Assessing and mitigating the impacts of whale-watching activities on humpback whales in Iceland

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Er nokkuð frjálsara, óháðara og hlutlausara en sjá hvali fara stefnur sínar á flötum hafsins.

(Is there anything more free, independent and unattached than whales going their way across the ocean plain.)

Johannes Kjarval, 1948

Abstract

Vessel-based whale-watching is a potential source of disturbance for target cetacean populations, with responses including avoidance and the disruption of key activities such as feeding, resting and communication. However, management of this industry to mitigate potential negative impacts is often undermined by a lack of site-specific ecological information regarding baseline population processes, the responses of whales to vessels and future ecosystem change. My thesis aims to address some of these knowledge gaps for humpback whales (*Megaptera novaeangliae* [Borowski 1781]) in Iceland, an important North Atlantic feeding ground with an established whale-watching industry, to inform future policy. From a conservation perspective, whale-watching activities in Iceland are currently under-regulated, with a voluntary code of conduct primarily informed by impact assessments from other regions.

First, I assessed the behavioural responses of humpback whales to variable whale-watching practices in Skjálfandi Bay, in which the second largest fleet in Iceland operates (**Chapter 2**). Over three summer seasons (633 hours of survey effort), visual observations and positional measurements were collected from 210 whales during 727 focal follows. These data were used to construct seven behavioural variables, while whale-watching vessel movements were quantified using a novel combination of coarse-scale AIS and fine-scale GPS positional data. I then applied generalised additive mixed models (GAMMs) to determine that whale behaviour was influenced by vessel speed, the number of vessels and encounter duration. For example, dive times increased with increasing vessel speed and when more than four whale-watching vessels were present, indicating vertical avoidance; while prolonged encounters led to changes in movement patterns, possibly representing horizontal avoidance, and feeding disruption. Meanwhile, compliance to speed–distance restrictions in the code of conduct appeared to limit behavioural disturbance.

However, behavioural observation may not capture the full short-term cost of whale-watching impacts. Therefore, I attempted to investigate the physiological stress response of whales to local whale-watching activity (**Chapter 3**), which could reveal population-level impacts before they occur. Samples of exhaled breath (blow) were collected using unoccupied aerial vehicles (UAVs), representing a potentially suitable but largely untested sample type for dynamic physiological assessment. I then used liquid chromatography–tandem mass spectrometry (LC–MS/MS) to explore and quantify a panel of steroid hormones, including the stress-related hormone cortisol. To my knowledge, this combination of sampling and analytical approaches was previously untested. Therefore, I first developed a protocol in 2018/19 for sample collection, extraction and analysis. In 2021, we then collected samples from four areas of varying whale-watching activity across North Iceland to address the ecological question. In total, we collected 87 samples ($n = 32$ in 2018/19, $n = 55$ in 2021) from at least 42 different whales. Using an optimised ethanol–water wash (3 mL, 50:50 v/v) to extract samples, I detected a variety of steroid hormones via LC–MS/MS, including cortisol (10/68 samples), cortisone (12/63

samples), DHEAS (48/63 samples), progesterone (58/63 samples) and testosterone (15/63 samples). Low detection rates at quantifiable levels, remaining methodological challenges and a lack of biological validation prevented ecological interpretation of steroid hormone contents. Nevertheless, these results advance the development of best practices for blow sample collection and analysis, and highlight the potential of this approach for comprehensive physiological monitoring.

Beyond assessing short-term responses to disturbance, rational policy also requires an understanding of baseline variability in species occurrence over space and time. Climate change has already altered whale distribution, and this is likely to continue in the future. Therefore, I explored the relationship between physical environmental predictors and humpback whale occurrence in offshore waters around North Iceland, and determined the temporal relationship between predicted offshore density and abundance at Skjálfandi Bay, to consider recent and potential future changes (**Chapter 4**). Using a generalised additive model (GAM) framework, which outperformed a boosted regression tree and ensemble model in terms of predictive ability, I applied a species distribution model to offshore sightings data collected between 1987 and 2015 (five survey years) to reveal the apparent sensitivity of humpback whales to environmental change in Icelandic waters. The final model explained 47.5% of deviance and retained 11 significant ($p < 0.05$) physical variables, including distance to coast, sea surface temperature, sea surface height and mixed layer depth. Model predictions suggested that offshore density declined between 2006 and 2019, particularly to the east of Iceland. Meanwhile, capture–recapture models (using an open robust design framework) applied to long-term photo-identification data indicated that the number of whales visiting Skjálfandi Bay in summer increased considerably during the same period, ranging from 30 (95% confidence interval 22–40) animals in 2010 to 183 (95% CI 155–203) in 2018. The significant negative relationship between these two time series suggests that coastal waters may be increasingly important for humpback whales around Iceland, and highlights the potential increased sensitivity of this population to whale-watching disturbance and other coastal stressors in the future as climate change accelerates.

Taken together, I can use these results to make several recommendations for responsible whale-watching management in Iceland (**Chapter 5**). The existing code of conduct confers some benefits but numeric changes to the maximum speed and maximum encounter duration, in addition to limiting the number of vessels per encounter, may reduce behavioural disturbance. Additionally, spatiotemporal management, such as marine protected areas and seasonal and time-of-day restrictions, may further limit cumulative exposure. These policies should be specified within an adaptive framework, enabling the industry to respond to future changes in whale occurrence and habitat use. By coupling these policies with inclusive, community-led governance and sustained research and monitoring, we can bolster the resilience of whale populations and a sustainable whale-watching industry to an uncertain future in Icelandic waters.

Lay Summary

Whale populations are facing an increasing intensity and variety of human activities, from fisheries to shipping traffic, whose effects are compounded by global climate change. Vessel-based whale-watching is recognised as an additional stressor in coastal areas around the world, with short-term disturbance and, possibly, long-term impacts on population dynamics and distribution. Whilst whale-watching is not the greatest population-level threat, it is essential to limit the impacts of any stressor to improve the resilience of populations to future change. Beyond conservation, any form of disturbance can also compromise animal welfare and should be minimised.

To mitigate whale-watching impacts, we need to characterise the response of whales to whale-watching vessels. This is commonly achieved with visual observation of surface behaviours, which has previously revealed that whales change dive patterns, respiration rate, swim speed and activity states during whale-watching encounters. However, important knowledge gaps remain. For example, the relative influence of different vessel practices (such as approach distance, speed and encounter duration) is understudied. Furthermore, the 'hidden' physiological stress response to encounters, which could compromise wellbeing and fitness, has not been characterised and may not be detectable by behavioural observation alone. Beyond general knowledge gaps, it is apparent that behavioural responses vary between species and locations — therefore, predicting the impacts of a local industry requires local fieldwork. We also often lack baseline information on whale populations at a regional scale. As climate change accelerates, this limits our ability to predict future changes in abundance and distribution, which could both have implications for whale-watching potential and impacts.

This thesis focuses on humpback whales in Iceland. In recent years, whale-watching has developed into an important part of the Icelandic tourism industry, and humpback whales are the primary target species. Limited research has revealed behavioural impacts of whale-watching vessels, but the effect of different vessel practices is unknown and activities are largely unregulated, with only a voluntary code of conduct and evidence of low compliance rates. We also lack baseline information for this population, including habitat use and sensitivity to environmental change. Therefore, this thesis aims to provide new information on whale-watching impacts and habitat use for humpback whales in Iceland, combining dedicated fieldwork with existing data sets. I focus on Skjálfandi Bay, home to the second largest whale-watching fleet in the country and established research collaborations with whale-watching operators.

I started by investigating the behavioural interactions between whales and whale-watching vessels in the Bay, conducting fieldwork over three summers. Whale-watching vessels were used as a research platform; I initially attempted to collect control data (in the absence of vessels) from land, but whales were generally too far from shore. Therefore, I focused on the influence of variable vessel behaviours – such as distance to whale, speed, number of vessels and encounter duration – in relation to seven variables of whale behaviour. Vessel movements were determined using fine-scale GPS data (which I recorded during fieldwork) and coarse-scale automatic identification system (AIS) data, which were

provided by the Icelandic coastguard for every vessel. Results were complex but highlighted several key components of vessel behaviour. Whales exhibited horizontal and vertical avoidance, as well as potentially more cautious surfacing behaviour, with increasing vessel speed and number; while the extension of encounters was related to reduced surface feeding. I also determined that compliance to IceWhale's speed guidelines appeared to limit behavioural disturbance.

Next, I attempted to deduce the chronic physiological stress response of humpback whales to whale-watching activities across North Iceland. Stress in mammals (including whales) is often inferred by elevated levels of the hormone cortisol. To minimise disturbance, I attempted to measure cortisol levels in samples of exhaled breath (blow) collected by an unoccupied aerial vehicle, or drone. Blow sampling has not previously been used to monitor stress in large whales, so I first developed protocols for sampling and analysis. Using samples collected in 2018 and 2019, I determined that an ethanol–water wash was suitable for extracting samples from Petri dishes. I then used liquid chromatography–tandem mass spectrometry, a sensitive method for measuring hormone levels, to screen samples for cortisol and other informative steroid hormones. I applied the resulting method in 2021, collecting samples from four areas around Iceland with varying whale-watching activity. In total, we collected 87 samples and I detected at least eight steroid hormones, including cortisol (10/68 samples), its inactive form cortisone (12/63), and DHEAS (48/63 samples), which has not been previously described in whales. Unfortunately, due to low detection rates, a lack of biological validation and inconsistencies in my method, I could not interpret these hormone contents to answer the question. Nevertheless, these results highlight the potential of blow sampling for physiological monitoring and provide guidance for its practical application.

Beyond whale-watching impacts, I examined humpback whale sightings and environmental data from North Iceland to provide baseline information on whale distribution and sensitivity to environmental change. Sightings from offshore surveys (conducted by the Icelandic government), collected during five summers spanning nearly 30 years (1987–2015) were combined with oceanographic data, including the outputs of a model of Atlantic Ocean circulation, to determine the relationship between humpback whale occurrence and their physical environment. From the resulting model, I determined that humpback whale distribution is influenced by static variables, such as distance to coast, and dynamic variables, such as sea surface temperature and elevation, as well as vertical mixing of surface layers. As a result, humpback whales are sensitive to climate change, and I used the model to predict that offshore density decreased between 2006 and 2019. I related these predicted densities to the abundance of whales in Skjálfandi Bay, determined using long-term sightings from whale-watching vessels, which increased during the same period. A negative relationship between these time series suggests that coastal areas such as Skjálfandi Bay may be increasingly important for Icelandic humpback whales. Therefore, as climate change accelerates, humpback whales may be more exposed to whale-watching and other coastal stressors, such as shipping traffic and proposed port constructions, around Iceland.

Finally, I used this information about whale-watching impacts, environmental sensitivity and potential future changes to consider its implications for whale-watching management in Iceland. Combining my results with the wider literature and existing whale-watching policies, I have several recommendations for future management. First, the existing IceWhale code is largely in line with other codes worldwide but a lower approach speed, shorter encounter duration and numeric limits on the number of vessels per encounter could improve its ability to mitigate disturbance. Second, temporal management of whale-watching activities may be considered: in particular, the high number of trips and low number of whales at the end of August may lead to greater per-animal impacts. Third, spatial management, such as the creation of marine protected areas (MPAs), may be used to limit disturbance in critical habitats, pending further baseline information. In light of residual knowledge gaps about humpback whales and whale-watching impacts in Iceland, I encourage a precautionary approach to management to account for uncertainty and adaptive policies to respond to a changing future. These recommendations should be integrated with other knowledge sources to guide responsible, inclusive whale-watching management.

Acknowledgements

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First, I have to thank the Whale Wise team: since 2018, we have worked together to plan fieldwork and collect data for various projects, including this PhD. To everyone who conducted fieldwork: the effort you have put into this project is truly humbling. I want to apologise for the times when I haven't made best use of the data you helped to collect – this is a painful part of the PhD journey – but most of all I am so grateful for your uplifting spirits, positive attitudes and dedication, to this work but also to the whales. You conducted behavioural observations and helped to collect blow samples, and you know who you are: Alyssa Stoller, Mark Romanov, Katy Maleta, Danny Kosiba, Amelie Laute, Flordespina Dodds, Abigail Robinson, Beverly Tan, Gabriele Negro, Benjamin Hildebrand, Synnøve Rosand and Jessica Ward. Alyssa, my partner in crime: you have been with me the whole way, and I would not have a thesis without you. Katy and Danny, we had a tough season together and I am amazed to this day that you stayed the course. To the most amazing drone pilots: Mark, thank you for collecting the 2018 blow samples and having a major impact on our blow sampling methods; Abigail, you built hurricanes from houses in 2019; and Flo and Amelie, some of the fastest learners out there, in 2021. Amelie and Beni deserve special mention for conducting behavioural observations in 2020, when I sadly could not travel to Iceland due to the COVID-19 pandemic, and Amelie and Beverly were also a huge help in transcribing those insanely long voice recordings. Also, thanks to Lucie Weber and Leonhard Balz for helping with data collection in 2021. Honestly, this paragraph does not do you all justice.

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Húsavík whale-watching: researchers and companies do not always get on, so I am grateful for your open minds. Speaking of this beautiful town, I am forever tied to Húsavík Whale Museum (Hvalasafnið): you have given me employment, support, friendship, a platform to share my work and so much more. Eva and Þröstur, I am excited to see what the future holds.

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Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified. The sightings data sets used in Chapter 4 were provided by the Icelandic Marine and Freshwater Research Institute (Hafrannsóknastofnun) and the University of Iceland (Háskóli Íslands).

Thomas James Grove

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Figures, Tables and Boxes

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Abbreviations

ACCOBAMS	Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area
ACTH	Adrenocorticotropic hormone
AEAC	Alber's equal area conic [planar coordinate system]
AIC	Akaike's Information Criterion
AICc	Akaike's Information Criterion corrected for small sample size
AIS	Automatic Identification System
AMOC	Atlantic Meridional Overturning Circulation
ANOVA	Analysis of variance
ASL	Airway surface liquid
BFSS	Beaufort sea state
BRT	Boosted regression tree
CBD	Convention on Biological Diversity
CI	Confidence interval
CJS	Cormack–Jolly–Seber capture–recapture model
CMS	Convention on Migratory Species
CR	Capture–recapture [data or model]
DHEA(S)	Dehydroepiandrosterone (sulphate)
DI	Directness index
DSLR	Digital single lens reflex [camera]
EBSA	Ecologically or Biologically Significant marine Area
EDF	effective degrees of freedom
EEZ	Exclusive economic zone
ESW	Effective strip width
GAM	Generalised additive model
GAMM	Generalised additive mixed model
GC	Glucocorticoid
GCM	General circulation model
GCV	Generalised cross validation
GEBCO	General Bathymetric Chart of the Oceans
GIS	Geographic information system
GLM	Generalised linear model
GPS	Global Positioning System
HPA	Hypothalamic–pituitary–adrenal [axis]
HSD	Hydroxysteroid dehydrogenase
HYCOM	HYbrid Coordinate Ocean Model

IBI	Inter-breath interval
IceWhale	The Icelandic Whale Watching Association
IDH	Individual detection heterogeneity
IFAW	International Fund for Animal Welfare
IMMA	Important Marine Mammal Area
IPCC	Intergovernmental Panel on Climate Change
IS	Internal standard
IUCN	The International Union for Conservation of Nature
IWC	International Whaling Commission
JSSA	Jolly–Seber–Schwarz–Arnason capture–recapture model
LAC	Limits of acceptable change
LC–MS/MS	Liquid chromatography–tandem mass spectrometry
MLE	Maximum likelihood estimation
MMPA	[USA] Marine Mammal Protection Act
MPA	Marine protected area
MSORD	Multi-state open robust design capture-recapture model
MSP	Marine spatial planning
MXL	Mised layer depth
NAO	North Atlantic Oscillation
NASS	North Atlantic Sightings Surveys
NOAA	National Oceanic and Atmospheric Administration [USA]
PCoD	Population consequences of disturbance
POP	Persistent organic pollutant
REML	Restricted maximum likelihood
RF	Random forest
RIB	Rigid inflatable boat
RMSE	Root mean square error [CV-RMSE: cross-validated RMSE]
ROMS	Regional Ocean Modeling System
SAB	Surface-active behaviour
SAC	Special area of conservation
SD	Standard deviation
SDM	Species distribution model
SE	Standard error
SFE	Surface feeding event
SNR	Signal-to-noise ratio
SPG	Sup-polar gyre
SSH	Sea surface height
SST	Sea surface temperature
UAV	Unoccupied aerial vehicle
UN	United Nations

VMS	Vessel monitoring system

Chapter 1

General introduction

1.1 Introduction

Human–wildlife interactions can be framed as a social–ecological system, in which humans and animals exert both positive and negative effects on each other (Dou and Day, 2020; Redman et al., 2004). For example, non-consumptive wildlife tourism can support conservation efforts and provide key ecosystem services to society (Buckley et al., 2016; Malinauskaite et al., 2021; Stronza et al., 2019), but its associated activities are also a source of disturbance for both terrestrial and marine animals (Bateman and Fleming, 2017). Tourism activities and human presence generally may be perceived as a predation risk by target animals, generating a 'landscape of fear' (Ciuti et al., 2012; Frid and Dill, 2002). The resulting behavioural responses can lead to disruption of critical activities such as foraging and resting (Christiansen et al., 2013a; Lusseau and Higham, 2004); and changes in energy acquisition and expenditure (Christiansen et al., 2014; Rode et al., 2007). Responses are not always observable; animals may exhibit a repeated acute or chronic physiological stress response to human presence without a clear behavioural change, with potential fitness consequences (Maréchal et al., 2011; Semeniuk et al., 2009; Watson et al., 2021). If exposure is sufficiently high, population-level impacts may occur, including changes in distribution, vital rates and abundance (Bain et al., 2014; Bejder et al., 2006; Carome et al., 2022). Therefore, wildlife tourism is of both welfare and conservation concern for wild animal populations (Moorhouse et al., 2015), and it is critical to characterise human–wildlife interactions to inform responsible management of sustainable tourism activities (Higham et al., 2015). Due to the variation in tourism practices and responses to disturbance between regions and taxa, site-specific assessments are necessary to determine local–regional impacts.

