ACCOBAMS

Best Practices

on Cetacean Population Genetics

Version 2, November 2023

ACCOBAMS Best Practices on Cetacean Population Genetics

Version 2, November 2023

This Best Practices document was drafted by the participants to the "ACCOBAMS workshop on Data Collection on Cetacean Population Genetics in the ACCOBAMS area", held in September 2022: Pauline Gauffier, Anna Schleimer, Inês Carvalho, Olfa Chaieb, Greg Donovan, Michael Fontaine, Natalia Fraija, Tilen Genov, Pavel Gol'din, Nik Lupše, Sandro Mazzariol, Paula Méndez Fernández, Cristina Panti, Céline Tardy, Arda Tonay, Karina Vishnyakova, with the support of the Secretariat (see report ACCOBAMS-MOP8/2022/Inf25). Version 1 of this document was presented at the 8th Meeting of the Parties to ACCOBAMS, 29 November - 2 December 2022, Malta (ACCOBAMS-MOP8/2022/Inf54). Resolution 8.11 on Cetacean Population Genetics invites Parties to apply these ACCOBAMS Best Practices on Cetacean Population Genetics. Minor edits were added in November 2023, such as updating the Appendix 2, and are included in this new version (Version 2, November 2023).

Some parts are extensively based on existing guidelines such as the ones developed by IWC (see references herein). Furthermore, Ralph Tiedemann, Amy Van Cise, Elena Valsecchi kindly provided useful comments and allowed that text and figures from their papers are reflected in these Best Practices document.

Due to the quick development of new analytical techniques, these Best Practices should be considered as a living document and be updated regularly.

All the pictures (unless otherwise specified) were downloaded under a Freepik Premium license (Unlimited use without attribution). Art direction and design by Irene Álvarez Vicente and Irem Özbek.

How to cite: ACCOBAMS 2023. ACCOBAMS Best Practices on Cetacean Population Genetics. Version 2, November 2023. By Gauffier, P., Schleimer, A., Carvalho, I., Chaieb, O., Donovan, G., Fontaine, M., Fraija, N., Genov, T., Gol'din, P., Lupše, N., Mazzariol, S., Méndez Fernández, P., Panti, C., Salivas, M., Tardy, C., Tonay, A., Vishnyakova, K. 98 pp.

Index

Introduction 4

01) Study design 6
A) Define study objectives/hypotheses 6
B) Choice of genetic/omic markers 7
C) Seeking collaborators 9

02) Permits for samples collection 10
A) National permits 10
B) Report to ACCOBAMS 11

03) Sample collection 12
A) Biopsy sampling 13
B) Stranded animals 15
C) Environmental DNA 16
D) Scrub sampling/skin swabbing 19
E) Faeces 19
F) Museum collections 19
G) Ancient DNA 20
H) Other techniques - Sloughed skin 21

04) Sample preservation 22
A) General recommendations 22
B) Long-term storage 25
C) Backup at tissue banks 25

05) Exchange of samples 27
A) Agreement or Memorandum of Understanding (MOU) 27
B) CITES Procedure 27
C) Nagoya Protocol on Access and Benefit-Sharing 30
D) Inform ACCOBAMS National Focal Point 32

06) Sample processing 33
A) Techniques/protocols for sample processing 33
B) IWC guidelines on quality control 34
C) Suitable genetic labs 35

07) Data analysis 36
A) IWC guidelines on data analysis 36
B) Environmental DNA/faeces 38

C) Computational resources for genomic data ... 38

08) Dissemination of results ... 39
A) ACCOBAMS Scientific Committee ... 39
B) Scientific community ... 39
C) ACCOBAMS National Focal Point ... 39
D) Other stakeholders ... 39
E) General public ... 39

09) Data archiving and collecting data from previously published studies ... 40

10) Forensic science ... 41
A) Special case of individual identification of captive individuals ... 41
B) Species identification for trade ... 41
11) Glossary of terms ... 42

References ... 46

Appendix 1 ... 54
01) Summary for each species ... 54
02) Summary table of scientific literature ... 62
03) Full reference list for species summaries ... 80

Appendix 2 ... 90
Suitable genetics labs in the ACCOBAMS area [November 2023] ... 90

Appendix 3 ... 92
Example laboratory protocols for DNA extraction from tissue ... 92
A) The Ammonium acetate precipitation method ... 92
B) The Phenol/Chloroform method ... 94

Appendix 4 ... 96
Example of Biological Material Transfer Agreement ... 96

Introduction

Understanding population structure and delimiting appropriate units-to-conserve (often, but not always 'biological populations') is essential to good conservation and management. Although such understanding requires integrating results from a suite of data types and analytical techniques, a fundamental component is population genetics. Two vital strands of the ACCOBAMS strategy for management are Conservation Management Plans (CMPs[1]) and the Long-Term Monitoring Programme (LTMP).

The overall goal of CMPs is to integrate scientific information to enable the management of human activities that affect a nominated species in a nominated area in order to maintain a favourable conservation status of that species. The first four being drafted are for fin whales, Risso's dolphins, bottlenose dolphins and common dolphins and all focus on the Mediterranean Sea.

To maintain favourable status of a species throughout the range requires determining the population structure within the range (*e.g.* the Mediterranean Sea) and determining the appropriate units-to-conserve (and their geographical and temporal boundaries). Whilst it is not impossible that there is only a single population of a species in the Mediterranean Sea with no geographical or temporal influx (or outflux) this is unlikely. It is not surprising that for all four draft CMPs, high priority Actions to determine appropriate management units have been developed. This will then allow determination of status and threats at the appropriate geographical scale for each management unit and facilitate any necessary mitigation measures.

The LTMP arose out of the success of the ACCOBAMS Survey Initiative in the Mediterranean Sea in 2018 and a similar effort in the Black Sea in 2019 in establishing baseline abundance estimates and distribution in summer for many species for the first time. Abundance (and trends in it) is a key parameter in determining status but interpreting the results of surveys requires knowledge of population structure and seasonal movements. In simple terms, assuming one population when there is more can lead to local depletions.

Whilst genetic studies can address a large number of issues related to cetaceans, the primary focus of these guidelines is on matters related to understanding population structure, abundance and movements in order for ACCOBAMS to meet its conservation and management objectives.

[1] See https://accobams.org/species_/conservation-plans

1. SET OVERALL OBJECTIVES OF STUDY

e.g. [contribute to] identifying units-to-conserve within a given area.

1

2. REVIEW AVAILABLE INFORMATION/SAMPLES

2.1) If possible and relevant, use available information (genetic and non-genetic) to postulate plausible hypotheses.
2.2) Identify if additional geographical and/or temporal coverage (samples) is required.
2.3) Determine whether collaboration is required (it usually is) and begin consultation early at the design stage, including relevant permits (Nagoya, CITES). This is true for field work. Lab work and analyses.

2

3. STUDY DESIGN - BEFORE YOU START!

3.1) Sampling strategy/method if new samples, including 'ancillary data', platform, sampling equipment, expertise, permits, short-term storage - sample size/distribution should be examined in conjunction with (3.4-3.6).

3.2) Explore permit situation if crossing borders ('old' and new samples).

3.3) Determine long-term storage/archiving strategy to allow new analyses.

3.4) Choose appropriate markers given objectives and resources. If using more than one lab ensure calibration where needed. Follow DNA quality guidelines.

3.5) As appropriate, decide null hypothesis/es.

3.6) Decide analytical techniques (normally more than one depending on markers) and look at potential power to meet objectives under various assumptions.

3

4. DO IT!

4.1) Complete fieldwork and analysis using recommended guidelines.
4.2) Write up report with full set of results.
4.3) Submit to ACCOBAMS Scientific Committee for review with focus on management implications.
4.4) If results not conclusive don't despair - science is iterative so return to Part 2!
4.5) Publish and disseminate results.

4

01) Study design

A) Define study objectives/hypotheses

Genetics can provide insights relevant to many aspects of conservation and management planning for cetaceans. For instance, genetic data can be used to:

- Identify and delimit species, subspecies, populations;
- Investigate the occurrence of hybridisation;
- Estimate effective population size;
- Identify individuals and track their contemporary movements;
- Characterise levels of genetic connectivity and differentiation among populations;
- Quantify genetic diversity within populations with insights into past demographic processes;
- Resolve population admixture and assign individuals to the population which they most likely originated from;
- Forensic science.

Within the ACCOBAMS area, CMPs often require input from genetic analyses. The definition of clear study objectives will determine the study design in terms of required sample sizes, genetic markers, spatial-temporal coverage, and collaborations. By quantifying expectations before the study begins, researchers can plan an optimal experimental study design. Waples *et al*. (2018) specify the IWC's approach to determining stock structure and discuss the use of threshold levels of population differentiation that require separate stock management.

Appendix 1 compiles a literature review on existing studies relating to the genetics of cetaceans in the ACCOBAMS area as of September 2022, and identifies knowledge gaps. **Future studies should aim to fill these existing gaps and update this information.**

B) Choice of genetic/omic markers

In population genetics we study the distribution in space and time of allele frequencies (patterns) resulting from certain evolutionary forces or processes. The characterisation of allele frequencies and distributions in a population enables inferences about processes (*e.g.* genetic drift, mutation, gene flow, and natural selection), which have shaped the patterns observed in a given population. A population genetic analysis consists of asking relevant biological questions, sampling individuals, determining frequencies of alleles at *loci* and using statistical approaches to infer patterns and processes.

One of the most important steps in a population genetic study is the choice of genetic markers to be analysed. This choice depends on several factors, such as the type of questions that one intends to answer, the available budget, the laboratory, or the technical capacity (human and computational resources) to analyse the results (Table 1). Some markers can be applied to non-model species (*e.g.* RAD sequencing) while other markers require a priori development of species-specific primers (*e.g.* microsatellite *loci*), although in some cases primers from closely related species are applicable. A thorough literature review should be undertaken to identify which markers have already been applied to the species of interest, and/or whether the development of new markers is required.

Molecular markers need to be chosen appropriately to be neutral/adaptive (depending on questions), reasonably polymorphic, reproducible, and provide insights at the right evolutionary scale. Markers with high mutation rates such as microsatellites (simple sequence repeats or SSRs) provide insights into recent divergence whereas mitochondrial, nuclear or other sequence *loci* provide inferences about the more distant evolutionary history given their slower mutation rates.

The minimum number of markers that should be used in a population genetic study varies with the genetic diversity of the population, scale of the study, and type of marker used.

Presently, genetic and genomic datasets can be used to estimate genetic diversity, population structure, and demographic history. Genome-scale data with an increased density of markers across the genome can provide more accurate estimation of these parameters, sometimes resulting in different conservation recommendations (Supple and Shapiro 2018).

The cost of sequencing continues to decrease; however, most conservation projects have a limited budget that allows genome-scale sequencing of only a small number of samples. The trade-off between the number of samples and the number of sequenced *loci* is a critical consideration, and the best approach in each case will depend on the research question and can often be investigated via simulation studies.

Another vital consideration is data analysis, specifically the resources and expertise available to analyse genomic data. For example, calling genotypes requires a reference genome, which may not be available for some cetacean species and analysis software is not always user-friendly. Moreover, analysis of genomic data requires high resolution computer power and storage capacity (see Section on **Computational resources**). Moreover, it is often difficult to interpret the results from whole-genome analyses and to translate them into conservation recommendations.

Table 1. Short summary presenting some characteristics of different genetic/genomic markers.

	mtDNA	MICROSATELLITES (SSR)	SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)	NUCLEAR GENE	WHOLE GENOME
Effect of selection	Neutral[a]	Neutral	Neutral or Adaptive[b]	Adaptive	Neutral and adaptive
Mode of inheritance	Maternal	Bi-parental	Bi-parental	Bi-parental	Bi-parental
Mutation rate	Low	High	Moderate-High	Low-moderate	Low-moderate
Temporal scale	Long	Short	Short	Variable	Variable
Genomic coverage	Small	Whole genome	Whole genome	Small	Whole genome
Amount of DNA required	Low	Medium (20-50 ng)	High (≥50 ng)	Low	High (≥50 ng)
Quality of DNA required	Low Medium	Medium	High	Low-Medium	High
Technically demanding	Low	Low	High	Low	High
Time demanding	Low	High	Low	Low	Medium-high
Cost	Low	Medium-high	Medium-high	Low	High

a – Assumed to be selectively neutral; b - May be located in or adjacent to regions of the genome under selection

For more details, researchers are advised to read the following literature: Allendorf *et al.* 2010, Shafer *et al.* 2015, Andrews *et al.* 2016, Hunter *et al.* 2018, Cabrera *et al.* 2021, Willi *et al.* 2022.

C) Seeking collaborators

For population studies investigating management units or units-to-conserve, there are two primary issues with respect to samples: (1) a sufficient number and (2) a sufficient geographical and seasonal spread. In an area as large as the ACCOBAMS region it is unlikely that a single institute/organisation will have sufficient samples to meet these requirements. It is therefore essential to develop a collaborative approach throughout the region as early as possible. This collaboration should extend to all stages of the process from obtaining, archiving and sharing samples to choice of markers, laboratories and analyses, and finally to publication. It is important to develop protocols for each of these stages to avoid any misunderstandings amongst collaborators. The importance of collaboration rather than working in isolation and the fact that it greatly strengthens our ability to develop wise conservation and management measures should be emphasised to all potential collaborators.

The ACCOBAMS workshop held in September 2022 on Data collection on cetacean population genetics in the ACCOBAMS Area[2] compiled a list of research Institutions collecting and storing samples in the ACCOBAMS area available on the ACCOBAMS website **https://accobams.org/population-genetics/**. The information includes institution names with the corresponding contact person, type of samples (stranded animals, remote biopsy, etc.) and number of samples per species. This is a living document that will be updated regularly with new information.

It is also important to consider collaborations with research groups outside the ACCOBAMS area, especially for species that might exchange individuals with adjacent areas or where the expertise for *e.g.* new laboratory or analytical techniques is outside the region.

[2] See report ACCOBAMS-MOP8/2022/Inf25 available at https://accobams.org/wp-content/uploads/2022/11/MOP8.Inf25_Report-of-the-ACCOBAMS-Workshop-on-Data-Collection-on-Cetacean-Population-Genetics.pdf

02) Permits for samples collection

A) National permits

The Conservation Plan (Annex 2 of the Agreement) binds the Parties to:

- Develop "systematic research programmes on dead, stranded, wounded or sick animals, to determine the main interactions with human activities and to identify present and potential threats" (paragraph 4.d);
- "Develop the systems for collecting data on observations, by-catches, strandings, epizootics and other phenomena related to cetaceans " (paragraph 5.a);
- "Establish, as appropriate, a sub-regional or regional data bank for the storage of information collected" (paragraph 5.e).

Collecting samples from stranded individuals might require a permit from the competent national authority. Following ACCOBAMS Resol ution 1.10 on Cooperation between national networks of cetacean strandings and the creation of a database[3], Parties are encouraged to create a stranding network. Following ACCOBAMS Resolution 3.9 on *Guidelines for the establishment of a system of tissue banks within the ACCOBAMS area and the ethical code*[4], "their activity must follow procedures approved by the competent State Authorities for treatment of live or dead animals under CITES. Accordingly, Tissue Banks must follow CITES procedures during the acquisition, processing and distribution of tissue fragments or bodily parts".

Moreover, Article II, paragraph 1, of ACCOBAMS prohibits any deliberate "taking" of cetaceans, including "harassment" and Article II, paragraph 2, of ACCOBAMS establishes the possibility for any Party to grant an exception to this prohibition for the purpose of non-lethal *in situ* research aimed at maintaining a favourable conservation status for cetaceans and after having obtained the advice of the Scientific Committee. According to Resolution 4.18 on *guidelines on the granting of exceptions to Article II, paragraph 1, for the purpose of non-lethal in situ research in the Agreement Area*[5], a permit is required for all research activities that involve potential harassment of cetaceans in breach of the prohibition on deliberate taking laid down by Article II.1 of the Agreement. Research activities that fall within this category include **sample collection via biopsy sampling (or scrub pad).** Competent national authorities are able to grant the relevant permit, following the Guidelines of Res 4.18.

[3] https://www.accobams.org/wp-content/uploads/2016/06/ACCOBAMS_MOP1_Res.1.10.pdf
[4] https://www.accobams.org/wp-content/uploads/2016/06/ACCOBAMS_MOP3_Res.3.9.pdf
[5] https://www.accobams.org/wp-content/uploads/2016/06/ACCOBAMS_MOP4_Res.4.18.pdf

When sampling live animals, project evaluation by an animal welfare committee might be needed before applying for the sampling permit (*e.g.* Directive 2010/63/EU and transpositions to national legislations). Additional required permits might include transportation of samples from the site of collection to the temporary or permanent storage facility (including national tissue banks) within a country and weapons permits to use crossbow/rifle for remote biopsy.

All these permits might be granted by different competent authorities (for ex: *In Spain, several ethics committee can evaluate project for live animals, Regional authorities are responsible to grant permits for strandings, National authorities for at-sea sampling and the "Guardia Civil" regulates the weapons licences required to use a crossbow or rifle*).

Researchers should contact competent national and regional authorities to make sure they follow all relevant legislation regarding cetacean sample collection in their country.

B) Report to ACCOBAMS

According to ACCOBAMS Resolution 4.18 on *guidelines on the granting of exceptions to Article II, paragraph 1, for the purpose of non-lethal in situ research in the Agreement Area*, Parties should report when granting these exceptions.

These exceptions should be included in the newly dedicated section on that matter in the national report format [see Paragraph 147 of MOP8 final report **ACCOBAMS-MOP8/2022/Doc31**[6]].

[6] https://accobams.org/wp-content/uploads/2022/05/MOP8.Doc31_Final-Report_ENG.pdf

03) Sample collection

Sterile sampling

Sample collection should always be conducted under clean and sterile conditions to minimise the possibility of contamination. In the field, potential sources of sample contamination include the marine environment, human handlers and processing location, as well as cross-contamination from other samples collected concurrently (Van Cise *et al.* 2022).

Field equipment, such as forceps/tweezers, biopsy tips, scalpels, should be thoroughly cleaned with hot water and detergent to remove visible debris, before rinsing with freshwater. It is essential to remove all traces of detergent as it can affect downstream extraction and analyses. Subsequently, sampling devices should be sterilised using for example a bleach and ethanol clean method, by Van Cise *et al.* (2022):

1. Soaking for 10 min in a 10% bleach solution,
2. Rinsing with potable water,
3. Rinsing with 95% ethanol or isopropanol,
4. Allowing it to air-dry before storage in an unused, sterile container for future use.

Labelling

We recommend wearing gloves and working on clean surfaces with sterile equipment whenever possible. Samples should be stored in a pre-labelled container, prefilled with appropriate storage buffer (if used). To avoid losing sample labels, it is recommended to double-label every vial with a waterproof pen and to avoid labels attached with tape as these may fall off. It is advisable to start with the vial with lowest number and to strictly follow numbers, such that they reflect order of sampling (Tiedemann *et al.* 2012). At least two samples's aliquots should be collected, one for the analysis and the other for tissue banking.

Associated data

At a minimum, metadata should include the date, time, sample number, geographic location (latitude, longitude), and species.
Then, if known, it is recommended to indicate: sex, size, weight and/or age class; field conditions including anatomical sample site, collection method, time from collection to preservation, in-field processing techniques, field storage method and number of freeze/thaw cycles before archiving (Van Cise *et al.* 2022). When sampling from stranded animals or carcasses, researchers should estimate the amount of time that has passed since death according the ACCOBAMS/ASCOBAMS Best Practice Document on Post-mortem investigations (ACCOBAMS-MOP7/2019/Doc 33)[7], as tissue degradation can affect data quality and downstream interpretability of results (Van Cise *et al.* 2022).

[7] Joint ACCOBAMS and ASCOBANS document on Best practice on cetacean post mortem investigation and tissue sampling available at https://accobams.org/wp-content/uploads/2019/04/MOP7.Doc33_Best-practices-on-cetacean-post-mortem-investigation.pdf

Maximise use of samples

Even though these guidelines pertain primarily to genetic studies, samples can be used for other types of analyses as well. The following non-exhaustive list might help researchers **optimise sample collection to meet different research purposes. Maximisation of the use of samples should be attempted whenever possible.**

Skin tissue can be subdivided for several purposes:

- Genetics/genomics and/or sex determination (20-50 mg, immediately frozen in liquid nitrogen, dry ice or -20°C; or stored in ethanol, DMSO or RNAlater)
- Gene expression/transcriptomics (RNA analysis)/protein analysis (20-50 mg, immediately frozen in liquid nitrogen or dry ice or stored in ethanol or RNAlater)
- Stable isotope analysis (20-50 mg, immediately frozen in liquid nitrogen, dry ice or -20°C)

Blubber tissue can be subdivided for several purposes:

- Contaminant analysis (>150mg, immediately frozen in liquid nitrogen, dry ice or -20°C)
    - Store in aluminium foil or glass vials for assessment of persistent organic contaminants (*e.g.* organochlorine contaminants), plastic additives, PFAS, etc.
    - Store in plastic vials for assessment of heavy metals
- Hormone analysis or fatty acids analysis (>100mg immediately frozen in liquid nitrogen, dry ice or -20°C)

A) Biopsy sampling

Biopsy sampling (remote) is the most common method for collecting tissue samples from live, free-ranging cetaceans (Noren and Mocklin 2012), as it avoids the need to physically capture the animals or have direct access to them. In addition to genetic population structure studies (*e.g.* Louis *et al.* 2014, Gaspari *et al*. 2015, Nykänen *et al*. 2019), the same samples can also be used for other analyses such as contaminants (*e.g.* Fossi *et al.* 2000, Ylitalo *et al*. 2001, Jepson *et al*. 2016) and foraging ecology studies (Kiszka *et al*. 2010a, Kiszka *et al.* 2014), or a combination of methods (Esteban *et al*. 2016, Giménez *et al*. 2018). While biopsy sampling typically elicits relatively minor and short-lived behavioural responses with no lasting injuries and is therefore considered 'safe' (Weller *et al*. 1997, Gorgone *et al*. 2008, Kiszka *et al*. 2010b, Giménez *et al*. 2011), it does have the potential to cause severe injury or death (Bearzi 2000) and should therefore be carried out with utmost care to ensure both animal and human safety.

Equipment

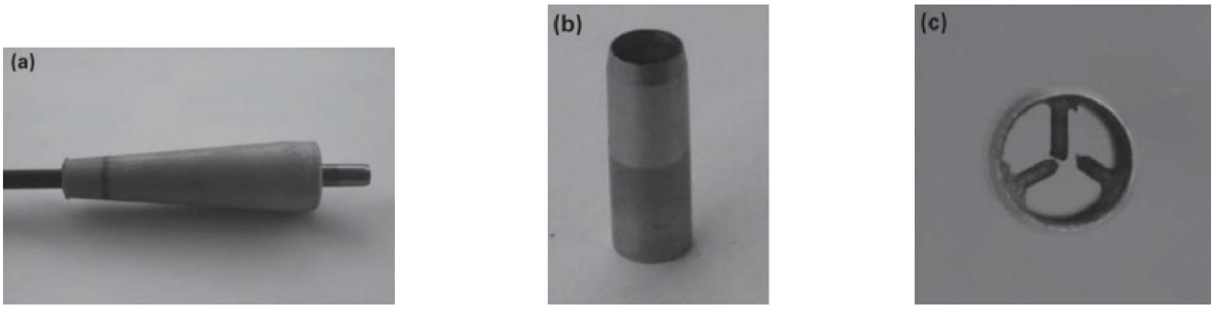


Figure 1. Biopsy darts used for sampling skin and blubber tissue of large delphinids. (a) Biopsy tip attached to the arrow. The stop collar is also visible. (b) Biopsy tip. (c) Inner of the biopsy tip, showing the tooth-like barbs to hold the sample material (Giménez et al. 2011, reproduced with permission)

Skin biopsies (epidermis and dermis/blubber) from free-ranging cetaceans can be obtained using an aluminium pole armed with biopsy tips for bowriding animals (Bilgmann *et al.* 2007) or remotely using a crossbow or modified rifle and darts armed with tips (Krützen *et al.* 2002, Gorgone *et al.* 2008, Giménez *et al.* 2011, Figures 1 and 2).

The choice of equipment may be guided by a number of considerations, including the target species and their typical behaviours (*e.g.* for species prone to bowriding, the pole system may be used, whereas for boat-shy species a remote system may be needed), the size of the target species (related to the choice of the power of the projectile delivery and the size of sampling tips), the vessel used, the costs and ease of obtaining various types of equipment, as well as local/national legislation related to the use of weapons/firearms. Typically, after recoiling from the sampled animal, the dart/bolt floats in the water and is collected by hand or by dip net. However, in certain conditions tethered darts may be used.

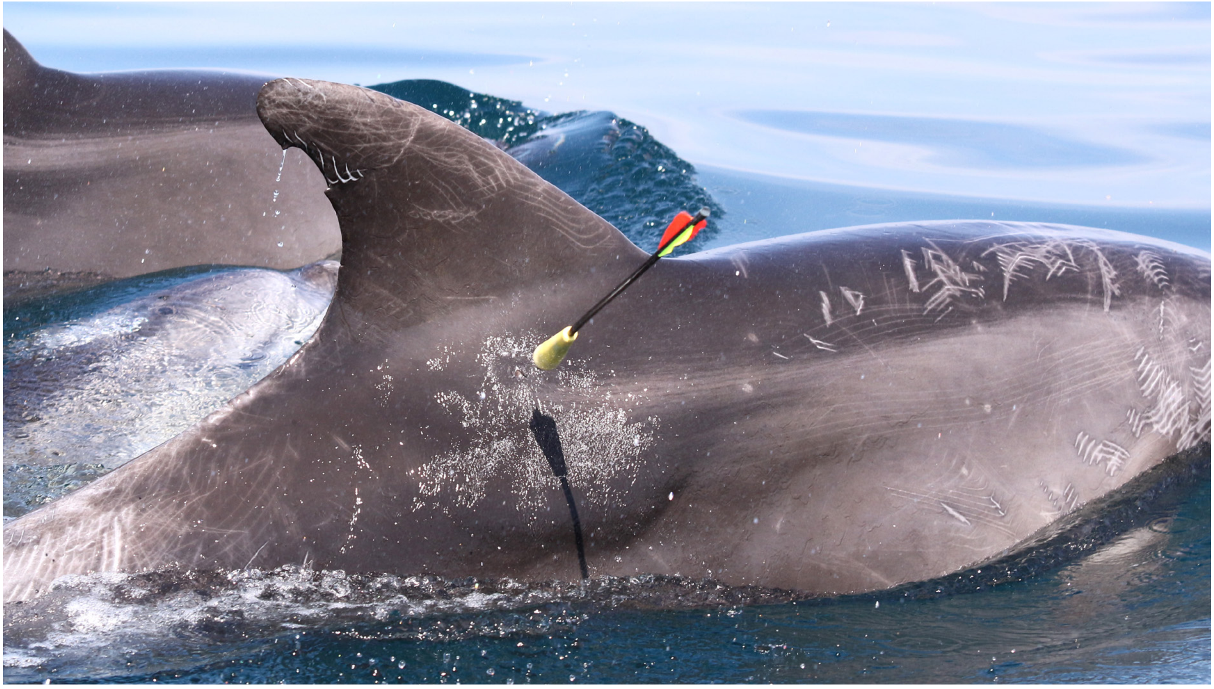


Figure 2. Biopsy sample collected from an adult free-ranging common bottlenose dolphin (Tursiops truncatus) in the Gulf of Trieste, northern Adriatic Sea, using a crossbow and a dedicated sampling dart (Picture © Morigenos).

Scrub sampling can also be used to collect epidermal tissue from bowriding animals (See section on Scrub sampling).

Safety

Human safety is a priority in any field work including biopsy sampling. This includes the use of the safety stop, avoiding pointing the crossbow/gun towards people and not leaving the sampling equipment unattended, especially when armed. Sampling should only be attempted on apparently healthy animals that do not show evidence of severe malnutrition, poor health or swimming difficulty. Calves or females accompanied by calves should not typically be targeted, although this may be species and study-dependent. The behaviour and movements of the animals should be taken into account, as erratic movements can present challenges to effective and safe sampling. Biopsy samples should ideally be obtained from the area immediately under the dorsal fin (Figure 2) or the flank between the dorsal fin and the upper part of the caudal peduncle, although the target area may be species-dependent. The head, rib cage, pectoral fins and ventral side should be avoided. Particular care should be taken when a non-target animal is likely to surface next to the target animal, which can result in accidental shots in the head. Animals should be approached with care to minimise disturbance, ideally from the side, converging with the predicted movement route of the animals, without crossing their movement path. The required speed and distance will depend on the animal behaviour and the species being sampled. As described above, to avoid the possibility of infection and cross-contamination, the sampling tips and tip mount on the bolts/darts need to be thoroughly cleaned and sterilised before use.

Sample storage

Biopsy samples typically consist of skin and blubber tissue. Individual samples can be immediately stored upon collection as a whole, or they can be subdivided into different aliquots immediately after the collection to avoid recurrent cycle of thawing (preferred), depending on the analysis to be performed (see section on Sample preservation).

Key data to be collected

As a minimum, the metadata mentioned above must be collected at each sampling event.
Whenever possible, additional information should also be recorded, which includes the equipment and platform used, group size and composition of the target group, behaviour and reaction to biopsy events of target and non-target animals, distance to the target animal and whether or not the sample was retained, and any additional auxiliary information. Information on reactions to biopsy (both individual and group) should be as detailed as possible, and should be collected irrespective of whether an attempt was a hit or a miss. Whenever possible, sampled animals should be photographed for individual identification, to (a) prevent the multiple sampling of the same individual unless there is strong scientific reason to do so, (b) to be able to integrate information from samples with various life history, demographic and other parameters (*e.g.* Ylitalo *et al*. 2001, Genov *et al*. 2019) and (c) assist in follow-up studies of healing in conjunction with subsequent photo-identification monitoring (Giménez *et al*. 2011). As much as possible, photographs should be taken at the exact moment of the sample being taken, so that the precise location of the biopsy wound is documented and can be easily found on subsequent pictures.

Staff training

Biopsy sampling should only be performed by trained, experienced and authorised individuals (Gales *et al*. 2009), under relevant permits (both scientific and for handling weapons) from competent authorities (See section on National permits). This pertains to both deploying biopsy darts/bolts and driving of the boat, as a skilful boat driver is crucial to the success of biopsy sampling. Proper training plays a fundamental role in the safe and successful biopsy sampling. Training of inexperienced people should be supervised by experienced samplers and should typically consist of a prolonged tiered approach, including practice shooting of inanimate objects on both land and at sea, as well as "shadowing" experienced samplers carrying out biopsy sampling in the field, before attempting sampling on live animals for the first time. Good communication between the sampler, boat driver and photographer is key to success.

