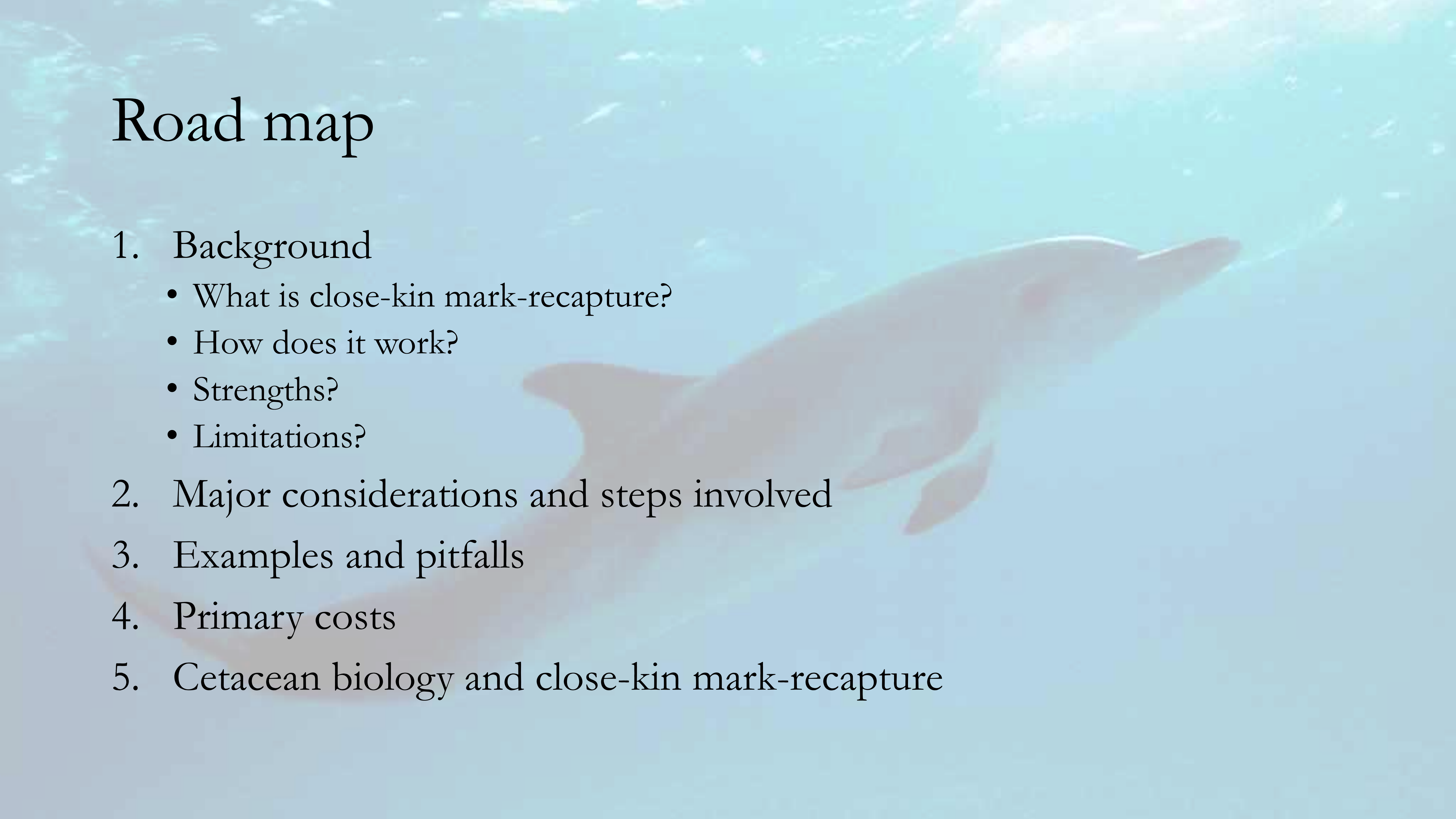
The potential for close-kin mark-recapture (CKMR) to inform monitoring of dolphin populations in the EPO

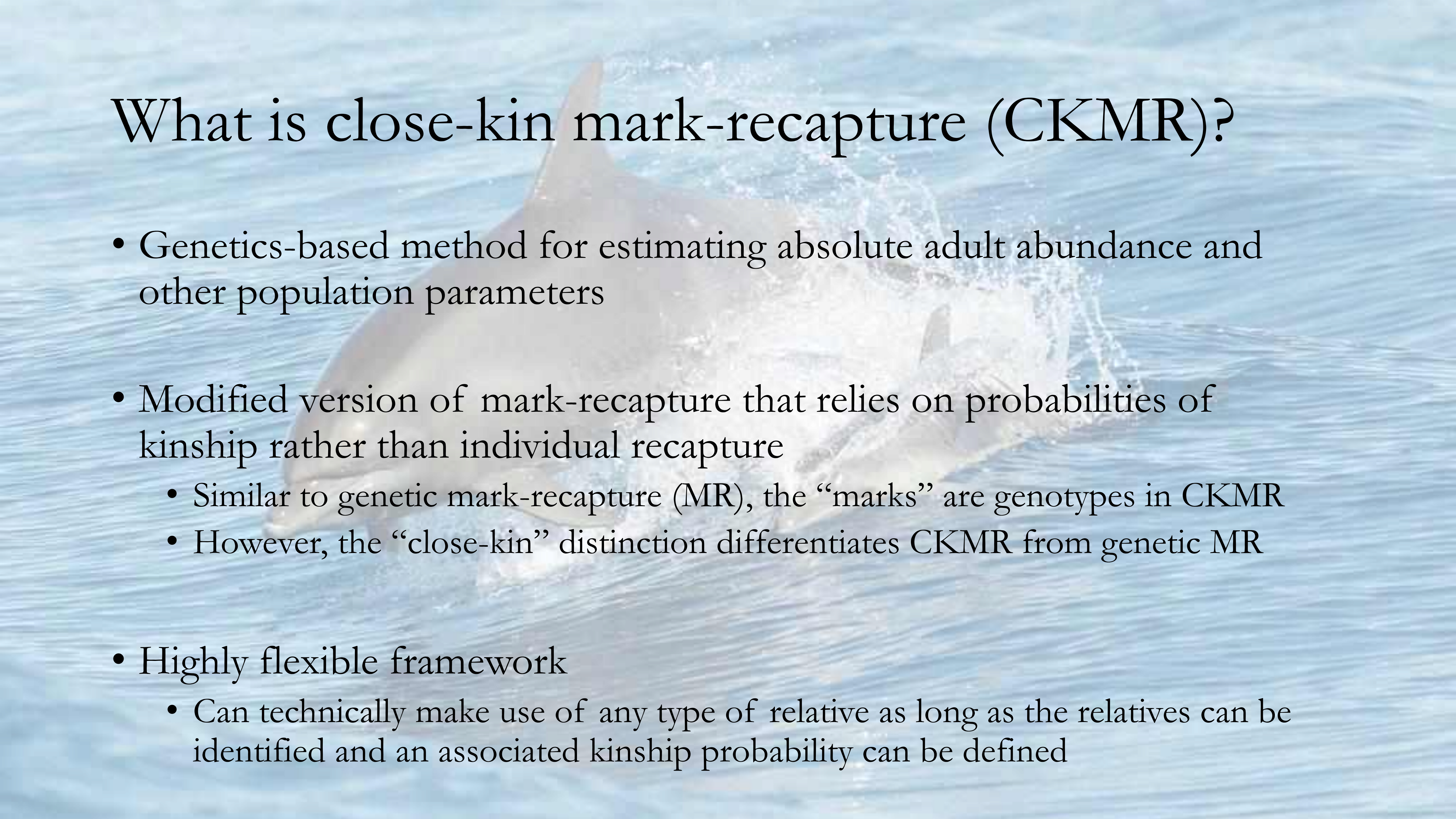
John Swenson, PhD candidate, University of Massachusetts



Road map

1. Background
    - What is close-kin mark-recapture?
    - How does it work?
    - Strengths?
    - Limitations?
2. Major considerations and steps involved
3. Examples and pitfalls
4. Primary costs
5. Cetacean biology and close-kin mark-recapture

What is close-kin mark-recapture (CKMR)?

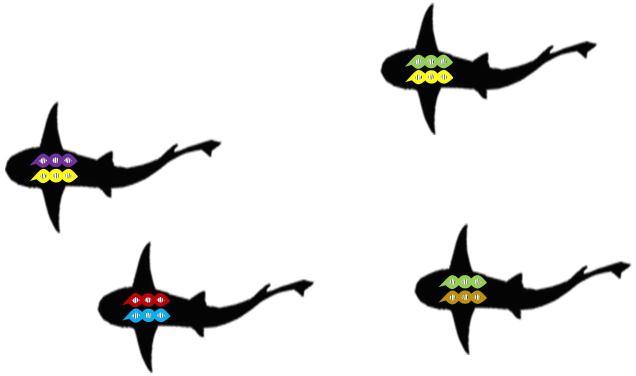


- Genetics-based method for estimating absolute adult abundance and other population parameters
- Modified version of mark-recapture that relies on probabilities of kinship rather than individual recapture
  - Similar to genetic mark-recapture (MR), the “marks” are genotypes in CKMR
  - However, the “close-kin” distinction differentiates CKMR from genetic MR
- Highly flexible framework
  - Can technically make use of any type of relative as long as the relatives can be identified and an associated kinship probability can be defined

Close-Kin Mark-Recapture (CKMR) uses probability of kinship to estimate population abundance

1. Tissue samples + data
    - Age (or length)
    - Sex
    - Location

2. DNA extraction, genotyping, and kinship assignment



3. Pairwise comparisons and kinship probability

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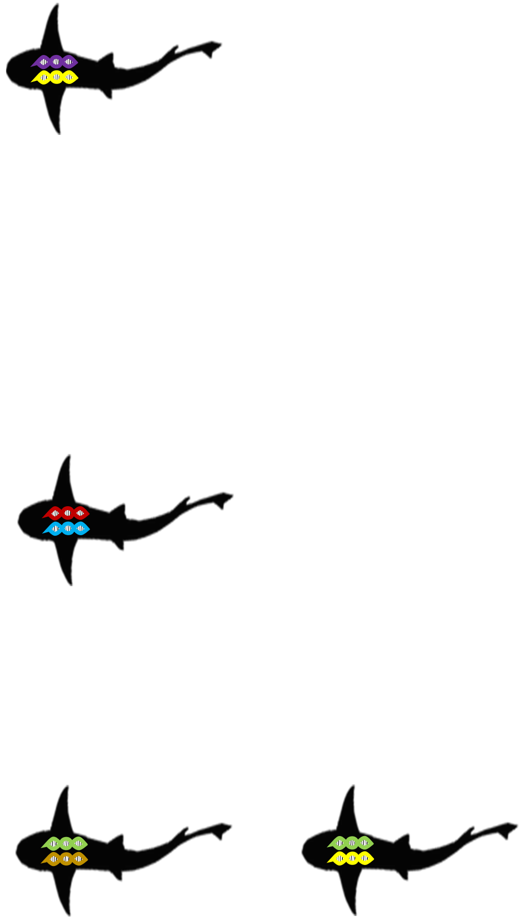

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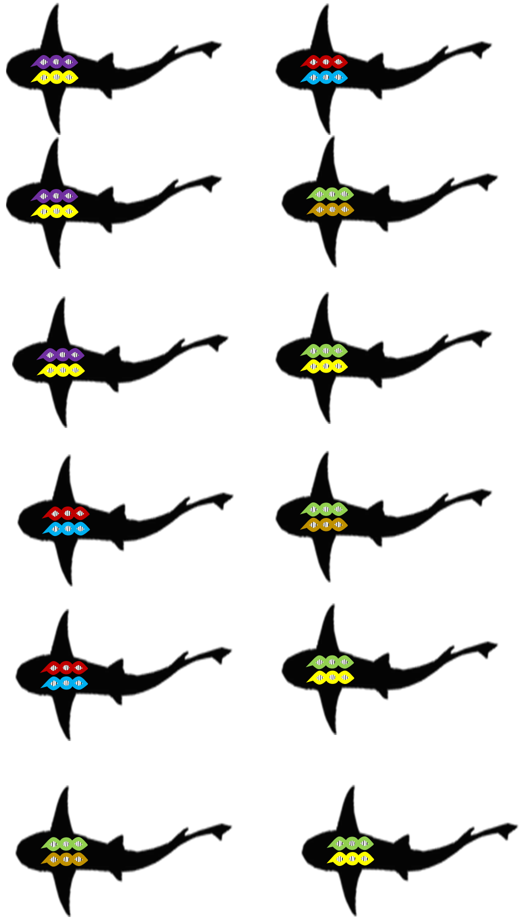
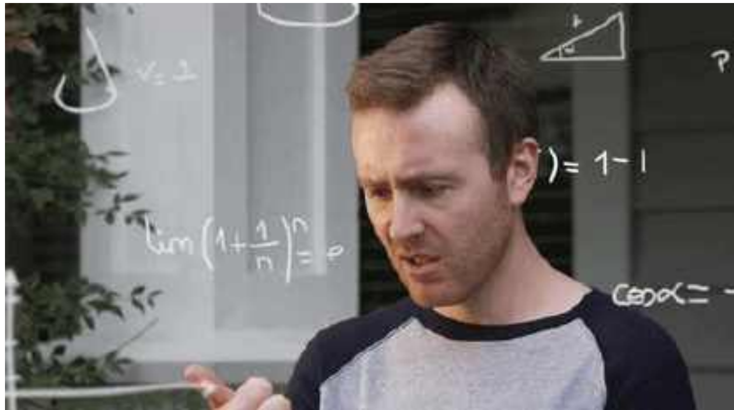


3. Pairwise comparisons and kinship probability

CKMR can use parent-offspring or half-sibling kinship probabilities to estimate adult abundance

$$P\{K_{i,j} = MPOP\} = \frac{I[y_i + \alpha \leq y_j]}{N_{\text{♀}(y_j)}}\, x \begin{cases} 1; & c_i > y_j \\ \phi_i^{(y_j - c_i)}; & c_i < y_j \end{cases}$$

$$P\{K_{i,j} = MHS\} = \frac{\phi^{(y_j - y_i)}}{N_{\text{♀}(y_j)}}$$



Equations from Bravington et. al. (2016)

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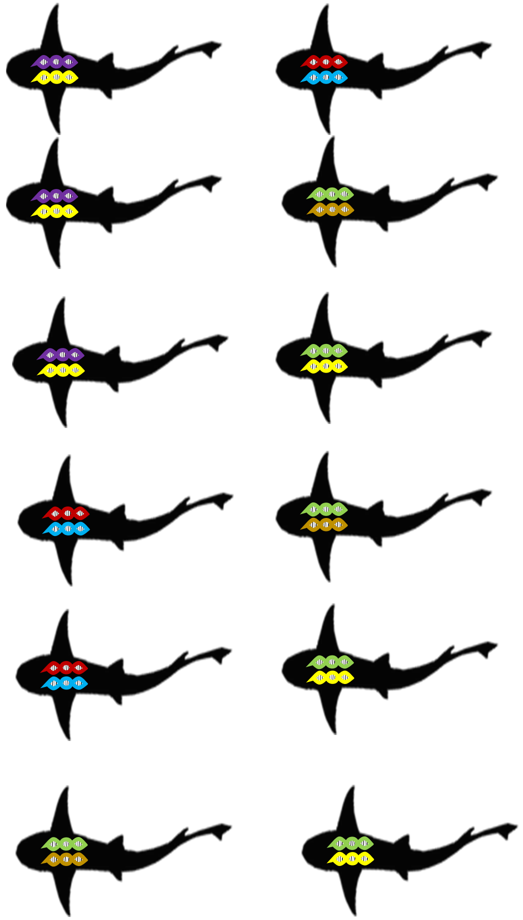