My thesis aims to elucidate the potential negative impacts of whale-watching activities on North Atlantic humpback whales (*Megaptera novaeangliae novaeangliae* [Borowski, 1781]) in Iceland. In this Chapter, I start by providing a broader overview of baleen whales, their benefits to human societies and principal anthropogenic threats. Following a summary of cetacean research and management efforts, I then outline our understanding of whale-watching impacts on cetaceans and existing knowledge gaps. Finally, I provide information on North Atlantic humpback whales, the study area of Iceland and whale-watching activities around the country, with a focus on Skjálfandi Bay (my primary study site).

1.2 Baleen whales

Whales, dolphins and porpoises belong to the marine mammal infraorder Cetacea [Brisson, 1762], and are some of the largest and longest-lived of all animals (Carwardine, 2019). Cetacean species have diverse life histories, including both capital and income breeding, and are characterised by complex behaviours, social structures and communication. All species are K-selected (Pianka, 1970) and slow maturing, relative to other mammalian taxa. Cetaceans are found throughout the global ocean, from tropical to polar and coastal to offshore habitats, and some of the world's large rivers (Carwardine, 2019). Despite their large size, cetacean species are still being discovered, with new species of baleen whale (Rice's whale, *Balaenoptera ricei* [Rosel et al., 2021]) and beaked whale (Ramari's beaked whale, *Mesoplodon eueu* [Carroll et al., 2021]) declared in 2021.

A total of 92 known extant species (Society for Marine Mammalogy, 2022) is split into two parvorders, Odontoceti (toothed whales) and Mysticeti (baleen whales), which diverged 35–30 million years ago (Sasaki et al., 2005). Mysticetes are filter-feeding cetaceans in which teeth have been replaced by keratinous baleen plates over evolutionary time (Peredo et al., 2017), and occupy intermediate trophic positions (Pauly et al., 1998). There are 11 species of baleen whales across five genera, ranging in size from pygmy right whales (*Caparea marginata*; average 6 m length and 5 tons), to blue whales (*Balaenoptera musculus* [Linnaeus, 1758]; average 25 m length and 150 tons). Mysticetes are generally migratory capital breeders, with energetically distinct feeding and breeding seasons; persistent annual movement between high-latitude feeding grounds and low-latitude breeding grounds; and consequent large habitat ranges (Carwardine, 2019). Reflecting the complexity of their environments, mysticetes possess multi-modal sensory systems, including vision, audition (passive and potentially active) and chemoreception (Bouchard et al., 2019; Torres, 2017).

1.2.1 The importance of baleen whales

Cetaceans play an important role in ecosystems, earth systems and human societies (Cook et al., 2020). Baleen whales, in particular, provide numerous ecosystem services (Daily et al., 1997) or nature's contributions to people (Pascual et al., 2017), broadly defined as gains in human wellbeing secured from the natural environment (Millennium Ecosystem Assessment, 2005). For example, whales maintain ecosystem function and influence nutrient cycling (Bowen, 1997; Pearson et al., 2022; Roman et al., 2021). The removal of great whales from an ecosystem (e.g., by historical commercial whaling) has previously led to sequential megafaunal and regional ecosystem collapse, underlining their contribution to trophic stability (Springer et al., 2003; Wilmers et al., 2012). Meanwhile, whales influence nutrient cycling in several ways (Pearson et al., 2022). Directly, great whales (mysticetes and sperm whales) serve as large carbon sinks by depositing carbon accumulated throughout their lifetime to the seafloor when they die (Pershing et al., 2010; Roman et al., 2014). Indirectly, they enhance primary productivity in their feeding grounds by consuming prey at depth and defecating near the surface, thereby concentrating nutrients such as nitrogen and iron in the photic zone (Lavery et al., 2010; Roman et al., 2014, 2016; Smith et al., 2013). The phytoplankton blooms that result from this 'whale pump' (Roman and McCarthy, 2010) sequester carbon dioxide from the atmosphere and ocean through photosynthesis (Roman et al., 2014), and are likely to increase productivity at higher trophic levels (Ratnarajah et al., 2016). Therefore, whales could play a considerable role in mitigating climate change through carbon sequestration, although there is limited knowledge about these processes through the global ocean (Meynecke et al., 2020). Nutrients can also be re-distributed latitudinally, with migratory whales feeding at high latitudes and subsequently defecating at nutrient-poor, low-latitude breeding grounds, constituting a 'great whale conveyor belt' (Roman et al., 2021).

In addition, whales provide direct benefits in terms of economic value and resources at local, regional and global scales, such that the per-animal value of large whales (primarily baleen whales) was estimated to be more than $2 million USD, based on their various activities (Chami et al., 2020, 2019). Beyond carbon sequestration, increases in primary productivity may increase commercial fish stocks (Roman et al., 2014). Meanwhile, whale-watching has developed into a multi-billion dollar industry and supports coastal economies globally (O'Connor et al., 2009). Furthermore, commercial and subsistence whaling provide food and other products that are used for sustenance or sold, supporting entire communities, particularly in the Arctic (Caulfield, 1993).

Baleen whales also play an important role in human cultures, identity and recreation (Malinauskaite et al., 2021). Watching whales constitutes a recreational activity, while educational benefits can be acquired from viewing whales, visits to education centres and scientific research, stimulating a wider interest in the natural world (Cook et al., 2020; Lopez and Pearson, 2017). Throughout recorded human history, whales have inspired various art forms, including music and film-making in recent years (Burnett, 2012; Ritts, 2017; Thomasson, 2005). Whales are depicted in lore and mythology, and often hold spiritual or religious value to societies, including Māori peoples in New Zealand and Inuit communities across the Arctic (Bodenhorn, 1988; Sakakibara, 2019; Wehi et al., 2013). Similarly, whales can contribute to community cohesiveness and cultural identity (Cook et al., 2020; Einarsson, 2009). Finally, societies value whales for their aesthetics (appreciation of their beauty) and existence, which may be considered non-use values (Edwards, 1986; Hausmann et al., 2017).

Beyond these services to humanity, from a ecocentric point of view, it can be argued that whales (and other components of the natural world) have additional, intrinsic value (Vilkka, 1997).

1.2.2 Anthropogenic threats to baleen whales

As slow-maturing marine predators with wide geographical ranges, baleen whales face numerous anthropogenic threats, with both lethal and sublethal impacts (Avila et al., 2018; National Academies, 2017). These threats do not act in isolation; rather, the effects of multiple stressors accumulate and interact over time and space, yielding a complex combined impact on cetacean populations (Gissi et al., 2021; Pirotta et al., 2022). Globally, marine mammal communities are at high risk from cumulative impacts in 47% of coastal waters (Avila et al., 2018); conversely, 70% of areas with high anthropogenic impact are within, or near to, key conservation sites for marine mammals (Pompa et al., 2011). Contemporary threats to great whales are exacerbated by historical whaling, with an estimated

2,894,094 animals caught in the 20th century alone (Rocha et al., 2014). Commercial and subsistence hunting of baleen whales still take place but at a far smaller scale (Clapham and Baker, 2018). Despite limited recovery, whale populations are still largely depleted and are vulnerable to an expanding human footprint on the global ocean (Halpern et al., 2015; Thomas et al., 2016; Tulloch et al., 2019).

Baleen whales are exposed to a diverse array of anthropogenic stressors, some of which have population-level consequences (Thomas et al., 2016). For example, entanglement in fishing gear (sometimes resulting in injuries or gear damage) or bycatch (getting caught in fishing gear and drowning) are a conservation concern for most baleen whale species (Avila et al., 2018; Saez et al., 2021). Entanglement can have lethal or sub-lethal consequences (Cassoff et al., 2011; Moore and van der Hoop, 2012); has limited the recovery of North Atlantic right whales, *Eubalaena glacialis* (Knowlton et al., 2012); is perhaps the greatest anthropogenic threat to humpback whales, (Benjamins et al., 2012; Carwardine, 2019); and has severe consequences for animal welfare (Cassoff et al., 2011; Moore and van der Hoop, 2012; Rolland et al., 2017). Both mortality and exposure rates can be high and variable at regional levels (Basran et al., 2019; Robbins, 2009), although the sub-lethal consequences for vital rates remain unknown at a population level. Meanwhile, bycatch is responsible for more than 300,000 cetacean deaths (primarily small odontocetes) each year (Read et al., 2006) and is still massively under-reported (Basran and Sigurðsson, 2021).

Baleen whales, particularly slower moving species, are also susceptible to vessel collisions, commonly known as ship strike (IWC, 2005; Peel et al., 2018; Schoeman et al., 2020). These events are likely to be fatal if the vessel is greater than 80 m long or travelling faster than 14 knots (Laist et al., 2001), and the risk of collisions is elevated in narrow passages (Williams and O'Hara, 2010). Until vessel speed restrictions were introduced, ship strike was the primary cause of death for North Atlantic right whales (Henry et al., 2012). Other commonly reported species suffering from ship strike include fin whales (*Balaenoptera physalus* [Linnaeus, 1758]) and humpback whales (Jensen and Silber, 2003; Neilson et al., 2012). Whales may be limited in their ability to avoid large vessels (McKenna et al., 2015), in part due to poor sound transmission near the surface and the acoustic shadow generated by the bow of the vessel (Allen et al., 2012). Collisions that are not fatal can still cause severe injuries (Hill et al., 2017), compromising individual fitness.

In addition to their physical presence, the noise generated by vessel engines and cavitation impacts baleen whale populations (Erbe et al., 2019; Nowacek et al., 2007; Tyack, 2008), and is now the dominant anthropogenic source of underwater noise globally (Hildebrand, 2009; Wilcock et al., 2014). Owing to the acoustic properties of water, cetaceans primarily sense their world, navigate and communicate through sound (passive and active audition; Tyack and Clark, 2000). Noise from large vessels overlaps with the frequency range of cetacean (particularly mysticete) vocalisations and hearing, thereby masking vocalisations, reducing communication space (Cholewiak et al., 2018; Putland et al., 2018; Bejder et al., 2019) and impairing hearing. As a result, vessels may influence calling behaviour, including rate (Fournet et al., 2018; Tsujii et al., 2018), amplitude (Parks et al., 2011), duration (Foote et al., 2004) and frequency (Parks et al., 2008), and impair communication generally (Dunlop, 2016; Erbe, 2002). Vessel traffic can also disrupt foraging (Blair et al., 2016), induce (possibly chronic) physiological stress

(Lemos et al., 2022; Pallin et al., 2022; Rolland et al., 2012), and even influence distribution (Pack et al., 2022). Other sound sources also elicit strong avoidance responses, including sonar (Sivle et al., 2016), offshore construction (Brandt et al., 2011) and seismic surveys used for oil and gas exploration (Kavanagh et al., 2017). Anthropogenic noise may have more severe direct consequences (Erbe et al., 2018): naval sonar has been attributed to mass strandings and unusual mortality events (Balcomb and Claridge, 2001; Bernaldo de Quirós et al., 2019); and underwater explosions can damage internal organs through acoustic trauma (Ketten, 1995).

Finally, anthropogenic climate change is a pervasive threat to most baleen whale species and is likely to worsen in the coming years (Cheng et al., 2019; Tittensor et al., 2021; Tulloch et al., 2019). The availability and distribution of baleen whale prey (zooplankton and pelagic fish) is dictated by spatial patterns in physical oceanography (Barlow et al., 2021; Santora et al., 2012). Whilst ocean systems exhibit natural variability in physical characteristics, such as temperature, anthropogenic climate change is increasing the magnitude and speed of these changes (Cheng et al., 2019). Ecological responses include shifts in distribution, such as the poleward shift of fish species (Kortsch et al., 2015), and biomass, such as the persistent decline of euphausiids in the Atlantic and Southern oceans (Edwards et al., 2021). Some whale populations are able to adapt to these changes through range shifts (Meynecke et al., 2020) and prey switching (Fleming et al., 2016). Contemporary climate change may even increase habitat availability for some species; for example, retreating summer sea ice and warming waters in both the Atlantic and Pacific sectors of the high Arctic are increasing oceanic productivity and literally opening up new foraging grounds for boreal and sub-Arctic cetaceans, such as humpback, common minke (*Balaenoptera acutorostrata* [Lacépède, 1804]), fin, sperm (*Physeter macrocephalus*) and killer (*Orcinus orca*) whales (Ferguson et al., 2010; Higdon and Ferguson, 2011; Moore, 2016; Posdaljian et al., 2022; Stafford et al., 2022). However, globally, the extent of this adaptation potential is uncertain and the carrying capacity of ecosystems for baleen whales may be increasingly limited (Pallin et al., 2023; Tulloch et al., 2019). Climate-driven regime shifts have been attributed to declining reproductive success in North Pacific humpback whales (Cartwright et al., 2019; Frankel et al., 2022) and the potential collapse of the North Atlantic right whale population (Meyer-Gutbrod et al., 2021, 2015). The effects of climate change may be exacerbated by regional stressors, such as the over-harvesting of prey stocks (Gissi et al., 2021; Pallin et al., 2023), and could threaten the future carbon benefits of great whale populations (Durfort et al., 2022).

1.3 Cetacean research and conservation

Goal 14 of the United Nations (UN) Sustainable Development Goals, ‘Life Below Water’, emphasises the need to “conserve and sustainably use the oceans, seas and marine resources” (United Nations, 2016), and changing public attitudes have rendered cetaceans a flagship for marine conservation (Bearzi et al., 2010; Mazzoldi et al., 2019). Moreover, as marine anthropogenic activities continue to expand and intensify globally (Halpern et al., 2015; O’Hara et al., 2021), there is a growing need for cetacean conservation efforts to preserve their wellbeing and contributions to humanity (Reynolds et al., 2009). Effective conservation, that makes efficient use of increasingly limited resources (Walls, 2018), requires i) scientific research to understand cetacean populations and their response to environmental pressures; and ii) evidence-based management to safeguard cetaceans and entire marine ecosystems (Berthinussen et al., 2021; Sutherland et al., 2004).

1.3.1 Overview of cetacean research

Generally, conservation requires an understanding of populations, communities or ecosystems (Kareiva and Marvier, 2012). However, cetacean research (and wildlife research generally) often begins with the targeted observation of individual animals and their characteristics. Individual variation in behaviour, physiology and health influence population dynamics and species responses to anthropogenic stressors (Baker, 2013). Therefore, by studying an adequate number of animals, it is possible to infer mean responses across an area or population, and the population consequences of disturbance (which are often difficult to determine; McHuron et al., 2017; National Academies, 2017; Pirotta et al., 2018). Owing to the challenges of sampling and studying free-ranging cetaceans, research often focuses on visually observable changes at the sea surface (Hunt et al., 2013). For example, visual observation of surface behaviours has revealed that: vessel traffic disrupts feeding and induces avoidance responses in diverse cetacean species (Christiansen et al., 2013a; Currie et al., 2021; Nowacek et al., 2001; Williams et al., 2009); small odontocetes show avoidance responses to unoccupied aerial vehicles (UAVs) flown below a certain height above water level (Fettermann et al., 2019; Palomino Gonzalez, 2019); and acoustic 'pingers' may be an effective entanglement mitigation tool for humpback whales (Basran et al., 2020). Meanwhile, suction cup tags have revealed foraging dynamics of baleen whales (Goldbogen et al., 2011; Hazen et al., 2009); demonstrated that beaked whales show strong avoidance responses to mid-frequency active sonar (Miller et al., 2015; Wensveen et al., 2019); and revealed that humpback whales reduce feeding rates in the presence of intense vessel noise (Ovide, 2017). Yet more studies have used acoustic monitoring to deduce that individual cetaceans alter communication strategy with increasing ambient noise (Dunlop et al., 2010; van Ginkela et al., 2017).