B) Stranded animals

Full post-mortem investigations should be encouraged whenever possible following the Joint ACCOBAMS and ASCOBANS document on Best practice on cetacean post mortem investigation and tissue sampling (ACCOBAMS-MOP7/2019/Doc 33). Samples from dead stranded animals for genetic and genomic studies can be collected even from decomposed carcasses, or carcasses for which a full post-mortem investigation is not possible (*e.g.* difficult access, impossible to transport to a specialised facility, lack of equipment/personnel etc.). In those cases, small samples of skin (or possibly muscle) can be collected with a minimally invasive procedure and equipment (gloves, sterile scalper, and sterile recipient frozen at -20ºC or stored in ethanol). Good results of DNA extraction have been obtained from desiccated skin from carcasses undergoing advanced decomposition or from samples dried in a stove (Fontaine *et al.* 2007, 2014). Indeed, bacterial decomposition tends to be slower in dried tissue. These samples can then be stored frozen at -20°C or in 70% ethanol. Otherwise, baleen plates, teeth and bone samples can be sampled following Museum specimens protocols (See section on **Museum specimens**). For further guidance on how to best store stranding samples for genetic purposes, see section on **Sample Preservation**.

As detailed above, basic tissue sampling of skin/muscles and blubber on stranded animals can serve several research purposes and should be encouraged whenever possible.

When more detailed post-mortem investigation can be performed on fresh animals, the procedures in the ACCOBAMS Best Practices Guide should be followed (**ACCOBAMS-MOP7/2019/Doc 33**).

C) Environmental DNA

Environmental DNA (eDNA), or the analysis of the genetic material pooled from an environmental sample (water, soil, faeces) has emerged as a powerful approach for characterising and monitoring the diversity in the marine realm. One of the major benefits of this non-invasive method is the capacity of using DNA traces for studying marine organisms, reducing the potential impact of sampling directly from sensitive organisms, and increasing the capacity for an early detection and tracking of rare or invasive species (Goldberg *et al.* 2016).

Advances and improved approaches for sampling, data generation by means of sequencing technologies and data analyses are responsible for the success of eDNA-based monitoring studies as shown by the exponential increase of related publications. However, for any study it is essential to consider at least the three major processes that affect the accurate detection and characterisation of eDNA: (1) production of eDNA according to the size, health, sex and density of organisms; (2) transport, diffusion rates and current effects on eDNA in water; and (3) eDNA degradation, affecting persistence and amount of DNA in the environment, mainly caused by temperature, pH and light (Goldberg *et al.* 2015). Therefore a sampling strategy should be carefully designed to reproduce a representative picture of the community to be studied and minimise the probability of contamination. Protocols must include negative field controls; decontamination of field equipment prior to use (*e.g.* 10% commercial bleach solution) and single-use supplies for eDNA collection. If supplies are to be used more than once, they should be cleaned with bleach and thoroughly rinsed before use (Goldberg *et al.* 2016).

In the ACCOBAMS area, eDNA studies specifically for cetaceans are in their infancy, with just a few studies exploring the potential of this methodology (*e.g.* Valsecchi *et al.* 2021, 2020). However, research developed elsewhere can provide useful examples for sample acquisition and downstream processing. In broad terms, an eDNA workflow will include: (1) environmental sample acquisition, (2) processing and preservation of samples and DNA extraction, followed by (3) eDNA sequencing library preparation, high-capacity sequencing and sequencing data analysis.

Diverse methods for water collection have been tested. For instance, Valsecchi *et al.* (2021) used ferries as an opportunistic platform for surveys while collecting water samples from the ferry engine room via a derivation pipe intercepting the marine cooling water upstream of the engine.

Alternatively, water can be obtained from Niskin or Nansen bottles released at different depths for a vertical stratification analysis (Closek *et al*. 2019). Other researchers have manually obtained water samples using pumps for water during or after a cetacean sighting (Parsons *et al*. 2018, Székely *et al*. 2021). The reported number of replicates (range 2-5) and volume of water collected (1 to 4L per sample) varies (Hunter *et al*. 2018, Juhel *et al*. 2021, Ma *et al*. 2016, Parsons *et al*. 2018).

It is recommended to filter samples as soon as possible to maximise eDNA retention and prevent DNA degradation. However, if not possible, 1-2 weeks between collection and filtration has been considered tolerable, keeping the water sample under cold and dark conditions, for which sterile foil laminated plastic containers have been shown suitable (see Figure 3 for a Standard Operational Procedure). Special care should be taken as DNA in water samples can easily degrade and be lost. In addition, researchers must be especially cautious to avoid cross contamination, as this will largely affect the outcome and validity of the results and conclusions obtained (Goldberg *et al*. 2016). Filtration methods can be diverse and they can be performed on-site of sample collection or at a filtration station. eDNA has been effectively collected from cellulose nitrate, glass fibre, polycarbonate, nylon, polyethersulfone and cellulose acetate filters (Djurhuus *et al*. 2017, Goldberg *et al*. 2016, Spens *et al*. 2017).

BOX 1. Standard Operational Procedure (SOP) for Commercial Vessel Transect eDNA Sampling.

• **(1) VOLUME OF SAMPLED WATER.** It would be good practice to filter large volumes of marine waters (up to membrane saturation), in order to retain as much eDNA as possible. Such a volume is however variable, depending on filter characteristics and on water density (*e.g.* day-time samples saturate the filters quickly, being rich in phytoplankton). According to our experience, from his study and in the analysis carried out in controlled environment (Valsecchi et al. 2020), we suggest the processing of **4–5 litres of marine water per filter.**

• **(2) FILTER POROSITY.** We did not find any significant difference between the three tested NC (nitrocellulose) filter types with porosity 0.22, 0.45, 0.8 μm. However, we suggest to exclude the 0.22 μm pore-size membrane, as filtration is very slow, and saturation is reached after 2–3 litres, without providing a better quantity/quality eDNA. Between the two remaining filter types, we recommend the use of **0.45 μm pore-size membranes,** in order to retain the smallest biological particles, consistentl with findings by Li et al. (2018).

• **(3) NUMBER OF REPLICATES** sample replicates are necessary for both a) increase the total amount of eDNA retrieved from each single sampling station (useful for future analyses) and b) to reduce the false positive and negative rate inbuilt in the metabarcoding technique (Ficetola et al. 2015). Thus, a **minimum of three replicas per station** is advisable (meaning a total of 12–13 litres collected from each sampling station).

• **(4) SAMPLE CONTAINER.** The **Bag in the Box Sampling System (BiBSS)** presents many advantages for the collection/preservation/storage of marine water samples for eDNA surveys (see **Box 2**).

• **(5) SAMPLING STATION DESIGN.** The selection of the geographic positions to locate fixed sampling stations (FSS) invariable over cruises, should aim to: (1) **sample spots of biological interest** based on previous observational/literature data; (2) **prioritize points on bathymetric maps indicating habitat changes** (*e.g.* edge of continental shelf); (3) **select roughly equidistant sampling sites** (about 35–45 nautical miles apart) along the designated shipping lanes, to cover the whole route and (contingent on vessel schedule) allow the FSSs to be sampled during both day and night time. For the same reason, in order to sample adjacent points at different time of the day, it is recommendable to number the stations following the sampling chronological order, meaning that on the map they will not appear in a consecutive order. For example, if six fix sampling stations are selected, and three will be sampled on the outward journey and three on the return journey, the order along the route, on the map, will be: PortA-FSS6- FSS 1-FSS 5-FSS 2-FSS 4-FSS 3-PortB, with the three underscored sampling stations surveyed in the return journey.

• **(6) TIME BEFORE FILTRATION.** Preferably the water contained in the BiB bags should be filtered **immediately after collection** to maximise eDNA retention, and to simplify sample transportation, by avoiding transfer of bulky water samples. However, if this is not possible, sample storage times of 1–2 weeks between collection and filtering is well tolerated, provided that the BiBs are kept at 4 degrees and away from exposure to the sun during transport. It is important to note that water samples should never be frozen to avoid breaking of cellular components that would result in the release of extracellular DNA which is more easily lost in filtration.

• **(7) TIME BEFORE EXTRACTION.** After filtration **filters should be frozen a.s.a.p.** The time before extraction does not seem to have a negative effect within the tested time interval, although it is advisable to perform DNA extraction a.s.a.p. after filtration.

• **(8) IMPORTANCE TO COMBINE MOLECULAR TO VISUAL/TAXONOMIC CENSUS.** eDNA methods do not have perfect taxon detection and resolution. Therefore it is important to incorporate visual observations to monitor detection efficacy and support the molecular identification of new species (followed by the sequencing of their mitogenome in order to fill reference sequences gaps in molecular databases).

Figure 3. Standard Operational Procedure (Valsecchi et al. 2021, reproduced with permission)

D) Scrub sampling/skin swabbing

Another alternative sampling method that does not require puncturing the skin is skin swabbing (Harlin *et al*. 1999, Gales *et al*. 2002). This procedure is only feasible for cetacean species that tend to approach boats (Farro *et al*. 2008), usually delphinids. An important consideration when deciding whether to use this method is that the amount of collected skin may be too low for certain analyses.

This method consists of attaching a synthetic fibre scrub pad to the tip of long sticks (Harlin *et al*. 1999). Samples are collected by friction of the scrub pad against the back of an approaching dolphin to remove and retain sloughed epidermal cells. Sometimes, the sample is not visible on the scrub pad, but is present, and genetic analysis can be undertaken. In the laboratory, the skin adhered to the scrub pad must be removed and DNA extracted using a standard protocol such as that for skin biopsies.

To avoid replicate samples, care must be taken to recognize previously sampled individuals.

Some individuals react to the scrub pad contact (Harlin *et al*. 1999). They swim faster, jumping or diving after being touched but, in general, they return rapidly to the bow. This suggests that the skin swabbing procedure usually only causes very short term disturbance for the animals.

E) Faeces

Collection and analysis of cetacean faecal samples is another non-invasive method to consider. However, cetacean faeces largely vary in consistency, ranging from well-formatted floating semi-solid clumps to more fluid and dispersal plume (*e.g.* Hermosilla *et al*. 2015, 2018). There is a limited time for faecal samples to be collected at the water surface before they sink. Samples can be collected manually, within a few seconds after animals' defecation, when they reach the sea surface and float using a fine nylon mesh net. For more fluid faeces, alternative containers such as plastic bags or buckets can be used, according to the consistency of the sample. Collected samples can be stored in separate plastic vials such as falcon tubes or large eppendorfs, or directly into plastic containers. After collection, samples should be stored at -20ºC or fixed in 70 to 96% ethanol (for further information see Sample Preservation section).

F) Museum collections

Collecting samples from field sites and museum collections also allows DNA research (Nakahama, 2021). Great care must be taken with regards to avoiding contamination, thermal degradation (heat, boiling or hot vapour processing), use of DNA damaging reagents (*e.g.* detergents, benzene vapour, etc), rinsing and washing procedures, and storage conditions. It is optimal to collect and store hard tissue samples unwashed (if possible), dry, cool (if not found frozen) and well isolated. Thermal processing is undesirable.

Among bone and teeth samples, the densest structures are preferable (*e.g.* teeth; tympanic bulla; periotic bone). However, the mass of the sample also matters and can be crucial, so large bones (*e.g.* whole vertebrae) and baleen should be collected as well. Dry skin samples can be important; however they are often subject to microbial contamination. Additionally, all sorts of pure ethanol preparations can be suitable for sampling. Other wet preparations and paraffin embedded samples can be considered under certain conditions (more details in Straube *et al*. 2021).

Sample collection from bone, tooth and baleen specimens is destructive sampling and should be done with minimal damage to specimens, especially to those of historical importance (Freedman *et* *al*. 2018). External examination of the specimen to choose the structure to be sampled is necessary, and sometimes CT scanning is required. Photography, photogrammetry, 3D surface scanning or CT scanning of the specimen is a good precondition before deciding to undertake any destructive sampling. Low speed drilling of small holes is recommended for extraction of bone powder to avoid external damage and heating of the sample. Areas where there was pre-existing damage to the specimen made by collectors are preferable (McDonough *et al*. 2018). The minimum mass of the bone powder depends on the age and preservation of the specimen.

G) Ancient DNA

Fulton and Shapiro (2019) have compiled recommendations on how to set up an ancient DNA (aDNA) lab to extract DNA from specimens dating back thousands of years (potentially up to 100,000 or 1,000,000 years). Limits to DNA survival, postmortem degradation and contamination pose a nontrivial challenge to laboratory practitioners.

For instance, Fulton and Shapiro (2019) state that:

"The most challenging complication of aDNA research stems from the small proportion of surviving copies of endogenous DNA in an extract, compared to the ubiquitous nature of DNA in the environment. The high sensitivity of PCR allows amplification to proceed from only one or a few starting copies of the target sequence but also often allows contaminating DNA to be amplified. Even when the level of contamination is extremely low, PCR will preferentially amplify modern DNA over damaged ancient molecules. Copies of the targeted fragment may contain blocking lesions, for example, which affect polymerase processing, or may simply be in low abundance so that PCR enters the exponential phase many cycles after the reaction has begun. If only a few contaminant molecules are present and amplified during the initial cycles of the PCR, these will rapidly outnumber (and outcompete) amplification of the authentic ancient DNA.

Contamination can occur at any stages of processing an aDNA sample. The sample itself may be contaminated. For example, bones and teeth are porous, and contamination may occur via adherence or uptake of exogenous DNA from microorganisms in the depositional environment. Contamination may also occur during collection; this is a particular problem for human and microbial studies, where the source of contamination is genetically similar to the target DNA. Contamination may also be introduced during downstream experimental processes, including DNA extraction, sequencing library preparation, or PCR setup. Laboratory personnel may introduce their DNA or any DNA carried into the lab such as on shoes or clothing, reagents may be contaminated with human or animal DNA, and airborne particulates may enter through the building air supply. Previously amplified DNA that is present in the laboratory environment is another potential source of contaminating DNA. Even the tiny amount of DNA that is aerosolized when a tube is opened is likely to contain over a million copies of template in a volume as small as 0.005 µl. This is potentially thousands of times the number of copies than that DNA which is preserved in an ancient sample. To avoid this problem, strict separation between the laboratory in which ancient samples are prepared and any laboratory where samples are processed after amplification should be maintained."

Guidelines for aDNA research (Fulton and Shapiro 2019)

1. Physical isolation of the pre-PCR ancient DNA facility and strict maintenance of a "one-way" rule of movement up the concentration gradient.
2. Negativ and PCR controls.
3. Appropriate molecular behaviour (Short DNA fragments are prevailing).
4. Reproducibility (Multiple extraction and sequencing rounds are involved).

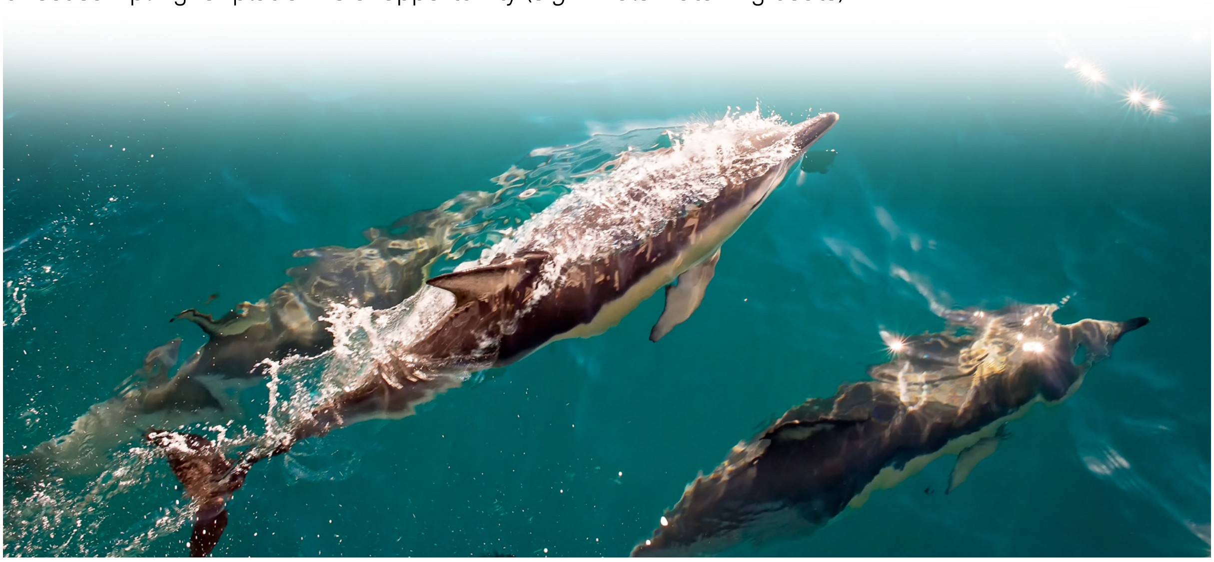
5. Cloning (Backup).
6. Independent replication.
7. Biochemical preservation.
8. Quantitation of starting material.
9. DNA from associated remains (esp. for microbial research).
10. Use of a "carrier DNA" negative in PCR-based assays.
11. Time-dependent or preservation-dependent pattern of DNA damage and sequence diversity.
12. Critical assessment of results (Phylogenetic sense or otherwise reasonable results).

H) Other techniques - Sloughed skin

DNA samples can also be obtained from free-ranging cetaceans through the collection of sloughed skin. For this non-invasive method, pieces of sloughed skin floating in the wake of cetaceans are collected either using a dipnet from a vessel, or, by snorkelers. Pieces of skin are removed from the dipnet using sterile stainless steel tweezers and preserved in DMSO or ethanol. The advantage of this method is that the required sampling equipment is minimal and little skill or training is needed.

The drawback of this method is that sloughed skin sinks quickly, leaving a short window to collect the sample. The origin of the skin can therefore generally be attributed to individuals in the immediate vicinity of the collection site. However, assigning a piece of sloughed skin to the actually sampled individual is difficult when several individuals are in close proximity, or have recently been in physical contact with one another (Whitehead *et al.* 1990). For that reason, samples of sloughed skin often cannot always be assigned to a given individual, which is an issue for many applications. Sloughed skin DNA is often degraded and its quality and quantity are highly variable (Amos *et al.* 1992). For example, 40mg is needed from sloughed skin for sperm whales to extract DNA (Drouot *et al.* 2004). In addition, the number of duplicate samples can be high, increasing the time and cost of the genetic analyses.

The frequency and circumstances of occurrence of sloughed skin varies considerably among species and individuals, and among study areas. The method of collecting sloughed skin has been used with large cetaceans, *e.g.* sperm whales (Amos *et al.* 1992), humpback whales (Valsecchi *et al.* 1998), and fin whales. Most sloughed skin samples have been successfully analysed, confirming that the samples contained enough DNA to perform genetic analyses (*e.g.* Hoelzel and Donovan 1991, Neveceralova *et al.* 2022). Sloughed skin is more efficient to determine gender than to study population structure. However, sloughed skin collection may constitute a viable alternative for some studies where biopsy sampling is either not permitted or otherwise considered undesirable. This method may be more appropriate than direct sampling for platforms of opportunity (*e.g.* whale watching boats).



04) Sample preservation

A) General recommendations

Sample preservation methods greatly influence the quality and quantity of genetic material available for analysis. Their objective is to prevent the degradation of DNA and RNA, thereby minimising downstream errors and maximising the scientific value of biological samples. To ensure minimal sample degradation, samples should be stored in adequate media immediately upon collection in the field.

The most appropriate sample preservation method depends on a number of factors, relating to the study design, logistics, availability and budgets. The 'gold standard' option for sample preservation may not always be feasible nor desirable given certain constraints and some compromises may have to be made. For instance, not all genotyping approaches require ultra-high quality DNA and may still yield good results from partially degraded samples. Van Cise and colleagues (2022) reviewed best practices for preserving marine mammal biological samples in relation to different 'omics technologies (*e.g.* genomics, metagenomics, metabarcoding, transcriptomics). A comparison of preservation media in relation to biospecimen type and targeted analyses is shown in Table 2.

Table 2. Comparison of common preservatives and fixatives used for biospecimen preservation for 'omics (and other) targeted analytical methods (Van Cise et al. 2022, reproduced with permission)

Biospecimen Type and Storage Methods																				
	SKIN/BLUBBER/ORGANS					**FECES/GASTROINTESTINAL CONTENT**					**BLOW/CHUFF**					**BIOFLUIDS (*e.g.* BLOOD)**				
	DMSO/5M NaCl (<-20°C)	-20°C	RNAlater (-20°C or -80°C)	Organic solvent (*e.g.* 95% EtOH, -20°C)	LN2 or -80°C	20% DMSO/5M Nacl (<-20°C)	-20°C (dry)	RNAlater (-20°C or -80°C)	95% EtOH (-20°C)	LN2 or -80°C	20% DMSO/5M NaCl (<-20°C)	-20°C	RNAlater (-20°C or -80°C)	95% EtOH (-20°C)	LN2 or -80°C	20% DMSO/5M NaCl (<-20°C)	-20°C	RNAlater (-20°C or -80°C)	EDTA vacutainer	LN2 or -80°C
Genetics*	1	2	1	2	3	2	2	2	2	3	1	2	2	2	3	-	2	1	1	3
Genomics**	1	2	1	1	3	0	1	2	1	3	-	2	?	1	3	-	2	1	1	3
Microbiomics***	0	2	1	1	3	0	1	2	1	3	0	1	2	1	3	-	1	2	1	3
Transcriptomics	0	0	2	0	3	0	0	2	0	3	0	0	2	0	3	-	0	2	-	3
Proteomics	0	0	?	0	3	0	0	?	0	3	0	0	?	0	3	-	0	?	-	3
Metabolomics	0	1	0	0	3	0	1	0	0	3	0	1	0	0	3	-	1	0	2	3
Epigenetics	1	2	?	2	3	-	-	-	-	-	?	?	?	?	?	-	2	?	1	3
Stable Isotopes	1	1	0	0	3	?	1	0	0	3	?	1	-	-	3	-	1	-	-	3
POPs	0	1	0	0	3	0	1	0	1	3	?	?	?	?	?	-	1	0	-	3
Steroid Hormones	0	1	0	0	3	0	1	0	0	3	0	1	0	0	3	-	1	0	?	3
Microplastics	?	3	?	2	3	?	3	?	2	3	?	3	?	2	3	-	3	?	-	3

Key: - analysis inappropriate for this tissue type; 0 unsuitable; 1 good; 2 better; 3 best; ? unknown

** Sanger sequencing, multilocus genotyping, genetic sex*
***2nd generation sequencing*
****amplicon sequencing, metagenomics*

The best available preservation option is to store biological samples in a portable dry shipper containing liquid nitrogen immediately upon collection, until the samples can be transferred to a longterm archival storage at or below -80°C. This kind of cryopreservation halts all chemical and biological processes causing degradation *e.g.* by inactivating enzymes such as DNAses, RNases, or proteinases. If flash-freezing in liquid nitrogen is not possible, a secondary option is to store samples on ice or in a -20°C freezer until they can be transferred to long-term archival conditions. Finally, storage in liquid preservatives, *e.g.* lab-grade ethanol, DMSO solutions, or RNAlater, can serve as an alternative method without immediate freezing for some types of studies. However, when using liquid preservatives, it is important to consider the potential downstream effects of these chemicals on subsequent analyses as detailed in Van Cise *et al*. (2022).

Additional recommendations outlined by Van Cise *et al*. (2022) include:

- Ideal conditions for long-term storage of biological samples are dry and ultracold (-80°C or below).
- In general, extracted molecules (*e.g.* DNA, RNA, proteins) stored in a molecule-specific buffer at -80°C are stable for longer periods of time than those stored in tissue.
- High-salt RNA/DNA preservatives will not penetrate frozen tissue unless specifically formulated for frozen tissue.
- The dehydrating effect of ethanol can cause the release of water from the tissue sample, thereby diluting the preservative; it is therefore recommended to replace with fresh ethanol one to two days after initial preservation.
- DNA quality is inversely correlated with the number of times a sample is thawed; researchers should therefore limit the number of times a sample becomes thawed after collection *e.g.* by dividing the sample into smaller aliquots.
- In terms of sample to preservative ratio, sample preservation should allow for at least five times the volume of the fixative to tissue.

Environmental DNA

Regarding environmental DNA, the most common way to preserve samples is under the form of eDNA on filters. Following filtration across a porous membrane, the eDNA concentrated on filtered can be preserved by freezing, storing in a liquid preservative, or drying using silica beads (Kumar *et al*. 2019). Direct filtration on-site has the advantage that samples can be immediately stored in an appropriate preservation medium. Under this form, eDNA on preserved filters may be sufficiently stable for months to years (Kumar *et al*. 2019).

Faecal samples

Faecal samples are best preserved by storing the samples at -80°C, but -20°C can be considered as a secondary option. In addition, samples can also be fixed in 70 to 96% ethanol.

Filter paper

FTA® paper is a commercial product (Whatman[8]) consisting of filter paper impregnated with a proprietary mix of chemicals which serve to lyse cells, to prevent growth of bacteria, and to protect the DNA in the sample. The basic premise of purifying DNA using FTA® paper is simple: biological

[8] http://www.whatman.co.uk/

samples are applied to the FTA® paper and air-dried. A small disc of the FTA® paper is then removed, and washed to remove any non-DNA material (the DNA remains entangled within the paper). Analysis can subsequently be performed on the DNA whilst still attached to the paper, or the DNA can be eluted prior to use. Blood, blood clots and tissues have been successfully sampled (Smith and Burgoyne, 2004). As long-term stability of the DNA once it has been eluted from the FTA® paper has not yet been investigated, it is preferable to only process the samples as required.

B) Long-term storage

To maximise availability of tissues and DNA extracts for future studies, it is recommended to create and store samples in an archive. The creation of a standardised tissue bank for each network assists sample identification and recovery, and simplifies CITES accreditation to facilitate exchanges of samples between networks. An effective sample archive is essential when dealing with rare species, as it may take several years to build up a sample size sufficient for statistically robust conclusions. Availability of a range of samples from multiple individuals and species in one place greatly facilitates long-term pathological, ecological and population studies (**ACCOBAMS-MOP7/2019/Doc 33**).

After all related diagnostic and other routine analyses have been performed, samples should be stored for long-term preservation. It is necessary to document samples well and extensively with detailed collecting/field information, collecting/export permit information. If necessary, samples should be double marked with long-life labels. The label should contain a unique number or identifier that makes it easy to find relevant metadata for the sample, *e.g.* in the archive database. Ideally, an updated database should be available on-line containing information on the animal and the tissues available.

Reference samples consist of well characterised samples for which a long term preservation and traceability is needed with a reduced number of accesses (3/4 time per year maximum). For these samples, specific pre-marked and labelled vials could be used which allow a proper traceability thanks to dedicated scanning system and software managing relevant information. Preservation should be at -80°C for frozen samples. These samples should be considered as reference for future research as a control, a negative or positive sample, or of very rare values, or from which it is possible to obtain cell cultures.

For small samples (including biopsy, scrub pad), a protocol should be developed to make sure that the whole sample is not consumed in one analysis and is still available for future (and potentially more powerful) analyses.

C) Backup at tissue banks

The Mediterranean Marine Mammal Tissue Bank[9]

This collects and preserves biological material sampled from marine mammals stranded along the Italian coasts of the Mediterranean Sea, in cooperation with the University of Padova, the Italian Ministry of the Environment, the Institutes for Animal Health, and with several other non-profit Italian

[9] The Mediterranean Marine Mammal Tissue Bank http://www.marinemammals.eu/index.php

organisations dedicated to marine mammal research. It is part of the International Environmental Specimen Bank (IEBS)[10] circuit and it has a permanent CITES permit for samples exchanges (IT020).

The Mediterranean Marine Mammal Tissue Bank collects, catalogues, preserves and distributes tissues free of charge, upon motivated request.

The Bank offers additional services, including: - diagnostic pathology - necropsy of whole specimens -age determination - parasite identification - histochemistry and immunohistochemistry - hormone essay in blood, urine and faeces - general info and specific bibliography on marine mammals.

The Ukrainian National Bank of Cetacean Samples

This was created in 2019 in Kyiv and is the first institution for storing samples from marine mammals in the Black Sea Basin. The main storage, established in the Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, comprises a 750 l freezer (-80°C) and an additional -20°C freezer, as well as places for storing dry and wet materials. Good practices guides in sample acquisition, storage and sample exchange were developed and introduced, and contact with the existing Mediterranean Marine Mammals tissue bank established. An agreement was signed on the transfer of samples from other Ukrainian institutions (UkrSCES). Currently, samples from Black Sea cetaceans and historical collections from other regions are stored in this sample bank.

The French national stranding network (RNE)

This is a structured and participatory science programme created in 1970 in charge of the monitoring of marine mammals stranded along the French coasts. For each stranding, data and samples are taken according to standardised protocols based on the Joint ACCOBAMS and ASCOBANS document on Best practice on cetacean post mortem investigation and tissue sampling (ACCOBAMS-MOP7/2019/Doc 33). Samples are then conditioned according to the analyses that will be carried out afterwards (*i.e.* frozen, in formalin, ethanol).

The RNE is scientifically coordinated by the Pelagis observatory[11] under the supervision of the French Ministry of Ecology. The RNE members receive scientific training for standard data collection protocol and a legal framework (the 'green card', *i.e.* a permit to collect and transport samples). The RNE governance is ensured by a steering committee of ~20 members reflecting the diversity of the RNE's stakeholders. The role of this committee is to evaluate and validate protocols, requests for the use of samples and new requests for authorisation to collect samples.

[10] The International Environmental Specimen Bank https://www.umweltbundesamt.de/en/topics/chemicals/international-environmental-specimen-bank-group
[11] Observatoire Pelagis https://www.observatoire-pelagis.cnrs.fr/?lang=en

05) Exchange of samples

A) Agreement or Memorandum of Understanding (MOU)

Before exchanging samples, it is very useful to draw up a sample agreement that addresses different aspects of the collaboration between researchers and/or institutions. The agreement can take the shape of a contract-type document that is signed by the relevant parties (this may also be a useful document to provide to the ABS National Focal Point to obtain Prior Informed Consent under the Nagoya Protocol, see section on Nagoya Protocol). Alternatively, these discussion points can also be addressed less formally in emails.

Elements that should be considered include:

- **Ownership of samples:** does the sender retain ownership and is left-over sample material returned or stored after initial usage (if any)?
- Which metadata are required
- **Usage of samples:** which studies will the provided samples feed into?
- **Coauthorship on scientific publications:** will the sample provider be included as a co-author on all scientific material that include their samples (and which co-authors will be included from the sample provider institution)
- **Paperwork:** Who will apply for the relevant CITES/Nagoya permits and inform ACCOBAMS NFPs/SC of the exchange?
- **Cost of sending samples/permits:** Who will cover the permit/exchange/laboratory costs associated with the sample exchange?
- **Feedback of results:** What kind of data/information will be provided to the sample provider?
- **Confidentiality**
- **Expected timeline:** When can the sample provider expect to see publishable results?

An example of Material Transfer Agreement is provided in *Appendix 4*. This is not supposed to be a one-fits-all model, rather a draft document that can be adapted to each specific collaboration if needed.

