Southern Ocean Research Partnership

South Pacific Whale Research Consortium

Photo courtesy
of P. Ensor

Accessing the molecular archive - wide and deep, past and future

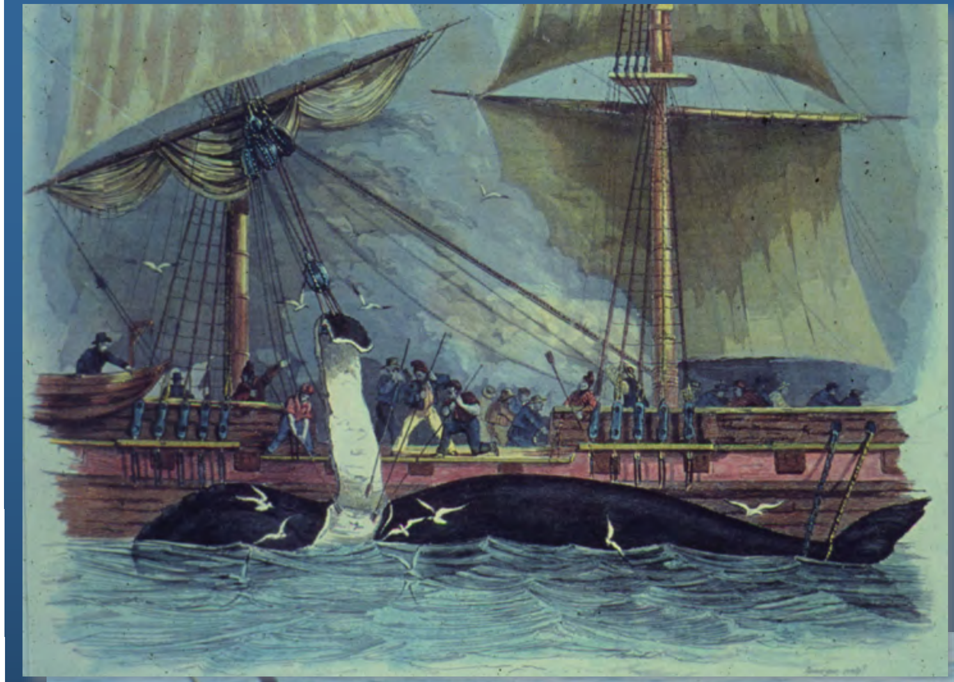
Scott Baker
Oregon State University and University of Auckland

"It is a great time to be a geneticist" *Peter and Rosemary Grant*

Photo courtesy of B. Skerry

“It is a great time to be a cetacean geneticist”

Photo courtesy of B. Skerry

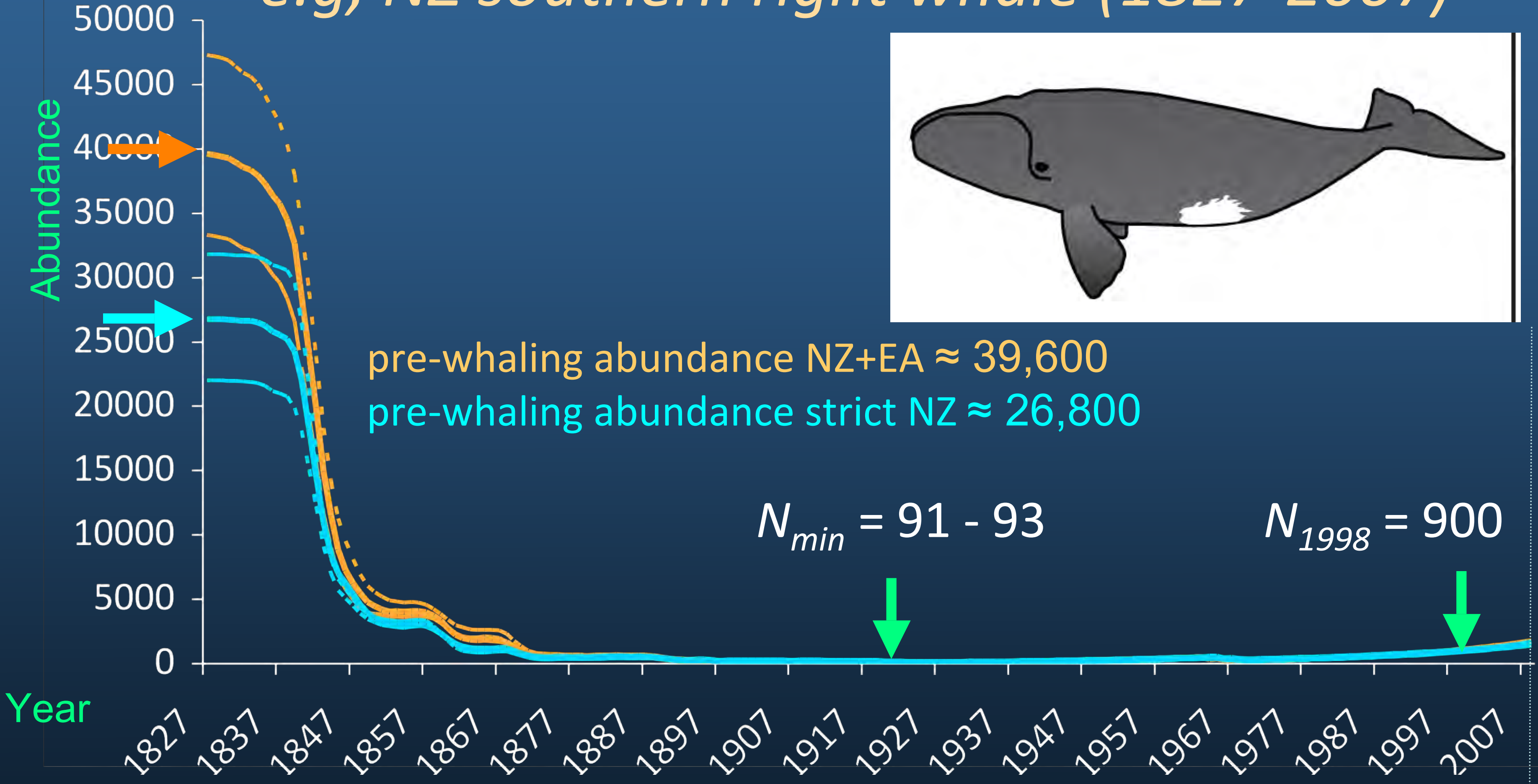


Two centuries of ‘remorseless havoc’ have ended

- 150,000 right whales killed from 1770-1870
  - Some local populations were extirpated and reducing others to >1% of former abundance
- 2,000,000 fin, blue, humpback and sperm whales killed from 1904-1972
  - Reducing some populations to >0.1% of former abundance

Recovery from the brink of extinction

e.g, NZ southern right whale (1827-2007)