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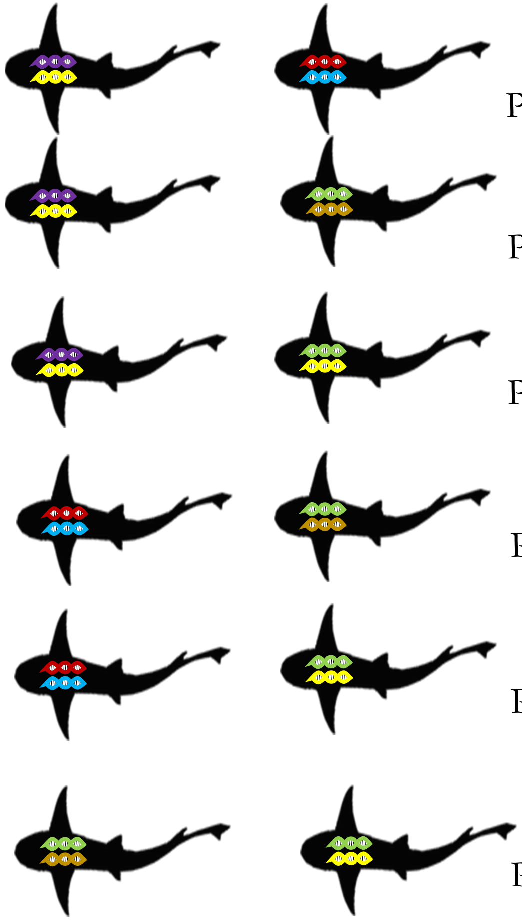

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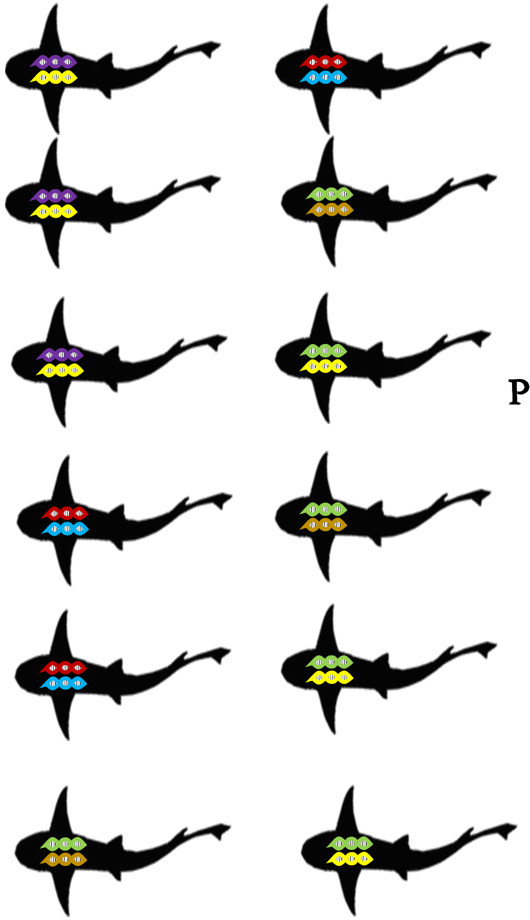

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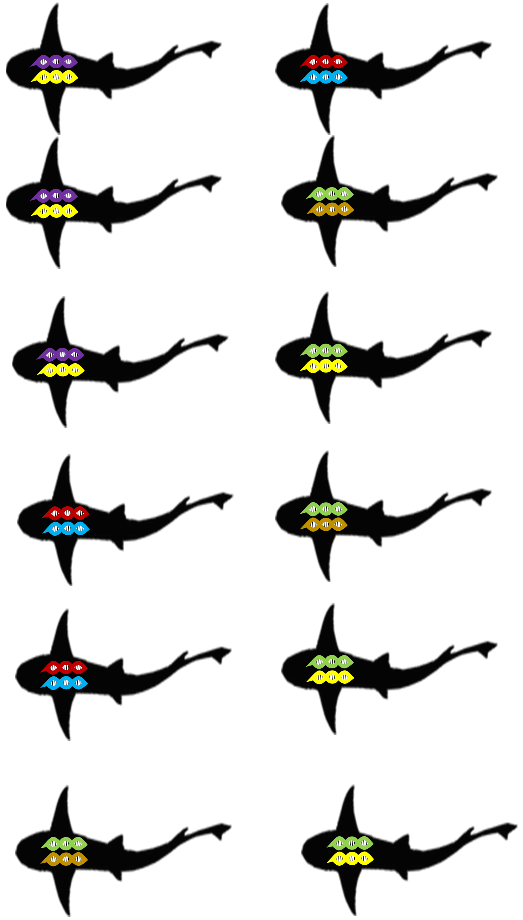

3. Pairwise comparisons and kinship probability

$$\frac{\phi^{(y_j-y_i)}}{N_{♀(y_j)}}$$

Close-Kin Mark-Recapture (CKMR) uses probability of kinship to estimate population abundance

$$Y\{K_{i,j} = MHSP\} \sim Binomial\left(\frac{\phi^{(y_j-y_i)}}{N_{\text{♀}(y_j)}}, \boxed{R_{(y_i, y_j)}}\right)$$

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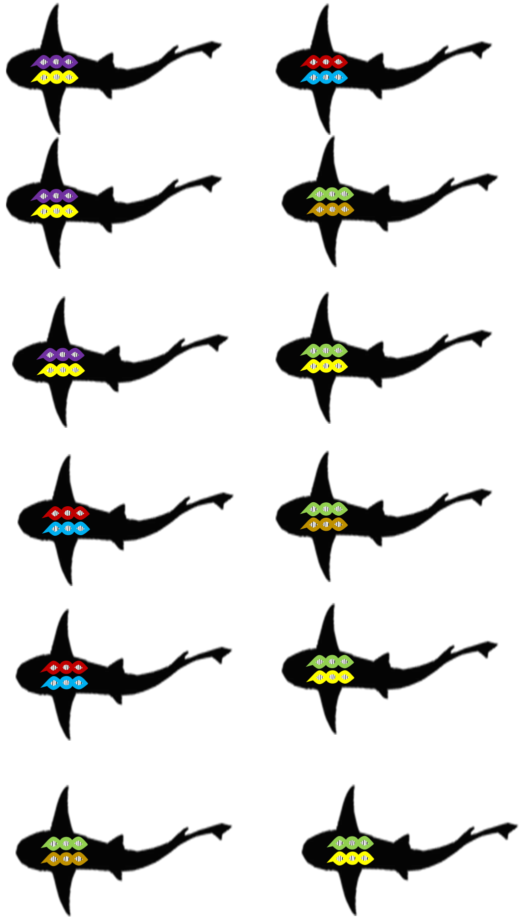

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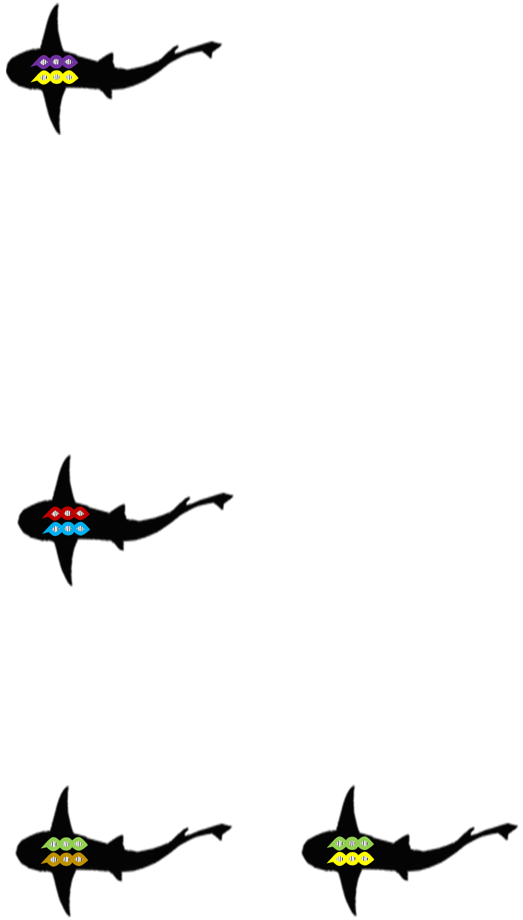

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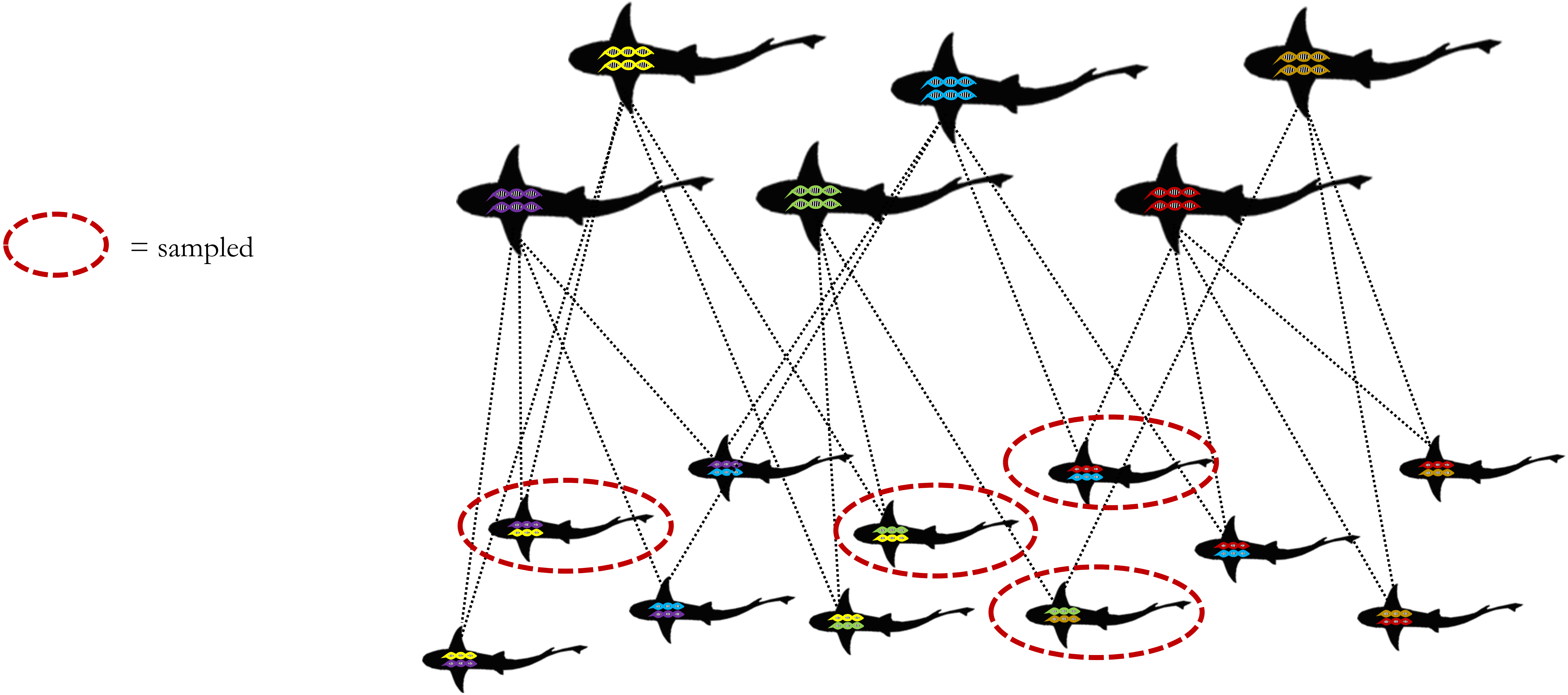

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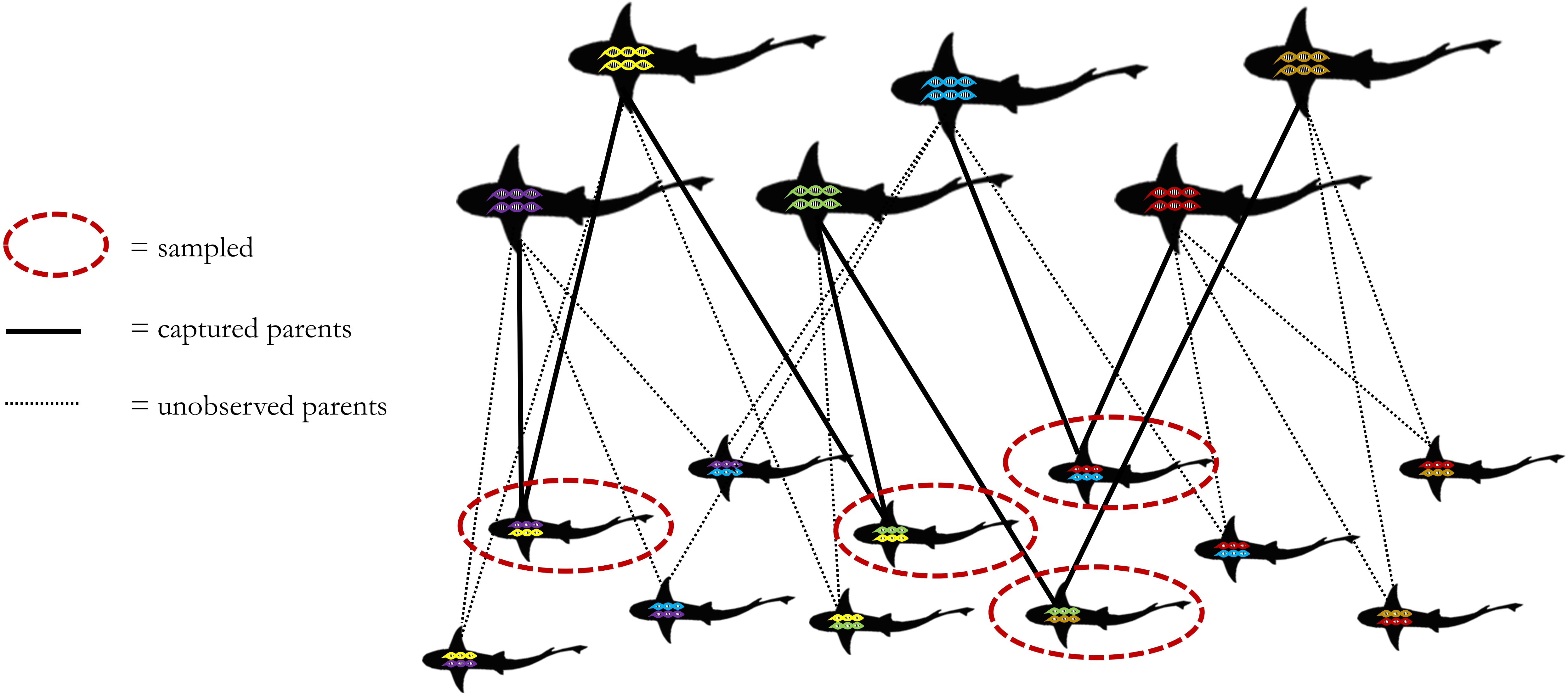

= sampled

= captured parents

= unobserved parents

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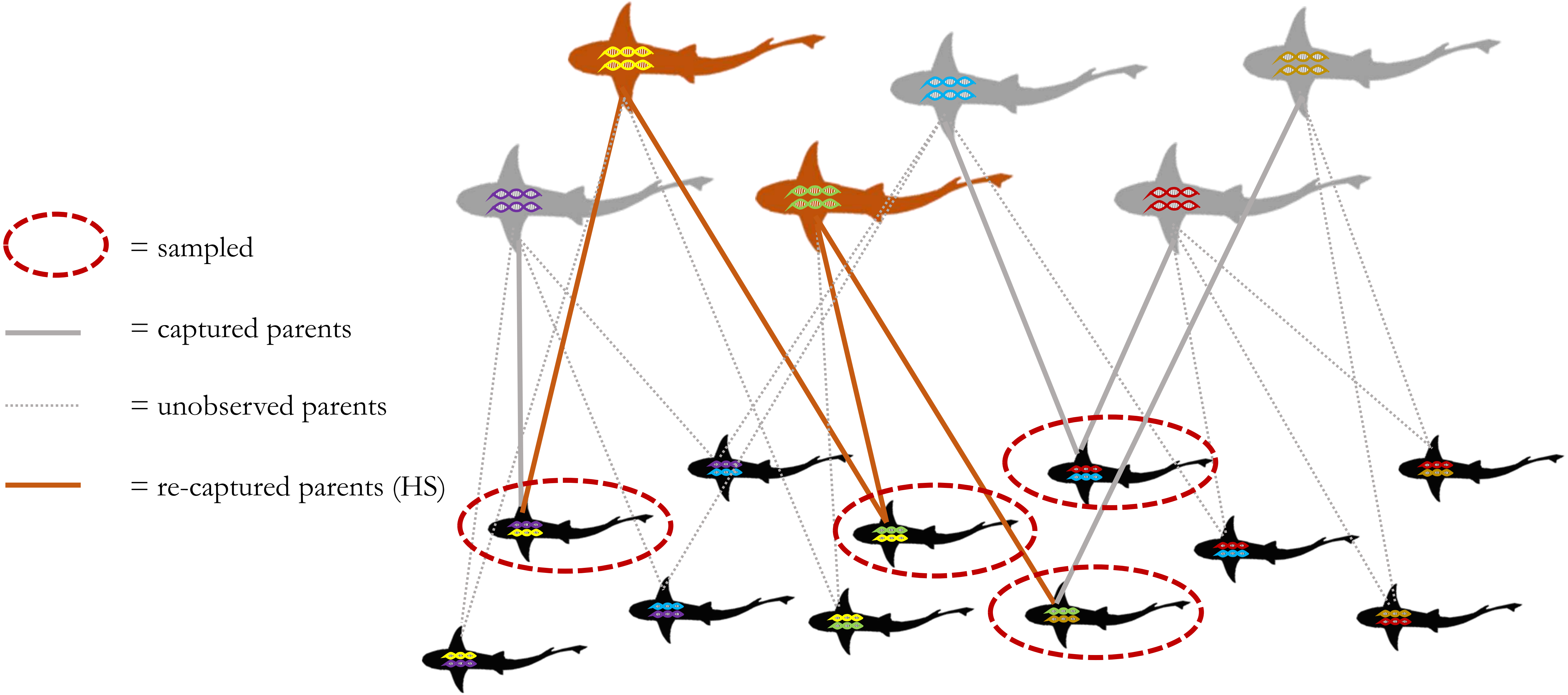