B) CITES Procedure

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulates the exchange of biological samples between member countries.

Here we consider the exchange of cetacean samples for non-commercial research purposes, including tissues collected from free-ranging cetaceans (skin and blubber biopsies, blow, sloughed skin, etc.), body parts from stranded cetaceans (tissues and skeletons) or extracted DNA products.

All regular cetacean species in the ACCOBAMS area belong to Appendix I or II of CITES (and Appendix A in EU legislation). According to CITES, as a general rule:

- Appendix I (or EU-A) contains species threatened with extinction for which CITES prohibits international trade except for non-commercial purposes, including scientific research. To exchange samples of these species, researchers need to apply for **import and export permits (or re-export certificates)** to CITES National Management Authorities.

- Appendix II contains species that require a controlled trade for which CITES might allow international trade if it is not detrimental to the survival of the species in the wild. To exchange samples of these species, researchers need to apply for **export permits (or re-export certificates)** to CITES National Management Authorities but no import permit is required.

- To grant export permits, documentation proving that the samples were collected legally might be required (see Paragraph on "*National permits*")

Some exemptions might apply to the general CITES procedures.

- **Scientific Exchange Exemption (SEE):**

Paragraph 6 of Article VII of the Convention includes an exemption known as 'the scientific exchange exemption' (*i.e.* non-commercial loan, donation, or exchange for scientific purpose) **allowing Registered Scientists or Scientific Institutions (RSI) to exchange CITES samples or specimens** without applying the requirements of Articles III, IV or V[12]. No CITES permits or certificate is required for such an exchange, although the samples must carry a label "CITES Biological Samples" issued or approved by a Management Authority of the State that registered the institution. The recommended conditions that apply for the exemption are further specified in Resolution Conf. 11.15 (Rev. CoP18)[13]. The scientists, scientific institutions and forensic research institutions must be **registered in the Register of the CITES Secretariat** (CITES Register[14]) by a Management Authority of their hosting State in accordance with certain standards and report annually on the use of the exemption.

This exemption could apply to the exchange of cetacean samples for research on population genetics in the ACCOBAMS area. However, some national laws do not recognise this exemption (ex: Portugal, Georgia) and some Parties have not officially registered any Institution at CITES (*ex: Georgia, Türkiye*). In those cases, the general procedure described above will apply.

- **Simplified procedure (SP) for biological samples:**

Unlike SEE, the simplified procedure is a simplified way to apply the normal requirements under CITES in situations of trade with **no or negligible impact** on the conservation of the species concerned. The procedures are set out in Resolution Conf. 12.3 (Rev. CoP19)[15] section XIII, paragraph 22. The simplified procedure allows the Management Authority to provide persons and bodies determined to be *bona fide* with partially completed permits and certificates, and hence to 'pre-authorize' trade under certain conditions. This procedure can be applied to the exchange of biological samples, such as tissues/pieces of tissues of 5-25 mm^3 (detailed in Annex 4 of Resolution Conf. 12.3 (Rev. CoP19)). Simililarly, according to Directive EU 338/9 to expedite this process, Article 18 of Regulation (EC) No 865/2006 provides for pre-issued permits and certificates with regard to certain trade in biological samples of specimens of species listed in the Annexes or the CITES Appendices. The type of samples covered by pre-issued permits and certificates and their use are specified in Annex XI of Regulation (EC) No 865/2006[16].

[12] https://cites.org/eng/disc/text.php#VII
[13] https://cites.org/sites/default/files/document/E-Res-11-15-R18.pdf
[14] https://cites.org/eng/common/reg/e_si.html
[15] https://cites.org/sites/default/files/documents/E-Res-12-03-R19.pdf
[16] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02006R0865-20220119

- **Introduction from the sea (IFS):**

Additionally, when collecting samples from the high seas (Areas Beyond National Jurisdiction), a special CITES Procedure might apply. Indeed, Introduction from the sea of specimens of species included in Appendix I and II is regulated by the Convention[17]. IFS is defined in Article 1 of the Convention as *transportation into a State of specimens of any species which were taken in the marine environment not under the jurisdiction of any State.* The Conference of the Parties has adopted additional guidance regarding the practical implementation of these provisions in Resolution Conf. 14.6 (Rev. CoP16)[18]. These include conditions to issue IFS certificates and import/export permits if the specimen/sample is taken by vessel, is registered in one State and is transported into a different State. This might be especially relevant for pelagic cetacean species whose distribution extends in off-shore areas beyond the ACCOBAMS adjacent Atlantic area.

- **Special case: within EU borders:**

According to EU legislation[19], no permit is required to exchange cetacean samples for non-commercial research purposes between two EU member countries.

However, some countries require the emission of an EU certificate (*ex: Portugal*), and/or documentation proving that the samples were collected legally (see Paragraph on "*National permits*").

Due to disparity between national legislations, researchers should contact the CITES Management Authority[20] of the exporting and importing country to ensure that they follow the appropriate procedure. ACCOBAMS Parties should facilitate the exchanges of samples for population genetics and diagnostic purposes by engaging CITES with IWC for a clear and well defined procedure.

[17] https://cites.org/eng/prog/ifs.php
[18] https://cites.org/eng/res/14/14-06R16.php
[19] https://eur-lex.europa.eu/eli/reg/1997/338/2022-01-19
[20] https://cites.org/eng/parties/country-profiles/es/national-authorities

C) Nagoya Protocol on Access and Benefit-Sharing

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation was adopted by the Conference of the Parties of the Convention on Biological Diversity at its tenth meeting in Nagoya, Japan in October 2010. It entered into force on 12 October 2014 and had 131 Parties as of July 2021.

Access and benefit-sharing (ABS) refers to the way in which genetic resources may be accessed, and how the benefits that result from their use are shared between the people or countries using the resources (users) and the people or countries that provide them (providers).

The Nagoya Protocol on ABS establishes an international legal framework based on three sections:

- **Access to genetic resources and their associated traditional knowledge with a view to their utilisation:** States can decide to make this access subject to their prior informed consent or to the consent of the traditional communities involved.

- **Benefit sharing:** the benefits must be shared fairly and equitably, subject to conditions established by mutually agreed terms between the user and the provider country or the traditional community involved.

- **Compliance:** the States Parties must adopt measures to ensure that access to genetic resources and to the associated traditional knowledge used under their jurisdiction complies with the internal regulations of the provider countries for access and benefit sharing.

This Protocol was negotiated in order to provide greater legal certainty and transparency for both providers and users of genetic resources and associated traditional knowledge:

- Establishing more predictable conditions for access to those resources and
- Helping to ensure benefit-sharing when genetic resources leave the contracting Party providing the genetic resources.

To gain access, users must first get permission **(=PIC)** from the provider country. In addition, the provider and the user must negotiate an agreement **(=MAT)** to share the resulting benefits equitably.

- **National Focal Points (NFPs):** NFPs are responsible for providing information to users on ABS, such as who to contact (national correspondent ABS-NC) and what the requirements and processes are in provider countries in order to gain access to genetic resources.

- **Competent National Authorities (CNAs):** CNAs are bodies established by governments and are responsible for granting access to users of their genetic resources, and representing providers on a local or national level. National implementation measures establish how CNAs work in a given country.

- **Prior informed consent (PIC):** Permission given from the CNAs of a provider country to a user (individual or institution) prior to accessing genetic resources, in line with an appropriate legal and institutional framework.

- **Mutually agreed terms (MAT):** An agreement reached between the providers of genetic resources and users on the conditions of access and use of the resources, and the benefits to be shared between both parties. Should include:

  - Type and quantity of genetic resources, and the geographical/ecological area of activity.
  - Any limitations on the possible use of material.
  - Whether the genetic resources can be transferred to third parties and under what conditions.
  - Recognition of the sovereign rights of the country of origin.
  - Capacity-building in various areas to be identified in the agreement.

- **Internationally Recognized Certificates of Compliance (IRCC):** issued by CNAs, as evidence that the genetic resources covered by the certificate have been accessed in accordance with PIC and that MAT have been established. Notification to the **ABS Clearing-House (ABS-CH)**[21].

Information regarding ABS National Focal Points, Competent National Authorities, Legislative, Administrative or Policy Measures, ABS Procedures, National Model Contractual Clauses, Internationally Recognized Certificates of Compliance, National Websites or Databases and Checkpoints of each Party are available on the **ABS countries profiles**[22].

Overview of the steps that prospective users of genetic resources should follow to be in compliance with ABS requirements (from Davis and Borisenko 2017):

1. Potential User finds out about Provider Country ABS rules, via ABS-Clearing House and NFP.

2. Potential User follows process for PIC and other permissions and negotiates MAT with Provider Country CNA, or other as authorised.

3. CNA grants PIC or issues evidence PIC was granted by other authorities/communities: CNA issues 'a permit or its equivalent' = national access permit ➜ User can now access genetic resources and begin to share benefits as agreed in MAT.

4. CNA submits a national permit to ABS-Clearing House. ABS-Clearing House generates IRCC with unique ID number.

5. User obtains and keeps IRCC number linked to genetic resources, derivatives and data that PIC and MAT cover; User provides IRCC # to other users if genetic resources are transferred (and allowed by PIC/MAT); New PIC/MAT may be needed for new uses/users.

6. User submits information, including IRCC #, to User Country checkpoints at key stages of utilisation, as determined by User Country rules.

7. User Country checkpoint submits information to ABS-Clearing House. ABS-Clearing House issues a checkpoint communiqué that is sent to the NFP and CNA of the Provider Country.

[21] https://absch.cbd.int
[22] https://absch.cbd.int/en/countries

The Nagoya Protocol might also apply between EU members.

Due to disparity between national legislations, researchers should contact the ABS National Focal Point of the providing country to understand which processes are required in order to gain access to genetic resources.

D) Inform ACCOBAMS National Focal Point

ACCOBAMS NFPs[23] should be informed of any sample exchanges to update the ACCOBAMS sample database.

[23] An up-to-date list of ACCOBAMS National Focal Points is available at https://accobams.org/about/partiesand-range-states/

06) Sample processing

A) Techniques/protocols for sample processing

With the advent of more affordable sequencing technologies, an increasing number of researchers are employing genetics and genomics as an integral part of their research. Before doing this, it is of essence for researchers to recognize the importance of high-quality (extracted) genetic material, especially so in the case of high-throughput sequencing (Cammen *et al*. 2016). Regardless of the specific aim of the study, be it within the scope of genetics (*e.g.* mtDNA, microsatellites) or even genomics (*e.g.* whole genome sequencing), the first laboratory-based step towards data generation is the DNA extraction (or in case of transcriptomics, RNA extraction), which can vary with sample type (*e.g.* blood, muscle).

A prerequisite for the extraction of genetic material is lysis of the tissue. This can be achieved *e.g.* through the incubation of the sample material in a proteinase K / SDS solution (Strauss 1998).

DNA extraction can be achieved using several available kits, such as the DNeasy Blood and Tissue kit (Qiagen) or NucleoSpin® Tissue kit (Macherey-Nagel, Germany), a phenol-chloroform extraction (Sambrook and Russell 2006) or a salting-out procedure as per Miller *et al*. (1988). For a variety of common extraction methods from skin biopsies, see Morin *et al*. (2015). The in-house formulations for DNA extractions have the advantage that they are considerably less expensive (<0.5€ per sample) compared to commercial kits (*e.g.* 4€ per sample for Qiagen's DNeasy Blood and Tissue kit). However, especially the phenol-chloroform extraction method involves acutely toxic chemicals and the protocol can be more time-consuming. In comparison, the ammonium-acetate precipitation method uses nontoxic and easily available reagents. Appendix 3 provides 2 protocols for DNA extraction.

Environmental samples

Specifically for eDNA samples, according to the type of filter used during sample collection and preparation a prior step before DNA extraction shall be considered. Comparative testing of extraction methods according to the type of filter used (Deiner *et al*. 2015, Liang and Keeley, 2013, Spens *et al*. 2017, Turner *et al*. 2014). "Open filters" require handling, filter funnel and vacuum pump, in contrast "enclosed filters" reduces unnecessary handling, and downstream DNA extraction takes place within the filter capsule substantially reducing the entrance of potential ways of contamination. eDNA capturing techniques from "open filters" have been comparatively tested in Liang and Keeley (2013), Turner *et al*. (2014) and Deiner *et al*. (2015), and from "enclosed filters" in Spens *et al*. (2017).

Cetacean faecal samples

DNA isolation from cetacean faecal samples can be performed using specialised kits for stool or soil (*e.g.* Promega Maxwell RSC Faecal Microbiome DNA Kit, QIAGEN QIAamp PowerFecal Pro DNA Kit, QIAGEN DNeasy PowerSoil Pro Kit), where an initial step including beads can help to break down larger particles. If available, samples can also be pre-processed on a sonication (or similar) device.

RNA extraction is the basis for transcriptome research which deals with gene expression. Several commercial kits are available. RNeasy micro or mini kit (Qiagen) can be used on the sample, depending on the expected RNA yield, or Aurum™ Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad) as a valid alternative for skin biopsies from free-ranging organisms.

In both types of extraction, genetic material is bound to the silica membrane in the spin columns by the addition of chaotropic salts and ethanol, and contaminants are removed through washing with different buffers. Cleanup and yield of pure extracts in the case of DNA is achieved with an RNase treatment, and in the case of RNA, with a DNase (Cammen *et al*. 2016).

Concentration and the integrity of DNA (or RNA) should be checked either on the nanodrop spectrophotometer (Desjardins and Conklin 2010) or using the *e.g.* Agilent bioanalyzer (Krupp 2005).

In case of genetic studies, **PCR (Polymerase Chain Reaction)** is necessary in order to amplify genetic material to such an extent that it can be then sequenced reliably (*e.g.* Sanger). PCR protocols vary, but generally involve extracted DNA, a premix/mastermix (*e.g.* the PPP Master Mix, Top-Bio s.r.o.; GoTaq G2 Green Mastermix, Promega, Madison, WI, USA), both forward and reverse primers and water. Instead of the so-called mastermix, one can also use a set of Taq DNA polymerase, dNTPs, MgCl2 and reaction buffers. The PCR template should then be exposed to a thermal cycle by *e.g.* using a Mastercycler Gradient 96-well system (Eppendorf, Hamburg, Germany). An example of a PCR reaction would be consisting of a initial denaturation at 94°C (30s), followed by 30 cycles of 94°C (30s), 60°C (30 s), and 72°C (30s), and a final extension step of 72°C (10min) (Rosel *et al*. 2005). In order to have greater success at amplifying genetic material, one can tweak the amplifying profile by either adjusting the timing of each step, number of steps or the exact (annealing) temperature. For an overview, please see Metzker and Caskey (2009).

To confirm amplification, PCR products can be electrophoresed on 1 or 2 % agarose gels (Foote *et al*. 2019).

Extracted and amplified DNA can then be purified using PCR Clean-up kit (Macherey-Nagel, Germany) or ExoSAP-IT PCR Product Cleanup Reagent (Applied Biosystems), for example, and sequenced either in-house on a *e.g.* a ABI Prism 3100xl Genetic Analyzer (Applied Biosystems) applying the BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) (Mulholland *et al*. 2015), or commercially-sequenced (*e.g.* Macrogen, USA; GATC Biotech, Germany).

When interested in **genome or transcriptome** level analyses, extracted DNA (or RNA) should then be prepared for next generation sequencing (NGS) on an *e.g.* Illumina platform. For this, the library preparation step is key. Just to give an example, RNAseq libraries can be constructed in-house using llumina's NEBNext Ultra II Directional RNA library preparation kit, NEBNext Multiplex Oligos and the NEBNext Poly(A) mRNA Magnetic Isolation Module, New England Biolabs (Morey *et al*. 2022). Libraries can also be outsourced (*e.g.* to Novogene, Singapore). As an alternative to RNAseq, one can also make use of qPCR; however, the latter can only detect known sequences (cost related pros and cons discussed in Nonis *et al*. 2014). See Cammen *et al*. (2016) for an overview of more general advances in high-through-put sequencing, and Foote *et al*. (2019) for a more detailed methodology concerning library building for genome analyses.

B) IWC guidelines on quality control

The Scientific Committee of the IWC has compiled guidelines for DNA data quality control and error rate estimation, for genetic studies relevant to IWC management advice (Tiedemann *et al*. 2012). These guidelines mainly deal with awareness, minimisation, and control of DNA typing errors. They emphasise the importance of reporting genotyping error rates (or inconsistencies in data sets). Errors can be introduced at various points of a DNA study (Figure 4) and the guidelines propose measures to minimise errors; the most important factors that contribute to errors will likely include mislabelled samples, data entry errors, etc. – sometimes called "handling errors". In contrast, "systematic errors" are associated with the tendency for particular genetic markers and/or sample types to be susceptible to errors due to their inherent characteristics.

Tiedemann *et al.* (2012) recommend that measures should be taken to reduce the overall error rate to around 1% for microsatellite data used in population studies and less than 1% for studies using SNPs (Bonin *et al.* 2004, Broquet and Petit 2004, Morin *et al.* 2009), even lower rates for parentage and genetic mark-recapture studies to reduce the number of false positives (Bonin *et al.* 2004, Hoffman and Amos 2005, Waits *et al.* 2001). In all cases, researchers should report the genotype error rates detected in the course of quality checks (ideally both locus-specific and overall error rates).

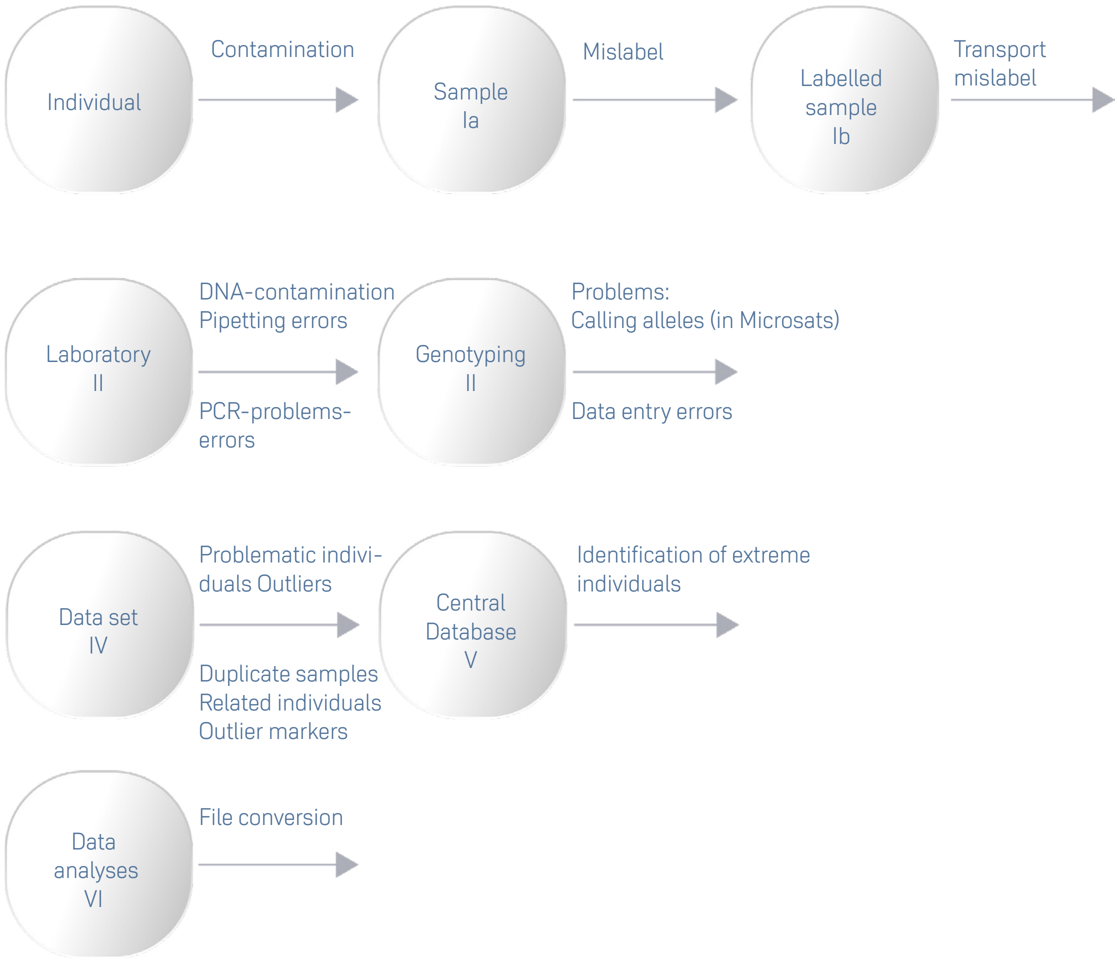


Figure 4. Flow chart on DNA analysis procedures and potential error sources (Tiedemann et al. 2012, reproduced with permission).

C) Suitable genetic labs

Genetic analyses require a substantial amount of laboratory processing requiring specific equipment (*e.g.* centrifuge, PCR machine, sequencer, freezers). It may therefore be necessary to seek collaborations with institutions that can provide the required facilities and expertise. Some institutions have specialised in particular kinds of analyses and can process samples for a fee, while others are open to collaborative projects. For consistency and whenever possible, all samples should be processed in the same laboratory, following the same protocol. If more than one laboratory is to be used, appropriate calibration must be carried out, depending on the markers.

A database with suitable genetic laboratories is available in **Appendix 2**. This list was created based on the information received from ACCOBAMS NFPs and workshop participants in September 2022. It was later updated in November 2023. It should be noted that the list is not exhaustive and other laboratories may also have suitable facilities.

07) Data analysis

A) IWC guidelines on data analysis

Providing detailed guidelines on all types of genetic/omic data analyses is beyond the scope of these best practices and tools are frequently being modified or added. The Scientific Committee of the IWC has already compiled in-depth material on different aspects of data analyses relevant to IWC management advice (Waples *et al*. 2018) and this is being updated at present. Specifically, Waples *et al*. (2018) discuss key data analysis aspects on:

1. Identify/delimit species, sub-species and populations;
2. Provide estimates of census population size (N) and effective population size (Ne);
3. Track contemporary movements of individuals;
4. Estimate long-term levels of connectivity among populations;
5. Quantify genetic diversity and provide insights into past bottlenecks and population expansions;
6. Help resolve mixtures of individuals originating from different breeding populations.

They also discuss some important analytical considerations regarding difficulties to identify appropriate threshold levels of population differentiation, demographic independence, and the interpretation of genetic results. The definition of a population in a management context is complex and can run along a continuum from isolation to panmixia. Ultimately, the importance from a conservation perspective is that management measures are robust to uncertainty in population structure. Demographic independence occurs when migration rates are low enough that population dynamic processes are determined primarily by local birth and death rates. Such units require separate management measures.

Several core principles emerge:

1. Clarify goals and objectives and quantify expectations before the study begins and determine one or more appropriate markers.
2. Follow appropriate data quality-control measures.
3. Test for agreement with Hardy-Weinberg equilibrium *e.g.* Fis.
4. Consider several indices of genetic diversity.
5. Statistical tests are a useful starting point, but a significant P value provides no information other than that the null hypothesis can be rejected. Use a variety of approaches and integrate the results (*e.g.* see Table 3 below).
6. Don't overinterpret point estimates.
7. Absence of evidence of structure is not evidence of absence - try to estimate the power of the tests you are using (not always easy).
8. Important to examine assumptions and possible biases (*e.g.* ascertainment bias, influence of multiple testing, influence of selection).
9. Consider the distinction between scientific results, conclusions, and recommendations.
10. Effects of linkage are important to consider in genomics studies.

Given the continued development of analytical methods, the primary literature should always be consulted as well as reviews by bodies such as the IWC. Researchers should publish their data and annotated scripts to ensure reproducibility of results and improve transparency of analytical approaches.

Table 3. Summary of some of the most common analysis tools used in population genetics investigating management units/units-to-conserve (after IWC, 2019) that do not involve the more traditional hypothesis testing approaches where putative populations are defined a priori. Note that a thorough study to identify management units will use several approaches and integrate the results and inferences from these, also with non-genetic analyses.

The program **STRUCTURE**[24] groups individuals such that departure from Hardy-Weinberg-Expectations (HWE) within groups is minimised. Until recently, STRUCTURE was the most common hypothesis-free assignment method based on genetics used to investigate population structure. The Simulation studies have shown that the program has relatively low power (typically finding structure only when FST is greater than approximately 0.02). While the number of genetic clusters present in the data (k) is an input parameter (as a range of possible values), STRUCTURE provides a likelihood for each given value of k. Whilst STRUCTURE may identify a number of genetic groups with high probability this does not necessarily mean that the identified groups may not have further sub-structure. Under scenarios of spatial overlap in the distribution of stocks, STRUCTURE may detect heterogeneity, but not allow for the assignment of individual specimens to putative 'additional' stocks.
GENELAND[25] is a landscape genetics program run in R that groups samples into homogeneous putative populations by assuming approximate Hardy Weinberg and linkage equilibrium, and by incorporating individual-specific spatial data. Although similar in approach to STRUCTURE, the spatially explicit component generally provides greater power (as long as stocks are not randomly mixed).
TESS[26] incorporates spatial information and conducts Bayesian clustering using tessellations (division of samples into best fit polygons), and thereby provides a landscape genetics method with a distinct methodology from GENELAND or STRUCTURE. The use of fractals in TESS means that some fine-grained elements of structure might be missed or identified out of place.
BAPS[27] uses Bayesian methods to capture genetic population structure by describing the molecular variation in each subpopulation using a separate joint probability distribution over the observed *loci*. This method is based on allele frequency distributions rather than equilibrium expectations, and so may not have the power to detect very recently diverged populations.
The **sPCA** (spatial Principal Component Analysis)[28] approach is based around two key elements – a spatial autocorrelation, implemented using Morin's I, and an assessment of allele frequency variance on global and local scales. Although informed by spatial data (which is incorporated into a network structure), it does not use spatial coordinates directly. The presence of multiple populations sampled in the same designated area could exaggerate local variance, potentially obscuring structure at the global scale. For this reason, spatially explicit models using equilibrium tests (as implemented in GENELAND) may be better at extracting structure on a local scale from a mixed assemblage of populations.

[24] https://web.stanford.edu/group/pritchardlab/structure.html
[25] https://i-pri.org/special/Biostatistics/Software/Geneland/
[26] http://membres-timc.imag.fr/Olivier.Francois/tess.html
[27] https://www.helsinki.fi/en/researchgroups/network-pharmacology-for-precision-medicine/software
[28] https://www.nature.com/articles/hdy200834

B) Environmental DNA/faeces

In any study, the choice of data analyses techniques should be driven by the scientific question to be resolved. For eDNA water samples, non-PCR methods (*e.g.* metagenomics) might not be the best approach for specifically studying selected taxa. Alternatively, traditional PCR from mixed templates (*e.g.* metabarcoding) allows a molecular marker selection to be specifically amplified and analysed. An important caveat in the selection of molecular markers is the amount of available reference sequences with which the studied sample will be compared. Currently, the mitochondrial 12S and 16S rRNA have been extensively used as reference sequences for cetacean biodiversity assessments (*e.g.* Valsecchi *et al.* 2021, 2020). In addition, quantitative (qPCR) methods have been also used to quantify and compare species-specific techniques using taxon-specific genetic markers and for studying population genetic structure of cetaceans (*e.g.* Parsons *et al.* 2018, Pinfield *et al.* 2019).

Similar approaches can be used when dealing with cetacean faecal samples. However, in this case a metagenomic approach can also be useful, as long as good quality reference sequences are available, for biodiversity assessment, and simultaneous characterization of different ecological components (*e.g.* diet, host, parasites). Similarly, metabarcoding and qPCR analytics can be resourceful for analysing this type of data.

C) Computational resources for genomic data

Determining which approach (genetic or genomic) is best in a particular case depends on many factors, including the resources available and the data required to address a specific scientific question. The advantage of using newer techniques is increased statistical power and resolution with more markers, and in many cases increased efficiency and cost-effectiveness. In recent years, next generation sequencing (NGS) used to produce genomic data has and continues to revolutionise the field of molecular ecology by allowing us to understand better (with higher resolution) the evolutionary history of populations and species, to delineate populations, detect cryptic population structure and to detect genomic regions that could be under selection. However, the economic and computational resources needed generate a trade-off between the number of *loci* that can be obtained and the number of populations or individuals that can be sequenced (Aguirre-Liguori et al. 2020).

NGS produces large amounts of data, normally generating an additional 100 Gigabytes of data per genome, which will require high computational power, storage, and bioinformatic processing, which can be economically challenging for many institutions. **Cloud computing is an option for researchers who currently lack the tools to make full use of this data type and represents a viable (but not free) way to analyse large datasets relatively quickly without having to maintain and upgrade servers.**

It is important to recognise that many bioinformatic pipelines and population genomics analyses require fairly advanced computer and programming skills, in addition to understanding population genetics concepts. Bioinformatics pipelines and guidelines for best practices have not yet been standardised. In recent years, significant progress is being made in the development of more userfriendly programmes and clear guidelines for collecting and applying genomics to wildlife biology and management (Gomez-Sanchez and Schlötterer 2018, Gruber *et al.* 2018, Ravindran *et al.* 2019).

Some information to keep in mind:

- The data size of a whole genome for one individual is between 20 and 60 GB depending on coverage and species (cetaceans genome sizes vary between ~2.3Gb-3Gb).
- The original data (RAW data) size for ~100 individuals sequenced with ddRAD-seq (with 20-30X coverage) is around 220GB.
- The initial analyses will typically generate X times (between 2-10 times) the original data size before you get your "final" usable file.
- To run population analyses (selection, demographic history, etc.) new files will be generated.

08) Dissemination of results

Dissemination of results in appropriate ways for the target audience is important to scientists, organisations, communities and policymakers. Effective dissemination can also be useful in fostering collaboration between partners and is essential for effective conservation and management measures to be developed and implemented.

A) ACCOBAMS Scientific Committee

The ACCOBAMS Scientific Committee is the primary scientific body providing advice to ACCOBAMS to allow it to develop effective conservation and management advice. It is advisable where possible to submit proposed population genetics studies to that body for comment and advice. It is particularly important to submit the results of population genetics studies to the Scientific Committee as soon as possible (*i.e.* before formal publication in a peer-reviewed journal) since this will allow the results to be incorporated into management advice in a timely manner.

B) Scientific community

Publishing in an Open access and preprints make research results visible and increase the number of citations. Also, beyond traditional academic publishing (journal articles, books, and conference presentations), digital dissemination can achieve more widespread research uptake and understanding. The use of social media accounts, researchers' identifiers, academic social networks (*e.g.* Academia.edu, ResearchGate, LinkedIn, Google Scholar) and wikis as a specific form of 'open notebook science' can boast millions of users.

C) ACCOBAMS National Focal Point

Main findings should be summarised in the national report. Pending adoption by MOP8, national reports must also include any sample exchange, samples being collected to feed ACCOBAMS sample database, potential genetic laboratories for cetacean analysis and list of scientific publications. Researchers should therefore inform their ACCOBAMS NFPs every three years of any updates in their sample collection/exchange.

