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Best Practices for Collecting and Preserving Marine Mammal Biological Samples in the 'Omics Era

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Abstract

The recent rise of " 'omics" (and other molecular-based research technologies) and improved techniques for tissue preservation have broadened the scope of marine mammal research. These techniques require varying levels of sample quality and preservation methods, and collecting biological samples from wild marine mammals is both logistically challenging and expensive; few research groups possess the required field expertise. To improve the power of marine mammal research, great effort has been made in both the field and the laboratory to collect, preserve, transport, process, and archive samples in a manner that supports their efficient use in a broad range of 'omics studies. However, as this technological toolkit continues to evolve and expand, simply identifying the best methods at each stage can be challenging. Standardization of best practices among marine mammal researchers could maximize the scientific value of biological samples, foster multi-institutional collaborative efforts across fields, and improve the quality of individual studies by removing potential sources of error from the collection, handling, and preservation processes. With these aims in mind, we summarize the relevant literature, current knowledge, and best practices in each of these steps as a resource for scientists interested in exploring collaborative studies and preserving samples in a suitable manner for a broad spectrum of analyses, emphasizing support for 'omics technologies.

Introduction

The field of marine mammal biology has seen recent dramatic growth in the use of biological samples - primarily tissue, blow, and feces - for emerging " 'omics" technologies (e.g., genomics, metagenomics, metabarcoding, transcriptomics, proteomics, metabolomics and epigenomics) and the analysis of exogenous and endogenous elements (pollutants, toxins, stable isotopes, and hormones) (Cammen et al., 2016; Mancia, 2018; Nelms et al., 2021) (Figure 1). This growth has advanced our capability to address evolutionary (Apprill et al., 2020; Foote et al., 2019; Gui et al., 2013; McGowen et al., 2020a, 2020b), ecological (Arregui et al., 2018; Hooper et al., 2019; Polanowski et al., 2014), health (Jepson et al., 1999; Lawson et al., 2020), and conservation (Apprill et al., 2017; Nelms et al., 2021) questions that were previously left largely unaddressed for non-model organisms. Concurrent advances in archival techniques, supporting the preservation of samples for long periods of time with little degradation, inspire new opportunities to maximize the scientific potential of tissue samples and biological collections.

One of the major limitations to studies relying on marine mammal biological samples is the logistical difficulty and expense associated with field sampling, which can severely limit sample size (Bowen and Iverson, 2013; Dantzer et al., 2014; Deyarmin et al., 2019; Khudyakov et al., 2017). Marine mammals often inhabit remote environments, necessitating costly transportation. Further, extreme weather conditions, elusive behavior of the study species and the expertise required for remote sample collection methods all serve to limit sample numbers. The often remote or resource limited field sites challenge sample handling, processing, and preserving). On the whole, these challenges and costs amplify the inherent value of every sample, especially in instances where the target species or population is on the verge of extinction.

Among the scientific research community, there is a strong desire to maximize the use of biological samples due to the logistical and financial costs associated with sampling marine mammals, and a conservative sampling approach out of consideration for the health and welfare of study species. Project leads often go to great lengths to maximize the use of individual biological samples, sampling and subsampling tissue biopsies to support multiple chemical and molecular studies (e.g. Bechmann et al., 2021). Many such studies require the collaboration of several institutions, both national and international, to achieve the sample size and geographic or taxonomic breadth needed to be successful (Apprill et al., 2020; Baker et al., 2013; Bik et al., 2016; Dudek et al., n.d.; Herman et al., 2008; Krahn et al., 2007; Morin et al., 2021b; Parsons et al., 2013; Van Cise et al., 2019). Further, interdisciplinary approaches are increasingly preferred to address research questions (e.g. population structure, evolution, individual or population health and fitness) (Bahamonde et al., 2016), as ensemble methods are often better suited to address function, mechanism and/or causation (Hasin et al., 2017). As methods advance, and novel approaches are conceived to address new questions, the development of a standardized set of best practices for the collection, processing, and long-term storage of marine mammal biological samples will allow scientists to capitalize on these developments to address complex questions and incorporate historical samples to increase the scale and relevance of marine mammal research.

In the field, multiple considerations are routinely evaluated in the course of sample handling, and the increasing sensitivity of ‘omics approaches warrant particular consideration to protect sample integrity and avoid sample contamination (Kühn et al., 2020; Sepulveda et al., 2020; Taberlet et al., 1999). Wherever possible, adherence to a set of best practices for sample collection, processing, and storage will increase the breadth of sample utility and support sample longevity. This review summarizes contemporary information from current literature and ongoing studies on best practices for handling and storing specimens collected from marine mammals for use in ‘omics and other studies, to support the development of best practices that will not only suit short-term research objectives but also maximize the potential use of these samples in future conservation research.

General considerations

Samples collected from marine mammals most commonly include blood and other biofluids, tissue, feces, and keratin structures (e.g. whiskers, fur, baleen). In the field, potential sources of sample contamination include the marine environment, human handlers, processing location, as well as cross-contamination from other samples that may be collected concurrently. The protocols for collecting, processing, and storing samples may differ according to the type of biological material as well as intended sample use. However, many best practice techniques for sterilizing collection equipment and storing samples until archiving can be generalized across most types of biological samples and most research methods. In this section we provide a general overview of the best practice techniques for collecting and processing samples in the field, as well as long-term sample archiving, in an effort to contribute to the standardization of these techniques across the field of marine mammal research.

Many institutions will have IACUC (Institutional Animal Care and Use Committee) guidelines detailing approaches that should be followed for sampling instruments or equipment that will penetrate the body of a research animal. These protocols should be reviewed and followed when available. In addition to these protocols, which were created with the aim of protecting the health of the animals being studied, separate but complementary protocols are necessary to protect the samples, to ensure that samples are collected and processed in a way that avoids contamination by exogenous materials such as DNA or RNA, microbes, or other cross-contaminants.

Sterile techniques: an overview

The value of sterile techniques (defined here as methods intended to prevent the contamination of biological samples with exogenous DNA or RNA, microbes, environmental or other cross-contaminants) should be emphasized during each step of sample collection and processing (Khan et al., 2021; Weiss et al., 2014; Wong et al., 2012). However, while best practices for sterility in controlled laboratory settings are typically well-defined and standard operating procedures are established within research groups, maintaining sterility in the field can be challenging. Cleaning protocols and sample handling protocols also have a tendency to drift towards laxity over time or through a lack of documentation. While field site constraints and sample requirements may shift over time, establishing minimum requirements for sampling

handling and cleaning of reusable sampling equipment (e.g. tissue biopsy darts) is key for protecting sample integrity and documenting potential analytical limitations. 'Omics tools are rapidly increasing in sensitivity with technological advances, and the ability to generate millions of genomic sequences for each sample simultaneously increases the power to detect contamination and confound downstream analyses. Developing protocols that adopt a conservative approach to equipment sterilization and sample handling in the field and in the lab, and adhering to these protocols across field projects, will decrease downstream errors, increase opportunities for collaboration and support our future selves as we strive to adopt new methods and technologies.

Sample collection

Prior to sample collection, all researchers involved in sample collection should establish or review standard protocols for sterile collection of samples and proper tracking of sample nomenclature and metadata. At a minimum, metadata should include the date, time, sample number, latitude, longitude, field conditions, species, sex (if known), age class (if known), 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. When sampling from carcasses, note the amount of time that has passed since death, as this will affect data quality and downstream interpretability of results from these samples. Noting unavoidable deviations from sample handling protocols in sample metadata is valuable for reference during downstream troubleshooting, identification of sample anomalies, and data analyses.

When preparing reusable field equipment for use (e.g. stainless steel biopsy tips or forceps), all devices should be sterilized using the best available sterilization techniques. It is generally recommended that reusable, stainless steel sampling devices are sterilized using gas or steam sterilization (e.g. autoclaving) when possible prior to field deployment. Before and between uses, all devices should be thoroughly cleaned with hot water and detergent (or enzymatic cleaners) to remove visible debris, and thoroughly rinsed with potable freshwater (e.g. distilled or deionized water). Detergent can affect downstream extraction and analyses, therefore it is important to ensure all sampling equipment is thoroughly rinsed after cleaning. Sampling devices should then be sterilized using a *bleach and ethanol clean method* (e.g. Sinclair et al., 2015), by (1) soaking for 10 min in a 10% bleach solution, (2) rinsing with potable water, (3) rinsing with 95% EtOH (ethanol) or isopropanol (isopropyl alcohol), and finally (4) allowed to air dry before being used or packaged in an unused, sterile container for future use. Other solvents, such as DNA away or RNase away, will remove target compounds but may not be sufficient to remove all exogenous biological material and completely sterilize the equipment. Field researchers often choose to store sample collection equipment in sterile, single-use Whirl-Pak[1] bags, which can double as a sterile barrier between ungloved hands and the sampling equipment.

Limiting the number of people involved in sample collection will facilitate tracking of possible contamination during the collection process. Sample handlers should wear disposable gloves and surgical masks to protect both the person and the sample, particularly during the collection of blow samples, and both work surfaces (e.g. *bleach and ethanol clean method*) and

[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

sample handling tools (e.g. forceps, scalpel handles, biopsy darts) should be sterilized between samples (e.g., autoclaving or *bleach and ethanol clean method*) (Bykowski & Stevenson 2008, 2020). If field conditions prevent the use of gloves or other PPE, care should be taken to avoid touching equipment that contacts the sample, and to ensure sampling equipment does not come in contact with any non-sterile field equipment. Once collected, biological samples should be stored in a sterile, single-use vessel (e.g. Whirl-Pak, Falcon tube, cell-free blood collection tubes) and preserved as quickly as possible according to intended sample use.

Biological samples intended for 'omics studies (except those intended for tissue culture; see below) should be maintained in a stasis that reflects the moment of sampling as accurately as possible. Limiting the degradation of DNA, RNA and proteins, growth or shifting of microbial communities, and alteration of proteins or metabolites in sampled tissues are critical to minimize downstream errors and ensure the sample accurately reflects the time of sampling. The best available practice is to store biological samples in a portable dry shipper containing liquid nitrogen (LN_2) immediately upon collection, until they can be transferred to long-term archival at or below -80°C. 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. However, common household freezers (which support cooling to -20°C) equipped with automatic defrost cycles are often not appropriate: subjecting samples to multiple freeze-thaw cycles may degrade their quality or skew the resulting data (Cardona et al., 2012; Koopman et al., 2002; Trana et al., 2015). Additional options, including liquid preservatives (e.g. RNAlater, dimethyl sulfoxide [DMSO] or EtOH) can be useful in preserving samples for specific study methods without immediate freezing (Figure 2, Supplementary Table S1 and S2). When using liquid preservatives, it is valuable (e.g. particularly for microbiome studies) to simultaneously collect and store a negative control of the liquid to detect potential contamination from the reagent itself (Pollock et al., 2018; Salter et al., 2014). Fluid preservative selection may dictate acceptable long-term storage temperatures, and preservative specifications should be referenced prior to freezing. Samples frozen in LN_2 or to -80°C can be used for most currently available analysis methods, with the exception of cell culture, as well as sample-specific processing that must occur before freezing samples for metabolomics (such as separating plasma from whole blood; see *Metabolomics* section).

Sample processing

It is generally preferable to conduct tissue sample processing and subsampling in a sterile laboratory setting. However, in some cases biological samples may require additional subsampling or processing in the field before they are transported to their archival locations. In these cases, samples should be removed from the freezer for the minimum amount of time needed for subsampling. Sample processing in the field should also follow best practices for sterile techniques. This includes cleaning the work space and re-usable equipment before processing begins using the *bleach and ethanol clean method* (and, if available, exposure to a UV sterilization lamp for ≥15 minutes). If multiple samples are being processed, individual sterile fields should be prepared for each sample (e.g. by cleaning individual pieces of aluminum foil with bleach and EtOH, or by using sterile, single-use working surfaces such as disposable weigh boats). Sterilization and sample processing should be performed wearing disposable gloves (for microbiome samples, a mask should be used as well). Equipment used to process

samples (e.g. forceps or razor blades) may be re-used if re-sterilized between samples using the *bleach and ethanol clean method*. All subsamples should be transferred to labeled sterile vials and returned to the freezer as soon as possible to minimize sample degradation. If pathogens or other potential health conditions are suspected or known to be present, users should minimize their exposure by performing all handling and processing in a biosafety level 2 cabinet in a laboratory setting.

Archiving and Curation

Many factors will affect the selection of long-term sample archiving methods and ultimately impact both the success of sample preservation and the potential for downstream analyses. Variables including sample age and tissue type, field conditions and available facilities, storage conditions, handling, and transport all factor into the decision of how best to preserve valuable biological samples (Keighley et al., 2021). Long-term storage methods should account for potential contamination of a sample by its immediate storage environment (e.g. chemical contamination or adulteration of sample due to vial type) or by environmental factors specific to the archive (Waller, 1994). Cryopreservation is considered to be the most efficient method for preserving genomic material as all chemical and biological processes are halted and enzymes that result in degradation (e.g. RNase, DNase, proteinase) are inactivated by flash-freezing in liquid nitrogen (Nagy, 2010; Shabihkhani et al., 2014; Wong et al., 2012). Ideal long-term sample archives preserve tissue samples dry, in ultracold (-80°C or below) conditions, as fluid preservatives can contribute to unanticipated complications in the preservation and analytical processes (e.g. De Wit et al., 2012; Gamble, 2014; Kotrba and Schilling, 2017; Nagy, 2010). Some sample types may experience RNA degradation during long-term storage (>7 years) at -80°C (Chu et al., 2002), so preserving sub-samples in LN_2 specifically for future potential analyses of gene expression may be prudent where possible despite the increased difficulties in cost, safety, and transport presented by storage below -80°C (Babel et al., 2020 and see *Transcriptomics* section). 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, so extracting the molecules from samples prior to long-term archival is ideal.

Where ultracold or cryogenic storage is not feasible due to cost, logistical challenges, lack of facilities and/or shipping restrictions, alternative liquid preservatives such as lab-grade EtOH, DMSO solutions, or RNA*later* have been used successfully and may be appropriate for long-term archival of samples for some types of studies (Amos, 1997; Kilpatrick, 2002; Seutin et al., 1991; Zimkus et al., 2018); several of these preservatives can be stored at -20°C for additional sample protection (see manufacturer specifications for more details). When using liquid preservatives, it is important to consider that not all preservatives are compatible with all downstream applications (Figure 2 and Supplementary Table S2). Additionally, high-salt RNA/DNA preservatives (e.g. RNA*later*) will not penetrate frozen specimens, leaving them surrounded by, rather than preserved by, fluid, unless they are specifically formulated for frozen tissue (e.g., RNA*later* ICE) (De Wit et al., 2012; Gamble, 2014). While EtOH is highly effective for tissue preservation, the dehydrating effect of EtOHl causes the release of water from the specimen—diluting the fluid preservative which can have detrimental results if the fluid is not replaced with fresh EtOH one to two days after initial preservation (Gamble, 2014; Nagy, 2010). Additionally, biospecimen quality (e.g. protein and DNA stability) is inversely correlated with the

number of times the sample is thawed; researchers should limit the number of times a sample is thawed after collection and divide samples into smaller aliquots so that only a portion is thawed for a given experiment (Shabihkhani et al., 2014). Regardless of the fixative of choice, of key importance when preserving biological samples in liquid preservatives is sample to preservative ratio. In general, sample preservation should allow for at least five times the volume of fixative to tissue, particularly for very dense tissues or those with very high water content (Nagy, 2010). For some applications, samples may be stored dry or in a liquid preservative at -20°C (see Supplementary Table S2); however, it is again important to remember that commercially available freezers equipped with freeze/thaw cycles are not appropriate for long-term sample storage (Cardona et al., 2012; Koopman et al., 2002; Trana et al., 2015).