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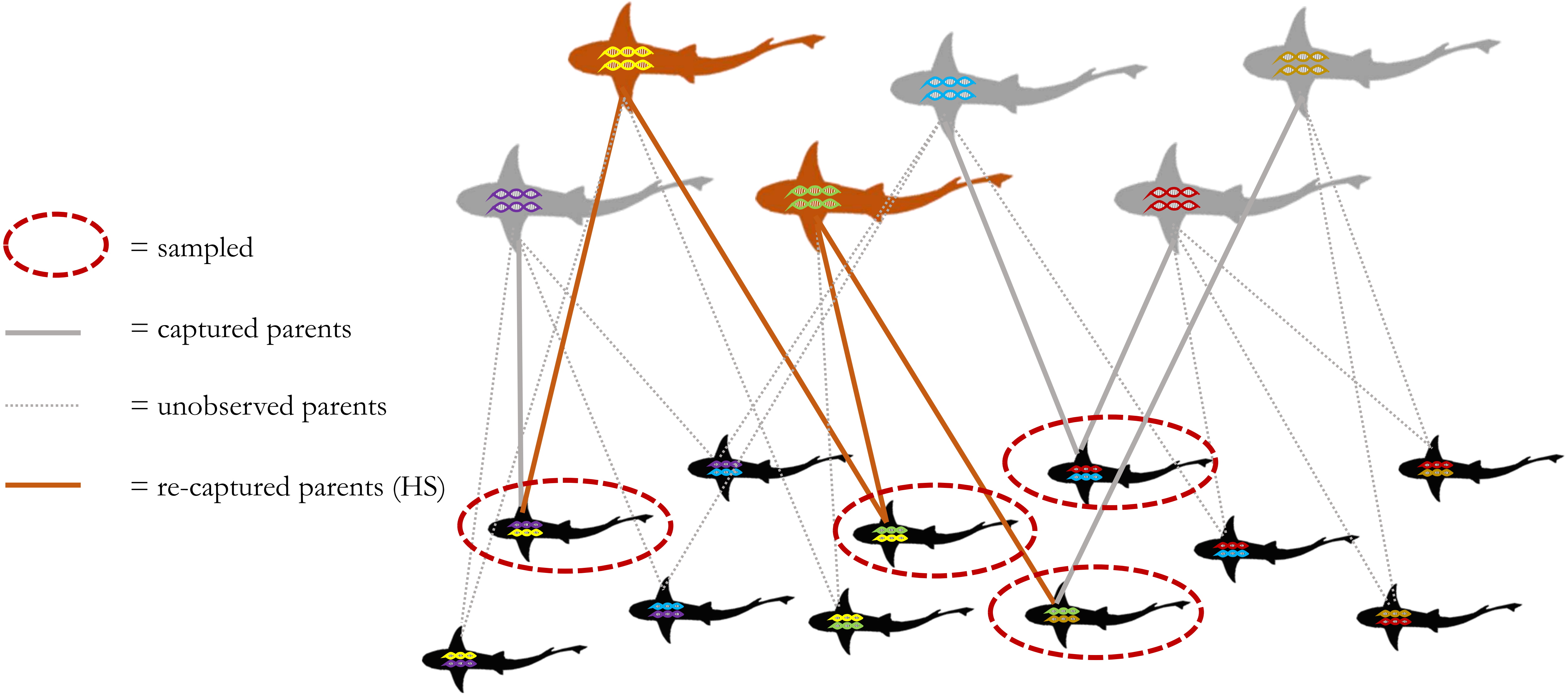


◯ = sampled

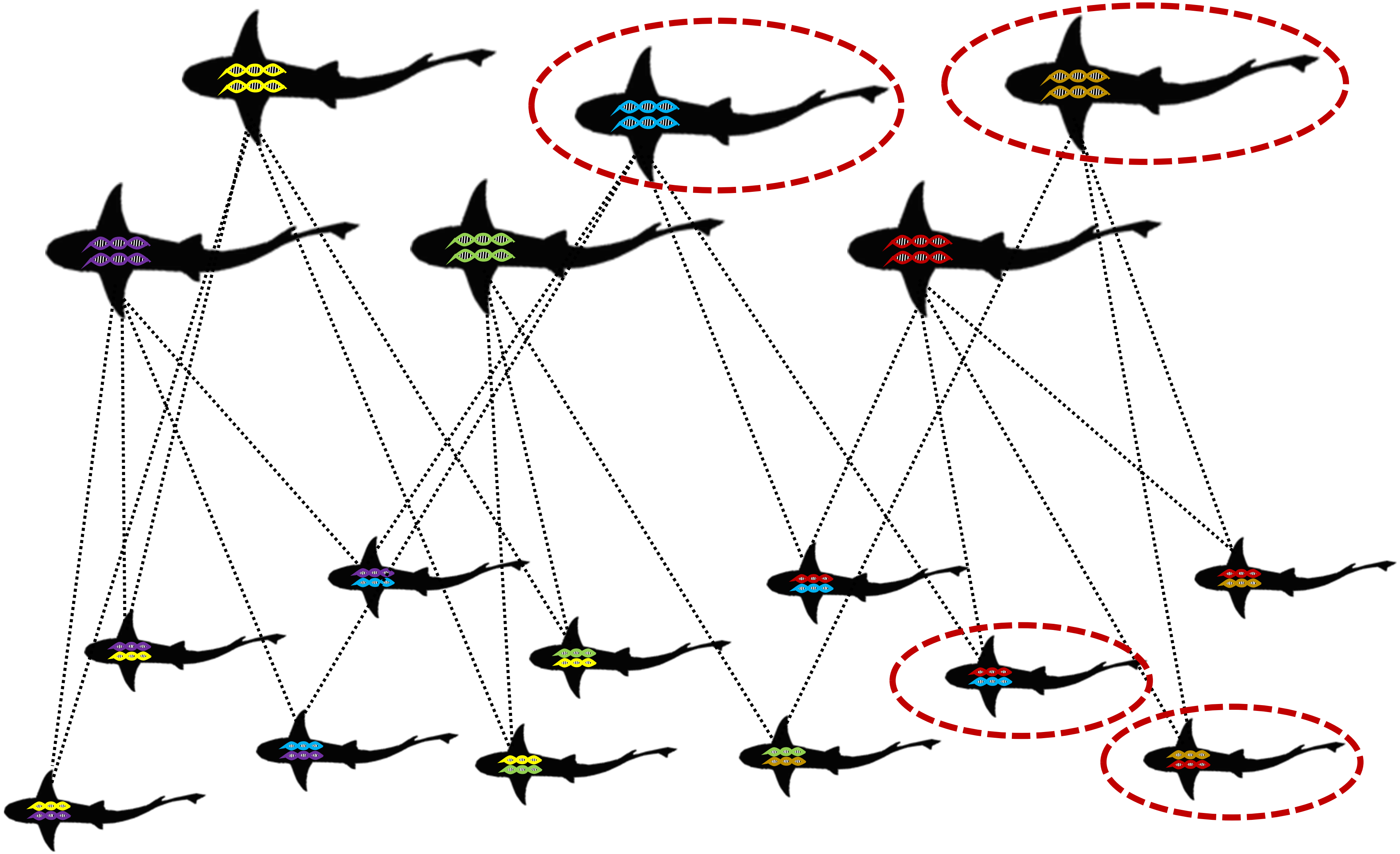
━ = captured parents

┈ = unobserved parents

━ = re-captured parents (HS)

Close-Kin Mark-Recapture (CKMR) uses probability of kinship to estimate population abundance

◯ = sampled



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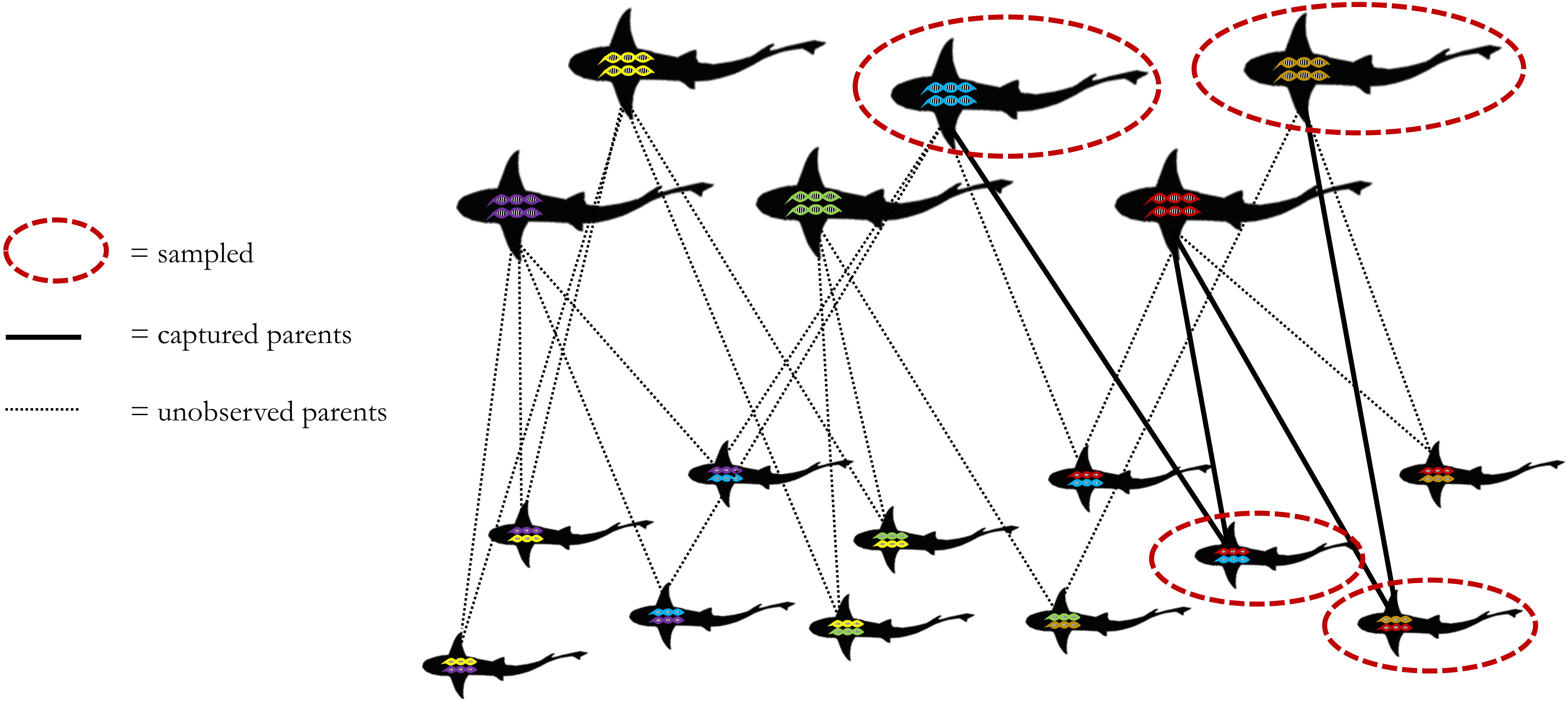

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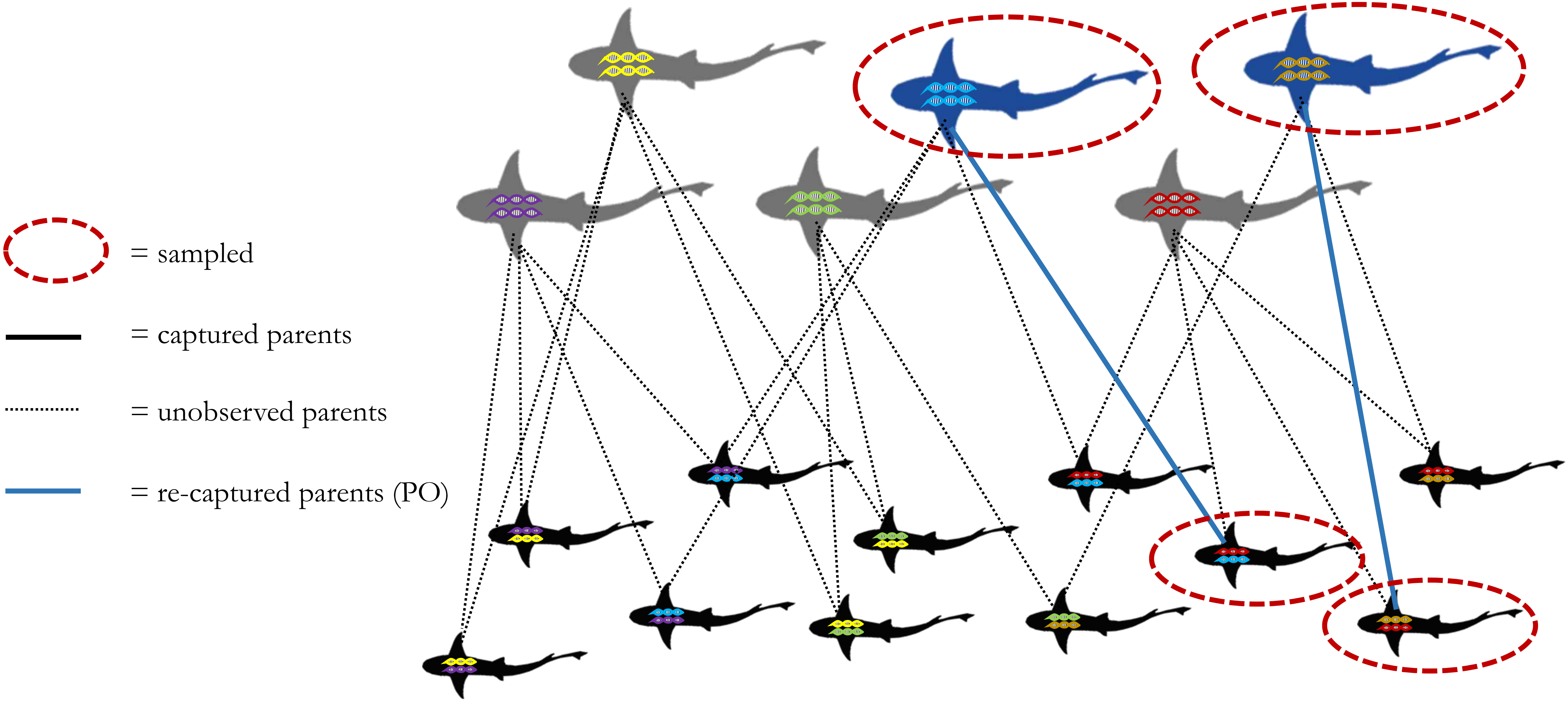