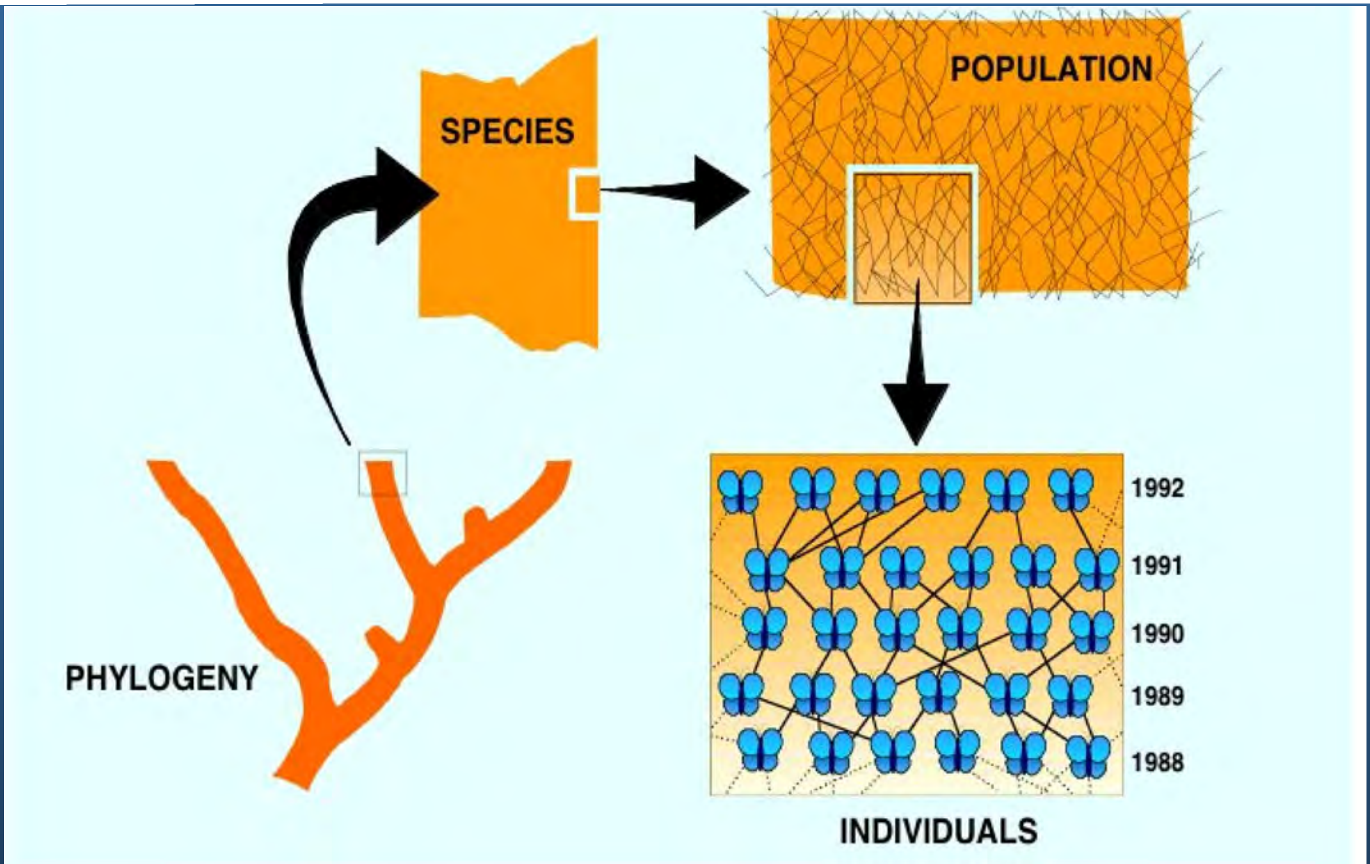
pre-whaling abundance NZ+EA ≈ 39,600

pre-whaling abundance strict NZ ≈ 26,800

N_{min} = 91 - 93

N_{1998} = 900

Jackson, Carroll, Smith Patenaude and Baker, ‘Taking Stock’



genes in time and space

Evolutionary

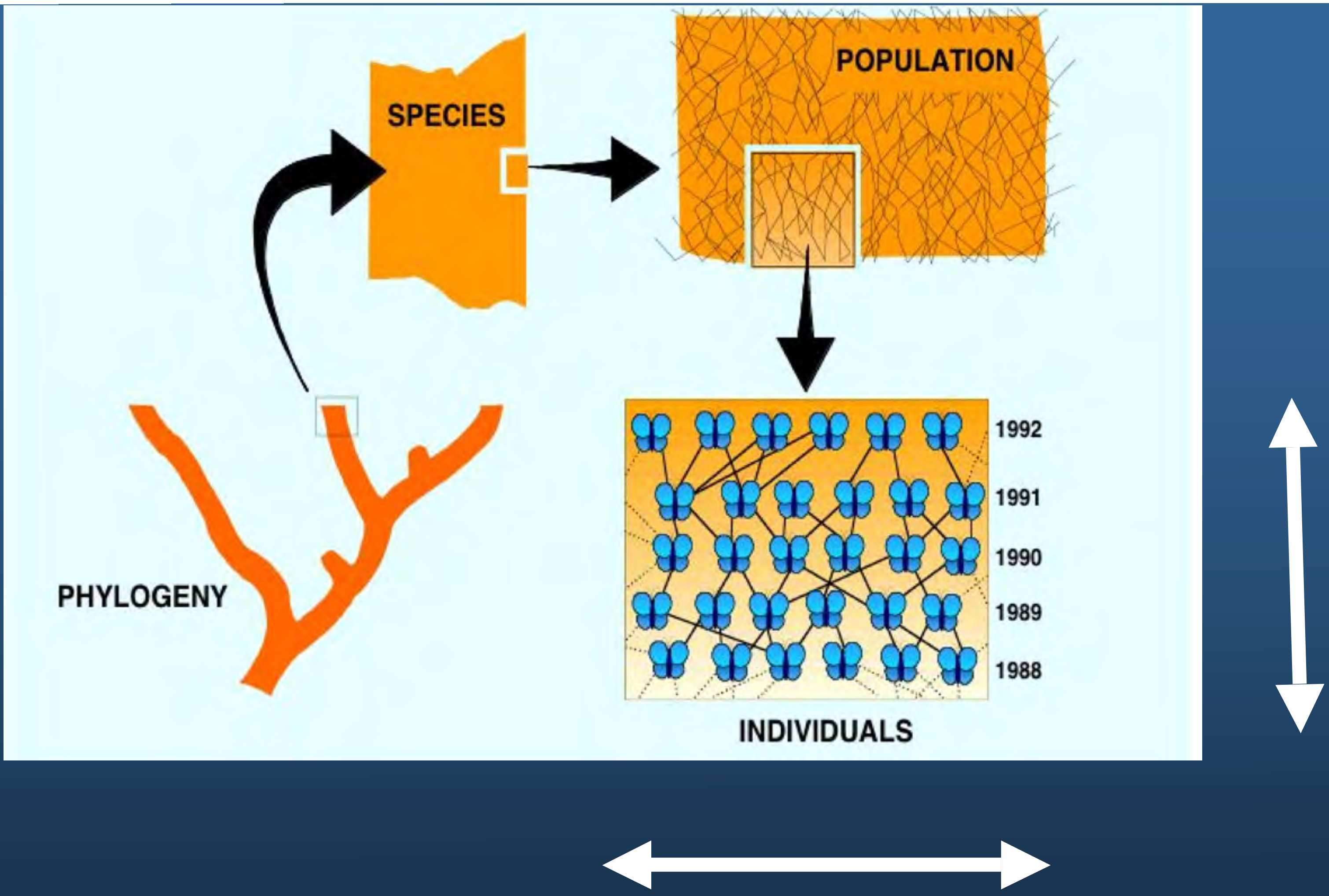
- Gene flow
- Long-term N_e
- Subspecies
- Species

Stocks

- Units of Management
- Units of Conservation

Demographic

- Individual identity
- Parentage
- Social organization
- Abundance (N)



Go wide or go deep?

i.e., more individuals or more genes?

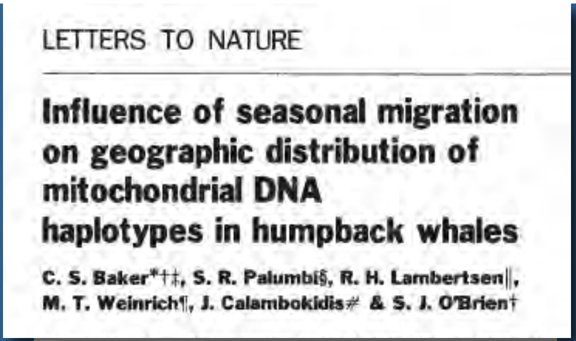
What could we learn?