There are exceedingly few studies directly evaluating preservation media and processes. Instead, the majority of sample preservation protocols result from commonplace, or historical, practices passed down by word of mouth and developed based on logistical and/or financial considerations (e.g. Simmons, 2014). The longest-duration preservation experiment using marine mammal tissue to date was conducted over a 9 yr period (Kiszka et al., 2014), which pales in comparison to the decades some samples have been stored at many long-term archival storage collection facilities (e.g. Marine Mammal and Sea Turtle Research (MMaSTR) Collection at Southwest Fisheries Science Center, La Jolla, CA was established in 1989). Long-term evaluations are needed to understand how preservation decisions (e.g. cryogenic, EtOH, DMSO, etc) impact the various analysis methods, including genomics, trace element chemistry, proteomics, and other fields of analysis, although initial explorations have been made in some fields (Amos and Hoelzel, 1991; Castellini et al., 1992; Geraci and Medway, 1974; Lesage et al., 2010; McCormack et al., 2020), for particular sample types (e.g. epidermal, microbial, blubber, muscle, bone).

Curated tissue collections are increasingly encouraging the archiving of associated sample metadata as their value becomes more apparent; sharing all sample metadata, including sampling protocol and any deviations from the standardized protocol, will improve the utility of samples in the future, preserve the sample-metadata connection and support efforts to share samples across institutions.

‘Omics:

Sample preservation for sequencing and other ‘omic analyses

Genetics/Genomics

Sanger sequencing, or first generation sequencing (Sanger et al., 1977), has been the bedrock of marine mammal genetics for over three decades for species identification, phylogenetics and population genetics. Sanger sequencing is a flexible and somewhat forgiving technique that allows high quality genetic sequences up to about 1000 base pairs (bp) to be generated from marine mammal tissue and body parts that are degraded and/or stored in a variety of media. While a sterile environment is not required for sample preservation if processing is limited to Sanger sequencing, a clean workspace and specific protocols should be in place to minimize contamination between samples and maximize future potential for tissue samples and extracted DNA. For short term preservation (8-12 hours), samples intended for

Sanger sequencing can be preserved in sterile vials, or vials with EtOH or a 20% DMSO salt saturated (5M NaCl) solution, and stored temporarily in a cooler on wet or dry ice (Michaud and Foran, 2011; Mulcahy et al., 2016). For long term preservation, sample vials should be stored frozen, without preservative, at -80°C (Nagy, 2010). If ultracold freezer storage is unavailable, samples should be preserved in EtOH at -20°C, although one sample preservation study using fish tissue found that tissue stored in 20% DMSO degraded less quickly than tissue stored in EtOH, at ambient temperature (Oosting et al., 2020).

DNA used in next generation sequencing studies - e.g. GTseq (Campbell et al., 2015), RADseq (Andrews et al., 2016), or mitogenome sequencing - generally needs to be of higher quality than DNA sequenced using traditional Sanger sequencing due to the exponential increase in sensitivity and quantity of output data. Sterile sampling and processing techniques are particularly important for molecular genetic or genomic techniques involving second generation sequencing to avoid contamination. GTseq and mitogenome sequencing can be performed on tissue stored long term in both EtOH or 20% DMSO as well as vials without preservative that are frozen at -80°C. Protocols such as RADseq that rely on restriction enzymes to cut DNA at very specific places in the genome, may be affected by tissues stored long term in 20% DMSO at ambient temperatures, and preliminary assessment of archived samples may be warranted in some cases.

Standards for reference genome assemblies have evolved rapidly as technologies for long-read sequencing (e.g., PacBio and Nanopore sequencing) and chromatin linkage methods (Hi-C (Burton et al., 2013), optical mapping (Weissensteiner et al., 2017)) have allowed highly contiguous, complete, chromosome-length assemblies (Rhie et al., 2021; Whibley, 2021; Whibley et al., 2021). These methods all require very high-quality tissue samples to yield the ultra-high-quality DNA and chromatin structure, as well as RNA (preferably from multiple tissues) that can be sequenced to improve annotation of genes. Current best practices still call for cryopreserved fresh tissues, stored in liquid nitrogen or ultra-cold (-80°C freezers) without thawing until extraction, or cultured cells (Blom, 2021; Dahn et al., 2021; Morin et al., 2021a). However, as large genome consortia strive to generate reference genomes from more species, multiple preservation methods have shown promise for at least short-term preservation of ultra-high quality DNA, in particular EtOH and salt-saturated 20% DMSO with simple refrigeration for initial storage and transport, though DNA quantity and quality can vary by tissue type and storage conditions (Dahn et. al., 2021). Commercial preservatives such as Allprotect® (Qiagen), RNA*later* (Invitrogen) and DNAgard® (Sigma-Aldrich) performed less well, and DNAgard is not suitable for use with Hi-C library preparation, but these preservatives may be preferable in some cases to provide greater ease of use and transport (Dahn et. al., 2021).

Transcriptomics

Gene expression and high throughput sequencing of the transcriptome can be used to reveal gene activity at a particular moment in time; additionally, transcriptomics provides an extensive genomic resource for biomarker discovery. While DNA can remain relatively stable for a short period of time post collection, RNA is prone to rapid degradation after tissue sampling and appropriate sample preservation is critical to preserve integrity. In addition, gene expression continues in isolated tissues until preservation, and preserving the tissue as soon as possible is essential for capturing the transcriptomic profile at the moment of collection. The “gold standard”

for RNA sample preservation, for both transcriptomics and targeted gene expression studies, is cryopreservation, flash-freezing in liquid nitrogen or nitrogen vapor in a dry shipper, followed by long-term storage at -80°C or colder (Salehi and Najafi, 2014; Wong et al., 2012).

The greatest variability in RNA quality is introduced at the pre-analytical stage immediately after tissue collection. Temperature during transport, time elapsed between collection and stabilization in a buffer or transfer to ultra-low temperatures are all important factors affecting RNA integrity (Caixeiro et al., 2016; Choi et al., 2016; Vincek et al., 2003). In general, tissues that are frozen immediately after collection, with or without stabilization buffer, are shown to yield the highest quality RNA (Choi et al., 2016; Wong et al., 2012), however, RNA quality and gene expression profiles have been shown to be stable for up to 16h post-collection, as long as the tissue is kept on ice (Micke et al., 2006).

Collection and preservation methods resulting in high-quality RNA in sufficient quantities have been challenging due to limitations commonly associated with fieldwork conditions (Camacho-Sanchez et al., 2013; Gallego Romero et al., 2014; Kono et al., 2016). To overcome limitations associated with flash-freezing, a number of commercially available RNA stabilizing buffers have been developed including RNA*later* (Ambion), Allprotect® tissue reagent (Qiagen) and DNA/RNA Shield™ (Zymo Research), the latter two with capabilities to preserve multiple molecular components. According to the manufacturer's protocols, RNA*later* stabilizes RNA at room temperature (25°C) for 1 week and at fridge temperatures (4°C) for 1 month. Allprotect® tissue reagent stabilizes DNA, RNA and protein at room temperature (15-25°C) for 1 week and at fridge temperatures (2-8°C) for 12 months, however, it is cost prohibitive and harder to work with due to its viscosity (Salehi and Najafi, 2014). On the other hand, DNA/RNA Shield™ stabilizes RNA and DNA at ambient temperatures (4-25°C) for at least 1 month. Additionally, all three of these preservatives can be used for long-term storage of the tissue at -20°C or colder. In particular, RNA*later* has revolutionized field collection of tissue samples by enabling RNA preservation at ambient temperatures often much longer than recommended by the manufacturer (Caixeiro et al., 2016, 2016; Camacho-Sanchez et al., 2013; Gayral et al., 2011; Mutter et al., 2004). Moreover, samples preserved in stabilizing buffers before freezing are largely protected against degradation occurring during often inevitable freeze-thaw cycles (Nagy, 2010). However, there is some evidence that RNA*later* may elicit a physiological response resulting in a bias in transcriptome profiles in the form of enrichment for functional categories that are involved in RNA processing when compared with flash-frozen samples (Passow et al., 2019).

The selection of a preservation buffer also depends on the tissue type. While RNA*later* has been successfully used for skin tissue (B. Neely et al., 2018; Trego et al., 2020; Unal et al., 2018), solution density may prevent effective precipitation of other tissue types such as blow/chuff or cell pellets for further processing. On the other hand, DNA/RNA Shield™ allows precipitation of the cells while inactivating infectious agents. Liquid samples that stay in suspension can also be readily processed in DNA/RNA Shield™ without reagent removal, and has successfully been utilized for sample preservation for blow collections from belugas (Unal, *unpublished data*).

Metabolomics

Metabolomics assesses the small-molecule (≲ 1500 Da), chemical intermediates of metabolic reactions, i.e. *metabolites* (Liu and Locasale, 2017). Relative to the other primary 'omic fields (genomics, transcriptomics, & proteomics), metabolomics lies nearest to the biological phenotype (Wishart, 2019) and has been used in a variety of marine mammal applications, including characterizing metabolic changes following food restriction (Houser et al., 2021; Olmstead et al., 2017), responses to environmental perturbation/disturbance (Pasamontes et al., 2017), and discovering biomarkers of health status (Borras et al., 2017). Metabolomic samples collected from marine mammals most commonly include blood and other biofluids, tissue, feces, and keratin structures (e.g. whiskers, fur, baleen). The critical aspect in metabolomics studies is consistency—each sample should be collected, processed, and stored in an identical manner to minimize the effect of post-sampling alterations—generally processing samples and freezing them as rapidly as possible to minimize any changes post-sampling (e.g. continued metabolism).

Several biofluids, including whole blood and serum can be used in metabolomic studies, but blood plasma (collected in EDTA vacutainers) is the most common sample type (Kennedy et al., 2021). Care is needed to keep blood samples cool, but unfrozen, to prevent lysing of erythrocytes by avoiding direct contact of ice with the walls of the vacutainer. Delayed separation of hematocytes from plasma affects the measured metabolome (Jain et al., 2017), so samples must be centrifuged promptly (≲ 2 hours), in the field if possible. Other biofluid samples (e.g. urine, milk, and respiratory blow) must be collected into clean (non-sterile is acceptable) containers compatible with metabolite analyses (e.g. clean polypropylene or glass vials) (Giskeødegård et al., 2019). Best practice is to rapidly freeze plasma and other biofluid samples immediately on dry ice or in a liquid nitrogen dewar and store frozen at -80 °C until subsequent analyses (Smith et al., 2020). Ideally, samples should be shipped overnight on dry ice to a metabolomics facility for final processing, however a recent study found that freeze-thaw cycles and extended thawing at 4 °C had little effect on plasma metabolite levels (Kennedy et al., 2021).

For skin, blubber, and muscle tissues destined for metabolomic analyses, sterile, disposable biopsy tools should be used, but properly cleaned instruments (refer to 'General Considerations' section) would be appropriate in many circumstances (Sikes, 2016). Target tissue samples are frequently 'contaminated' with surrounding tissue at the time of extraction (e.g. blood, connective tissue); as with other methods such as transcriptomic and proteomics, it may be best to perform a rough cleaning of the sample tissue using dissection tools or rinsing in physiological saline before transferring to cryosafe vials (e.g. polypropylene tubes). This handling protocol should be consistent across all samples within the study and standard collection protocols should be used. Tissue samples are highly metabolically active and flash freezing in liquid nitrogen (preferred) or on dry ice and storing samples at -80 °C until analysis is necessary to stop cell metabolism.

A variety of fecal collection and storage methods have been used in metabolomic studies of non-marine mammals, with varying influences on metabolomic measurements (Karu et al., 2018). Marine mammal fecal collection is often opportunistic and generally occurs under varying field conditions. The method of fecal sample collection and processing (e.g. endoscopically sampled vs *ex vivo* sampling; processing in the field vs in the lab) can affect the measured metabolome, so consistent collection methods are important across a study (Couch

et al., 2013). To the extent possible, entire fecal samples should be collected using clean (non-sterile is acceptable) collection equipment free of contamination from previous samples, and stored in a clean container. Note that fecal samples can be heterogeneous, so collecting a partial fecal sample may not be representative of the entire sample (Santiago et al., 2014). After collecting a fecal sample, the entire bolus should be homogenized to avoid within-sample variance (Santiago et al., 2014), and sub-sample(s) transferred to a storage vial (e.g. clean polypropylene or glass vials). Samples can be stored in 95% EtOH at room temperature (Karu et al. 2018 recommend a 1:5 feces:EtOH (95%) ratio), or at ≤ -10 °C if available; the important factor is that all samples in a study be stored consistently. Samples can then be transported for analysis on dry ice (see Figure 1 and S1 in Karu et al. 2018). To our knowledge, metabolomics has not yet been performed on marine mammal fur, vibrissae, or other keratin compounds. Few specific sample collection demands would be required, but substantial development of appropriate washing and extraction techniques would be needed.

Microbiomes and metagenomics

A growing body of research highlights the value of examining the structure of microbial communities (microbiomes) associated with marine mammals, and their functional potential (metagenomes), to infer relationships between microorganisms, animal health and ecology (Apprill, 2017; Bik et al., 2016; Dudek et al., 2017; Nelson et al., 2015; Sanders et al., 2015; Van Cise et al., 2020). Adhering to best practices, including attention to sterile or aseptic technique, is essential for microbiome analysis, to prevent sample contamination from the marine environment (e.g., from seawater and sand), human handlers (e.g. from skin or exhalates), processing location, and other samples collected concurrently.

Careful study design is important in microbiome and metagenomics studies due to a greater number of potential sources of bias and variation. Study design will include a precise definition of the sample site, as subtle differences in body biogeography may influence microbial community composition (Costello et al., 2009; Donaldson et al., 2016; Jones et al., 2018; Proctor et al., 2018; Simón-Soro et al., 2013), or recoverable microbial biomass which affects susceptibility to contamination (Eisenhofer et al., 2019; Quince et al., 2017). Host species is also an important consideration to meet study objectives; whereas feces (frozen without preservative) has been successfully used to characterize the fecal metagenome of baleen whales (Sanders et al 2015), similar attempts in sea otters were instead reflective of the host's diet (Dudek, et al, 2022). Furthermore, collection methods as well as sampling approaches can influence the microbial community. For example, fecal samples collected from seawater will contain seawater-associated microbiota, and swabs collected from dead, anesthetized or captive animals may have altered community composition (Garber et al., 2020; Roy et al., 2021; Serbanescu et al., 2019; Wang et al., 2019).

To minimize sample contamination and protect sample integrity for microbiome analyses, sample handlers should wear disposable gloves and face masks and sterilize sampling surfaces and equipment between samples (Bykowski and Stevenson, 2020); e.g. using the *bleach and ethanol clean method* if autoclave or Bunsen burners are not available. During sampling campaigns, negative sampling controls should be collected to detect potential microbial contamination (Apprill, 2017; Apprill et al., 2014; Eisenhofer et al., 2019). For example, collecting samples from adjacent seawater and dietary items can facilitate microbial

source-tracking downstream (Apprill et al., 2014; Bik et al., 2016; Sanders et al., 2015). Laboratory negatives, in which no sample is present but otherwise all reagents and handling protocols are used, may also help account for reagent or other lab-based contamination that can be otherwise difficult to discern, especially in low-biomass samples.

The gold standard for preserving community structure for microbiome studies is immediate flash-freezing of samples on dry ice, liquid nitrogen or liquid nitrogen vapors without preservative, followed by storage at -80°C or lower (Choo et al., 2015; Goodrich et al., 2014; Rissanen et al., 2010). Freezing at -20°C will yield similar results (Bundgaard-Nielsen et al., 2018), but care should be taken to minimize the number of freeze-thaw cycles, as this can alter community composition and degrade DNA (Sergeant et al., 2012). If necessary, samples may be held frozen on dry or wet ice and transferred to a freezer immediately upon return to the lab (Apprill et al., 2014; Bik et al., 2016). Short-term refrigeration of samples (72 hrs) has been shown to yield comparable results to flash-freezing (Choo et al., 2015); however, a recent study suggested that refrigeration may alter community structure (Poulsen et al., 2021). Immediate processing of fresh samples, while considered by some as another best-practice technique, is not a practical option for most marine mammal researchers, so freezing prior to DNA extraction is recommended to promote comparability between studies (Poulsen et al., 2021).

