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Ex vivo and *in vitro* methods as a platform for studying anthropogenic effects on marine mammals: four challenges and how to meet them

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Marine mammals are integral to global biodiversity and marine health through their roles in coastal, benthic, and pelagic ecosystems. Marine mammals face escalating threats from climate change, pollution, and human activities, which perturb their oceanic environment. The diverse biology and extreme adaptations evolved by marine mammals make them important study subjects for understanding anthropogenic pressures on marine ecosystems. However, ethical and logistical constraints restrict the tractability of experimental research with live marine mammals. Additionally, studies on the effects of changing ocean environments are further complicated by intricate gene-environment interactions across populations and species. These obstacles can be overcome with a comprehensive strategy that involves a systems-level approach integrating genotype to phenotype using rigorously defined experimental conditions *in vitro* and *ex vivo*. A thorough analysis of the interactions between the genetics of marine mammals and their exposure to anthropogenic pressures will enable robust predictions about how global environmental changes will affect their health and populations. In this perspective, we discuss four challenges of implementing such non-invasive approaches across scientific fields and international borders: 1) practical and

ethical limitations of *in vivo* experimentation with marine mammals, 2) accessibility to relevant tissue samples and cell cultures; 3) open access to harmonized methods and datasets and 4) ethical and equitable research practices. Successful implementation of the proposed approach has the potential impact to inspire new solutions and strategies for marine conservation.

KEYWORDS

marine mammals, systems biology, toxicology, physiology, climate change, predictive modeling, functional genomics, endocrine-metabolic disruptors

1 Introduction

Marine mammals play essential roles in coastal, benthic, and pelagic ecosystems. Many species occupy high trophic levels and contribute to carbon sequestration, nutrient cycling, and primary productivity. Some marine mammals dive deeply or migrate long distances, enabling vertical and horizontal movement of nutrients. Therefore, declines in marine mammal populations compromise the stability and biodiversity of ocean ecosystems and serve as warning signs of declines in ecosystem health (Fortuna et al., 2024; Estes et al., 2016). Furthermore, the accumulation of contaminants and microplastics in marine mammal tissues provides a bioindicator of pollution in marine food webs and its effects on large, long-lived mammals (Bossart, 2011). This diverse group, comprising whales and dolphins (cetaceans), seals and sea lions (pinnipeds), manatees and dugongs (sirenians), and polar bears and sea otters (marine fissipeds) has convergently evolved adaptations to the aquatic environment on a relatively short time scale, including extreme body size, extraordinary diving ability, and extended fasting capacity (Foote et al., 2015; Jones et al., 2009). During the Anthropocene, marine mammals increasingly confront mounting pressures impacting their health, reproduction, and population viability (Parsons et al., 2015; O'Hara et al., 2021; Halpern et al., 2015; Schaap et al., 2023; Morrison et al., 2022; National Academy of Sciences, Engineering, and Medicine, 2017). As vanguards in a changing world, a more profound understanding of marine mammal adaptations and vulnerabilities to anthropogenic pressures is essential to global health and will allow modeling responses to further change.

Direct and indirect anthropogenic pressures threaten marine mammal populations' health. Over 40% of the 134 recognized marine mammal species are classified at high risk of global extinction by the International Union for Conservation of Nature (IUCN), an increase from only 25% in 2021 (Nelms et al., 2021; IUCN, 2023). This is likely an underestimate as many species and populations (102 out 209 (IUCN, 2023)) are data-deficient due to uncertain distribution, population status, or taxonomic assignment, and may have an especially high extinction risk (Braulik et al., 2023; Borgelt et al., 2022). While regulatory actions have contributed to the recovery of some populations (Valdivia et al., 2019; Lowry et al., 2014; Herr et al., 2022), others remain under threat due to direct and indirect effects of incidental catch, harvesting, pollution, marine traffic, tourism, urban development, and climate change (Nelms et al., 2021; Davidson et al., 2012; Avila et al., 2018). This is concerning as marine mammals offer diverse ecosystem services critical to ocean health, such as nutrient cycling and food web stability (Estes et al., 2009; McCauley et al., 2015; Hammerschlag et al., 2019; Roman and McCarthy, 2010).

Bycatch in fisheries is a significant threat to whales and dolphins (Cetacea), with at least 300,000 individuals ensnared and killed annually (Read et al., 2006; Elliott et al., 2023). In addition, some fisheries compete with marine mammals for prey, and both fisheries and shipping traffic can cause physical encounters, injuries, and fatalities to marine mammals (Schoeman et al., 2020; International Whaling Commission, 2023; Demaster et al., 2001). Although the number of harvested marine mammals has dropped, several marine mammal species are still consumed worldwide, and direct harvesting is still a threat to tens of species (Avila et al., 2018; Skern-Mauritzen et al., 2022).