In addition to the targeted study of individual characteristics, large-scale surveys of occurrence and demographics can be used to determine population parameters such as abundance and survival, habitat use and interactions with the wider environment (Carretta et al., 2022; El-Gabbas et al., 2021a; Hammond et al., 2021). While these surveys provide less information about each individual, baseline population parameters such as abundance are essential to inform conservation status and consider the implications of individual responses to stressors (Lawton, 1993; Mace et al., 2008). For example, visual sighting surveys from vessel or aerial platforms can cover large spatial areas, are frequently used to estimate regional abundance (Desportes et al., 2019; Hammond et al., 2021) and can be combined with spatially resolved oceanographic data to determine the environmental drivers of distribution (Becker et al., 2019; El-Gabbas et al., 2021a). Meanwhile, long-term acoustic deployments provide longitudinal information on species occurrence, habitat use and behaviour (Davis et al., 2020; Stafford et al., 2012). Population dynamics can also be determined from sighting histories of recognisable animals, which is largely achieved through photo-identification of unique markings for cetaceans (Franklin et al., 2020), via capture–recapture methods (Grove et al., 2023; Somerford et al., 2022).

Both targeted individual research and population-level surveys are conducted from a variety of research platforms. Increasingly, the value of 'platforms of opportunity', which are not specifically provided for research purposes, is realised for the dedicated study of cetaceans, enabling cost-effective monitoring (Stack and Currie, 2022). Whale-watching vessels, in particular, are regularly used for studies of behaviour, photo-identification, population biology and anthropogenic impacts (Basran et al., 2019; Christiansen et al., 2013a; Grove et al., 2020; Palazzo et al., 2004). Furthermore, emerging and repurposed technologies are enabling the collection and analysis of a broader range of sample types. For example, UAVs have become a standard tool for photogrammetric measurement of large whale body length and condition (e.g., Christiansen et al., 2021; Hirtle et al., 2022), and are additionally used to provide an aerial perspective on cetacean behaviour (Ejrnæs et al., 2021; Torres et al., 2018); collect samples of exhaled breath (blow) for endocrinological, microbial and genetic studies (Atkinson et al., 2021; Pirotta et al., 2017); and even monitor disease (Leslie et al., 2023). UAVs represent a less obtrusive and cheaper alternative to other research platforms such as vessels (Christiansen et al., 2016b). Meanwhile, gold standard laboratory assays, such as liquid chromatography–tandem mass spectrometry (LC–MS/MS), are being adopted from other fields of research (such as clinical studies) to extract more information from biological samples (Dalle Luche et al., 2019; Hayden et al., 2017).

Beyond field monitoring, other sources of data are increasingly available, allowing us to better understand the relationships between cetaceans and their physical, biotic and human environment. For example, vessel positional data, such as automatic identification system (AIS) or vessel monitoring system (VMS) positions, are now readily accessible, enabling the mapping of anthropogenic activity at local-to-global scales (Nesdoly et al., 2022; Pirotta et al., 2019), and the identification of key stressors (Nesdoly et al., 2022). Meanwhile, satellite sensors are monitoring oceanographic conditions at the sea surface at high spatial resolution, e.g., Moderate Resolution Imaging Spectroradiometer (MODIS, 1km resolution; Chin et al., 2017). As an alternative, general circulation models (GCMs) can provide accurate hindcasts of oceanographic conditions, filling gaps in physical data such as temperature, salinity and sea surface height, which are often not possible to collect remotely or *in situ* at a basin scale (Biastoch et al., 2021; Chassignet et al., 2007). These data types are frequently used in species distribution models (Becker et al., 2019; Redfern et al., 2006), and real-time environmental monitoring and oceanographic forecasts can be used predict future changes in abundance and distribution (Barlow and Torres, 2021; Becker et al., 2019; Torres et al., 2013). In addition, a wide variety of modelling approaches are now available to ecologists, including ensemble frameworks that combine the strengths of multiple model types (Claro et al., 2020; El-Gabbas et al., 2021a). For example, Abrahms et al. (2019) applied an ensemble framework (combining a generalised additive model and boosted regression tree) to blue whale satellite tag positional data and Regional Ocean Modelling System (ROMS; Haidvogel et al., 2008) environmental data to determine the seasonal spatial overlap between blue whale distribution and shipping lanes in southern California. Altogether, cetacean research is becoming a more efficient and impactful field.

1.3.2 Management bodies and conservation policies

The outputs of cetacean research can be used to inform evidence-based management of whale populations (Meek et al., 2011). Owing to the contribution of whales to human societies, the magnitude and extent of anthropogenic threats and changing public perceptions about cetaceans and their welfare (Papastavrou et al., 2017), marine mammal conservation is a key component of the management of marine resources. As such, whales benefit from legal protection at multiple levels of government, internationally and in some countries (Reeves, 2018). Internationally, cetaceans are protected by several treaties and organisations (Jefferies, 2016). Some are broader conservation agreements, including the Convention on Biological Diversity (CBD[1]) and the Convention on Migratory Species (CMS[2]), which are both UN treaties and include provisions for cetaceans. However, other bodies were created specifically to manage cetaceans, including the International Whaling Commission (IWC[3]; constituted through the International Convention on the Regulation of Whaling), which aims to regulate whaling and conserve whale stocks, and the Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas[4]. Some international groups do not have legislative power but promote cooperation between nations for marine mammal conservation and provide relevant advice. For example, the IWC also hosts workshops and informs conservation related to entanglement, ship strike, bycatch and strandings; the North Atlantic Marine Mammal Commission (NAMMCO[5]) aims to promote cooperation on the conservation, management and study of marine mammals in the Northeast Atlantic; and the IUCN Marine Mammal Protected Areas Taskforce[6] provides a platform for collaboration and data-sharing of marine mammal protected areas.

Beyond multilateral treaties and organisations, some legal instruments exist at a national level to specifically safeguard cetacean or marine mammal populations. The first was the USA Marine Mammal Protection Act (MMPA; 16 U.S.C. 1421h), constituted in 1972, which aims to “prevent marine mammal species and population stocks from declining beyond the point where they ceased to be significant functioning elements of the ecosystems of which they are a part”. Since the MMPA, other nations have developed their own provisions to protect cetaceans, including the Canadian Marine Mammal Regulations under the Fishery Act (SOR/93-56) and Australian Whale Protection Act (No. 92 of 1980).

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1. Convention on Biological Diversity: https://www.cbd.int/
2. Convention on Migratory Species: https://www.cms.int/
3. International Whaling Commission: https://iwc.int/en/
4. Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas (ASCOBANS): https://www.ascobans.org/
5. North Atlantic Marine Mammal Commission: https://nammco.no/
6. IUCN Marine Mammal Protected Areas Taskforce: https://www.marinemammalhabitat.org/

The specific policies that act to conserve populations can take several forms, depending on spatial scale and the species or anthropogenic threats of concern (Nelms et al., 2021). Policies may constitute mandatory regulations or voluntary guidelines (Carlson, 2012). Commonly, marine protected areas (MPAs) are used to protect core cetacean habitats from a variety of local stressors (Giakoumi et al., 2018). For example, Glacier Bay National Park enforces vessel speed and number restrictions in a key humpback whale foraging ground in Alaska, USA (US National Park Service, 2022); Robson Bight Ecological Reserve includes voluntary guidelines to discourage all marine human activity in a critical killer whale habitat in British Columbia, Canada (BC Parks, 2007); and the Pelagos sanctuary spans the exclusive economic zones (EEZ) of three Mediterranean countries and aims to minimise the impact of all anthropogenic activities in a region with high cetacean diversity and habitat use (Sanctuaire Pelagos, 2019). Additionally, management may include a temporal component, reflecting the variability of cetacean occurrence and behaviour, such as seasonal restrictions on vessel speed in Cape Cod, USA, to mitigate ship strike incidents involving North Atlantic right whales (Merrick, 2005; NOAA, 2008). Beyond spatiotemporal management, guidelines may be used to control specific human activities at local, regional or national levels, such as the behaviour of commercial or recreational vessels in close proximity to cetaceans (Carlson, 2012).

Management plans may incorporate several key principles to improve their benefits to cetacean conservation and human societies. First, policies may adopt a precautionary approach, taking conservation action in the absence of further evidence (Cooney, 2004; Fumagalli et al., 2021), which is often advocated by international organisations (e.g., IWC, NAMMCO). Furthermore, management may take an ecosystem-based approach (strongly advocated by the CBD), ensuring the protection of cetaceans and the wider environment (Cook et al., 2019; Pauly et al., 2002). MPAs are a key component of ecosystem-based management. Policies may also be adaptive, reflecting inherent uncertainty about our interactions with cetaceans, such as Dynamic Management Areas for North Atlantic Right whales to mitigate ship strike (NOAA, 2008). Finally, policies are most effective and equitable through co-management, i.e., governance by multiple stakeholders. This ensures that multiple sources of information are included, such as natural science, social science, local ecological knowledge and other stakeholder perspectives (Bentz et al., 2016; IWC, 2021).

1.4 Whale-watching: impact and management

The whale-watching industry is at the nexus of our complex positive, negative and investigative relationship with cetaceans, as part of a wider social–ecological system (Figure 1.1; Malinauskaite et al., 2021). Whale-watching can be defined as the commercial practice of observing whales in their natural habitat, typically as part of an organised tourist activity (Hoyt, 2018; IWC, 1994). Modern whale-watching targets a variety of species, from porpoises to large whales; can take place from vessel, aerial or land-based platforms; and is either recreational or commercial (Hoyt, 2018). Vessel-based whale-watching may be considered a form of eco-tourism, visiting fragile natural environments while supporting conservation through public education and engagement (Orams, 1995; García-Cegarra and Pacheco, 2017). The

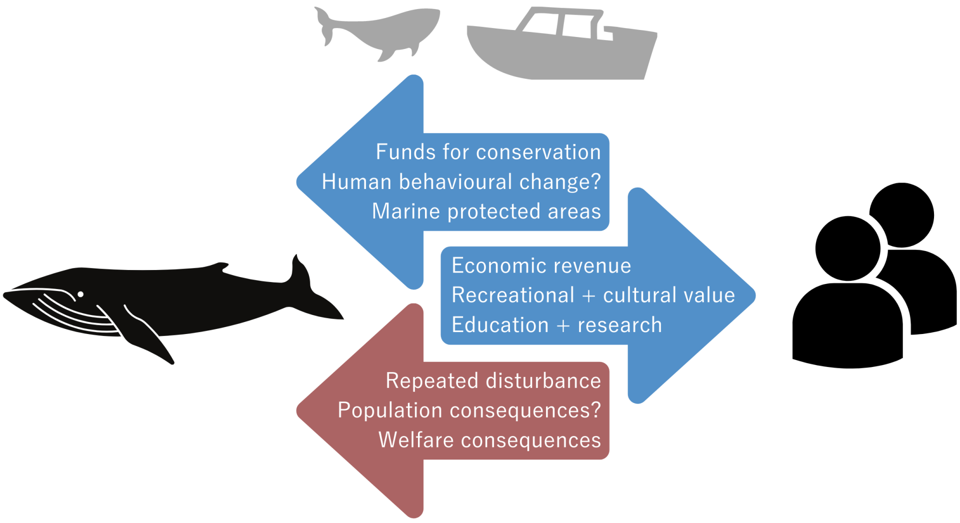

Figure 1.1: Summary of the positive (blue) and negative (red) impacts of whale-watching on whale populations and human societies. Question marks indicate limited evidence for a given impact.

economic practice started in the 1950s in California, offering $1 USD tours to see migrating grey whales (*Eschrichtius robustus*). In the wake of changing public perceptions (Hoyt, 2018; Mazzoldi et al., 2019) and the 1986 moratorium on commercial whaling (Herrera and Hoagland, 2006; IWC, 2020), whale-watching has since expanded into a global industry, with nearly 13 million people taking part annually as of 2008 (O'Connor et al., 2009). Moreover, there is considerable potential for expansion economically and geographically (Cisneros-Montemayor et al., 2010). Primary target species include humpback whales, bottlenose dolphins (*Tursiops* spp.), orcas and grey whales (IWC, 2019b).

1.4.1 Benefits of whale-watching

Many whale ecosystem services can be obtained through whale-watching. The economic value of the industry has grown rapidly, from $1 billion USD in 1998 (Hoyt, 2001) to $2.1 billion USD in 2008 (O'Connor et al., 2009), supporting more than 13,000 jobs, with the potential for continued economic expansion (Cisneros-Montemayor et al., 2010). Whilst the largest industry still exists in North America, strong growth has taken place in Central and South America, Asia, Oceania and Europe (O'Connor et al., 2009). Whale-watching is a significant contributor to national economies such as Iceland and Tonga (Icelandic Tourist Board, 2020; O'Connor et al., 2009; Orams, 2013), and now supports coastal communities worldwide (e.g., Einarsson, 2009). Furthermore, whale-watching provides recreational, spiritual and educational benefits, serving as a platform for wider environmental engagement (Cook et al., 2020; Lopez and Pearson, 2017). Finally, commercial whale-watching activities now constitute an important part of the cultural heritage of some coastal communities, providing a focus for community cohesion and identity (Malinauskaite et al., 2021). This has contributed to the development of whale festivals and celebrations around the world, such as the Pacific Rim Whale Festival (Canada), Hermanus Whale Festival (South Africa) and Maui Whale Festival (Hawai'i).

The benefits of this relationship can also take place in the reverse direction. Whale-watching was born out of a love for whales, and has long been viewed as the antithesis to whaling and a driver of marine conservation (Neves, 2010; Parsons, 2012). As with other forms of ecotourism (Fennell, 2020), it can indeed foster pro-conservation intentions (Andersen and Miller, 2008; García-Cegarra and Pacheco, 2017; Lopez and Pearson, 2017), although there is debate about consequent conservation action (Ballantyne et al., 2009; Orams, 1996). Furthermore, whale-watching can lead to the designation of 'whale sanctuaries' to preserve the industry and populations (Cook et al., 2019), and can be used to generate funds for wider conservation work (Chalcobsky et al., 2017). In addition, vessel-based whale-watching provides an excellent platform of opportunity to study whales, extending the spatiotemporal coverage of cetacean research (Palazzo et al., 2004; Stack and Currie, 2022). For example, analytical methods such as capture–recapture modelling and line-transect sampling have been applied to sightings and photographic data from whale-watching vessels to determine species habitat use (Currie et al., 2018) and local–regional population parameters, including survival (Boys et al., 2019; Verborgh et al., 2009) and abundance (Bertulli et al., 2018; Chandra et al., 2021; Johannessen et al., 2022). These population estimates can yield comparable accuracy to those derived from dedicated research platforms (Henderson et al., 2023).

1.4.2 Negative impacts of whale-watching

Despite these mutual benefits, research over several decades has revealed that large whales and other cetaceans are negatively impacted by whale-watching vessels in various ways (Higham et al., 2015; IWC, 2006; Parsons, 2012; Senigaglia et al., 2016). The assumption of sustainability (as the antithesis to whaling) allowed for whale-watching to remain largely unregulated (Higham et al., 2015; Neves, 2010). As such, tourism and recreational activities now threaten the conservation status of 21% of marine mammal species (Bejder et al., 2022). Impacted areas include important feeding and breeding grounds for endangered populations and species (Bejder et al., 1999; Schaffar et al., 2010; Williams et al., 2006b), and whale-watching disturbance is of particular conservation concern for populations with low abundance and restricted ranges (Bejder et al., 2006; Lusseau et al., 2006). As a result, whale-watching impact assessments are conducted with increasing frequency (Gray et al., 2022).

The majority of studies has focused on the visually observable behavioural response of cetaceans to whale-watching vessels, which typically involves focal follows (Altmann, 1974) of individual whales (or groups) in the presence and absence of vessels. Common responses include changes in dive time (Christiansen et al., 2013a; Lusseau, 2003; Schaffar et al., 2010), respiration rate (Christiansen et al., 2014; Currie et al., 2021), swim speed (Currie et al., 2021), path directness (Corbelli, 2006; Williams and Ashe, 2007) and surface-active behaviours (Di Clemente et al., 2018; Corkeron, 1995). Furthermore, activity budgets and state transition probabilities have been used to show that animals change the proportion of time spent in particular behavioural states in the presence of whale-watching vessels (Di Clemente et al., 2018; Lusseau, 2003). For example, in the presence of vessels: common minke whales in Iceland and orcas in western Canada decreased foraging and increased travelling behaviours (Christiansen et al., 2013a,b; Williams et al., 2011, 2006b); resting and socialising behaviours of bottlenose dolphins in New Zealand were disrupted (Lusseau, 2003); and Risso's dolphins (*Grampus griseus*) in the Azores exhibited less resting activity (Visser et al., 2011b). As with other forms of disturbance, these behavioural changes have been interpreted as a stress response and the perception of vessels as a (possibly predatory) threat (Christiansen and Lusseau, 2014; Christiansen et al., 2013a; Wirsing et al., 2008). Moreover, by disrupting energy acquisition and increasing energy expenditure, these responses may compromise individual fitness (Lind and Cresswell, 2005).