While liquid preservative buffers may be used to stabilize the samples chemically at room temperature, there may be undesired side effects such as altering community composition and inhibiting potential future metabolomic analyses (Goodrich et al., 2014; Gorzelak et al., 2015). Kit-based OMNIgene-Gut is an appropriate alternative to flash freezing for metagenomic studies (Anderson et al., 2016; Ilett et al., 2019), although the Copan FLOQSwab-ADT method produced more robust results (Pribyl et al., 2021). RNAlater (Invitrogen) performed well for short-term storage at room temperature but lost stability after two weeks (Song et al., 2016) and has been shown to degrade DNA (Gorzelak et al., 2015). Long-term preservation in 95% EtOH is an acceptable and cost-efficient option but may result in reduced DNA yield. For fecal samples, fecal occult blood test cards (FOBT) are also an acceptable and accessible option (Byrd et al., 2020). Regardless of the chosen preservation approach, it is essential that all samples compared in an analysis are treated in a consistent manner.

Epigenetics

The study of how cells control gene activity without altering the genetic sequence, epigenetics, is a growing field providing insight into the effects of both environmental and behavioral stressors. Although still an emerging field in marine organisms, epigenetic analyses in marine mammals can extend our understanding of phenotypic plasticity or health and disease, and provide useful biomarkers of age and stress (Beal et al., 2019; Bors et al., 2021; Crossman et al., 2021; Horvath, 2013; Maegawa et al., 2010; Polanowski et al., 2014; Tanabe et al., 2020; Weaver et al., 2004).

The various carriers of epigenetic information include DNA methylation, histone modifications and non-coding RNAs, and best practices for sample handling and processing depend upon marker selection. *DNA methylation* refers to the addition of a methyl group to a cytosine residue in DNA that occurs almost exclusively at CpG dinucleotides (i.e., a cytosine located 5′ of a guanine). This strong covalent carbon-to-carbon bond is stable and relatively robust to degradation, and best practices for sampling are the same as those for genomic DNA

(see *Genetics/Genomics* section). In humans, DNA methylation has been shown to be stable in genomic DNA isolated from decades old dried blood spots (Staunstrup et al., 2016), up to 30 year old formalin-fixed paraffin-embedded (FFPE) tissues (Kristensen et al., 2009) and in human placental tissue at room temp for up to 24 hours (Vilahur et al., 2013). However, it should be noted that for some genome-wide approaches, DNA methylation quantification can be affected by DNA quality (Vilahur et al., 2013) and should be evaluated prior to use in epigenomic applications. *Histone modifications* are chemical tags attached to histone proteins, and are evaluated using chromatin immunoprecipitation combined with high-throughput sequencing (ChIP-Seq). ChIP-Seq approaches have been applied in marine mammals (Villar et al., 2015), but studies are sparse. It is considered best practice to crosslink freshly sampled tissues, although protocols have been developed for performing ChIP-Seq on FFPE samples (Cejas et al., 2016). As such, field sampling for histone modification analysis without laboratory support remains technically challenging as tissue samples must be processed prior to freezing or storing; processing these samples requires a multi-step, sterile protocol that is best performed in a laboratory setting (Schmidt et al., 2009). *Non-coding RNAs* (ncRNAs) play a role in modulating gene expression and can be isolated from tissues that have been preserved following best practices for sterile sampling (to avoid contamination) and processing of total RNAs (see *Transcriptomics*). In addition, total RNA that has been stored according to best practices (see *Transcriptomics*) can also be processed to analyze ncRNAs.

Proteomics

Proteomics provides a tissue-specific, proteome-wide survey of translated proteins in a sample, and is the study of protein “expression” to provide insight into an organism’s health and physiology at the time of sampling. Non-invasive sampling from living tissue can limit the hypotheses tested with this technology, but researchers have successfully applied proteomics in marine mammals to investigate, for example, health impacts of disease (e.g., Nelly et al., 2018), the impacts of captivity on physiology (e.g., Tian et al., 2020), and the impact of physical activity on physiology (e.g., Miller et al., 2017). Since proteins are the molecules that directly influence phenotypic change, proteomics is a powerful tool for better understanding marine mammal physiology.

Acquiring samples for proteomics from marine mammals – especially wild specimens – requires careful consideration of available tissues and how their analysis can support scientific inquiry. Similar to metabolomics, transcriptomics and epigenomics, proteomic profiles are tissue-specific since each tissue has its own specific function supporting the whole organism, and care must be taken to sample only the tissue of interest, avoiding contamination from other tissues. For most wild marine mammals, this narrows the tissues available for analysis to skin (B. Neely et al., 2018), blubber (Deyarmin et al., 2019; Kershaw et al., 2018; Khudyakov et al., 2018; B. Neely et al., 2018), chuff (Bergfelt et al., 2018), or urine/feces (B. A. Neely et al., 2018). For a very small number of species/populations with intensive health monitoring programs, muscle (Shero et al., 2019; Voisinet et al., 2015), blood/serum (Desoubeaux et al., 2019; Lazensky et al., 2021; Miller et al., 2017; Neely et al., 2013, 2015a; P. Sobolesky et al., 2016; P. M. Sobolesky et al., 2016; Tian et al., 2020), and cerebrospinal fluid (Neely et al., 2015b) provide additional sample types suitable for proteomics. Recently deceased animals can be sampled for proteomics (Kershaw et al., 2018), but proteins rapidly degrade in dead and dying

tissue and may not provide a high quality or accurate dataset. Similar to other methods, tools used to remove tissue samples for proteomics must be thoroughly cleaned and disinfected using the *bleach and ethanol clean method* between samples.

To preserve the proteome in its closest state to *in vivo* function, tissue specimens should be immediately flash frozen in liquid nitrogen or in an EtOH/dry ice slurry and then transferred to a -80°C freezer for long-term storage. Many *ex vivo* proteins degrade quickly, altering the proteome from its natural, functioning state, and inhibiting accurate interpretation of the specimen's physiological status. Protease inhibitors can be added to the tissue to decrease protein degradation rates. Alternatively, recent work in mammals and plants suggests that incubation in RNA*later* at room temperature prior to freezing at -80°C has minimal impact on the proteome (e.g. Bae et al., 2019; Bennike et al., 2016; Kruse et al., 2017). Tissue storage at -20°C has been successful in some marine mammal proteomics studies (Desoubeaux et al., 2019; Kershaw et al., 2018; B. A. Neely et al., 2018), but -80°C is the biomedical standard and storage at warmer temperatures measurably alters the proteome (Lee et al., 2010). Repeated freeze-thaw cycles can lead to sample degradation, but long-term storage, at least for up to four years, does not appear to impact proteome profiles in mammal serum (Mitchell et al., 2005) and proteomic profiles seem to be unimpacted for up to 30 years in paraffin-embedded human tissue (Balgley et al., 2009).

Beyond 'Omics:

Sample preservation for chemical, microparticle and small molecule analyses

Hormones

The vast majority of endocrine investigation of free-ranging marine mammals has focused on the relatively stable steroid class of hormones that are derived from cholesterol, including sex steroids (progesterone, androgens, estrogens), and corticosteroids (e.g. cortisol, corticosterone, aldosterone) (Champagne et al., 2018; Kellar et al., 2015, 2009; Mello et al., 2017; Mingramm et al., 2019). These lipophilic molecules are frequently studied in blubber (Kellar et al., 2015), but are also detectable in numerous alternative sample types including feces (Hunt et al., 2019; Rolland et al., 2012), skin (Bechmann et al., 2021), respiratory blow (Burgess et al., 2018, 2016), claws (Karpovich et al., 2020), vibrissae (Keogh et al., 2021), and can be collected from deceased subjects or museum specimens in baleen (Hunt et al., 2017, 2014), earplugs (Trumble et al., 2018), and teeth and bone (Charapata et al., 2018; Hudson et al., 2021). For brevity, this section will focus primarily on the collection and storage of blubber samples, currently the most common bio-sample used for hormone analyses.

Anatomical sample site can affect expected blubber hormone concentrations (Aguilar and Borrell, 1990; Deslypere et al., 1985; Kershaw et al., 2017; Koopman et al., 1996). Hormone concentrations in blubber reflect an average over the previous tens of minutes to tens of hours depending on the species and conditions associated with the individual prior to sampling (Beaulieu-McCoy et al., 2017; Champagne et al., 2018; Kellar et al., 2006). It is worth noting that, particularly with stress-related hormones (e.g., cortisol), sampling activities can alter the detected hormone levels if samples are not collected rapidly, i.e. within 60 minute or less (Champagne et al., 2018).

Blubber and fecal samples should be collected in clean (non-sterile is ok) collection jars or vials and placed immediately on dry ice or in liquid nitrogen (LN_2) dry shippers (Kley and Rick, 1984; Toone et al., 2013). If this is not possible, samples may be stored at temperatures below 0°C for up to 8 hours (e.g. in coolers with reusable ice packs), the duration of which will depend on the ambient temperature (Lemos et al., 2020). Respiratory hormones are best detected when blow is collected in polystyrene dishes and rinsed with EtOH before storing cold and later transferring to long-term storage at -20°C or -80°C (Burgess et al., 2018, 2016). To minimize problems associated with sample thawing and seeping of lipids, tissue biopsy subsampling is best conducted in a laboratory setting where tissues can be subsampled on a cold surface (4°C). Steroid hormone measurements are likely less sensitive to common contamination processes compared to other 'omics techniques described above (do Rego and Vaudry, 2016; Fang et al., 2016). Nonetheless, it is recommended to follow best practices to minimize sample contamination as there are common anthropogenic compounds that can bind to steroid receptors (Lilienthal et al., 2006).

The relative stability of steroid hormones allows some flexibility across a range of sample storage conditions (Bolelli et al., 1995; Hunt et al., 2017; Kellar et al., 2006). For interim storage and long-term archiving, blubber samples should be maintained at -80°C or below, although several studies have demonstrated usability of samples stored at -20°C for multiple years particularly for applications targeting large differences in hormone concentration (Kellar et al., 2009, 2006; Kley and Rick, 1984; Toone et al., 2013). Less is understood about the suitability of samples stored in hyper-concentrated salt solutions with DMSO, EtOH, or other preservative solutions and as such they are not recommended as these solutions may pull target hormones away from the tissue (Sheridan, 2004).

Stable Isotopes

Stable isotopic analyses are a powerful means to examine trophic ecology, inferring dietary preferences and trophic shifts predictably reflected in the ratio of stable isotopes in a consumer's tissues (Fry, 2006). Carbon stable isotope ratios (i.e., $^{13}C/^{12}C$ or $\delta^{13}C$), for example, change very little between diet and consumer, which makes this ratio a convenient tracer of habitats the consumer moved through. In contrast, nitrogen stable isotope ratios (i.e., $^{15}N/^{14}N$ or $\delta^{15}N$) become more enriched at each trophic level making it suitable for estimating a consumer's trophic position (Post, 2002). Recent advances in compound specific (i.e., amino and fatty acids) stable isotope analyses are further improving the understanding of metabolic processes and trophic relationships, providing increased resolution for specific amino acids (Dale et al., 2011).

Samples intended for stable isotope analyses should follow sterile handling techniques (see *General Considerations*) to avoid cross contamination among individuals or tissues (in the case when multiple tissue types are collected, e.g., blubber, organs, etc., from the same individual). Sterile handling techniques should also be used when subsampling and homogenizing tissue for stable isotope analysis, and modified to include rinsing all working surfaces and equipment with acetone prior to processing (Gates et al., 2020). To prevent tissue degradation during subsampling, thawing should be minimized by working on one sample at a time, on an ice block covered by acetone-rinsed aluminum foil, and returned immediately to a

-80°C freezer for a minimum of 1 hr prior to lyophilization, or for long-term storage (Gates et al., 2020).

Tissues collected for stable isotopic analyses are best stored frozen (Busquets-Vass et al., 2017; Kaehler and Pakhomov, 2001; Newsome et al., 2010). Burrows et al. (2014) reported that stable isotope values of killer whale skin remained stable for up to 14 days at 4°C or lower, and values were stable for at least a year when skin and blubber samples were stored at -20°C or -80°C. Although only minor advantages were reported when storing samples at -80°C rather than -20°C, -80°C is recommended for long-term storage. For animal carcasses, confidence in stable isotope values will be low without a known time of death or visual evidence indicating little or no postmortem tissue decay, as decomposition has been observed to significantly change δ^{15}N within 3 days (Burrows et al., 2014). Slight, non significant differences in stable isotope values were reported for lipid extracted cetacean skin samples when comparing samples that were frozen without preservative to those preserved in a 20% DMSO-salt solution and stored either frozen or at room temperature (Burrows et al., 2014; Lesage et al., 2010). However, these small effects may mask isotopic differences among population groups or species and it is advisable to avoid comparing isotopic signatures across samples stored using different methods (Burrows et al., 2014; Ruiz-Cooley et al., 2011).

Toxic Contaminants

Analysis of contaminants in marine mammal tissues is routinely conducted for legacy contaminants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (refs). In recent years, interest has increased in other types of pollutants such as perfluorinated compounds, often referred to as contaminants of emerging concern (CEC). The primary concern in tissue sampling and storage for contaminant analysis is preventing degradation of pollutant molecules by enzymatic and non-enzymatic pathways. This is best achieved by freezing samples as soon as possible after collection. Short-term (< 1 month) storage at -20°C is usually acceptable, with longer term storage at -80°C most desirable. If a sample cannot be immediately frozen upon collection (-80°C or LN_2), temporary storage in a 1°C cooler for a few hours is acceptable. In general, the use of preservative solutions such as formalin or RNA*later* should be avoided as they may leach or structurally alter analytes from the tissue (ref).

Additional important considerations for both sampling and storage include preventing contamination during collection, and ensuring uniformity and representativeness of the sample. Contamination can occur from deposition of airborne particulates and leaching of chemicals from the storage container. The latter can be prevented by thoroughly rinsing and cleaning all sampling and processing equipment prior to use. Aluminum foil is frequently used to wrap solid tissue samples because it is easily rinsed with solvent (e.g. acetone) and can be baked at 450° C for several hours prior to use. All commercially purchased tubes or other sampling supplies should be rinsed with solvent (e.g. acetone) and oven baked at 450° (if possible) prior to coming in contact with the sample.

The distribution of contaminants within a tissue is typically heterogeneous (ref); therefore, consideration of the sample representativeness is warranted and careful documentation of sampling location relative to the animal's body is key to ensure consistency with past and ongoing sampling efforts. If study resources permit, collecting multiple samples

from different regions of a large animal is helpful to assess the overall pattern of contaminant distribution among tissues.

Microplastics

The ingestion of microplastics (plastic particles < 5 mm in size) has been heavily documented in a wide range of marine species, from the base of the food chain to top predators (Cole et al., 2013; Maes et al., 2020; Nelms et al., 2019a; Setälä et al., 2014). Recent methodological advances allow microplastics analyses to be seamlessly integrated with fecal prey metabarcoding to investigate the relationship between microplastic ingestion, diet and trophic transfer, better informing our understanding of trophic level interactions (Nelms et al., 2019b).

Collecting fecal or gut content (during postmortem processing) samples for microplastics analyses requires strict contamination control protocols to ensure accurate results and avoid particle contamination in the field or laboratory. Key sampling practices to support the isolation of microplastic particles include (1) using non-plastic sampling equipment and ensuring all sampling equipment is free of both microplastics and biological contaminants that may affect metabarcoding, and thoroughly rinsed with pre-filtered Milli-Q water (2) limiting possible exogenous microplastic contamination during sample collection and/or accounting for it with field collection of negative controls, (3) monitoring airborne contamination by exposing damp filter paper to sample collection conditions, (4) processing samples in a laboratory setting with a laminar flow hood, and finally (5) storing scat or gut contents at -20°C or below for metabarcoding purposes (see Zantis et al., 2021 for a comprehensive review). Field scientists collecting samples intended for microplastic analysis should avoid wearing clothing with plastics or synthetic fibers to the extent possible during sample collection to limit sources of field contamination (Gwinnett and Miller, 2021). Generating a reference library of synthetic materials and clothing worn during sample collection may help to identify microparticle contamination downstream. Nelms et al (2019b) provides a practical framework for parallel isolation of microplastics and DNA for prey metabarcoding, demonstrating the potential value of extending the use of fecal samples collected from wild marine mammals beyond ‘omics.

Analysis of archived fecal samples can be challenging if protocols to avoid exogenous contamination were not adopted at the time of sample collection. However, archived and opportunistically collected samples represent valuable opportunities to examine temporal and spatial changes in microplastic particle exposure and consumption. Acknowledging potential sources of field contamination, maximizing sample numbers to minimize sampling artifacts, and capitalizing on opportunities for collecting contemporary samples using comparable methods that also incorporate field/lab controls provide avenues for minimizing the effects of contamination while generating valuable data on microplastics consumption by marine mammals.

