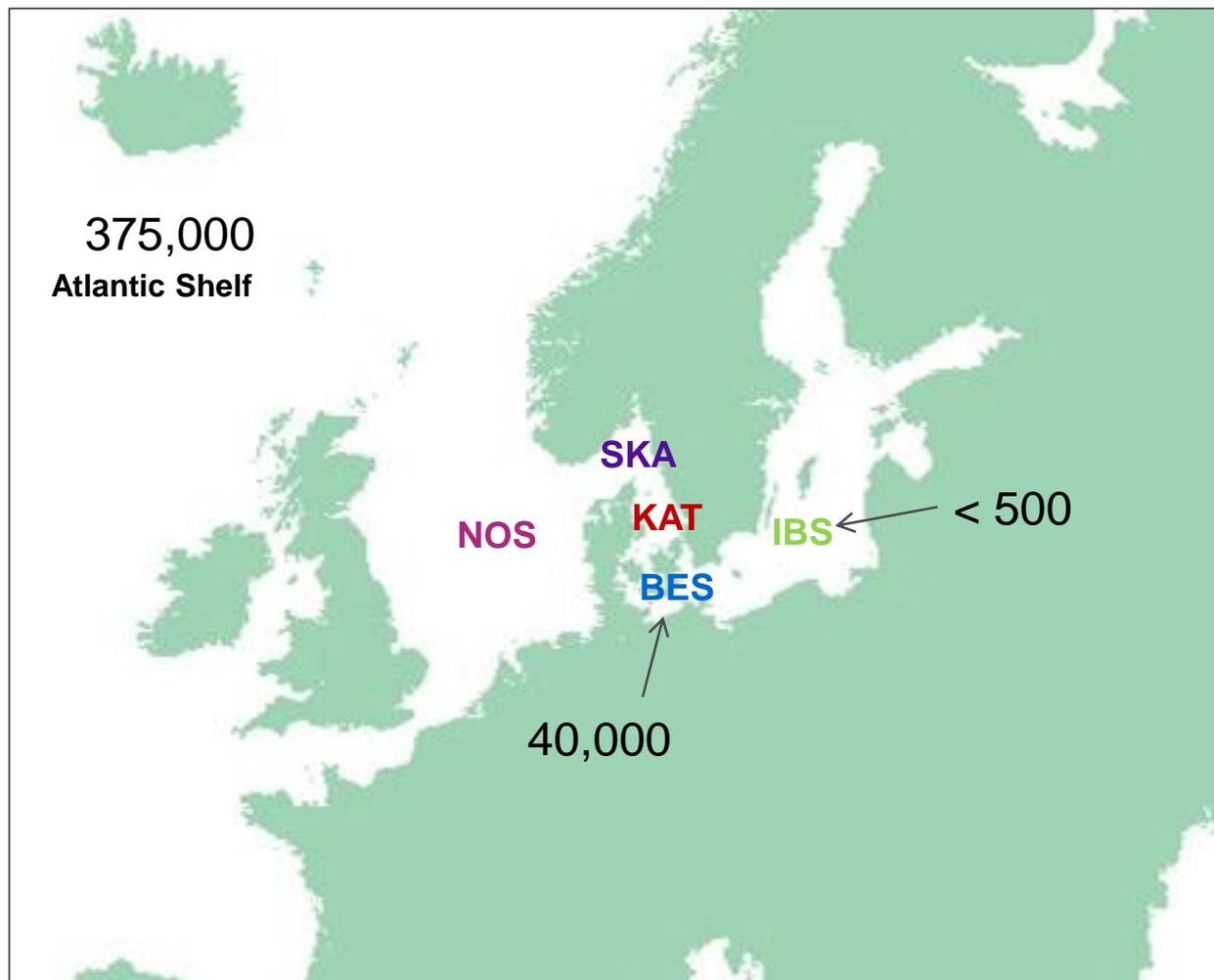
North Sea - **NOS**
Skagerrak - **SKA**
Kattegat - **KAT**
Belt Sea - **BES**
Inner Baltic Sea - **IBS**

Baltic harbour porpoise **CR**
(Phocoena phocoena)

HARBOR PORPOISE IN THE BALTIC SEA

Short Communication

Oryx 2008

Conservation genetics without knowing what to

PLOS ONE   2017
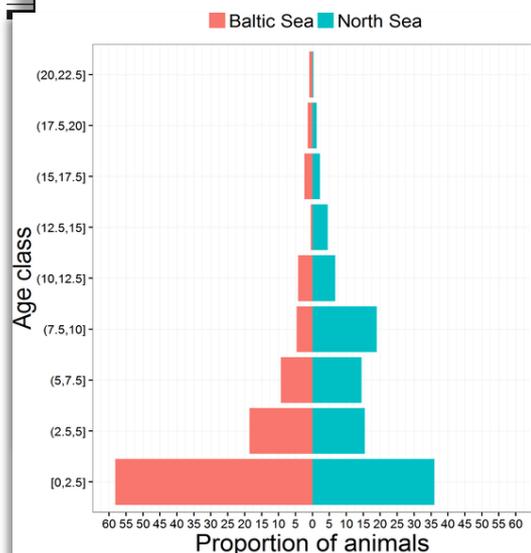
RESEARCH ARTICLE

Coming of age: - Do female harbour porpoises (*Phocoena phocoena*) from the North Sea and Baltic Sea have sufficient time to reproduce in a human influenced environment?