Short-term impacts are not limited to visually observable changes. For example, acoustic monitoring suggests that humpback whales call less frequently in the presence of vessels (Fournet et al., 2018; Laute et al., 2022) and the underwater noise levels of whale-watching vessels are likely to shrink the communication space of cetaceans (Arranz et al., 2021; Erbe, 2002; Rey-Baquero et al., 2021). Meanwhile, tagging data have revealed a decline in humpback whale foraging behaviour in close proximity to whale-watching vessels (Ovide, 2017).

Despite a large body of evidence of disturbance, the influence of whale-watching vessels on whale behaviour is inconsistent (Senigaglia et al., 2016). Different species may exhibit different responses. For example, where minke whales and orcas showed a decrease in foraging and increase in travelling activity (Christiansen et al., 2013a,b; Williams et al., 2006b), humpback whales in Southeast Alaska showed no change in foraging behaviour and a decrease in travelling behaviour in the presence of vessels (Di Clemente et al., 2018). Differences also exist within a species, often according to location. During whale-watching encounters, humpback whale dive times increased in New Caledonia and Australia breeding grounds (Schaffar et al., 2009; Stamation et al., 2010), did not change in Alaska, Canada and Iceland feeding grounds (Corbelli, 2006; Ovide, 2017; Schuler et al., 2019) and decreased in the Hawai'i breeding ground (Currie et al., 2021). This may be attributed to variation in vessel practices, with variables such as distance to whale, vessel size and speed, approach angle and number of vessels affecting behavioural response (Di Clemente et al., 2018; Corkeron, 1995; Schuler et al., 2019; Sullivan and Torres, 2018; Williams and Ashe, 2007; Williams et al., 2009; Fiori et al., 2019), or regional differences in behaviour and habitat use. Alternatively, variation in behavioural responses could be driven by local habituation to vessel presence in some areas, i.e., a gradual decline in behavioural response over time (Allaby, 2020). Species including bottlenose dolphins (Acevedo, 1991) and belugas (*Delphinapteras leucas*)(Krasnova et al., 2020; Malcolm and Penner, 2011) have shown signs of habituation to whale-watching activity, although there is also evidence of sensitisation in some circumstances (Bejder et al., 1999; Constantine, 2001; Filby et al., 2014). Within these responses, there is high inter-individual variability in behavioural change (Oliveira et al., 2022) and individual or group identification is often the most significant explanatory variable in whale-watching impact assessments (Senigaglia et al., 2016). Some individuals even exhibit behaviours indicative of curiosity or play towards whale-watching vessels, including grey whales in Mexico (Dahlheim et al., 1981) and humpback whales in several areas (Watkins, 1986), which could represent positive behavioural change.

Attempts have been made to characterise the translation of short-term responses into long-term changes in population dynamics, as outlined by the Population Consequences of Disturbance framework (National Research Council, 2005; New et al., 2014; Pirotta et al., 2018). For example, Christiansen and Lusseau (2015) and Weinrich and Corbelli (2009) determined that short-term whale-watching disturbance of minke whales in Iceland and humpback whales in New England, respectively, did not have population-level consequences for reproductive success. In contrast, small, isolated populations of odontocetes may be more susceptible to population-level impacts. A decline in bottlenose dolphin abundance (Bejder et al., 2006) and female reproductive success (Bejder, 2005) in Shark Bay, Australia (relative to an adjacent control site), was attributed to concurrent growth in dolphin-watching activities; and a long-term shift in the summer distribution of Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand was attributed to an increase in cruise ship tourism (Carome et al., 2022). For bottlenose dolphins in Fiordland, New Zealand, although changes in individual behavioural budgets (Lusseau, 2004a, 2003) were not significant at a population level (Lusseau, 2005), long-term avoidance of the area (Lusseau, 2005) and tour boat collisions (Lusseau et al., 2002) led to the conclusion that dolphin-watching activities were unsustainable (Lusseau et al., 2006).

1.4.3 Knowledge gaps for whale-watching impacts

Despite extensive research on whale-watching impacts, several knowledge gaps remain, limiting our ability to inform effective management (Bejder et al., 2022). First, whilst behavioural responses to vessel presence have been observed for numerous cetacean species and whale-watching areas (Senigaglia et al., 2016), there is little information regarding the drivers of variability in behavioural response. In particular, the relative influence of vessel practices such as approach distance, speed, encounter duration and number of vessels remains understudied. High-resolution positional data for whale-watching vessels are often unavailable, and vessel movement metrics are difficult to measure from visual observation. Similar to vessel presence, for studies which have included these variables, behavioural responses vary between species and areas. For example, the number of vessels was significantly related to avoidance behaviour of killer whales in Canada (Williams and Ashe, 2007) and surface-active behaviour of humpback whales in Alaska (Di Clemente et al., 2018) but had no influence on the behavioural response of humpback whales in Hawai'i (Currie et al., 2021). Similarly, vessel distance to whale has varying impacts on humpback whales in different breeding grounds (Currie et al., 2021; Schaffar et al., 2013; Stamation et al., 2010). As a result, local impact assessments are required to determine behavioural responses to whale-watching practices (Higham et al., 2014). This information could be directly used to inform responsible vessel practices around cetaceans (Currie et al., 2021; Schuler et al., 2019), particularly if there are apparent thresholds of vessel behaviour that elicit whale responses. For instance, Risso's dolphins in the Azores spent less time resting when more than five vessels were present (Visser et al., 2011b) and humpback whales in New Caledonia exhibited avoidance responses when vessels approached closer than 335 m (Schaffar et al., 2013). Local vessel practices could also influence habituation to vessel presence and long-term behavioural monitoring (i.e., repeated observation of the same animals over time) could provide further information on this learning process (Bejder et al., 2009; Lusseau and Bejder, 2007).

Second, whilst a stress response is often assumed from behaviour, the physiological impact of whale-watching activities has seldom been investigated. Physiological stress is often not reflected by behavioural evidence (Walker et al., 2005a; Ditmer et al., 2015). Chronic stress can negatively affect individual fitness (Bonier et al., 2009; Sheriff et al., 2009) and physiological responses may be detectable in individuals before population-level effects on health or fecundity (Hunt et al., 2013). Stress in wild animals is typically monitored via concentrations of stress-related steroid hormones, particularly the glucocorticoids cortisol and corticosterone, in biological samples (Dickens and Romero, 2013). For example, faecal glucocorticoid responses increased in response to individual tourist encounters in macaques (Maréchal et al., 2011), and related to the level of tourism (human presence) at a timescale of days in elk (Creel et al., 2002), seasons in pine martens and tigers (Barja et al., 2007; Tyagi et al., 2019), and years in wolves (Creel et al., 2002). Similar responses have been detected in response to spatial variation in tourism pressure (Piñeiro et al., 2012; Walker et al., 2005b). Collecting biological samples in baleen whales is more challenging, owing to their underwater lifestyle and large size (Hunt et al., 2013). Nevertheless, potential physiological responses to vessel traffic have been detected, including elevated blubber cortisol (Pallin et al., 2022) and faecal glucocorticoids (Lemos et al., 2022; Rolland et al., 2012). However, these samples either require invasive collection (blubber) or are infrequently available (faeces). Therefore, there is growing interest in whale blow, which is predictably available and can now be collected using unobtrusive UAVs (Pirotta et al., 2017). Steroid hormones have been detected in blow (Atkinson et al., 2021; Burgess et al., 2018; Hogg et al., 2009), although the high, variable dilution of this biological sample has prevented robust physiological monitoring (Mingramm et al., 2019b). Compared with established immunoassays, analytical approaches such as LC–MS/MS may increase the number of steroids that can be quantified in these samples and their detection rates (Dalle Luche et al., 2019; Dunstan et al., 2012; Seger and Salzmann, 2020).

Third, the link between short-term responses and long-term impacts remains poorly understood (Bejder et al., 2022; Pirotta et al., 2018). In particular, there is little evidence that behavioural disturbance during whale-watching encounters has population-level impacts for wide-ranging mysticetes. Elucidating these linkages requires an improved understanding of: changes in activity budgets due to whale-watching vessel presence and practices (Christiansen et al., 2013a); the bio-energetic consequences of behavioural changes (Christiansen et al., 2013b); and the per-animal cumulative exposure to whale-watching vessels at a population level (Christiansen et al., 2015; Pirotta et al., 2018). Within this, it is important to characterise inter-individual variability in behavioural response, whale-watching exposure and fitness, which could alter mean long-term population impacts (New et al., 2015).

Beyond direct impact assessments, there is a general lack of information about the baseline occurrence and habitat use of cetaceans (Parsons et al., 2015; Redfern et al., 2006). Large-scale sighting surveys often provide patchy spatial coverage (Hammond et al., 2021; Pike et al., 2020a), and do not allow interrogation of fine-scale distribution (Baines and Weir, 2020). Furthermore, the environmental drivers of distribution may be unknown or poorly characterised at a regional level, limiting our ability to identify critical habitats and forecast future shifts in response to dynamic and changing ocean systems (Embling et al., 2010; IPCC, 2021). For example, humpback whale species distribution models (SDMs) generally have low explanatory power or predictive ability, and key environmental predictors vary between regions (Bassoi et al., 2020; Chavez-Rosales et al., 2019; Dalla Rosa et al., 2012). In addition, large-scale SDMs are often fitted to offshore sightings data, and the resulting model and available environmental data may not accurately predict distribution in highly complex coastal environments (Dalla Rosa et al., 2012). These knowledge gaps are important because the changing distribution of whale populations is likely to alter i) individual exposure to whale-watching activities and, thus, the population consequences of disturbance (New et al., 2015); and ii) the availability of whales for whale-watching activities, which may impact the viability of local industry and the future of coastal communities (Lambert et al., 2010; Meynecke et al., 2017; Richards et al., 2021). Therefore, an improved understanding of the interactions between cetaceans and their environment will facilitate proactive sustainable management (Barlow and Torres, 2021).

1.4.4 Whale-watching management

Despite these knowledge gaps, our existing understanding of whale-watching impacts has been used to inform and introduce policies worldwide to minimise potential negative effects (Carlson, 2012; IWC, 2019b). Most commonly, a set of guidelines or a code of conduct is used to guide vessel operators as to how to behave around cetaceans (Garrod and Fennell, 2004; Inman et al., 2016). Codes can be voluntary or mandatory, at international to local levels, and specific guidelines include minimum approach distance, maximum encounter duration, approach speeds and the maximum number of vessels in an area (Carlson, 2012). These guidelines can be accompanied by licensing schemes (mandatory) or accreditation schemes (voluntary) to ensure that vessels comply with regulations, and often include an educational component to maximise the positive effects of whale-watching (García-Cegarra and Pacheco, 2017; Lopez and Pearson, 2017). Codes of conduct may constitute regulations within MPAs, which can employ additional policies to mitigate whale-watching disturbance, such as MPA speed limits or permit requirements (Government of Canada, 2002; IWC, 2021; US National Park Service, 2022). Management may also include seasonal and diel (time-of-day) restrictions of whale-watching activity (IWC, 2021; Washington Department of Fish and Wildlife, 2021).

While some efforts have been met with compliance and success (Allen et al., 2007; Parsons and Woods-Ballard, 2003), others have faced opposition from operators and local communities, and as such are not followed (Kessler and Harcourt, 2013; Lusseau, 2004b). Guidelines may be difficult to enforce or perceived as unrealistic (Chalcobsky et al., 2017), and the conversion of voluntary codes into mandatory regulations is often recommended (Parsons, 2012). Low compliance rates may be further exacerbated when relevant stakeholder perspectives are not considered – for example, operators in Argentina regularly violated an existing code due to the shifting seasonal occurrence of southern right whales (*Eubalaena australis*), but this local knowledge had not yet been incorporated into regional policy (Chalcobsky et al., 2017). In contrast, ex-whalers in the Azores (who owned whale-watching companies) felt that whale-watching legislation provided insufficient protection for target whale populations because their local ecological knowledge was not integrated into policy development (Neves-Graça, 2004). This has led to calls for co-management of whale-watching activities by all relevant parties (Fumagalli et al., 2021). Furthermore, guidelines may not mitigate disturbance even when followed (Visser et al., 2011b), such that ongoing impact assessments are necessary to inform future regulations (Currie et al., 2021). For this reason, adaptive management is often recommended by natural scientists (Tyne et al., 2014), enabling changes to policy in response to updated information. However, this can be difficult to implement in practice (Fumagalli et al., 2021) and hindered by poor communication between natural scientists and policy makers (Alm, 2002).

1.5 Study system

1.5.1 North Atlantic humpback whales

Humpback whales belong to the mysticete family Balaenopteridae (commonly known as rorquals), which also contains common minke whale, Antarctic minke whale (*Balaenoptera bonaerensis* [Burmeister, 1867]), sei whale (*Balaenoptera borealis* [Lesson, 1828]), Bryde's whale (*Balaenoptera edeni* [Anderson, 1878]), Rice's whale, fin whale, blue whale and Omura's whale (*Balaenoptera omurai* [Wada, Oishi and Amada, 2003]). A key characteristic of rorqual species is lunge feeding, a unique two-step feeding mode which involves engulfing prey, followed by expulsion of excess water out of the buccal cavity via baleen filtration (Pivorunas, 1979; Shadwick et al., 2019). Humpback whales are a cosmopolitan species, found in all the world's oceans (Carwardine, 2019). This single species consists of at least 14 distinct population segments (DPS; NOAA, 2016), although genetic evidence supports the delineation of three subspecies (Jackson et al., 2014). Adults typically grow to a length of 16 m and a weight of 30–40 tons (Carwardine, 2019; Johnson and Wolman, 1984). Humpback whales are generalist feeders amongst rorquals, with a diet including euphausiids, other zooplankton and small schooling fish (Carwardine, 2019; Clapham, 2018).

Like other large mysticetes, humpback whales are highly migratory (Carwardine, 2019; Mackintosh, 1946; Stevick et al., 2011; Stone et al., 1990). In the North Atlantic, the principal breeding grounds are the northern Caribbean, southeastern Caribbean and Cape Verde; and the principal feeding grounds are the Gulf of Maine, eastern Canada, West Greenland, Iceland and north of Norway (Reeves et al., 2002; Smith et al., 1999; Stevick et al., 2003b, 2018). Migration routes are primarily oceanic, although stopover sites include Bermuda, the Azores and Norway (Cucuzza et al., 2015; Grove et al., 2023; Narganes Homfeldt et al., 2022; Ramm, 2020). A satellite-tagged female completed a round-trip migration of 18,300 km between the Barents Sea and the northern Caribbean, one of the longest migrations of any mammal (Kettemer et al., 2022). Whilst the majority of North Atlantic humpback whales appear to undergo regular migration, there is some evidence that a proportion of the population over-winters in feeding grounds (Kowarski et al., 2018; Magnúsdóttir et al., 2014; Martin et al., 2021; Palsbøll et al., 1997).

Following extensive commercial hunting in the 19th and 20th centuries (Reeves et al., 2002; Smith and Reeves, 2010; Stevick et al., 2003a) and a commercial whaling ban in 1955 (Best, 1993), the abundance of North Atlantic humpback whales steadily increased through the end of the 20th century (Punt et al., 2006), at 1.2–3.1% per annum (Stevick et al., 2003b), and may have stabilised in recent decades. This reflects population trends across the species' global range, such that humpback whales were listed as 'endangered' in the 1980s, 'vulnerable' in the 1990s and are currently listed as 'least concern' by the IUCN Red List of Threatened Species (Cooke, 2018b). There are now an estimated 84,000 humpback whales globally (Cooke, 2018b), although the North Atlantic estimate of 10,752 for the primary breeding ground was derived from sightings data up to 1992 (Stevick et al., 2003b). This is thought to be far below the pre-exploitation population size (Ruegg et al., 2013), although the current carrying capacity is not known. Recent abundance estimates are lacking for the population and the IWC recommended an in-depth assessment of North Atlantic humpback whales (IWC, 2019a), although regional evidence suggests that some parts of the population are continuing to increase (Grove et al., 2023; Robbins and Pace, 2018).

Contemporary threats to North Atlantic humpback whales are typical of the global threats to other baleen whales, although research has primarily been conducted in the western North Atlantic. Mortality rates due to entanglement are estimated to be as high as 5% annually in the western North Atlantic, with up to 16% of entangled animals dying due to entanglement (Volgenau et al., 1995); and entanglement rates determined from scar analysis range from 25% in Iceland (Basran et al., 2019) to 50% in the Gulf of Maine (Robbins, 2009). Vessel collisions are a considerable threat and have increased in recent years (Hill et al., 2017; van der Hoop et al., 2015). Underwater noise is increasing in the North Atlantic (Širović et al., 2016) and impacts on foraging behaviour have been demonstrated in the western North Atlantic (Blair et al., 2016). Persistent organic pollutants (POPs) have been detected in humpback whales in the western (Baugh et al., 2023; Elfes et al., 2010) and eastern (Ryan et al., 2013) North Atlantic, and polychlorinated biphenyl concentrations in juveniles in the Gulf of Maine may cause adverse effects (Baugh et al., 2023; Jepson et al., 2016). Humpback whales are still subject to whaling in the North Atlantic, with quotas of up to 28 animals in St Vincent and Grenadines and 10 in West Greenland per annum (IWC, 2022), but this is unlikely to be a population-level threat. Climate change is arguably the greatest future threat to humpback whale populations (Meynecke et al., 2020), although responses to oceanographic variability in the North Atlantic remain understudied (Víkingsson et al., 2015; Weinrich et al., 1997). Whilst declining reproductive success of humpback whales in eastern Canada was attributed to climate change via changes in prey availability, recent expansion of foraging grounds into Arctic areas such as East Greenland (Hansen et al., 2018) and Svalbard (Storrie et al., 2018) suggests that retreating sea ice and warming waters in these regions could be providing new foraging opportunities. Finally, humpback whales are the primary target of whale-watching activities across the North Atlantic (IWC, 2019b), with limited evidence of behavioural disturbance in Newfoundland (Corbelli, 2006) and Iceland (Laute et al., 2022).