Discussion

Technological advances in the lab allow scientists to derive ever greater amounts of information from the diverse array of biological samples collected from marine species. These advances consistently trend toward improved sensitivity and resolution; promising enhanced

ability to address previously intractable questions relevant to the conservation, management and health of wildlife populations. Concomitantly, this increased sensitivity exacerbates the risk of contamination during the sample handling process that can introduce irrevocable bias into a dataset. Adhering to and documenting sterile methods, both in the field and in the lab, goes a long way to ensure that sample quality is not compromised and that sample use can be maximized in current or future studies without fear of bias. Here, we summarize the best practice methods for preserving and archiving marine mammal biospecimens used in some of the most commonly used or innovative molecular methods. However, this list is by no means comprehensive and analytical approaches not listed here (e.g. targeted gene expression and fatty acid analysis) may require specific considerations for sample collection and preservation. Further, many preservative methods have not yet been rigorously validated for all analytical approaches; as the 'omics fields continue to evolve, so does the body of literature evaluating suitability of various preservatives for long-term tissue storage.

In the field, the use of sterile collection equipment is key both for the safety of the animal as well as to avoid contamination from other sources. Similarly, equipment used to process samples in the field should be sterilized with both bleach and EtOH (not one or the other) using the *bleach and ethanol clean method* before processing begins, and between each sample. The *Sterile Techniques* section above outlines an accessible procedure for sterilizing equipment prior to use, and method-specific recommendations are included in each methodological overview section. Personal protective equipment, such as disposable gloves, should be worn on both hands while working with samples in order to avoid contaminating the samples, and special consideration is warranted to protect the integrity of samples at high risk for contamination, such as samples used in microbiome or metabolomics studies. For most end-point methods, freezing samples without preservatives is recommended nearly universally (see Figure 2 and supplemental table for additional detail). Under ideal conditions, samples are frozen using LN_2 or a -80° C freezer immediately upon collection, and removed as briefly as possible for processing and sub-sampling. Although accessibility to dry shippers and sources of LN_2 are increasing, remote field sites, limited research budgets, and long-term field deployments often limit access to such resources. If immediate freezing is not possible, many analytical approaches are still possible using samples that are stored for short periods of time at -20°C (Figure 2). Preserving segments of unprocessed samples in appropriate liquid preservatives may support a suite of future analyses for samples archived at room temperature (see Figure 2 and method-specific sections for more details).

Through collaborative research and the standardization of sample archiving methods, the marine mammal research community can capitalize on technological developments that promise to increase the breadth and depth of marine mammal research. Proactive consideration of sample collection and preservation methods is especially important for critically endangered marine mammal species and populations where sampling efforts are increasingly difficult and time is limited. Considering the substantial investment in time and resources involved in marine mammal fieldwork, maximizing the use of biospecimens is most often a key consideration for research centers maintaining sample archives, and working towards standardized and documented collection and processing protocols supports these goals promising generalizable results and increasing opportunities for collaborative and interdisciplinary efforts.

A robust system for tracking samples, methods, and metadata, while not immediately related to sterile sampling techniques, is equally important in facilitating the use of samples in future studies. Many marine mammal studies make use of dozens if not hundreds of samples contributed by multiple collaborating research organizations (Nelson et al., 2012; Bik et al., 2016; Bierlich et al., 2017; Apprill et al., 2020; Vendl et al., 2020; and others). Integrating disparate sample sets can be challenging, and equally important to standardized sample collection and archiving are the metadata accompanying each sample (Kolker et al., 2014; Rajesh et al., 2021). Ideally, metadata will include (but are not limited to): detailed description of field conditions and location, collection method (including anatomical sample site), time lapsed before freezing, in-field processing techniques (noting deviations from standardized sterile techniques), duration and method of short-term storage, and the location and method for long-term archiving.

Capitalizing on sampling investments by collecting samples in a way that is compatible with new and emerging ‘omics technologies offers numerous avenues for expanding ongoing research studies and increasing resolution through interdisciplinary efforts. Rapid developments in molecular and chemical assays offer new insights into marine mammal health and physiology and, through direct integration with ‘omics data, support a holistic approach to conservation and management goals. By highlighting the opportunities and limitations associated with specific techniques across the spectrum of ‘omics and small molecule analyses, we hope to support the growing breadth of research projects, highlight the potential future value of archived samples and contribute to the development of best practices for the collection, processing and archiving of biospecimens in the marine mammal community.

References

Aguilar, A., Borrell, A., 1990. Patterns of Lipid Content and Stratification in the Blubber of Fin Whales (Balaenoptera physalus). J. Mammal. 71, 544–554. https://doi.org/10.2307/1381793

Amos, W., 1997. Marine mammal tissue sample collection and preservation for genetic analyses, in: Molecular Genetics of Marine Mammals. Society for Marine Mammalogy, Lawrence, KS, pp. 107–113.

Amos, W., Hoelzel, A., 1991. Long-term preservation of whale skin for DNA analysis, in: Genetic Ecology of Whales: Incorporating the Proceedings of the Workshop on the Genetic Analysis of Cetacean Populations. International Whaling Commission, Cambridge, pp. 99–104.

Anderson, E.L., Li, W., Klitgord, N., Highlander, S.K., Dayrit, M., Seguritan, V., Yooseph, S., Biggs, W., Venter, J.C., Nelson, K.E., Jones, M.B., 2016. A robust ambient temperature collection and stabilization strategy: Enabling worldwide functional studies of the human microbiome. Sci. Rep. 6, 31731. https://doi.org/10.1038/srep31731

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

Apprill, A., 2017. Marine Animal Microbiomes: Toward Understanding Host–Microbiome Interactions in a Changing Ocean. Front. Mar. Sci. 4, 222. https://doi.org/10.3389/fmars.2017.00222

Apprill, A., Miller, C.A., Moore, M.J., Durban, J.W., Fearnbach, H., Barrett-Lennard, L.G., 2017. Extensive Core Microbiome in Drone-Captured Whale Blow Supports a Framework for

Health Monitoring. mSystems 2. https://doi.org/10.1128/mSystems.00119-17
Apprill, A., Miller, C.A., Van Cise, A.M., U'Ren, J.M., Leslie, M.S., Weber, L., Baird, R.W., Robbins, J., Landry, S., Bogomolni, A., Waring, G., 2020. Marine mammal skin microbiotas are influenced by host phylogeny. R. Soc. Open Sci. 7, 192046. https://doi.org/10.1098/rsos.192046
Apprill, A., Robbins, J., Eren, A.M., Pack, A.A., Reveillaud, J., Mattila, D., Moore, M., Niemeyer, M., Moore, K.M.T., Mincer, T.J., 2014. Humpback Whale Populations Share a Core Skin Bacterial Community: Towards a Health Index for Marine Mammals? PLOS ONE 9, e90785. https://doi.org/10.1371/journal.pone.0090785
Arregui, M., Borrell, A., Víkingsson, G., Ólafsdóttir, D., Aguilar, A., 2018. Stable isotope analysis of fecal material provides insight into the diet of fin whales. Mar. Mammal Sci. 34, 1059–1069. https://doi.org/10.1111/mms.12504
Babel, M., Mamilos, A., Seitz, S., Niedermair, T., Weber, F., Anzeneder, T., Ortmann, O., Dietmaier, W., Brochhausen, C., 2020. Compared DNA and RNA quality of breast cancer biobanking samples after long-term storage protocols in − 80 °C and liquid nitrogen. Sci. Rep. 10, 14404. https://doi.org/10.1038/s41598-020-71441-9
Bae, J., Kim, S.-J., Lee, S.-E., Kwon, W., Kim, H., Han, Y., Jang, J.-Y., Kim, M.-S., Lee, S.-W., 2019. Comprehensive proteome and phosphoproteome profiling shows negligible influence of RNAlater on protein abundance and phosphorylation. Clin. Proteomics 16, 18. https://doi.org/10.1186/s12014-019-9239-z
Bahamonde, P.A., Feswick, A., Isaacs, M.A., Munkittrick, K.R., Martyniuk, C.J., 2016. Defining the role of omics in assessing ecosystem health: Perspectives from the Canadian environmental monitoring program: Omics for ecosystem health. Environ. Toxicol. Chem. 35, 20–35. https://doi.org/10.1002/etc.3218
Baker, C.S., Steel, D., Calambokidis, J., Falcone, E., Gonzalez-Peral, U., Barlow, J., Burdin, A., Clapham, P., Ford, J., Gabriele, C., Mattila, D., Rojas-Bracho, L., Straley, J., Taylor, B., Wade, P., Weller, D., Witteveen, B., Yamaguchi, M., 2013. Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. Mar. Ecol. Prog. Ser. 494, 291–306. https://doi.org/10.3354/meps10508
Balgley, B.M., Guo, T., Zhao, K., Fang, X., Tavassoli, F.A., Lee, C.S., 2009. Evaluation of archival time on shotgun proteomics of formalin-fixed and paraffin-embedded tissues. J. Proteome Res. 8, 917–925. https://doi.org/10.1021/pr800503u
Beal, A.P., Kiszka, J.J., Wells, R.S., Eirin-Lopez, J.M., 2019. The Bottlenose Dolphin Epigenetic Aging Tool (BEAT): A Molecular Age Estimation Tool for Small Cetaceans. Front. Mar. Sci. 6, 561. https://doi.org/10.3389/fmars.2019.00561
Beaulieu-McCoy, N.E., Sherman, K.K., Trego, M.L., Crocker, D.E., Kellar, N.M., 2017. Initial validation of blubber cortisol and progesterone as indicators of stress response and maturity in an otariid; the California sea lion (Zalophus californianus). Gen. Comp. Endocrinol. 252, 1–11. https://doi.org/10.1016/j.ygcen.2017.07.003
Bechmann, N., Watts, D., Steenblock, C., Wallace, P.W., Schürmann, A., Bornstein, S.R., Wielockx, B., Eisenhofer, G., Peitzsch, M., 2021. Adrenal Hormone Interactions and Metabolism: A Single Sample Multi-Omics Approach. Horm. Metab. Res. 53, 326–334. https://doi.org/10.1055/a-1440-0278
Bennike, T.B., Kastaniegaard, K., Padurariu, S., Gaihede, M., Birkelund, S., Andersen, V., Stensballe, A., 2016. Comparing the proteome of snap frozen, RNAlater preserved, and formalin-fixed paraffin-embedded human tissue samples. EuPA Open Proteomics 10, 9–18. https://doi.org/10.1016/j.euprot.2015.10.001
Bergfelt, D.R., Lippolis, J., Vandenplas, M., Davis, S., Miller, B.A., Madan, R., Kline, M., Martinez, M., Sanchez-Okrucky, R., de Almeida, A.M., 2018. Preliminary Analysis of the Proteome of Exhaled Breath Condensate in Bottlenose Dolphins (Tursiops truncatus). Aquat. Mamm. 44, 256–266. https://doi.org/10.1578/AM.44.3.2018.256

Bik, E.M., Costello, E.K., Switzer, A.D., Callahan, B.J., Holmes, S.P., Wells, R.S., Carlin, K.P., Jensen, E.D., Venn-Watson, S., Relman, D.A., 2016. Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. Nat. Commun. 7, 10516. https://doi.org/10.1038/ncomms10516

Blom, M.P.K., 2021. Opportunities and challenges for high-quality biodiversity tissue archives in the age of long-read sequencing. Mol. Ecol. 30, 5935–5948. https://doi.org/10.1111/mec.15909

Bolelli, G., Muti, P., Micheli, A., Sciajno, R., Franceschetti, F., Krogh, V., Pisani, P., Berrino, F., 1995. Validity for epidemiological studies of long-term cryoconservation of steroid and protein hormones in serum and plasma. Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol. 4, 509–513.

Borras, E., Aksenov, A.A., Baird, M., Novick, B., Schivo, M., Zamuruyev, K.O., Pasamontes, A., Parry, C., Foutouhi, S., Venn-Watson, S., Weimer, B.C., Davis, C.E., 2017. Exhaled breath condensate methods adapted from human studies using longitudinal metabolomics for predicting early health alterations in dolphins. Anal. Bioanal. Chem. 409, 6523–6536. https://doi.org/10.1007/s00216-017-0581-6

Bors, E.K., Baker, C.S., Wade, P.R., O'Neill, K.B., Shelden, K.E.W., Thompson, M.J., Fei, Z., Jarman, S., Horvath, S., 2021. An epigenetic clock to estimate the age of living beluga whales. Evol. Appl. 14, 1263–1273. https://doi.org/10.1111/eva.13195

Bowen, W.D., Iverson, S.J., 2013. Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Mar. Mammal Sci. 29, 719–754. https://doi.org/10.1111/j.1748-7692.2012.00604.x

Bundgaard-Nielsen, C., Hagstrøm, S., Sørensen, S., 2018. Interpersonal Variations in Gut Microbiota Profiles Supersedes the Effects of Differing Fecal Storage Conditions. Sci. Rep. 8, 17367. https://doi.org/10.1038/s41598-018-35843-0

Burgess, E.A., Hunt, K.E., Kraus, S.D., Rolland, R.M., 2018. Quantifying hormones in exhaled breath for physiological assessment of large whales at sea. Sci. Rep. 8, 10031. https://doi.org/10.1038/s41598-018-28200-8

Burgess, E.A., Hunt, K.E., Kraus, S.D., Rolland, R.M., 2016. Get the most out of blow hormones: validation of sampling materials, field storage and extraction techniques for whale respiratory vapour samples. Conserv. Physiol. 4, cow024. https://doi.org/10.1093/conphys/cow024

Burrows, D.G., Reichert, W.L., Bradley Hanson, M., 2014. Effects of decomposition and storage conditions on the δ13C and δ15N isotope values of killer whale (Orcinus orca) skin and blubber tissues. Mar. Mammal Sci. 30, 747–762. https://doi.org/10.1111/mms.12076

Busquets-Vass, G., Newsome, S.D., Calambokidis, J., Serra-Valente, G., Jacobsen, J.K., Aguíñiga-García, S., Gendron, D., 2017. Estimating blue whale skin isotopic incorporation rates and baleen growth rates: Implications for assessing diet and movement patterns in mysticetes. PLOS ONE 12, e0177880. https://doi.org/10.1371/journal.pone.0177880

Bykowski, T., Stevenson, B., 2020. Aseptic Technique. Curr. Protoc. Microbiol. 56, e98. https://doi.org/10.1002/cpmc.98

Byrd, J.B., Greene, A.C., Prasad, D.V., Jiang, X., Greene, C.S., 2020. Responsible, practical genomic data sharing that accelerates research. Nat. Rev. Genet. 21, 615–629. https://doi.org/10.1038/s41576-020-0257-5

Caixeiro, N.J., Lai, K., Lee, C.S., 2016. Quality assessment and preservation of RNA from biobank tissue specimens: a systematic review. J. Clin. Pathol. 69, 260–265. https://doi.org/10.1136/jclinpath-2015-203384

Camacho-Sanchez, M., Burraco, P., Gomez-Mestre, I., Leonard, J.A., 2013. Preservation of RNA and DNA from mammal samples under field conditions. Mol. Ecol. Resour. 13, 663–673. https://doi.org/10.1111/1755-0998.12108

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., Van Cise, A.M., 2016. Genomic Methods Take the Plunge: Recent Advances in High-Throughput Sequencing of Marine Mammals. J. Hered. 107, 481–495. https://doi.org/10.1093/jhered/esw044

Campbell, N.R., Harmon, S.A., Narum, S.R., 2015. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Mol. Ecol. Resour. 15, 855–867. https://doi.org/10.1111/1755-0998.12357

Cardona, S., Eck, A., Cassellas, M., Gallart, M., Alastrue, C., Dore, J., Azpiroz, F., Roca, J., Guarner, F., Manichanh, C., 2012. Storage conditions of intestinal microbiota matter in metagenomic analysis. BMC Microbiol. 12, 158. https://doi.org/10.1186/1471-2180-12-158

Castellini, M.A., Castellini, J.M., Kirby, V.L., 1992. Effects of standard anticoagulants and storage procedures on plasma glucose values in seals. J. Am. Vet. Med. Assoc. 201, 145–148.