Marine mammals are also adversely impacted by the physiological stress responses induced by other oceanic and coastal activities. For example, ecotourism - driven by a fascination with marine mammals - exposes millions of people to these animals globally and is a threat to 21% of the marine mammal species included in the IUCN Red List of Threatened Species (Bejder et al., 2022; IUCN, 2023). Similarly, noise pollution from shipping, naval activities, sonar, and seismic operations is another increasing threat to marine mammals. Noise pollution affects marine mammals' underwater echolocation and communication strategies, inducing neuroendocrine stress responses that impact energy budgets, diving, foraging, social interactions, reproduction, and survival, and has been linked to whale strandings (Erbe et al., 2019; NOAA, 2023; Southall et al., 2021; Williams et al., 2017).

Atmospheric and oceanic pollution is a worsening global problem impacting marine mammal health (Landrigan et al., 2020). Legacy and emerging chemical pollutants accumulate in marine mammals, particularly affecting predators at high trophic levels, such as polar bears and orcas (Remili et al., 2023). Biomagnification and preferential consumption of fatty tissues that accumulate pollutants such as liver and blubber increase the risk of endocrine and metabolic disruption (Guo et al., 2023;

Mortimer and Batley, 2023; Sanganyado et al., 2021; Weijs and Zaccaroni, 2016; Fair and Houde, 2023). Thus, chemical pollutants acting as endocrine and metabolic disruptors impair pre- and post-natal development, reproduction, metabolic and immune health, longevity, and may have further impacts on population dynamics in marine mammals (Landrigan et al., 2020; Sanganyado et al., 2021; Weijs and Zaccaroni, 2016; Desforges et al., 2018; Dietz et al., 2019). Furthermore, plastic pollution - adding around 500,000 metric tons to the oceans annually - poses additional threats through entanglement and ingestion and as a carrier of chemical pollutants (Commission, I.W, 2020; Fossi et al., 2020; Kaandorp et al., 2023). Methodologies to delineate and prioritize chemical pollutant drivers of biological processes are under development but have not yet reached "prime time" regarding policy changes.

Beyond pollution, global climate change is likely to exacerbate anthropogenic impacts and physiological stress in marine mammals by intensifying habitat loss, altering ocean productivity, causing shifts in prey range and abundance, releasing pollutants stored in the sea ice, and increasing the frequency and severity of toxic algal blooms and pathogen outbreaks (Read, 2023; Davidson et al., 2012; Albouy et al., 2020; Gobler, 2020; Mahon et al., 2024). Conversely, the loss of marine mammals may exacerbate the effects of climate change on ecosystems, as large-bodied species play a role in carbon sequestration, nutrient cycling, and other vital ecosystem services (Pearson et al., 2023). Diverse stressors interact in complex ways, as is the case for mixtures of chemical pollutants, challenging the study of their combined effects (Wilson et al., 2016; Bestley et al., 2020; Romero, 2004; Wada, 2019; Tartu et al., 2017; Erbe et al., 2018). Understanding the impacts of multiple stressors on marine mammals is crucial for their conservation (Parsons et al., 2015; National Academy of Sciences, Engineering, and Medicine, 2017). As *in vivo* experimentation with marine mammals is restricted due to logistic and ethical concerns, correlative field studies investigating the relationships between exposure to a stressor and specific health biomarkers have been widely used (Pallin et al., 2022; Trego et al., 2019; Schaap et al., 2023). These studies have inherent limitations in establishing cause-and-effect relationships and mechanistic understanding (Foote et al., 2015; Dietz et al., 2019).

The extraordinary biology of marine mammals captivates many fields, including evolutionary biology, physiology, and toxicology (Manger, 2022); at the union of these disciplines lie the solutions to understanding and predicting marine mammals' response and resilience to global change. For example, genomic insights can unravel adaptations to cardiovascular and metabolic diseases and the evolution of cancer resistance in large-bodied, long-lived mammals such as whales (Steimle and Moskowitz, 2017; Sun et al., 2022; Nagy et al., 2007; Silva et al., 2023; Vazquez et al., 2022). The Earth Biogenome Project aims to sequence the genomes of all eukaryotic species to understand the evolution of life at all scales and to drive solutions for preserving biodiversity and sustaining human societies (Lewin et al., 2022). The sequencing of several marine mammal genomes is ongoing worldwide, which increases our knowledge of their adaptive potential and facilitates species' conservation (Theissinger et al., 2023).