D) Other stakeholders

Regular and ongoing contact with partners can support the spread of knowledge. Within ACCOBAMS, the information can be shared through the "NETCCOBAMS" platform where updated information and main findings are continuously uploaded. Researchers should also make an effort to notify affected stakeholders through appropriate channels.

E) General public

Research results presented in complex and technical jargon should be translated to non-technical language that the general public will find easier to understand. This approach includes communication in the form of popular science magazines and science shows on television and the radio or a press release. Digital technologies offer new online formats for interaction with the wider public and for reaching citizens who would otherwise remain out of reach for traditional methods of communication. These approaches include TED talks broadcasted on YouTube and blogs that often receive millions of views.

09) Data archiving and collecting data from previously published studies

In order to contribute to open and transparent science, and to secure reproducibility all raw and processed data is recommended to become available after publication or during the reviewing process. These procedures will secure a broad dataset to be available for future studies and reviews. Currently, diverse platforms are available for data storage (*e.g.* EMBO, GenBank, Dryad, Obis) that can be cross-linked to favour a connection between data usage and publications.

10) Forensic science

A) Special case of individual identification of captive individuals

According to document of "Taking of Cetaceans, Dolphinaria and Quasi-Dolphinaria: A Legal Analysis Relating to Accobams Parties" (**ACCOBAMS-MOP7/2019/Inf 09**), concerns as regards the question of taking of cetaceans and dolphinaria have been expressed by the ACCOBAMS Scientific Committee that remarked "the illegality of live removals of cetaceans from the Black Sea" and called for "an inventory and thorough assessment of individual identity of all bottlenose dolphins kept in captivity by means of genetic, morphological and photo-ID methods", as well as for the provision of "appropriate administrative measures in order to prevent substitution of dolphins that die in captivity from animals taken from the wild" (Recommendation 8.2). Following recommendations by the Scientific Committee, the Fifth Meeting of ACCOBAMS Parties adopted **Resolution 5.14** about Live Removals of Bottlenose Dolphins in the Black Sea (*Tursiops truncatus*).

At its 17th meeting, the Conference of the Parties to CITES (Johannesburg, 2016) further dealt with the above-mentioned species (Recommendations 17.299-301). Parties are now encouraged to use genetic analysis to confirm the origin of the animals prior to the issuance of export permits. Furthermore, Parties are encouraged to establish national or regional repositories where relevant genetic identification data are stored and to make them accessible on-line, as well as to report to the CITES Animals Committee on exports of *Tursiops truncatus ponticus* and their origins.

A template of genetic passport for different cetacean species kept in captivity is currently being developed, in collaboration with the ACCOBAMS Advisory Committee on Captivity related issues.

B) Species identification for trade

Additionally, wildlife forensic genetic science is becoming accepted as a recognised discipline. The teeth of sperm whale Physeter macrocephalus (CITES Appendix I) or killer whale *Orcinus* orca (CITES Appendix II) can be objects of worldwide illegal trade (Baker *et al*. 2020). They can be scrimshawed or superficially carved, thus retaining their original shapes as morphologically recognizable objects. But for pieces lacking species diagnostic morphological characters, genetic analyses can be powerful tools in their identification. In these cases ancient/museum DNA study protocol is applicable. Silica-based extraction of low volumes (0.01–0.02g) of dentine-cementum powder of sperm whale teeth and scrimshaws, obtained without significant damage to the specimen, can provide sufficient DNA by sequencing of mitochondrial *loci* (Pichler *et al*. 2001).

11) Glossary of terms

The glossary was adapted from Waples et al. (2018).

adaptation: the process by which the frequency of alleles that enhance the survival and/or reproductive success (*i.e.* the fitness) of individuals in a given environment increases over time.

admixture: the result of interbreeding and gene flow between genetically-differentiated populations.

allele: one of two or more alternative forms of a gene or nucleotide sequence at a given locus.

allele frequency: the proportion of all alleles at a given locus that are of a specific type within the group being sampled.

assignment test: a statistical method using multi-locus genotypes to assign individuals to the population from which they most likely originated.

autosome: a chromosome that is not a sex chromosome.

balancing selection: a form of natural selection that acts to maintain polymorphism at a locus within a population.

connectivity: the degree of exchange between two or more groups or populations. Connectivity can be demographic, in which case it relates to the degree to which population growth and vital rates are affected by dispersal; or genetic, in which case it refers to the exchange of genes (*i.e.* **gene flow**).

demographic: pertaining to processes that affect the size of a population (*e.g.* birth, death, dispersal).

diploid: having two sets of chromosomes. In sexually reproducing populations, one set is inherited from the mother and one from the father. At a given diploid locus, an individual can have two different alleles (**heterozygous**) or two identical alleles (**homozygous**). *Loci* with autosomal inheritance patterns are diploid (see **haploid**).

dispersal: movement of an individual away from its natal population and into another population. As used in this document, dispersal usually implies that the dispersing individual subsequently reproduces with members of the new population, resulting in gene flow; however, that is not always the case. In many species, but not cetaceans, passive dispersal of gametes or larvae is common.

effective population size *Ne*: the size of an 'ideal' population that would experience the same rate of genetic drift as the population in question. In an ideal population (also called a Wright-Fisher population), generations are discrete, mating is random, and every individual has an equal probability of contributing genes to the next generation—in which case *Ne* = *N*. In most species, including cetaceans, *Ne* is typically smaller than the number of individuals in a population (see **population size**).

F_{IS}: a measure of whether the genotypic frequencies observed in a sample are compatible with those expected under Hardy-Weinberg equilibrium. Positive F_{IS} values indicate a deficiency of heterozygotes compared to HWE, while negative values indicate a deficiency of homozygotes compared to HWE.

F_{ST}: a measure of the decrease in heterozygosity, relative to that expected under random mating, that occurs as a result of population structure. Low values of F_{ST} indicate that allele frequencies are similar among the groups being compared, while higher values indicate more genetic differentiation between groups.

gene flow: exchange of genes between populations or groups. Gene flow can result from an individual moving to a new population/group and successfully reproducing with members of that group, or through interbreeding between individuals of different populations or groups without any permanent movement of individuals (only gametes) between groups.

genetic differentiation: the accumulation of genetic differences (allele frequencies or sequence substitutions) between groups. Genetic differentiation can occur due to limited gene flow as well as to natural selection on non- neutral genes in sympatric groups.

genetic diversity: genetic variation that occurs within individuals, within populations, and among populations.

genetic drift: random change in allele frequencies from one generation to the next. Drift is expected to have a greater effect as the effective population size of the population decreases.

genotype: the genetic makeup (allelic composition) of an individual, either of the entire genome or more commonly of a certain locus or set of *loci* (see **phenotype**).

haploid: having a single set of chromosomes, such that only a single copy of an allele or sequence exists at a given locus. In cetaceans, mtDNA is an example of a haploid marker, as it is inherited only from the mother. Sex-specific markers, such as Y-chromosome markers, also exhibit a haploid inheritance pattern (see **diploid**).

haplotype: the combination of alleles at *loci* that are found on a single chromosome or DNA molecule and thus tend to be inherited together. In cetaceans, haplotype typically refers to the mitochondrial DNA sequence held by an individual. Phased nuclear alleles, *e.g.* SNP variants physically located on the same chromosome, also constitute a haplotype.

Hardy-Weinberg equilibrium (HWE): an idealised state under which the genotypic frequencies in a population are simple products of allele frequencies. In theory, HWE is achieved in randomly-mating populations of infinite size that do not experience migration, natural selection, or mutation.

heterogeneity: the presence of multiple genetically or demographically distinct groups within a set of samples.

heterozygous: having two different alleles at a gene locus (*e.g.* Aa)

homogeneity: the absence of multiple genetically or demographically distinct groups within a set of samples.

homozygous: having two copies of the same allele at a gene locus (*e.g.* AA).

hybridisation: mating between individuals from two genetically distinct populations or species.

Inbreeding: mating between individuals that are more closely related than by chance alone. Inbreeding is expected to increase homozygosity because there is a greater probability that the genotype of an inbred individual will contain alleles that are identical by descent (inherited from a common ancestor).

linkage: a measure of the degree to which alleles of two *loci* do not assort independently. Two *loci* in close proximity on a chromosome have a higher probability of being inherited together than do two *loci* that are further apart and hence are said to be linked. Nonrandom associations of alleles at different *loci* can also occur by natural selection, migration, or genetic drift without physical linkage.

linkage disequilibrium (LD): the nonrandom association of alleles between *loci*, often because the *loci* are located close together on the same fragment of DNA. Also known as gametic disequilibrium. Random LD also occurs in all populations due to genetic drift, with magnitude inversely proportional to effective population size.

locus (plural *loci*): a stretch of DNA at a particular place on a particular chromosome; often used to refer to a gene.

microsatellite: a genetic marker composed of short DNA sequence units that are repeated multiple times (*e.g.* ATATATATAT). Although microsatellites can be found on sex chromosomes and in mitochondrial DNA, use of this term in cetacean population genetics typically refers to *loci* that are biparentally inherited and of nuclear origin. Microsatellite alleles are usually labelled according to the number of repeated units (and thus the size) contained in a given allele, as opposed to being directly sequenced.

migration: this term is commonly used in two different ways, to refer to: a) seasonal movements between two geographical areas that are related to the population's reproductive cycle, changes in their physical environment (*e.g.* ice formation), and/or prey availability; and b) movement of individuals between groups or populations, which might or might not result in successful reproduction and gene flow. Unless otherwise specified, as used in this document 'migration' implies both movement between populations and gene flow.

mitochondrial DNA (mtDNA): a small, circular DNA molecule (in animals ~16–20 kbp long) found in the mitochondria (*i.e.* outside of the nucleus) of a cell. In cetaceans, mtDNA is inherited only from the mother and is thus an example of haploid inheritance.

mutation: a change to the genetic material of a cell. Mutations can include single nucleotide changes, deletions, and insertions, as well as duplications, losses, inversions, and translocations of segments of DNA sequence.

natural selection: differential contribution of genotypes to the next generation due to differences in survival and/or reproduction.

nuclear DNA (nDNA): DNA found in a cell's nucleus. In cetaceans, autosomal nuclear DNA is biparentally inherited, such that an individual's genotype at a given locus contains one allele inherited from its mother and another allele inherited from its father. Nuclear DNA also includes DNA found on sex chromosomes.

nucleotide diversity: a measure of genetic variation calculated from DNA sequence data, which measures the average proportion of differences between all DNA sequences (*i.e.* the average difference between two randomly taken sequences) in a group.

neutral: not influenced by natural selection.

non-model species: species that have not been subject to extensive research and for which markers or reference genomes may not be available.

phylogenetic: a term used to describe evolutionary relationships among taxa.

phylogeography: the study of how the genetic lineages of a taxon are distributed across the landscape, in order to better understand its evolutionary history (its origin and spread).

polymorphic: having more than one allele at a locus. This term is typically used to refer to a group/ population rather than to an individual, which is considered to be heterozygous if more than one allele is present.

population: a group of individuals that co-occur in space and time and freely interbreed. Terms that are often used synonymously with 'population' include 'subpopulation' and 'stock,' although the latter can also refer to units of management convenience that do not imply interbreeding.

population size (N): the number of individuals in a population, often denoted as the census size (Nc). Commonly used to refer either to all individuals or only adults (see **effective population size**).

primer: locus-specific short sequence (oligonucleotides) that is complementary to the regions flanking the targeted microsatellite pattern.

Restriction site Associated DNA (RAD) sequencing: sequencing of DNA libraries comprising regions adjacent to restriction sites.

single nucleotide polymorphism (SNP): DNA sequence variation that occurs when a single nucleotide (A, T, C, or G) differs at a specific site among individuals or within an individual (for diploid markers).

sterile: free from bacteria or other living microorganisms that could cause contamination.

References

Aguirre-Liguori, JA, Luna-Sanchez JÁ, Gasca-PIneda J., Eguiarte LE (2020) Evaluation of the minimum sampling design for population genomic and microsatellite studies: an analysis based on Wild Maize. Frontiers in Genetics, 11: 870. https://doi.org/10.3389/fgene.2020.00870

Allendorf, F., Hohenlohe, P. and Luikart, G. Genomics and the future of conservation genetics. Nat Rev Genet 11, 697–709 (2010). https://doi.org/10.1038/nrg2844

Amos, W., Whitehead, H., Ferrari, M. J., Glockner-Ferrari, D. A., Payne, R., and Gordon, J. (1992). Restrictable DNA from sloughed cetacean skin; its potential for use in population analysis. Marine Mammal Science, 8(3), 275-283. https://doi.org/10.1111/j.1748-7692.1992.tb00409.x

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., and Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. Nature Reviews Genetics, 17(2), 81– 92. https://doi.org/10.1038/nrg.2015.28

Bearzi G (2000) First report of a common dolphin (*Delphinus delphis*) death following penetration of a biopsy dart. Journal of Cetacean Research and Management 2:217-222. https://doi.org/10.47536/jcrm.v2i3.910

Bilgmann K, Griffiths OJ, Allen SJ, Möller LM (2007) A biopsy pole system for bow-riding dolphins: sampling success, behavioral responses, and test for sampling bias. Marine Mammal Science 23:218-225. https://doi.org/10.1111/j.1748-7692.2006.00099.x

Bonin A, Bellemain E, Bronken Eidesen P, Brochmann C, Taberlet P. (2004) How to track and assess genotyping errors in population genetics studies. Molecular Ecology 13, 3261-3273. https://doi.org/10.1111/j.1365-294X.2004.02346.x

Broquet T, Petit E (2004) Quantifying genotyping errors in noninvasive population genetics. Molecular Ecology 13, 3601-3608. https://doi.org/10.1111/j.1365-294X.2004.02352.x

Cabrera A.A, Bérubé M, Lopes X.M, Louis M., Oosting T., Rey-Iglesia A, Rivera-León V.E., Székely D., Lorenzen E.D, Palsbøll P.J. (2021) A genetic perspective on Cetacean Evolution. Annual Review of Ecology, Evolution, and Systematics 2021 52:1, 131-151. https://doi.org/10.1146/annurev-ecolsys-012021-105003

Cammen, K. M., Andrews, K. R., Carroll, E. L., Foote, A. D., Humble, E., Khudyakov, J. I., Louis, M., McGowen, M. R., Olsen, M. T. and Van Cise, A. M. (2016). Genomic methods take the plunge: recent advances in high-throughput sequencing of marine mammals. Journal of Heredity, 107(6), 481-495. https://doi.org/10.1093/jhered/esw044

Closek, C.J., Santora, J.A., Starks, H.A., Schroeder, I.D., Andruszkiewicz, E.A., Sakuma, K.M., Bograd, S.J., Hazen, E.L., Field, J.C., Boehm, A.B., 2019. Marine Vertebrate Biodiversity and Distribution Within the Central California Current Using Environmental DNA (eDNA) Metabarcoding and Ecosystem Surveys. Front. Mar. Sci. 6, 1–17. https://doi.org/10.3389/fmars.2019.00732

Davis, K., Borisenko A. 2017. Introduction to Access and Benefit-Sharing and the Nagoya Protocol: What DNA Barcoding Researchers Need to Know. Pensoft Publishers, Sofia, 37 pp. https://doi.org/10.3897/ab.e22579

Deiner, K., Walser, J.-C., Mächler, E., Altermatt, F., 2015. Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. Biol. Conserv. 183, 53–63. https://doi.org/10.1016/j.biocon.2014.11.018

Desjardins, P., and Conklin, D. (2010). NanoDrop microvolume quantitation of nucleic acids. JoVE (Journal of Visualized Experiments), (45), e2565. https://dx.doi.org/10.3791/2565

Djurhuus, A., Port, J., Closek, C.J., Yamahara, K.M., Romero-Maraccini, O., Walz, K.R., Goldsmith, D.B., Michisaki, R., Breitbart, M., Boehm, A.B., Chavez, F.P., 2017. Evaluation of Filtration and DNA Extraction Methods for Environmental DNA Biodiversity Assessments across Multiple Trophic Levels. Front. Mar. Sci. 4. https://doi.org/10.3389/fmars.2017.00314

Drouot, V., Bérubé, M., Gannier, A. , Goold, J. C., Reid, R. J., and Palsboll, P. J. (2004). A note on genetic isolation of Mediterranean sperm whales (*Physeter macrocephalus*) suggested by mitochondrial DNA. Journal of Cetacean Research and Management, 6(1), 29-32. https://doi.org/10.47536/jcrm.v6i1.787

Esteban R, Verborgh P, Gauffier P, Giménez J, Martín V, Pérez-Gil M, *et al*. 2016. Using a multi-disciplinary approach to identify a critically endangered killer whale management unit. Ecol Indic;66:291–300. http://dx.doi.org/10.1016/j.ecolind.2016.01.043

Farro, A. P. C., Rollo Jr, M. M., Silva Jr, J. M., and Marino, C. L. (2008). A simple protocol for a low invasive DNA accessing in *Stenella longirostris* (Cetacea: *Delphinidae*). Pan-American Journal of Aquatic Sciences 3(2): 130-134 http://panamjas.org/pdf_artigos/PANAMJAS_3(2)_130-134.pdf

Fontaine, M.C., Tolley, K.A., Siebert, U. *et al*. (2007) Long-term feeding ecology and habitat use in harbour porpoises *Phocoena phocoena* from Scandinavian waters inferred from trace elements and stable isotopes. BMC Ecol 7, 1 . https://doi.org/10.1186/1472-6785-7-1

Fontaine, M.C., Roland, K., Calves, I., Austerlitz, F., Palstra, F.P., Tolley, K.A., Ryan, S., Ferreira, M., Jauniaux, T., Llavona, A., Öztürk, B., Öztürk, A.A., Ridoux, V., Rogan, E., Sequeira, M., Siebert, U., Vikingsson, G.A., Borrell, A., Michaux, J.R. and Aguilar, A. (2014). Postglacial climate changes and rise of three ecotypes of harbour porpoises, *Phocoena phocoena*, in western Palearctic waters. Molecular Ecology, 23: 3306-3321. https://doi.org/10.1111/mec.12817

Foote AD, Martin MD, Louis M, Pacheco G, Robertson KM, Sinding MS, Amaral AR, Baird RW, Baker CS, Ballance L, Barlow J, Brownlow A, Collins T, Constantine R, Dabin W, Dalla Rosa L, Davison NJ, Durban JW, Esteban R, Ferguson SH, Gerrodette T, Guinet C, Hanson MB, Hoggard W, Matthews CJD, Samarra FIP, de Stephanis R, Tavares SB, Tixier P, Totterdell JA, Wade P, Excoffier L, Gilbert MTP, Wolf JBW, Morin PA. (2019). Killer whale genomes reveal a complex history of recurrent admixture and vicariance. Molecular Ecology, 28(14), 3427-3444. https://doi.org/10.1111/mec.15099

Fossi M, Marsili L, Neri G, Casini S, Bearzi G, Politi E, Zanardelli M, Panigada S (2000) Skin biopsy of Mediterranean cetaceans for the investigation of interspecies susceptibility to xenobiotic contaminants. Marine Environmental Research 50:517-521. https://doi.org/10.1016/S0141-1136(00)00127-6

Freedman, J., van Dorp, L.B. and Brace, S., 2018. Destructive sampling natural science collections: an overview for museum professionals and researchers. Journal of Natural Science Collections, 5, pp.21-34. https://www.natsca.org/article/2440

Fulton, T.L., Shapiro, B. (2019). Setting Up an Ancient DNA Laboratory. In: Shapiro, B., Barlow, A., Heintzman, P., Hofreiter, M., Paijmans, J., Soares, A. (eds) Ancient DNA. Methods in Molecular Biology, vol 1963. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-9176-1_1

Gales, N. J., Bowen, W. D., Johnston, D. W., Kovacs, K. M., Littnan, C. L., Perrin, W. F., Reynolds III, J. E., & Thompson, P. M. (2009). Guidelines for the treatment of marine mammals in field research. Marine Mammal Science, 25(3), 725-736. https://doi.org/10.1111/j.1748-7692.2008.00279.x

Gales, N. J., Dalebout, M. L., and Bannister, J. L. (2002). Genetic identification and biological observation of two free-swimming beaked whales: Hector's beaked whale (*Mesoplodon hectori*, Gray, 1871), and Gray's beaked whale (*Mesoplodon grayi*, von Haast, 1876). Marine Mammal Science, 18(2), 544-551. https://doi.org/10.1111/j.1748-7692.2002.tb01055.x

Gaspari S, Scheinin A, Holcer D, Fortuna C, Natali C, Genov T, Frantzis A, Chelazzi G, Moura AE (2015) Drivers of population structure of the bottlenose dolphin (*Tursiops truncatus*) in the Eastern Mediterranean Sea. Evolutionary Biology 42:177-190. https://doi.org/10.1007/s11692-015-9309-8

Genov T, Jepson PD, Barber JL, Hace A, Gaspari S, Centrih T, Lesjak J, Kotnjek P (2019) Linking organochlorine contaminants with demographic parameters in free-ranging common bottlenose dolphins from the northern Adriatic Sea. Science of the Total Environment 657:200-212. https://doi.org/10.1016/j.scitotenv.2018.12.025

Giménez J, De Stephanis R, Gauffier P, Esteban R, Verborgh P (2011) Biopsy wound healing in long-finned pilot whales (*Globicephala melas*). Veterinary Record 168:101. https://doi.org/10.1136/vr.c5284

Giménez J, Louis M, Barón E, Ramírez F, Verborgh P, Gauffier P, Esteban R, Eljarrat E, Barceló D, Forero MG, de Stephanis R (2018) Towards the identification of ecological management units: a multidisciplinary approach for the effective management of bottlenose dolphins in the southern Iberian Peninsula. Aquatic Conservation: Marine and Freshwater Ecosystems 28:205-215. https://doi.org/10.1002/aqc.2814

Goldberg, C.S., Strickler, K.M., Pilliod, D.S., 2015. Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. Biol. Conserv. 183, 1–3. https://doi.org/10.1016/j.biocon.2014.11.040

Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., Spear, S.F., McKee, A., Oyler-McCance, S.J., Cornman, R.S., Laramie, M.B., Mahon, A.R., Lance, R.F., Pilliod, D.S., Strickler, K.M., Waits, L.P., Fremier, A.K., Takahara, T., Herder, J.E., Taberlet, P., 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol. Evol. 7, 1299–1307. https://doi.org/10.1111/2041-210X.12595

Gomez-Sanchez, D., and Schlötterer, C. (2018). ReadTools: A universal toolkit for handling sequence data from different sequencing platforms. Molecular Ecology Resources, *18, 676–680. https://doi.org/10.1111/1755-0998.12741*

Gorgone AM, Haase PA, Griffith ES, Hohn AA (2008) Modeling Response of Target and Nontarget Dolphins to Biopsy Darting. Journal of Wildlife Management 72:926-932. https://doi.org/10.2193/2007-202

Gruber, B., Unmack, P. J., Berry, O. F., and Georges, A. (2018). DARTR: An R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. Molecular Ecology Resources, *18, 691–699. https://doi.org/10.1111/1755-0998.12745*

Harlin, A. D., Würsig, B., Baker, C. S., and Markowitz, T. M. (1999). Skin swabbing for genetic analysis: application to dusky dolphins (*Lagenorhynchus obscurus*). Marine Mammal Science, 15(2), 409-425. https://doi.org/10.1111/j.1748-7692.1999.tb00810.x

Hermosilla, C., Hirzmann, J., Silva, L.M.R., Brotons, J.M., Cerdà, M., Prenger-Berninghoff, E., Ewers, C., Taubert, A., 2018. Occurrence of anthropozoonotic parasitic infections and faecal microbes in free-ranging sperm whales (*Physeter macrocephalus*) from the Mediterranean Sea. Parasitol. Res. 117, 2531–2541. https://doi.org/10.1007/s00436-018-5942-3

Hermosilla, C., Silva, L.M.R., Prieto, R., Kleinertz, S., Taubert, A., Silva, M.A., 2015. Endo- and ectoparasites of large whales (Cetartiodactyla: *Balaenopteridae, Physeteridae*): Overcoming difficulties in obtaining appropriate samples by non- and minimally-invasive methods. Int. J. Parasitol. Parasites Wildl. 4, 414–420. https://doi.org/10.1016/j.ijppaw.2015.11.002

Hoelzel, A. R., and Donovan, G. P. (1991). Report of the workshop on the genetic analysis of cetacean populations. Genetic Ecology of Whales and Dolphins. Edited by AR Hoelzel. Rep. Int. Whaling Comm. Spec. Issue, (13).

Hoffman JI, Amos W. (2005) Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Molecular Ecology 14, 599-612.
https://doi.org/10.1111/j.1365-294x.2004.02419.x

Hunter, ME, Hoban, SM, Bruford, MW, Segelbacher, G, Bernatchez, L.(2018) Next-generation conservation genetics and biodiversity monitoring. Evol Appl.; 11: 1029– 1034. https://doi.org/10.1111/eva.12661

Hunter, M., Meigs-Friend, G., Ferrante, J., Takoukam Kamla, A., Dorazio, R., Keith-Diagne, L., Luna, F., Lanyon, J., Reid, J., 2018. Surveys of environmental DNA (eDNA): a new approach to estimate occurrence in Vulnerable manatee populations. Endanger. Species Res. 35, 101–111.
https://doi.org/10.3354/esr00880

Jepson PD, Deaville R, Barber JL, Aguilar À, Borrell A, Murphy S, Barry J, Brownlow A, Barnett J, Berrow S, Cunningham AA, Davison NJ, ten Doeschate M, Esteban R, Ferreira M, Foote AD, Genov T, Giménez J, Loveridge J, Llavona Á, Martin V, Maxwell DL, Papachlimitzou A, Penrose R, Perkins MW, Smith B, de Stephanis R, Tregenza N, Verborgh P, Fernandez A, Law RJ (2016) PCB pollution continues to impact populations of orcas and other dolphins in European waters. Scientific Reports 6:18573.
https://doi.org/10.1038/srep18573

Juhel, J., Marques, V., Polanco Fernández, A., Borrero-Pérez, G.H., Mutis Martinezguerra, M., Valentini, A., Dejean, T., Manel, S., Loiseau, N., Velez, L., Hocdé, R., Letessier, T.B., Richards, E., Hadjadj, F., Bessudo, S., Ladino, F., Albouy, C., Mouillot, D., Pellissier, L., 2021. Detection of the elusive Dwarf sperm whale (Kogia sima) using environmental DNA at Malpelo island (Eastern Pacific, Colombia). Ecol. Evol. 11, 2956–2962. https://doi.org/10.1002/ece3.7057

Kiszka J, Oremus M, Richard P, Poole M, Ridoux V (2010a) The use of stable isotope analyses from skin biopsy samples to assess trophic relationships of sympatric delphinids off Moorea (French Polynesia). Journal of Experimental Marine Biology and Ecology 395:48-54.
https://doi.org/10.1016/j.jembe.2010.08.010

Kiszka J, Simon-Bouhet B, Charlier F, Pusineri C, Ridoux V (2010b) Individual and group behavioural reactions of small delphinids to remote biopsy sampling. Animal Welfare 19:411-417.
https://doi.org/10.1017/S0962728600001895

Kiszka JJ, Méndez-Fernandez P, Heithaus MR, Ridoux V (2014) The foraging ecology of coastal bottlenose dolphins based on stable isotope mixing models and behavioural sampling. Marine Biology 161:953-961. https://doi.org/10.1007/s00227-014-2395-9

Krupp, G. (2005). Stringent RNA quality control using the Agilent 2100 bioanalyzer. Agilent technologies application notes.

Krützen M, Barré LM, Möller LM, Heithaus MR, Simms C, Sherwin WB (2002) A biopsy system for small cetaceans: Darting success and wound healing in *Tursiops spp.* Marine Mammal Science 18:863-878. https://doi.org/10.1111/j.1748-7692.2002.tb01078.x

Kumar, G., Eble, J. E., and Gaither, M. R. (2020). A practical guide to sample preservation and pre-PCR processing of aquatic environmental DNA. Molecular Ecology Resources, 20(1), 29–39. https://doi.org/10.1111/1755-0998.13107

Liang, Z., Keeley, A., 2013. Filtration Recovery of Extracellular DNA from Environmental Water Samples. Environ. Sci. Technol. 47, 9324–9331. https://doi.org/10.1021/es401342b

Louis M, Viricel A, Lucas T, Peltier H, Alfonsi E, Berrow S, Brownlow A, Covelo P, Dabin W, Deaville R, De Stephanis R, Gally F, Gauffier P, Penrose R, Silva MA, Guinet C, Simon-Bouhet B (2014) Habitat-driven population structure of bottlenose dolphins, *Tursiops truncatus*, in the North-East Atlantic. Molecular Ecology 23:857-874. https://doi.org/10.1111/mec.12653

Ma, H., Stewart, K., Lougheed, S., Zheng, J., Wang, Y., Zhao, J. (2016) Characterization, optimization, and validation of environmental DNA (eDNA) markers to detect an endangered aquatic mammal. Conserv. Genet. Resour. 8, 561–568. https://doi.org/10.1007/s12686-016-0597-9

McDonough, M.M., Parker, L.D., Rotzel McInerney, N., Campana, M.G. and Maldonado, J.E., 2018. Performance of commonly requested destructive museum samples for mammalian genomic studies. Journal of Mammalogy, 99(4), pp.789-802. https://doi.org/10.1093/jmammal/gyy080

Metzker, M.L. and Caskey, C.T. (2009). Polymerase Chain Reaction (PCR). In eLS, John Wiley and Sons, Ltd (Ed.)