⬭ = sampled

— = captured parents

┈ = unobserved parents

— = re-captured parents (PO)

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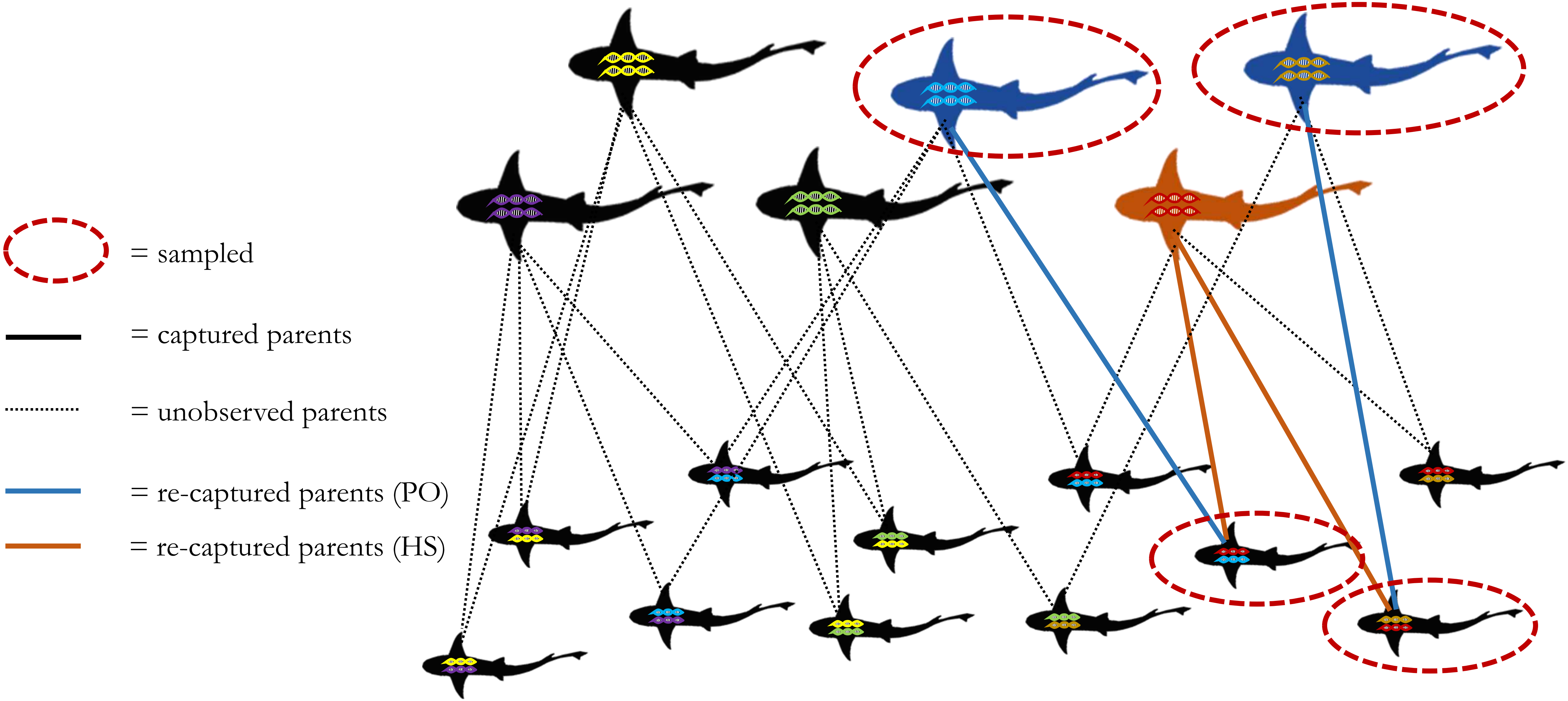


= sampled

= captured parents

= unobserved parents

= re-captured parents (PO)

= re-captured parents (HS)

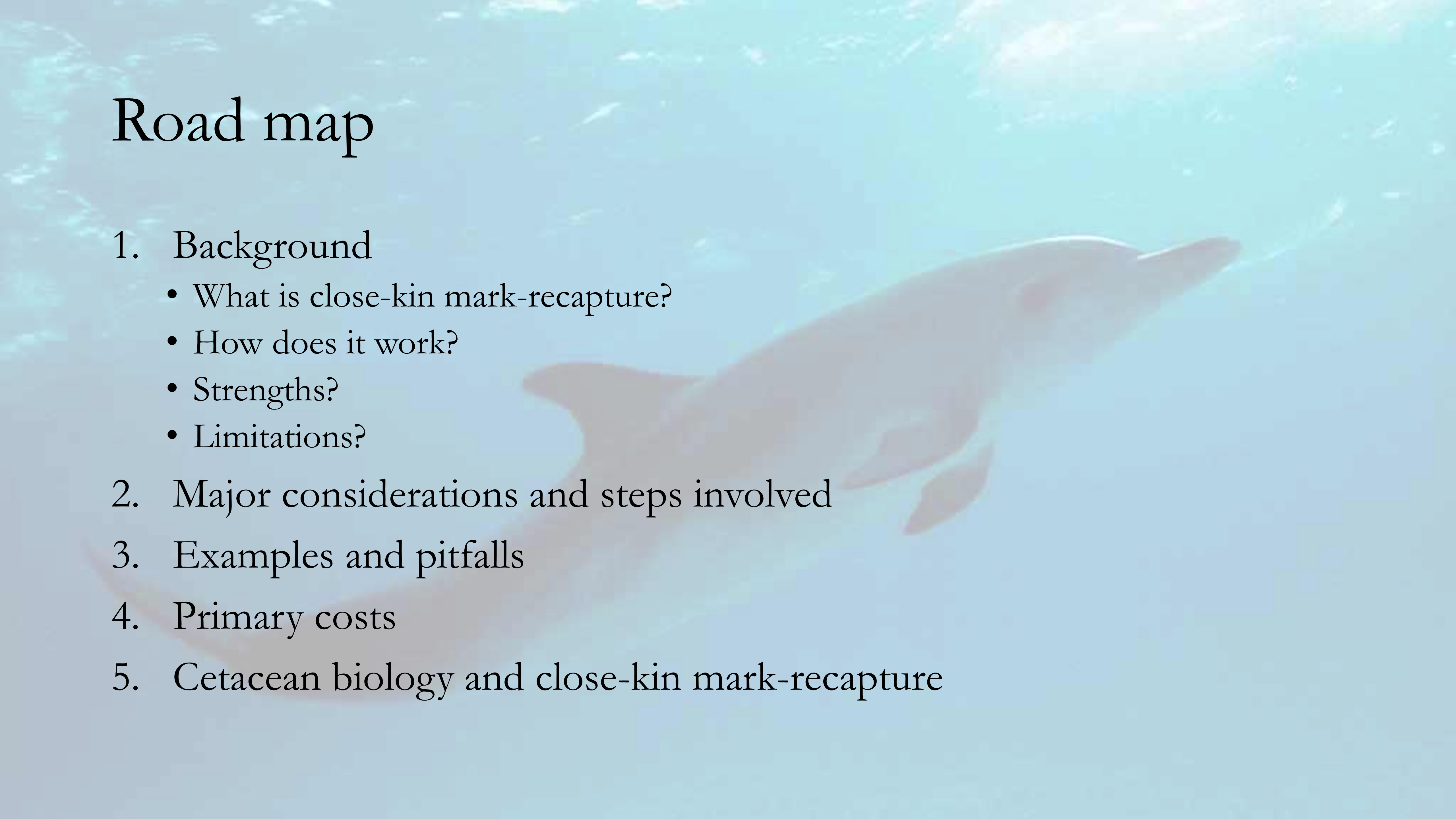
CKMR strengths

- No need for individual recapture
- No issues with tag loss or tag reporting
- No need to observe adults at all
- Costs and effort to maintain a CKMR program are reasonable following initial project setup

CKMR limitations

- Substantial initial investment needed
- Fundamentally can only inform on the adults
- Requires substantial biological knowledge to apply properly
- In most cases requires age data or, at a minimum, a reliable age-length curve

Road map



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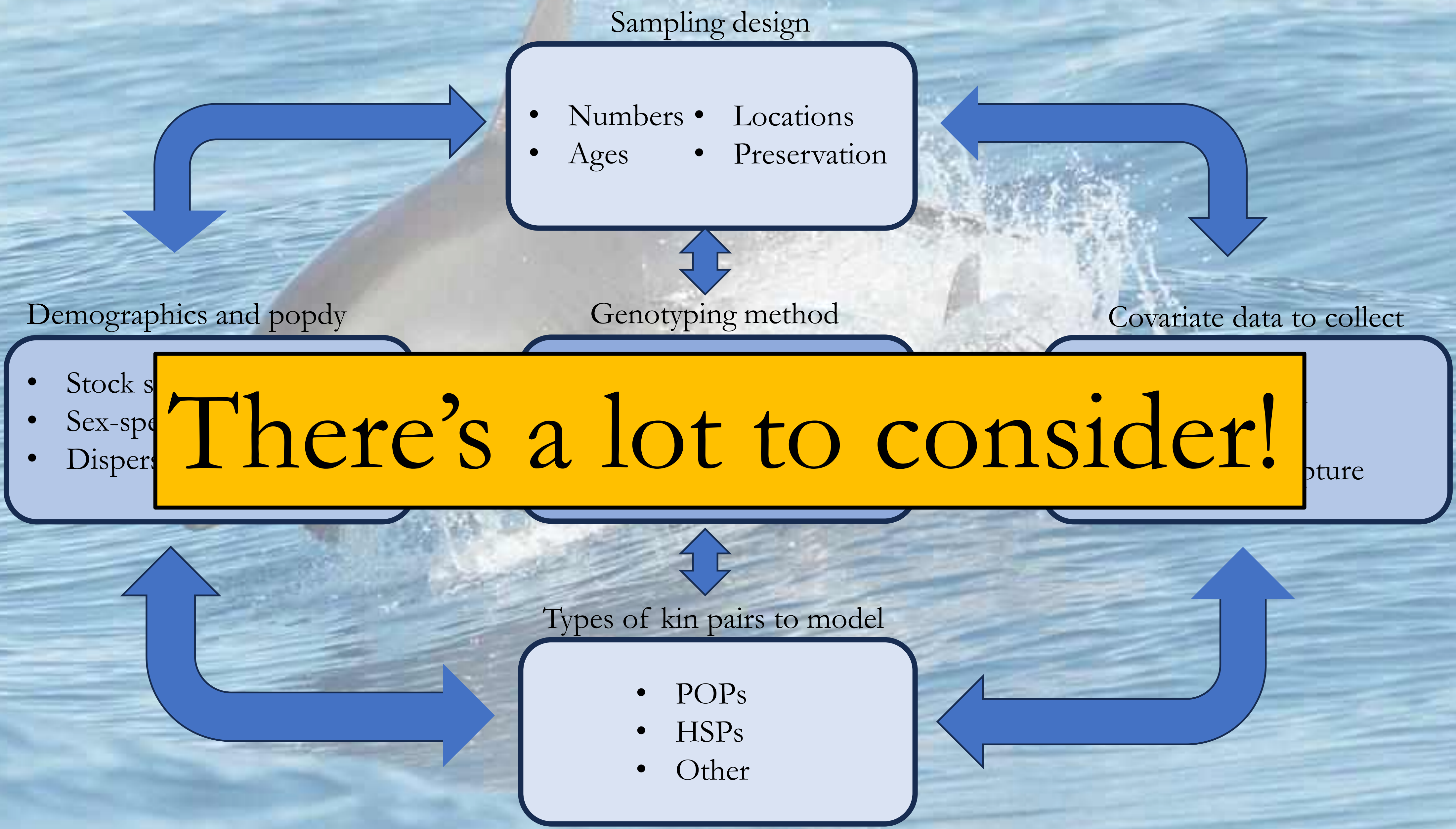
Major considerations for CKMR projects