Cejas, P., Li, L., O'Neill, N.K., Duarte, M., Rao, P., Bowden, M., Zhou, C.W., Mendiola, M., Burgos, E., Feliu, J., Moreno-Rubio, J., Guadalajara, H., Moreno, V., García-Olmo, D., Bellmunt, J., Mullane, S., Hirsch, M., Sweeney, C.J., Richardson, A., Liu, X.S., Brown, M., Shivdasani, R.A., Long, H.W., 2016. Chromatin immunoprecipitation from fixed clinical tissues reveals tumor-specific enhancer profiles. Nat. Med. 22, 685–691. https://doi.org/10.1038/nm.4085

Champagne, C.D., Kellar, N.M., Trego, M.L., Delehanty, B., Boonstra, R., Wasser, S.K., Booth, R.K., Crocker, D.E., Houser, D.S., 2018. Comprehensive endocrine response to acute stress in the bottlenose dolphin from serum, blubber, and feces. Gen. Comp. Endocrinol. 266, 178–193. https://doi.org/10.1016/j.ygcen.2018.05.015

Charapata, P., Horstmann, L., Jannasch, A., Misarti, N., 2018. A novel method to measure steroid hormone concentrations in walrus bone from archaeological, historical, and modern time periods using liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 32, 1999–2023. https://doi.org/10.1002/rcm.8272

Choi, S., Ray, H.E., Lai, S.-H., Alwood, J.S., Globus, R.K., 2016. Preservation of Multiple Mammalian Tissues to Maximize Science Return from Ground Based and Spaceflight Experiments. PloS One 11, e0167391. https://doi.org/10.1371/journal.pone.0167391

Choo, J.M., Leong, L.E., Rogers, G.B., 2015. Sample storage conditions significantly influence faecal microbiome profiles. Sci. Rep. 5, 16350. https://doi.org/10.1038/srep16350

Chu, T.-Y., Hwang, K.-S., Yu, M.-H., Lee, H.-S., Lai, H.-C., Liu, J.-Y., 2002. A research-based tumor tissue bank of gynecologic oncology: characteristics of nucleic acids extracted from normal and tumor tissues from different sites. Int. J. Gynecol. Cancer 12, 171. https://doi.org/10.1136/ijgc-00009577-200203000-00006

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic Ingestion by Zooplankton. Environ. Sci. Technol. 47, 6646–6655. https://doi.org/10.1021/es400663f

Costello, E.K., Lauber, C.L., Hamady, M., Fierer, N., Gordon, J.I., Knight, R., 2009. Bacterial Community Variation in Human Body Habitats Across Space and Time. Science 326, 1694–1697. https://doi.org/10.1126/science.1177486

Couch, R.D., Navarro, K., Sikaroodi, M., Gillevet, P., Forsyth, C.B., Mutlu, E., Engen, P.A., Keshavarzian, A., 2013. The Approach to Sample Acquisition and Its Impact on the Derived Human Fecal Microbiome and VOC Metabolome. PLoS ONE 8, e81163. https://doi.org/10.1371/journal.pone.0081163

Crossman, C.A., Barrett-Lennard, L.G., Frasier, T.R., 2021. An example of DNA methylation as a means to quantify stress in wildlife using killer whales. Sci. Rep. 11, 16822. https://doi.org/10.1038/s41598-021-96255-1

Dahn, H.A., Mountcastle, J., Balacco, J., Winkler, S., Bista, I., Schmitt, A.D., Pettersson, O.V.,

Formenti, G., Oliver, K., Smith, M., Tan, W., Kraus, A., Mac, S., Komoroske, L.M., Lama, T., Crawford, A.J., Murphy, R.W., Brown, S., Scott, A.F., Morin, P.A., Jarvis, E.D., Fedrigo, O., 2021. Benchmarking ultra-high molecular weight DNA preservation methods for long-read and long-range sequencing. https://doi.org/10.1101/2021.07.13.451380

Dale, J.J., Wallsgrove, N.J., Popp, B.N., Holland, K.N., 2011. Nursery habitat use and foraging ecology of the brown stingray Dasyatis lata determined from stomach contents, bulk and amino acid stable isotopes. Mar. Ecol. Prog. Ser. 433, 221–236. https://doi.org/10.3354/meps09171

Dantzer, B., Fletcher, Q.E., Boonstra, R., Sheriff, M.J., 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? Conserv. Physiol. 2. https://doi.org/10.1093/conphys/cou023

De Wit, P., Pespeni, M.H., Ladner, J.T., Barshis, D.J., Seneca, F., Jaris, H., Therkildsen, N.O., Morikawa, M., Palumbi, S.R., 2012. The simple fool's guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. Mol. Ecol. Resour. 12, 1058–1067. https://doi.org/10.1111/1755-0998.12003

Deslypere, J.P., Verdonck, L., Vermuelen, A., 1985. Fat Tissue: A Steroid Reservoir and Site of Steroid Metabolism. J. Clin. Endocrinol. Metab. 61, 564–570.

Desoubeaux, G., Piqueras, M.D.C., Le-Bert, C., Fravel, V., Clauss, T., Delaune, A.J., Daniels, R., Jensen, E.D., Flower, J.E., Bossart, G.D., Bhattacharya, S.K., Cray, C., 2019. Labeled quantitative mass spectrometry to study the host response during aspergillosis in the common bottlenose dolphin (Tursiops truncatus). Vet. Microbiol. 232, 42–49. https://doi.org/10.1016/j.vetmic.2019.03.030

Deyarmin, J.S., McCormley, M.C., Champagne, C.D., Stephan, A.P., Busqueta, L.P., Crocker, D.E., Houser, D.S., Khudyakov, J.I., 2019. Blubber transcriptome responses to repeated ACTH administration in a marine mammal. Sci. Rep. 9, 2718. https://doi.org/10.1038/s41598-019-39089-2

do Rego, J.L., Vaudry, H., 2016. Comparative aspects of neurosteroidogenesis: From fish to mammals. Gen. Comp. Endocrinol. 227, 120–129. https://doi.org/10.1016/j.ygcen.2015.05.014

Donaldson, G.P., Lee, S.M., Mazmanian, S.K., 2016. Gut biogeography of the bacterial microbiota. Nat. Rev. Microbiol. 14, 20–32. https://doi.org/10.1038/nrmicro3552

Dudek, N.K., Sun, C.L., Burstein, D., Kantor, R.S., Aliaga Goltsman, D.S., Bik, E.M., Thomas, B.C., Banfield, J.F., Relman, D.A., 2017. Novel Microbial Diversity and Functional Potential in the Marine Mammal Oral Microbiome. Curr. Biol. 27, 3752-3762.e6. https://doi.org/10.1016/j.cub.2017.10.040

Dudek, N.K., Switzer, A.D., Costello, E.K., Murray, M.J., Tomoleoni, J.A., Staedler, M.M., Tinker, M.T., Relman, D.A., n.d. Characterizing the oral and distal gut microbiota of the threatened southern sea otter (Enhydra lutris nereis) to enhance conservation practice. Conserv. Sci. Pract. n/a, e12640. https://doi.org/10.1111/csp2.12640

Eisenhofer, R., Minich, J.J., Marotz, C., Cooper, A., Knight, R., Weyrich, L.S., 2019. Contamination in Low Microbial Biomass Microbiome Studies: Issues and Recommendations. Trends Microbiol. 27, 105–117. https://doi.org/10.1016/j.tim.2018.11.003

Fang, T.Y., Praveena, S.M., deBurbure, C., Aris, A.Z., Ismail, S.N.S., Rasdi, I., 2016. Analytical techniques for steroid estrogens in water samples - A review. Chemosphere 165, 358–368. https://doi.org/10.1016/j.chemosphere.2016.09.051

Foote, A.D., Martin, M.D., Louis, M., Pacheco, G., Robertson, K.M., Sinding, M.-H.S., Amaral, A.R., Baird, R.W., Baker, C.S., Ballance, L., Barlow, J., Brownlow, A., Collins, T., Constantine, R., Dabin, W., Dalla Rosa, L., Davison, N.J., Durban, J.W., Esteban, R., Ferguson, S.H., Gerrodette, T., Guinet, C., Hanson, M.B., Hoggard, W., Matthews, C.J.D., Samarra, F.I.P., de Stephanis, R., Tavares, S.B., Tixier, P., Totterdell, J.A., Wade,

P., Excoffier, L., Gilbert, M.T.P., Wolf, J.B.W., Morin, P.A., 2019. Killer whale genomes reveal a complex history of recurrent admixture and vicariance. Mol. Ecol. 28, 3427–3444. https://doi.org/10.1111/mec.15099
Fry, B., 2006. Stable isotope ecology. Springer, New York, NY.
Gallego Romero, I., Pai, A.A., Tung, J., Gilad, Y., 2014. RNA-seq: impact of RNA degradation on transcript quantification. BMC Biol. 12, 42. https://doi.org/10.1186/1741-7007-12-42
Gamble, T., 2014. Collecting and preserving genetic material for herpetological research. Society for the Study of Amphibians and Reptiles, Salt Lake City.
Garber, A., Hastie, P., Murray, J.-A., 2020. Factors Influencing Equine Gut Microbiota: Current Knowledge. J. Equine Vet. Sci. 88, 102943. https://doi.org/10.1016/j.jevs.2020.102943
Gates, J.B., Chittaro, P.M., Veggerby, K.B., 2020. Standard Operating Procedures for Measuring Bulk Stable Isotope Values of Nitrogen and Carbon in Marine Biota by Isotope Ratio Mass Spectrometry (IRMS) (No. NMFS-NWFSC-PR-2020-04). Northwest Fisheries Science Center (U.S.).
Gayral, P., Weinert, L., Chiari, Y., Tsagkogeorga, G., Ballenghien, M., Galtier, N., 2011. Next-generation sequencing of transcriptomes: a guide to RNA isolation in nonmodel animals. Mol. Ecol. Resour. 11, 650–661. https://doi.org/10.1111/j.1755-0998.2011.03010.x
Geraci, J.R., Medway, W., 1974. Simulated field blood studies in the bottle-nosed dolphin Tursiops truncatus. 3. Changes in hematology and chemistry during blood and plasma storage. J. Wildl. Dis. 10, 410–419. https://doi.org/10.7589/0090-3558-10.4.410
Giskeødegård, G.F., Andreassen, T., Bertilsson, H., Tessem, M.-B., Bathen, T.F., 2019. The effect of sampling procedures and day-to-day variations in metabolomics studies of biofluids. Anal. Chim. Acta 1081, 93–102. https://doi.org/10.1016/j.aca.2019.07.026
Goodrich, J.K., Di Rienzi, S.C., Poole, A.C., Koren, O., Walters, W.A., Caporaso, J.G., Knight, R., Ley, R.E., 2014. Conducting a microbiome study. Cell 158, 250–262. https://doi.org/10.1016/j.cell.2014.06.037
Gorzelak, M.A., Gill, S.K., Tasnim, N., Ahmadi-Vand, Z., Jay, M., Gibson, D.L., 2015. Methods for Improving Human Gut Microbiome Data by Reducing Variability through Sample Processing and Storage of Stool. PLOS ONE 10, e0134802. https://doi.org/10.1371/journal.pone.0134802
Gui, D., Jia, K., Xia, J., Yang, L., Chen, J., Wu, Y., Yi, M., 2013. De novo Assembly of the Indo-Pacific Humpback Dolphin Leucocyte Transcriptome to Identify Putative Genes Involved in the Aquatic Adaptation and Immune Response. PLOS ONE 8, e72417. https://doi.org/10.1371/journal.pone.0072417
Gwinnett, C., Miller, R.Z., 2021. Are we contaminating our samples? A preliminary study to investigate procedural contamination during field sampling and processing for microplastic and anthropogenic microparticles. Mar. Pollut. Bull. 173, 113095. https://doi.org/10.1016/j.marpolbul.2021.113095
Hasin, Y., Seldin, M., Lusis, A., 2017. Multi-omics approaches to disease. Genome Biol. 18, 83. https://doi.org/10.1186/s13059-017-1215-1
Herman, D., Matkin, C., Durban, J., Hanson, M., Dahlheim, M., Straley, J., Wade, P., Tilbury, K., Boyer, R., Pearce, R.W., Krahn, M.M., 2008. Assessing age distributions of killer whale Orcinus orca populations from the composition of endogenous fatty acids in their outer blubber layers. https://doi.org/10.3354/MEPS07709
Hooper, R., Brealey, J.C., van der Valk, T., Alberdi, A., Durban, J.W., Fearnbach, H., Robertson, K.M., Baird, R.W., Bradley Hanson, M., Wade, P., Gilbert, M.T.P., Morin, P.A., Wolf, J.B.W., Foote, A.D., Guschanski, K., 2019. Host-derived population genomics data provides insights into bacterial and diatom composition of the killer whale skin. Mol. Ecol. 28, 484–502. https://doi.org/10.1111/mec.14860
Horvath, S., 2013. DNA methylation age of human tissues and cell types. Genome Biol. 14,

3156. https://doi.org/10.1186/gb-2013-14-10-r115
Houser, D.S., Derous, D., Douglas, A., Lusseau, D., 2021. Metabolic response of dolphins to short-term fasting reveals physiological changes that differ from the traditional fasting model. J. Exp. Biol. 224, jeb238915. https://doi.org/10.1242/jeb.238915
Hudson, J.M., Matthews, C.J.D., Watt, C.A., 2021. Detection of steroid and thyroid hormones in mammalian teeth. Conserv. Physiol. 9, coab087. https://doi.org/10.1093/conphys/coab087
Hunt, K., Robbins, J., Buck, C., Bérubé, M., Rolland, R., 2019. Evaluation of fecal hormones for noninvasive research on reproduction and stress in humpback whales (Megaptera novaeangliae). Gen. Comp. Endocrinol. 280. https://doi.org/10.1016/j.ygcen.2019.04.004
Hunt, K.E., Lysiak, N.S., Robbins, J., Moore, M.J., Seton, R.E., Torres, L., Buck, C.L., 2017. Multiple steroid and thyroid hormones detected in baleen from eight whale species. Conserv. Physiol. 5, cox061. https://doi.org/10.1093/conphys/cox061
Hunt, K.E., Stimmelmayr, R., George, C., Hanns, C., Suydam, R., Brower, H., Rolland, R.M., 2014. Baleen hormones: a novel tool for retrospective assessment of stress and reproduction in bowhead whales (Balaena mysticetus). Conserv. Physiol. 2, cou030–cou030. https://doi.org/10.1093/conphys/cou030
Ilett, E.E., Jørgensen, M., Noguera-Julian, M., Daugaard, G., Murray, D.D., Helleberg, M., Paredes, R., Lundgren, J., Sengeløv, H., MacPherson, C., 2019. Gut microbiome comparability of fresh-frozen versus stabilized-frozen samples from hospitalized patients using 16S rRNA gene and shotgun metagenomic sequencing. Sci. Rep. 9, 13351. https://doi.org/10.1038/s41598-019-49956-7
Jain, M., Kennedy, A.D., Elsea, S.H., Miller, M.J., 2017. Analytes related to erythrocyte metabolism are reliable biomarkers for preanalytical error due to delayed plasma processing in metabolomics studies. Clin. Chim. Acta Int. J. Clin. Chem. 466, 105–111. https://doi.org/10.1016/j.cca.2017.01.005
Jepson, P.D., Bennett, P.M., Allchin, C.R., Law, R.J., Kuiken, T., Baker, J.R., Rogan, E., Kirkwood, J.K., 1999. Investigating potential associations between chronic exposure to polychlorinated biphenyls and infectious disease mortality in harbour porpoises from England and Wales. Sci. Total Environ. 243–244, 339–348. https://doi.org/10.1016/s0048-9697(99)00417-9
Jones, R.B., Zhu, X., Moan, E., Murff, H.J., Ness, R.M., Seidner, D.L., Sun, S., Yu, C., Dai, Q., Fodor, A.A., Azcarate-Peril, M.A., Shrubsole, M.J., 2018. Inter-niche and inter-individual variation in gut microbial community assessment using stool, rectal swab, and mucosal samples. Sci. Rep. 8, 4139. https://doi.org/10.1038/s41598-018-22408-4
Kaehler, S., Pakhomov, E., 2001. Effects of storage and preservation on the 13C and 15N signatures of selected marine organisms. Mar Ecol Prog Ser 219, 299–304.
Karpovich, S.A., Horstmann, L.A., Polasek, L.K., 2020. Validation of a novel method to create temporal records of hormone concentrations from the claws of ringed and bearded seals. Conserv. Physiol. 8, coaa073. https://doi.org/10.1093/conphys/coaa073
Karu, N., Deng, L., Slae, M., Guo, A.C., Sajed, T., Huynh, H., Wine, E., Wishart, D.S., 2018. A review on human fecal metabolomics: Methods, applications and the human fecal metabolome database. Anal. Chim. Acta 1030, 1–24. https://doi.org/10.1016/j.aca.2018.05.031
Keighley, X., Bro-Jørgensen, M.H., Ahlgren, H., Szpak, P., Ciucani, M.M., Sánchez Barreiro, F., Howse, L., Gotfredsen, A.B., Glykou, A., Jordan, P., Lidén, K., Olsen, M.T., 2021. Predicting sample success for large-scale ancient DNA studies on marine mammals. Mol. Ecol. Resour. 21, 1149–1166. https://doi.org/10.1111/1755-0998.13331
Kellar, N.M., Catelani, K.N., Robbins, M.N., Trego, M.L., Allen, C.D., Danil, K., Chivers, S.J., 2015. Blubber cortisol: A potential tool for assessing stress response in free-ranging dolphins without effects due to sampling. PLOS ONE 10, e0115257.