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, Stefan Bräger[1,2], Michael Dähne[1,4], Anja Gallus[1], Sophie Hansen[1], Christopher G. Honnef[1], Martin Jabbusch[1], Jens C. Koblitz[1,*], Kathrin Krügel[1], Alexander Liebschner[3], Ingo Narberhaus[3], Ursula K. Verfuß[1,5]

INDICATION FOR POPULATION STRUCTURING

- subpopulations arose during end of the last glacial period (~7000yra)
  - → North Sea populations recolonized the Baltic Sea

<u>acoustic data</u>
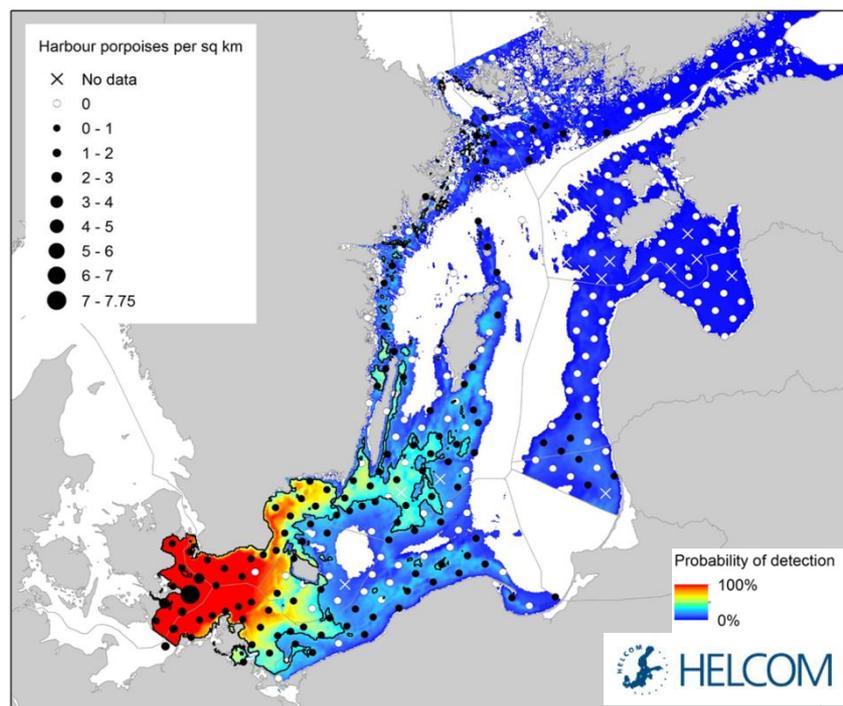
<u>morphometric data</u>

- NOS-KAT vs. Belt Sea
- Belt Sea vs. IBS

<u>behavioral data</u>

habitat use:
- food availability
- activity patterns

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INDICATION FOR POPULATION STRUCTURING

GENETIC DATA ?

- mtDNA, microsatellites:
  - pooled data: structure between population detectable
  - individual level: no clear pattern

- mtDNA haplotypes:
  - inner Baltic Sea private haplotypes (HT)
 - indicative haplotype HT7 for Belt Sea (Wiemann et al. 2010)

AIM

further investigate inner Baltic population structure with SNP markers

One panmictic Baltic population   *vs*   different subpopulations ?

INITIAL STUDY - SNP ANALYSIS DETECTING SUBTLE POPULATION STRUCTURE

main findings

- SNPs (~1000) outperform microsatellites (~15)

- three main groupings:
  - Black Sea
  - North Atlantic
  - Baltic Sea

- notable distinction between **Belt Sea** and **Inner Baltic Sea**
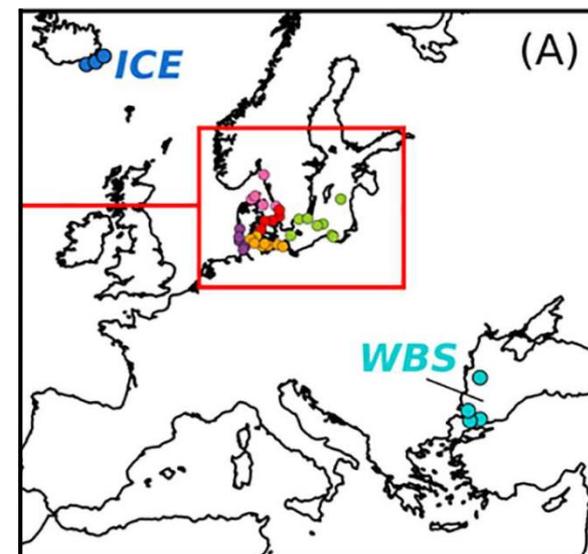
SUB-REGIONS	SAMPLES
Western Black Sea (WBS)	4
Iceland (ICE)	3
North Sea (NOS)	6
Skagerrak-Kattegat (SK1)	5
Kattegat-Belt Sea 1 (KB1)	6
Belt Sea 2 (BES2)	10
Inner Baltic Sea (IBS)	10

Lah et al. 2016

STUDY SET UP

- **samples**: 196+37=233
  after filtering: 109 (+37)

- **markers**:
  - mtDNA haplotypes
  - 15 microsatellites
  - 2518 new SNP loci
    (+1874 SNPs)
    present in 95% of individuals
    (≥ 10reads/locus/individual)