Sampling design

- Numbers
- Ages
- Locations
- Preservation

Demographics and popdy

- Stock s
- Sex-spe
- Dispers

Genotyping method

Covariate data to collect

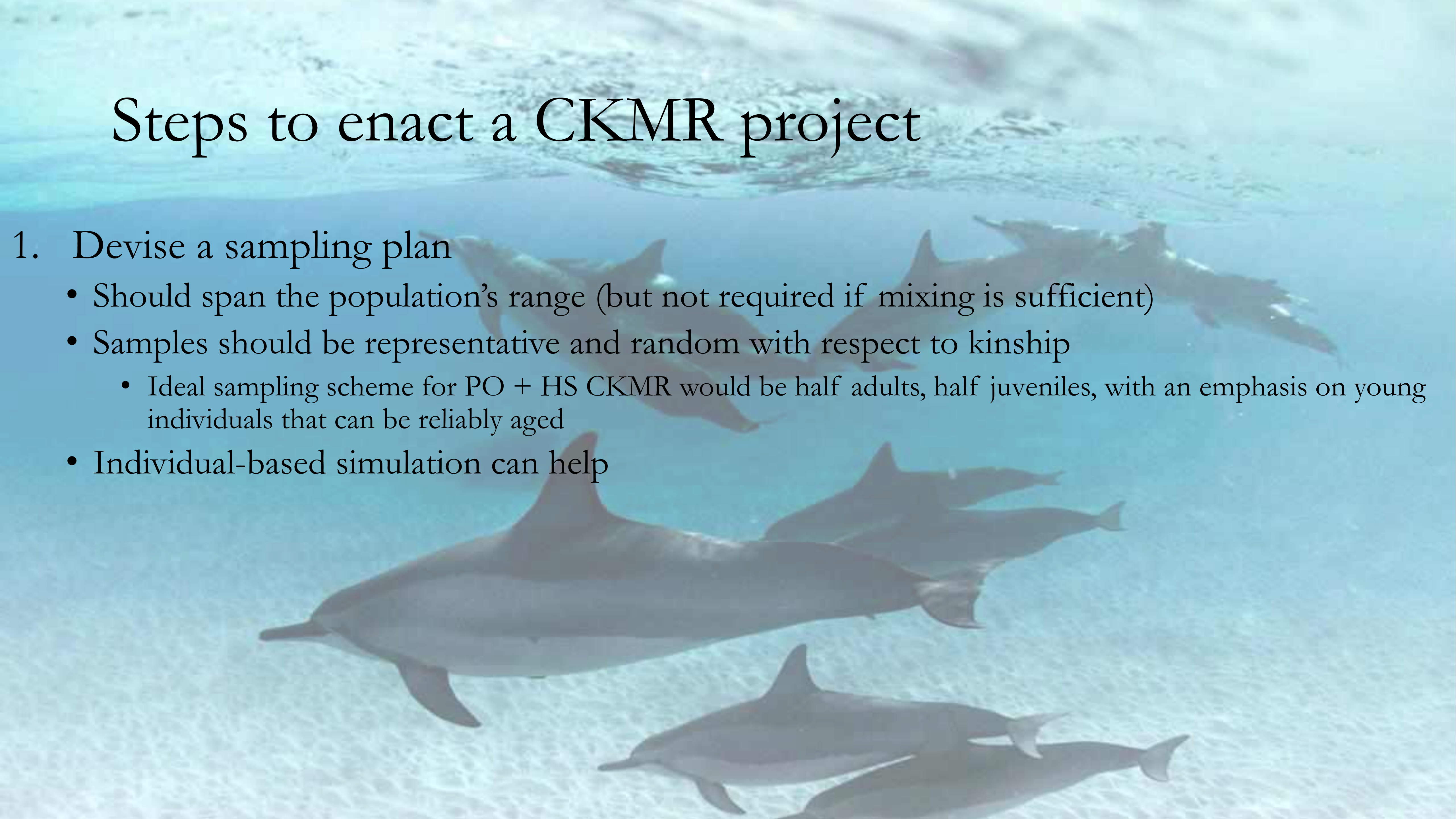
pture

Types of kin pairs to model

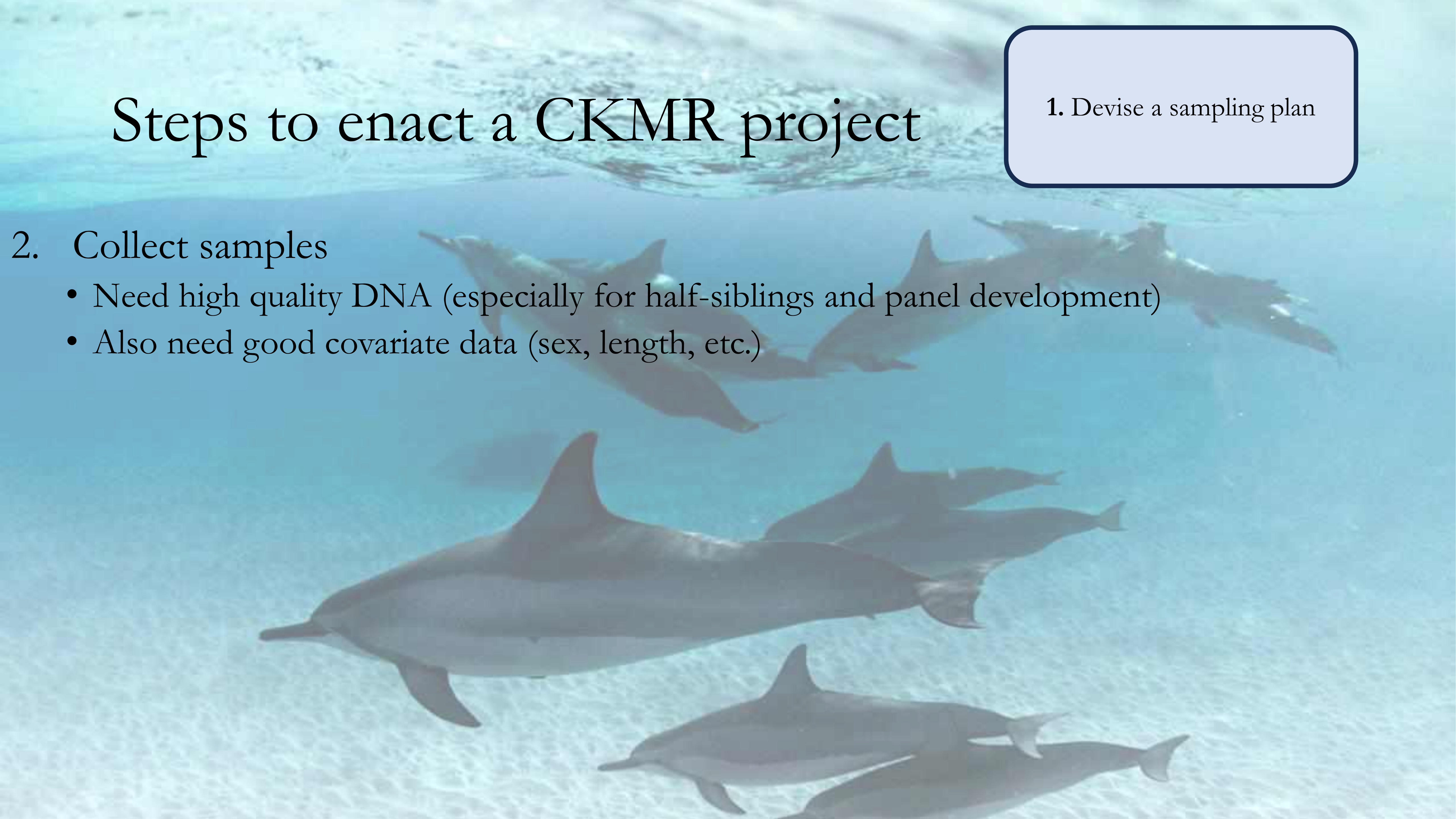
- POPs
- HSPs
- Other

There's a lot to consider!

Steps to enact a CKMR project



1. Devise a sampling plan
   - Should span the population’s range (but not required if mixing is sufficient)
   - Samples should be representative and random with respect to kinship
     - Ideal sampling scheme for PO + HS CKMR would be half adults, half juveniles, with an emphasis on young individuals that can be reliably aged
   - Individual-based simulation can help

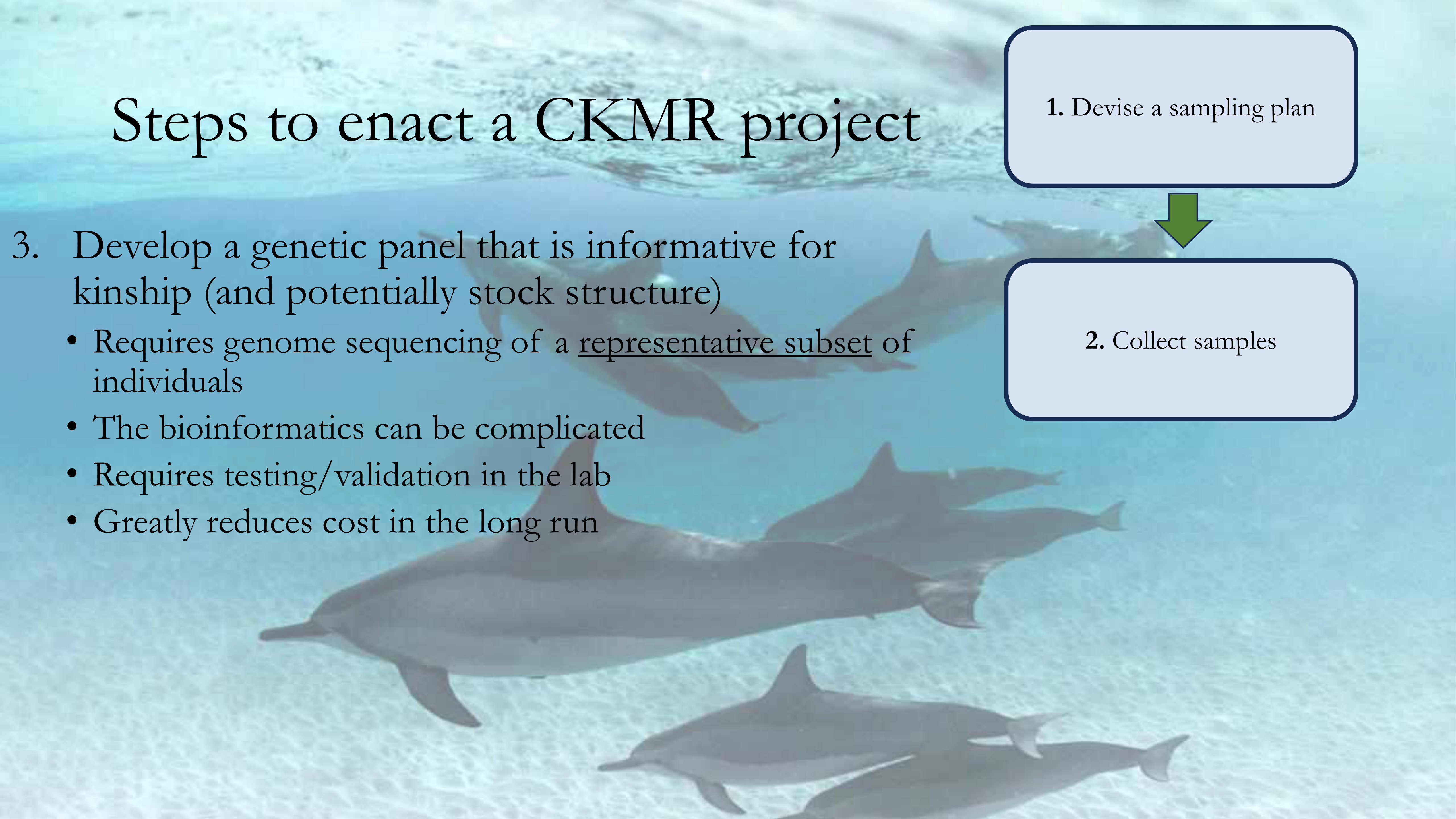


Steps to enact a CKMR project

1. Devise a sampling plan

2. Collect samples
    - Need high quality DNA (especially for half-siblings and panel development)
    - Also need good covariate data (sex, length, etc.)

Steps to enact a CKMR project

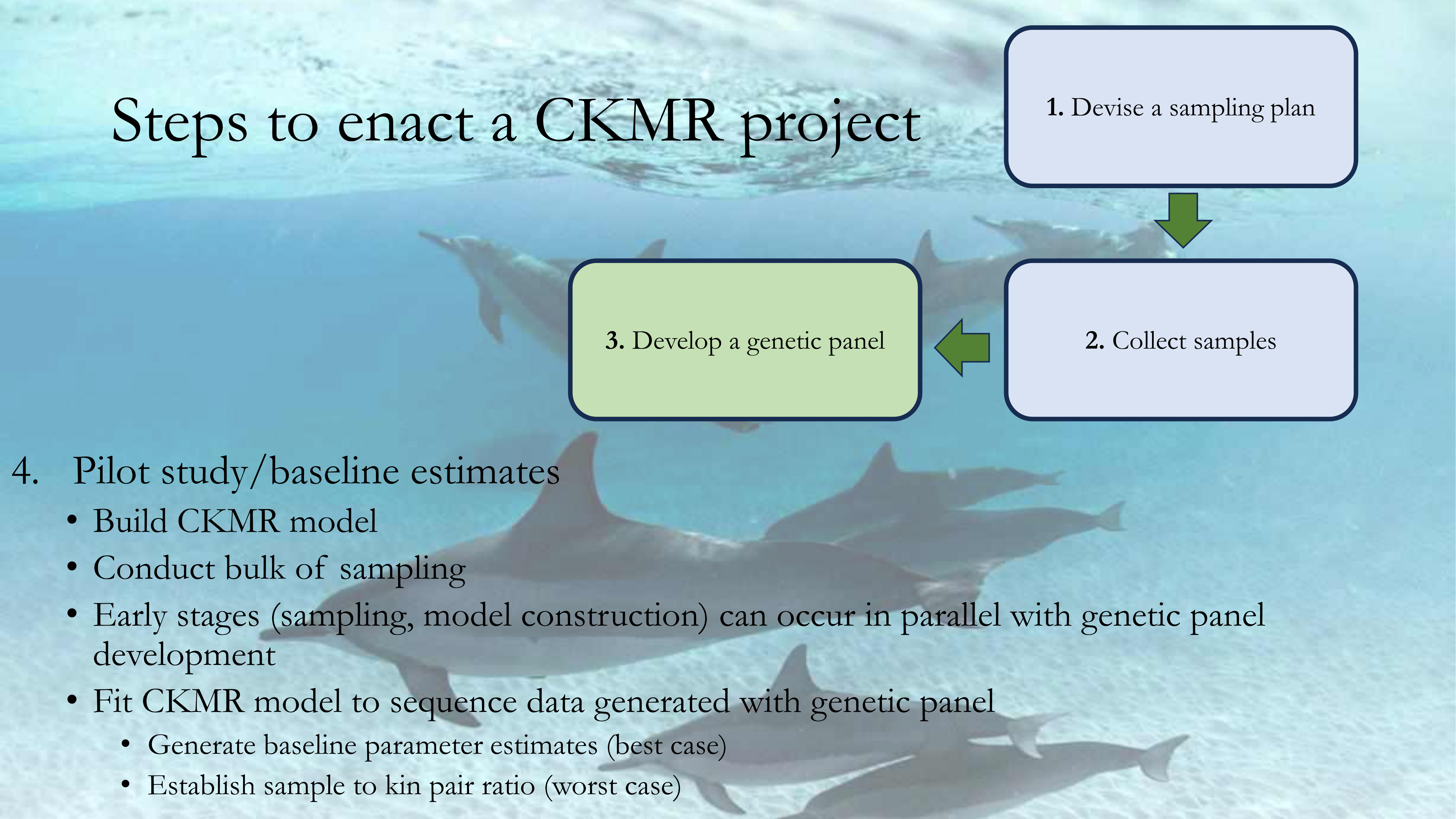


1. Devise a sampling plan

2. Collect samples

3. Develop a genetic panel that is informative for kinship (and potentially stock structure)
   - Requires genome sequencing of a representative subset of individuals
   - The bioinformatics can be complicated
   - Requires testing/validation in the lab
   - Greatly reduces cost in the long run

Steps to enact a CKMR project



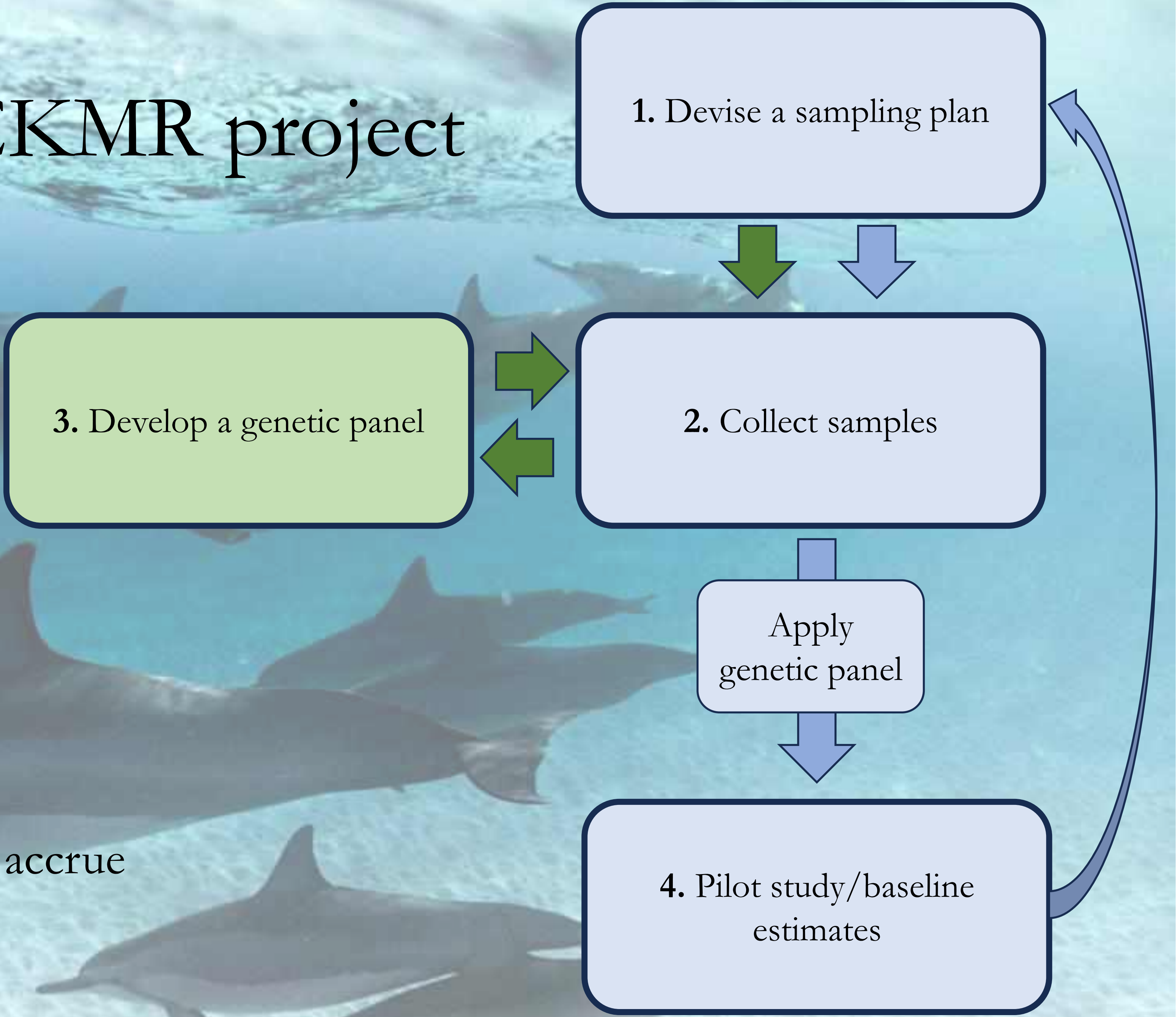
1. Devise a sampling plan

2. Collect samples

3. Develop a genetic panel

4. Pilot study/baseline estimates
   - Build CKMR model
   - Conduct bulk of sampling
   - Early stages (sampling, model construction) can occur in parallel with genetic panel development
   - Fit CKMR model to sequence data generated with genetic panel
     - Generate baseline parameter estimates (best case)
     - Establish sample to kin pair ratio (worst case)