To maximize the impact of marine mammal and environmental research at all levels, we suggest using an approach that simultaneously leverages new and innovative ways to study top-level (phenotype and environmental response) and bottom-level (genomics and molecular biology) phenomena. This "middle-out" approach includes *in vitro* studies with marine mammal cells, *ex vivo* experiments with tissue slices and explants, and *in silico* genome mining and modeling. These approaches can help resolve physiological paradoxes, assess interactions between and impacts of multiple stressors, and have many applications in conservation biology (Schaap et al., 2023; Weijs and Zaccaroni, 2016; Lam et al., 2020; Torres-Velarde et al., 2021; Allen et al., 2024; Goksøyr, 2022). The prospects and challenges involved in implementing such less-invasive approaches are discussed below.

2 Challenge #1: practical and ethical limitations of *in vivo* experimentation with marine mammals

Conducting *in vivo* experiments with marine mammals presents significant challenges driven by logistical and ethical constraints (Parsons et al., 2015; Williams and Hindle, 2021; Hawkins et al., 2017; Hunt et al., 2013). Additionally, experimental manipulations are restricted to captive animals, limited field interventions (Lam et al., 2020; Ensminger et al., 2021a), and hormone and contaminant profiling (O'Hara and Hart, 2018; Ross et al., 1995; De Swart et al., 1996). Our proposal to circumvent these limitations consists of a "middle-out" approach that combines *ex vivo*, *in vitro*, and *in silico* studies, along with comparative functional genomics and computational methods to gain an integrated, systems-level understanding of basic and applied physiology and toxicology of marine mammals (Figure 1) (Torres-Velarde et al., 2021; Bories et al., 2021; Bjørneset et al., 2023; Khudyakov et al., 2022; Penso-Dolfin et al., 2020; Godard et al., 2004; Hindle et al., 2019). This strategy minimizes our impact on animals while facilitating experiments involving multiple treatments and stressors under precisely controlled conditions (Weijs and Zaccaroni, 2016).

2.1 Ethics of sampling marine mammals

A common paradox in biology is that the more endangered the species, the more dire the need for invasive studies to understand and manage their challenges. Over 40% of marine mammal species are at high risk of global extinction (Nelms et al., 2021; IUCN, 2023). Hence, this paradox is especially critical due to the pressing need to understand how environmental hazards threatening marine mammals continue to expand in scope and scale. Handling marine mammals for purposes such as tissue sampling for toxicological studies and measurements for physiological determinations - or even piloting a boat to the sampling site - can induce stress responses that may adversely impact their health (Southall et al., 2021). Moreover, different species present different ethical challenges, which adds another layer of complexity. As such, any approaches that reduce or eliminate intensive *in vivo* sampling would simultaneously further conservation goals.