Miller, S., Dykes, D., and Polesky, H. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acids res, 16(3), 1215. https://doi.org/10.1093/nar/16.3.1215

Morey, J. S., Balmer, B. C., Zolman, E. S., Takeshita, R., De Guise, S., Rowles, T. K., Smith, C.R, Wells, R.S and Schwacke, L. H. (2022). Transcriptome profiling of blood from common bottlenose dolphins (*Tursiops truncatus*) in the northern Gulf of Mexico to enhance health assessment capabilities. Plos one, 17(8), e0272345. https://doi.org/10.1371/journal.pone.0272345

Morin P.A., LeDuc R.G., Archer F.I., Martien K.K., Huebinger R., Bickham J.W. and Taylor B.L. 2009. Significant deviations from Hardy-Weinberg equilibrium caused by low levels of microsatellite genotyping errors. Mol Ecol Res 9:498-504. https://doi.org/10.1111/j.1755-0998.2008.02502.x

Morin, P. A., Parsons, K. M., Archer, F. I., Avila-Arcos, M. C., Barrett-Lennard, L. G., Dalla Rosa, L., Duchêne, S., Durban, J. W., Ellis, G. M., Ferguson, S. H., Ford, J. K., Ford, M. J., Garilao, C., Gilbert, M. T. P., Kaschner, K., Matkin, C. O., Petersen, S. D., Robertson, K. M., Visser, I. N., Wade, P. R., Ho S. Y. W. and Foote, A. D. (2015). Geographic and temporal dynamics of a global radiation and diversification in the killer whale. Molecular Ecology, 24(15), 3964-3979. https://doi.org/10.1111/mec.13284

Mulholland, V., Elliot, M., and Green, S. Diagnostics of Tree Diseases Caused by Phytophthora Species. https://doi.org/10.1007/978-1-4939-2620-6_5

Nakahama, N., 2021. Museum specimens: An overlooked and valuable material for conservation genetics. Ecological Research, 36(1), pp.13-23. https://doi.org/10.1111/1440-1703.12181

Neveceralova, P., Carroll, E. L., Steel, D., Vermeulen, E., Elwen, S., Zidek, J., Stafford, J.K., Chivell, W., Hulva, P. (2022). Population changes in a whale breeding ground revealed by citizen science noninvasive genetics. Global Ecology and Conservation, 37, e02141. https://doi.org/10.1016/j.gecco.2022.e02141

Nonis, A., De Nardi, B. & Nonis, A. (2014) Choosing between RT-qPCR and RNA-seq: a back-of-the-envelope estimate towards the definition of the break-even-point. Anal Bioanal Chem 406, 3533–3536. https://doi.org/10.1007/s00216-014-7687-x

Noren DP and Mocklin JA (2012) Review of cetacean biopsy techniques: Factors contributing to successful sample collection and physiological and behavioral impacts. Marine Mammal Science 28:154-199. https://doi.org/10.1111/j.1748-7692.2011.00469.x

Nykänen M, Louis M, Dillane E, Alfonsi E, Berrow S, O'Brien J, Brownlow A, Covelo P, Dabin W, Deaville R, de Stephanis R, Gally F, Gauffier P, Ingram SN, Lucas T, Mirimin L, Penrose R, Rogan E, Silva MA, Simon-Bouhet B, Gaggiotti OE (2019) Fine- scale population structure and connectivity of bottlenose dolphins, *Tursiops truncatus*, in European waters and implications for conservation. Aquatic Conservation: Marine and Freshwater Ecosystems 29:197-211. https://doi.org/10.1002/aqc.3139

Parsons, K.M., Everett, M., Dahlheim, M., Park, L., (2018) Water, water everywhere: environmental DNA can unlock population structure in elusive marine species. R. Soc. Open Sci. 5, 180537. https://doi.org/10.1098/rsos.180537

Pichler, F. B., Dalebout, M. L., and Baker, C. S. (2001). Nondestructive DNA extraction from sperm whale teeth and scrimshaw. Molecular Ecology Notes, 1(1- 2), 106-109. https://doi.org/10.1046/j.1471-8278.2001.00027.x

Pinfield, R., Dillane, E., Runge, A.K.W., Evans, A., Mirimin, L., Niemann, J., Reed, T.E., Reid, D.G., Rogan, E., Samarra, F.I.P., Sigsgaard, E.E., Foote, A.D., 2019. False-negative detections from environmental DNA collected in the presence of large numbers of killer whales (*Orcinus orca*). Environ. DNA 1, 316–328. https://doi.org/10.1002/edn3.32

Ravindran, P. N., Bentzen, P., Bradbury, I. R., and Beiko, R. G. (2019). RADProc: A computationally efficient de novo locus assembler for population studies using RADseq data. Molecular Ecology Resources, 19, 272–282. https://doi.org/10.1111/1755-0998.12954

Rosel, P. E., Forgetta, V., and Dewar, K. (2005). Isolation and characterization of twelve polymorphic microsatellite markers in bottlenose dolphins (*Tursiops truncatus*). Molecular Ecology Notes, 5(4), 830-833. https://doi.org/10.1111/j.1471-8286.2005.01078.x

Sambrook, J., and Russell, D. W. (2006). Purification of nucleic acids by extraction with phenol: chloroform. Cold Spring Harbor Protocols, 2006(1), pdb-prot4455.

Shafer AB, Wolf JB, Alves PC, Bergström L, Bruford MW, Brännström I, Colling G, Dalén L, De Meester L, Ekblom R, Fawcett KD, Fior S, Hajibabaei M, Hill JA, Hoezel AR, Höglund J, Jensen EL, Krause J, Kristensen TN, Krützen M, McKay JK, Norman AJ, Ogden R, Österling EM, Ouborg NJ, Piccolo J, Popović D, Primmer CR, Reed FA, Roumet M, Salmona J, Schenekar T, Schwartz MK, Segelbacher G, Senn H, Thaulow J, Valtonen M, Veale A, Vergeer P, Vijay N, Vilà C, Weissensteiner M, Wennerström L, Wheat CW, Zieliński P. (2015a). Genomics and the challenging translation into conservation practice. Trends in Ecology and Evolution, 30(2), 78– 87. https://doi.org/10.1016/j.tree.2014.11.009

Smith LM, Burgoyne LA. Collecting, archiving and processing DNA from wildlife samples using FTA databasing paper. BMC Ecol. 2004 Apr 8;4:4.https://doi.org/10.1186/1472-6785-4-4

Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S.T., Sigsgaard, E.E., Hellström, M., 2017. Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. Methods Ecol. Evol. 8, 635–645. https://doi.org/10.1111/2041-210X.12683

Straube, N., Lyra, M.L., Paijmans, J.L., Preick, M., Basler, N., Penner, J., Rödel, M.O., Westbury, M.V., Haddad, C.F., Barlow, A. and Hofreiter, M., 2021. Successful application of ancient DNA extraction and library construction protocols to museum wet collection specimens. Molecular Ecology Resources, 21(7), pp.2299-2315. https://doi.org/10.1111/1755-0998.13433

Strauss, W. M. (1998). Preparation of genomic DNA from mammalian tissue. *Current protocols in molecular biology,* 42(1), 2-2. https://doi.org/10.1002/0471142727.mb0202s42

Supple, MA, and B, Shapiro (2018) Conservation of biodiversity in the genomics era. Genome Biol. 11;19(1). https://doi.org/10.1186/s13059-018-1520-3

Székely, D., Corfixen, N.L., Mørch, L.L., Knudsen, S.W., McCarthy, M.L., Teilmann, J., Heide-Jørgensen, M.P., Olsen, M.T., 2021. Environmental DNA captures the genetic diversity of bowhead whales (*Balaena mysticetus*) in West Greenland. Environ. DNA 3, 248–260. https://doi.org/10.1002/edn3.176

Tiedemann, R., Cipriano, F., Morin, P. a, Hoelzel, R. a, Palsbøll, P., Waples, R. S., Natoli, A., Bachmann, L., Postma, L., Double, M., Pampoulie, C., Skaug, H., and Baker, C. S. (2012). Updated guidelines for DNA data quality control and error rate estimation, for genetic studies relevant to IWC management advice. IWC-SC60, SC/64/SD2. Available at Link.

Turner, C.R., Miller, D.J., Coyne, K.J., Corush, J., 2014. Improved Methods for Capture, Extraction, and Quantitative Assay of Environmental DNA from Asian Bigheaded Carp (*Hypophthalmichthys spp.*). PLoS One 9, e114329. https://doi.org/10.1371/journal.pone.0114329

Valsecchi, E., Arcangeli, A., Lombardi, R., Boyse, E., Carr, I.M., Galli, P., Goodman, S.J., 2021. Ferries and environmental DNA: Underway sampling from commercial vessels provides new opportunities for systematic genetic surveys of marine biodiversity. Front. Mar. Sci. 8, 1136. https://doi.org/10.3389/fmars.2021.704786

Valsecchi, E., Bylemans, J., Goodman, S.J., Lombardi, R., Carr, I., Castellano, L., Galimberti, A., Galli, P., 2020. Novel universal primers for metabarcoding environmental DNA surveys of marine mammals and other marine vertebrates. Environ. DNA 2, 460–476. https://doi.org/10.1002/edn3.72

Valsecchi, E, Glockner-Ferrari, D. , Ferrari, M., and Amos, W. (1998). Molecular analysis of the efficiency of sloughed skin sampling in whale population genetics. Molecular Ecology, 7(10), 1419-1422. https://doi.org/10.1046/j.1365-294x.1998.00446.x

Van Cise, A., Switzer, A., Apprill, A., Champagne, C., Chittaro, P., Dudek, N., Gavery, M., Hancock-Hanser, B., Harmon, A., Kellar, N., Miller, C., Morin, P., Nelms, S., Robertson, K., Schultz, I., Timmins-Schiffman, E., Unal, E., and Parsons, K. (2022/under review). Best Practices for Collecting and Preserving Marine Mammal Biological Samples in the 'Omics Era'. SC/68D/SD and DNA/03. Available at Link.

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10, 249-256. https://doi.org/10.1046/j.1365-294X.2001.01185.x

Waples, R. S., Rus Hoelzel, A., Gaggiotti, O., Tiedemann, R., Palsbøll, P. J., Cipriano, F., Jackson, J., Bickham, J. W., and Lang, A. R. (2018). Guidelines for genetic data analysis. Journal of Cetacean Research and Management, 18, 33–80. Available at Link.

Weller DW, Cockcroft VG, Würsig B, Lynn SK, Fertl D (1997) Behavioral responses of bottlenose dolphins to remote biopsy sampling and observations of surgical biopsy wound healing. Aquatic Mammals 23:49-58. http://digitalcommons.unl.edu/usdeptcommercepub/132

Whitehead, H., Gordon, J., Mathews, E. A., and Richard, K. R. (1990). Obtaining skin samples from living sperm whales. Marine Mammal Science, 6(4), 316-326. https://doi.org/10.1111/j.1748-7692.1990.tb00361.x

Willi Y, Kristensen TN, Sgrò CM, Weeks AR, Ørsted M, Hoffmann AA. (2022) Conservation genetics as a management tool: The five best-supported paradigms to assist the management of threatened species. Proc Natl Acad Sci USA. 4;119(1):e2105076119. https://doi.org/10.1073/pnas.2105076119

Ylitalo GM, Matkin CO, Buzitis J, Krahn MM, Jones LL, Rowles T, Stein JE (2001) Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, AK. Science of the Total Environment 281:183-203. https://doi.org/10.1016/S0048-9697(01)00846-4

Appendix 1

Existing knowledge on population genetics of cetaceans in the ACCOBAMS area (September 2022)

The information presented below is based on scientific literature available in September 2022. This review covers the ACCOBAMS area and the proposed extension to the Exclusive Economic Zones of mainland Spain and Portugal (Res. A/4.[29]), hereafter *ACCOBAMS extension area.*

1) Summary for each species

Minke whale – *Balaenoptera acutorostrata*

The minke whale is a cosmopolitan species, widely distributed across the northern hemisphere and occasionally observed throughout the Mediterranean Sea (more frequently in its western part), with a single historical record in the Black Sea. Calves are consistently recorded among the stranded animals, and a calving ground in the area can be suggested. At least two specimens were reported to be genetically analysed: the control region of the mtDNA (343 and 500 bp) was used (Pastene *et al*. 2007, Maio *et al*. 2016). The haplotype of the first sample collected in the Mediterranean Sea was identical to the most common haplotype in the North Atlantic (Pastene *et al*. 2007), and the haplotype of another specimen also was identical to a North Atlantic haplotype (Maio *et al*. 2016).

➔ Not a priority species - mostly a vagrant species in ACCOBAMS area

Fin whale - *Balaenoptera physalus*

The fin whale is present in the North Atlantic, in the central and western Mediterranean and is rare in the southern and eastern parts of the Sea (Cooke 2018). Its occurrence is variable depending on the season and the area (Notarbartolo-di-Sciara *et al*. 2003). Most studies are focused on the genetic differentiation between the Mediterranean Sea and North Atlantic populations. Most studies employed mitochondrial control region DNA sequences (200-400bp) and microsatellite *loci* (6-29 *loci*) to assess population genetic structure, with samples from both free-ranging and stranded animals. Sample sizes ranged between 10 and 500, mostly from the western Mediterranean Sea. Early studies suggested that the small Mediterranean fin whale population (<1,700 individuals; Tardy *et al*. submitted, ACCOBAMS 2021, Notarbartolo-di-Sciara *et al*. 2003) was likely resident and genetically distinct from Atlantic individuals through mitochondrial and nuclear DNA analyses (Bérubé *et al*. 1998, Notarbartolo-di-Sciara *et al*. 2003). A limited gene flow and movement of some individuals were identified between the Mediterranean Sea and the Atlantic (Palsbøll *et al*. 2004, Bentaleb *et al*. 2011, Gauffier *et al*. 2018). In the Mediterranean population, the overall nucleotide diversity is two times lower than those reported for fin whale populations in other oceans. The presence of two private haplotypes in the Mediterranean Sea supports the genetic isolation hypothesis (Tardy 2021). Demographic histories suggested that North Atlantic fin whales underwent a post-glacial population expansion whereas the Mediterranean Sea fin whale population declined during this period (Schleimer 2021, Tardy 2021). Despite the small size of the Mediterranean population and its particular habitat, the population does not suffer from inbreeding depression (Tardy 2021). Inside the Mediterranean Sea, the population does not demonstrate a regional structure (Tardy 2021). Furthermore, the range of Mediterranean Sea fin whales includes the Strait of Gibraltar (Schleimer 2021).

[29] https://www.accobams.org/wp-content/uploads/2016/06/ACCOBAMS_MOP4_Res.A-4.1.pdf

Genetic results highlight that all individuals contribute more or less equally in maintaining the genetic diversity of the Mediterranean fin whale population (Tardy 2021), which is congruent with the solitary behaviour of the fin whale (Notarbartolo-di-Sciara *et al*. 2003).

In the Mediterranean fin whale population, fullsiblings were identified. Further research is needed to better understand the reproductive strategy of this population, and to quantify the gene flow between Atlantic and Mediterranean populations.

- Quantify gene flow between Atlantic and Mediterranean populations
- Delimit the range of the Mediterranean population
- Integrate genetic and non-genetic data
- Increase sampling effort in winter and central and eastern Mediterranean Sea and adjacent North Atlantic
- Increase sample sizes by integrating data from bone/baleen
- *Also see CMP on Mediterranean fin whales*[30]

Common dolphin - *Delphinus delphis*

The common dolphin has a wide distribution that includes a series of geographically separate subpopulations, with evidence of some population structure across its range (Jefferson and Van Waerebeek 2002, Amaral *et al*. 2007, Mirimin *et al*. 2009, Stockin *et al*. 2014) probably driven by prey distribution and habitat preferences (Amaral *et al*. 2012). Few studies had been done about the population structure of common dolphins in the Mediterranean (Amaral *et al*. 2007, Natoli *et al*. 2008, Moura *et al*. 2013). Most studies employed mtDNA control region and cytochrome b sequences (400-1121bp) and microsatellite *loci* (9-15 *loci*) to assess population genetic structure, with samples (skin and teeth) from free-ranging, bycaught and stranded animals. Sample sizes ranged between 10 and 500, mostly from the ACCOBAMS extension area, some from the Strait of Gibraltar and from the western Mediterranean Sea. In the Mediterranean Sea, the Almería-Orán thermohaline front has been identified as an environmental boundary that drives genetic differentiation between the Mediterranean population of common dolphins occurring east of the front, and a north-eastern Atlantic population that also utilises the Mediterranean waters of the Alboran Sea (Natoli *et al*. 2008, Moura *et al*. 2013). Common Dolphins occurring east of the Almería-Orán front differ genetically from those occurring west of the front (Natoli *et al*. 2008, Moura *et al*. 2013). More studies are needed using more samples and markers to have a fine detail of the population substructure of this species in the region.

- Increase markers to refine population genetic structuring, identify local/regional populations
- Assess how decline is affecting genetic diversity
- *Also see CMP on Mediterranean common dolphins*

Long-finned pilot whale - *Globicephala melas*

There is little literature on long-finned pilot whale genetics encompassing their whole distribution in the ACCOBAMS area. One study used mitochondrial control region DNA sequences (800bp) and 11 microsatellite *loci* on samples from free-ranging and stranded animals from the western Mediterranean Sea (80), the Strait of Gibraltar (90), the extension area (50) (Verborgh *et al*. 2016, in prep.). It found strong genetic differentiation between these areas with both markers. Results also suggested potentially substructure within the western Mediterranean Sea, between the Alboran Sea and the Ligurian Sea/Gulf of Lion, however sample size was low in the latter (15). Genetic diversity was lower in the Mediterranean and Strait of Gibraltar samples, and the latter showed higher levels

[30] Once adopted, all CMPs will be available at https://accobams.org/species_/conservation-plans/

of inbreeding. Another study compared stranded individuals from the extension area (Portugal mainland and north of Spain) with the rest of the North Atlantic using mitochondrial DNA (400 bp) (Monteiro *et al*. 2015). The authors found high and significant levels of differentiation between the extension area and the rest of northeastern Atlantic. Haplotype diversity is very low in this species, as only 7 haplotypes have been described in the whole North Atlantic Ocean and 15 worldwide (Kraft et 2020), including one private to the Mediterranean Sea and Gibraltar, three from the extension area and one shared (Verborgh *et al*. 2016, in prep., Monteiro *et al*. 2015). Although the genetic analyses do not reach the subspecies threshold, the divergence between the populations from the two hemispheres suggest they should be considered Demographically independent populations (Kraft et al. 2020). Another study used the same samples as Monteiro *et al*. (2015) but looked at adaptive genetic diversity and selective processes with two Major Histocompatibility Complex adaptive *loci* (MHC DRA and DQB) (Monteiro *et al*. 2016). Overall nucleotide diversities were relatively low for MHC *loci* in the North Atlantic, but comparable to other populations. There were significant differences in allele frequencies within the North Atlantic. Patterns of diversity and divergence were consistent with the long-term effects of balancing selection operating on the MHC *loci*, potentially mediated through the effects of host-parasite coevolution. Future research should include new samples from the northwestern Mediterranean Sea to confirm possible substructure within the Mediterranean Sea. Due to the overall low diversity, it might also require increasing the number of microsatellites or using NGS techniques.

- Evaluate substructure within the Mediterranean Sea
- Include samples from northwestern Mediterranean Sea
- Assess how abundance decline is affecting genetic diversity

Risso's dolphin - *Grampus griseus*

Available data on the genetics and population structure of Risso's dolphin in the ACCOBAMS area are limited and mostly focus on nuclear and mitochondrial DNA data. Accordingly, Mediterranean Risso's dolphin is a well-differentiated population from those in UK waters based on eight selected microsatellite *loci* ($F_{ST} = 0.0296$ $P<0.05$) and mtDNA control region ($F_{ST} = 0.260$ $P<0.001$) (Gaspari, 2004, Gaspari *et al*. 2007). Among the analyses performed, the microsatellite diversity was measured by the level of polymorphisms, testing for linkage disequilibrium and deviations from expected HW genotypic frequencies. In addition, 619bp from the mtDNA control region were analysed under a population genetic landscape by measuring nucleotide diversity, testing for neutrality and performing phylogenetic analyses.

Free ranging samples (n= 24) from Ligurian Sea were used for kinship analysis, suggesting a kin structure with a trend from female philopatry and male dispersal (Gaspari, 2004). Nucleotide diversity within the mitochondrial 16S rDNA has shown that this could be considered a potential molecular marker suitable for studying individual genetic structuring and differentiation among Risso's dolphin populations (Sönmez *et al*. 2012). Further sampling throughout the distribution area of the species in the Mediterranean Sea and additional research is needed to clarify internal population structuring.

- Samples from throughout species range to gain a more in-depth understanding on substructuring
- Integrate existing samples
- *Also see CMP on Risso's dolphins*

Killer whale – *Orcinus orca*

Genetic research on killer whales in the ACCOBAMS area has been done on the subpopulation inhabiting waters in and around the Strait of Gibraltar (SoG) with the aim of assessing genetic differentiation between the SoG and North Atlantic (Foote *et al*. 2011, Esteban *et al*. 2016), or on a more global scale (Foote *et al*. 2019). Former studies employed mtDNA control region (989-bp), complete (~16,390-bp) and partial mitogenomes (12 814–14 689 bp), and up to 19 microsatellite markers to assess population genetic structure, with samples obtained mostly from free-ranging animals, but also a stranded individual (Foote *et al*. 2011, Esteban *et al*. 2016). Existing literature shows pod-specific haplotypes which are in line with the matrifocal social structure that is otherwise observed in killer whales (Esteban *et al*. 2016), low gene flow and no close kinship between the SoG and Canary Islands (CI) subpopulations (Esteban *et al*. 2016). On a broader scale (NE Atlantic), genetic and ecological differentiation has been observed between the "population C" (which includes both SoG and CI killer whales) and higher latitude populations, A and B (Foote *et al*. 2011). Foote *et al*. (2019) also provided a reference SoG killer whale genome, which they included in their global data set of genomes with the aim of examining worldwide population structure of killer whales. Overall, it has been shown that SoG killer whales represent a genetically and ecologically distinct subpopulation that should be treated as a separate management unit in order to preserve genetic, cultural and ecological diversity within this subpopulation of killer whales (Esteban *et al*. 2016).

- Evaluate inbreeding
- Assess effect of stress/pollution on gene expression (transcriptomics) *e.g.* in comparison with other killer whale populations
- Delimit population ranging patterns (space and time)

Harbour porpoise - *Phocoena phocoena*

Harbour porpoises from the ACCOBAMS area include on the east side the isolated population(s) from the Balck Sea and adjacent waters also recognized as a distinct subspecies (P. p. relicta). On the west side, porpoises are mostly absent from the Mediterranean Sea, although vagrant individuals likely originating from the Atlantic coasts of Iberia and NW African were reported venturing along the Mediterranean coasts of Spain. Porpoises from the Iberia and Mauritania reach larger sizes than those from further north within Europe or in the Black Sea (*e.g.* Smeenk *et al*. 1992, Donovan and Bjørge 1995, Sequeira 1996, López Fernández 2003, López-Fernández and Martínez-Cedeira 2011). As genetic evidence has been amassed using microsatellite and mitochondrial data, it has become clear that Iberian porpoises form a morphologically and genetically distinct, largely isolated, population (Fontaine *et al*. 2007, 2010, 2014) closely related to the population in Northwest Africa (Fontaine et al. 2014). Fontaine *et al*. (2014; see also the review by Fontaine 2016) proposed that the Iberian and NW African porpoises together represent a distinct ecotype adapted to upwelling systems.Considering their phylogenetic divergence from the subspecies described in the North Atlantic (P. p. phocoena) and in the Black Sea (P. p. relicta), their allopatric distribution, and their morphological and ecological distinctiveness, it was proposed to raise this distinct ecotype as a separate subspecies with the name P. p. meridionalis (Fontaine *et al*. 2014, Fontaine 2016, Ben Chehida *et al*. 2021a,b). Genetic diversity at nuclear microsatellite and mtDNA markers of the Iberian population was lower than in all other documented harbour porpoise populations except that of the Black Sea. Phylogeographic analyses and paleo-habitat modellling suggested that the upwelling ecotype of harbour porpoise present in the Iberian Peninsula and Mauritania descended from a now extinct paleo-population living in the Western Mediterranean Sea during the last glacial maximum (~20,000 years before present (Fontaine *et al*. 2014, Ben Chehida *et al*. 2021a,b). Porpoises likely entered the Mediterranean Sea from populations in the Northeast Atlantic and split from them within the past ~30,000 years.

Porpoises subsequently disappeared from the Mediterranean during the postglacial warming period, but these lineages gave rise to the Black Sea and "upwelling" groups, around 15,000 years ago, with the latter giving rise to the Iberian and NWt African groups. Sequencing one quarter of the mitogenome for individuals collected over a 30-years time period (1990-2020) revealed a dramatic decline in diversity, but also identified haplotypes that were distinct from the typical Iberian mitotypes. These were more closely related to the Mauritanian clade, but still distinct from it (Ben Chehida *et al*. 2021b). This suggests that cryptic genetically distinct population(s) might exist between the Iberian Mauritanian populations. Further studies improving on the sampling and the amount of genetic markers (whole genomes and also amplicon sequencing, Morin *et al* 2021) are required to shed light in these enigmatic groups existing close to the Gibraltar Strait, but also to assess the extent of local adaptation in these populations, as well as their demographic trends.

- Increase sampling along the NW African coast and Southern Iberian coast to extend the geographic coverage, but also include time series to assess the population trends.
- Develop genomic surveys to screen cryptic genetic structure, assess the extent of gene flow and local adaptation in harbour porpoise populations, assess demographic trends.
- Whole genome sequencing analyses of modern and ancient samples.

Sperm whale - *Physeter macrocephalus*

Existing literature on sperm whale genetics in the ACCOBAMS area has mostly focused on the genetic differentiation between the Mediterranean Sea and North Atlantic populations. Most studies employed mitochondrial control region DNA sequences (200-400bp) and microsatellite *loci* (3-16 *loci*) to assess population genetic structure, with samples from both free-ranging and stranded animals (Drouot *et al*. 2004, Engelhaupt *et al*. 2009). Sample sizes ranged between 4 and 116, mostly from the western Mediterranean Sea. The sex ratio of females to males was 0.5:1 which is significantly different from an expected ratio of 1:1 (Engelhaupt *et al*. 2009). Worldwide, mitochondrial DNA diversity is very low in sperm whales, compared to other cetaceans, consistent with a recent population expansion (Alexander *et al*. 2016, Morin *et al*. 2018). Within the Mediterranean Sea, all sampled individuals carried the same mitochondrial haplotype, precluding further analysis on mitochondrial diversity (Drouot *et al*. 2004, Engelhaupt *et al*. 2009, Alexander *et al*. 2016). Morin *et al*. (2018) reported two mitogenome haplotypes for their four Mediterranean samples. Overall, levels of nuclear differentiation are lower than mitochondrial differentiation, which was hypothesised to result from female philopatry and male-biased dispersal. Violi *et al*. (under review) employed 5000-10,000 SNP *loci* from RAD sequencing to assess the population genetic structure within the Mediterranean Sea (west vs east). Their results suggest significant differentiation between eastern and western Mediterranean sperm whales. The dispersal between Mediterranean and North Atlantic sperm whales has not been quantified and the demographic independence of the Mediterranean population has not been confirmed. Given the influence of social groups on genetic differentiation in other oceans, future studies should also assess whether fine-scale genetic structuring exists within the Mediterranean Sea.

- Increase sample coverage in eastern/southern areas and North Atlantic adjacent areas
- Delimit the range of the Mediterranean population
- Quantify gene flow between North Atlantic and Mediterranean Sea populations
- Focus on local substructuring (*e.g.* in relation to social groups/ vocal clans)

Striped dolphin - *Stenella coeruleoalba*

The majority of the studies focus on Mediterranean samples (mostly from Western part of the basin), compared to North Atlantic and Pacific samples. The research mainly focuses on: (1) genetic diversity and population structure of striped dolphins in the Mediterranean Sea (Bourret *et al*. 2007, Gaspari *et al*. 2007); (2) Social kin associations (Gaspari *et al*. 2007); (3) Spatio-temporal patterns of genetic diversity in the Mediterranean (also related to epizootic outbreaks) (Gaspari *et al*. 2019); (4) Biogeography and temporal evolution of striped dolphin population (Med vs Atlantic) (Gkafas *et al*. 2017). Most of the studies used microsatellites (from 5 to 15 *loci*) and mtDNA control region. Recent studies suggest the existence of a separate subpopulation in the Gulf of Taranto (Italy, Ionian Sea, Ciccarese *et al*. 2019).

Gaps remain mainly to study the eastern Mediterranean population, to focus the studies on the possible implications of epizootics outbreaks and other potential stressors in genetic diversity and population resilience. Genomic studies are lacking so far.

- Increase sampling effort in eastern Mediterranean Sea
- Use genomic markers to resolve fine-scale structuring
- Focus on the possible implications of epizootics outbreaks and other potential stressors on genetic diversity and population resilience (transcriptomics)

Rough-toothed dolphin – *Steno bredanensis*

The information on the rough-toothed dolphin in the Mediterranean is very limited, particularly so with respect to genetic origin and population structure. Based on limited sample size (3 from Israel and one from Aegean Sea, Greece) and analysis of mtDNA sequences, Mediterranean samples appear to cluster strongly with Atlantic ones. Main data gaps relate to general information on the distribution and abundance of the species, and the lack of samples across the ACCOBAMS area.

- More samples, increase coverage
- Confirm origin of the population
- Generate reference information for non-invasive sampling

Bottlenose dolphin - *Tursiops truncatus*

Bottlenose dolphins in the Northeast Atlantic and Mediterranean Sea inhabit a wide range of habitats throughout their range. Several genetic studies identified in these areas a clear population structuring based on mitochondrial (control region), nuclear microsatellites (9-25 *loci*) and Single Nucleotide Polymorphisms (~26,000 SNPs) with varying geographical scales (*e.g.* Natoli *et al*. 2005, Louis *et al*. 2014a, Nykänen *et al*. 2019, Moura *et al*. 2020). Populations typically segregate between lineages inhabiting pelagic and coastal environments (Louis *et al*. 2014b, Gaspari *et al*. 2015, Nykänen *et al*. 2019), the divergence estimated between these two ecotypes likely occurred between the Last Glacial Maxima and the post-glacial period (~10,320 yr BP; Louis *et al*. 2014b). Pelagic dolphins from the Atlantic and Mediterranean Sea likely diverged during a period of high productivity in the Mediterranean Sea (Louis *et al*. 2014a). On coastal environments, bottlenose dolphin populations commonly consist of distinct social communities that display fine-scale behavioural differentiation, resulting from localised adaptations on small spatial scales resulting in fine scale genetic structuring (Natoli *et al*. 2005, Fernández *et al*. 2011, Louis *et al*. 2014a, Nykänen *et al*. 2019). Its population structure appears to correlate strongly with environmental differences (Natoli *et al*. 2005, Louis *et al*. 2014a, b). Data shows evidence of fine scale population structure within the Mediterranean basin, with a clear population division within the Adriatic and the Levantine Seas (Gaspari *et al*. 2013, Gaspari *et al*. 2015).

- Include samples from Iberian area and winter sampling
- Integrate genetic and non-genetic data
- *Also see CMP on bottlenose dolphins*

Cuvier's beaked whale - *Ziphius cavirostris*

Cuvier's beaked whales are deep diving pelagic cetaceans. They are encountered throughout the Mediterranean Sea, and are confined to deeper regions of high slope. Despite Cuvier's beaked whales life history parameters being still poorly known, information on diving behaviour, habitat preferences and distribution in the Mediterranean Sea are available. Much of the knowledge has come from stranding data and Ziphius initiative undertaken under the ACCOBAMS. The species is listed as vulnerable in the Mediterranean and contains fewer than 10,000 mature individuals. Very few genetic analyses are available, and mtDNA (300 bp) analyses indicated a high degree of differentiation from the Atlantic population and low maternal gene flow among ocean basins. It was suggested that Cuvier's beaked whales in the Mediterranean Sea should be considered as a separate Evolutionarily Significant Unit, distinct from the other populations worldwide. The sample size in the mediterranean was very low (n=12 Greece + 05 from levantine and Aegean Seas) and individuals were characterised by only two private mtDNA haplotypes T3 and T4 (Dalebout *et al*. 2005, Tonay et al 2019). There have been 33 Ziphius haplotypes globally identified; New markers such as ddRAD are being tested to assess the population structure for this species (Carroll *et al*. 2016). A new study including samples using nuclear ddRAD SNPs (n=33) and full mitogenomes (n=3) found that Mediterranean Sea samples have the lowest levels of diversity, indicate population contraction and diverged from the North Atlantic approximately 0.5 mya (Onoufriou *et al*. 2022). The authors also identified substructure between the eastern (east of Sicily) and western (Ligurian Sea) basins that they consider 2 ESUs (Onoufriou *et al*. 2022).