Steps to enact a CKMR project



1. Devise a sampling plan

2. Collect samples

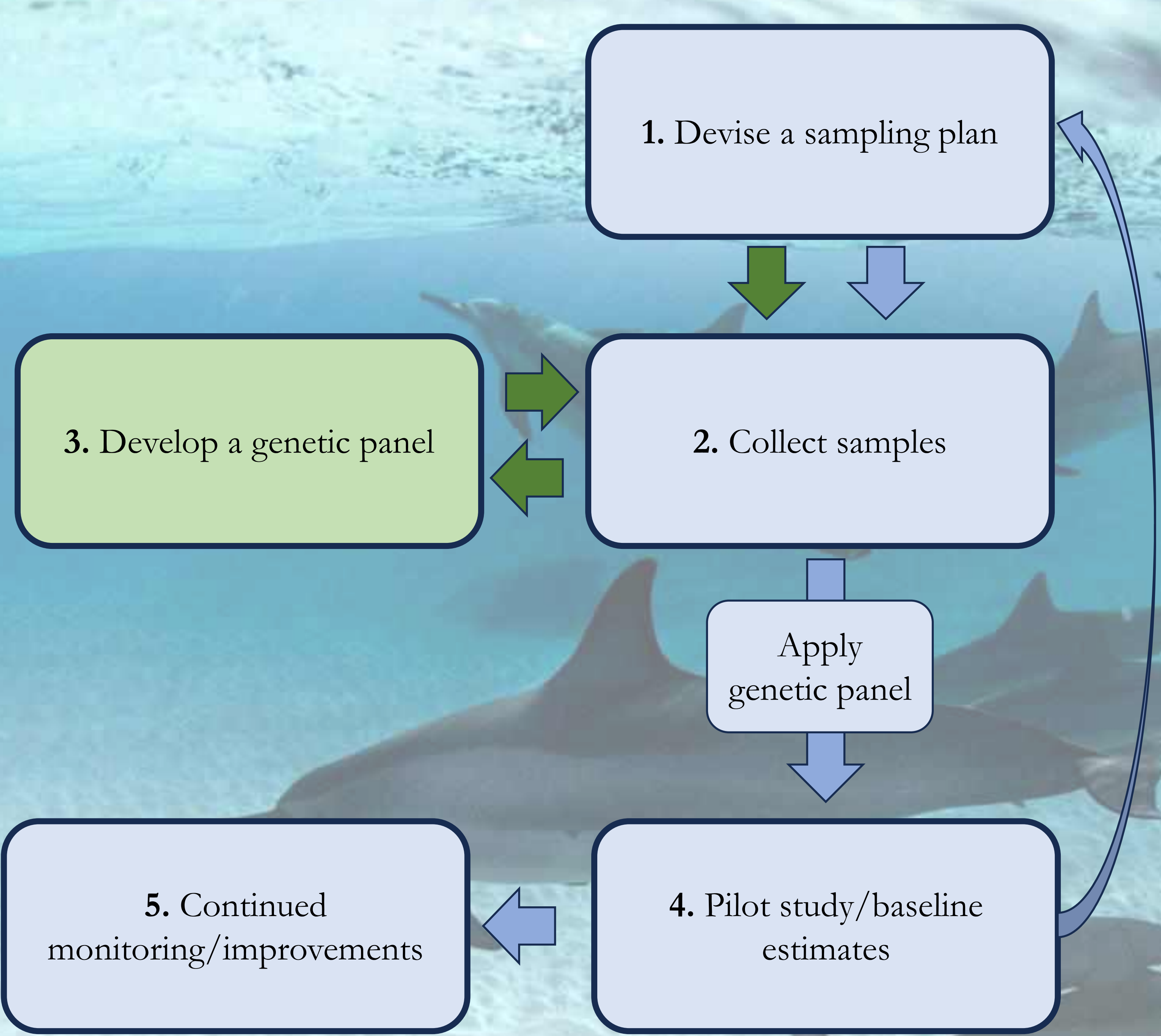
3. Develop a genetic panel

Apply genetic panel

4. Pilot study/baseline estimates

5. Continued monitoring
   - Precision will improve as samples accrue
   - Less costly to maintain

Steps to enact a CKMR project



1. Devise a sampling plan

2. Collect samples

3. Develop a genetic panel

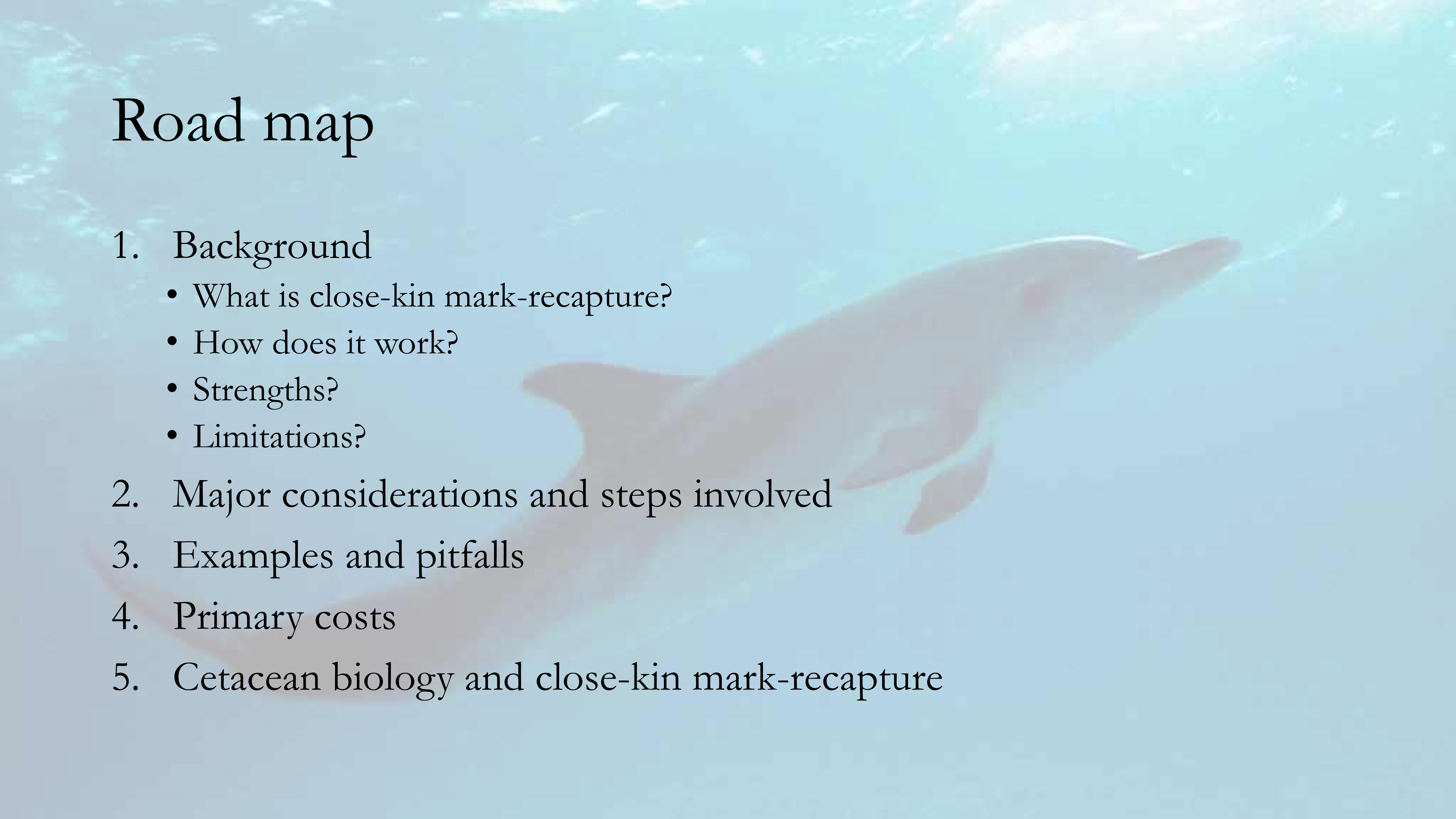
Apply genetic panel

4. Pilot study/baseline estimates

5. Continued monitoring/improvements

Assess feasibility and conduct design study to determine sample needs before starting

Road map



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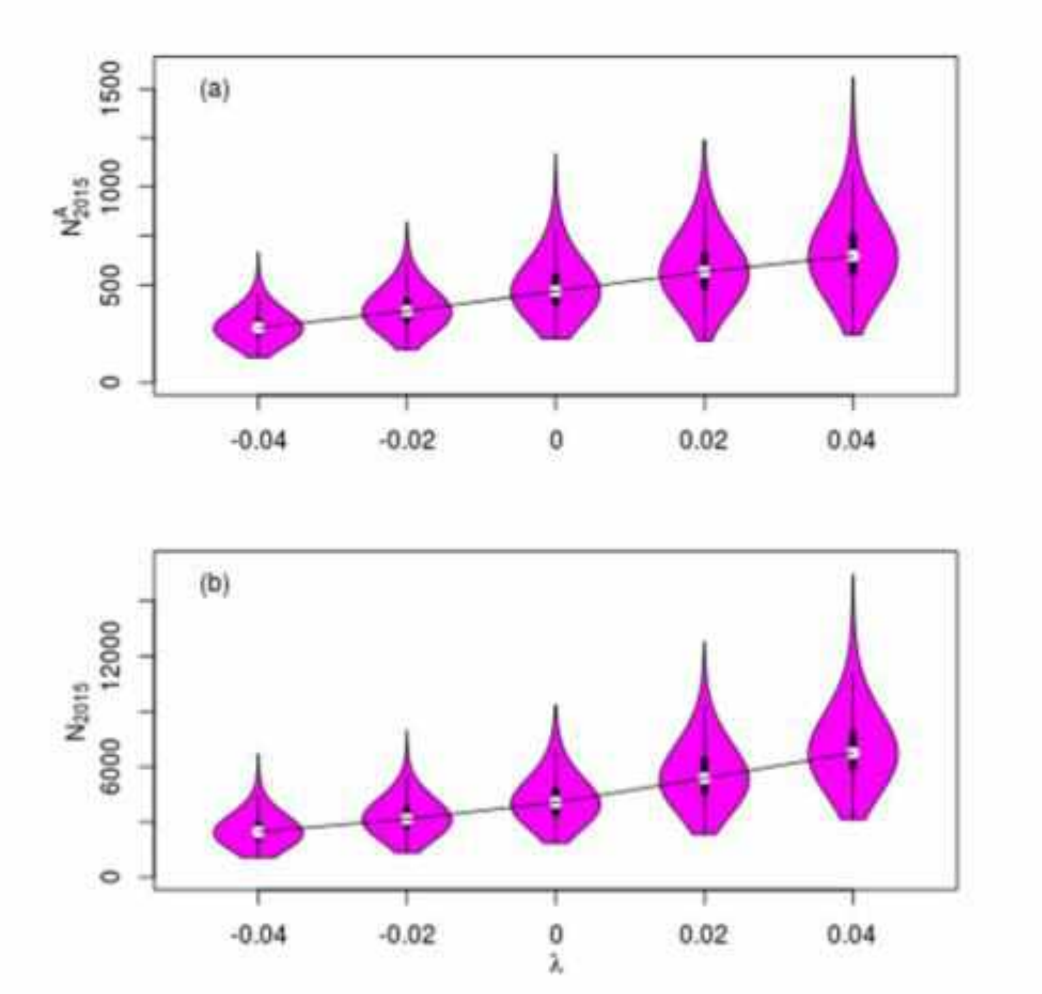
White sharks

- Used SNPs and half-sibling CKMR
- Estimated juvenile survival from acoustic data
- Combined acoustic tag data, CKMR, and alternative data sources on fecundity and YOY survival to estimate total population abundance



Hillary et al. 2018

Speartooth sharks

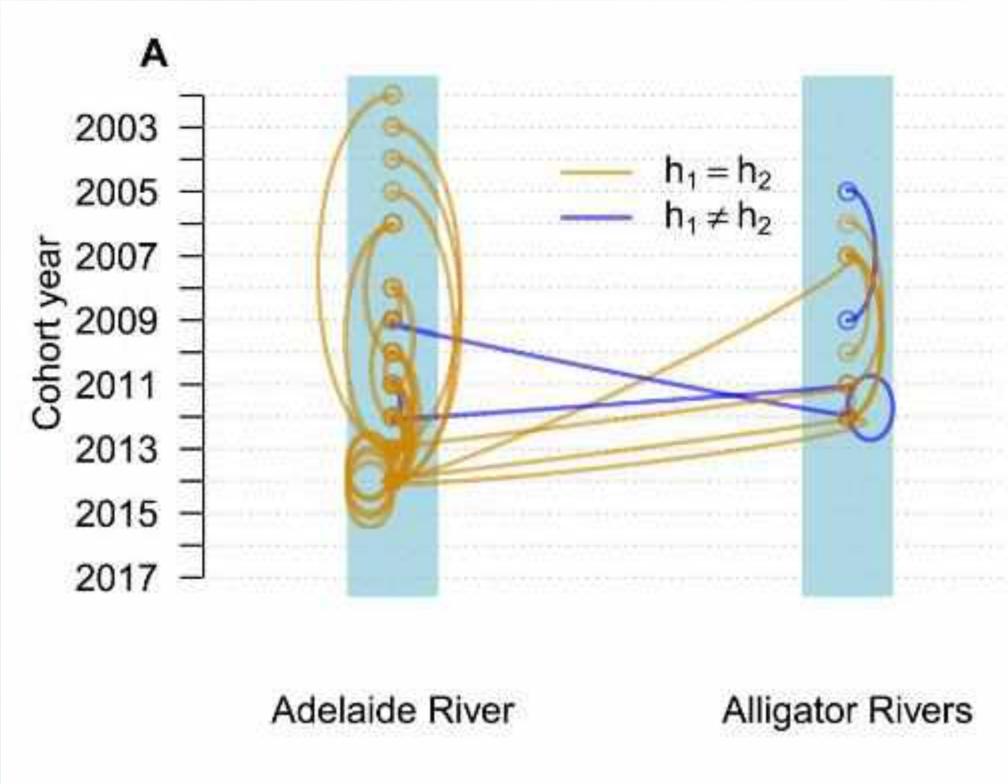
SCIENCE ADVANCES | RESEARCH ARTICLE

ECOLOGY

Rapid assessment of adult abundance and demographic connectivity from juvenile kin pairs in a critically endangered species

Toby A. Patterson[1]†*, Richard M. Hillary[1]†, Peter M. Kyne[2], Richard D. Pillans[3], Rasanthi M. Gunasekera[1], James R. Marthick[4], Grant J. Johnson[5], Pierre Feutry[1]

- Used SNPs and half-sibling/full-sibling CKMR
- Estimated abundance and connectivity
- Also adult survival, sex ratio, lucky litter effect, effective female mates per male
- Only needed four years of sampling