1.5.2 Iceland

Icelandic waters are some of the most biologically productive in the North Atlantic (Astthorsson et al., 2007) and an important summer feeding ground for humpback whales and other cetaceans (Carwardine, 2019; Pike et al., 2020b). Around Iceland, transverse topographic ridges and energetic atmospheric circulation create boundaries between diverse water masses and generate persistent, productive frontal regions with seasonal phytoplankton blooms (Astthorsson and Vilhjálmsson, 2002; Thórdardóttir, 1994). In summer, approximately 10,000 humpback whales inhabit Icelandic waters (Pike et al., 2019, 2020a). Recent stable isotope analyses suggest that humpback whales primarily feed on euphausiids (>60% of diet), followed by capelin and herring (García-Vernet et al., 2021), in contrast to previous estimates that 60% of their diet was capelin (Sigurjónsson and Víkingsson, 1997; Stefansson et al., 1997). Humpback whales are primarily sighted around North Iceland, on a wide continental shelf that extends 100–170 km offshore (Klotz et al., 2017; Pike et al., 2019), in areas of higher primary productivity and lower sea surface temperature (Paxton et al., 2009). Hydrographically, North Iceland is influenced by: Atlantic-derived water from the west (Irminger Current and North Icelandic Irminger Current); Arctic-derived water from the north (East Greenland Current and East Iceland Current); and a freshwater-induced coastal current (Icelandic Coastal Current; Astthorsson and Vilhjálmsson, 2002; Stefánsson, 1962; Figure 1.2).

Icelandic waters have experienced large and sudden changes in oceanography and biological communities during the last few decades, in line with basin-scale variability across the wider North Atlantic (Beaugrand et al., 2015). A regime shift in the mid-1990s was attributed to a declining sub-polar gyre, increasing North Atlantic Oscillation (NAO) and progressive 'Atlantification' of coastal and offshore waters (Alheit et al., 2019; Häkkinen and Rhines, 2004; Hátún et al., 2009). In the 21st century, Icelandic waters have experienced some of the greatest increases in heatwave intensity and frequency globally (Oliver et al., 2018). The associated increases in temperature and salinity (Figure 1.2B–C), as well as changes in sea surface height and mixed layer depth, appear to have altered regional primary productivity (McGinty et al., 2016) and zooplankton stocks (Gislason et al., 2021; Silva et al., 2014). As a result, warm-water fish species such as mackerel have moved northwards into Icelandic waters (Olafsdottir et al., 2019) and cold-water species such as capelin have retreated northwards out of the Icelandic EEZ (Carscadden et al., 2013; Vilhjalmsson, 2007).

The abundance and distribution of humpback whales (and other cetaceans; Pike et al., 2020b) have also changed during this period. Between 1987 and 2007, total abundance in Icelandic and adjacent waters increased from 1,722 (95% confidence interval 1,061–2,795; Pike et al., 2005) to 14,553 (95% CI 5,819–27,906; Pike et al., 2020a), before declining to 6,643 (95% CI 3,543–12,456) in 2015 (Pike et al., 2019). Meanwhile, in some coastal areas, humpback whale abundance and sighting rates have increased

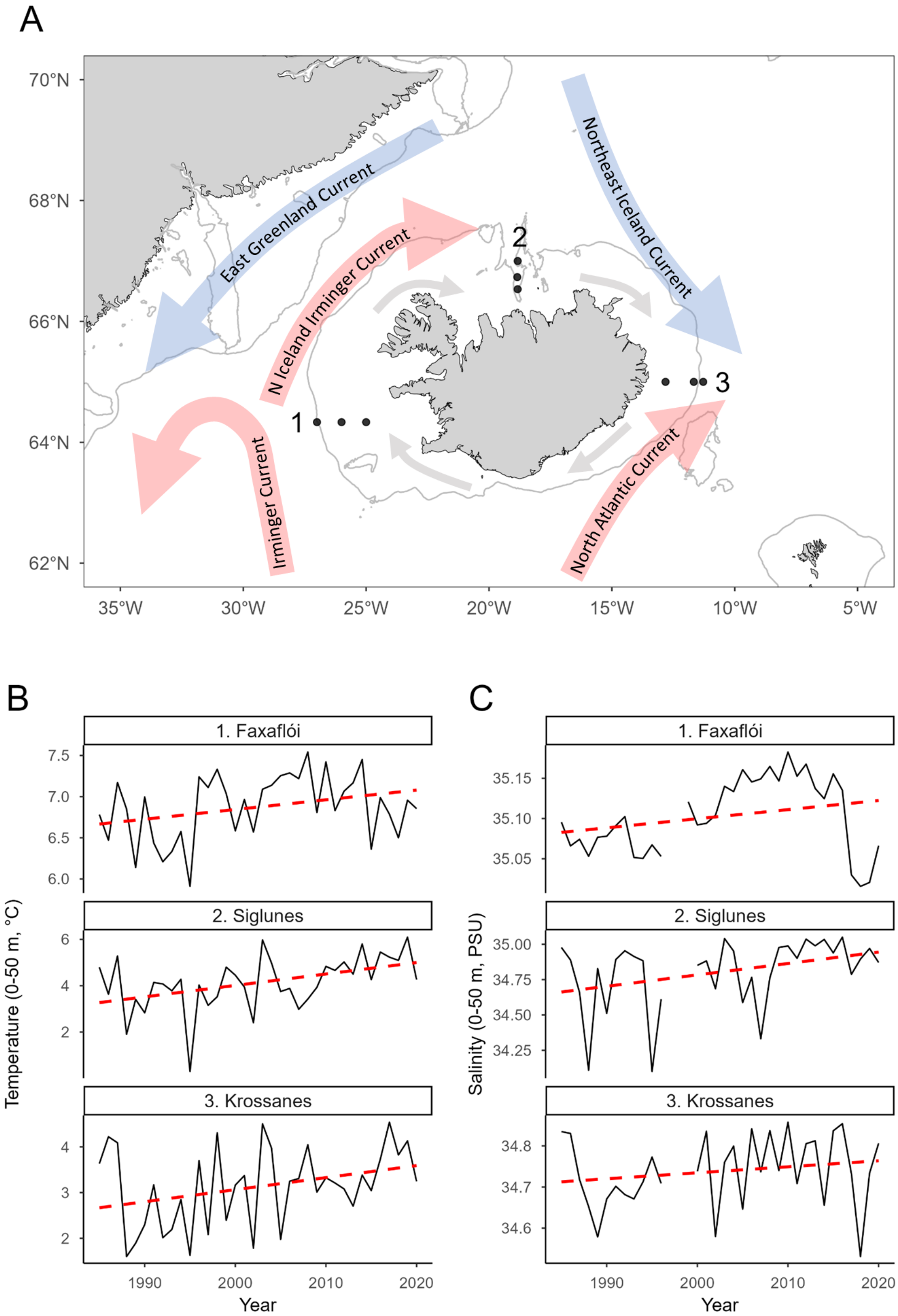

Figure 1.2: Hydrography around Iceland. A) Principal currents (Arctic origin in blue, Atlantic origin in red, Icelandic Circumpolar Current in grey) and three hydrographic transects around Iceland: Faxaflói (1), Siglunes (2) and Krossanes (3). The 400 m depth contour (a proxy for the edge of the continental shelf) is denoted by a grey line. Lower plots display summer time series of B) temperature and C) salinity between 1986 and 2020 for select hydrographic transects from plot A, averaged over at least two stations and all samples from 0 to 50 m depth. The linear trend is denoted by the dashed red line. Time series data available from the Icelandic Marine and Freshwater Research Institute (Hafrannsóknastofnun) at: https://sjora.hafro.is/ (Accessed 1 December 2022).

during the last 15 years (Bertulli et al., 2018; Klotz et al., 2017; Malinauskaite et al., 2022). Despite concurrent environmental shifts, the drivers of estimated changes in humpback whale occurrence are still uncertain (Malinauskaite et al., 2022; Víkingsson et al., 2015) and have not been linked to prey availability. Changes may, in part, be attributed to population recovery from commercial whaling. Furthermore, humpback whales appear to be associated with lower sea surface temperatures and shallower depths in Icelandic waters (Paxton et al., 2009). In other feeding grounds, distribution is influenced by numerous environmental variables, including depth, distance to depth contours, water temperature and chlorophyll concentration (Bassoi et al., 2020; Chavez-Rosales et al., 2019; Dalla Rosa et al., 2012; El-Gabbas et al., 2021a; Zerbini et al., 2016), although relationships are inconsistent (and even contrasting) across studies (Meynecke et al., 2021).

1.5.3 Whale-watching in Iceland

Reliable whale occurrence in several coastal areas has led to the progressive development of a whale-watching industry in Iceland, encouraged by international organisations such as the International Fund for Animal Welfare (IFAW) as an alternative to commercial hunting of minke and fin whales (Rasmussen, 2014). From the first whale-watching trips in the 1991, the number of passengers increased consistently in Iceland (Figure 1.3), with 14% annual growth between 1998 (30,330) and 2008 (114,500), resulting in $17 million USD expenditure annually in 2008 (O'Connor et al., 2009). This growth continued until a peak of 368,000 passengers in 2017, representing 18% of all tourists that visited Iceland (Icelandic Tourist Board, 2020). Since 2006, this growth has taken place alongside commercial whaling operations in southwest Iceland, with scepticism that the two industries could co-exist (Bertulli et al., 2016). Currently, organised vessel-based whale-watching trips take place in five areas: Faxaflói (operating out of Reykjavík) in the southwest; the Snæfellsnes Peninsula (operating primarily out of Ólafsvík) in the west; and Ísafjarðardjúp (operating out of Ísafjörður), Steingrímsfjörður (within the larger bay of Húnaflói, operating out of Hólmavík), Eyjafjörður (operating out of several ports) and Skjálfandi Bay (operating out of Húsavík) in the north (Figure 1.4). Humpback whales are the primary target species in North Iceland and are sighted in all whale-watching areas, whereas minke whales are the primary target in Faxaflói. The size of the whale-watching operations varies considerably between regions, ranging from one vessel in Steingrímsfjörður to 26 whale-watching vessels (eight companies) in Faxaflói and 17 vessels (three companies) in Skjálfandi Bay.

1.5.4 Húsavík and Skjálfandi Bay

Known as the "Whale Watching Capital of Europe" (Cunningham et al., 2011), Húsavík is home to the second-largest whale-watching fleet in Iceland (O'Connor et al., 2009). The town has 2,382 inhabitants (as of 2021) and lies 58 km south of the Arctic Circle (Statistics Iceland, 2021). All trips take place in and around Skjálfandi Bay, a feeding ground for migratory and resident cetacean species, including humpback whales, blue whales, minke whales and white-beaked dolphins (*Lagenorhynchus albirostris*)(Cecchetti, 2006). Despite the near year-round occurrence of cetaceans, whale-watching activities are highly seasonal, with a peak in May–September (Annisius, 2014). Starting with a single

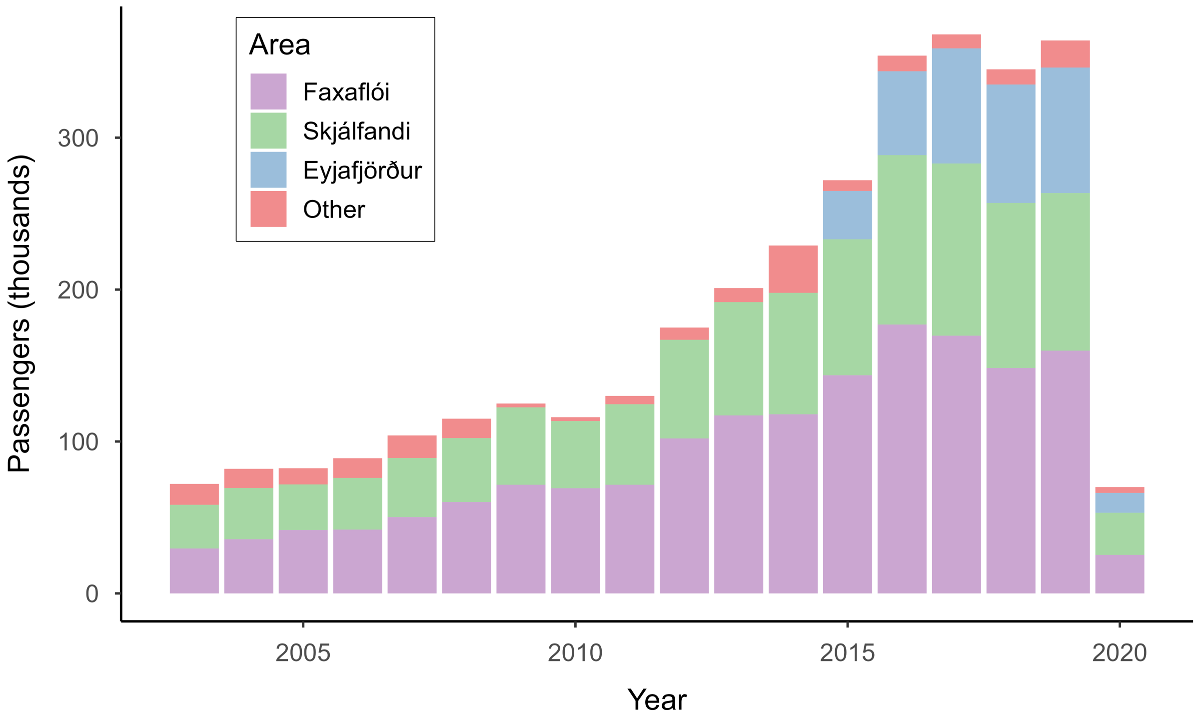

Figure 1.3: Annual number of whale-watching passengers in Iceland, stratified by region. Data available from the Icelandic Tourist Board at: https://www.maelabordferdathjonustunnar.is/is/afthreying/hvalaskodun (Accessed 3 January 2023).

company in 1995 (North Sailing), the growth of this local industry has provided new economic opportunities to the town, reversing the drain of human capital following the decline of local fisheries (Einarsson, 2009; Norðurþing Municipality, 2020). Whale-watching also provides other whale ecosystem services, including recreation and cultural identity, with whales becoming a symbolic part of the town (Guðmundsdóttir and Ívarsson, 2008; Malinauskaite et al., 2021; Nicosia and Perini, 2016).

Whale-watching in Húsavík has experienced steady growth in recent years, with the number of passengers increasing from 29,000 in 2003 to approximately 113,000 in 2017, on up to 50 trips per day run by four operators, representing 30% of all Icelandic whale-watching passengers (Figure 1.3; (Icelandic Tourist Board, 2020). The number of passengers remained high (>100,000) until the COVID-19 pandemic in 2020, with travel restrictions leading to a major decline in whale-watching activities (Figure 1.3) and global tourism generally (Rutz et al., 2020). One company ended operations during the pandemic, with three remaining operators as of 2022. Moreover, the future of this local industry is uncertain due to the potential susceptibility of both cetacean occurrence and whale-watching vessels to a changing climate (Malinauskaite et al., 2022; Vallejo, 2013).