- Population structure
  - Extended kinship relations
- Effective population sizes
  - Exploitation ‘bottlenecks’ and N_{min}
- Genetics of recovery
  - Selection and drift
- Recombination
  - Demographic histories
- Phylogenetic and taxonomy
  - Species and speciation
- Hybridization
  - Introgression and adaptation

Photo courtesy
of P. Ensor

But first,
narrow and shallow

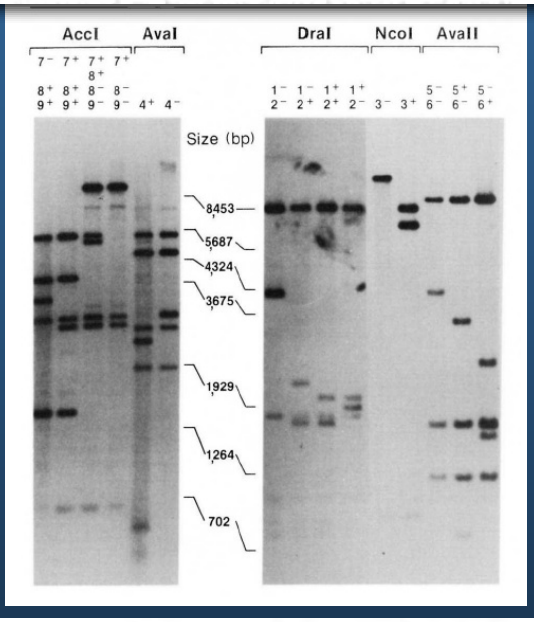
- Two oceans
  - 84 individuals
- 18 restriction enzymes
  - 9 polymorphic sites
- Distinct haplotype frequencies
  - No fixed differences

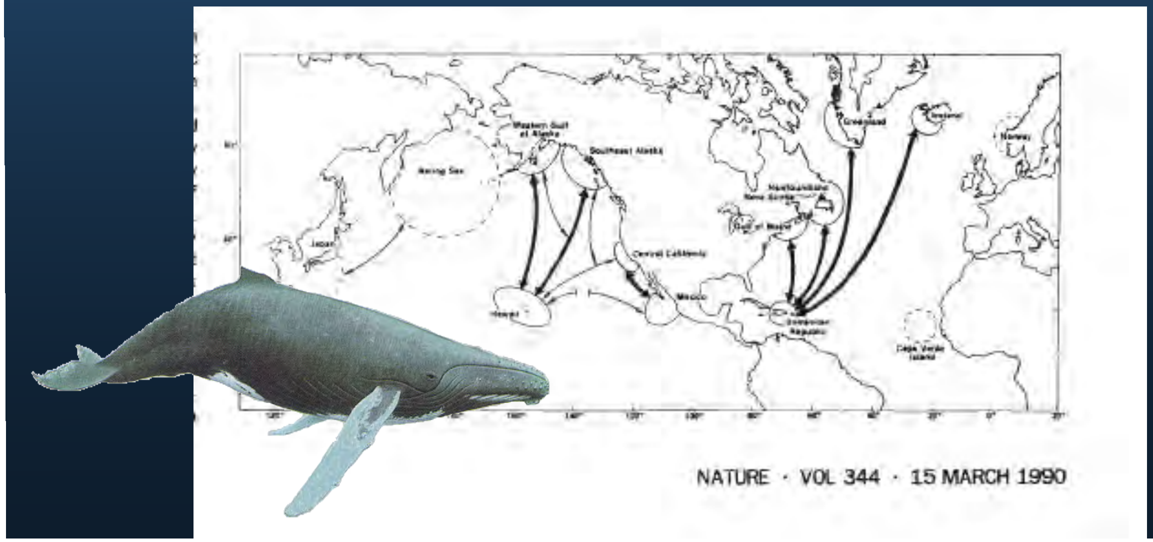


LETTERS TO NATURE

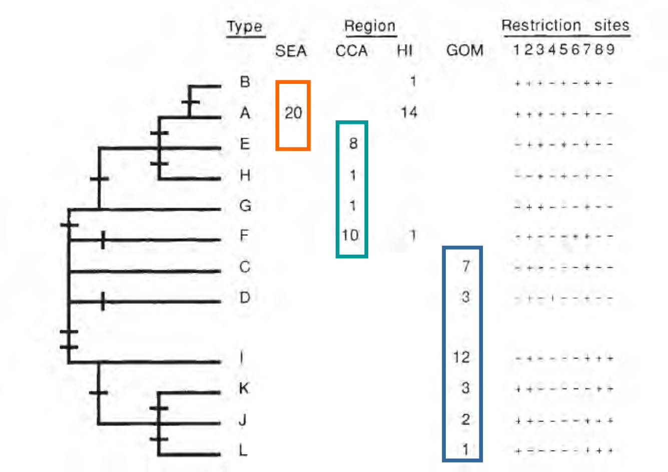
Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales

C. S. Baker*†‡, S. R. Palumbi§, R. H. Lambertsen‖, M. T. Weinrich¶, J. Calambokidis# & S. J. O'Brien†



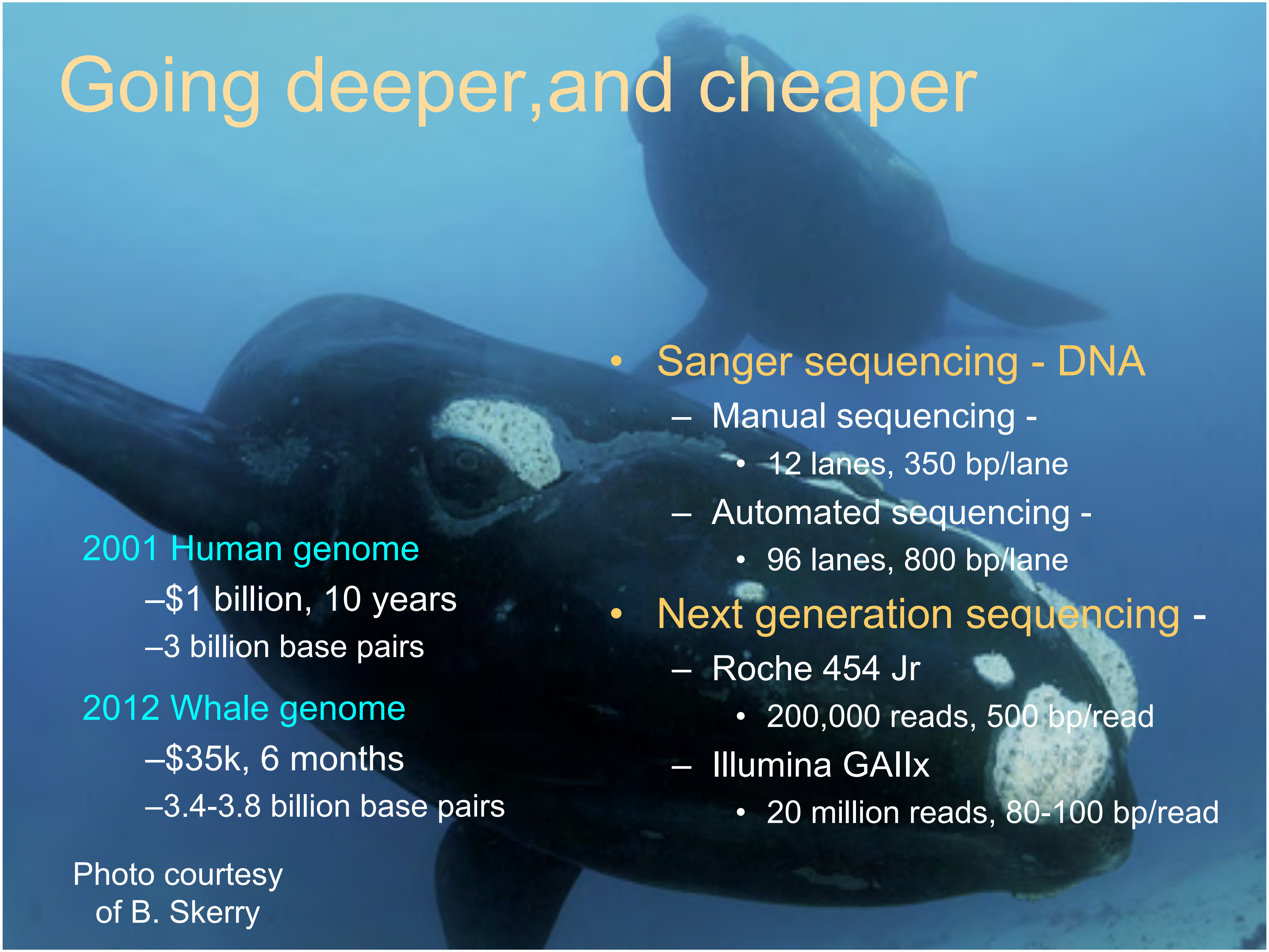


NATURE · VOL 344 · 15 MARCH 1990



Type	SEA	CCA	HI	GOM	Restriction sites 1 2 3 4 5 6 7 8 9
B			1		+ + + − + − + + −
A	20		14		+ + + − + − + − −
E		8			− + + − + − + − −
H		1			− − + − + − + − −
G		1			− + + − − − + − −
F		10	1		− + − − − + + − −
C				7	− + − − − − + − −
D				3	− + − + − − + − −
I				12	− + − − − − + + +
K				3	+ + − − − − − + +
J				2	+ + − − − − + + +
L				1	+ − − − − − + + +