- Increase sample size and coverage to further understand population structuring within the Mediterranean Sea.

Black Sea harbour porpoise – *Phocoena phocoena relicta*

Black Sea harbour porpoises are frequently seen in the Azov and Black Seas and the Turkish Straits System (TSS, which includes Marmara Sea, Istanbul and Çanakkale Straits) and are rarely observed in the Aegean Sea. The Black Sea harbour porpoise is differentiated morphologically and genetically from the Atlantic ones. It was estimated that Black Sea and North Atlantic harbour porpoises have diverged within the last 7000 years ago and followed independent evolutionary paths (Fontaine *et al*. 2010, 2014). The divergence between the western and eastern populations in the Mediterranean Sea likely occurred around ca. 14 kyr BP (Fontaine *et al*. 2014). Most studies were carried out using mitochondrial control region DNA sequences (344-5085bp) and microsatellite *loci* (10-13 *loci*) to assess population genetic structure, with samples from both stranded and bycaught animals (Rosel et al. 1995, 2003, Viaud-Martinez *et al*. 2007, Fontaine *et al*. 2007, 2010, 2012, 2014, Tonay *et al*. 2012, 2017, Llavona *et al*. 2014, Lah *et al*. 2016, Uzun *et al*. 2017, 2018, Ben Chehida *et al*. 2020). In addition, double digest RAD-sequencing methods were used to analyse the nuclear DNA (2872-4924 SNPs) of Black Sea harbour porpoises, with the sample sizes ranging between 3 and 102 (Lah *et al*. 2016, Uzun *et al*. 2018). Black Sea is the source for the Aegean Sea porpoises (Rosel *et al*. 2003, Viaud-Martinez et al. 2007, Fontaine *et al*. 2012, Tonay *et al*. 2017). Despite morphological heterogeneity, the genetic homogeneity found in the Black Sea and adjacent waters, supporting a single population (Ben Chehida *et al*. 2020). However the possibility of locallyan isolated harbour porpoise populations in the TSS or in the Azov Sea has also been suggested (Tonay *et al*. 2017, Uzun *et al*. 2017, 2018) and could be associated with selective processes involved in local adaptation (Ben Chehida *et al*. 2020). Fontaine *et al*. (2012) revealed a strong population reduction (~90%) that occurred within the past 50 decades, due to massive killing and bycatch of the species. In addition to these, there is a different study on performance of several biomolecular methods for species identification in 800 to 1600 years old odontocete bone samples (Biard *et al*. 2017).

- Increasing genomic coverage because genetic diversity is low.
- Selection pressures/adaptation.
- Whole genome sequencing analyses of modern and ancient samples.
- Time series genetic analyses to investigate demographic and selective changes.
- Impact of the Ukrainian conflict on the harbour porpoise population in the Black Sea.

Black Sea common dolphin – *Delphinus delphis ponticus*

Black Sea common dolphins are frequently seen in the Black Sea and the Turkish Straits System (TSS, which includes Marmara Sea, Istanbul and Çanakkale Straits). Several studies of the Black Sea common dolphin in the ACCOBAMS region are currently available, focusing on genetic differences between Atlantic, Mediterranean and Black Sea populations. The studies employed mitochondrial control region DNA sequences (404-428bp), cytochrome b (360bp) and microsatellite *loci* (9 *loci*) to assess population genetic structure, with samples from stranded animals (Rosel *et al*. 1994, Natoli *et al*. 2008, Tonay *et al*. 2020). Sample sizes ranged between 4 and 37. Rosel *et al*. (1994) and Natoli *et al*. (2008) suggested that differences do exist between Black Sea and Mediterranean common dolphins, although differentiation was not significant due to small sample size. However, such differentiation was not observed by mitochondrial DNA analyses comparing samples from Mediterranean Sea, TSS and the Black Sea (Tonay *et al*. 2020). In comparison to the Atlantic Ocean, the haplotype and nucleotide diversity values were lower in the Black Sea, TSS, and western Mediterranean Sea, suggesting the migration of Atlantic populations into these two seas (Tonay *et al*. 2020). The protection of open seas and narrow straits to improve connectivity may be crucial for common dolphins, which have high dispersal potential (Tonay *et al*. 2020). It will be necessary to carry out genetic research on nuclear and mitochondrial DNA with a greater number of samples to better understand the phylogeny and genetic connectivity between subpopulations of the species.

- Increase sample size to re-assess differentiation between Black Sea and Mediterranean Sea
- Integrate existing samples (*e.g.* from museums) to increase sample size
- Add genomic analyses/coverage, including a reference genome
- *Also see CMP on common dolphins*

Black Sea bottlenose dolphin – *Tursiops truncatus ponticus*

Black Sea bottlenose dolphins inhabit most of the Black Sea and the Turkish Straits System (TSS, which includes Marmara Sea, Istanbul and Çanakkale Straits) and are seasonally observed in the Azov Sea. The Black Sea bottlenose dolphins are morphologically differentiated from Mediterranean ones. They belong to at least two different genetic lineages originating in the Mediterranean Sea (Natoli *et al*. 2005, Viaud-Martinez *et al*. 2008, Moura *et al*. 2013) and these groups split from the Mediterranean clades ca. 10 000 years ago possibly showing two colonisation events and a founder effect (Moura *et al*. 2013). Moreover, presence of these two lineages was shown in ancient bone samples 800 to 1600 years old (Biard *et al*. 2017). Several studies were carried out using mitochondrial control region DNA sequences (404-630 bp), complete mitogenomes and microsatellite *loci* (9 *loci*) to assess population genetic structure, with samples from stranded and captive animals (Rosel *et al*. 1994, Natoli *et al*. 2005, Viaud-Martinez *et al*. 2008), only stranded animals (Tonay *et al*. 2018) and on aDNA (Biard *et al*. 2017). Low genetic diversity is clear and intra Black Sea structure can be suggested (Moura *et al*. 2013, Tonay *et al*. 2018), as well as female dispersal and gene flow from the marginal habitat (Natoli *et al*. 2005, Moura *et al*. 2013). In overall, differentiation of Black Sea bottlenose dolphin population from the adjacent populations and low levels of genetic diversity indicates a conservation concern (Tonay *et al*. 2018).

- Increase sampling throughout Black Sea basin, with focus on local populations.
- Genomics to assess demographic history, origin, relationship with Mediterranean populations.
- Delimit ranging patterns in relation to adjacent areas, gene flow among populations.
- Identify origin of captive bottlenose dolphins, assignment to wild populations and develop marker guidelines suitable for individual identification.
- *Also see CMP on bottlenose dolphins.*

02) Summary table of scientific literature

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Balaenoptera physalus	Bérubé *et al.* 1998	Mediterranean Sea, North Atlantic, Sea of Cortez	Population genetic structure	Tissue from stranded and biopsy animals	74	mtDNA-CR 288 bp 6 microsatellite *loci*	Existence of several recently diverged populations in the NA and Med. Some limited gene flow.
	Palsbøll *et al.* 2004	Mediterranean Sea, North Atlantic	Discerning between recurrent gene flow and recent divergence	Reusing samples from Bérubé *et al.* 1998	72	mtDNA-CR	Favouring a model of recurrent gene flow. Migration rate at 2 females/generation.
	Tardy *et al.* 2020	Mediterranean Sea	Characterised new microsatellite markers	Tissue from stranded and biopsy animals	50	39 microsatellites	25 new microsatellites. Successful cross-amplification.
	Schleimer *et al.* 2021	Mediterranean Sea, North Atlantic	Population genetic structure	Tissue from stranded and biopsy animals	Med: 154 Gib: 53	mtDNA-CR 450 bp 20 microsatellite *loci*	Contemporary connectivity between Med and NA. The range of Med Sea fin whales includes the Strait of Gibraltar. NA fin whales underwent a post-glacial population expansion whereas the Med Sea fin whale population declined during this period.
	Tardy 2021	Mediterranean Sea	Population genetic structure	Tissue from stranded and biopsy animals	495	29 microsatellites mtDNA-CR 465 bp	Population size at 1,300 individuals. Effective population size: 400-500 individuals. Population composed of numerous families. No inbreeding depression.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Balaenoptera acutorostrata	Maio *et al.* 2016	Mediterranean Sea	Identify origin of the stranded specimen	Stranding	1	mtDNA control region (343 bp)	The haplotype was identical to a haplotype from the North Atlantic
	Pastene *et al.* 2007	Mediterranean Sea	Genetic population structure	Stranding	1	mtDNA control region (500 bp)	The haplotype of the single sample collected in the Mediterranean Sea was identical to the most common haplotype in the North Atlantic.
Delphinus delphis	Amaral *et al.* 2007	NE Atlantic	Population structure in NE Atlantic; phylogenetic relationship within the genus *Delphinus*	Tissue and tooth samples from stranded animals	55 ACCOBAMS extension area + 13 NE Atlantic	mtDNA (630 bp control region, 1121 bp cyt b)	Evidence of sex-biased population structure in NE Atlantic. Some highly divergent groups in the Iberian Peninsula.
	Natoli *et al.* 2008	Mediterranean sea and ENE Atlantic.	Population structure of Mediterranean population and gene flow with Atlantic population.	Tissue from stranded and biopsy animals	53 MED + 5 Black sea + 47 ACCOBAMS extension area	9 microsatelites + mtDNA control region (428 bp)	Small population differentiation between E and W MED. Directional gene flow suggests movements of females out of MED. Possible isolation of Black sea population from MED population (small sample size).
	Moura *et al.* 2013	European waters and Mediterranean	Population structure of EU common dolphins	Tissue from stranded, bycaught and biopsy animals	515 samples (253 from ACCOBAMS extension area, 17 Gibraltar; 26 MED)	15 microsatelites	Panmixia across most of the range. Eastern Mediterranean (Greek waters) is differentiated from the rest.
	Ball *et al.* 2017	Portugal	Kinship structure	Biopsy samples	204 Portugal	15 microsatellites	Groups with close kin were found in the same area suggesting some level of site fidelity.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Delphinus delphis	Lee *et al.* 2018	-	Report the complete mitogenome of common dolphins	Tissue from bycatch	1 sample	16,386 bp of mtDNA (complete genome)	Multigene phylogeny revealed that *D. delphis* was most closely related to *S. coeruleoalba.*
Globicephala melas	Miralles *et al.* 2013	Global	Interspecific hybridization in *Globicephala spp.*	stranded animals, biopsies and museum collections	7 ACCOBAMS extension area + 50 NE Atl	8 microsatellites + mtDNA control region (703 bp)	Introgressive hybridization, current temperature increases and lower genetic variation in *G. melas* suggest that this species could be at risk in its northern range.
	Monteiro *et al.* 2015	North Atlantic Ocean	Population differentiation in in the North Atlantic	Skin from stranded animals	32 from ACCOBAMS extension area (+ 134 from North Atlantic)	mtDNA control region (400 bp) (*+ fatty acids and stable isotopes*)	High and significant levels of differentiation among the NE Atlantic. 3 haplotypes in ACCOBAMS extension area (total 6 in NA).
	Monteiro *et al.* 2016	North Atlantic Ocean	Population differentiation in in the North Atlantic	Skin from stranded animals	119 from North Atlantic, including 26 from ACCOBAMS extension area	Major Histocompatibility Complex genes (MHC DRA and DQB)	Significant differentiation between ACCOBAMS extension area and rest of the NA.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Globicephala melas	Verborgh 2015, Verborgh *et al.* 2016	North Atlantic Ocean + Mediterranean Sea	Population structure	Skin from free ranging + stranded animals	Strait of Gibraltar (90), Medite-rranean (80 = 65 Alboran + 15 Ligurian), ACCOBAMS extension area (50) +NE Atlantic (28)	11 microsatellites + mtDNA control region (800 bp)	Diversity is lower in MED/GIB. 4 subpop : 1 Strait of Gibraltar + 1 inner MED (possible substructure between Alboran and Ligurian) + 2 NE Atlantic.
	Kraft *et al.* 2020	Global	Global phylogeography and genetic diversity	Reusing samples from previous studies (including Monteiro 2013 and Verborgh 2015) + rest of the world	All samples from Monteiro *et al.* 2015, 2016, Verborgh *et al.* 2016	15 microsatellites + mtDNA control region (345 bp)	- Haplotype diversity is very low, as only 7 haplotypes have been described in the whole North Atlantic Ocean and 15 worldwide. -The divergence between the populations from the two hemispheres suggests they should be considered Demographically independent populations.
Grampus griseus	Gaspari *et al.* 2004	Ligurian and Mediterranean	Assess the differentiation between populations from the North Atlantic and the Mediterranean Sea; individual relatedness between groups	Skin of free ranging (24) and strandings (6)	30	Microsatellite diversity analyses	The Mediterranean population showed a higher level of variability than the NE Atlantic population.
	Gaspari *et al* 2007	Ligurian and Mediterranean Sea	Genetic population structure	Free ranging tissues and strandings	33	Microsatellite diversity analyses	High genetic diversity.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Grampus griseus	Sönmez *et al.* 2012	Türkyie	Genetic structure of the individual	muscle tissue	1	Mitochondrial 16S rDNA	Alignment of 529 bp length of 16S achieved.
Orcinus orca	Foote *et al.* 2011	Strait of Gibraltar (+ North Sea)	Characterization of population structure of killer whales in the North Atlantic	Skin biopsies (10) + necropsy (1)	11	mtDNA control region (989 bp) + complete (16,390 bp) and partial mitogenomes (12 814-14 689 bp) + 17 microsatellite markers	Resource specialisation leads genetic differentiation in the absence of physical barriers to gene flow.
	Esteban *et al.* 2016	Strait of Gibraltar SoG (+ Canary Islands CI)	Level of gene flow and ecological diffe-rences between SoG and CI populations	Skin biopsies (11) + necropsy (1)	12	mtDNA control region (989 bp) + complete mitogenomes (~16,390 bp) for a subset of individuals + 19 microsatellite markers	Pod-specific haplotypes, low gene flow between the SoG and CI populations, ecological differences.
	Foote *et al.* 2019	Strait of Gibraltar (+ global)	Global population structure	Skin biopsies	1	WGS	Genetic homogenisation at lower latitudes and greater differentiation at high latitudes.
Phocoena phocoena	Fontaine *et al.* 2007 (BMC Biol)	Black Sea + Iberian Peninsula (+European/Nordic waters)	Genetic structure + seascape genetics	Skin / muscle / other samples (standings / bycatch)	752 (78 Black Sea; 30 Iberian Peninsula; 642 European/Nordic waters)	10 microsatellites	Three major genetic groups with Black Sea as a genetically well distinct and homogenous group. Seascape feature impact individual dispersal, with Isolation by distance, but not in the Black Sea.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Phocoena phocoena	Fontaine *et al.* 2012 (PNAS)	Black Sea (BS) + Marmara Sea (MS) + Northern Aegean Sea (AeS)	Genetic structure + demographic/ phylogeographic history	Skin / muscle / other samples (standings / bycatch)	89 (11 AeS, 3 MS, 75 BS)	10 microsatellite markers + mtDNA control region	Genetic homogeneity between BS, MS, and AeS; Founding effect ~7kyr ago when BS reopened onto the Mediterranean Sea; Genetic signal of population decline by 90%, consistent with estimates of cetacean hunting in until the 1980's.
	Fontaine *et al.* 2014 (Mol Ecol)	Iberian Peninsula+ NW Africa + Black Sea	Phylogeographic history, and eco-type / sub-species isolation	Muscle / bones / teeth / skin	78 Black Sea + 31 Iberian Peninsula+ 15 NW Africa +	10 microsatellite markers + ¼ mitogenome	Identification of three genetically well distinct and equally divergent groups corresponding to the subspecies *P.p. relicta* and *P. p. phocoena* and a new lineage unnamed subspecies possibly *P. p. meridionalis* in the Iberian Peninsula and NW Africa. Divergence during the LGM related to paleo-mediterranean populations of harbour porpoises.
	Ben Chehida *et al.* 2020 (Heredity)	Black Sea (BS)+ Azov Sea (AzS) + Marmara Sea (MS) + Aegean Sea (AeS)	Genetic structure related to morphological heterogeneity?	Skin / muscle from standings and bycatch porpoises	144 (11 AeS, 3 MS, 87 BS, 32 AzS)	10 microsatellite markers + ¼ mitogenome	No genetic structure detected, not even considering the documented morphological heterogeneity between BS and AzS. Modelling shows that analyses had adequate power. Modelling shows that substructure may still be possible, assuming a lag between demography and genetics, or if the phenotypic differences are driven by natural selection involving non-neutral genetic markers not sampled in the study.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Physeter macrocephalus	Drouot *et al.* 2004	Eastern North Atlantic and Mediterranean Sea (Tyrrhenian Sea, Ionian Sea, North western Basin, Balearic Sea)	Assess genetic differentiation between North Atlantic and Mediterranean	Sloughed skin for MED, tissue from strandings for North Atlantic	13 (MED)	mtDNA control region (200 bp), 3 microsatellite *loci*	Different mitochondrial haplotype frequency between MED and North Atlantic.
	Engelhaupt *et al.* 2009	Gulf of Mexico, North Atlantic, North Sea, and Mediterranean Sea	Test the hypothesis that coastal basins represent isolated gene pools of matrifocal social units	Biopsies and sloughed skin	44 (MED)	mtDNA control region (399 bp), 16 microsatellite *loci*	No mt diversity in MED; significant differentiation between MED and other regions for both mt and usat markers.
	Alexander *et al.* 2016	Global; Pacific, Indian Ocean, Atlantic, Mediterranean	Test whether pop expansion explains low mtDNA diversity; influence of geographic regions vs social groups on genetic structure	Mix; used MED samples from Engelhaupt *et al.* 2009	40 (MED)	mtDNA control region (394 bp), 13 microsatellite *loci*	Low mtDNA diversity likely result of recent population expansion; differentiation among social groups, and among geographic regions in some oceans.
	Morin *et al.* 2018	Global; Pacific, Indian Ocean, Atlantic, Mediterranean	Understand which mechanisms (demography/ selection) contribute to low mtDNA diversity	Live biopsy and stranded animals	4 (NW MED)	Mitogenomes	Ocean-specific mitogenome haplotypes; 2 haplotypes in MED; population expansion and ocean-basin divergence since the last interglacial period.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Physeter macro	Violi *et al.* 2023	Mediterranean Sea (East + West) and NE Atlantic	Study population structure, demography, gene flow, kinship within the Mediterranean	Stranded and free-ranging	116 MED (34 from East; 82 from West)	5000-10,000 SNP *loci* (RADseq)	Recent founding of MED population, around last glacial maximum; differentiation between East and West MED populations.
Stenella coeruleoalba	Bourret *et al.* 2007	Central and western Mediterranean Sea and North Atlantic Ocean; North Pacific (as outgroup)	Genetic differentiation and levels of genetic diversity	Muscle, skin and blubber, liver, or kidney from stranded organisms	78 (MED)	5 microsatellite *loci*	Mediterranean population showed the lowest allelic richness and expected heterozygosity. Higher nuclear genetic diversity within the Atlantic than within the Mediterranean.
	Gaspari *et al.* 2007	Mediterranean Sea (West + East) and NE Atlantic	Social kin associations and genetic structu-ring of populations	Free-ranging and stranded animals (skin, liver,muscle, and heart)	149 (MED)	8 microsatellite polymorphic *loci*	- Differentiation between MED and North Atlantic - Differences over small geographical scales within the MED and among the Ligurian Sea between inshore and offshore. - The kin structure (Ligurian pop.) greater association among female than among male kin.
	Gkafas *et al.* 2017	NE Atlantic Ocean and Mediterranean Sea	Population genetic structure to identify the causes of genetic divergence, the effect of past climate change on demography and population connectivity	Stranded and bycatch Skin and muscle tissue	140	20 microsatellite *loci*	Directional gene flow from NE to south and west in the North Atlantic, and from west to east in the Mediterranean. Division between the North Atlantic and MED populations during the middle Pleistocene, and within the MED between the east and west basins towards the end of the Pleistocene.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Stenella coeruleoalba	Gaspari *et al.* 2019	Mediterranean Sea	Spatio-temporal patterns of genetic diversity in the Mediterranean	Stranded and free-ranging	368	mtDNA control Rregion (919 bp) and 15 microsatellites	Weak geographical differentiation in the MED (recent expansion) Cyclical fluctuations in genetic composition, which correspond with recurrent morbillivirus epizootics.
	Ciccarese *et al.* 2019	Gulf of Taranto (Ionian Sea)	Local differentiation of a subpopulation	Skin swabbing	25	mtDNA cytochrome b (421 bp)	High nucleotide diversity and heterogeneity in the Gulf of Taranto samples 2 separate lineage in the MED, one specific to the Gulf of Taranto.
Steno bredanensis	Kerem *et al.* 2016	NE Atlantic Ocean (Canary Islands) + E Mediterranean (Israel)	Determine genetic population structure and origin of the Mediterranean population	Skin tissue from strandings	3 MED (+6 Canary Islands)	mtDNA control region (450 bp)	Strong evidence for an Atlantic origin of the MED population. MED population basal within the Atlantic cluster. 9 samples, 9 haplotypes.
	Albertson *et al.* 2022	Global	Describe worldwide phylogeography	Skin tissue and teeth, from strandings, bycatch and biopsy samples	3 MED (same as Kerem *et al.* 2016) + 333 globally	mtDNA control region (n = 360), mitogenomes (n = 19), and six nuclear introns (n = 35)	Mediterranean samples clearly clustered with Atlantic sequences.
	Komnenou *et al.* 2022 (ECS)	Aegean Sea, Greece	Case study on live stranded individual	Skin(?)	1	mtDNA sequences from D-loop region and Cox1 gene	Close proximity to Atlantic haplotypes.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Tursiops truncatus	Natoli *et al.* 2004	NE Atlantic, Mediterranean and others	Fine scale population structure in the North Atlantic	Skin tissue from standings, bycatch and biopsy samples	27 NE Atlantic 45 MED	mtDNA control region (297 bp); 9 microsatellites	Significant population differentiation suggesting restricted gene flow for both males and females. Differentiation between coastal and pelagic populations.
	Natoli *et al.* 2005	NE Atlantic, Mediterranean and Black Sea	Large and fine scale population structure and gene flow	Skin tissues from stranding, biopsy and scrub samples	35 ACCOBAMS extension area; 42 W MED; 32 E MED; 16 Black Sea	mtDNA control region (630 bp); 9 microsatellites	Clear population structure over the geographical range. Strong differentiation between MED and Black Sea.
	Fernandez *et al.* 2011	Iberian Peninsula	Fine scale population structure	Skin tissues from stranding and bycatch samples	60 Spain (Galicia; Basque country and Canary Islands) 31 Portugal (mainland, Sado and Azores)	mtDNA control region (549 bp); 10 microsatellites	Fine scale population differentiation between the resident populations (South Galicia and Sado) and the other regions.
	Moura *et al.* 2013	Global; Mediterranean; Black Sea	Population differentiation and phylogenetic analysis	Skin tissue from standings, bycatch and biopsy samples	8 NE Atlantic 10 MED 10 Black Sea	75 mtDNA complete mitogenome	Coastal populations are differentiated from pelagic populations in the NE Atlantic. Radiation in pelagic environments was recent, and was likely followed by a return to coastal habitat.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Tursiops truncatus	Louis *et al.* 2014a	NE Atlantic and Mediterranean	Population structure	Skin tissue from standings, and biopsy samples	405 samples (~52 from MED; samples from Galicia, Gibraltar/ Cadiz)	mtDNA control region (682 bp); 25 microsatellites	Clear population structure between coastal and pelagic populations; fine scale population structure within these groups. Differentiation between Atlantic and Mediterranean populations.
	Louis *et al.* 2014b	NE Atlantic and Mediterranean	Population structure; ecotype differentiation, demographic history	Skin tissue from standings, and biopsy sampless	405 samples (~52 from MED; samples from Galicia, Gibraltar/ Cadiz)	mtDNA control region (682 bp); 25 microsatellites	Coastal populations were likely founded by the Atlantic pelagic population after the LGM. Pelagic dolphins from Atlantic and MED likely diverged during a period of high productivity in the MED.
	Gaspari *et al.* 2015a	Mediterranean	Genetic differentiation and dispersal	Skin tissues from standings, and biopsy samples	89 samples (63 Adriatic sea, 6 Ionian; 6 Aegean sea; 14 Tyrrhenian sea)	mtDNA complete control region (920 bp); 12 microsatellites	Genetic diff. among all the pops. Fine-scale pop structure within the Adriatic. High gene flow from N Adriatic to adjacent waters.
	Gaspari *et al.* 2015b	Mediterranean	Population structure phylogeography in Mediterranean	Skin tissues from strandings, and biopsy samples	194 samples (87 Adriatic sea; 10 Aegean; 16 Tyrrhenian; 14 Ionian; 68 Levantine basin	mtDNA complete control region (920 bp); 12 microsatellites	Genetic diff between pelagic and coastal populations. Fine scale pop division within the Adriatic and the Levantine Seas.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Tursiops truncatus	Gonzalvo *et al.* 2016	Mediterranean (Gulf of Ambracia)	Genetic differentiation of bottlenose dolphins in the Gulf of Ambracia	Skin tissues were obtained with "skin swabbing" method; biopsies	19 Gulf of Ambracia	mtDNA control region (442 bp)	Unique haplotypes and lower genetic diversity for population of Gulf of Ambracia. Population differentiation between this population and the others.
	Brotons *et al.* 2019	Mediterranean (Balearic Islands)	Population structure around the islands	Skin tissues from strandings, and biopsy samples	50 samples (26 Gimnèsies; 22 Pitiuses; 9 Comunitat Valenciana)	mtDNA control region (660 bp); 11 microsatellites	Genetic differentiation between balearic islands and coastal region (Peninsula).
	Nykanen *et al.* 2019	NE Atlantic	Fine scale population structure and connectivity	Skin tissues from strandings, and biopsy samples	33 Gibraltar/ Cadiz 33 N Spain (Galicia)	14 microsatellites	Fine scale population structure for coastal populations, low levels connectivity between these populations.
	Moura *et al.* 2020	Global, including Mediterranean and Black Sea	Phylogenetic relationship of the genus *Tursiops*	Skin tissue from standings, bycatch and biopsy samples	8 NE Atlantic 10 MED 10 Black Sea	RAD seq data (26720 SNPs)	Monophyly for the genus *Tursiops*; extensive gene flow between european coastal and pelagic ecotypes. Differentiation between Atlantic pelagic and Mediterranean + Black Sea but with some gene flow between them.
Ziphius cavirostris	Carroll *et al.* 2016	Ligurian Sea (1), Canary Islands (2), Scotland (1)	To assess the utility of ddRAD sequencing in identifying specific SNPs for ecological and evolutionary studies	Skin biopsy	4	ddRAD markers	10,000 *loci* would be sufficient to detect population structure. However additional analyses are needed.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Ziphius cavirostris	Dalebout *et al.* 2005	Mediterranean: Greece + Croatia (+North Atlantic, South hemisphere, North Pacific)	Phylogeography	Stranding tissues	12: Greece + 2 Croatia	mtDNA control region (300 bp)	Strong phylogeographic structure among ocean basins with Mediterranean population being isolated (two private haplotypes T3 and T4). *Ziphius* in the Mediterranean to be considered a separate evolutionarily significant unit (ESU).
	Tonay *et al.* 2019	Aegean Sea, Eastern Mediterranean.	Genetic population structure	tissue from stranding	5	mtDNA control region (444 bp) cytochrome b (424 bp)	Control region haplotypes were identical to two previously identified ones from the Ionian (Greece) and Adriatic (Croatia) Seas.
	Onoufriou *et al.* 2022	Mediterranean Sea (eastern basin + Ligurian Sea) Global Oceans	Phylogeography and genomic population structure	Skin from stranded and biopsied individuals	33 (19 W + 14 E) for SNPs 3 for mtDNA	30,479 SNPs Full mitogenome (15,219 bp) mtDNA control region (860 bp)	Strong phylogeographic structure among ocean basins. The Mediterranean populations diverged 0.5 mya from the Atlantic Ocean. W MED and E MED to be considered a separate Evolutionarily Significant Unit (ESU).
Delphinus delphis ponticus	Biard *et al.* 2017	Black Sea	Performance of three biomolecular methods for species identification in a mixed assemblage of 800 to 1600 years old odontocete bone samples	Excavated zooarchaeological material	10	Cyt b (43 bp); full mitogenome (72-100% coverage)	- First mitogenome data obtained. - The combination of ZooMS, mtDNA and shotgun sequencing provides a powerful tool for species ID in aDNA/eDNA studies.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Delphinus delphis ponticus	Rosel *et al.* 1994	Black Sea	Genetic population structure	Tissue from stranding	4	mtDNA control region (404 bp), cytochrome b (360 bp)	Genetic differentiation between BS, California and Pacific populations.
	Natoli *et al.* 2008	Black Sea	Genetic population structure	Tissue from stranding	5	mtDNA control region (428 bp) 9 microsatellite *loci*	Suggest isolation from the MED population.
	Tonay *et al.* 2020	Black Sea, TSS, Aegean Sea	Genetic population structure	Tissue from stranding	17+19*+1	mtDNA control region (428 bp)	- Differentiation was observed between the Atlantic Ocean, and the MED, TSS and the Black Sea but not between MED and Black Sea. - Multidirectional colonisation events of the Med. Sea from the eastern Atlantic Ocean.
Tursiops truncatus ponticus	Biard *et al.* 2017	Black Sea	Performance of three biomolecular methods for species identification in a mixed assemblage of 800 to 1600 years old odontocete bone samples	Excavated zooarchaeological material	11	Cyt b (43 bp); full mitogenome (72-100% coverage)	- Ancient haplotypes are present in modern population. - The combination of ZooMS, mtDNA and shotgun sequencing provides a powerful tool for species ID in aDNA/ eDNA studies.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Tursiops truncatus ponticus	Moura *et al.* 2013	Black Sea, Mediterranean Sea	Genetic population structure	tissue from stranding, biopsy, scrub sampling	10+10 (the same as Natoli *et al.* 2005)	full mitogenome (16,386 bp)	Separation between Eastern Mediterranean and Black Sea was visible in two independent lineages, both splitted from the Mediterranean clades *ca.* 10 kyr ago.
	Natoli *et al.* 2005	Black Sea, Mediterranean Sea	Genetic population structure, sex segregation	tissue from stranding, biopsy, scrub sampling	16+74	mtDNA control region (630 bp), 9 microsatellite *loci*	-Two isolated lineages in the Black Sea showing the founder effect. -Directional effect for gene flow, suggesting the emigration of females.
Tursiops truncatus ponticus	Tonay *et al.* 2018	Black Sea, TSS, Aegean Sea, Mediterranean Sea	Genetic population structure	tissue from stranding	31+31*+7+5	mtDNA	BS bottlenose dolphin population differentiation from the adjacent populations and low levels of genetic diversity indicates a conservation concern.
	Viaud-Martinez *et al.* 2008	Black Sea, Mediterranean Sea	Genetic population structure	tissue from stranding, biopsy, scrub sampling	43+31 (partly the same as Natoli *et al.* 2005)	mtDNA control region (442 bp)	Low genetic diversity in the Black Sea coupled with significant differentiation and some shared haplotypes.
Phocoena phocoena relicta	Ben Chehida *et al.* 2020	Black Sea, Azov Sea	Genetic population structure	tissue from stranding	55	mtDNA control region 3904 bp 10 microsatellite *loci*	The genetic homogeneity in the Black Sea porpoises at the mtDNA and microsatellites, despite morphological heterogeneity.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Phocoena phocoena relicta	Biard *et al.* 2017	Black Sea	Performance of three biomolecular methods for species identification in a mixed assemblage of 800 to 1600 years old odontocete bone samples	excavated zooarchaeological material	10	Cyt b (43 bp); full mitogenome (72-100% coverage)	- ancient haplotypes are present in modern population. - The combination of ZooMS, mtDNA and shotgun sequencing provides a powerful tool for species ID in aDNA/ eDNA studies.
	Fontaine *et al.* 2007	Black Sea, Marmara Sea	Genetic population structure	tissue from stranding and bycaught	75+3*	10 microsatellite *loci*	Strong barriers to gene flow were detected in the SE part of the range.
	Fontaine *et al.* 2010	Black Sea, Marmara Sea	Genetic population structure	tissue from stranding and bycaught	75+3* (same with Fontaine *et al.* 2007)	10 microsatellite *loci*	Black Sea and North Atlantic harbour porpoises have diverged within the last 7000 years ago.
	Fontaine *et al.* 2012	Black Sea, Marmara Sea, Aegean Sea	Genetic population structure	tissue from stranding and bycaught	75+3*+11 (same with Fontaine *et al.* 2007, 2010 except Aegean Sea)	mtDNA control region (705 bp), 10 microsatellite *loci*	A strong population reduction (~90%) that occurred within the past 5 decades, due to massive killing and bycatch.
	Fontaine *et al.* 2014	Black Sea, Marmara Sea	Genetic population structure	tissue from stranding and bycaught	75+3* (same with Fontaine *et al.* 2007, 2010)	mtDNA control region (5085 bp), 10 microsatellite *loci*	The divergence between the western and eastern populations in the Mediterranean Sea likely occurred during the postglacial period, around ca. 14 kyr BP.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Phocoena phocoena relicta	Lah *et al.* 2016	Black Sea	Genetic population structure	tissue from stranding and bycaught	4	ddRAD - 2872 (SNPs), 13 microsatellite *loci*, mtDNA-CR- 414 bp	BS porpoises are most clearly separated based on nuclear as well as private and divergent mitochondrial markers.
	Llavona *et al.* 2014	Black Sea	Genetic population structure	tissue from stranding and bycaught	16	10 microsatellite *loci*	Aegean, Marmara and Black Seas appear to form a distinct group.
	Rosel *et al.* 1995	Black Sea	Genetic population structure	tissue from stranding and bycaught	9	mtDNA control region (394 bp)	No shared haplotypes were found among the three ocean basins, and the estimated sequence divergence among them was high.
	Rosel *et al.* 2003	Aegean Sea	Genetic population structure	tissue from stranding	2	mtDNA control region (344 bp)	Movement of porpoises out of the Black Sea and into the Aegean Sea.
	Tonay *et al.* 2012	Black Sea, Marmara Sea, Aegean Sea	Genetic population structure	tissue from stranding and bycaught	1+1*+1	mtDNA control region (364 bp)	Possibility of isolated group in TSS.
	Tonay *et al.* 2017	Black Sea, TSS, Aegean Sea	Genetic population structure	tissue from stranding and bycaught	58+11*+1	mtDNA control region (358 bp)	BS harbour porpoises dispersed into the Aegean through the TSS. Possibility of isolated group in TSS.
	Uzun *et al.* 2017	Black Sea, TSS, Aegean	Genetic population structure	tissue from stranding and bycaught	57+15*+2	mtDNA control region (364bp)	Possibility of isolated group in TSS.