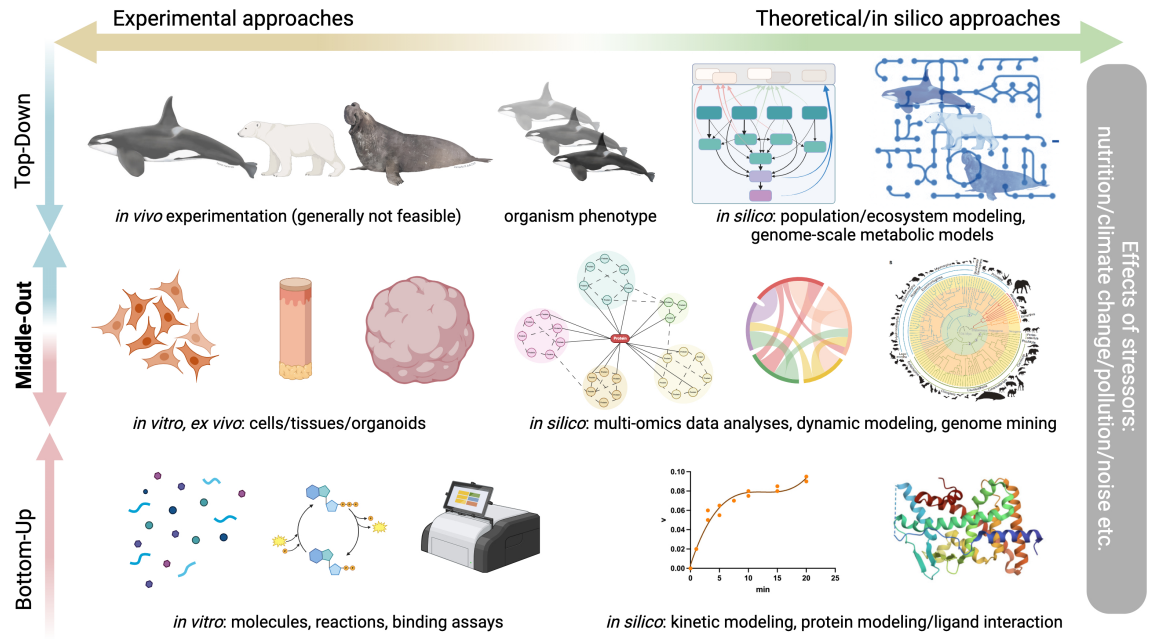

FIGURE 1
"Middle-out" systems approach, which combines *ex vivo*, *in vitro*, and *in silico* methods. The proposed approach is crucial to conduct research with marine mammals where *in vivo* experimentation is generally not applicable. In this way, an understanding of how marine mammals will respond to anthropogenic pressures such as climate change, noise pollution, and contaminants at the individual and population levels can be achieved through *in vitro* and *ex vivo* experimentation and *in silico* modeling. The proposed approach may be implemented as follows: First, levels of stress hormones, metabolites, or contaminants of interest are measured in tissues from live or recently deceased animals to establish reference ranges (Desforges et al., 2018; Tartu et al., 2020, 2017; Guo et al., 2021). Next, primary cells or tissues are used to test the effects of varying stressor concentrations and combinations on cell and tissue function (Routti et al., 2016; Torres-Velarde et al., 2021; Kashiwabara et al., 2023; Desforges et al., 2018; Rajput et al., 2021; Lühmann et al., 2020). These functional tests may also incorporate other stressors exacerbated by climate change, such as alterations in nutrient levels, oxygen tension, temperature, and pH. The role of specific pathways may be tested using functional tools such as gene overexpression, knockdown, and pharmacological manipulations (Torres-Velarde et al., 2021; Lam et al., 2020). In some cases, *in vitro* and *ex vivo* studies can be integrated with *in vivo* experimentation in amenable systems to determine whether cell and tissue responses are scalable to the organismal level (Ensminger et al., 2021a; McCormley et al., 2018). Functional experiments may also be conducted using *in silico* modeling to predict responses to genetic and environmental variations (Hanna et al., 2020). Data generated by this approach may be incorporated into models such as the Population Consequences of Multiple Stressors (PCoMS) to predict the impacts on individual survival and reproduction and, by extension, on population stability (Pirotta et al., 2023; Keen et al., 2021). Potential limitations to the proposed approach include the use of primary cells and tissues, which have a finite lifespan, the use of few tissue and cell types due to the limitations of sampling live marine mammals and species-specific differences in tissue structure, amenability to culture, and response to stressors (Lambilotte, 2024). The latter impacts the ability to understand how tissue responses are integrated at the organismal level but may be overcome by coupling *in vitro* and *ex vivo* studies with *in vivo* experimentation in select systems. Furthermore, contaminant and hormone exposure experiments do not always recapitulate the complexity of pollutant cocktails and signaling molecules likely experienced by marine mammals *in vivo* but offer the advantage of disentangling the interactions between individual components. Figure generated in BioRender; icons from BioRender or noaa.org (orca and elephant seal); phylogeny from (Christmas et al., 2023); crystal structure from Protein Data Bank (2O9I).

2.2 Logistics involved in sampling marine mammals

In addition to ethical limitations, there are significant logistical challenges to studying marine mammals. These include regional and international regulations that demand specialized knowledge and dedicated efforts to procure, maintain, and coordinate research permits. These regional regulatory apparatuses balance the need for conservation while reducing research redundancy; however, distinct regulatory regimes across regions lead to asymmetric research capacity across the globe. Similarly, some marine mammal populations sometimes span entire oceans and the resources necessary to reach most marine mammals in oceanic environments are either prohibitive or intractable. Even if the resources are in place, logistical challenges often constrain timely sample preservation and laboratory processing. For marine mammal studies specifically and to maximize research efforts more generally, it is critical to facilitate the open sharing of material and develop novel systems to enable mechanistic marine mammal research.

3 Challenge #2: accessibility to relevant tissue samples and cell cultures

Lack of access to tissue samples and cell cultures severely limits research on the impacts of chemical and other stressors on marine mammals. Critical issues include sample collection, exchange, storage logistics, incomplete source animal data, and research permit requirements. Low animal accessibility, climate, weather patterns, and distance to laboratory facilities often hinder sample collection. Quality and type of tissues and reliable animal access

differ among live, stranded, by-caught, euthanized, and hunted animals. Suitable and reproducible storage and preservation of samples, tissues, and cell cultures is critical for research but depends on field logistics and laboratory equipment availability. While metadata from the source animal (e.g., species, sex, age class, body condition, reproductive state, health status, photo ID, specific population, and past exposure to contaminants or other stressors) are essential for functional data interpretation, they are often limited or unavailable. Permits required for sample collection and exchange vary by country and species, usually creating bureaucratic challenges (Parsons et al., 2015). Finally, financial resources needed to provide reliable and equitable storage and access, such as ultralow-temperature freezer equipment, dedicated personnel responsible for inventory and user management, shipping costs, and assistance and training of low-income or traditionally underrepresented researchers or institutions, present an additional challenge.