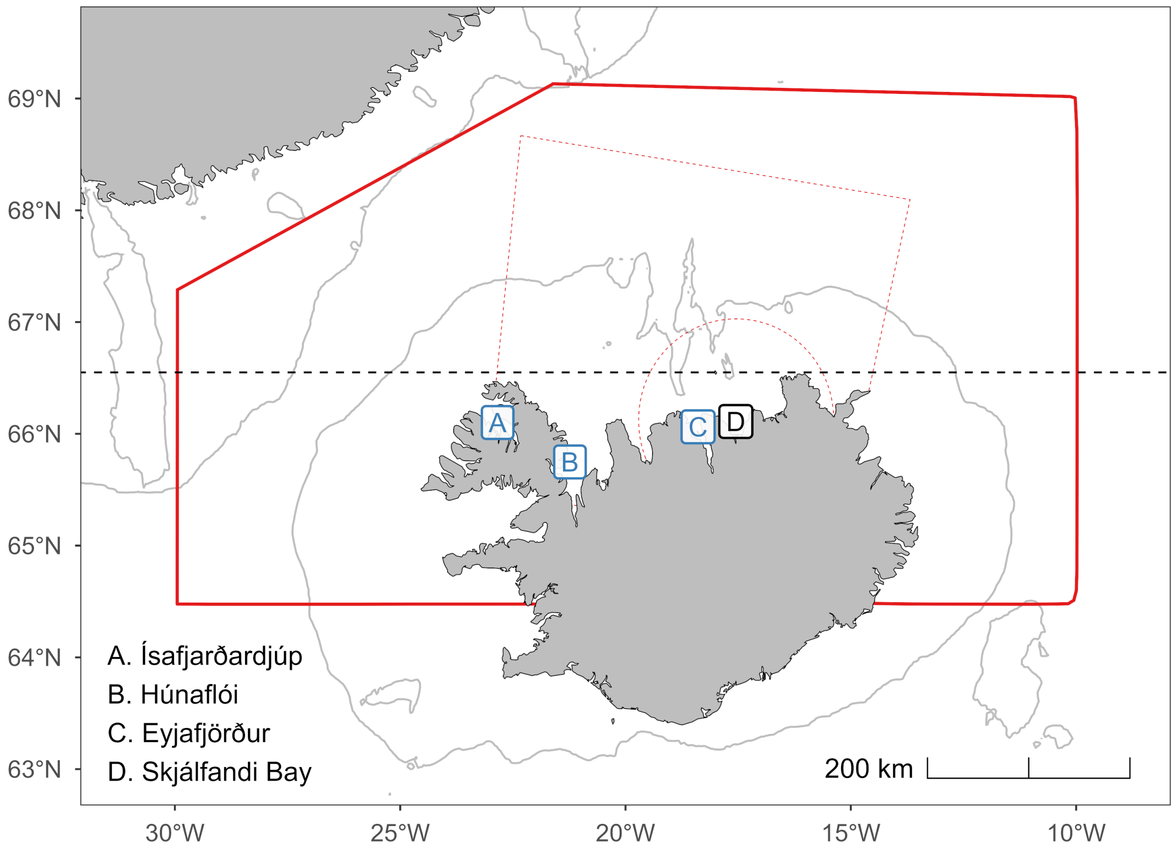

Figure 1.4: Study sites in this thesis, including four coastal areas (Ísafjarðardjúp, Húnaflói, Eyjafjörður and Skjálfandi Bay) and offshore North Iceland (solid red line). Chapter 2 used behavioural observations from Skjálfandi Bay; Chapter 3 used blow samples collected from all four coastal areas; and Chapter 4 used sightings data from offshore North Iceland (areas of particular interest in red dashed lines) and Skjálfandi Bay. The Arctic Circle is denoted by the black dashed line and the 400 m depth contour (a proxy for the edge of the continental shelf) is denoted by a grey line. Dedicated commercial whale-watching took place in Húnaflói (Steingrímsfjörður), Eyjafjörður and Skjálfandi Bay during the study period.

1.5.5 Whale-watching research and management in Iceland

Previous research in Iceland has revealed that humpback and minke whales exhibit behavioural responses to whale-watching vessels. In Faxaflói, a series of studies led by Dr Fredrik Christiansen examined the link between short-term disturbance and population-level vital rates for minke whales (Christiansen et al., 2015; Christiansen and Lusseau, 2015; Christiansen et al., 2013a,b, 2014), collecting focal follow behavioural data from whale-watching vessels (test data) and from land (control data). In the presence of vessels, animals exhibited shorter dives and more sinuous movement, suggesting a reduction in foraging and an increase in travelling activity states (Christiansen et al., 2013a). The rate of surface-feeding events (SFEs) also decreased in the presence of vessels. Simultaneously, swim speeds were higher in the presence of vessels, indicating an increase in energy expenditure (Christiansen et al., 2014). However, due to low per-animal cumulative exposure to whale-watching vessels (Christiansen et al., 2015), these responses did not significantly impact foetal growth (a vital rate) at a population level (Christiansen and Lusseau, 2015).

Impacts have also been demonstrated for humpback whales in Skjálfandi Bay. Ovide (2017) used suction cup acoustic tags to show that high noise intensity from vessels (indicating close passes), which masks vocalisations, was related to a reduction in foraging attempts and deeper dives, indicating potential disruption of foraging behaviour. Meanwhile, long-term acoustic deployments were paired with automatic identification system (AIS) vessel positions and visual observations to show that humpback whales reduced their calling effort in the presence of vessel sound, independent of overall ambient sound levels (Laute et al., 2022). Whale-watching vessel traffic declined in 2020, during the COVID-19 pandemic, relative to 2018, with a two-fold increase in the acoustic presence of humpback whales (Laute et al., 2022).

Despite apparent whale-watching disturbance, there is currently no legal regulation of whale-watching activities (regionally or nationally) in Iceland, concerning the number and distribution of vessels or vessel behaviour. Southern Faxaflói, Eyjafjörður and Skjálfandi Bay are designated as 'whale-watching sanctuaries' by the Icelandic government, but these MPAs only provide protection from commercial whaling (Government of Iceland, 2017). Iceland is a Party to the CBD and a Member of the IWC and NAMMCO, but these confer no specific regulation of whale-watching activities. However, there is a voluntary code of conduct maintained by IceWhale, the Icelandic Whale Watching Association (https://icewhale.is/). Signed in 2015 by Icelandic whale-watching companies during a workshop in Reykjavík, the code was developed in collaboration with whale-watching staff and international experts (IceWhale, 2017). This replaced older codes, for example produced by the Húsavík Whale Museum and North Sailing (Carlson, 2009; Martin, 2012). The IceWhale code has the aims of:

- "Minimising impact on cetacean for the future and the sustainability of whale watching operation in Iceland."
- "Ensuring the best possible encounter, both for animal welfare and passenger enjoyment."
- "Increasing development, understanding and awareness of appropriate practices when watching cetaceans."

The code contains various guidelines to encourage responsible whale-watching, including minimum approach distance (50 m) and angle, vessel speed and communication between vessels (Table 1.1). Three zones of distance to whale are delineated: searching zone (3,000–300 m radius), approaching zone (300–50 m) and encounter zone (50 m). The code also contains guidance about behavioural signs of disturbance in a more detailed operators manual[7].

Table 1.1: Key guidelines from the IceWhale code of conduct for commercial whale-watching in Icelandic waters. Three zones of distance to whale are delineated: searching (3,000–300 m), approaching (300–50 m) and encounter (<50 m). Code of conduct available at: https://icewhale.is/ (Accessed 1 December 2022)

Zone	Guideline
Searching zone (3,000–300 m)	• Keep a dedicated lookout and stay in radio contact with other vessels. • Avoid sudden or excessive noises or disturbances. • Avoid sudden speed or course changes (approaching or departing). • Continuously assess cetacean behaviour during interactions, and avoid repeated attempts to interact with animals that are showing signs of distress.
Approaching zone (300–50 m)	• Stop the main propellor (max speed $\sim$5-6 mph). If the cetacean is travelling fast you can speed up a little (up to $\sim$8 mph) but not directly towards the animal. • Avoid following behind and never deliberately approach in front of the cetacean. Vessel movements should be parallel, approaching cautiously at an oblique angle (from behind). • Do not move closer if there is another boat in the approaching zone, unless the other boat gives way or signals that it is safe to approach. • Take turns if there are more boats in the area, preferably each boat shouldn't spend more than 20–30 minutes with the same cetacean. • Never (deliberately) sail through pods of concentrated cetaceans.
Encounter zone (<50 m) "area of cetacean choice", no intentional approach by any vessel	• When possible, stop the propellor if any cetaceans approach the vessel and do not re-engage propulsion until all cetaceans are observed to be well clear of your vessel. • Do not touch, swim with or feed cetaceans.

7. IceWhale's code of conduct for responsible whale watching - operators manual: https://s3-eu-west-1.amazonaws.com/wwhandbook/guideline-documents/Iceland_IceWhale-CoC-OperatorsManual.pdf

At present, 11 operators in Iceland (out of at least 18) publicly abide by the code of conduct (IceWhale, 2017). Compliance rates at a national level are unknown. However, Martin (2012) used vessel- and land-based observations to characterise whale-watching practices and assess compliance in Skjálfandi Bay between 2009 and 2011. Up to 39% of approaches to humpback whales were within 50 m (the current minimum approach distance) and close approach speeds were up to 17 km/h (10.6 mph). Moreover, approach speeds increased across the period. The study concluded that observed approaches were frequently inconsistent with existing guidelines. However, contemporary compliance rates to the Ice-Whale code are unknown. Furthermore, the effectiveness of guidelines in reducing behavioural impacts has not been studied.

1.6 Thesis objectives

As our human footprint expands throughout the global ocean, cetaceans face an increasing array of threats. Whilst local stressors such as vessel-based whale-watching are not generally considered a significant population threat to wide-ranging cetaceans such as baleen whales, it is critical to limit the cumulative impacts on whales to increase population resilience to more severe threats such as climate change. Mitigating local threats requires targeted research of the interaction between the stressor and the species of interest, and consideration of how these interactions may change in the future as a function of changing social and ecological conditions. This information can be used to guide suitable policies, with residual uncertainties supporting a precautionary approach, paired with inclusive governance.

This thesis aims to improve our understanding of the interactions between humpback whales and whale-watching activities in North Iceland to inform responsible practices. I focus on Skjálfandi Bay, home to a large-scale industry with no formal regulation and evidence of low compliance rates. Long-term monitoring of cetaceans has been conducted in Skjálfandi Bay since 2006, primarily led by the University of Iceland. As such, longitudinal data on cetacean occurrence and sighting histories are available for the Bay and there are established, authentic collaborations between research institutes and whale-watching operators (Klotz et al., 2017; Malinauskaite et al., 2022). Previous research suggests that local whale-watching activities disturb whale behaviour, but the influence of different vessel practices is poorly understood and, as with other whale-watching areas, the hidden physiological impacts of vessels remain unknown. Moreover, despite preliminary evidence that cetaceans are vulnerable to the effects of climate change in the Bay, regional spatiotemporal variability in occurrence and habitat use remain poorly characterised, hindering our ability to consider future changes. As a result, Skjálfandi Bay is an excellent case study for whale-watching management in North Iceland and whale-watching research more broadly.

This thesis has four key questions:

1. How do whale-watching encounters and variation in vessel practices impact humpback whale behaviour? [Chapter 2]

2. Is it possible to determine the physiological response of whales to whale-watching activity? [Chapter 3]
3. Are humpback whales sensitive to past and future environmental change in Icelandic waters, and could this influence their interactions with coastal whale-watching? [Chapter 4]
4. What are the key considerations for future whale-watching management in Iceland, based on the results for chapters 2–4? [Chapter 5]

Within the thesis, both established and emerging technologies were used to collect (and process) behavioural, physiological and sightings data. Sampling methods highlight and support the use of affordable and less obtrusive platforms of opportunity (e.g., whale-watching vessels) and land bases to study cetacean ecology. These field data were combined with existing data bases of sightings, physical oceanography and vessel positions to answer key questions, using hypothesis- and data-driven approaches. Whilst standard statistical methods were generally used (including a variety of model frameworks), data were often processed in novel ways to extract important information. In this way, I aimed to both inform regional management and advance the field of cetacean research. Importantly, the aims of this thesis were achieved through a series of key collaborations, involving academic institutions and international projects.

Chapter 2 examines the behavioural interactions between humpback whales and whale-watching vessels in Skjálfandi Bay (Figure 1.4). Focal follows of individual whales were conducted from whale-watching vessels to construct seven behavioural metrics (response variables), including the position-derived variables of swim speed and directness index that have inherent measurement errors. Meanwhile, fine-scale GPS and coarse-scale AIS vessel position data were used to construct vessel movement metrics (explanatory variables). Series of generalised additive mixed models (GAMMs) were then used to investigate the potential influence of vessel practices on whale behaviour, accounting for inter-individual variability and considering the bi-directional interactions between these two agents of change (whales and vessels). I also tested the influence of non-compliance with the code of conduct on behaviour. Access to vessel platforms and existing data sets were provided by the University of Iceland and North Sailing, a whale-watching operator.

Chapter 3 explores the use of blow sampling with UAVs, paired with LC–MS/MS for steroid hormone analysis, to monitor the hidden, physiological stress response of humpback whales to whale-watching vessels and vessel traffic at a regional scale (North Iceland; Figure 1.4). The primary steroid hormone of interest was cortisol (the dominant stress-related hormone in many mammals), but I screened for a wider panel of steroid hormones in an attempt to derive hormone ratios (overcoming variable dilution of blow samples) and explore the potential of blow sampling for broader endocrinological monitoring. Despite recent advances (e.g., Burgess et al., 2018), the use of blow sampling for dynamic physiological assessment of large whales (particularly related to stress) remains unvalidated. Therefore, methodological development constituted a large portion of this chapter and remaining barriers to steroid hormone measurements and biological interpretation were discussed. Sample analyses were performed at the University of Edinburgh Clinical Research Facility.

Chapter 4 examines the physical environmental predictors of humpback whale occurrence in offshore Iceland, as well as temporal changes in habitat use in coastal and offshore waters, using two distinct modelling approaches. A species distribution model (SDM) was used to relate offshore sightings data spanning 28 years (provided by the Icelandic Marine and Freshwater Research Institute) to static and dynamic environmental data obtained from multiple sources, including the VIKING20X general circulation model (Biastoch et al., 2021). Three SDM frameworks were examined for explanatory and predictive ability: generalised additive model (GAM), boosted regression tree (BRT) and a GAM–BRT ensemble. The final SDM was used to consider key environmental variables and predict summer whale density for each year during 2006–2019. Meanwhile, a capture–recapture (CR) model was applied to humpback whale photo-identification sightings data from Skjálfandi Bay to estimate the number of whales using this coastal area each summer during the same period. Again, three model frameworks were considered: Cormack–Jolly–Seber (CJS), Jolly–Seber–Schwarz–Arnason (JSSA) and multi-state open robust design (MSORD) models. The two resulting time series (offshore density and coastal abundance) were then related with weighted linear regression, to consider recent changes in habitat use around North Iceland. Using these results, I discuss the sensitivity of humpback whales to past and future climate change in Icelandic waters, and potential consequences for whale-watching exposure and impacts. This chapter builds upon work that I conducted as part of the iAtlantic project[8] with the University of Edinburgh.

Chapter 5 combines a synthesis of chapters 2–4 with the precedent of existing policies worldwide to inform future whale-watching management in Iceland. In line with existing management schemes, I consider changes to the existing IceWhale code of conduct, and the potential benefits of spatial, temporal and adaptive management in Icelandic waters. Within this, I conduct a systematic literature search of existing codes of conduct, visualise seasonal variability in whale sightings and whale-watching intensity, and consider the spatial structure of whale-watching in Iceland. Suggestions largely follow a precautionary approach in the absence of further evidence. Finally, I discuss the importance of appropriate governance strategies, as well as comprehensive education and training, to ensure compliance and the inclusion of multiple stakeholder perspectives to encourage sustainable whale-watching management.

In addition to informing whale-watching policy in Iceland, this thesis aims to advance impact assessments of wildlife tourism (and other forms of human disturbance) for other marine species. In particular, marine megafaunal groups, including pinnipeds, sirenians, some elasmobranchs, turtles and seabirds, are the focus of global vessel-based tourism industries (Gallagher and Hammerschlag, 2011; O'Malley et al., 2013), and perceived negative behavioural responses have been observed across these groups (Andersen et al., 2012; Hodgson and Marsh, 2007; Montero-Quintana et al., 2020). Despite some common traits among marine megafauna (including mobility, migratory behaviours and similar influences on ocean ecosystems), the ecological results from this thesis – in terms of responses to whale-watching vessels and spatiotemporal patterns in distribution and occurrence – should not be

8. iAtlantic: https://iatlantic.eu/

generalised to other marine species; even within cetaceans, there is inconsistency in tourism impacts and species–environment relationships between species. However, the methods developed in this thesis (for humpback whales) can be adapted to study other marine megafauna. For example, the use of GPS data in Chapter 2 to characterise vessel movement could be expanded to other vessel-based wildlife tourism systems to better understand the impact of variable vessel practices on animal behaviour. Despite evidence of physiological stress responses to anthropogenic disturbance (Beaulieu-McCoy et al., 2017; Walker et al., 2005b; Williard et al., 2015), generally physiological monitoring remains limited across marine species owing to shared challenges of sample collection (Fleming et al., 2018; Trave et al., 2017), and the endocrinological assessment of minute samples (e.g., exhaled breath; Chapter 3) could inform assessments of comparable sample types in other marine species. Climate change is a global stressor for the majority of marine megafaunal taxa (Grémillet and Descamps, 2023) and novel approaches to compare local and regional changes in abundance and distribution (Chapter 4) could advance similar comparisons in other species, informing adaptive management (Fuentes et al., 2016). As a result, the methodological developments presented throughout this thesis could be applied to other marine megafaunal taxa to inform more comprehensive regulation of marine wildlife tourism.

Chapter 2

Assessing the behavioural response of humpback whales to variable vessel practices in Skjálfandi Bay, Iceland

2.1 Abstract

Repeated disturbance of animal behaviour can have consequences for individual welfare, fitness and long-term population dynamics. Whale-watching vessels are known to impact whale behaviour, but responses vary between species and areas, and little is known about the contribution of variable vessel practices to disturbance, limiting our ability to inform responsible whale-watching guidelines.