- **methods**:
  - PCA and sPCA
  - STRUCTURE
  - association study

- **aim**: **BES** vs. **IBS**

SKA (10)

KAT (19)

NOS (20)

Denmark

IBS (21)

BES (39)

North Sea - **NOS**
Skagerrak - **SKA**
Kattegat - **KAT**
Belt Sea - **BES**
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SNP discovery using RADseq

A

RAD sequencing

✗ Rare cut site
✗ Common cut site
Genomic interval present in library
Sequence reads

Individual 1
Genomic DNA
Individual 2

B

double digest RADseq

Individual 1
Genomic DNA
Individual 2

a

b
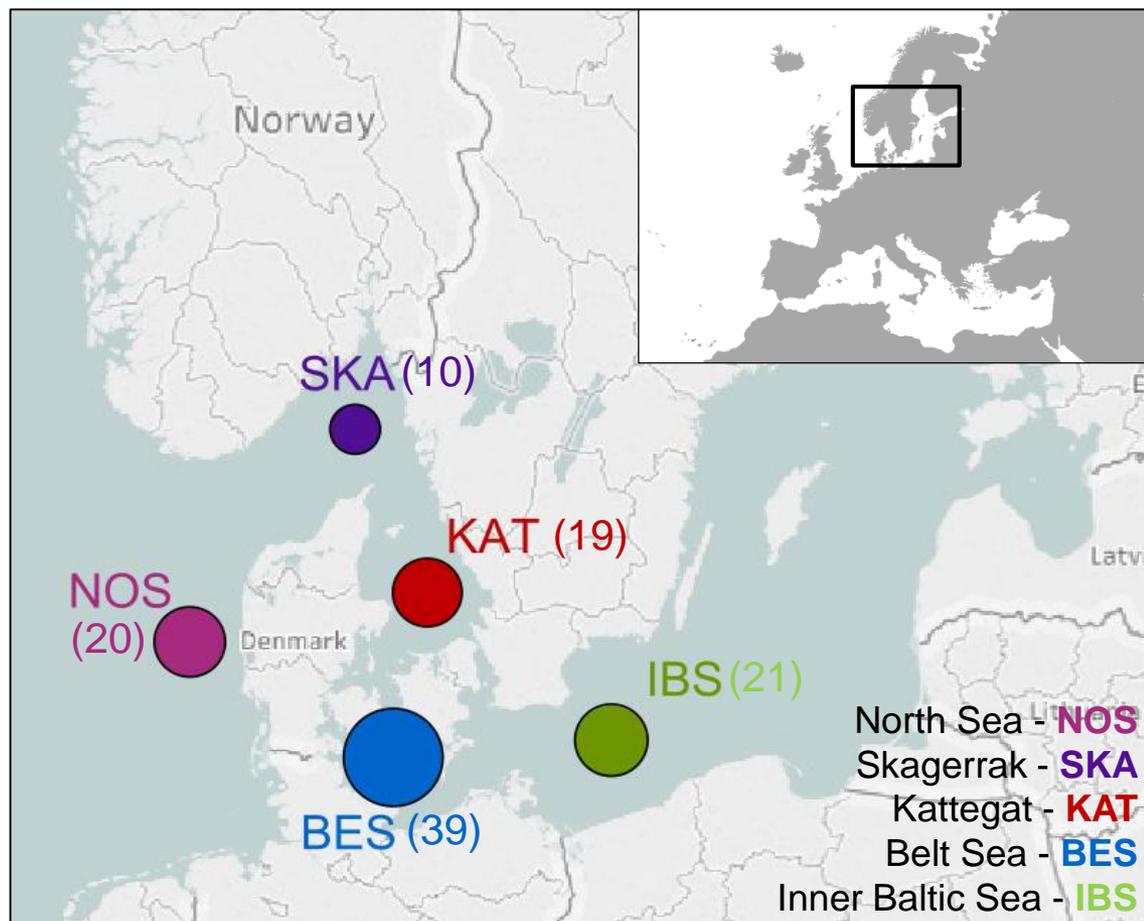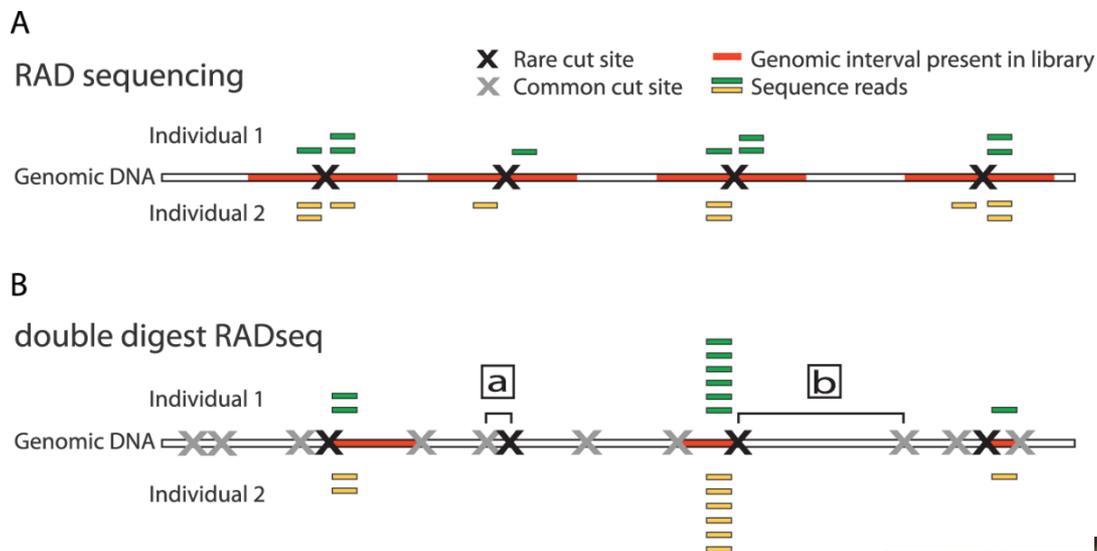
Double digest RAD sequencing improves efficiency and robustness while minimizing cost.