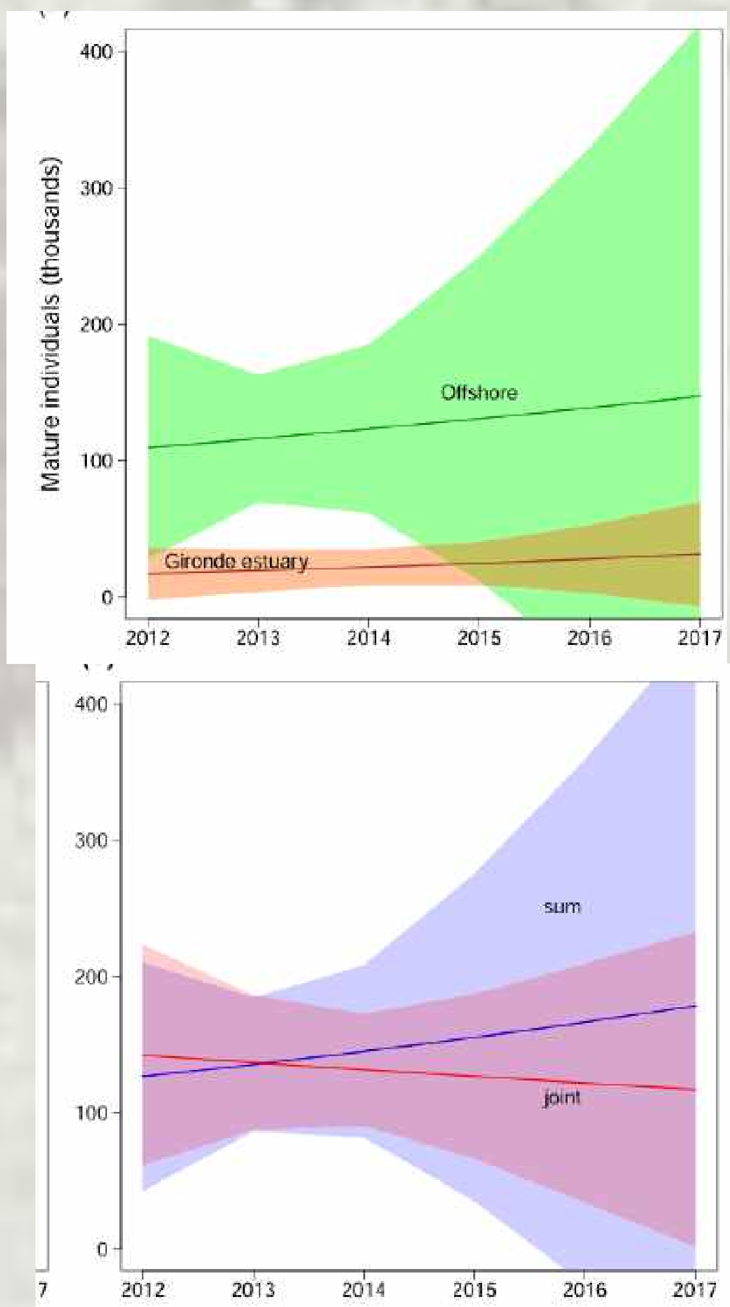


Patterson et al. 2022

Challenging applications

- Trenkel et al. (2022) applied CKMR to the thornback ray (*Raja clavata*).
    - Planned to use both HSPs and POPs, but had to abandon HSPs for abundance estimates
    - Still used HSPs to define metapopulation structure
    - Found unexpected metapopulation structure, so had to generate multiple abundance estimates
    - Estimates of population growth were imprecise

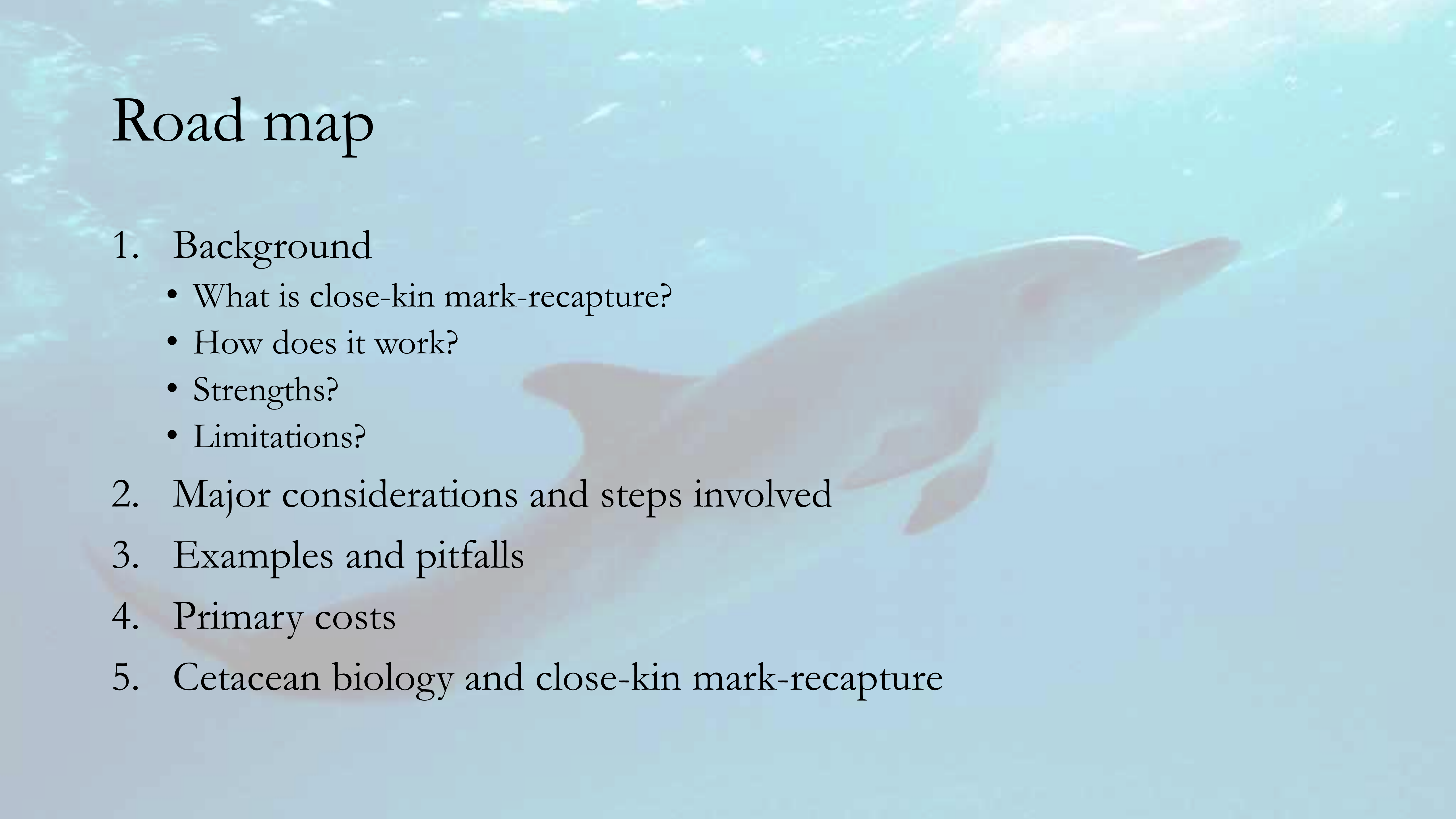
- No clear example (that I'm aware of) of successful estimation of population growth rate.



Other applications

- Bluefin tuna (Bravington et al. 2016)
- Trout (Marcy-Quay et al., 2020, Ruzzante et al. 2019)
- Salmon (Wacker et al. 2021)
- Northern river shark (Bravington et al. 2018)
- Grey nurse shark (Bradford et al. 2018)
- Blue skate (Delaval et al. 2023)
- Arctic grayling (Prystupa et al. 2021)
- Christmas Island flying fox (Lloyd-Jones et al. 2023)

Microsatellites
SNPs



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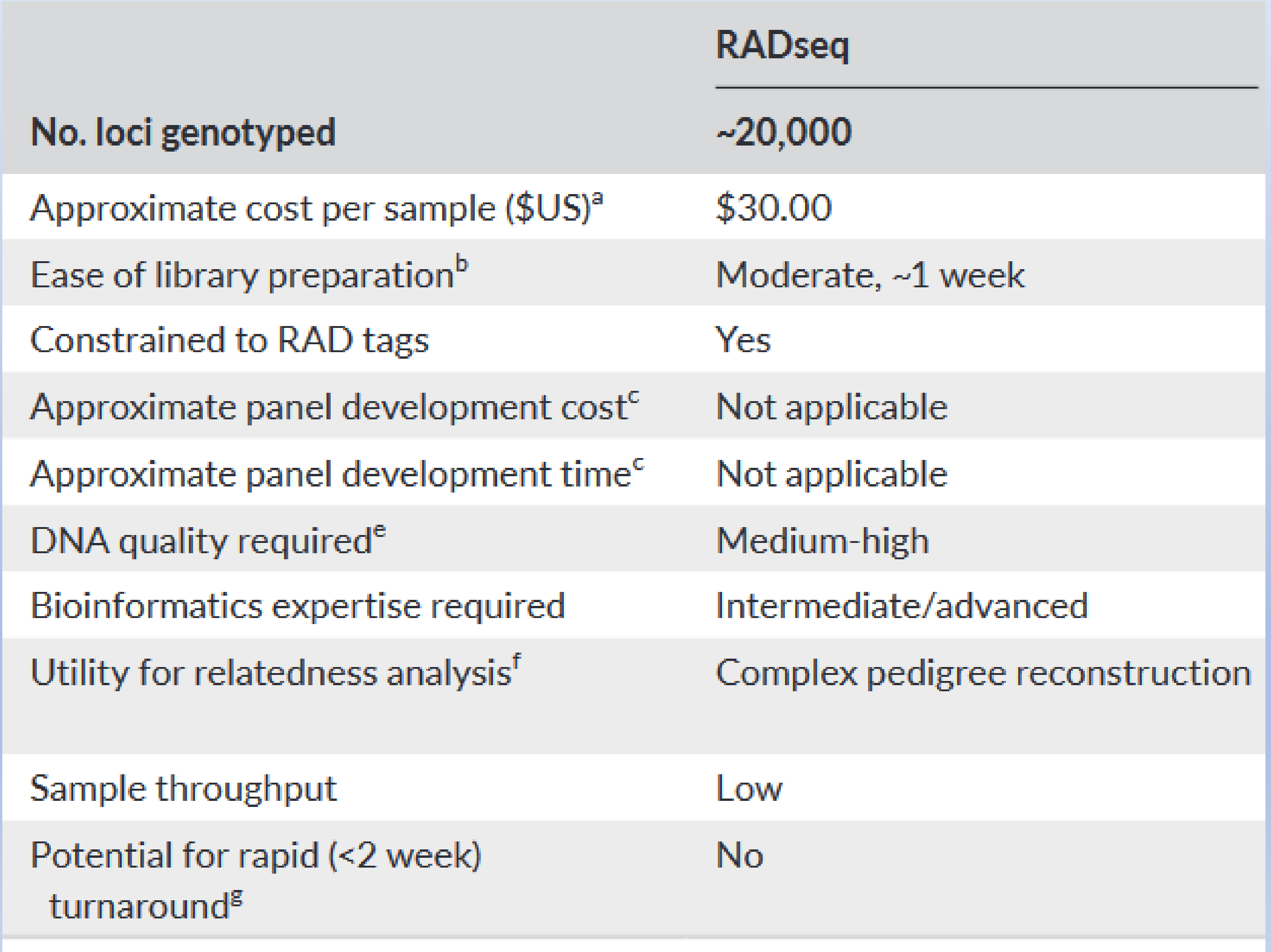
Primary costs

- Project design (Personnel)
    - Perhaps 6 months (full time) to 1 year (part time) of personnel costs
- Sampling (Supplies)
    - Most expensive supply (other than boat time) is disposable biopsy, if used (~$2 per sample)
    - Reusable biopsy also available, but would require cleaning between samples
- Labwork/genotyping (Supplies/Services)
    - Most expensive part other than personnel
    - Includes:
        - Initial sequencing of subset of samples and development of a genetic panel
        - High-throughput sequencing of remaining samples (with genetic panel)
    - There are options …

Primary costs

Initial sequencing

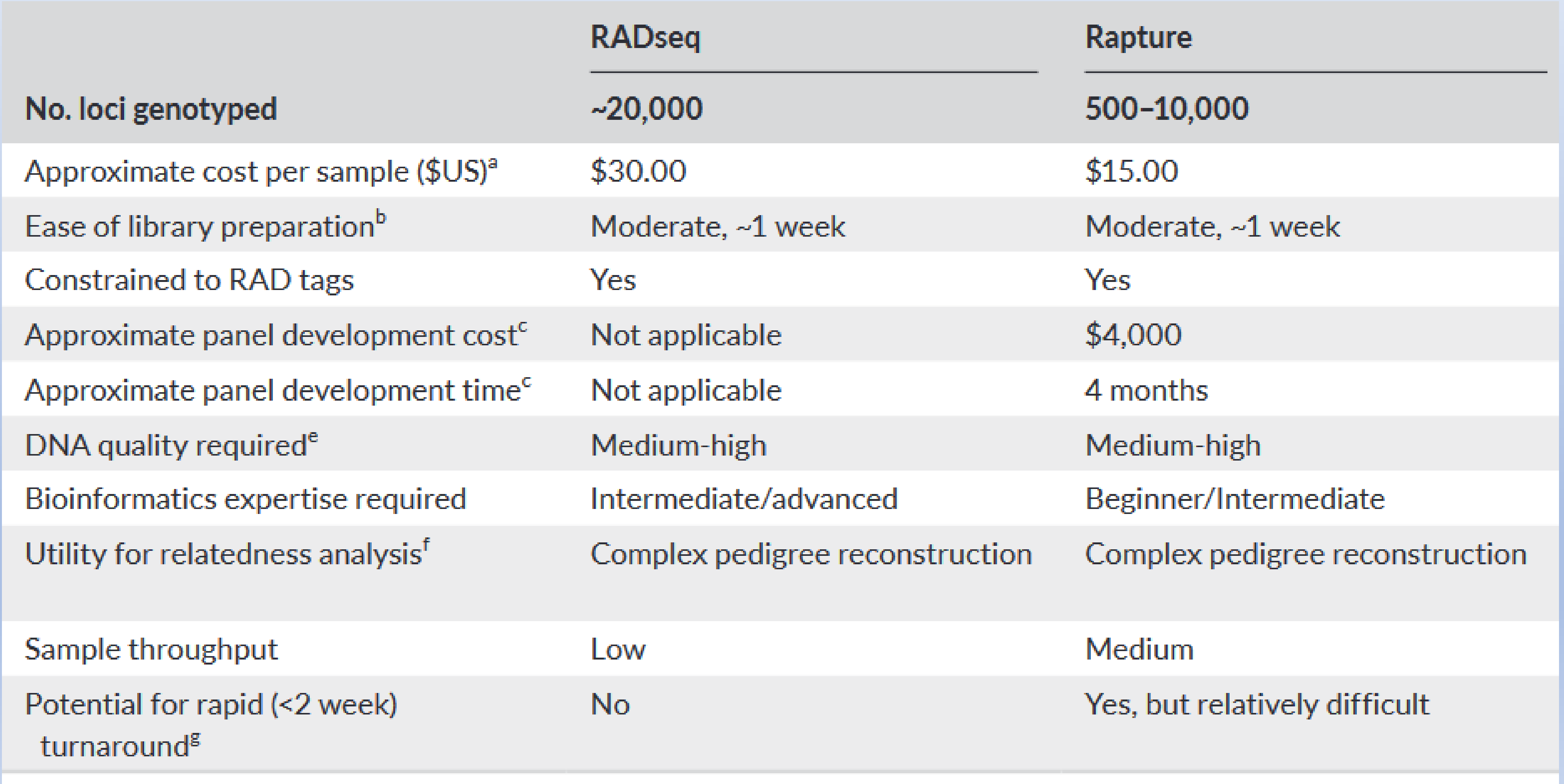


	RADseq
No. loci genotyped	**~20,000**
Approximate cost per sample ($US)[a]	$30.00
Ease of library preparation[b]	Moderate, ~1 week
Constrained to RAD tags	Yes
Approximate panel development cost[c]	Not applicable
Approximate panel development time[c]	Not applicable
DNA quality required[e]	Medium-high
Bioinformatics expertise required	Intermediate/advanced
Utility for relatedness analysis[f]	Complex pedigree reconstruction
Sample throughput	Low
Potential for rapid (<2 week) turnaround[g]	No