To be feasible and reproducible at scale, a middle-out approach for endangered marine mammals requires a strategic plan to generate, store, and access a collection of tissues and cells within a framework that allows equitable and cooperative material exchange. Experimentation in cells and tissues offers a bridge between *in vivo* techniques and functional molecular biology (Godard-Codding et al., 2011; Lam et al., 2020; Madelaire et al., 2022; Debier et al., 2020; Godard et al., 2006; Godard-Codding and Fossi, 2018; Boroda, 2017), providing unique insights into the impacts of contaminant exposure and response to stressors in marine mammals (Kanter et al., 2002). The establishment of tissue cultures, such as precision-cut adipose tissue slices (Debier et al., 2020; Kashiwabara et al., 2023) and other organotypic models would enable the investigation of responses that are a closer reflection of *in vivo* conditions (Khudyakov et al., 2017), and unveil physiological mechanisms, such as metabolism, fasting, stress physiology while providing a holistic understanding of multi-cellular complexity, extracellular interactions, and organ structure and function (Khudyakov et al., 2022; Godard et al., 2004; Debier et al., 2020; Godard et al., 2006; Tranganida et al., 2023; Deyarmin et al., 2020, 2019; Khudyakov et al., 2018).

Cell, explant, and organotypic cultures can be derived from most marine mammal tissues and have a variety of downstream applications (Lam et al., 2020; Godard et al., 2004, 2006; Kashiwabara et al., 2023; Khudyakov et al., 2017). While dermal fibroblasts are the most used cultures due to skin tissue accessibility, fibroblasts obtained from other tissues, adipocytes, stem cells, myoblasts, endothelial cells, and trophoblasts have been cultured successfully, increasing research opportunities (Torres-Velarde et al., 2021; Allen et al., 2024; Routti et al., 2016; Louis et al., 2015; Griffeth et al., 2014; Johnson et al., 2012). Most marine mammal cell cultures are primary monocultures, but the field is evolving to include directly reprogrammed and immortalized cell lines, induced pluripotent stem cells, organoids, explants, and organotypic (tissue slice) cultures (Godard et al., 2004; Debier et al., 2020; Kashiwabara et al., 2023; Tranganida et al., 2023; Bennett et al., 2017; Robinson et al., 2018). Nevertheless, the scope of available cultures remains limited in the type of source organs and species, while validation and standardization of newer methodologies are still ongoing. Additionally, primary cell cultures, explants, and organotypic cultures have a finite lifespan, limiting experimentation and resource sharing. Developing collaborative research pipelines between researchers with funding and access to animals and laboratories that can derive, store, and share cells and tissues may democratize access to marine mammal tissues for experimentation.

4 Challenge #3: open access to harmonized methods and datasets

In silico studies, such as simulations and computational genomics, have transformed our ability to approach traditional physiological, toxicological, and evolutionary research questions. However, these studies depend on the availability of the prerequisite data, such as genomic, epigenetic, and experimental data. Furthermore, functional and computational studies highly depend on the experimental methods' robustness and reproducibility. As the field progresses toward new frontiers, standardizing and generating open-source methods become increasingly crucial.

The need for standardization and standard reference sets extends beyond biological samples to wet and dry lab methodologies. Standardized methods expand the impact of research products across disciplines. Endeavors such as ENCODE, GTEx, and HuBMAP have developed standardized reference datasets across a range of tissues, enabling an era of exponential research and development by democratizing access to high-quality functional data and by standardizing best practices for future data collection (ENCODE Consortium, 2012; Carithers et al., 2015; Jain et al., 2023). Similarly, creating and standardizing bioinformatic datasets and methods across tissues and species for marine mammals will provide a necessary resource for future physiological and toxicological investigations.

Open and collaborative protocol development and sharing would optimize researchers' time and resources. For example, optimal conditions for establishing and culturing marine mammal cell lines and tissues often deviate from traditional protocols due to their unique metabolic adaptations and tissue structures (Lam et al., 2020; Routti et al., 2016; Louis et al., 2015; Boroda et al., 2020; Burkard et al., 2015; De Miranda et al., 2012). While some modifications are necessary due to cell type- and species-specific quirks, heterogeneity due to trivial technical factors, such as reagent sources, lot-to-lot variability, and environmental controls, results in wasted research effort. Foreseeing these failures requires a space where researchers can share finalized, successful methods and their failed attempts.