Challenges:
Sample and DNA quality

SNP discovery using RADseq

233 samples
100 bp Paired End reads
CUTTERS: MspI, PstI
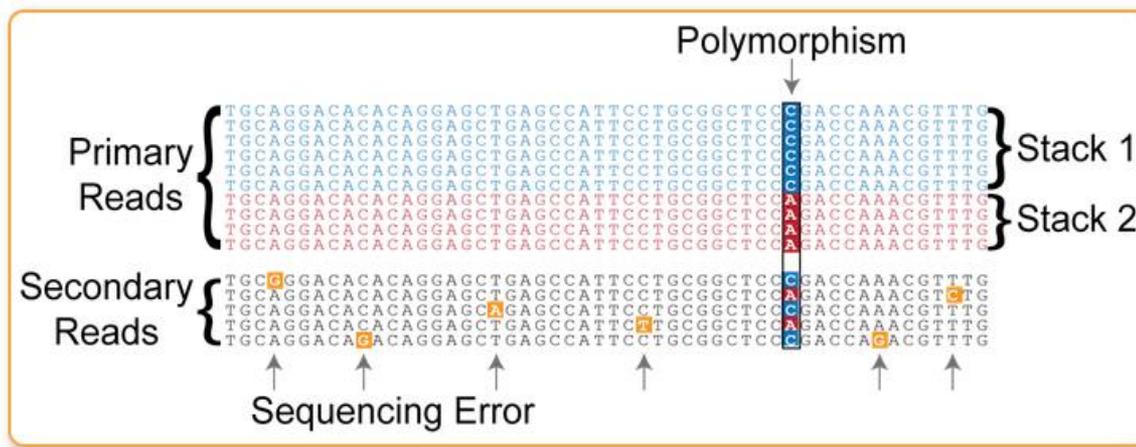
FIXATION COEFFICIENTS BASED ON 2518 SNPs

	all data (109)	females (74)	reproduction time (74)
between areas	0.022 ***	0.013*	0.013 *
NOS vs. SKA	0.018*	0.010	0.012
SKA vs. KAT	0.024**	0.002	0.002
KAT vs. BES	0.021***	0.011(*)	0.013(*)
BES vs. IBS	0.019***	0.006	0.006

significance: (*) $p<0.1$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

➢ differentiation shown in SNPs, also in microsatellites and mtDNA
(Wiemann et al. 2000, Lah et al. 2016)

# STRUCTURE PLOTS

All 5 populations

BES vs. IBS

all data
(n= 109 | 60)
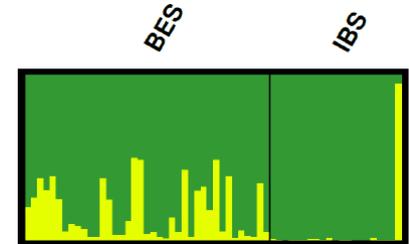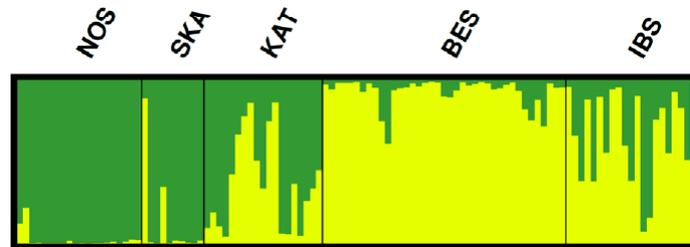
only female
(n= 74 | 36)
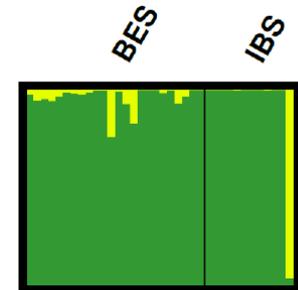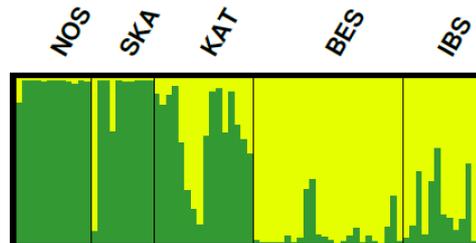
only reproduction
time (April-September)
(n= 74 | 40)
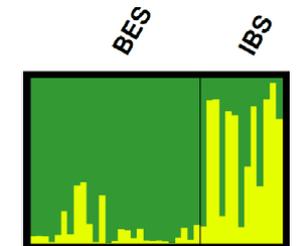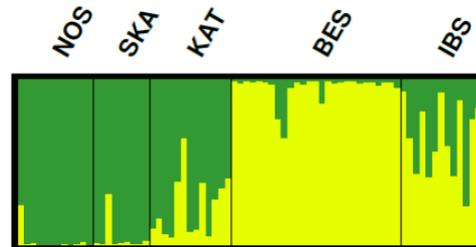
SPATIAL PRINCIPAL COMPONENT ANALYSIS