SPECIES	REFERENCE	REGION	OBJECTIVE	SAMPLE TYPE	SAMPLE NO.**	MARKER	KEY FINDINGS
Phocoena phocoena relicta	Uzun *et al.* 2018	Black Sea, TSS, Aegean	Genetic population structure	tissue from stranding and bycaught	37+17*+1	ddRAD-4924 (SNPs)	Highest genetic diversity in the Western Black Sea and TSS, possibility of isolated group in TSS.
	Viaud-Martinez *et al.* 2007	Black Sea, Marmara Sea, Aegean Sea, Gibraltar area	Degree of morphological and genetic differentiation	tissue from stranding and bycaught	95+3*+4+4	mtDNA control region (364 bp)	Genetically differentiated and recognized as the subspecies *P. p. relicta*.

**Turkish Straits System (Marmara Sea, Istanbul and Çanakkale Straits) is not in the ACCOBAMS area. **Number of samples included from the ACCOBAMS area.*

03) Full reference list for species summaries

Albertson, G. R., Alexander, A., Archer, F. I., Caballero, S., Martien, K. K., Hemery, L. G., Baird, R. W., Oremus, M., Poole, M. M., Duffield, D. A., Brownell Jr, R. L., Kerem, D., Mignucci-Giannoni, A. A. and Baker, C. S. Worldwide phylogeography of rough-toothed dolphins (*Steno bredanensis*) provides evidence for subspecies delimitation. Marine Mammal Science. https://doi.org/10.1111/mms.12933

Alexander, A., Steel, D., Hoekzema, K., Mesnick, S. L., Engelhaupt, D., Kerr, I., Payne, R., and Baker, C. S. (2016). What influences the worldwide genetic structure of sperm whales (*Physeter macrocephalus*)? Molecular Ecology, 25(12), 2754–2772. https://doi.org/10.1111/mec.13638

Amaral, A. R., Beheregaray, L. B., Bilgmann, K., Boutov, D., Freitas, L., Robertson, K. M., Sequeira, M., Stockin, K. A., Coelho, M. M., and Möller, L. M. (2012). Seascape genetics of a globally distributed, highly mobile marine mammal: The short-beaked common dolphin (genus *Delphinus*). PLoS ONE, 7(2), e31482. https://doi.org/10.1371/journal.pone.0031482

Amaral, A.R., Sequeira, M., Martínez-Cedeira, J. and Coelho, M.M., 2007. New insights on population genetic structure of *Delphinus delphis* from the northeast Atlantic and phylogenetic relationships within the genus inferred from two mitochondrial markers. Marine Biology, 151(5), pp.1967-1976. https://doi.org/10.1007/s00227-007-0635-y

Archer, F. I., Brownell, R. L. Jr., Hancock-Hanser, B. L., Morin, P. A., Robertson, K. M., Sherman, K. K., Calambokidis, J., Urbán R, J., Rosel, P. E., Mizroch, S. A., Panigada, S., Taylor, B. L. (2019). Revision of fin whale *Balaenoptera physalus* (Linnaeus, 1758) subspecies using genetics. J. Mammal. 100, 1653–1670. https://doi.org/10.1093/jmammal/gyz121

Archer, F. I., Morin, P. A., Hancock-Hanser, B. L., Robertson, K. M., Leslie, M. S., Bérubé, M., Panigada, S., Taylor, B. L. (2013). Mitogenomic phylogenetics of fin whales (*Balaenoptera physalus spp.*): genetic evidence for revision of subspecies. PLoS One 8:e63396. https://doi.org/10.1371/journal.pone.0063396

Ball, L., Shreves, K., Pilot, M., Moura, A. E. (2017) Temporal and geographic patterns of kinship structure in common dolphins (*Delphinus delphis*) suggest site fidelity and female-biased long-distance dispersal. Behav Ecol Sociobiol 71, 123. https://doi.org/10.1007/s00265-017-2351-z

Ben Chehida Y, Stelwagen T., Hoekendijk J., Ferreira M, Eira C,., Pereira AT, Nicolau L., Thumloup J., Fontaine M. (bioRxiv). Harbor porpoise losing its edges: genetic time series suggests a rapid population decline in Iberian waters over the last 30 years. https://doi.org/10.1101/2021.08.19.456945

Ben Chehida Y, Thumloup J, Vishnyakova K, Gol'din P, Fontaine MC. 2020. Genetic homogeneity in the face of morphological heterogeneity in the harbor porpoise from the Black Sea and adjacent waters (*Phocoena phocoena* relicta). Heredity 124, 469–484 https://doi.org/10.1038/s41437-019-0284-1

Ben Chehida, Y., Loughnane, R., Thumloup, J., Kaschner, K., Garilao, C., Rosel, P. E., and Fontaine, M. C. (2021). No leading-edge effect in North Atlantic harbor porpoises: Evolutionary and conservation implications. Evolutionary applications, 14(6), 1588-1611. https://doi.org/10.1111/eva.13227

Ben Chehida, Y., Thumloup, J., Schumacher, C., Harkins, T., Aguilar, A., Borrell, A., Ferreira, M., Rojas-Bracho, L., Robertson, K. M., Taylor, B. L., Víkingsson, G. A., Weyna, A., Romiguier, J., Morin, P. A. and Fontaine, M. C. (2020). Mitochondrial genomics reveals the evolutionary history of the porpoises (*Phocoenidae*) across the speciation continuum. Sci Rep 10, 15190.
https://doi.org/10.1038/s41598-020-71603-9

Bentaleb, I., Martin, C., Vrac, M., Mate, B., Mayzaud, P., Siret, D., De Stephanis, R., and Guinet, C. (2011). Foraging ecology of Mediterranean fin whales in a changing environment elucidated by satellite tracking and baleen plate stable isotopes. Marine Ecology Progress Series, 438, 285–302.
https://doi.org/10.3354/meps09269

Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo Di Sciara, G., Sears, R., Sigurjónsson, J., Urban-R., J., and Palsbøll, P. J. (1998). Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): Analysis of mitochondrial and nuclear *loci*. Molecular Ecology, 7, 585–599. https://doi.org/10.1046/j.1365-294x.1998.00359.x

Biard, V., Gol'din, P., Gladilina, E., Vishnyakova, K., McGrath, K., Vieira, F.G., Wales, N., Fontaine, M.C., Speller, C. and Olsen, M.T., 2017. Genomic and proteomic identification of Late Holocene remains: Setting baselines for Black Sea odontocetes. Journal of Archaeological Science: Reports, 15, pp.262-271. https://doi.org/10.1016/j.jasrep.2017.07.008

Bourret, V., Macé, M. and Crouau-Roy, B. 2007. Genetic variation and population structure of western Mediterranean and northern Atlantic Stenella coeruleoalba populations inferred from microsatellite data. Journal of the Marine Biological Association of the UK 87(1): 265–269.
https://doi.org/10.1017/S0025315407054859

Brotons, J. M., Islas-Villanueva, V., Alomar, C., Tor, A., Fernández, R., and Deudero, S. (2019). Genetics and stable isotopes reveal non-obvious population structure of bottlenose dolphins (*Tursiops truncatus*) around the Balearic Islands. Hydrobiologia, 7. https://doi.org/10.1007/s10750-019-04038-7

Carroll EL, Reyes C, Gaggiotti OE, Olsen MT, Maaholm DJ, Rosso M, Davison N, Martin V, Schiavi A, Aguilar de Soto N. (2016). Pilot study to assess the utility of ddRAD sequencing in identifying species-specific and shared SNPs among Blainville's (*Mesoplodon densirostris*) and Cuvier's (*Ziphius cavirostris*) beaked whales. IWC report SC/66b/DNA/03. https://doi.org/10.13140/RG.2.1.2286.5527

Chen, I., Nishida, S., Chou, L.-S., Tajima, Y., Yang, W.-C., Isobe, T., Yamada, T.K., Hartman, K. and Hoelzel, A.R. (2018). Concordance between genetic diversity and marine biogeography in a highly mobile marine mammal, the Risso's Dolphin. Journal of Biogeography 45(9): 2092–2103.
https://doi.org/10.1111/jbi.13360

Ciccarese S, Carlucci R, Ciani E, Corcella E, Cosentino A, Fanizza C, Linguiti G, Antonacci R. (2019). Cytochrome b marker reveals an independent lineage of *Stenella coeruleoalba* in the Gulf of Taranto. Chiang T-Y, editor. PLoS One, 14(3):e0213826. https://dx.plos.org/10.1371/journal.pone.0213826

Cooke, J. G. (2018). *Balaenoptera physalus* (Fin whale). The IUCN Red List of Threatened Species 2018: E.T2478A50349982. https://doi.org/10.1007/978-1-4612-9824-3_33

Dalebout ML, Robertson KM, Frantzis A, Engelhaupt D, Mignucci AA, Rosario Delestre RJ, Baker CS. (2005). Worldwide structure of mtDNA diversity among Cuvier's beaked (*Ziphius cavirostris*): implications for threatened populations. Mol Ecol.;14:3353–71. https://doi.org/10.1111/j.1365-294x.2005.02676.x

Donovan, G. P., and Bjørge, A. (1995). Harbour porpoises in the North Atlantic: edited extract from the report of the IWC Scientific Committee, Dublin 1995. Reports of the International Whaling Commission, Special Issue, 16, 3-26.

Drouot, V., Bérubé, M., Gannier, A., Goold, J. C., Reid, R. J., and Palsbøll, P. J. (2004). A note on genetic isolation of Mediterranean sperm whales (*Physeter macrocephalus*) suggested by mitochondrial DNA. Journal of Cetacean Research and Management, 6(1), 29–32. https://doi.org/10.47536/jcrm.v6i1.787

Engelhaupt, D., Hoelzel, A. R., Nicholson, C., Frantzis, A., Mesnick, S., Gero, S., Whitehead, H., Rendell, L., Miller, P., De Stefanis, R., Cañadas, A., Airoldi, S., and Mignucci-Giannoni, A. A. (2009). Female philopatry in coastal basins and male dispersion across the North Atlantic in a highly mobile marine species, the sperm whale (*Physeter macrocephalus*). Molecular Ecology, 18(20), 4193–4205.
https://doi.org/10.1111/j.1365-294X.2009.04355.x

Esteban R, Verborgh P, Gauffier P, Giménez J, Martín V, Pérez-Gil M, Tejedor M, Almunia J, Jepson PD, García-Tíscar S, Barrett-Lennard LG, Guinet C, Foote AD and de Stephanis R. (2016). Using a multi-disciplinary approach to identify a critically endangered killer whale management unit. J Appl Ecol 66: 291–300. https://doi.org/10.1016/j.ecolind.2016.01.043

Esteban R., P. Verborgh, P. Gauffier, D. Alarcón, J.M. Salazar-Sierra, J. Giménez, A.D. Foote, R. de Stephanis. 2016. Conservation Status of Killer Whales, Orcinus orca, in the Strait of Gibraltar, Eds: Giuseppe Notarbartolo Di Sciara, Michela Podestà, Barbara E. Curry, Advances in Marine Biology, 75: 141-172,
https://doi.org/10.1016/bs.amb.2016.07.001

Fernández, R., Santos, M. B., Pierce, G. J., Llavona, A., Lopez, A., Silva, M. A., Ferreira, M., Carrillo, M., Cermeño, P., Lens, S. and Piertney, S. B. (2011). Fine-scale genetic structure of bottlenose dolphins, *Tursiops truncatus*, in Atlantic coastal waters of the Iberian Peninsula. Hydrobiologia, 670, 111–125.
https://doi.org/10.1007/s10750-011-0669-5

Fontaine MC (2016). Harbour porpoises, *Phocoena phocoena*, in the Mediterranean Sea and Adjacent Regions: Biogeographic Relicts of the Last Glacial Period, 1st edn. Elsevier Ltd.
https://doi.org/10.1016/bs.amb.2016.08.006

Fontaine MC, Baird SJE, Piry S, Ray N, Tolley KA, Duke S, Birkun A, Ferreira M, Jauniaux T, Llavona Á, Öztürk B, A Öztürk A, Ridoux V, Rogan E, Sequeira M, Siebert U, Vikingsson GA, Bouquegneau J-M, Michaux JR (2007) Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. BMC Biology 5.
https://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-5-30

Fontaine MC, Roland K, Calves I, Austerlitz F, Palstra FP, Tolley KA, Ryan S, Ferreira M, Jauniaux T, Llavona A, Öztürk B, Öztürk AA, Ridoux V, Rogan E, Sequeira M, Siebert U, Vikingsson GA, Borrell A, Michaux JR, Aguilar A (2014) Postglacial climate changes and rise of three ecotypes of harbour porpoises, *Phocoena phocoena*, in western Palearctic waters. Molecular Ecology 23, 3306-3321.
https://doi.org/10.1111/mec.12817

Fontaine MC, Snirc A, Frantzis A, Koutrakis E, Ozturk B, Ozturk AA and Austerlitz F (2012). History of expansion and anthropogenic collapse in a top marine predator of the Black Sea estimated from genetic data. Proc Natl Acad Sci 109: E2569–E2576. https://doi.org/10.1073/pnas.1201258109

Fontaine MC, Thatcher O, Ray N, Piry S, Brownlow A, Davison NJ, Jepson P, Deaville R, Goodman SJ (2017) Mixing of porpoise ecotypes in southwestern UK waters revealed by genetic profiling. R Soc Open Sci 4, 160992. http://dx.doi.org/10.1098/rsos.160992

Fontaine, M. C., Tolley, K. A., Michaux, J. R, Birkun, J.A., Ferreira, M., Jouniaux, T., Llavona, A., Öztürk, B., Ayaka, A.Ö., Ridoux, V., Rogan, E., Sequeira, M., Bouquegneau, J.M., Baird, S.J.E. (2010). Genetic and historic evidence for climate-driven population fragmentation in a top cetacean predator: the harbour porpoises in European water, Proceedings of the Royal Society of London B: Biological Sciences, 277, 2829-2837. https://doi.org/10.1098/rspb.2010.0412

Foote AD, Martin MD, Louis M, Pacheco G, Robertson KM, Sinding MS, Amaral AR, Baird RW, Baker CS, Ballance L, Barlow J, Brownlow A, Collins T, Constantine R, Dabin W, Dalla Rosa L, Davison NJ, Durban JW, Esteban R, Ferguson SH, Gerrodette T, Guinet C, Hanson MB, Hoggard W, Matthews CJD, Samarra FIP, de Stephanis R, Tavares SB, Tixier P, Totterdell JA, Wade P, Excoffier L, Gilbert MTP, Wolf JBW, Morin PA. (2019). Killer whale genomes reveal a complex history of recurrent admixture and vicariance. Mol Ecol. 28(14):3427-3444. https://doi.org/10.1111/mec.15099

Foote AD, Newton J, Ávila-Arcos MC, Kampmann M-L, Samaniego JA, Post K, Rosing-Asvid A, Sinding M-HS,Gilbert MTP. (2013). Tracking niche variation over millennial timescales in sympatric killer whale lineages. Proc R Soc B 280: 20131481. http://dx.doi.org/10.1098/rspb.2013.1481

Foote AD, Vilstrup JT, De Stephanis R, Verborgh P, Abel Nielsen SC, Deaville R, Kleivane L, Martín V, Miller PJ, Oien N, Pérez-Gil M, Rasmussen M, Reid RJ, Robertson KM, Rogan E, Similä T, Tejedor ML, Vester H, Víkingsson

GA, Willerslev E, Gilbert MT, Piertney SB. (2011). Genetic differentiation among North Atlantic killer whale populations. Mol Ecol. 20(3):629-41. https://doi.org/10.1111/j.1365-294X.2010.04957.x

Fossi, M. C., Panti, C., Marsili, L., Maltese, S., Spinsanti, G., Casini, S., Caliani, I., Gaspari, S., Muñoz-Arnanz, J., Jimenez, B. and Finoia, M. G. (2013). The Pelagos Sanctuary for Mediterranean marine mammals: Marine Protected Area (MPA) or marine polluted area? The case study of the striped dolphin (*Stenella coeruleoalba*). Marine pollution bulletin, 70(1-2), 64-72.
https://doi.org/10.1016/j.marpolbul.2013.02.013

Gaspari S, Airoldi S, Hoelzel AR. 2007. Risso's dolphins (*Grampus griseus*) in UK waters are differentiated from a population in the Mediterranean Sea and genetically less diverse. Conservation Genetics 8(3):727-732. https://doi.org/10.1007/s10592-006-9205-y

Gaspari, S (2004) Social and population structure of striped and Risso's dolphins in the Mediterranean Sea, Durham theses, Durham University. Available at Durham E-Theses Online:
http://etheses.dur.ac.uk/3051/

Gaspari, S., Azzellino, A., Airoldi, S. and Hoelzel, A.R. 2007. Social kin associations and genetic structuring of striped dolphin populations (*Stenella coeruleoalba*) in the Mediterranean Sea. Molecular Ecology 16(14): 2922-2933. https://doi.org/10.1111/j.1365-294x.2007.03295.x

Gaspari, S., Holcer, D., Mackelworth, P., Fortuna, C., Frantzis, A., Genov, T., Vighi, M., Natali, C., Rako, N., Banchi, E., Chelazzi, G. and Ciofi, C. (2015a). Population genetic structure of common bottlenose dolphins (*Tursiops truncatus*) in the Adriatic Sea and contiguous regions: implications for international conservation. Aquatic Conservation: Marine and Freshwater Ecosystems, 25(2), 212-222.
https://doi.org/10.1002/aqc.2415

Gaspari, S., Marsili, L., Natali, C., Airoldi, S., Lanfredi, C., Deeming, C. and Moura, A.E. (2019). Spatio-temporal patterns of genetic diversity in the Mediterranean striped dolphin (*Stenella coeruleoalba*). Journal of Zoological Systematics and Evolutionary Research 57(3): 721-734.
https://doi.org/10.1111/jzs.12265

Gaspari, S., Scheinin, A., Holcer, D.,Fortuna, C., Natali, C., Genov, T., Frantzis, A., Chelazzi, G., Moura, A.E. (2015b). Drivers of Population Structure of the Bottlenose Dolphin (*Tursiops truncatus*) in the Eastern Mediterranean Sea. Evolutionary Biology, 42: 177–190. https://doi.org/10.1007/s11692-015-9309-8

Gauffier, P., 2020. Ecología y conservación del rorcual común en el Mediterráneo español. PhD thesis. Universidad de Cádiz.

Gauffier, P., Verborgh, P., Gauffier, P., Verborgh, P., Giménez, J., Esteban, R., Manuel, J., Sierra, S., and de Stephanis, R. (2018). Contemporary migration of fin whales through the Strait of Gibraltar. Marine Ecology Progress Series, 588, 215–228. https://doi.org/10.3354/meps12449

Gkafas, G.A., Exadactylos, A., Rogan, E., Raga , J.A., Reid, R. and Hoelzel, A.R. 2017. Biogeography and temporal progression during the evolution of striped dolphin population structure in European waters. Journal of Biogeography 44(12): 2681-2691 https://doi.org/10.1111/jbi.13079

Gonzalvo, J., Lauriano, G., Hammond, P.S., Viaud-Martinez, K.A., Fossi, M.C., Natoli, A., Marsili, L. (2016). The Gulf of Ambracia's Common Bottlenose Dolphins, *Tursiops truncatus*: A Highly Dense and yet Threatened Population. Adv. Mar. Biol. 75, 259–296. https://doi.org/10.1016/bs.amb.2016.07.002

Jefferson, T.A. and Waerebeek, K.V. (2002). The taxonomic status of the nominal dolphin species *Delphinus tropicalis* van Bree, 1971. Marine Mammal Science, 18: 787-818.
https://doi.org/10.1111/j.1748-7692.2002.tb01074.x

Kerem, D., Goffman, O., Elasar, M., Hadar, N., Scheinin, A., and Lewis, T. (2016). The rough-toothed dolphin, *Steno bredanensis*, in the eastern Mediterranean Sea: a relict population? Advances in Marine Biology, 75, 233-258. https://doi.org/10.1016/bs.amb.2016.07.005

Komnenou, A. Gkafas, G., Drougas, A., Sarris, P., Kofidou, E., Sarantopoulou, J., Exadactylos, A., Garcia-Hartman, M. (2022). First documented alive stranding of a *Steno bredanensis* in Salamina Island, Aegean Sea, Greece. 33rd Conference of the European Cetacean Society, Ashdod, Israel, April 2022.

Kraft, S., Pérez-Álvarez, MJ., Olavarría, C. and Poulin, E. 2020. Global phylogeography and genetic diversity of the Long-finned pilot whale *Globicephala melas*, with new data from the southeastern Pacific. Scientific Reports 10(1): 1769. https://doi.org/10.1038/s41598-020-58532-3

Lah, L., Benke, H., Berggren, P., Gunnlaugsson, Þ., Lens, S., Lockyer, C., Öztürk, A. A., Öztürk, B., Pawliczka, I., Roos, A., Siebert, U., Skóra, K., Tiedemann, R. 2016 Spatially explicit analysis of genome-wide SNPs detects subtle population structure in a mobile marine mammal, the harbor porpoise, PLoS ONE, 11(10): e0162792. https://doi.org/10.1371/journal.pone.0162792

Lee, K., Lee, J., Cho, Y., Sohn, H., Choi, Y.-M., Lim, S. R., Yoon, S.-W., Jeong, D. G., Kim, J. H. (2018). Characterization of the complete mitochondrial genome and phylogenetic analysis of the common dolphin *Delphinus delphis* (Cetacea: *Delphinidae*). Mitochondrial DNA Part B, 3, 632–633
https://doi.org/10.1080/23802359.2018.1473720

Llavona A. (2018) Population parameters and genetic structure of the harbour porpoise (*Phocoena phocoena*, L. 1758) in the Northwest the Iberian Peninsula. PhD thesis. Aveiro University.
https://ria.ua.pt/handle/10773/24058?mode=full

Llavona, V.A., Ferreira, M., López, A., Vingada, J.V., Pierce, G.J., Dabin, W., Deaville, R., Jauniaux, T., Rogan, E., Tonay, A. M., Dede, A., Brownlow, A., Laria, L., Fernández, C., Cermeño, P., Piertney, S.B. 2014 Population genetic structure of harbour porpoise *Phocoena phocoena* across Europe: implications for management, 28th Annual Conf. European Cetacean Society, Liege, Belgium, 77.
https://www.europeancetaceansociety.eu/sites/default/files/28th%20conference%20Liege%20abstract_book.pdf

López A (2003). Estatus dos pequenos cetáceos da plataforma de Galicia. PhD Thesis. Universidade de Santiago de Compostela, Spain

López-Fernández, A. and Martínez-Cedeira, J.A. (2011). Marsopa – *Phocoena phocoena*. En: Enciclopedia Virtual de los Vertebrados Españoles. Salvador, A. and Cassinello, J. (Eds.). Museo Nacional de Ciencias Naturales, Madrid. http://www.vertebradosibericos.org/

Louis, M., Fontaine, M. C., Spitz, J., Schlund, E., Dabin, W., Deaville, R., Caurant F., Cherel, Y., Guinet, C., and Simon-Bouhet, B. (2014b). Ecological opportunities and specializations shaped genetic divergence in a highly mobile marine top predator. Proceedings of the Royal Society B: Biological Sciences, 281, 20141558–20141558. https://doi.org/10.1098/rspb.2014.1558

Louis, M., Viricel, A., Lucas, T., Peltier, H., Alfonsi, E., Berrow, S. D., Brownlow, A., Covelo, P., Dabin, W., Deaville, R., De Stephanis, R., Gally, F., Gauffier, P., Penrose, R., Silva, M. A., Guinet, C., and Simon-Bouhet, B. (2014a). Habitat-driven population structure of bottlenose dolphins, *Tursiops truncatus*, in the North-East Atlantic. Molecular Ecology, 23(4), 857–874. https://doi.org/10.1111/mec.12653

Maio, N., Giovannotti, M., Caputo Barucchi, V., Petraccioli, A., Pollaro, F., Guarino, F. M., Splendiani, A., De Stasio, R., Odierna, G. (2016). Haplotype characterization of a young stranded Common Minke Whale (*Balaenoptera acutorostrata* Lacépède, 1804): is the Mediterranean Sea a potential calving or nursery ground for the species?. Hystrix, the Italian Journal of Mammalogy, 27(2), 205-208.
https://doi.org/10.4404/hystrix-27.2-11661

Miralles, L., Oremus, M., Silva, M. A., Planes, S., and Garcia-Vazquez, E. (2016). Interspecific hybridization in pilot whales and asymmetric genetic introgression in northern *Globicephala melas* under the scenario of global warming. PLoS ONE, 11(8), 1–15. https://doi.org/10.1371/journal.pone.0160080

Mirimin, L., Westgate, A., Rogan, E, Rosel, P., Read, A., Coughlan, J. and Cross., T. (2009) Population structure of short-beaked common dolphins (*Delphinus delphis*) in the North Atlantic Ocean as revealed by mitochondrial and nuclear genetic markers. Mar Biol 156, 821–834.
https://doi.org/10.1007/s00227-008-1120-y

Monteiro S.S., Mendez-Fernandez P., Piertney S., Moffat C.F., Ferreira M., Vingada J.V., Lopez A., Brownlow A., Jepson P., Mikkelsen B., Niemeyer M., Carvalho J.C., Pierce G.J. (2015) Long-finned pilot whale population diversity and structure in Atlantic waters assessed through biogeochemical and genetic markers. Marine Ecology Progress Series, 536 243-257. https://doi.org/10.3354/meps11455

Monteiro S.S., Vingada J.V., Lopez A., Pierce G.J., Ferreira M., Brownlow A., Mikkelsen B., Niemeyer M., Deaville R.J., Eira C., Piertney S. (2016) Major Histocompatibility Complex (MHC) class II sequence polymorphism in long-finned pilot whale (*Globicephala melas*) from the North Atlantic. Marine Biology Research, 12 (6), 595-607. https://doi.org/10.1080/17451000.2016.1174266

Monteiro, S. (2014) Population ecology of long-finned pilot whales (*Globicephala melas*) off the western coast of the Iberian Peninsula. PhD Thesis. Aveiro University http://repositorium.sdum.uminho.pt/handle/1822/34429