5 Challenge #4: ethical and equitable research practices - community interaction and training

We have a responsibility to center ethical and equitable practices in our work. When research is ingrained with these

values, its quality and impact are greatly improved; however, scientific systems do not always incentivize ethical practices, and, in many cases, cell culture and genetics work have enacted harm (Beskow, 2016; Forsberg et al., 2013; Watson, 2014; Botkin et al., 2012; Couzin-Frankel, 2010; Mello and Wolf, 2010; Millum, 2010; Beattie et al., 2011; Mackey and Liang, 2012; Tanner et al., 2021). As research with marine mammal cell cultures expands, we must ensure ethics and equitability are ingrained into every aspect of our studies, from engagement with local communities to fostering an inclusive research community.

5.1 Community interaction and outreach

Scientists must obtain tissue samples to establish marine mammal cell cultures, which may rely on engagement with local communities through fieldwork partnerships. It is imperative to develop equitable rather than extractive relationships with researchers from low-income countries and local Indigenous communities in which input is solicited while generating ideas and before beginning data collection, with the expectation of a bidirectional exchange of knowledge and acknowledgment of contributions (Mackey and Liang, 2012; Hosseini et al., 2022; Buck and Hamilton, 2011). The data and resources generated during these collaborations should be available through open-access platforms and in addition to publishing journal articles, these data should be disseminated in accessible formats such as maps, outreach initiatives, and infographics. As charismatic megafauna, marine mammals have a strong potential to attract public attention and raise environmental awareness (Albert et al., 2018). Science communicators and stakeholders can foster belonging and engagement by identifying shared values.

5.2 Minimizing wildlife disturbance

A constant challenge for wildlife biologists is obtaining data while minimizing harm to the animals and environments studied. Over the past several decades, the ability to collect biological samples from marine mammals with decreasing levels of disturbance has increased (Danovaro et al., 2016; Gilbey et al., 2021; Stat et al., 2017; Suarez-Bregua et al., 2022). These developments include non-invasive sampling of matrices other than tissues for contaminant and hormone analyses and remote (dart) biopsy collection, which does not require direct animal handling (Hunt et al., 2013). This growing field should consider whether to establish consistent ethical practices and norms surrounding sample collection. For example, many tissues are only accessible from freshly deceased individuals, which may be acquired through whaling or Indigenous harvest (Pugliares et al., 2007; Becker et al., 1997). The marine mammal research community has an ethical obligation to consider the impact of its participation in these institutions, and it may be necessary for the community to delineate which sources and circumstances meet the ethical standards of responsible research. Moreover, establishing and sharing cell lines through a research network would allow the opportunity to reduce sampling, environmental footprint, and potential disturbance to focal animals.

5.3 Equitable recruiting and training practices

Current systems for recruiting and retaining diverse researchers fall short, hindering research development and innovation. Many researchers in marine mammal science begin their careers with unpaid positions, such as full-time internships. Marginalized groups are particularly disadvantaged and burdened by uncompensated work, leading to high rates of attrition (Millum, 2010; Beattie et al., 2011; Mackey and Liang, 2012). Providing fair compensation is critical for increasing the retention of early career scientists; thus, funding for stipends and financial support is crucial to fill these gaps (Spencer et al., 2005; Bruthers et al., 2021; Grady, 2005; Dutz et al., 2023; McGee et al., 2012). Establishing a research coordination network would provide funding support for research and training, connect trainees with mentors within the network, and improve retention for diverse early-career researchers.

6 How and when do we get there?

A middle-out approach for studying the complex relationship between genotype, environment, and phenotype solves some of the challenges associated with the study of marine mammals while enabling new avenues of experimental research that shine a light on the current and future response of these species to a changing global environment. Recent studies focusing on the biology of the northern elephant seal (*Mirounga angustirostris*, Inset 1) and the polar bear (*Ursus maritimus* Inset 2) serve as examples of the success of this approach in addressing complex questions in marine mammal physiology and toxicology. To this end, we propose the creation of repositories for the establishment and distribution of not only validated tissue samples, cells, and other reagents but of detailed and standardized methods and protocols to maximize the utility of these samples.

The goals of the repository are four-fold: 1) promote inter-institutional collaboration; 2) facilitate entry into and research in all facets of marine mammal biology; 3) accelerate the application of cutting-edge techniques to marine mammal species; and 4) maximize the field's ability to characterize and predict ongoing and future responses of marine mammals to global ocean changes. This repository, and the community supporting it, would detail best practices for isolation, management, and characterization of marine mammal tissue cultures, host comprehensive protocols with detailed information on unfruitful approaches, identify specific reagents in support of these practices, and link samples with tissue- and species-specific omics-level data. Additionally, by coordinating the collection, preservation, storage, and distribution of samples, this community will strengthen the utility of each sample while minimizing redundant or wasteful collection efforts.