https://doi.org/10.1371/journal.pone.0115257
Kellar, N.M., Trego, M.L., Marks, C.I., Chivers, S.J., Danil, K., Archer, F.I., 2009. Blubber testosterone: A potential marker of male reproductive status in short‐beaked common dolphins. Mar. Mammal Sci. 25, 507–522.
Kellar, N.M., Trego, M.L., Marks, C.I., Dizon, A.E., 2006. Determining Pregnancy from Blubber in Three Species of Delphinids. Mar. Mammal Sci. 22, 1–16. https://doi.org/10.1111/j.1748-7692.2006.00001.x
Kennedy, A.D., Ford, L., Wittmann, B., Conner, J., Wulff, J., Mitchell, M., Evans, A.M., Toal, D.R., 2021. Global biochemical analysis of plasma, serum and whole blood collected using various anticoagulant additives. PLOS ONE 16, e0249797. https://doi.org/10.1371/journal.pone.0249797
Keogh, M.J., Charapata, P., Fadely, B.S., Zeppelin, T., Rea, L., Waite, J.N., Burkanov, V., Marshall, C., Jones, A., Sprowls, C., Wooller, M.J., 2021. Whiskers as a novel tissue for tracking reproductive and stress-related hormones in North Pacific otariid pinnipeds. Conserv. Physiol. 9, coaa134. https://doi.org/10.1093/conphys/coaa134
Kershaw, J.L., Botting, C.H., Brownlow, A., Hall, A.J., 2018. Not just fat: investigating the proteome of cetacean blubber tissue. Conserv. Physiol. 6. https://doi.org/10.1093/conphys/coy003
Kershaw, J.L., Sherrill, M., Davison, N.J., Brownlow, A., Hall, A.J., 2017. Evaluating morphometric and metabolic markers of body condition in a small cetacean, the harbor porpoise (Phocoena phocoena). Ecol. Evol. 7, 3494–3506. https://doi.org/10.1002/ece3.2891
Khan, S., Shih, T., Shih, S., Khachemoune, A., 2021. Reappraising Elements of the Aseptic Technique in Dermatology: A Review. Dermatol. Pract. Concept. e2020126–e2020126. https://doi.org/10.5826/dpc.1101a126
Khudyakov, J.I., Champagne, C.D., Meneghetti, L.M., Crocker, D.E., 2017. Blubber transcriptome response to acute stress axis activation involves transient changes in adipogenesis and lipolysis in a fasting-adapted marine mammal. Sci. Rep. 7, 42110. https://doi.org/10.1038/srep42110
Khudyakov, J.I., Deyarmin, J.S., Hekman, R.M., Pujade Busqueta, L., Maan, R., Mody, M.J., Banerjee, R., Crocker, D.E., Champagne, C.D., 2018. A sample preparation workflow for adipose tissue shotgun proteomics and proteogenomics. Biol. Open bio.036731. https://doi.org/10.1242/bio.036731
Kilpatrick, C.W., 2002. Noncryogenic preservation of mammalian tissues for DNA extraction: an assessment of storage methods. Biochem. Genet. 40, 53–62. https://doi.org/10.1023/a:1014541222816
Kiszka, J., Lesage, V., Ridoux, V., 2014. Effect of ethanol preservation on stable carbon and nitrogen isotope values in cetacean epidermis: Implication for using archived biopsy samples. Mar. Mammal Sci. 30, 788–795. https://doi.org/10.1111/mms.12058
Kley, H.K., Rick, W., 1984. The effect of storage and temperature on the analysis of steroids in plasma and blood. J. Clin. Chem. Clin. Biochem. Z. Klin. Chem. Klin. Biochem. 22, 371–378.
Kolker, E., Özdemir, V., Martens, L., Hancock, W., Anderson, G., Anderson, N., Aynacioglu, S., Baranova, A., Campagna, S.R., Chen, R., Choiniere, J., Dearth, S.P., Feng, W.-C., Ferguson, L., Fox, G., Frishman, D., Grossman, R., Heath, A., Higdon, R., Hutz, M.H., Janko, I., Jiang, L., Joshi, S., Kel, A., Kemnitz, J.W., Kohane, I.S., Kolker, N., Lancet, D., Lee, E., Li, W., Lisitsa, A., Llerena, A., MacNealy-Koch, C., Marshall, J.-C., Masuzzo, P., May, A., Mias, G., Monroe, M., Montague, E., Mooney, S., Nesvizhskii, A., Noronha, S., Omenn, G., Rajasimha, H., Ramamoorthy, P., Sheehan, J., Smarr, L., Smith, C.V., Smith, T., Snyder, M., Rapole, S., Srivastava, S., Stanberry, L., Stewart, E., Toppo, S., Uetz, P., Verheggen, K., Voy, B.H., Warnich, L., Wilhelm, S.W., Yandl, G., 2014. Toward More

Transparent and Reproducible Omics Studies Through a Common Metadata Checklist and Data Publications. OMICS J. Integr. Biol. 18, 10–14. https://doi.org/10.1089/omi.2013.0149
Kono, N., Nakamura, H., Ito, Y., Tomita, M., Arakawa, K., 2016. Evaluation of the impact of RNA preservation methods of spiders for de novo transcriptome assembly. Mol. Ecol. Resour. 16, 662–672. https://doi.org/10.1111/1755-0998.12485
Koopman, H.N., Iverson, S.J., Gaskin, D.E., 1996. Stratification and age-related differences in blubber fatty acids of the male harbour porpoise (Phocoena phocoena). J. Comp. Physiol. B 165, 628–639. https://doi.org/10.1007/BF00301131
Koopman, H.N., Pabst, D.A., McLellan, W.A., Dillaman, R.M., Read, A.J., 2002. Changes in blubber distribution and morphology associated with starvation in the harbor porpoise (Phocoena phocoena): evidence for regional differences in blubber structure and function. Physiol. Biochem. Zool. PBZ 75, 498–512. https://doi.org/10.1086/342799
Kotrba, M., Schilling, L.-H., 2017. Measurement of pH in Ethanol, Distilled Water, and their Mixtures: On the Assessment of pH in Ethanol-Based Natural History Wet Collections and the Detrimental Aspects of Dilution with Distilled Water. Collect. Forum 31, 84–101. https://doi.org/10.14351/0831-4985-31.1.84
Krahn, M.M., Hanson, M.B., Baird, R.W., Boyer, R.H., Burrows, D.G., Emmons, C.K., Ford, J.K.B., Jones, L.L., Noren, D.P., Ross, P.S., Schorr, G.S., Collier, T.K., 2007. Persistent organic pollutants and stable isotopes in biopsy samples (2004/2006) from Southern Resident killer whales. Mar. Pollut. Bull. 54, 1903–1911. https://doi.org/10.1016/j.marpolbul.2007.08.015
Kristensen, L.S., Wojdacz, T.K., Thestrup, B.B., Wiuf, C., Hager, H., Hansen, L.L., 2009. Quality assessment of DNA derived from up to 30 years old formalin fixed paraffin embedded (FFPE) tissue for PCR-based methylation analysis using SMART-MSP and MS-HRM. BMC Cancer 9, 453. https://doi.org/10.1186/1471-2407-9-453
Kruse, C.P.S., Basu, P., Luesse, D.R., Wyatt, S.E., 2017. Transcriptome and proteome responses in RNAlater preserved tissue of Arabidopsis thaliana. PloS One 12, e0175943. https://doi.org/10.1371/journal.pone.0175943
Kühn, S., van Franeker, J.A., O'Donoghue, A.M., Swiers, A., Starkenburg, M., van Werven, B., Foekema, E., Hermsen, E., Egelkraut-Holtus, M., Lindeboom, H., 2020. Details of plastic ingestion and fibre contamination in North Sea fishes. Environ. Pollut. 257, 113569. https://doi.org/10.1016/j.envpol.2019.113569
Lawson, T.M., Ylitalo, G.M., O'Neill, S.M., Dahlheim, M.E., Wade, P.R., Matkin, C.O., Burkanov, V., Boyd, D.T., 2020. Concentrations and profiles of organochlorine contaminants in North Pacific resident and transient killer whale (Orcinus orca) populations. Sci. Total Environ. 722, 137776. https://doi.org/10.1016/j.scitotenv.2020.137776
Lazensky, R., Silva-Sanchez, C., Kroll, K.J., Chow, M., Chen, S., Tripp, K., Walsh, M.T., Denslow, N.D., 2021. Investigating an increase in Florida manatee mortalities using a proteomic approach. Sci. Rep. 11, 4282. https://doi.org/10.1038/s41598-021-83687-y
Lee, D.H., Kim, J.W., Jeon, S.Y., Park, B.K., Han, B.G., 2010. Proteomic analysis of the effect of storage temperature on human serum. Ann. Clin. Lab. Sci. 40, 61–70.
Lemos, L.S., Olsen, A., Smith, A., Chandler, T.E., Larson, S., Hunt, K., Torres, L.G., 2020. Assessment of fecal steroid and thyroid hormone metabolites in eastern North Pacific gray whales. Conserv. Physiol. 8, 19.
Lesage, V., Morin, Y., Rioux, È., Pomerleau, C., Ferguson, S.H., Pelletier, É., 2010. Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. Mar. Ecol. Prog. Ser. 419, 249–265. https://doi.org/10.3354/meps08825
Lilienthal, H., Hack, A., Roth-Härer, A., Grande, S.W., Talsness, C.E., 2006. Effects of developmental exposure to 2,2 ,4,4 ,5-pentabromodiphenyl ether (PBDE-99) on sex

steroids, sexual development, and sexually dimorphic behavior in rats. Environ. Health Perspect. 114, 194–201. https://doi.org/10.1289/ehp.8391
Liu, X., Locasale, J.W., 2017. Metabolomics - a primer. Trends Biochem. Sci. 42, 274–284. https://doi.org/10.1016/j.tibs.2017.01.004
Maegawa, S., Hinkal, G., Kim, H.S., Shen, L., Zhang, L., Zhang, J., Zhang, N., Liang, S., Donehower, L.A., Issa, J.-P.J., 2010. Widespread and tissue specific age-related DNA methylation changes in mice. Genome Res. 20, 332–340. https://doi.org/10.1101/gr.096826.109
Maes, T., van Diemen de Jel, J., Vethaak, A.D., Desender, M., Bendall, V.A., van Velzen, M., Leslie, H.A., 2020. You Are What You Eat, Microplastics in Porbeagle Sharks From the North East Atlantic: Method Development and Analysis in Spiral Valve Content and Tissue. Front. Mar. Sci. 7, 273. https://doi.org/10.3389/fmars.2020.00273
Mancia, A., 2018. Chapter 11 - New Technologies for Monitoring Marine Mammal Health, in: Fossi, M.C., Panti, C. (Eds.), Marine Mammal Ecotoxicology. Academic Press, pp. 291–320. https://doi.org/10.1016/B978-0-12-812144-3.00011-5
McCormack, M.A., Jackson, B.P., Dutton, J., 2020. Effects of Formalin Fixation on Trace Element Concentrations in Bottlenose Dolphin (Tursiops truncatus) Tissues. Environ. Toxicol. Chem. 39, 1149–1164. https://doi.org/10.1002/etc.4709
McGowen, M.R., Tsagkogeorga, G., Álvarez-Carretero, S., dos Reis, M., Struebig, M., Deaville, R., Jepson, P.D., Jarman, S., Polanowski, A., Morin, P.A., Rossiter, S.J., 2020a. Phylogenomic Resolution of the Cetacean Tree of Life Using Target Sequence Capture. Syst. Biol. 69, 479–501. https://doi.org/10.1093/sysbio/syz068
McGowen, M.R., Tsagkogeorga, G., Williamson, J., Morin, P.A., Rossiter, and S.J., 2020b. Positive Selection and Inactivation in the Vision and Hearing Genes of Cetaceans. Mol. Biol. Evol. 37, 2069–2083. https://doi.org/10.1093/molbev/msaa070
Mello, D., Colosio, A., Marcondes, M., Viau, P., Oliveira, C., 2017. Feasibility of using humpback whale blubber to measure sex hormones. J. Exp. Mar. Biol. Ecol. 486, 32–41. https://doi.org/10.1016/j.jembe.2016.09.010
Michaud, C.L., Foran, D.R., 2011. Simplified field preservation of tissues for subsequent DNA analyses. J. Forensic Sci. 56, 846–852. https://doi.org/10.1111/j.1556-4029.2011.01771.x
Micke, P., Ohshima, M., Tahmasebpoor, S., Ren, Z.-P., Östman, A., Pontén, F., Botling, J., 2006. Biobanking of fresh frozen tissue: RNA is stable in nonfixed surgical specimens. Lab. Invest. 86, 202–211. https://doi.org/10.1038/labinvest.3700372
Miller, B.A., Nanni, P., Fortes, C., Grossmann, J., Arreola, M.R., Vences, M., Canales, R., Sanchez-Okrucky, R., de Almeida, A.M., Bergfelt, D.R., 2017. Plasma Proteome and Clinical Biochemistry Associated with Performance-Based Physical Activity in Bottlenose Dolphins (Tursiops truncatus). Aquat. Mamm. 43, 453–464. https://doi.org/10.1578/AM.43.5.2017.453
Mingramm, F., Keeley, T., Whitworth, D., Dunlop, R., 2019. Relationships Between Blubber and Respiratory Vapour Steroid Hormone Concentrations in Humpback Whales (Megaptera novaeangliae). Aquat. Mamm. https://doi.org/10.1578/am.45.5.2019.465
Mitchell, B.L., Yasui, Y., Li, C.I., Fitzpatrick, A.L., Lampe, P.D., 2005. Impact of freeze-thaw cycles and storage time on plasma samples used in mass spectrometry based biomarker discovery projects. Cancer Inform. 1, 98–104.
Morin, P.A., Archer, F.I., Avila, C.D., Balacco, J.R., Bukhman, Y.V., Chow, W., Fedrigo, O., Formenti, G., Fronczek, J.A., Fungtammasan, A., Gulland, F.M.D., Haase, B., Peter Heide-Jorgensen, M., Houck, M.L., Howe, K., Misuraca, A.C., Mountcastle, J., Musser, W., Paez, S., Pelan, S., Phillippy, A., Rhie, A., Robinson, J., Rojas-Bracho, L., Rowles, T.K., Ryder, O.A., Smith, C.R., Stevenson, S., Taylor, B.L., Teilmann, J., Torrance, J., Wells, R.S., Westgate, A.J., Jarvis, E.D., 2021a. Reference genome and demographic history of the most endangered marine mammal, the vaquita. Mol. Ecol. Resour. 21,