## complete dataset (5 areas)

among areas

within areas

only BES and IBS

among areas
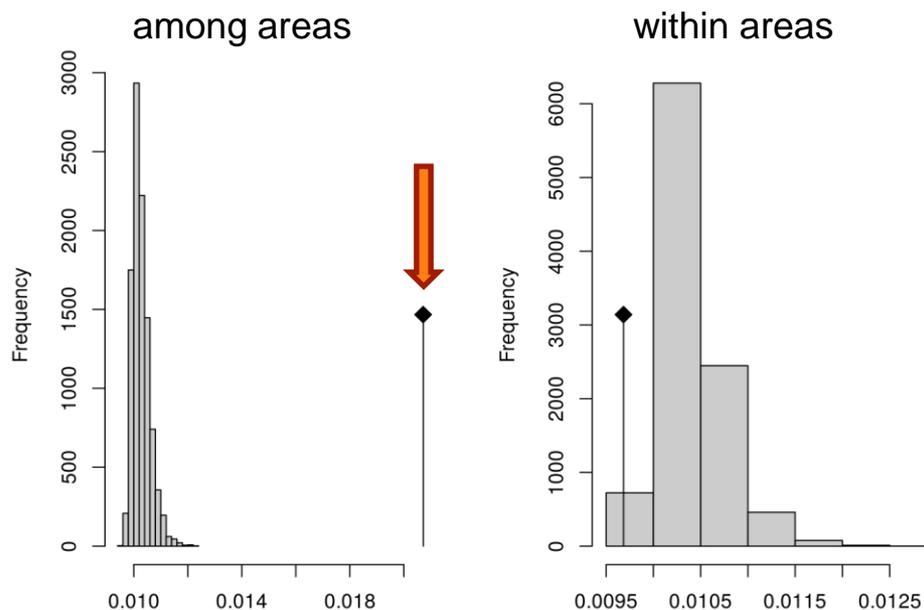
within areas

- ➤ significant structure only between areas
- ➤ no structure within areas detectable
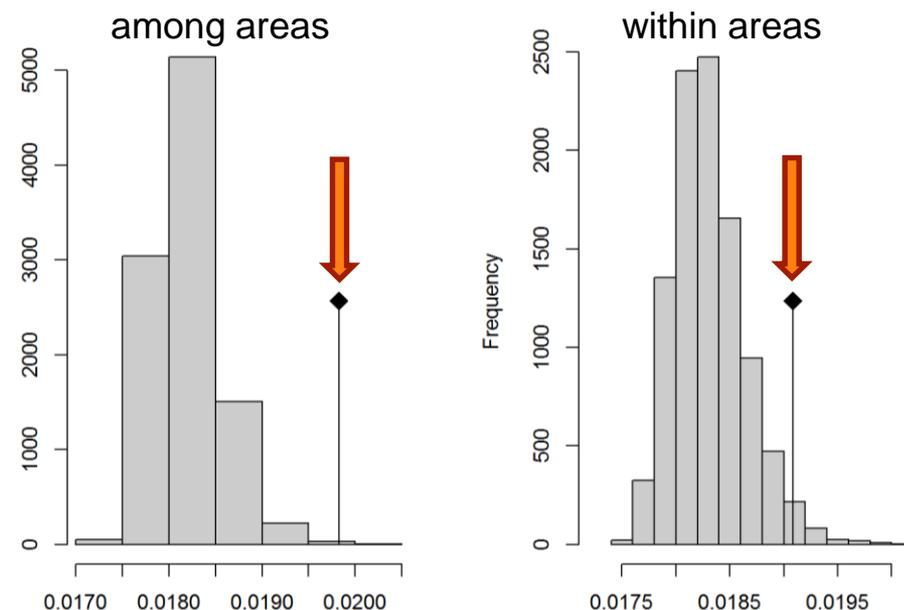
- ➤ significant **between** and **within** areas
- ➤ indicates two subpopulations and seasonal migration

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PCA ANALYSIS OF ALL 5 POPULATIONS