I assessed the influence of whale-watching vessel presence, positioning and movement on humpback whale behaviour in Skjálfandi Bay, Iceland – an area with a large-scale industry but limited information on behavioural impacts. Using whale-watching vessels as an opportunistic research platform, surface behaviours and movement tracks were measured in the absence of true control data. Positional errors were explored and propagated into subsequent modelling. Vessel presence and movement variables were constructed from a novel combination of coarse-scale AIS and fine-scale GPS data, and were related to seven whale behavioural variables through a series of generalised additive mixed models (GAMMs). Furthermore, the influence of encounter duration and potential benefit of speed compliance to a voluntary code of conduct were explored.

The behavioural interactions between whale-watching vessels and humpback whales were complex and GAMM relationships were often difficult to interpret. Nevertheless, key predictors of whale behaviour included vessel speed, the number of vessels and encounter duration. For example, increasing vessel speed was related to elevated dive times, the presence of more vessels was associated with changes in surfacing patterns and surface movements became less sinuous as encounters progressed. These results suggest potential horizontal and vertical avoidance, in addition to more cautious surface behaviours, in a more intense vessel environment and with prolonged exposure, potentially due to the perception of increased risk. Furthermore, surface feeding behaviour decreased significantly with apparent non-compliance to the code of conduct's speed guideline.

The observed behavioural responses suggest that the decisions made by captains during whale-watching encounters could have consequences for the energy budget and welfare of individual humpback whales. As the whale-watching industry in Iceland continues to expand geographically, I encourage a review of existing policies to minimise potential behavioural disturbance. Despite caveats associated with data collection, and a lack of information on long-term impacts, the results of this study may be used to inform changes to the current code of conduct and guide future targeted behavioural research.

2.2 Introduction

2.2.1 Behavioural disturbance

Animal behaviour refers to an individual interacting with its physical, biological and social environment (Huntingford, 1984). An animal's fitness is determined by its behaviour, such that repeated behavioural disturbance can negatively impact survival and reproduction (Lusseau et al., 2006). Human activities may disturb marine mammal behaviour through perception of a predation risk, generating a 'seascape of fear' (Beale and Monaghan, 2004; Frid and Dill, 2002; Wirsing et al., 2008). This disturbance may have an energetic cost (Williams et al., 2006a) and disrupt critical activities such as foraging, resting and socialising, potentially compromising long-term fitness (Christiansen and Lusseau, 2015; Christiansen et al., 2014; Lusseau et al., 2006). Therefore, persistent and widespread disturbance can influence population dynamics and threaten the conservation status of marine mammals (Bejder et al., 2006; New et al., 2014).

Behavioural disturbance is also important from a welfare perspective, i.e., the balance of positive and negative states experienced by an individual (Mellor et al., 2009). Behaviour is likely a direct and significant indicator of welfare state in cetaceans (Clegg and Butterworth, 2017; Waples and Gales, 2002), and defined behaviours are commonly related to emotional and physiological state. Therefore, behavioural disturbance likely has negative welfare consequences (Clegg and Butterworth, 2017), which may be further exacerbated by long-term impacts on fitness.

2.2.2 Behavioural response to whale-watching

Vessel-based whale-watching may be considered a form of ecotourism, visiting fragile natural environments while supporting conservation through public education and engagement (García-Cegarra and Pacheco, 2017; Orams, 1995). However, whale-watching encounters can also negatively impact cetacean behaviour (Senigaglia et al., 2016). As a large and expanding global industry (O'Connor et al., 2009), whale-watching activities are a considerable source of anthropogenic disturbance for coastal whale populations.

The impact of whale-watching on cetaceans, at both an individual and population level, is most commonly assessed through behavioural observation (Senigaglia et al., 2016), with two common questions.

1. How does cetacean behaviour differ in the presence and absence of vessels?

2. How do variations in encounter characteristics, such as the number and speed of vessels, influence behavioural responses?

Whale-watching impact studies primarily seek to address question 1, observing behaviour in the presence and absence of vessels. This may be achieved using a combination of land-based monitoring, enabling the collection of true control data (Stamation et al., 2010), and observations from the whale-watching vessels themselves (Fiori et al., 2019). Observers may record surface behaviours and measure the position of focal animals at every surfacing, enabling characterisation of horizontal movement patterns (Christiansen et al., 2013a; Currie et al., 2021). Broadly, most studies detect a behavioural response to whale-watching vessels, including changes in surface-active behaviours (Di Clemente et al., 2018; Corkeron, 1995), dive time (Schaffar et al., 2009), swim speed (Currie et al., 2021) and path directness (Corbelli, 2006; Williams et al., 2006b); increased travelling activity (Christiansen et al., 2013b); decreased foraging or resting activity (Morete et al., 2007); and general avoidance responses (Fiori et al., 2019). As with other forms of anthropogenic disturbance, these changes have been interpreted as an anti-predator response (Christiansen et al., 2013b; Wirsing et al., 2008), and may have population-level consequences if exposure is sufficiently high (Bain et al., 2014; Williams et al., 2006a).

Despite consistently observing some form of behavioural response to the presence of whale-watching vessels, the direction, shape and magnitude of this relationship is variable between locations and studies (Senigaglia et al., 2016). For example, during whale-watching encounters, humpback whale dive times increased in New Caledonia, Peru and Australia breeding grounds (Garcia-Cegarra et al., 2019; Schaffar et al., 2009; Stamation et al., 2010), did not change in a Canadian feeding ground (Corbelli, 2006) and decreased in the Hawai'i breeding ground (Currie et al., 2021). Meanwhile, humpback whale resting behaviour was affected by vessel presence in a Brazilian breeding ground (Morete et al., 2007) but not in an Alaskan feeding area (Di Clemente et al., 2018). Therefore, it is difficult to use results from one site-specific assessment to predict impacts in another region. High variability in behaviour and responses also exists within studies, highlighting the importance of large sample sizes (Williams et al., 2006b).

Differences in behavioural responses between and within studies may, in part, be attributed to variable vessel fleet dynamics and individual vessel practices (question 2). Captains manoeuvre vessels around whales to provide high-quality encounters (Chion et al., 2011; Orams, 2000), and vessel practices may vary between locations and captains due to various social and ecological factors, including target species, individual motivation, the behaviour of other vessels and local whale-watching guidelines (Carlson, 2012; Chion et al., 2011). In turn, whale behaviour can be influenced by numerous encounter variables (Corkeron, 1995; Steckenreuter et al., 2011; Williams and Ashe, 2007; Baker and Herman, 1989). For example, humpback whales exhibit lower swim speeds during closer vessel approaches in Hawai'i (Currie et al., 2021); perform longer dives with extending encounter duration in Alaska (Schuler et al., 2019); exhibit higher avoidance rates during direct (as opposed to parallel) vessel approaches in Tonga (Fiori et al., 2019); and increase swim speed in the presence of more vessels in Peru (Villagra et al., 2021). Vessel type may also influence behavioural response (Koroza and Evans, 2022; Sullivan and Torres, 2018). However, responses to different vessel environments are also variable: the number of vessels during an encounter influenced humpback whale behaviour in Canada (Corbelli, 2006), Alaska (Di Clemente et al., 2018; Baker and Herman, 1989) and Peru (Villagra et al., 2021), but not in Hawai'i (Currie et al., 2021). Vessel behaviour is often not included in whale-watching impact assessments: whale-watching activities can be challenging to monitor, with numerous vessels moving independently of each other around a single whale; and variables such as vessel speed and path predictability require high-resolution positional data. Nevertheless, understanding the relationship between whale and vessel behaviour is critical to inform responsible whale-watching guidelines for a profitable, sustainable industry (Currie et al., 2021; Garrod and Fennell, 2004; Schuler et al., 2019; Villagra et al., 2021).

2.2.3 Humpback whales in Skjálfandi Bay

In this study, I aim to characterise the behavioural responses of humpback whales to variable vessel practices (question 2) in Skjálfandi Bay, North Iceland, an important local foraging area within the wider Icelandic feeding ground (Pike et al., 2009; Cecchetti, 2006; Rasmussen, 2009). As humpback whale abundance has increased around Iceland (Víkingsson et al., 2015) and in Skjálfandi Bay (Bertulli et al., 2018), following a moratorium on commercial whaling (Smith and Reeves, 2010), this species has become the primary target for local and national whale-watching activities. From Húsavík, three whale-watching companies and up to 17 vessels operate in Skjálfandi Bay (as of 2022).

Due to the intensity of whale-watching activities and the large number of humpback whales in Skjálfandi Bay (Basran et al., 2019; Klotz et al., 2017; Rasmussen, 2009) and North Iceland generally, there is considerable potential for behavioural disturbance. Tag data have revealed that close vessel passes, inferred by elevated levels of vessel noise, disrupt foraging and increase dive depth (Ovide, 2017); while the general acoustic presence of vessels is associated with a decrease in calling rate (Laute et al., 2022) in Skjálfandi Bay. However, the influence of variable vessel practices on different aspects of whale behaviour has not been determined. As a result, the IceWhale (2017) code of conduct for responsible whale-watching, which is publicly adopted by all companies operating in the Bay, is largely informed by research from other areas and species, including minke whale-watching in southwest Iceland (Christiansen et al., 2013a,b). Given that behavioural disturbance in feeding grounds may compromise individual fitness (Bejder et al., 2019; Christiansen and Lusseau, 2014), understanding the influence of vessel practices on humpback whale behaviour in Skjálfandi Bay is essential to mitigate the potential negative impacts of local whale-watching activities.

2.2.4 Chapter aim

In this study, the behavioural interactions between humpback whales and whale-watching vessels in Skjálfandi Bay were observed using the vessels themselves as opportunistic, cost-effective research platforms (Klotz et al., 2017; Robbins, 2000). Lacking control data, I examined the effect of vessel variables such as number, distance and speed, rather than presence, on whale behaviour. Vessel variables were derived from two positional data sets; while whale surface behaviours were documented in detail and movement patterns were reconstructed using range finder measurements and digital single lens reflex (DSLR) camera images. Within this, I estimated measurement errors and propagated these through the statistical workflow. Whale behaviour was related to encounter variables through a series of generalised additive mixed models (GAMMs). Additionally, given evidence of poor compliance rates to previous whale-watching guidelines in Skjálfandi Bay (Martin, 2012), the influence of speed compliance to the IceWhale code of conduct on whale behaviour was determined.

First, I outline the methods used in terms of whale behavioural observation (including positional measurements), vessel positional data collection and analytical approaches. Following a description of results, I then discuss the underlying causes of observed behavioural changes, implications for responsible management and avenues for future research in Skjálfandi Bay.

2.3 Methods

To investigate the behavioural response of humpback whales to variable vessel practices, three primary data sets were collected – whale behaviour, fine-scale focal vessel positions and coarse-scale AIS vessel positions. Response variables were determined and two sets of GAMMs were applied to relate whale behaviour to broad-scale vessel environment (AIS GAMMs) and focal vessel behaviour (focal vessel GAMMs). In addition, simple statistical tests were used to compare whale behaviour when vessels were compliant or non-compliant with a speed guideline in the IceWhale code of conduct.

The workflow of data collection, processing and analysis is outlined in Figure 2.1. Data processing and analyses were performed using R v4.0 (R Core Team, 2020) and plots were primarily produced using the *ggplot* package (Wickham, 2022).

2.3.1 Behavioural observation

Focal follows of individual humpback whales (Altmann, 1974) were conducted in Skjálfandi Bay during the summers of 2018, 2019 and 2020 (Table 2.1 and Figure 2.2). Observations were conducted from whale-watching vessels belonging to North Sailing (access provided by the University of Iceland) during dedicated whale-watching tours departing from Húsavík. Nine different vessels of varying sizes and characteristics were used (Table 2.2) and observation platforms (decks) were often shared with whale-watching passengers. Tours took place between the hours of 08:30 and 23:00, tour duration was generally 2.5–3.5 hours and all observations were conducted in Beaufort sea states of 4 or less to minimise the probability of missing whale surfacings.

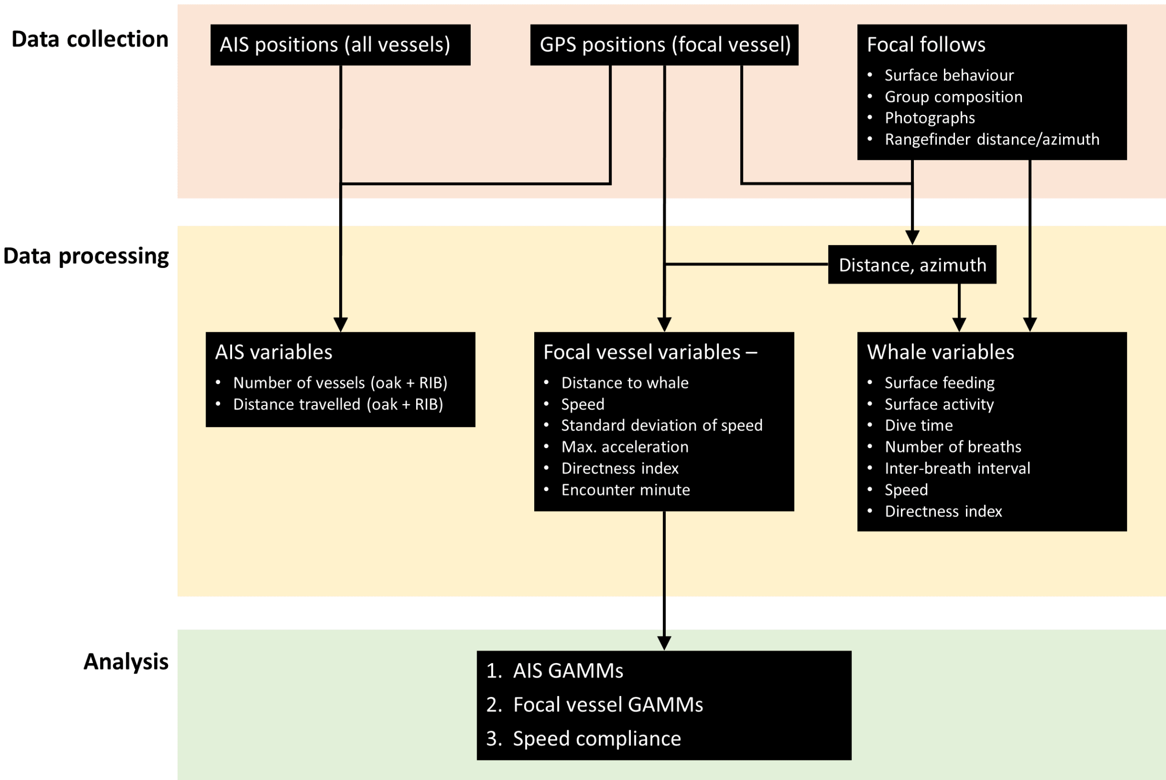

Figure 2.1: Summary of the workflow of data collection, processing and analysis for Chapter 2.

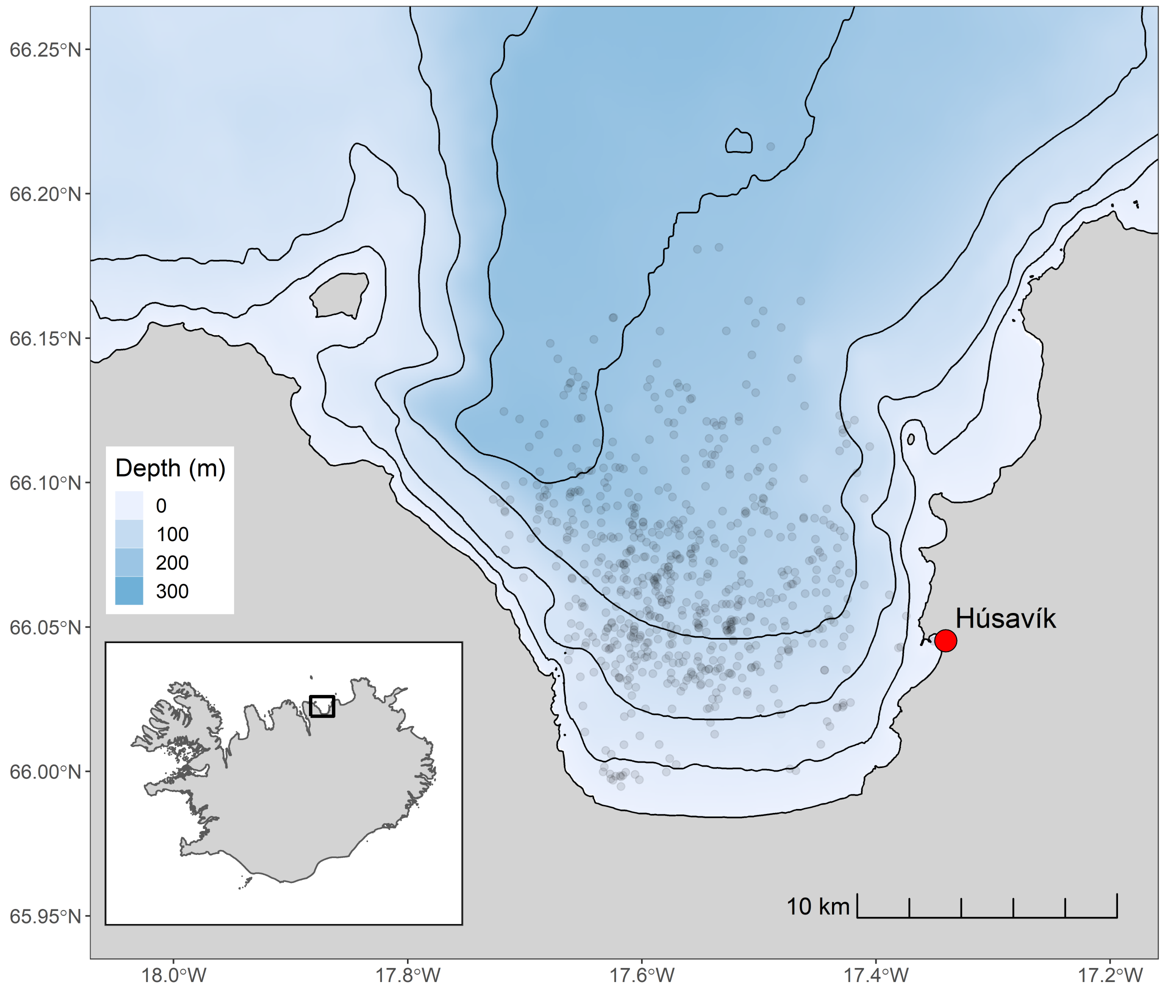

Figure 2.2: The starting position of all focal follows ($n = 727$, grey circles) conducted in Skjálfandi Bay, North Iceland (inset). All whale-watching trips departed from Húsavík.