1008–1020. https://doi.org/10.1111/1755-0998.13284
Morin, P.A., Forester, B.R., Forney, K.A., Crossman, C.A., Hancock-Hanser, B.L., Robertson, K.M., Barrett-Lennard, L.G., Baird, R.W., Calambokidis, J., Gearin, P., Hanson, M.B., Schumacher, C., Harkins, T., Fontaine, M.C., Taylor, B.L., Parsons, K.M., 2021b. Population structure in a continuously distributed coastal marine species, the harbor porpoise, based on microhaplotypes derived from poor-quality samples. Mol. Ecol. 30, 1457–1476. https://doi.org/10.1111/mec.15827
Mulcahy, D.G., Macdonald, K.S., Brady, S.G., Meyer, C., Barker, K.B., Coddington, J., 2016. Greater than X kb: a quantitative assessment of preservation conditions on genomic DNA quality, and a proposed standard for genome-quality DNA. PeerJ 4, e2528. https://doi.org/10.7717/peerj.2528
Mutter, G.L., Zahrieh, D., Liu, C., Neuberg, D., Finkelstein, D., Baker, H.E., Warrington, J.A., 2004. Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays. BMC Genomics 5, 88. https://doi.org/10.1186/1471-2164-5-88
Nagy, Z., 2010. A hands-on overview of tissue preservation methods for molecular genetic analyses. Org. Divers. Evol. https://doi.org/10.1007/s13127-010-0012-4
Neely, B., Carlin, K., Arthur, J., McFee, W., Janech, M., 2013. Ratiometric Measurements of Adiponectin by Mass Spectrometry in Bottlenose Dolphins (Tursiops truncatus) with Iron Overload Reveal an Association with Insulin Resistance and Glucagon. Front. Endocrinol. 4, 132. https://doi.org/10.3389/fendo.2013.00132
Neely, B., Ellisor, D., Davis, W., 2018. Proteomics as a metrological tool to evaluate genome annotation accuracy following de novo genome assembly: a case study using the Atlantic bottlenose dolphin (Tursiops truncatus). https://doi.org/10.1101/254250
Neely, B.A., Ferrante, J.A., Chaves, J.M., Soper, J.L., Almeida, J.S., Arthur, J.M., Gulland, F.M.D., Janech, M.G., 2015a. Proteomic Analysis of Plasma from California Sea Lions (Zalophus californianus) Reveals Apolipoprotein E as a Candidate Biomarker of Chronic Domoic Acid Toxicosis. PLOS ONE 10, e0123295. https://doi.org/10.1371/journal.pone.0123295
Neely, B.A., Prager, K.C., Bland, A.M., Fontaine, C., Gulland, F.M., Janech, M.G., 2018. Proteomic Analysis of Urine from California Sea Lions (Zalophus californianus): A Resource for Urinary Biomarker Discovery. J. Proteome Res. 17, 3281–3291. https://doi.org/10.1021/acs.jproteome.8b00416
Neely, B.A., Soper, J.L., Gulland, F.M.D., Bell, P.D., Kindy, M., Arthur, J.M., Janech, M.G., 2015b. Proteomic analysis of cerebrospinal fluid in California sea lions (Zalophus californianus) with domoic acid toxicosis identifies proteins associated with neurodegeneration. Proteomics 15, 4051–4063. https://doi.org/10.1002/pmic.201500167
Nelms, S.E., Alfaro-Shigueto, J., Arnould, J., Avila, I., Bengtson Nash, S., Campbell, E., Carter, M., Collins, T., Currey, R., Domit, C., Franco-Trecu, V., Fuentes, M., Gilman, E., Harcourt, R., Hines, E., Hoelzel, A., Hooker, S., Johnston, D., Kelkar, N., Kiszka, J., Laidre, K., Mangel, J., Marsh, H., Maxwell, S., Onoufriou, A., Palacios, D., Pierce, G., Ponnampalam, L., Porter, L., Russell, D., Stockin, K., Sutaria, D., Wambiji, N., Weir, C., Wilson, B., Godley, B., 2021. Marine mammal conservation: over the horizon. Endanger. Species Res. 44, 291–325. https://doi.org/10.3354/esr01115
Nelms, S.E., Barnett, J., Brownlow, A., Davison, N.J., Deaville, R., Galloway, T.S., Lindeque, P.K., Santillo, D., Godley, B.J., 2019a. Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? Sci. Rep. 9, 1075. https://doi.org/10.1038/s41598-018-37428-3
Nelms, S.E., Parry, H.E., Bennett, K.A., Galloway, T.S., Godley, B.J., Santillo, D., Lindeque, P.K., 2019b. What goes in, must come out: Combining scat-based molecular diet analysis and quantification of ingested microplastics in a marine top predator. Methods Ecol. Evol. 10, 1712–1722. https://doi.org/10.1111/2041-210X.13271

Nelson, T.M., Apprill, A., Mann, J., Rogers, T.L., Brown, M.V., 2015. The marine mammal microbiome: current knowledge and future directions. Microbiol. Aust. 36, 8. https://doi.org/10.1071/MA15004

Newsome, S.D., Clementz, M.T., Koch, P.L., 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Mar. Mammal Sci. 26, 509–572. https://doi.org/10.1111/j.1748-7692.2009.00354.x

Olmstead, K.I., La Frano, M.R., Fahrmann, J., Grapov, D., Viscarra, J.A., Newman, J.W., Fiehn, O., Crocker, D.E., Filipp, F.V., Ortiz, R.M., 2017. Insulin induces a shift in lipid and primary carbon metabolites in a model of fasting-induced insulin resistance. Metabolomics Off. J. Metabolomic Soc. 13, 60. https://doi.org/10.1007/s11306-017-1186-y

Oosting, T., Hilario, E., Wellenreuther, M., Ritchie, P.A., 2020. DNA degradation in fish: Practical solutions and guidelines to improve DNA preservation for genomic research. Ecol. Evol. 10, 8643–8651. https://doi.org/10.1002/ece3.6558

Parsons, K.M., Durban, J.W., Burdin, A.M., Burkanov, V.N., Pitman, R.L., Barlow, J., Barrett-Lennard, L.G., LeDuc, R.G., Robertson, K.M., Matkin, C.O., Wade, P.R., 2013. Geographic Patterns of Genetic Differentiation among Killer Whales in the Northern North Pacific. J. Hered. 104, 737–754. https://doi.org/10.1093/jhered/est037

Pasamontes, A., Aksenov, A.A., Schivo, M., Rowles, T., Smith, C.R., Schwacke, L.H., Wells, R.S., Yeates, L., Venn-Watson, S., Davis, C.E., 2017. Noninvasive Respiratory Metabolite Analysis Associated with Clinical Disease in Cetaceans: A Deepwater Horizon Oil Spill Study. Environ. Sci. Technol. 51, 5737–5746. https://doi.org/10.1021/acs.est.6b06482

Passow, C.N., Kono, T.J.Y., Stahl, B.A., Jaggard, J.B., Keene, A.C., McGaugh, S.E., 2019. Nonrandom RNAseq gene expression associated with RNAlater and flash freezing storage methods. Mol. Ecol. Resour. 19, 456–464. https://doi.org/10.1111/1755-0998.12965

Polanowski, A.M., Robbins, J., Chandler, D., Jarman, S.N., 2014. Epigenetic estimation of age in humpback whales. Mol. Ecol. Resour. 14, 976–987. https://doi.org/10.1111/1755-0998.12247

Pollock, J., Glendinning, L., Wisedchanwet, T., Watson, M., 2018. The Madness of Microbiome: Attempting To Find Consensus “Best Practice” for 16S Microbiome Studies. Appl. Environ. Microbiol. 84, e02627-17. https://doi.org/10.1128/AEM.02627-17

Post, D.M., 2002. Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. Ecology 83, 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Poulsen, C.S., Kaas, R.S., Aarestrup, F.M., Pamp, S.J., 2021. Standard Sample Storage Conditions Have an Impact on Inferred Microbiome Composition and Antimicrobial Resistance Patterns. Microbiol. Spectr. 9, e01387-21. https://doi.org/10.1128/Spectrum.01387-21

Pribyl, A.L., Parks, D.H., Angel, N.Z., Boyd, J.A., Hasson, A.G., Fang, L., MacDonald, S.L., Wills, B.A., Wood, D.L.A., Krause, L., Tyson, G.W., Hugenholtz, P., 2021. Critical evaluation of faecal microbiome preservation using metagenomic analysis. ISME Commun. 1, 1–10. https://doi.org/10.1038/s43705-021-00014-2

Proctor, D.M., Fukuyama, J.A., Loomer, P.M., Armitage, G.C., Lee, S.A., Davis, N.M., Ryder, M.I., Holmes, S.P., Relman, D.A., 2018. A spatial gradient of bacterial diversity in the human oral cavity shaped by salivary flow. Nat. Commun. 9, 681. https://doi.org/10.1038/s41467-018-02900-1

Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J., Segata, N., 2017. Shotgun metagenomics, from sampling to analysis. Nat. Biotechnol. 35, 833–844. https://doi.org/10.1038/nbt.3935

Rajesh, A., Chang, Y., Abedalthagafi, M.S., Wong-Beringer, A., Love, M.I., Mangul, S., 2021. Improving the completeness of public metadata accompanying omics studies. Genome Biol. 22, 106. https://doi.org/10.1186/s13059-021-02332-z

Rhie, A., McCarthy, S.A., Fedrigo, O., Damas, J., Formenti, G., Koren, S., Uliano-Silva, M., Chow, W., Fungtammasan, A., Kim, J., Lee, C., Ko, B.J., Chaisson, M., Gedman, G.L., Cantin, L.J., Thibaud-Nissen, F., Haggerty, L., Bista, I., Smith, M., Haase, B., Mountcastle, J., Winkler, S., Paez, S., Howard, J., Vernes, S.C., Lama, T.M., Grutzner, F., Warren, W.C., Balakrishnan, C.N., Burt, D., George, J.M., Biegler, M.T., Iorns, D., Digby, A., Eason, D., Robertson, B., Edwards, T., Wilkinson, M., Turner, G., Meyer, A., Kautt, A.F., Franchini, P., Detrich, H.W., Svardal, H., Wagner, M., Naylor, G.J.P., Pippel, M., Malinsky, M., Mooney, M., Simbirsky, M., Hannigan, B.T., Pesout, T., Houck, M., Misuraca, A., Kingan, S.B., Hall, R., Kronenberg, Z., Sović, I., Dunn, C., Ning, Z., Hastie, A., Lee, J., Selvaraj, S., Green, R.E., Putnam, N.H., Gut, I., Ghurye, J., Garrison, E., Sims, Y., Collins, J., Pelan, S., Torrance, J., Tracey, A., Wood, J., Dagnew, R.E., Guan, D., London, S.E., Clayton, D.F., Mello, C.V., Friedrich, S.R., Lovell, P.V., Osipova, E., Al-Ajli, F.O., Secomandi, S., Kim, H., Theofanopoulou, C., Hiller, M., Zhou, Y., Harris, R.S., Makova, K.D., Medvedev, P., Hoffman, J., Masterson, P., Clark, K., Martin, F., Howe, Kevin, Flicek, P., Walenz, B.P., Kwak, W., Clawson, H., Diekhans, M., Nassar, L., Paten, B., Kraus, R.H.S., Crawford, A.J., Gilbert, M.T.P., Zhang, G., Venkatesh, B., Murphy, R.W., Koepfli, K.-P., Shapiro, B., Johnson, W.E., Di Palma, F., Marques-Bonet, T., Teeling, E.C., Warnow, T., Graves, J.M., Ryder, O.A., Haussler, D., O'Brien, S.J., Korlach, J., Lewin, H.A., Howe, Kerstin, Myers, E.W., Durbin, R., Phillippy, A.M., Jarvis, E.D., 2021. Towards complete and error-free genome assemblies of all vertebrate species. Nature 592, 737–746. https://doi.org/10.1038/s41586-021-03451-0

Rissanen, A.J., Kurhela, E., Aho, T., Oittinen, T., Tiirola, M., 2010. Storage of environmental samples for guaranteeing nucleic acid yields for molecular microbiological studies. Appl. Microbiol. Biotechnol. 88, 977–984. https://doi.org/10.1007/s00253-010-2838-2

Rolland, R.M., Parks, S.E., Hunt, K.E., Castellote, M., Corkeron, P.J., Nowacek, D.P., Wasser, S.K., Kraus, S.D., 2012. Evidence that ship noise increases stress in right whales. Proc. R. Soc. B Biol. Sci. 279, 2363–2368. https://doi.org/10.1098/rspb.2011.2429

Roy, D., Tomo, S., Purohit, P., Setia, P., 2021. Microbiome in Death and Beyond: Current Vistas and Future Trends. Front. Ecol. Evol. 9, 630397. https://doi.org/10.3389/fevo.2021.630397

Ruiz-Cooley, R.I., Garcia, K.Y., Hetherington, E.D., 2011. Effects of lipid removal and preservatives on carbon and nitrogen stable isotope ratios of squid tissues: Implications for ecological studies. J. Exp. Mar. Biol. Ecol. 407, 101–107. https://doi.org/10.1016/j.jembe.2011.07.002

Salehi, Z., Najafi, M., 2014. RNA Preservation and Stabilization. Biochem Physiol 03, 126. https://doi.org/10.4172/2168-9652.1000126

Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., Turner, P., Parkhill, J., Loman, N.J., Walker, A.W., 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 12, 87. https://doi.org/10.1186/s12915-014-0087-z

Sanders, J.G., Beichman, A.C., Roman, J., Scott, J.J., Emerson, D., McCarthy, J.J., Girguis, P.R., 2015. Baleen whales host a unique gut microbiome with similarities to both carnivores and herbivores. Nat. Commun. 6, 8285. https://doi.org/10.1038/ncomms9285

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467. https://doi.org/10.1073/pnas.74.12.5463

Santiago, A., Panda, S., Mengels, G., Martinez, X., Azpiroz, F., Dore, J., Guarner, F., Manichanh, C., 2014. Processing faecal samples: a step forward for standards in microbial community analysis. BMC Microbiol. 14, 112.