Accordingly, this endeavor requires a synchronized effort across disciplines to ensure the construction of complete and accessible

methods and sample repositories. Challenges in this process include addressing intellectual property issues, as methods are often developed or applied years before their resulting publications. Additionally, we envision a collaborative research and training network through which the sharing of methodological knowledge and resources will facilitate interdisciplinary efforts to streamline and accelerate the publication of marine mammal cell culture resources. Ultimately, a coordinated approach to solutions founded in principles of open science will provide a path toward a standardized, robust, and equitable international research program poised to address threats and inform regulatory solutions to marine mammals and global ocean health.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

JV: Writing – original draft, Writing – review & editing. JK: Writing – original draft, Writing – review & editing. CM: Writing – original draft, Writing – review & editing. CG-C: Writing – original draft, Writing – review & editing. HR: Writing – original draft, Writing – review & editing. EL: Writing – original draft, Writing – review & editing. EP: Writing – original draft, Writing – review & editing. GM: Writing – original draft, Writing – review & editing. JW: Writing – original draft, Writing – review & editing. KA: Writing – original draft, Writing – review & editing. JC: Writing – original draft, Writing – review & editing. PB: Writing – original draft, Writing – review & editing. DS: Writing – original draft, Writing – review & editing. AG: Writing – original draft, Writing – review & editing. JV-M: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix

Inset 1: The middle-out approach and its use to understand elephant seal physiology

Northern elephant seals (*Mirounga angustirostris*) provide unparalleled research accessibility among marine mammals. Since their recovery from a near total population collapse in the 1800s, northern elephant seals have re-colonized much of their historical range along the California coast (Lowry et al., 2014). Fortuitously for Challenge 1, animals established large coastal rookeries near major research institutions (Año Nuevo State Reserve, Point Reyes National Seashore) in the mid to late 20th century (Le Boeuf et al., 2011). By providing easy access to these animals, these rookeries have facilitated extensive research on elephant seal behavior, physiology, and life history while also enabling conservation management and monitoring of responses to changing environmental conditions.

Comprehensive *in vivo* studies of elephant seal physiology have provided a wealth of data for future work, including biologging, endocrine and metabolic challenges, isotopic tracer and metabolic flux experiments, contaminant analyses, translocation, and various "omic" investigations (Crocker et al., 2016; Green and Larson, 2016; Williams and Ponganis, 2021; Blix, 2018; Andrews and Enstipp, 2016; Allen and Vázquez-Medina, 2019; Ensminger et al., 2021b). Research in elephant seals over the past decade has also examined variables influencing baseline variability in stress hormone levels and organismal and tissue-specific responses to stress and low oxygen levels during various life history stages (Ensminger et al., 2021a; Deyarmin et al., 2019; Vázquez-Medina et al., 2011; Meir et al., 2009; Peterson et al., 2023; Pujade Busqueta et al., 2020; Jelincic et al., 2017; Northey et al., 2023; McCormley et al., 2018; Ensminger et al., 2014; Khudyakov et al., 2015, 2017).

More recently, *in vivo* studies in elephant seals have been complemented by *in vitro* and *ex vivo* approaches, which have enabled genetic manipulations, subcellular resolution of stress pathways and contaminant responses, and combinatorial stress exposure experiments (Lam et al., 2020; Debier et al., 2020; Del Águila-Vargas et al., 2020; Pirard et al., 2023). For example, the function of a cortisol-responsive gene previously identified *in vivo* was examined in cultured elephant seal muscle cells (myotubes) using RNA-seq, pharmacological manipulations, and siRNA gene knockdowns, and found to regulate mitochondrial function and organelle dynamics during prolonged glucocorticoid elevations, providing a mechanistic explanation underlying the resilience of elephant seal muscle tissue to corticosteroids (Torres-Velarde et al., 2021; Khudyakov et al., 2015). Similarly, previously identified cytoprotective pathways conferring hypoxemic tolerance to elephant seals were found to be induced during experimental hypoxia exposure in cultured vascular endothelial cells (Allen et al., 2024; Allen and Vázquez-Medina, 2019; Vázquez-Medina et al., 2011). Moreover, precision-cut adipose tissue slices were recently used to disentangle the role of cortisol and epinephrine in the transcriptional response of blubber to stress, which was previously described *in vivo*, as well as to determine how the presence of bisphenol contaminants alters such responses (Kashiwabara et al., 2023; Pirard et al., 2023; Khudyakov et al., 2017).