SNPs: 1. and 2. PCA-axis
(mean values & standard error)
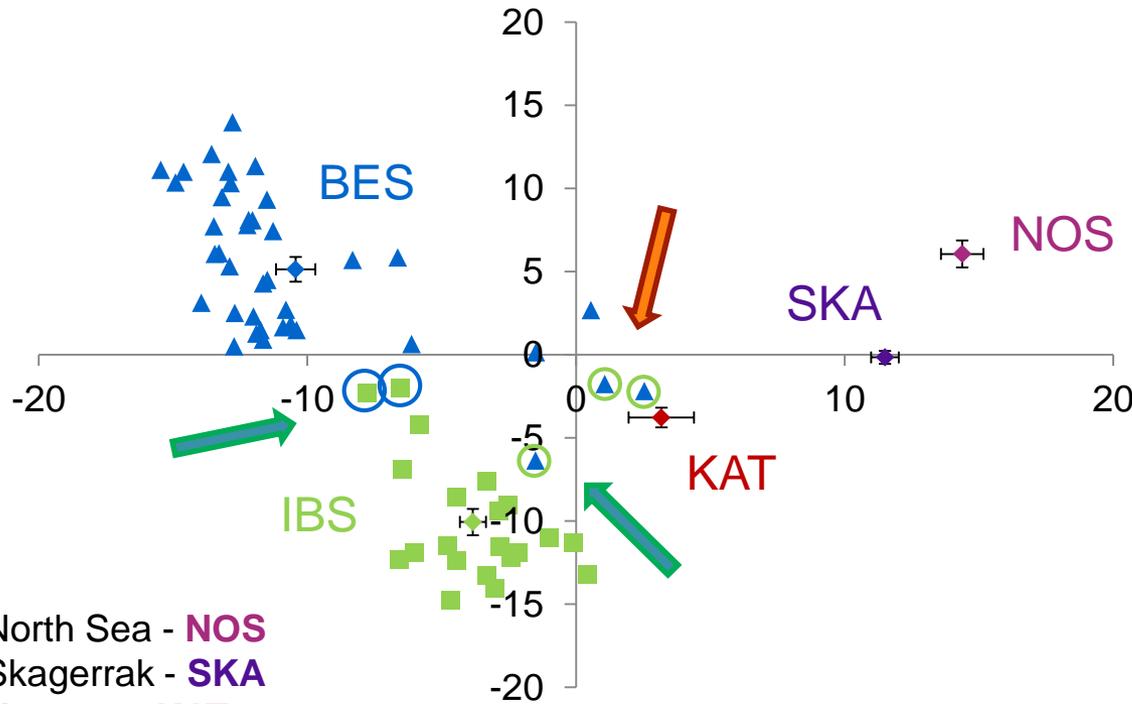
**SNPs: 1. and 3. PCA-axis**
(mean values & standard error)

SNPs: 2. and 3. PCA-axis
(mean values & standard error)

North Sea - **NOS**
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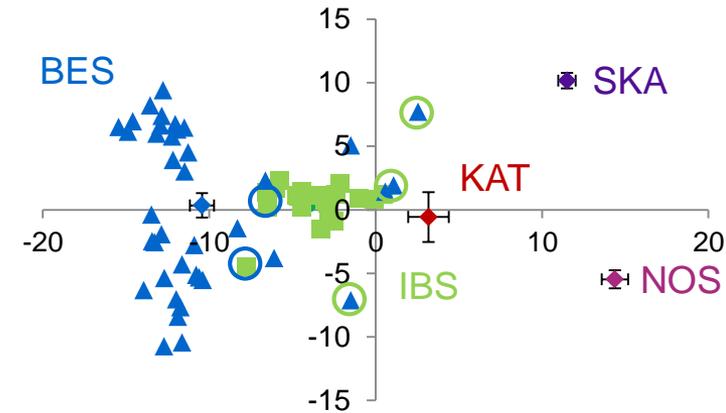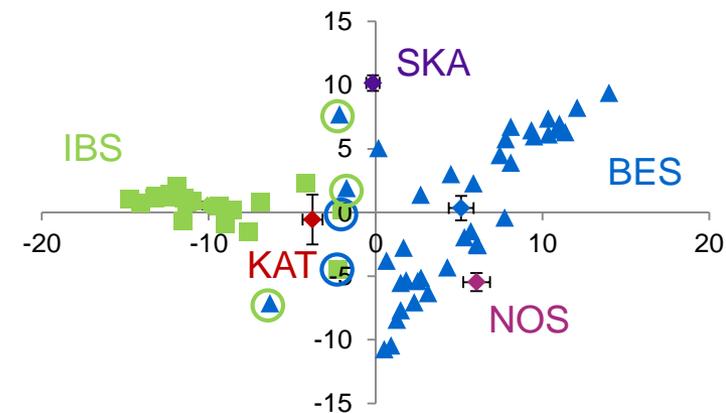
ANALYSIS FOR ONLY IBS -BES

- analysis of 60 Samples from the Baltic Sea
  - accociation analysis
  - sPCA analysis