Going deeper,and cheaper



2001 Human genome

–$1 billion, 10 years

–3 billion base pairs

2012 Whale genome

–$35k, 6 months

–3.4-3.8 billion base pairs

- Sanger sequencing - DNA
  - Manual sequencing -
    - 12 lanes, 350 bp/lane
  - Automated sequencing -
    - 96 lanes, 800 bp/lane
- Next generation sequencing -
  - Roche 454 Jr
    - 200,000 reads, 500 bp/read
  - Illumina GAIIx
    - 20 million reads, 80-100 bp/read

Photo courtesy
of B. Skerry

Maximum resolution, the complete genome

GENOME 10K ©

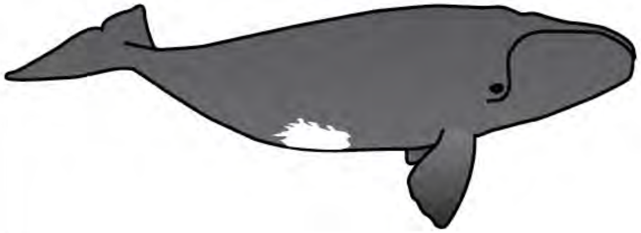
Whale genome sequencing projects underway

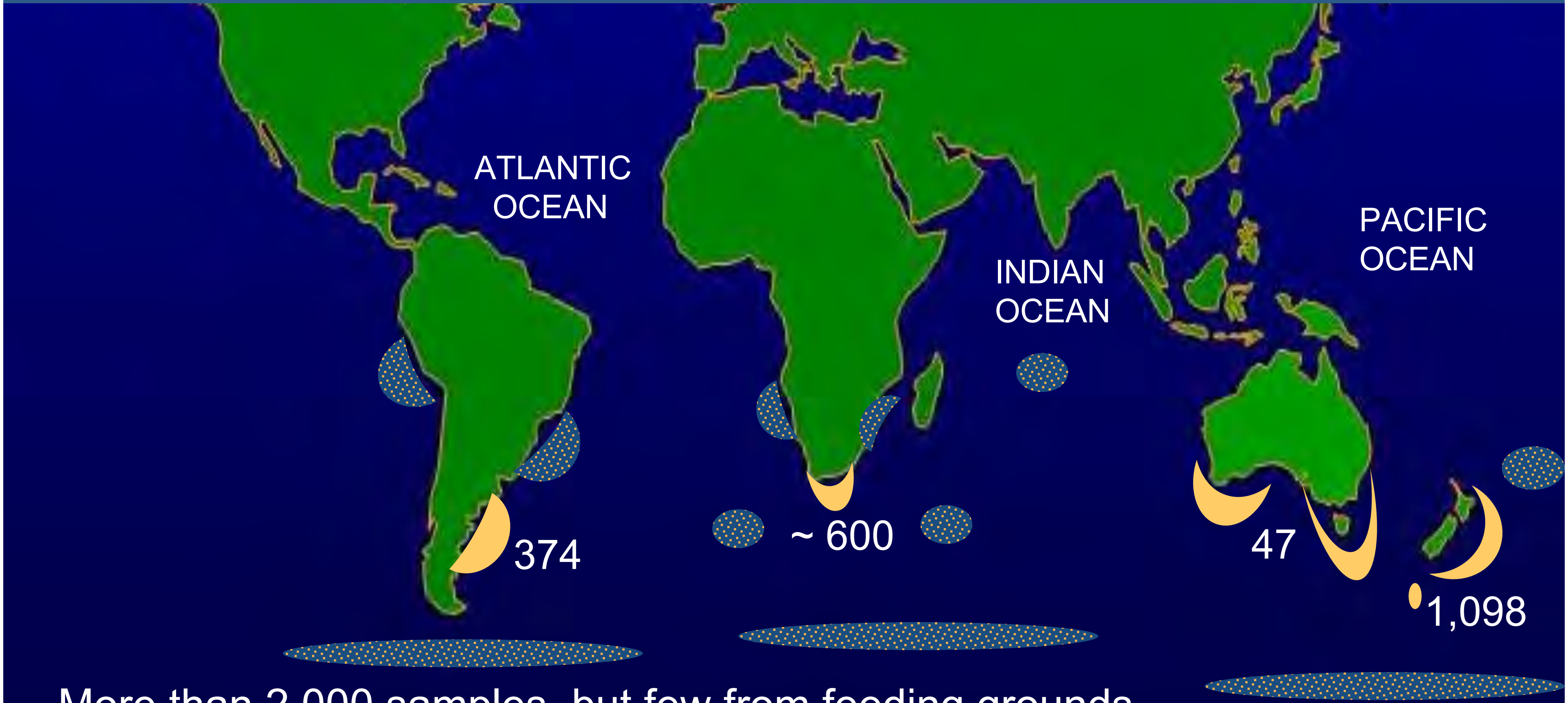
Bowhead whale - Broad Institute, MIT

Baiji, common minke whale and Chinese white dolphin - Beijing Genomic Institute/Broad Institute

Humpback whale and Narwhal - UC Santa Cruz

Going wide, southern right whales



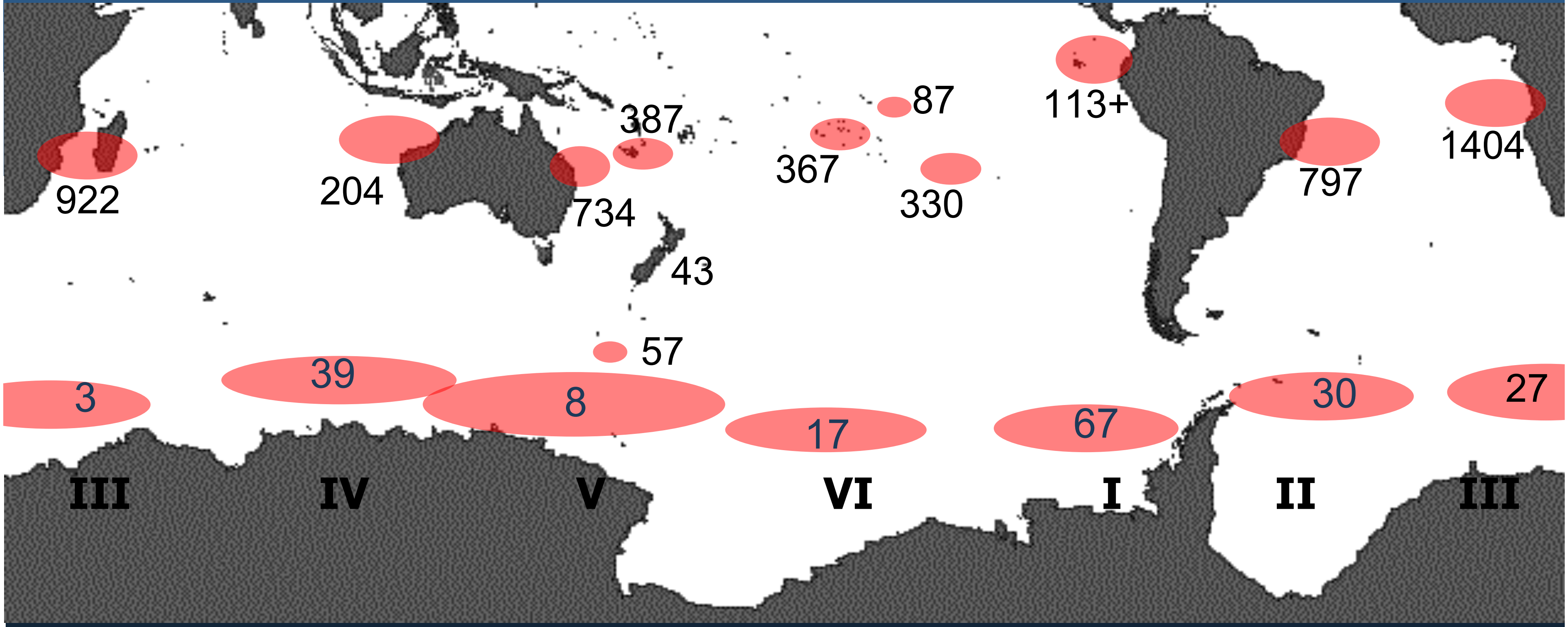


More than 2,000 samples, but few from feeding grounds

sources, L. Valenzuela, J. Seger, P. Best, J. Bannister, R. Harcourt, N. Patenaude, E. Carroll, C.S Baker