Morin PA, Archer FI, Avila CD, Balacco JR, Bukhman YV, Chow W, Fedrigo O, Formenti G, Fronczek JA, Fungtammasan A, Gulland FMD, Haase B, Peter Heide-Jorgensen M, Houck ML, Howe K, Misuraca AC, Mountcastle J, Musser W, Paez S, Pelan S, Phillippy A, Rhie A, Robinson J, Rojas-Bracho L, Rowles TK, Ryder OA, Smith CR, Stevenson S, Taylor BL, Teilmann J, Torrance J, Wells RS, Westgate AJ, Jarvis ED. (2021). Reference genome and demographic history of the most endangered marine mammal, the vaquita. Molecular Ecology Resources, 21(4), 1008-1020. https://doi.org/10.1111/1755-0998.13284

Morin, PA, Foote, AD, Baker, CS, Hancock-Hanser, BL, Kaschner, K, Mate, BR, Mesnick, SL, Pease, VL, Rosel, PL, Alexander, A (2018). Demography or selection on linked cultural traits or genes? Investigating the driver of low mtDNA diversity in the sperm whale using complementary mitochondrial and nuclear genome analyses. Mol Ecol. 27: 2604– 2619. https://doi.org/10.1111/mec.14698

Moura AE, Nielsen SCA, Vilstrup JT, Moreno-Mayar JV, M. Gilbert TP, Gray HWI, Natoli A, Möller L, Hoelzel R. 2013. Recent Diversification of a Marine Genus (*Tursiops spp.*) Tracks Habitat Preference and Environmental Change. Systematic Biology 62 (6): 865–877, https://doi.org/10.1093/sysbio/syt051

Moura AE, Shreves K, Pilot M, Andrews KR, Moore DM, Kishida T, Möller L, Natoli A, Gaspari S, McGowen M, Chen I, Gray H, Gore M, Culloch RM, Kiani MS, Willson MS, Bulushi A, Collins T, Baldwin R, Willson A, Minton G, Ponnampalam L, Hoelzel AR. (2020). Phylogenomics of the genus Tursiops and closely related Delphininae reveals extensive reticulation among lineages and provides inference about eco-evolutionary drivers. Mol. Phylogenet. Evol. 146, 106756. https://doi.org/10.1016/j.ympev.2020.106756

Moura, A. (2010). Investigating the relative influence of genetic drift and natural selection in shaping patterns of population structure in Delphinids (*Delphinus delphis; Tursiops spp.*), Durham theses, Durham University. http://etheses.dur.ac.uk/755/

Moura, A.E., Natoli, A., Rogan, E., and Hoelzel, A.R. (2013). Atypical panmixia in a European dolphin species (*Delphinus delphis*): Implications for the evolution of diversity across oceanic boundaries. Journal of Evolutionary Biology 26: 63-75. https://doi.org/10.1111/jeb.12032

Natoli A, Peddemors VM, Hoelzel AR. 2004. Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. J Evol Biol. 17: 363-375. https://doi.org/10.1046/j.1420-9101.2003.00672.x

Natoli, A., Birkun, A., Aguilar, A., Lopez, A. and Hoelzel, A. R. 2005. Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). Proceedings of the Royal Society of London B Biological Sciences 272: 1217-1226. https://doi.org/10.1098/rspb.2005.3076

Natoli, A., Cañadas, A., Vaquero, C., Politi, E., Fernandez-Navarro, P., and Hoelzel, A. R. (2008). Conservation genetics of the short-beaked common dolphin (*Delphinus delphis*) in the Mediterranean Sea and in the eastern North Atlantic Ocean. Conservation Genetics, 9(6), 1479–1487. https://doi.org/10.1007/s10592-007-9481-1

Notarbartolo di Sciara, G., Zanardelli, M., Jahoda, M., Panigada, S., and Airoldi, S. (2003). The fin whale *Balaenoptera physalus* (L. 1758) in the Mediterranean Sea. Mammal Review, 33(2), 105–150. https://doi.org/10.1046/j.1365-2907.2003.00005.x

Nykänen M, Louis M, Dillane E, Alfonsi E, Berrow S, O'Brien J, Brownlow A, Covelo P, Dabin W, Deaville R, de Stephanis R, Gally F, Gauffier P, Ingram SN, Lucas T, Mirimin L, Penrose R, Rogan E, Silva MA, Simon-Bouhet B, Gaggiotti OE. 2019. Fine-scale population structure and connectivity of bottlenose dolphins, *Tursiops truncatus,* in European waters and implications for conservation. Aquat Conserv. 29: 197-211. https://doi.org/10.1002/aqc.3139

Onoufriou AB, Gaggiotti OE, de Soto NA, McCarthy ML, Morin PA, Rosso M, Dalebout, M, Davison, N, Baird, RW, Baker, CS, Berrow, S, Brownlow, A, Burns, D, Caurant, F, Claridge, D, Constantine, R, Demaret, F, Dreyer, S, Đuras, M, Durban, JW, Frantzis, A, Freitas, L, Genty, G, Galov, A, Hansen, SS, Kitchener, AC, Martin, V, Mignucci-Giannoni, AA, Montano, V, Moulins, A, Olavarría, C, Poole, MM, Reyes Suárez, C, Rogan, E, Ryan, C, Schiavi, A, Tepsich, P, Urban R., J, West, K, Olsen, MT, Carroll, EL. 2022. Biogeography in the deep: Hierarchical population genomic structure of two beaked whale species. Global Ecology and Conservation 40: e02308. https://doi.org/10.1016/j.gecco.2022.e02308

Palsbøll, P. J., Bérubé, M., Aguilar, A., Notarbartolo-Di-Sciara, G., and Nielsen, R. (2004). Discerning between recurrent gene flow and recent divergence under a finite-site mutation model applied to North Atlantic and Mediterranean Sea fin whale (*Balaenoptera physalus*) populations. Evolution., 58(3), 670. https://doi.org/10.1554/02-529

Pastene, L.A., Goto, M., Kanda, N., Zerbini, A.N., Kerem, D.A.N., Watanabe, K., Bessho, Y., Hasegawa, M., Nielsen, R., Larsen, F. and Palsböll, P.J., 2007. Radiation and speciation of pelagic organisms during periods of global warming: the case of the common minke whale, *Balaenoptera acutorostrata.* Molecular Ecology, 16(7), pp.1481-1495. https://doi.org/10.1111/j.1365-294X.2007.03244.x

Pierce GJ, Petitguyot MAC, Gutierrez-Muñoz P, Fariñas-Bermejo A, Fernández-Fernández D, Dolman S, Fontaine MC, Hernández-González A, López M, Read FL, Martínez-Cedeira J, Covelo P, Ivaylova S, Llavona A, López A, Méndez-Fernández P, Pérez Fernández B, Saavedra C, Santos MB, Verutes GM, Viñas L, Weir CR (2022) An endangered population of harbour porpoise *Phocoena phocoena* hidden in plain sight: biology, ecology and conservation of the Iberian porpoise. Oceanography and Marine Biology - An Annual Review, submitted (preprint available via MC Fontaine).

Rosel PE, Dizon AE, Heyning JE. 1994. Genetic analysis of sympatric morphotypes of common dolphins (*genus Delphinus*). Mar Biol. 119(2): 159–167. https://doi.org/10.1007/BF00349552

Rosel, P.E., Dizon, A.E. Haygood, M.G. 1995 Variability of the mitochondrial control region in populations of the harbour porpoise, *Phocoena*, on interoceanic and regional scales, Canadian Journal of Fisheries and Aquatic Sciences, 52(6), 1210-1219. https://doi.org/10.1139/f95-118

Rosel, P.E., Frantzis, A., Lockyer, C., Komnenou, A. 2003 Source of Aegean Sea harbour porpoises, Mar. Ecol. Prog. Ser., 247: 257-261. https://doi.org/10.3354/meps247257

Schleimer, A (2021). Population biology of fin whales: applying demographic and evolutionary approaches to studying populations. PhD thesis. University of St Andrews and University of Groningen. Available at: https://research.rug.nl/en/publications/da510173-1b02-4b0e-a6ff-7afc7d1cb09e

Sequeira M. Harbour porpoises, *Phocoena phocoena*, in Portuguese waters. Rep Int Whaling Comm. 1996;46:583–6.

Smeenk, C., Leopold, M. F., and Addink, M. J. (1992). Note on the harbour porpoise *Phocoena phocoena* in Mauritania, West Africa. Lutra, 35, 98-104.

Sönmez,R., Tuncer, S., Yokeş, B. Genetic analysis of a stranded Risso's dolphin from the Turkish North Aegean coast of the Eastern Mediterranean. 2012. Journal of Agriculture and Rural Development. https://uard.bg/files/custom_files/files/documents/New%20knowledge/year1_n4/paper_sonmez_y1n4.pdf

Stockin, K.A., Amaral, A.R., Latimer, J., Lambert, D.M. and Natoli, A. (2014), Population genetic structure and taxonomy of the common dolphin (*Delphinus sp.*) at its southernmost range limit: New Zealand waters. Mar Mam Sci, 30: 44-63. https://doi.org/10.1111/mms.12027

Tardy C, Planes S, Jung JL, Ody D, Boissin E. (2020). Characterization of 25 new microsatellite markers for the fin whale (*Balaenoptera physalus*) and cross-species amplification in other cetaceans. Mol Biol Rep. 47(9):6983-6996. https://doi.org/10.1007/s11033-020-05757-0

Tardy, C (2021). Étude des grands mammifères marins en Méditerranée pour mieux adapter les politiques de conservation. PhD thesis. Ecole Pratique des Hautes Etudes and Centre de recherches insulaires et observatoire de l'environnement et WWF-France.

Tardy, C., Ody, D., Gimenez, O., and Planes, S., 2023. Abundance of fin whales (*Balaenoptera physalus*) in the north-western Mediterranean Sea, using photo-identification and microsatellite genotyping. Marine Ecology, 00, e12737. https://doi.org/10.1111/maec.12737

Tolley KA and Rosel PE (2006). Population structure and historical demography of eastern North Atlantic harbour porpoises inferred through mtDNA sequences. Marine Ecology Progress Series 327: 297-308. https://doi.org/10.3354/meps327297

Tonay AM, Dede A, Maracı Ö, Bilgin R. 2012. A preliminary genetic study on the harbour porpoise (*Phocoena phocoena*) in the Turkish Seas. J. Black Sea/Mediterr. Environ. 18:83-89 https://blackmeditjournal.org/volumes-archive/vol18-2012/vol-18-2012-no-1/a-preliminary-genetic-study-on-the-harbour-porpoise-phocoena-phocoena-in-the-turkish-seas/

Tonay AM, Karaman K, Dede A, Danyer E, Aytemiz Danyer I, Uzun B, Meray Y, Deval C, Ozturk Amaha A, Bilgin R. 2019. Genetic investigation on Cuvier's beaked whale, *Ziphius cavirostris* on the coast of Turkey and Northern Cyprus, based on mtDNA sequences. World Marine Mammals Conference, 9-12 December 2019, Barcelona, 437pp.
https://drive.google.com/file/d/1O9TlRRCh0aO___eOkd51WTWts02DHraS/view?usp=sharing

Tonay AM, Uzun B, Dede A, Amaha Öztürk A, Danyer E, Aytemiz Danyer I, Bilgin S, Öztürk B, Bilgin R. 2020. Population genetic structure of the short-beaked common dolphin from the Black Sea and the Turkish Straits System. Mitochondrial DNA A DNA Mapp Seq Anal. 31(6):257-264
https://doi.org/10.1080/24701394.2020.1788008

Tonay AM, Uzun B, Dede A, Danyer E, Aytemiz Danyer I, Öztürk AA, Bilgin R. 2018. Genetic structure of the bottlenose dolphin (*Tursiops truncatus*) in the Turkish waters based on mtDNA sequences. 5th Ecology and Evolutionary Biology Symposium 18 -20 July 2018, İzmir Türkyie, 20-21pp.

Tonay AM, Yazıcı Ö, Dede A, Bilgin S, Danyer E, Aytemiz I, Maracı Ö, Öztürk AA, Öztürk B, Bilgin R. (2017). Is there a distinct harbor porpoise subpopulation in the Marmara Sea? Mitochondrial DNA. Part A, DNA Mapping, Sequencing, and Analysis. 28(4):558-564. https://doi.org/10.3109/24701394.2016.1155118

Uzun B, Tonay AM, Dede A, Danyer E, Danyer Aytemiz I, Öztürk Amaha A, Öztürk B, Bilgin R. 2018. Genetic investigation on the population structure of the harbour porpoises living in Turkish waters by double digest restriction associated DNA (ddRAD) sequencing. 32. Conference of European Cetacean Society, 6-10 April 2018, La Spezia, Italy. 79pp.
https://www.europeancetaceansociety.eu/sites/default/files/27253%20ABSTRACT%20BOOK_nuovo_LR.pdf

Uzun, B., Tonay, A.M., Dede, A., Danyer, E., Aytemiz, I., Bilgin, S., Öztürk, A.A., Öztürk, B., Bilgin, R. (2017) Do we need to conserve the harbour porpoise (*Phocoena phocoena*) population in the Marmara Sea separately? 31st European Cetacean Society Conference, Middelfart, Denmark. 154p.
https://www.europeancetaceansociety.eu/sites/default/files/AbstractBook_1.pdf

Valsecchi E., William Amos, Juan Antonio Raga, Michela Podesta, and William Sherwin. 2004. The effects of inbreeding on mortality during a morbillivirus outbreak in the Mediterranean striped dolphin (*Stenella coeruleoalba*). Animal Conservation, 7: 139–146. https://doi.org/10.1017/S1367943004001325

Verborgh, P. 2015. Demografía y estructura de las poblaciones de calderones comunes (*Globicephala melas*) en el Mediterráneo español. PhD Thesis. Universidad de Las Palmas de Gran Canaria.
https://accedacris.ulpgc.es/handle/10553/24377

Verborgh, P., Gauffier, P., Esteban, R., Giménez, J., Cañadas, A., Salazar-Sierra, J. M. and de Stephanis, R. 2016. Conservation Status of Long-Finned Pilot Whales, *Globicephala melas*, in the Mediterranean Sea. In: Notarbartolo Di Sciara, G., Podestà, M. and Curry, B. E. (eds), Mediterranean Marine Mammal Ecology and Conservation. Advances in Marine Biology, vol. 75, pp. 173-203. Academic Press, Amsterdam.
https://doi.org/10.1016/bs.amb.2016.07.004

Verborgh, P., Gauffier, P., García Tiscar, S., Salazar, J.M., Esteban, R., Minvielle-Sebastia, L., Ridoux, V., Dabin, W., Martínez Cedeira, J.A., López, A., Ipiña, E.M., Monteiro, S.S., Ferreira, M., Berrow, S., Fossi, M.C., Marsili, L., Laran, S., Praca, E., Cañadas, A., Murcia, J.L., García, P. and de Stephanis, R. in prep. Population structure of long-finned pilot whales in Europe. Marine Ecology Progress Series.

Viaud-Martinez KA, Brownell Jr RL, Kommenou A, Bohonak AJ. 2008. Genetic isolation and morphological divergence of Black Sea bottlenose dolphins. Biol Conserv. 141: 1600-1611.
https://doi.org/10.1016/j.biocon.2008.04.004

Viaud-Martínez KA, Martínez Vergara M, Goldin PE, Ridoux V, Öztürk AA, Öztürk B, Rosel PE, Frantzis A, Komnenou A, Bohonak AJ (2007) Morphological and genetic differentiation of the Black Sea harbour porpoise *Phocoena phocoena*. Marine Ecology Progress Series 338, 281-294.
https://doi.org/10.3354/meps338281

Viaud-Martínez, K.A., Vergara, M.M., Gol'din, P.E., Ridoux, V., Öztürk, A.A., Öztürk, B., Rosel, P.E., Frantzis, A., Komnenou, A. and Bohonak, A.J., 2007. Morphological and genetic differentiation of the Black Sea harbour porpoise *Phocoena phocoena*. Marine Ecology Progress Series, 338, pp.281-294.
https://doi.org/10.3354/meps338281

Violi, B., de Jong, M. J., Frantzis, A., Alexiadou, P., Tardy, C., Ody, D., de Stephanis, R., Giménez, J., Lucifora, G., e Silva, M. A., Oliveira, C., Alves, F., Dinis, A., Tejedor, M., Fernández, A., Arregui, M., Arbelo, M., Lopez, A., Covelo, P., and Hoelzel, A. R. (2023). Genomics reveals the role of admixture in the evolution of structure among sperm whale populations within the Mediterranean Sea. *Molecular Ecology*, 32, 2715–2731. https://doi.org/10.1111/mec.16898

Appendix 2

Suitable genetics labs in the ACCOBAMS area [November 2023]

Note: this non-exhaustive list was based on the information received from ACCOBAMS NFPs and workshop participants until October 2022, and updated in November 2023 (other laboratories might be suitable in each Party)

PARTY	LABORATORY FACILITIES	CONTACT
Croatia	Department of Biology, Faculty of Science, University of Zagreb, Zagreb	Ana Galov: ana.galov@biol.pmf.hr
Egypt	Zoology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo	hamdy.ali.hamdy@gmail.com
France	Laboratoire de recherche: CRIOBE, UAR3278 - CRIOBE - CNRS - EPHE - UPVD, Perpignan	Serge Planes: planes@univ-perp.fr
	Laboratoire de recherche: MIVEGEC UMR IRD224 - CNRS5290 - University of Montpellier, Montpellier	Michael Fontaine: michael.fontaine@cnrs.fr
Greece	Molecular Biology of Marine Mammals Conservation and Fish stocks, Dept. of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, Volos	Georgios A. Gkafas : gkafas@uth.gr
Italy	University of Siena, Departments of Department of Environmental, Earth and Physical Sciences, Siena	Maria Cristina Fossi: fossi@unisi.it Cristina Panti: panti4@unisi.it
	University of Padova, Department of Comparative Biomedicine and Food Science - Mediterranean Marine Mammals Tissue Bank, Padova	Cinzia Centelleghe: marinemammals.bca@unipd.it
Malta	Conservation Biology Research Group, Department of Biology, University of Malta, Msida	Adriana Vella: adriana.vella@um.edu.mt
Portugal	Instituto Gulbenkian de Ciência (IGC), Population and Conservation Genetics group, Oeiras	Lounes Chikhi: chikhi@igc.gulbenkian.pt Inês Carvalho: carvalho.inesc@gmail.com
	Centro de Estudos do Ambiente e do Mar (CESAM), Campus Universitário de Santiago, Aveiro	geral@ua.pt
	Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Porto University, Matosinhos	Filipe Castro: filipe.castro@ciimar.up.pt
Slovenia	Morigenos – Slovenian Marine Mammal Society, Piran	Tilen Genov: tilen.genov@gmail.com ,morigenos@morigenos.org

PARTY	LABORATORY FACILITIES	CONTACT
Spain	Central Service for Experimental Research, University of Valencia, Valencia	Amparo Martinez: amparo.martinez@uv.es
	Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia	Juan Antonio Raga: toni.raga@uv.es
	Department of Functional Biology, University of Oviedo, Oviedo	Álvaro Jesús Obaya González: dpto.biofun@uniovi.es
	[COMMERCIAL COMPANY] All Genetics and Biology SL, A Coruña	info@allgenetics.eu
	[COMMERCIAL COMPANY] Macrogen SPAIN, Madrid	info-spain@macrogen.com
Tunisia	Le Laboratoire de Biodiversité Marine, Institut National des Sciences et Technologies de la Mer (INSTM), centre de Monastir, Monastir	Olfa Chaieb: offachaieb@yahoo.fr
Türkiye	Istanbul University Faculty of Aquatic Sciences, İstanbul	Arda M. Tonay: atonay@istanbul.edu.tr
	Zonguldak Bulent Ecevit University, Faculty of Science and Arts, Department of Biology	Mustafa Sözen: spalaxtr@hotmail.com mustafasozen@beun.edu.tr
Ukraine	Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv	Pavel Gol'din: pavelgoldin412@gmail.com
	Mechnikov Odesa National University, Odesa	Sabina Chebotar: kafgen@onu.edu.ua
	Karazin Kharkiv National University, Kharkiv	Oleksandr Zinenko: oleksandrzinenko@gmail.com

Appendix 3

Example laboratory protocols for DNA extraction from tissue

A) The Ammonium acetate precipitation method

1. Place a small piece of tissue sample (exact amount varies by tissue type and target DNA amount) in a 1.5 ml flip-top tube; make sure the sample is at the bottom of the tube, centrifuge if needed
2. Add 125 l DigSol buffer and Proteinase K mix to the sample (the mix should have a ratio of 250 µl Digsol buffer and 1 0µl Proteinase K (10 mg/ml)); close lid and centrifuge briefly
3. Place in an oven at 56°C for digestion *e.g.* overnight
4. Once digested, briefly centrifuge and add 300 µl 4M ammonium acetate to each sample for precipitation of proteins
5. Place sample tubes/plates on a shaker or vortex over a period of at least 15 minutes at room temp. to precipitate the proteins
6. Label new tubes used for transfer in the following steps
7. Centrifuge samples for 10 minutes at 15,000 rpm
8. Aspirate supernatant (clear liquid containing the DNA) into clean labelled 1.5 ml flip-top tubes (discard the precipitated protein stuff which usually pellets on the bottom although could be floating on the top)
9. Add 1 ml 100% ethanol
10. Close lids and invert tubes gently several times (20x) to precipitate DNA
11. Centrifuge for 10 minutes at 15,000 rpm
12. Pour off ethanol taking care not to lose DNA pellet
13. Add 500 µl 70% ethanol and invert several times to rinse pellet
14. If the pellet dislodges from the bottom of the tube centrifuge for 5 minutes at 15,000 rpm
15. Pour off ethanol and stand tubes upside-down on clean tissue (approx. 30-60 minutes)
16. Once fully dry add approx. 100 µl T10 E0.1 (the amount added is dependent on the size of the pellet)
17. Flick sample to dislodge pellet
18. Place tubes in waterbath or oven for 30 minutes (50°C) to dissolve pellet (flicking/vortexing every 10 mins)
19. Store at -20°C degrees (long term) or 4°C degrees (short term)

Preparation of Solutions

1M Tris-base (mol. wt. 121.1 g) pH 8.0
For 200 ml:

- Dissolve 24.22 g in distilled water by stirring
- pH should be about 8.0
- Autoclave to sterilise

0.5M EDTA (mol. wt. 372.2 g) pH 8.0
For 200 ml:

- Dissolve 37.2 g in distilled water by stirring
- Will need to pH solution with NaOH whilst it is dissolving (in order for all EDTA to solubilise)

20% SDS
For 100ml:

- Add 20 g SDS (use autoclaved water as end solution cannot be autoclaved)
- Use a fume hood and wear a mask when weighing this powder

Digsol (Digestion Solution) pH 8.0 (Bill Amos and Josephine Pemberton)

RECIPE	STOCK	FOR 1000ml	FOR 200ml
20 mM EDTA	EDTA (0.5 M, pH 8.0)	40 ml	8 ml
120 mM NaCl	NaCl	6.85 g	1.37 g
50 mM Tris	Tris (1 M, pH 8.0)	50 ml	10 ml
Distilled water		810 ml	172 ml

- Warm all constituents until dissolved
- Autoclave to sterilise
- Add SDS

SDS (20%)		50ml	10ml

- pH with HCl if necessary

4M Ammonium Acetate pH 7.5
For 100 ml:

- Dissolve 30.83 g Ammonium acetate in distilled water
- Autoclave to sterilise
- If necessary pH with Glacial acetic acid

NB: Ammonium acetate is hydrophilic and therefore most of the stock chemical is very wet, however this does not seem to affect the extraction process.

T10 E0.1 (Low EDTA T.E. Buffer) pH 7.5-8.0
For resuspending DNA which will be used in PCR
Recipe for 400 ml
10 mM Tris → 4 ml of 1 M Tris (pH 8.5)
0.1 mM EDTA → 80 µl of 0.5 M EDTA (pH 8.5)

- pH if necessary
- Autoclave to sterilise

10 mg/ml Proteinase K

- In 1 ml aliquots in -20°C freezer

B) The Phenol/Chloroform method

[copied from https://www.thermofisher.com/]

Materials required

- Glycogen (20 µg/µL)
- 7.5 M NH_4OAc (ammonium acetate)
- Ice bucket
- Phenol:chloroform:isoamyl alcohol (25:24:1)
- 100% ethanol
- Dry ice or a -80°C freezer
- 70% ethanol

Protocol - Phenol | Chloroform extraction

1. Add one volume of phenol:chloroform:isoamyl alcohol (25:24:1) to your sample, and vortex or shake by hand thoroughly for approximately 20 seconds.

2. Centrifuge at room temperature for 5 minutes at 16,000 × g. Carefully remove the upper aqueous phase, and transfer the layer to a fresh tube. Be sure not to carry over any phenol during pipetting.

Proceed to "Ethanol precipitation", below.

Protocol - Ethanol precipitation

REAGENT	VOLUME
Glycogen (20 µg/µL)	1 µL
7.5 M NH4OAc	0.5 × volume of sample
100% ethanol	2.5 × (volume of sample + NH4OAc)

3. Add the reagents to the aqueous phase, in the listed order in the above table.

4. Place the tube at -20°C overnight to precipitate the DNA from the sample. Note: If you wish to continue with the protocol, place the tube in dry ice or at –80°C for at least 1 hour.

5. Centrifuge the sample at 4°C for 30 minutes at 16,000 × g to pellet the cDNA.

6. Carefully remove the supernatant without disturbing the cDNA pellet.

7. Add 150 µL of 70% ethanol. Centrifuge the sample at 4°C for 2 minutes at 16,000 × g. Carefully remove the supernatant.

8. Repeat Step 3 once. Remove as much of the remaining ethanol as possible.

9. Dry the cDNA pellet in a Thermo Scientific™ SpeedVac ™ concentrator for 2 minutes or at room temperature for 5–10 minutes.

10. Resuspend the cDNA pellet in 300 µL of TEN buffer by pipetting up and down 30–40 times.

11. Centrifuge briefly to collect the sample, and place the tube on ice.

Appendix 4

Example of Biological Material Transfer Agreement

Subject:

The purpose of this agreement is to set out the rules by which cetacean samples are exchanged for non-commercial research between the following two institutions.

The parties to this agreement are:

The **lending institution**, also referred to as the **'Provider':**

Represented by:

and the **receiving institution**, also referred to as the **'Recipient':**

Represented by:

A) The Provider agrees to lend to the Recipient the Biological Material (hereafter referred to as the 'Material') described below, to be used for the purpose described in (B and C):

- Number of samples and species
- Type of sample (skin, muscle, etc.)
- Preservation buffer/method
- Identification codes and sample information
- Methods for sample collection (including national sampling permits)

B) The Recipient agrees to perform the analysis described below for the purpose described in (C) and to ensure the safe custody of the Material until their full consumption or safe return to the lending institution.

- Type of analysis (ex: stable isotopes, genetics, contaminants, cause of death, disease etc.)

C) Purpose of the Biological Material Transfer:

1/ A concise description of the research project is provided below.
- Project name
- Project Reference and Funding Agency (when appropriate)
- Short summary

2/ A summary of the scientific methodology applied on samples is provided below. *[It should stipulate if the samples will be partially or completely consumed, or if the samples will be modified or transformed (for example, DNA or RNA extraction) or if a product will be derived (eg, cell culture)].*

D) Both parties agree to the following conditions:

3/ If particular protocols are needed before providing the Material [briefly describe], preparation cost will be supported by _____________ [*the **Provider** and/or the **Recipient***].

4/ The **Material** remains a property of the **Provider**/is donated to the **Recipient** [choose the appropriate option].

5/ The **Material** may only be used for the non-commercial research purpose described in (B) and (C). If the agreed purpose was to change after signing this Agreement, the **Recipient** would consult with the **lending institution** and seek its written approval, that will be annexed to this Agreement.

6/ If the **Material** has to be shipped, shipping fees will be supported by ____________ [*the **Provider** and/or the **Recipient***].

7/ **Both parties** agree to provide all relevant documentation for the legal exchange of biological samples for non-commercial research purposes, including but not limited to relevant CITES permits, and Nagoya protocol procedures.

9/ Any portion of the **Material** that was not used for the purpose specified in (B) and (C) must be returned to the **Provider**/will remain in storage at the r**eceiving institution** [*choose the appropriate option*].

When appropriate, the **Material** must be adequately packed and shipped to insure their safe return by registered or insured mail. The **lending institution** must be contacted before shipping. Shipping fees are chargeable to the **Recipient**. If no parts of the **Material** remain after the investigation, the **lending institution** must be notified accordingly.

10/ The **Recipient** agrees to ensure that Code labels should always be associated with the respective **Material** and not get lost.

11/ The **Recipient** is an "end-user" meaning that no part of the the **Material**, product of the the Material and data related to the the **Material** (species, origin, age, sex, lesions, ...) may be forwarded to a Third party, except after consultation and written approval of the **Provider**. This transfer might require specific authorizations.

12/ The **Recipient** is responsible for the safekeeping of the **Material** described in (A). The loss or damage of the **Material** must be immediately reported to the **Provider**.

13/ Co-authorship is the most correct way of acknowledging other people's contribution. The **Recipient** formally agrees to which researcher(s)/staff member(s) from the **lending institution** must be considered as co-author(s) in all reports, presentations and papers. All publications and reports should stipulate that the **Material** was provided by the **lending institution** including the projects/Funding Agencies to be acknowledged.

- Expected outcomes [*including BSc/MSc/PhD dissertations, scientific publications, conference proceedings, reports to National or International authorities, etc.*]
- Co-authors from the lending institution to be included in all publications, presentations and reports resulting from this project: Dr./Mrs./Mr.
- Projects/Funding Agencies to be acknowledged
- Other people to be acknowledged by name

14/ Published results should be communicated to the **lending institution**; a digital copy of all papers should be sent to the **lending institution**.

15/ In order to avoid duplication of future work, all raw data should be sent to the **lending institution** (*e.g.* – results of pollutant analysis, results of isotopic signatures, genetic sequences, etc). The **Recipient** will retain co-authorship of these data. Any institutional use of these data (*e.g.* National or International reports requested by State Authorities) will be preceded by an authorization request made by the **lending institution** to the **Recipient** that was responsible for the data production.

16/ The **Recipient** will provide training to the Provider on [subject].

This agreement is effective on the date of ___________________ and will terminate on (1) completion of the research project, (2) on return of the samples to the **lending institution**, (3) upon any breach of the terms of this agreement by the **Recipient**, or (4) upon any request by the **lending institution** for the return of the samples [*choose appropriate options*].

Date:

From the **lending institution**:
Person:
Affiliation:
Phone:
Email:

Legally Represented by:
Affiliation:
Mail:
Signature of the legal represent of the Institution:

From the **receiving institution**:
Person:
Affiliation:
eMail:
Phone:

Legally Represented by:
Affiliation:
Mail:
Signature of the legal represent of the Institution:

ACCOBAMS Best Practices on Cetacean Population Genetics