→

↓

● BES
● IBS
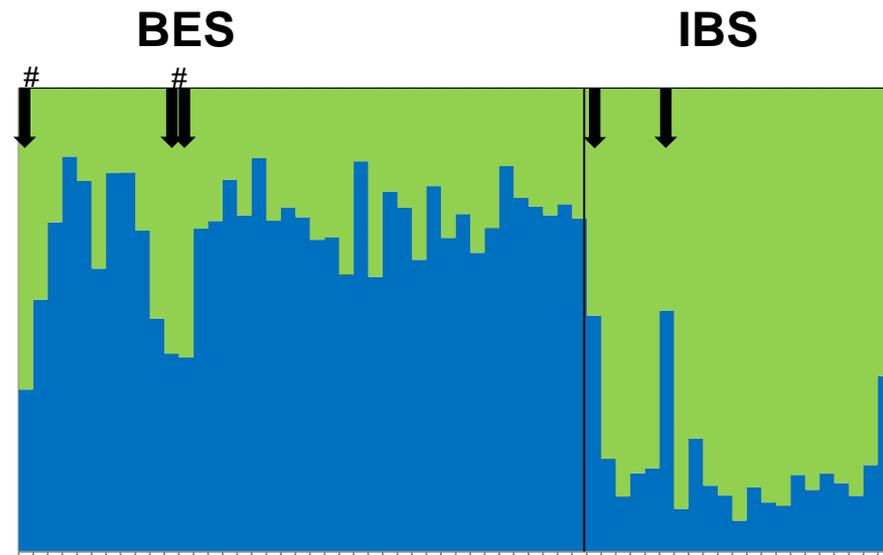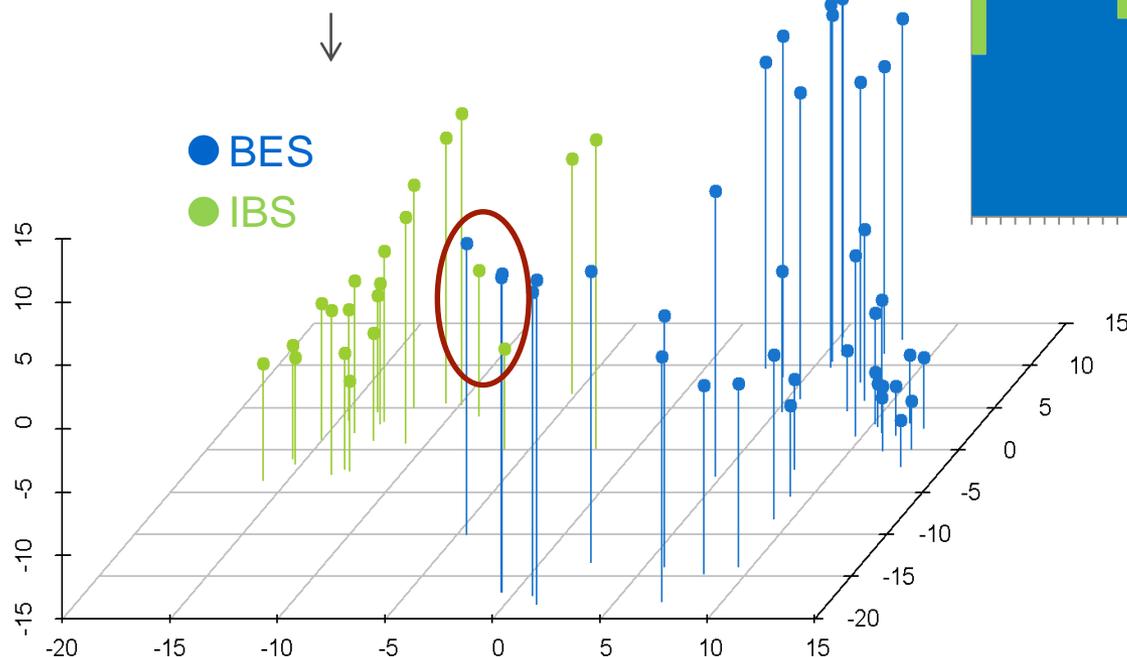
**BES**   **IBS**

→ two groups can be distinguished

→ Migrants can be detected

BES-IBS INDIVIDUAL ASSIGNMENT BASED ON SNP DATA

assignment	Occurrence		mitochondrial haplotype	
	BES	IBS	haplotype 7	other haplot.
Alle Daten				
west Baltic subpopulation	**39 (49)**	2 (7)	**19 (28)**	12 (19)
central Baltic subpopulation	0 (0)	**14 (17)**	4 (5)	**9 (11)**
Fisher's exact test:	p<0.001*** (p<0.001***)		p=0.050* (p=0.036*)	
Only females				
west Baltic subpopulation	**24 (34)**	2 (4)	**13 (21)**	7 (12)
central Baltic subpopulation	0 (0)	**8 (10)**	2 (3)	**5 (6)**
Fisher's exact test:	p<0.001*** (p<0.001***)		p=0.094(*) (p=0.084(*))	
only reproductive season (April-September)				
west Baltic subpopulation	**27 (36)**	1 (3)	**12 (18)**	9 (14)
central Baltic subpopulation	0 (0)	**8 (11)**	2 (3)	**6 (8)**
Fisher's exact test:	p<0.001*** (p<0.001***)		p=0.106ns (p=0.074(*))	

BES-IBS INDIVIDUAL ASSIGNMENT BASED ON SNP DATA

total = #78
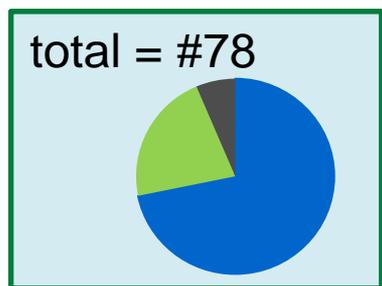
sampling location

mtDNA haplotype occurrence

SNP-BES | SNP-IBS

SNP-BES | SNP-IBS

all data

***

*
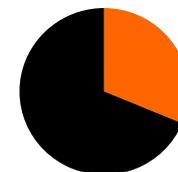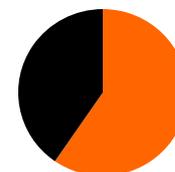
only female

***

(*)
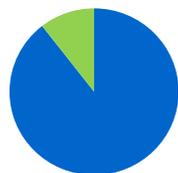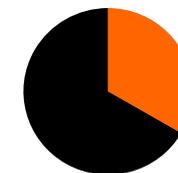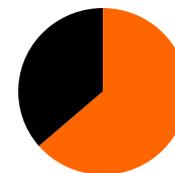
only reproduction time (April-September)