https://doi.org/10.1186/1471-2180-14-112
Schmidt, D., Wilson, M.D., Spyrou, C., Brown, G.D., Hadfield, J., Odom, D.T., 2009. ChIP-seq: using high-throughput sequencing to discover protein-DNA interactions. Methods San Diego Calif 48, 240–248. https://doi.org/10.1016/j.ymeth.2009.03.001
Sepulveda, A.J., Hutchins, P.R., Forstchen, M., Mckeefry, M.N., Swigris, A.M., 2020. The Elephant in the Lab (and Field): Contamination in Aquatic Environmental DNA Studies. Front. Ecol. Evol. 8, 440. https://doi.org/10.3389/fevo.2020.609973
Serbanescu, M.A., Mathena, R.P., Xu, J., Santiago-Rodriguez, T., Hartsell, T.L., Cano, R.J., Mintz, C.D., 2019. General Anesthesia Alters the Diversity and Composition of the Intestinal Microbiota in Mice. Anesth. Analg. 129, e126–e129. https://doi.org/10.1213/ANE.0000000000003938
Sergeant, M.J., Constantinidou, C., Cogan, T., Penn, C.W., Pallen, M.J., 2012. High-throughput sequencing of 16S rRNA gene amplicons: effects of extraction procedure, primer length and annealing temperature. PloS One 7, e38094. https://doi.org/10.1371/journal.pone.0038094
Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013
Seutin, G., White, B.N., Boag, P.T., 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69, 82–90. https://doi.org/10.1139/z91-013
Shabihkhani, M., Lucey, G.M., Wei, B., Mareninov, S., Lou, J.J., Vinters, H.V., Singer, E.J., Cloughesy, T.F., Yong, W.H., 2014. The procurement, storage, and quality assurance of frozen blood and tissue biospecimens in pathology, biorepository, and biobank settings. Clin. Biochem. 47, 258–266. https://doi.org/10.1016/j.clinbiochem.2014.01.002
Sheridan, M.L., 2004. Determining pregnancy status of extant and ancient whales by quantifying progesterone in blubber biopsies and bone (masters). Memorial University of Newfoundland.
Shero, M.R., Reiser, P.J., Simonitis, L., Burns, J.M., 2019. Links between muscle phenotype and life history: differentiation of myosin heavy chain composition and muscle biochemistry in precocial and altricial pinniped pups. J. Comp. Physiol. B 189, 717–734. https://doi.org/10.1007/s00360-019-01240-w
Sikes, R.S., 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J. Mammal. 97, 663–688. https://doi.org/10.1093/jmammal/gyw078
Simmons, J., 2014. Fluid Preservation: A Comprehensive Reference.
Simón-Soro, A., Tomás, I., Cabrera-Rubio, R., Catalan, M.D., Nyvad, B., Mira, A., 2013. Microbial geography of the oral cavity. J. Dent. Res. 92, 616–621. https://doi.org/10.1177/0022034513488119
Sinclair, C., Sinclair, J., Zolman, E.S., Martinez, A., Balmer, B., Barry, K., 2015. Remote biopsy field sampling procedures for cetaceans used during the Natural Resource Damage Assessment of the MSC252 Deepwater Horizon oil spill. https://doi.org/10.7289/V5CC0XN0
Smith, L., Villaret-Cazadamont, J., Claus, S.P., Canlet, C., Guillou, H., Cabaton, N.J., Ellero-Simatos, S., 2020. Important Considerations for Sample Collection in Metabolomics Studies with a Special Focus on Applications to Liver Functions. Metabolites 10, 104. https://doi.org/10.3390/metabo10030104
Sobolesky, P., Parry, C., Boxall, B., Wells, R., Venn-Watson, S., Janech, M.G., 2016. Proteomic Analysis of Non-depleted Serum Proteins from Bottlenose Dolphins Uncovers a High Vanin-1 Phenotype. Sci. Rep. 6, 33879. https://doi.org/10.1038/srep33879
Sobolesky, P.M., Harrell, T.S., Parry, C., Venn-Watson, S., Janech, M.G., 2016. Feeding a Modified Fish Diet to Bottlenose Dolphins Leads to an Increase in Serum Adiponectin

and Sphingolipids. Front. Endocrinol. 7, 33. https://doi.org/10.3389/fendo.2016.00033
Song, S.J., Amir, A., Metcalf, J.L., Amato, K.R., Xu, Z.Z., Humphrey, G., Knight, R., 2016. Preservation Methods Differ in Fecal Microbiome Stability, Affecting Suitability for Field Studies. mSystems. https://doi.org/10.1128/mSystems.00021-16
Staunstrup, N.H., Starnawska, A., Nyegaard, M., Christiansen, L., Nielsen, A.L., Børglum, A., Mors, O., 2016. Genome-wide DNA methylation profiling with MeDIP-seq using archived dried blood spots. Clin. Epigenetics 8, 81. https://doi.org/10.1186/s13148-016-0242-1
Taberlet, P., Waits, L.P., Luikart, G., 1999. Noninvasive genetic sampling: look before you leap. Trends Ecol. Evol. 14, 323–327. https://doi.org/10.1016/S0169-5347(99)01637-7
Tanabe, A., Shimizu, R., Osawa, Y., Suzuki, M., Ito, S., Goto, M., Pastene, L.A., Fujise, Y., Sahara, H., 2020. Age estimation by DNA methylation in the Antarctic minke whale. Fish. Sci. 86, 35–41. https://doi.org/10.1007/s12562-019-01371-7
Tian, J., Du, J., Han, J., Bao, X., Song, X., Lu, Z., 2020. Proteomics reveals the preliminary physiological states of the spotted seal (Phoca largha) pups. Sci. Rep. 10, 18727. https://doi.org/10.1038/s41598-020-75759-2
Toone, R.J., Peacock, O.J., Smith, A.A., Thompson, D., Drawer, S., Cook, C., Stokes, K.A., 2013. Measurement of steroid hormones in saliva: Effects of sample storage condition. Scand. J. Clin. Lab. Invest. 73, 615–621. https://doi.org/10.3109/00365513.2013.835862
Trana, M.R., Roth, J.D., Tomy, G.T., Anderson, W.G., Ferguson, S.H., 2015. Influence of sample degradation and tissue depth on blubber cortisol in beluga whales. J. Exp. Mar. Biol. Ecol. 462, 8–13. https://doi.org/10.1016/j.jembe.2014.10.010
Trego, M.L., Whitehead, A., Kellar, N.M., Lauf, M., Lewison, R.L., 2020. Tracking transcriptomic responses to endogenous and exogenous variation in cetaceans in the Southern California Bight. Conserv. Physiol. 7. https://doi.org/10.1093/conphys/coz018
Trumble, S.J., Norman, S.A., Crain, D.D., Mansouri, F., Winfield, Z.C., Sabin, R., Potter, C.W., Gabriele, C.M., Usenko, S., 2018. Baleen whale cortisol levels reveal a physiological response to 20th century whaling. Nat. Commun. 9, 4587. https://doi.org/10.1038/s41467-018-07044-w
Unal, E., Goertz, C.E.C., Hobbs, R.C., Suydam, R., Romano, T., 2018. Investigation of molecular biomarkers as potential indicators of health in wild belugas (Delphinapterus leucas). Mar. Biol. 165, 182. https://doi.org/10.1007/s00227-018-3439-3
Van Cise, A.M., Baird, R.W., Baker, C.S., Cerchio, S., Claridge, D., Fielding, R., Hancock-Hanser, B., Marrero, J., Martien, K.K., Mignucci-Giannoni, A.A., Oleson, E.M., Oremus, M., Poole, M.M., Rosel, P.E., Taylor, B.L., Morin, P.A., 2019. Oceanographic barriers, divergence, and admixture: Phylogeography and taxonomy of two putative subspecies of short-finned pilot whale. Mol. Ecol. 28, 2886–2902. https://doi.org/10.1111/mec.15107
Van Cise, A.M., Wade, P.R., Goertz, C.E.C., Burek-Huntington, K., Parsons, K.M., Clauss, T., Hobbs, R.C., Apprill, A., 2020. Skin microbiome of beluga whales: spatial, temporal, and health-related dynamics. Anim. Microbiome 2, 39. https://doi.org/10.1186/s42523-020-00057-1
Vilahur, N., Baccarelli, A.A., Bustamante, M., Agramunt, S., Byun, H.-M., Fernandez, M.F., Sunyer, J., Estivill, X., 2013. Storage conditions and stability of global DNA methylation in placental tissue. Epigenomics 5, 10.2217/epi.13.29. https://doi.org/10.2217/epi.13.29
Villar, D., Berthelot, C., Aldridge, S., Rayner, T.F., Lukk, M., Pignatelli, M., Park, T.J., Deaville, R., Erichsen, J.T., Jasinska, A.J., Turner, J.M.A., Bertelsen, M.F., Murchison, E.P., Flicek, P., Odom, D.T., 2015. Enhancer Evolution across 20 Mammalian Species. Cell 160, 554–566. https://doi.org/10.1016/j.cell.2015.01.006
Vincek, V., Nassiri, M., Knowles, J., Nadji, M., Morales, A.R., 2003. Preservation of Tissue RNA in Normal Saline. Lab. Invest. 83, 137–138. https://doi.org/10.1097/01.LAB.0000047490.26282.CF

Voisinet, M., Christina, V., Elowe, C., Crocker, D., Tomanek, L., 2015. Proteomic Response of Elephant Seal Pups, Mirounga angustriorstris, to Prolonged Fasting, in: Symposium Student Journal of Science & Math of CalPoly. pp. 15–23.
Waller, R., 1994. Conservation risk assessment: a strategy for managing resources for preventive conservation. Stud. Conserv. 39, 12–16. https://doi.org/10.1179/sic.1994.39.Supplement-2.12
Wang, L., Yang, X., Wu, H., 2019. Juvenile Rats Show Altered Gut Microbiota After Exposure to Isoflurane as Neonates. Neurochem. Res. 44, 776–786. https://doi.org/10.1007/s11064-018-02707-y
Weaver, I.C.G., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., Meaney, M.J., 2004. Epigenetic programming by maternal behavior. Nat. Neurosci. 7, 847–854. https://doi.org/10.1038/nn1276
Weiss, S., Amir, A., Hyde, E.R., Metcalf, J.L., Song, S.J., Knight, R., 2014. Tracking down the sources of experimental contamination in microbiome studies. Genome Biol. 15, 564. https://doi.org/10.1186/s13059-014-0564-2
Whibley, A., 2021. Genome insights give cause for optimism in the ongoing battle to save the vaquita. Mol. Ecol. Resour. 21, 1005–1007. https://doi.org/10.1111/1755-0998.13345
Whibley, A., Kelley, J.L., Narum, S.R., 2021. The changing face of genome assemblies: Guidance on achieving high-quality reference genomes. Mol. Ecol. Resour. 21, 641–652. https://doi.org/10.1111/1755-0998.13312
Wishart, D.S., 2019. Metabolomics for Investigating Physiological and Pathophysiological Processes. Physiol. Rev. 99, 1819–1875. https://doi.org/10.1152/physrev.00035.2018
Wong, P.B., Wiley, E.O., Johnson, W.E., Ryder, O.A., O'Brien, S.J., Haussler, D., Koepfli, K.-P., Houck, M.L., Perelman, P., Mastromonaco, G., Bentley, A.C., Venkatesh, B., Zhang, Y., Murphy, R.W., 2012. Tissue sampling methods and standards for vertebrate genomics. GigaScience 1, 8. https://doi.org/10.1186/2047-217X-1-8
Zantis, L.J., Carroll, E.L., Nelms, S.E., Bosker, T., 2021. Marine mammals and microplastics: A systematic review and call for standardisation. Environ. Pollut. 269, 116142. https://doi.org/10.1016/j.envpol.2020.116142
Zimkus, B., Hassapakis, C., Houck, M., 2018. Integrating current methods for the preservation of amphibian genetic resources and viable tissues to achieve best practices for species conservation. Amphib. Amp Reptile Conserv. 12, 1–27.

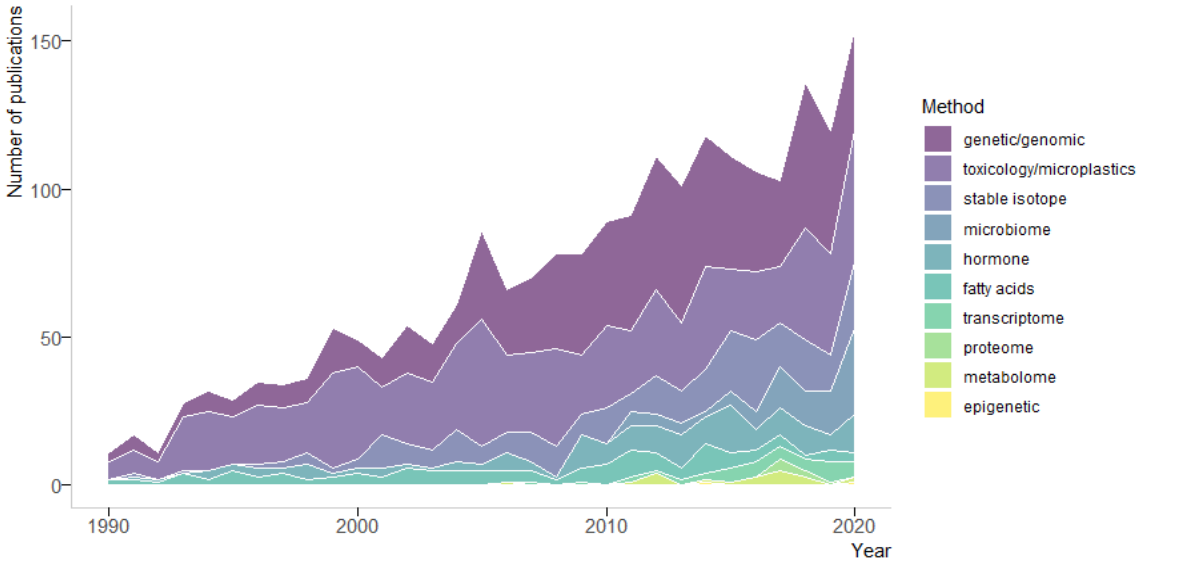

Figure 1. The number of studies relying on marine mammal biological samples published annually since 1990, stratified by primary study methodology. Counts were based on a Google Scholar search for “marine mammal” + each keyword listed in the legend, and manually filtered by study title to ensure extraneous studies were removed.

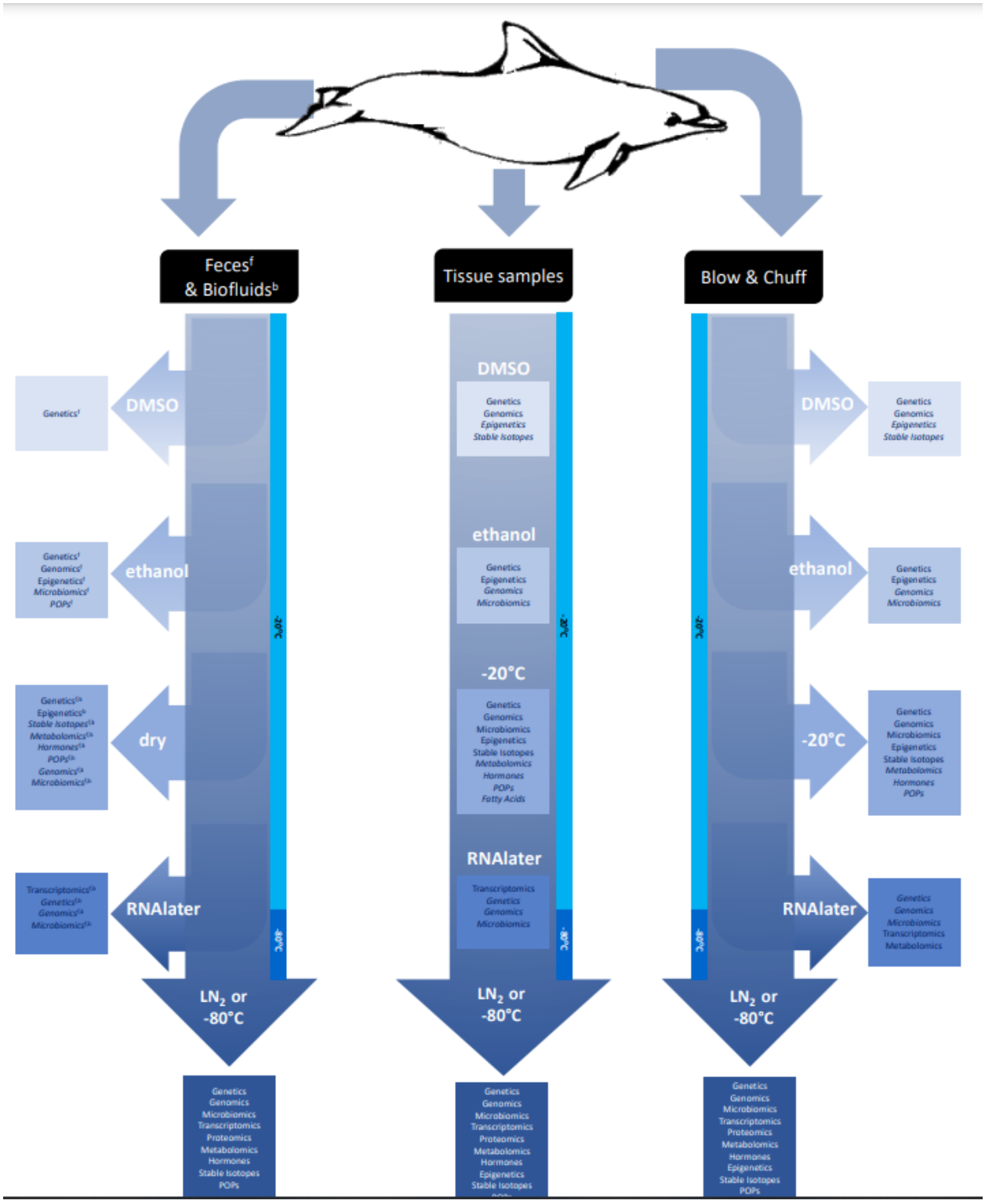

Figure 2. A schematic representation of common types of biospecimens collected from marine mammals and sample preservation methods suitable for a suite of analytical approaches including 'omics, hormones, stable isotopes, microplastics isolation and toxic contaminants.

Supplementary Information:

Table S1. Detailed comparison of common preservatives and fixatives used for long-term storage of marine mammal biological samples.

Table S2. Ranking of commonly used methods of biospecimen preservation for ‘omics (and other) targeted analytical methods.