Going wide,

humpback whales

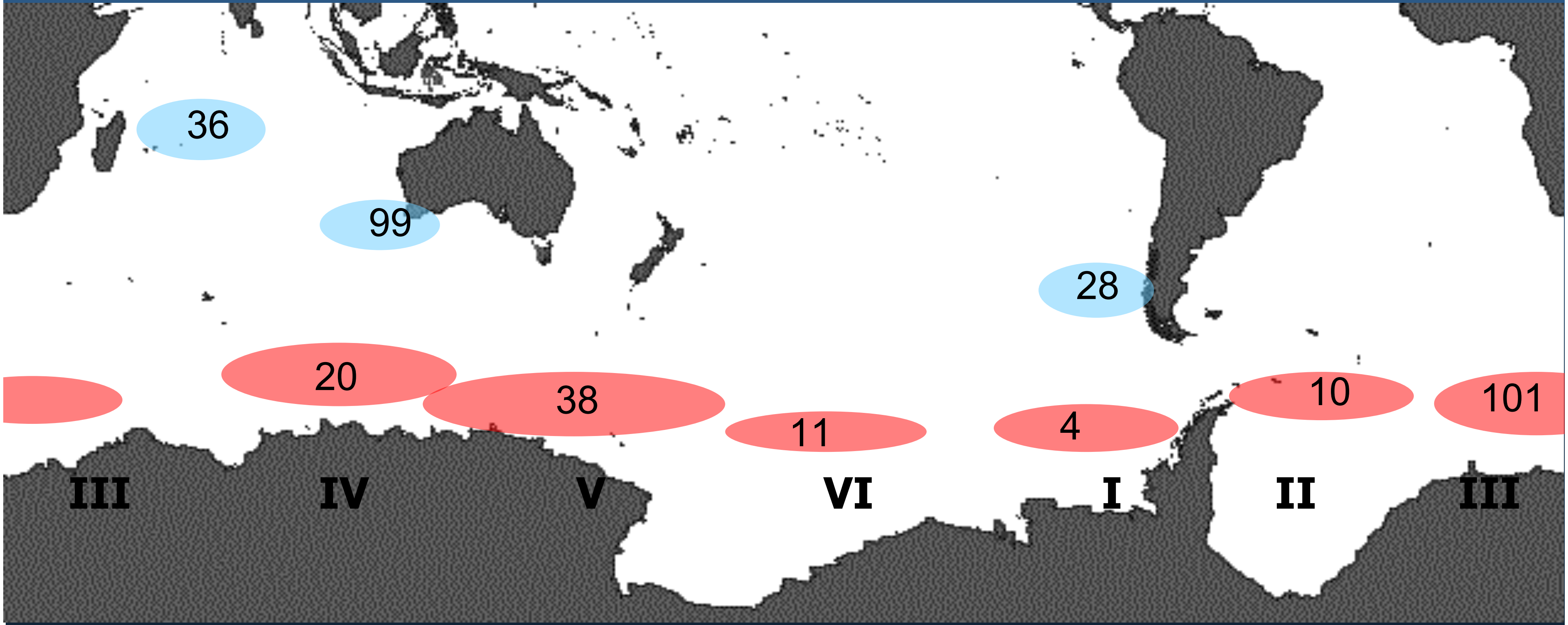


More the 6,000 samples, but few from Antarctic (excluding JARPA)

sources, H Rosenbaum, A. Cypriano, SPWRC, SCU, IDCR/SOWER, INACH, GLOBEC, AMMC-AWE