These and other studies in elephant seals may be used as a proof-of-concept to illustrate the biological relevance of *in vitro* and *ex vivo* systems in marine mammal research (e.g., many of the same stress-responsive genes were identified using both *in vivo* and *in vitro*/*ex vivo* experiments). The accessibility of free-ranging elephant seals for tissue sampling facilitates the development of cell lines, genetic manipulation techniques, and tissue culture approaches relevant to Challenge 2. These resources, which should be broadly disseminated to the marine mammal community (Challenge 3), can then be applied to other marine mammal systems from which tissue collection is limited. Most importantly, the accessibility of elephant seals on beaches near large population centers and universities, rather than in remote locations or at sea, facilitates the inclusion of diverse researchers, including undergraduate students as outlined in Challenge 4. Involving undergraduate students in research in ecology and other fields - including elephant seal research - has resulted in significant gains in inclusivity and diversity in post-graduate research programs and STEM fields more broadly (Jones et al., 2010; Brint and Cantwell, 2010; Linn et al., 2015; Awad and Brown, 2021; Stanfield et al., 2022). However, as stipulated in Challenge 4, continuous efforts must be made to minimize research impacts on these animals, and all work with elephant seals must be conducted in agreement with Indigenous communities and state and national parks in which their rookeries are located.

Inset 2: Studying endocrine-disruptive effects of chemical pollutants in polar bears using the middle-out approach

The polar bear (*Ursus maritimus*) offers another compelling example of the value of the middle-out approach in advancing our understanding of how pollutants disrupt endocrine function in marine mammals. The polar bear is a top predator with a circumpolar distribution, inhabiting ice-covered Arctic waters. Classified as vulnerable by the IUCN Red List of Threatened Species, polar bears face numerous stressors, including climate change, pollution, pathogens, and increasing Arctic resource exploration and development (Wiig et al., 2015). Despite logistical and practical challenges in studying polar bears (Challenge 1), sample collection has been possible due to national population monitoring programs and subsistence harvest.

Related to Challenge 2, skin biopsies collected during polar bear monitoring in Svalbard, Norway, allowed the establishment of adipose tissue-derived stem cells (Routti et al., 2016). Further studies showed that exposure to chemical mixtures influenced the transition of these cells to adipocytes (Routti et al., 2016). In addition, skin biopsies from live-captured bears and liver samples from subsistence harvests were instrumental in mechanistic *in vitro* studies on nuclear receptors, which act as transcription factors that regulate essential physiological processes, including metabolism, the endocrine and immune systems, and reproduction (Zhao et al., 2015). Research on polar bears has revealed that various chemical pollutants

modulate nuclear receptors *in vitro*, particularly those involved in lipid metabolism and detoxification (Lille-Langøy et al., 2015; Routti et al., 2019; Hwang et al., 2019). Comparative testing on human transcription factors showed species-specific differences, further supported by *in silico* protein structure modeling (Lille-Langøy et al., 2015; Routti et al., 2019). The binding affinities of pollutants to polar bear nuclear receptors have also been predicted using *in silico* methods followed by *in vitro* testing (Routti et al., unpublished (Hwang et al., 2019).

In parallel with *in vitro* experiments, samples were collected from over a hundred free-ranging adult female polar bears to explore the relationship between pollutant exposure and various parameters related to energy metabolism, including gene expression, circulating hormones, lipids and lipoproteins, and metabolites (Tartu et al., 2017). Additionally, transcriptomic analyses were performed on adipose tissue samples from polar bear mother-cub pairs (Herst et al., 2020). Also, a study in male polar bears from Canada investigated changes in the liver metabolome in relation to contaminant exposure (Morris et al., 2019). Altogether, these findings suggest that pollutant exposure disrupts lipid metabolism in polar bears. Both *in vitro* and correlative field approaches provide strong evidence of pollutant-mediated thyroid disruption in these animals (Braathen et al., 2004; Gutleb et al., 2010; Simon et al., 2011; Bytingsvik et al., 2013; Bourgeon et al., 2017).

Training the next generation to develop and utilize *in vitro* tools is crucial for polar bear studies (Challenge 4). Young researchers have been trained to analyze complex field data, whereas experienced researchers have primarily carried out sample collection following the guidelines of national authorities. In conclusion, these studies highlight how *in vitro* and *in silico* research on nuclear receptors, experimental studies on stem cells, and correlative field studies provide valuable insights into the mechanisms by which pollutants impact polar bears and their physiological condition in the wild. Ongoing research on cellular models and the development of tissue-based cultures will further enhance our understanding of how pollutant exposure disrupts biological processes in polar bears while providing resources and material for the wider marine mammal community (Challenge 3).