Primary costs

Initial sequencing

Panel options



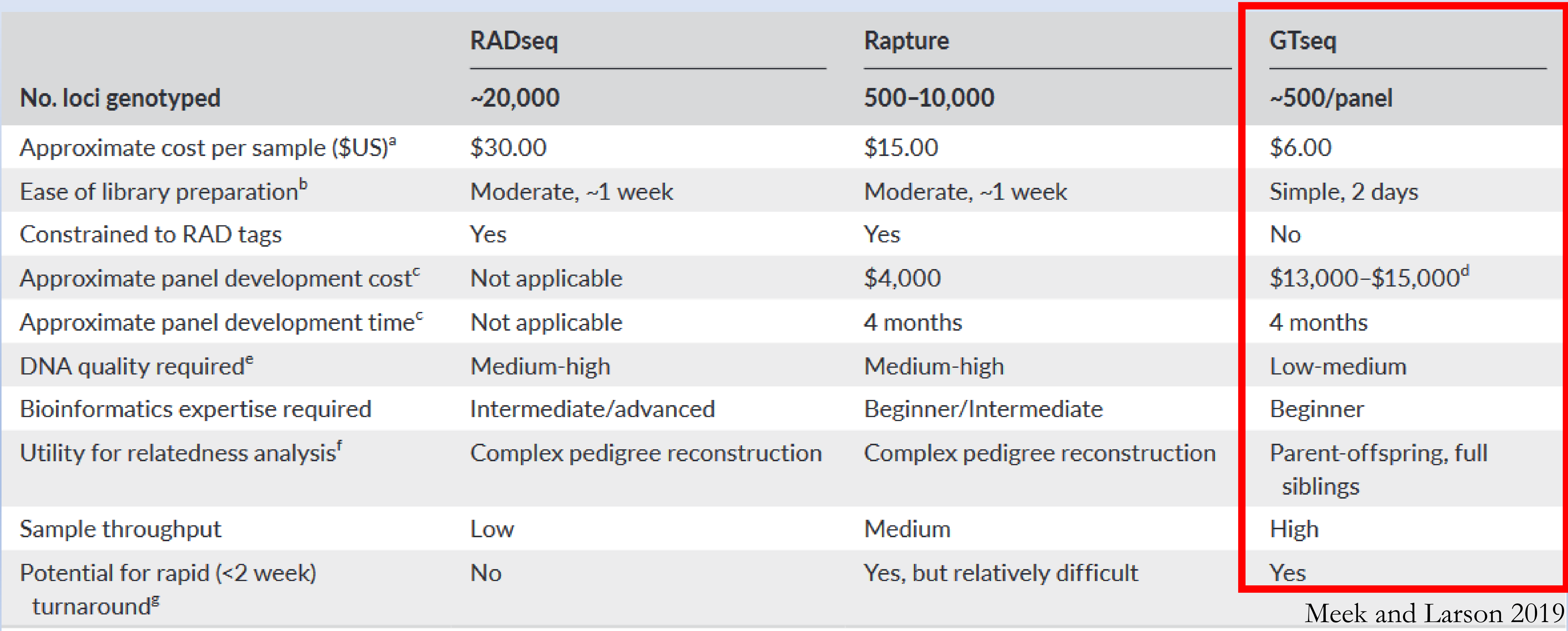
	RADseq	**Rapture**
No. loci genotyped	**~20,000**	**500–10,000**
Approximate cost per sample ($US)[a]	$30.00	$15.00
Ease of library preparation[b]	Moderate, ~1 week	Moderate, ~1 week
Constrained to RAD tags	Yes	Yes
Approximate panel development cost[c]	Not applicable	$4,000
Approximate panel development time[c]	Not applicable	4 months
DNA quality required[e]	Medium-high	Medium-high
Bioinformatics expertise required	Intermediate/advanced	Beginner/Intermediate
Utility for relatedness analysis[f]	Complex pedigree reconstruction	Complex pedigree reconstruction
Sample throughput	Low	Medium
Potential for rapid (<2 week) turnaround[g]	No	Yes, but relatively difficult

Meek and Larson 2019

Primary costs

Initial sequencing

Panel options



	RADseq	Rapture	GTseq
No. loci genotyped	**~20,000**	**500–10,000**	**~500/panel**
Approximate cost per sample ($US)[a]	$30.00	$15.00	$6.00
Ease of library preparation[b]	Moderate, ~1 week	Moderate, ~1 week	Simple, 2 days
Constrained to RAD tags	Yes	Yes	No
Approximate panel development cost[c]	Not applicable	$4,000	$13,000–$15,000[d]
Approximate panel development time[c]	Not applicable	4 months	4 months
DNA quality required[e]	Medium-high	Medium-high	Low-medium
Bioinformatics expertise required	Intermediate/advanced	Beginner/Intermediate	Beginner
Utility for relatedness analysis[f]	Complex pedigree reconstruction	Complex pedigree reconstruction	Parent-offspring, full siblings
Sample throughput	Low	Medium	High
Potential for rapid (<2 week) turnaround[g]	No	Yes, but relatively difficult	Yes

Meek and Larson 2019

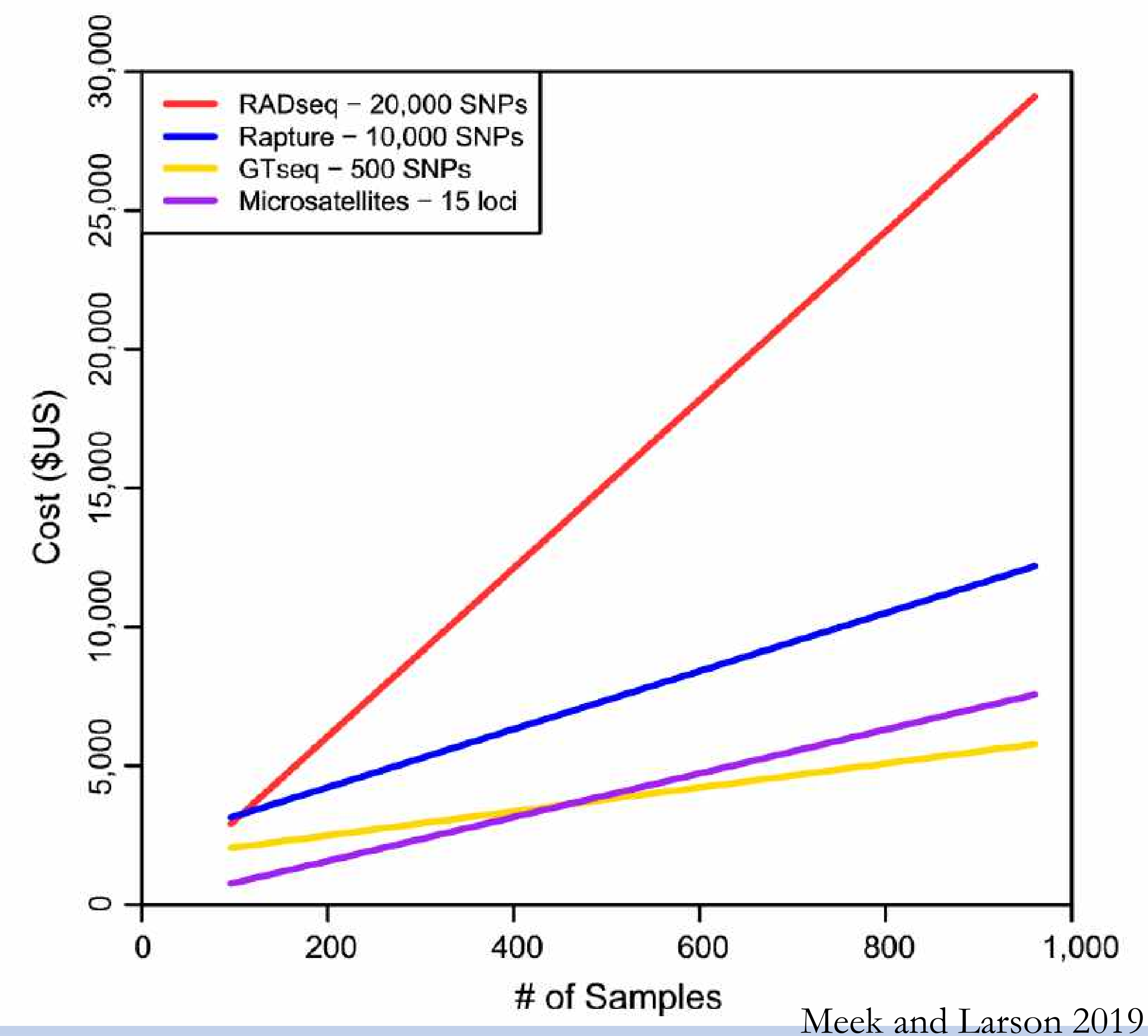
Primary costs

Personnel

- Sampling
- Labwork
- Bioinformatics and kinship
- Population dynamics modeling

Project maintenance

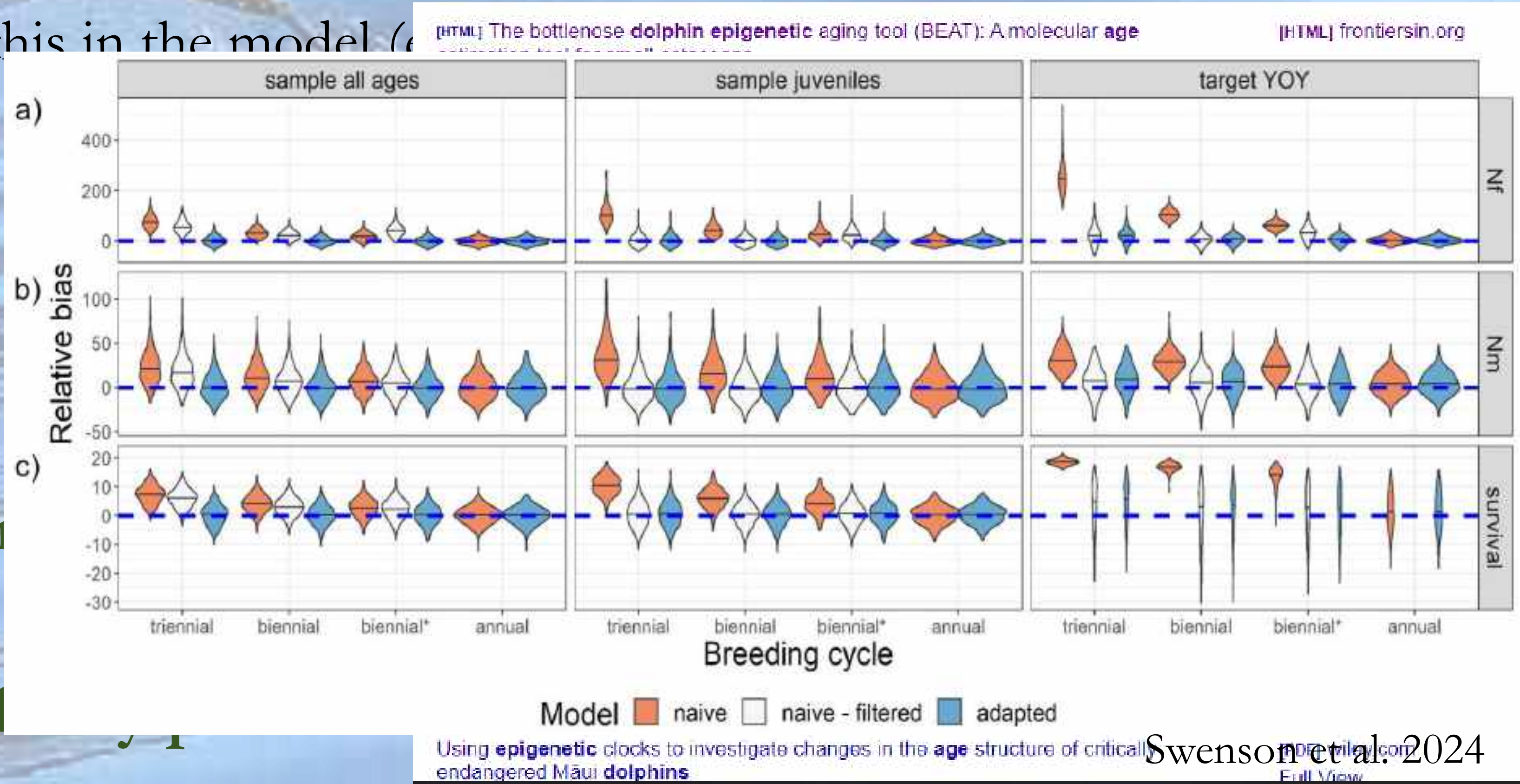
- Continued sampling
- Continued genotyping (with genetic panel)



Meek and Larson 2019

Considerations for application to cetaceans

- **Sampling bias**
  - Probability of sampling a parent **should not** be correlated with the probability of sampling its offspring
  - Could potentially account for this in the model (e
    capture?)
- **Intermittent breeding**
  - Straightforward to account for
- **Good genomic resources (i**
- **Epigenetic age estimation l**



[HTML] The bottlenose **dolphin epigenetic** aging tool (BEAT): A molecular **age** [HTML] frontiersin.org

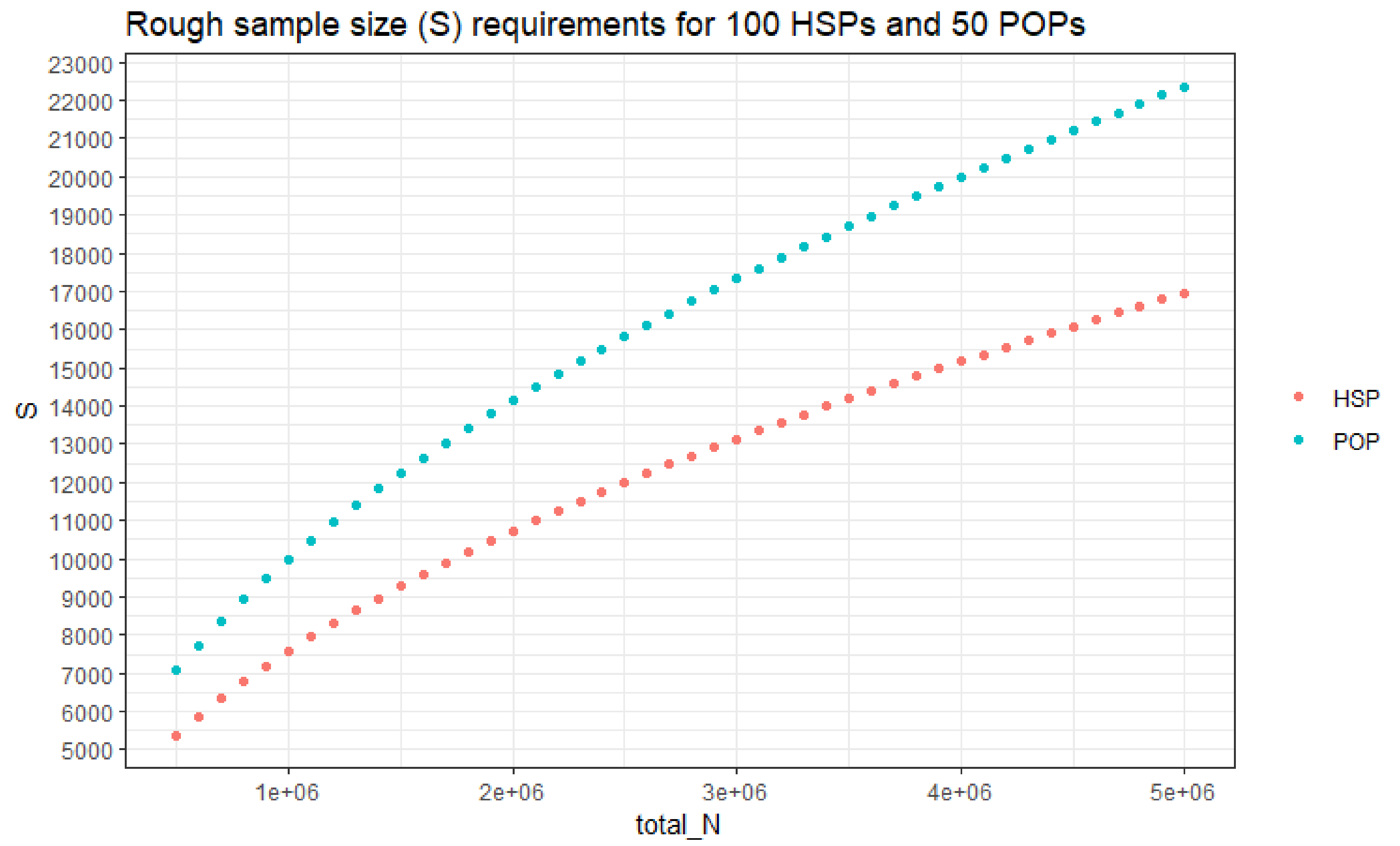
Using **epigenetic** clocks to investigate changes in the **age** structure of critically endangered Māui **dolphins**

Swenson et al. 2024

ROUGH sample size estimator

- Buckland et al. (2016) estimated that, based on an abundance of 1.3 million offshore spotted dolphins, tissue samples from ~17,000 dolphins would be needed for conventional MR

- For PO CKMR, they estimated 9,000 samples



Thank you!

Photo sources:

https://www.animalia.bio/atlantic-spotted-dolphin
https://www.pexels.com/photo/dolphins-swimming-underwater-9638689/
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