Going wide,

Antarctic and pygmy blue whales

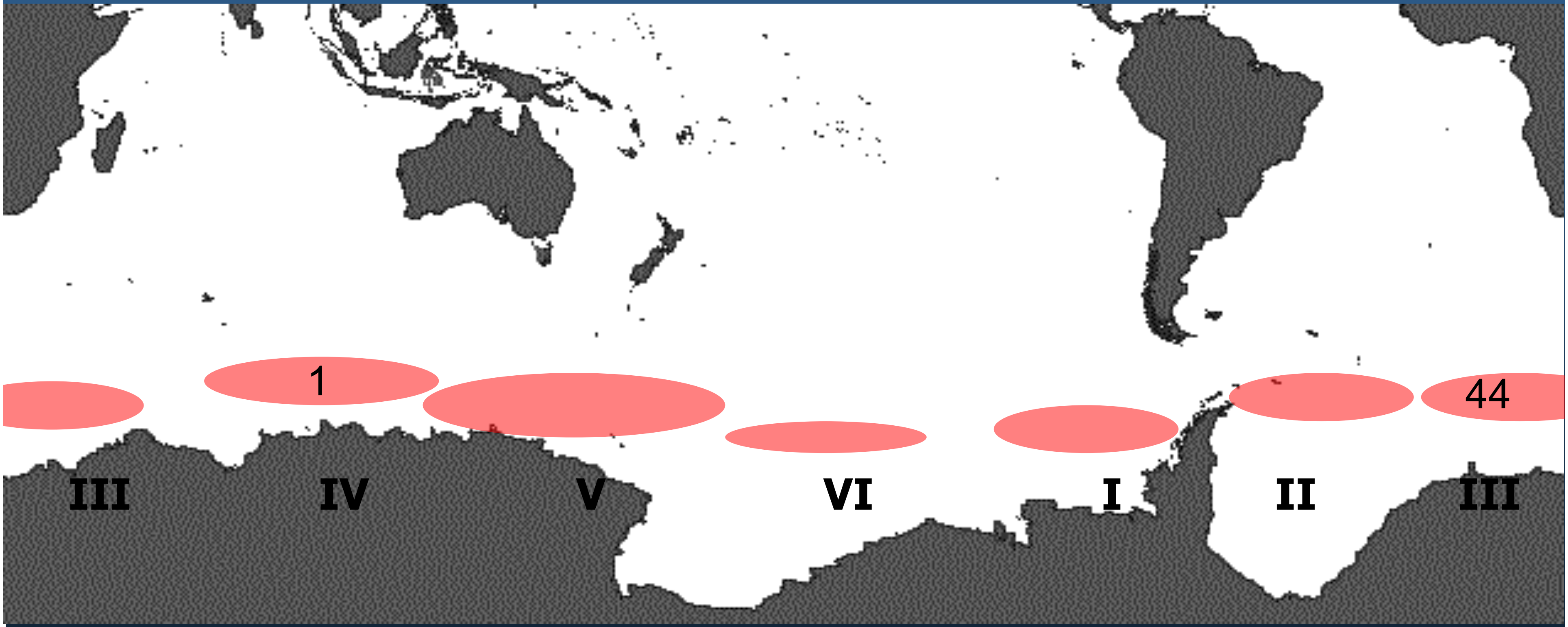


About 200 samples each, none from breeding grounds of Antarctic blue whales

See posters Leduc et al 2007.; Sremba et al. 2012 and Attard et al. 2010

Not so wide,

fin whale



Only 45 samples from a population that suffered removal of more than 750,000

courtesy, E. Archer, B. Taylor

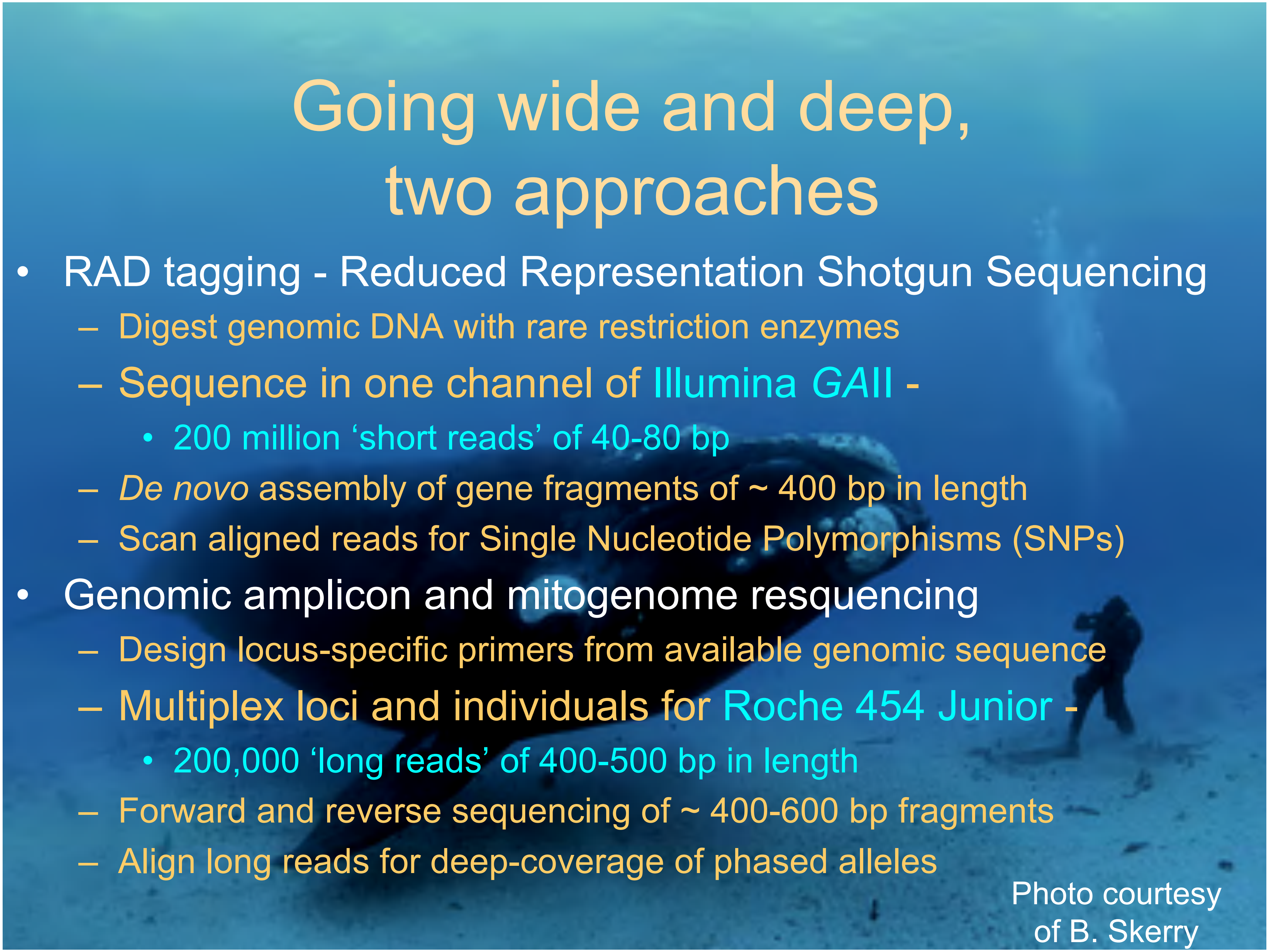
Maintaining the archive

- Archiving and access to samples are critical:
  - – SWFSC Marine Mammal and Turtle Molecular Research Sample Collection
  - – New Zealand Cetacean Tissue Archive
    - University of Auckland

Jackson J., et al. (2011) Guidelines for collecting and maintaining archives for genetic monitoring. *Conservation Genetics Resources*, 1-10

Photo courtesy of P. Ensor

Going wide and deep, two approaches



- RAD tagging - Reduced Representation Shotgun Sequencing
  - Digest genomic DNA with rare restriction enzymes
  - Sequence in one channel of Illumina *GAII* -
    - 200 million ‘short reads’ of 40-80 bp
  - *De novo* assembly of gene fragments of ~ 400 bp in length
  - Scan aligned reads for Single Nucleotide Polymorphisms (SNPs)
- Genomic amplicon and mitogenome resquencing
  - Design locus-specific primers from available genomic sequence
  - Multiplex loci and individuals for Roche 454 Junior -
    - 200,000 ‘long reads’ of 400-500 bp in length
  - Forward and reverse sequencing of ~ 400-600 bp fragments
  - Align long reads for deep-coverage of phased alleles

Photo courtesy
of B. Skerry

RAD tagging of fin and blue whales

- 2.65 Mb of sequence for fin
  - 1,242,459 reads
  - de novo assemble of 7,088 fragments
    - average 375 bp in length, 15x depth
- 1.5 Mb of sequence for blue
  - 569,451 reads for blue whale
  - de novo assemble of 3,995 fragments
    - average 354 bp in length, 15x depth

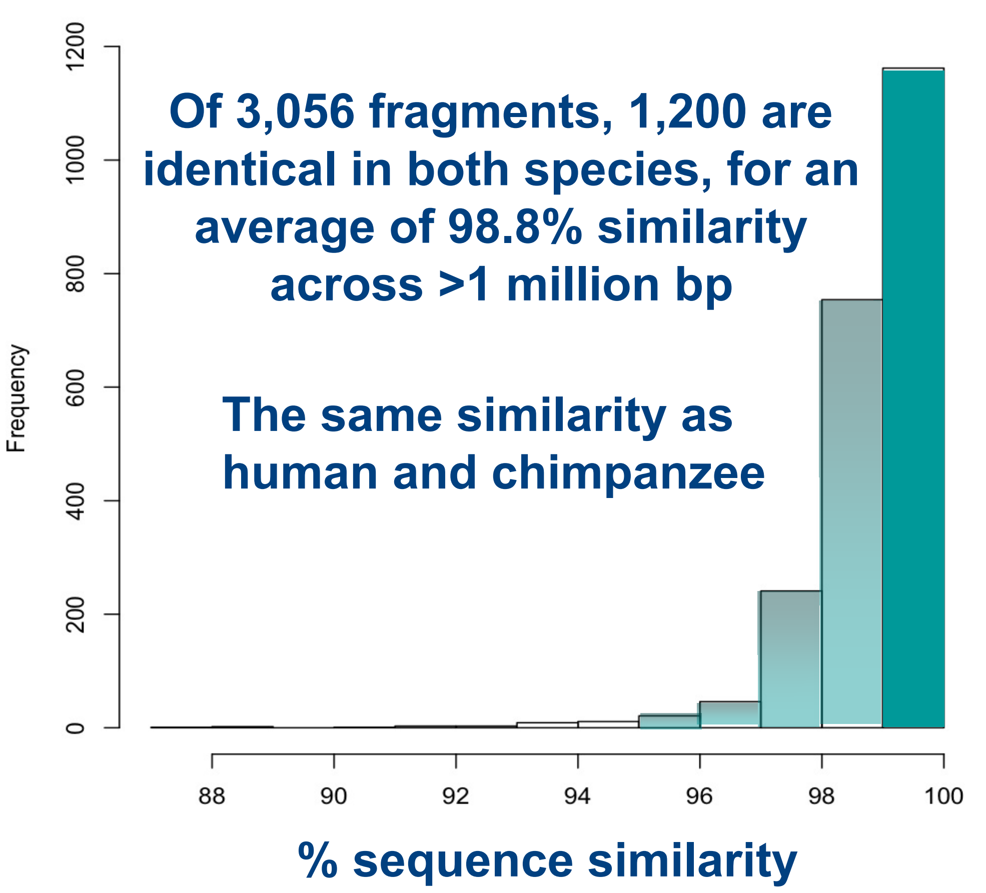
- Two species that hybridize in nature
  - Hybrids can be of either sex
  - Hybrids can backcross
  - Hybridization not uncommon

Courtesy, J. Calambokidis, Cascadia Research

Genomic similarity of fin and blue whales

Courtesy, Cascadia Research

- BLAST search
  - Fin database
    - 7,088 fragments
  - Blue query
    - 3,995 fragments
  - 3,056 likely homologues
    - 75% overlap