***

(*)
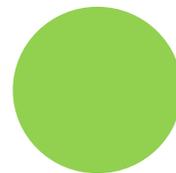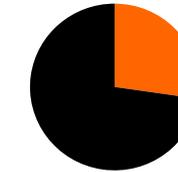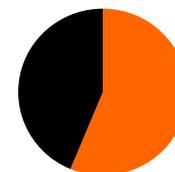
significance: (*) p≤0.1; * p≤0.05; *** p≤0.001

sampled in BES: ■ IBS: ■

HT 7: ■ other HT: ■

NEXT STEPS

- identify candidate SNPs for SNP panel (for ca. 200+ additional samples)

GATK	• mapping RAD-Seq - per individual
Fluidigm	• design initial Fluidigm's SNPtype™ assays
SNP panel	• decide on final SNP panel → apply to new samples
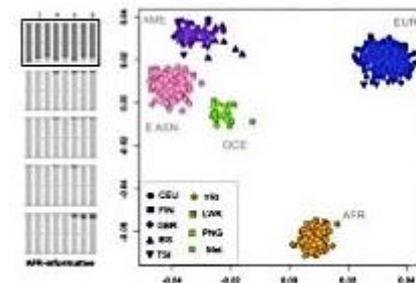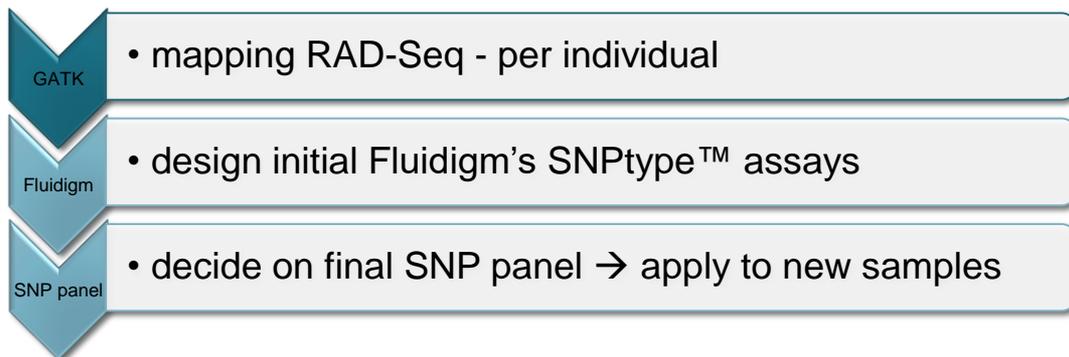
- different ecotypes North Atlantic vs Baltic Sea
  - ➤ local adaptation → underling genes ?

consensus	• extract SNP-ID + sequence from STACKS
BLAST	• identify coding region and gene identity
PAML	• search for selection → SNP
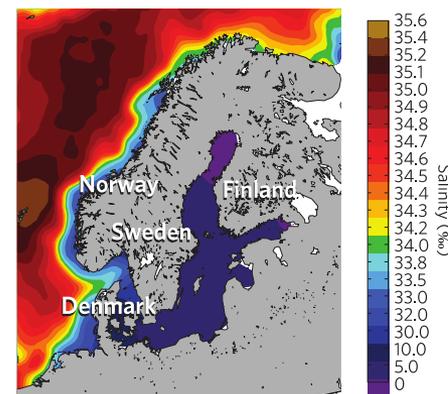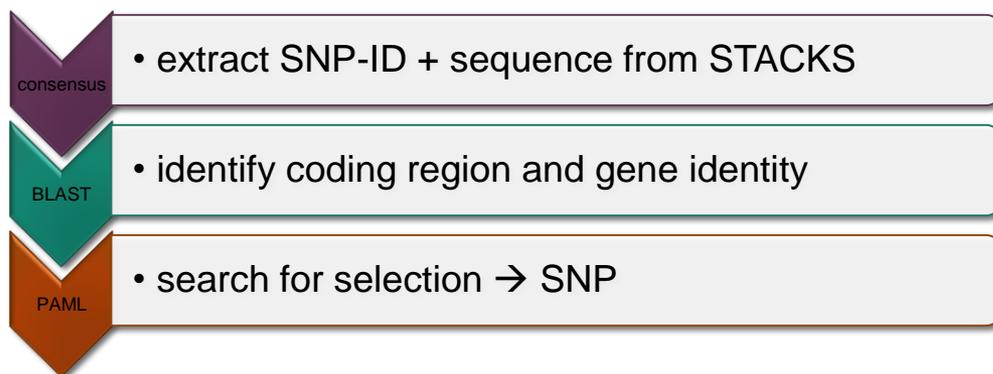
Paasche et al. 2015

CONCLUSION

SNP as genetic marker

- superior resolution

  ➤ underscores value of conservation genomics

- provides detailed information for any individual

  ➤ inference of migrants/residents

  ➤ assessment of admixture/genetic exchange

the Baltic harbour porpoise

- splits: Kattegat - Belt Sea - inner Baltic Sea
  - all data
  - only females
  - only reproduction time

  ➤ genetics support Inner Baltic population split

  ➤ conservation measures specific for IBS needed

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THANK YOU FOR YOUR ATTENTION …

… QUESTIONS OR COMMENTS ?

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