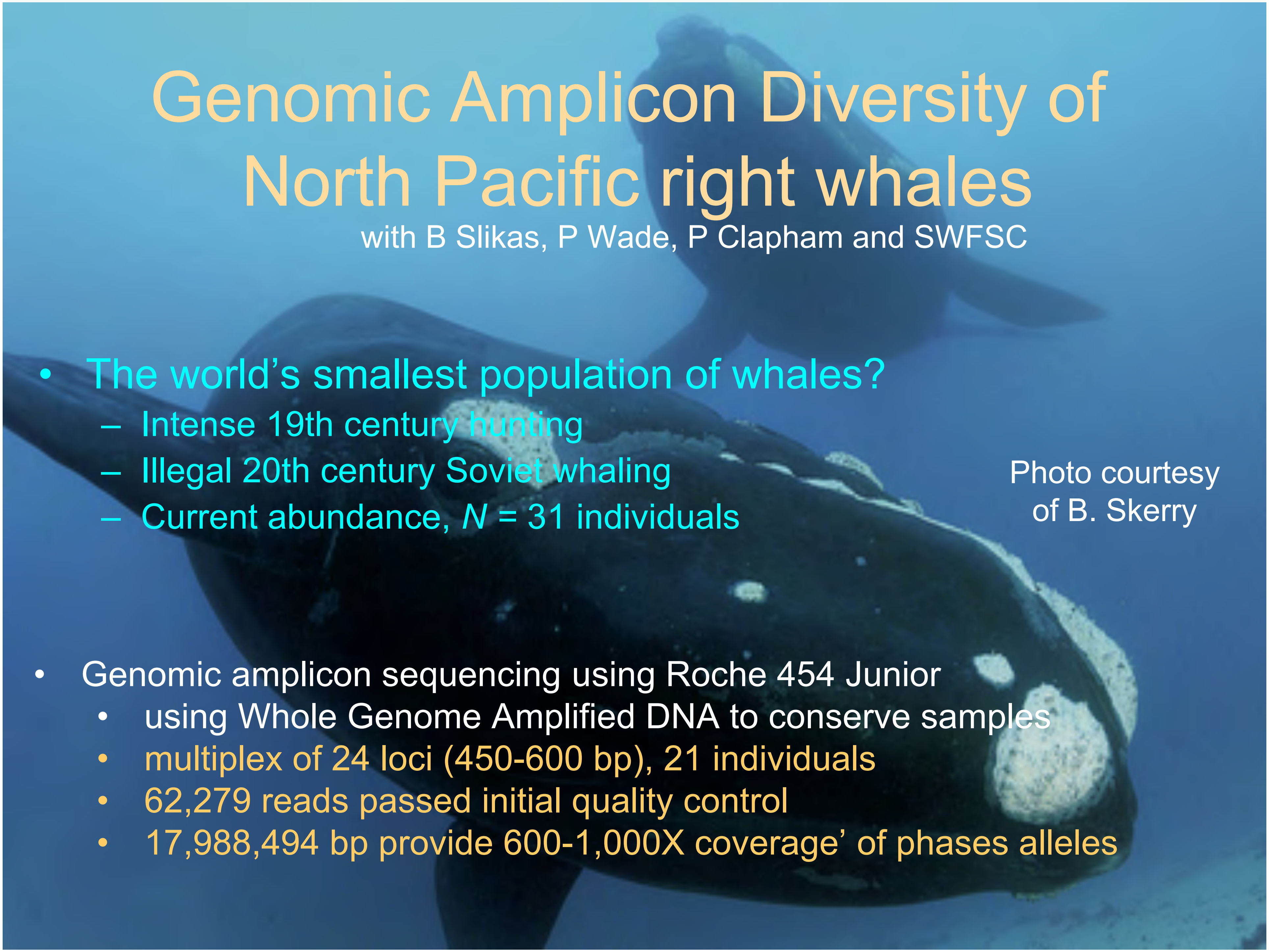


Of 3,056 fragments, 1,200 are identical in both species, for an average of 98.8% similarity across >1 million bp

The same similarity as human and chimpanzee

Genomic Amplicon Diversity of North Pacific right whales

with B Slikas, P Wade, P Clapham and SWFSC



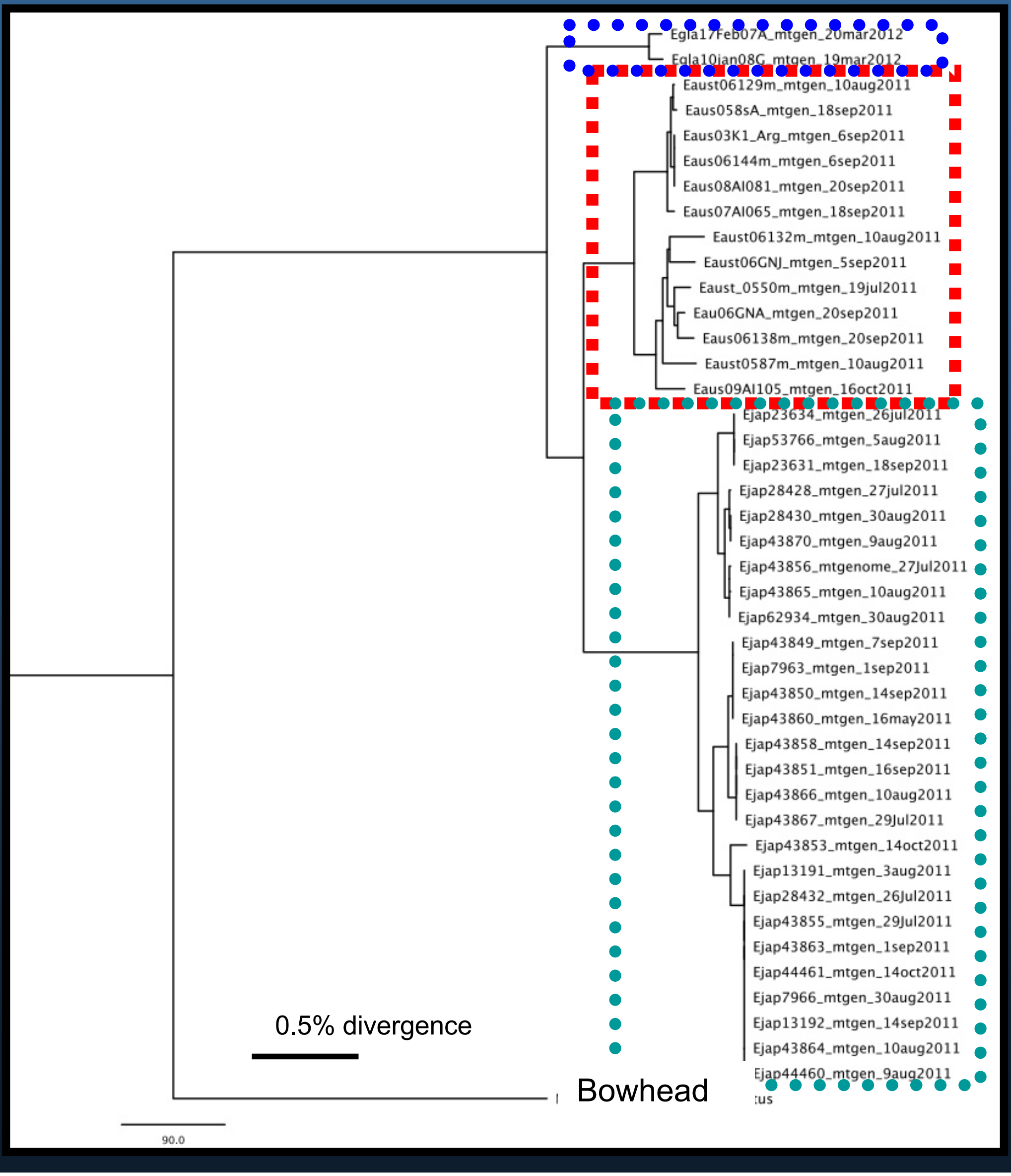
- The world's smallest population of whales?
  - Intense 19th century hunting
  - Illegal 20th century Soviet whaling
  - Current abundance, *N* = 31 individuals

Photo courtesy
of B. Skerry

- Genomic amplicon sequencing using Roche 454 Junior
  - using Whole Genome Amplified DNA to conserve samples
  - multiplex of 24 loci (450-600 bp), 21 individuals
  - 62,279 reads passed initial quality control
  - 17,988,494 bp provide 600-1,000X coverage' of phases alleles

Worldwide mitogenomic diversity

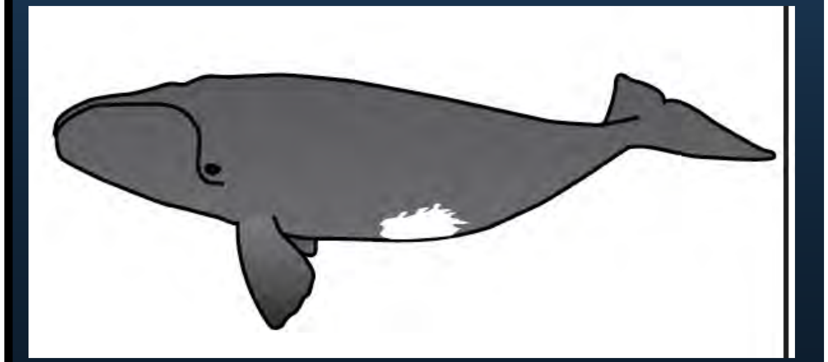
with B Slikas, L Valenzuela, J Seger, E Carroll, T Frasier and L Conger



North Atlantic right whale
n = 2 (more to come)

Southern right whale
n = 14, 12 haplotypes

North Pacific right whales
n = 28, 9 haplotypes



Future directions - look to the past

- The bones of South Georgia Island and other whaling stations are a repository of the former genetic diversity of whales in the Southern Ocean
  - More than 175,000 humpback, blue and fin were processed at this station, leaving countless bones

with Angie Sremba and Tony Martin

Diversity lost or retained?

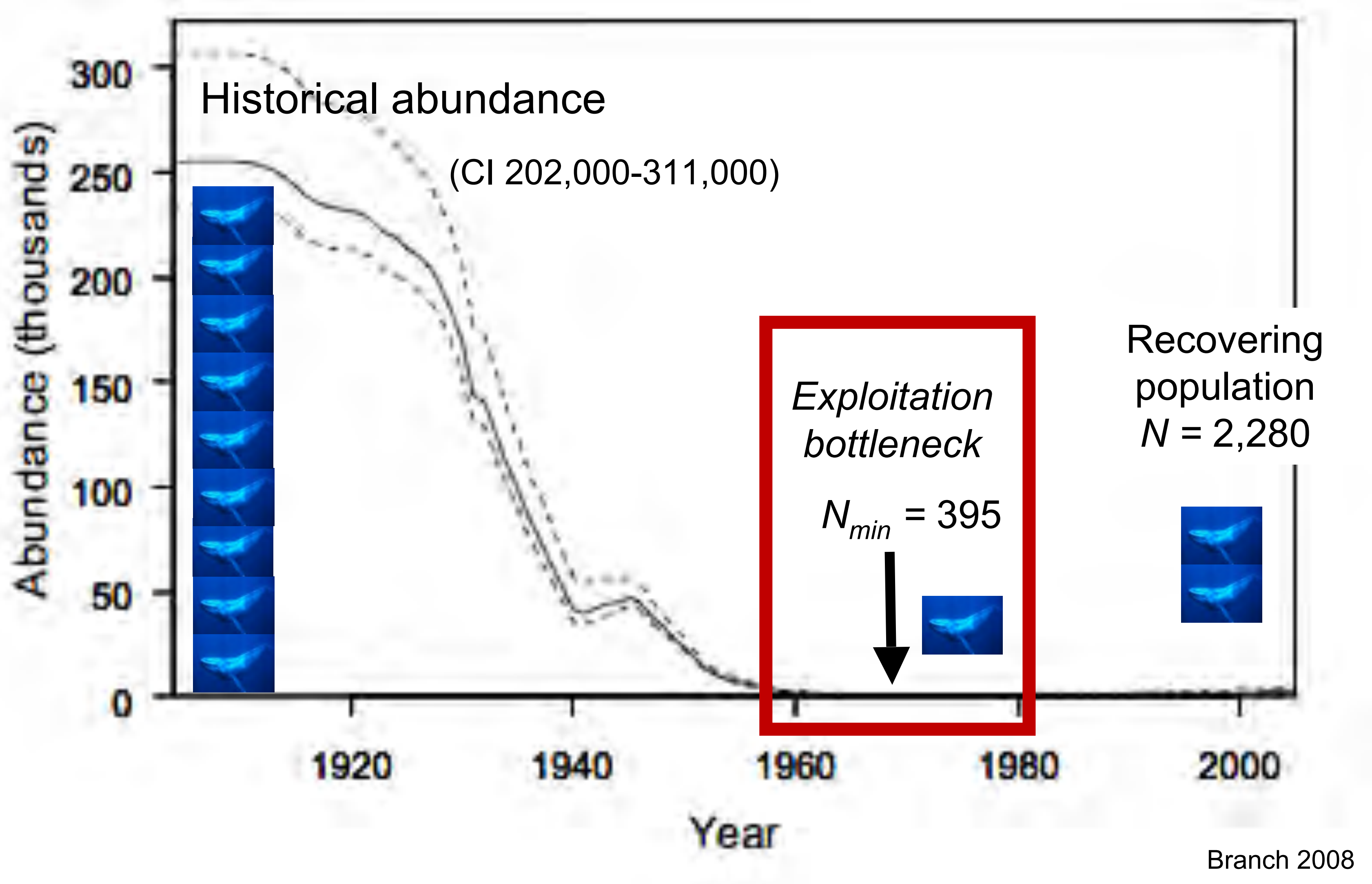
Species identity and mtDNA diversity from 223 of 281 bones

- using conventional PCR and sequencing of control region

	Humpback	Fin	Blue
Sample size (n)	153	49	18
Haplotypes	64	34	16
Haplotype diversity	0.980	0.982	0.987

Sremba et al. in prep.

Near Extinction of the Antarctic blue whale



Historical abundance

(CI 202,000-311,000)

Exploitation bottleneck

$N_{min} = 395$

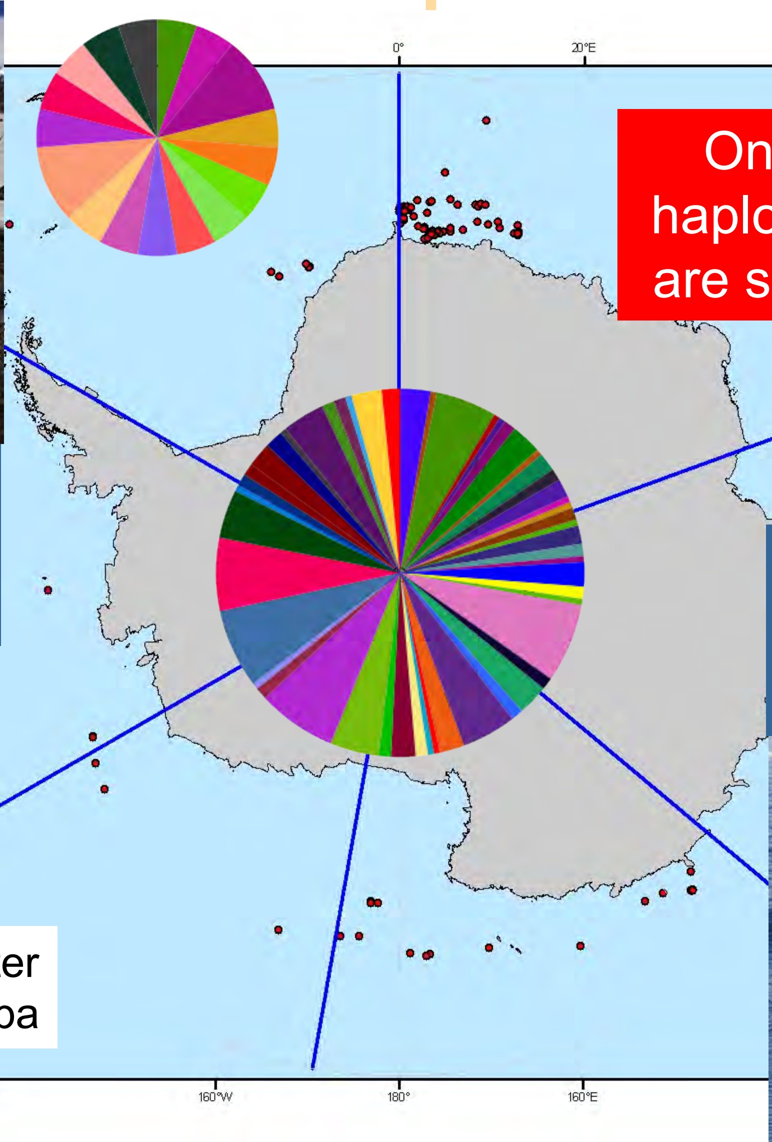
Recovering population

$N = 2{,}280$

Branch 2008

Comparing the past to the present

South Georgia bones
18 samples
16 haplotypes



Only 5 haplotypes are shared

See poster by Sremba

Southern Ocean
187 individuals
51 haplotypes

- Conclusions
  - Go wide
  - Go deep
  - Reconstruct the past
  - Assess the present
  - Hope for the future

With thanks to our host,
the Government of Chile
Local convener, Barbara Galletti
Absent coordinator, Debbie Steel
Australian Marine Mammal Centre
Beth Slikas, Angie Sremba
Photographs courtesy of P Ensor, B Skerry