Table 2.1: Summary of survey effort and follow data per season (year): effort start and end date, amount of survey effort and the number of follows and identifiable whales.

Year	Start date	End date	Survey effort (hours)	Follows	Identifiable whales
2018	08-May	19-Aug	342	467	151
2019	05-Jun	16-Sep	228	187	64
2020	30-Jul	06-Sep	63	73	28

Table 2.2: Specifications for all whale-watching vessels used as opportunistic research platforms. Information available at: https://www.northsailing.is/the-boats/ (Accessed 1 May 2022). Surveys primarily took place on board Náttfari (241 hours), Garðar (148 hours) and Sæborg (128 hours).

Vessel	Capacity (passengers)	Tonnage	Length (m)	kW	Horsepower
Andvari	75	33	18.4	119	
Bjössi Sör	56	30	16.4	221	300
Garðar	146	109	28.0	373	500
Haukur	46	20	15.6	155	210
Hildur	50	35	18.0	105	141
Knörrinn	46	20	15.2	183	249
Náttfari	90	57	23.0	269	366
Opal	60	69	24.0	120	
Sæborg	70	36	18.8		300

Whale encounters

Humpback whales were the primary target for whale-watching tours and the target animal was selected by the captain and guide (pers. obs.). During each tour, we scanned for visual cues of humpback whale presence: tall, bushy blow, black body, small dorsal fin and raised tail flukes (Carwardine, 2019). Upon locating a whale, the vessel generally approached the animal, leading to an encounter of variable duration. If multiple whales were present in the bay, the captain generally targeted animals that were closer to the vessel, more surface-active (e.g., breaching, surface feeding), easier to watch (e.g., shorter dives, more breaths per surfacing interval), in larger groups and/or surrounded by fewer vessels. From personal observation, encounter duration was dependent on various factors, including focal whale behaviour, the number of whales in the area, weather conditions and time constraints. All whale-watching vessels were in regular, cooperative radio contact with each other to increase the probability of observing present, 'viewable' and 'interesting' animals.

Focal follows

Here, a focal follow is defined as the observation (and accurate time recording) of at least two consecutive surfacings of a single animal. Each follow was conducted by two trained observers. To allow real-time collection of numerous parameters, voice recorders (one per observer) were used to store information during observation effort. One voice recording was produced per observer per trip, running continuously for the duration of the trip, and these were manually transcribed. To facilitate efficient transcription, photographs were taken whenever pertinent information was recorded to provide a time stamp. Automated transcription software (primarily Google Speech-to-Text API[1]) was considered and trialled, but was ineffective due to loud, variable background noise.

Observation height

During each trip, focal follows were often conducted from multiple platforms (vessel decks), of varying height, in response to the distribution of whale-watching passengers on the vessel. Therefore, observers recorded their specific platform location for each surfacing and observer heights were subsequently determined.

Observation height can influence whale visibility and is essential for determination of whale position (Gordon, 2001; see Appendix A). Therefore, the height of each platform (in centimetres) was determined by photogrammetric measurements at Húsavík harbour. Photographs were taken from land of a person standing (or sitting) on each vessel platform, holding an object of known length (60 cm stick), using a DSLR camera. From this, platform height was calculated in two stages. First, the actual length of an image pixel (P_{cm}) was calculated as:

$$P_{cm} = \frac{O_{cm}}{O_{px}}$$

Where O_{px} is object height in pixels and O_{cm} is true object height in cm. Second, platform height (H) was calculated as:

$$H = Tot_{px} * P_{cm} - obs_{cm}$$

Where Tot_{px} is the total height between the water surface and the observer eyeline in pixels, and obs_{cm} is observer height (to eyeline) in cm. The heights of 140 platform positions were determined in this way, ranging from 1.45 to 7.91 m, although >99% of observations were made from platforms higher than 3 m.

1. Google Speech-to-Text API: https://cloud.google.com/speech-to-text

Figure 2.3: Examples of ventral fluke images of four different humpback whales used for photo-identification.

Selecting an animal

Within a trip, individual whales were distinguished from each other with recognisable features: scarring patterns on the back; shape of the dorsal fin; and black-and-white patterning of the ventral side of the tail flukes. Photographs of these features were taken with a DSLR camera (Canon 77D or 70D, 100–400 mm lens). These images were subsequently used for photo-identification and compared to a catalogue of animals that had previously been observed in the study (e.g., Figure 2.3). Photo-identification was performed using standard and accepted methods, which have been exhaustively validated (Franklin et al., 2020; Katona et al., 1979; Stevick et al., 2001). Only images with adequate sharpness, contrast and feature visibility were used (Calambokidis et al., 2001). If the animal had not been previously observed, it was added to the catalogue as a new record.

Ideally, recognisable animals should be selected randomly for focal follows, irrespective of behaviour and distinctiveness of markings. However, to investigate behavioural responses to whale-watching activities, whales actively targeted by the vessel (observation platform) were preferentially followed. This is likely to bias the data set as animals targeted by whale-watching encounters often perform more 'interesting' or 'viewable' behaviour (e.g., breaching, surface feeding, curious behaviour, shorter dives). Therefore, this data set should not be interpreted as baseline behaviour at a population level, even in the absence of close vessels. The reason that each animal was selected for an encounter was not documented, but behaviour was recorded during approaches when possible.

At large distances, the target whale for whale-watching was unclear. In this case, the nearest visible whale was selected for focal observation. If a group of two or more animals was encountered, which is not uncommon for humpback whales (Wray et al., 2021), the first animal observed with identifiable features was selected. If the vessel subsequently targeted a different animal, the focal follow was terminated and a new follow of the target animal commenced. Follows were also terminated if a surfacing was missed by the observers or if it was not possible to distinguish between multiple animals in a group.

Field data collection

During each follow, to relate whale behaviour to vessel practices, four key pieces of information were collected.

Observer (vessel) position was recorded every second using a smart device with inbuilt GPS, both to determine vessel movement patterns and calculate whale position. For the first two weeks in 2018, position was only recorded every 5 seconds and positions were only used for subsequent calculation if they were recorded within 2 seconds of the surfacing. In 2018 and 2019, a Samsung Galaxy Tablet was used with PocketGIS[2] software. However, the software frequently crashed during data collection, resulting in lost GPS data. Therefore, in 2020, a Samsung mobile phone was used with the GPS Logger Pro[3] application, which was far more reliable in the field.

Whale surface behaviour can provide direct information on activity state and possible energy acquisition expenditure. For example, surface feeding lunges indicate feeding activity, whereas behaviours such as breaching are clear signs of non-feeding behaviour and high energy expenditure (Segre et al., 2020). Surface behaviours were categorised according to an ethogram adapted from previous studies (Appendix B). This includes 'blow', a visible breath. In addition, group size was recorded. Here, a group is defined as two or more animals surfacing within approximately 15 seconds and 50 metres of each other.

Identification images of the target animal were taken each surfacing. If the target animal surfaced as part of a group, photographs were taken of all animals in the group and subsequently matched to the photo-identification catalogue.

Whale positional information was recorded each surfacing to characterise movement patterns, which are crucial metrics of behaviour, reflecting bio-energetic balance, activity state, avoidance responses (Braithwaite et al., 2015; Christiansen et al., 2013b) and possibly welfare. Latitude and longitude positions were derived from measurements of horizontal distance and azimuth between the observer and the target animal, based on a combination of DSLR images (following Kinzey and Gerrodette, 2003)

2. PocketGIS software: http://pocket.co.uk/
3. GPS Logger Pro Android application: https://apkgk.com/com.peterhohsy.gpsloggerpro

and laser range finder readings; see Appendix A for full details. To account for uncertainty in positional data, I modified the approach of Christiansen et al. (2013a), using calibration and follow data to estimate realistic errors, which were very small for DSLR-derived azimuths and distances, as well as range finder distances, but errors were larger than expected for range finder azimuths (Appendix A).

2.3.2 Vessel positional data

To relate whale behaviour to whale-watching vessel practices and the broader vessel 'environment', two vessel positional data sets were collected. First, AIS positions for every whale-watching vessel in Skjálfandi Bay were provided by the Icelandic Coastguard[4], at a temporal resolution of 2–4 minutes and within the coordinate range 65.95–66.27° N, 17.25–17.82° W. In the original data set, vessel identities were anonymous and whale-watching vessels were not distinguished from other vessel types. To recover vessel identities, I matched AIS positions to incidental photographs of whale-watching vessels at sea and GPS observer tracks. After filtering out stationary vessels in Húsavík harbour, this data set provided information on the number of vessels and their coarse movement patterns in the vicinity of a whale surfacing (e.g., Figure 2.4).

Second, GPS observer positions were recorded for the duration of each whale-watching trip, enabling the calculation of various movement metrics, such as vessel speed and directness index, in a time period prior to each whale surfacing. For these purposes, the time resolution of the GPS data set was reduced from 1 second to 5 seconds (e.g., Figure 2.4) to minimise the influence of inherent GPS positional errors and the movement of observers around the vessel on movement metrics at very low vessel speeds.

2.3.3 Statistical analysis overview

To determine behavioural responses to variable vessel practices, three separate analyses were performed, using whale focal follows and vessel movement data.

1. AIS GAMMs to relate whale behaviour to the coarse-scale presence and movement of whale-watching vessels
2. Focal vessel GAMMs to relate whale behaviour to the fine-scale movement of the focal vessel (observation platform) and encounter progress
3. Simple statistical tests (Chi-squared tests and ANOVAs) to compare whale behaviour when vessels were compliant or non-compliant with IceWhale speed guidelines

4. Landhelgigæsla Islands [Icelandic Coastguard]: https://www.lhg.is/

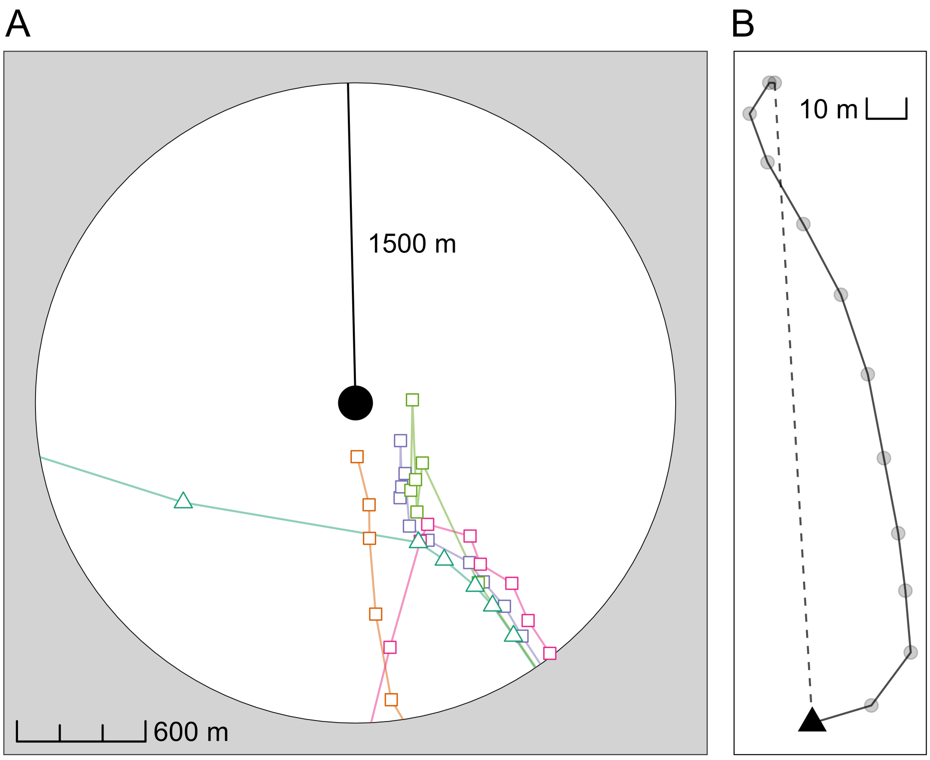

Figure 2.4: Examples of AIS and GPS vessel positions related to a single whale surfacing. A) AIS vessel positions within the preceding 30 minutes and 1,500 metres of an example whale surfacing (black circle; the time-distance frame over which AIS variables were calculated). Each colour represents the track of a different whale-watching vessel. Squares denote oak boats and triangles denote RIBs. B) Example of a GPS track preceding a whale surfacing. The black triangle denotes the vessel position at the time of a whale surfacing, and grey circles denote vessel positions at 5-second intervals for the preceding 60 seconds (the time frame over which focal vessel variables were calculated). The straight-line distance over the 60-second period is denoted by the dashed line.

Response variables

Each analysis involved the same set of response variables. The unit of response was the surfacing (or surfacing interval), rather than the follow or encounter, because vessel movement varies extensively within a single encounter (e.g., Figure 2.5), as part of a complex series of whale and vessel behaviours. The surfacing, rather than the entire follow, also allows inclusion of a greater proportion of the behavioural data set since positional measurements are often incomplete across an entire follow. This unit prevents an assessment of changes in broader activity state (e.g., foraging, travelling), although this would have been challenging (Christiansen et al., 2013a), particularly given the heterogeneous surface behaviours and movements of humpback whales. Because the unit of response was the surfacing, a duration threshold was not applied to follow data and variables that would have been influenced by duration, such as respiration rate, were not derived.

Seven response variables were constructed, adapted from previous studies (Christiansen et al., 2013a; Di Clemente et al., 2018; Currie et al., 2021) and selected based on their potential linkages to individual welfare, bio-energetics and fitness (Christiansen and Lusseau, 2015; Clegg and Butterworth, 2017; New et al., 2014).

1. **Surface-active behaviour (SAB):** the presence or absence of the following surface behaviours for each surfacing i: breach, tail lob, tail/pectoral fin slap/wave, head lunge (Appendix B).
2. **Surface feeding event (SFE):** the presence of full or partial surface feeding lunges for each surfacing i (Appendix B).
3. **Dive time:** the elapsed time between a clear dive (surfacing i) and subsequent re-surfacing $i+1$, in seconds. Here, a clear dive is defined by two criteria: a clear arch or fluke at the first surfacing of a dive; and an inter-breath interval of at least 30 seconds between the two surfacings.
4. **Breaths per surfacing interval:** the number of breaths across a surfacing interval. A surfacing interval is defined as the sequence of breaths between two clear dives, with the inter-breath interval between two consecutive breaths no greater than 30 seconds. Respiration rate was also considered as a metric but not chosen due to the large number of surfacing intervals with very few breaths (1–2).
5. **Mean inter-breath interval (IBI) per surfacing interval:** the mean time difference between consecutive breaths in a surfacing interval, in seconds.
6. **Swim speed:** horizontal speed between a pair of surfacings (i and $i+1$), in km/h, calculated as the distance between the surfacings divided by the time between them. To facilitate comparison with other studies, I refer to horizontal speed as swim speed.
7. **Directness index (DI)** was calculated following Christiansen et al. (2013a); Williams et al. (2006b). DI reflects the linearity of movement of each surfacing in the track and, for surfacing i was calculated by dividing the straight-line distance (L) between surfacings $i-1$ and $i+1$ by the sum of distances between $i-1$ and i, (l_1) and i and $i+1$ (l_2; Figure 2.6A). DI values equal to 1 indicate a straight path, and DI values equal to 0 indicate a path that returns to its starting point.

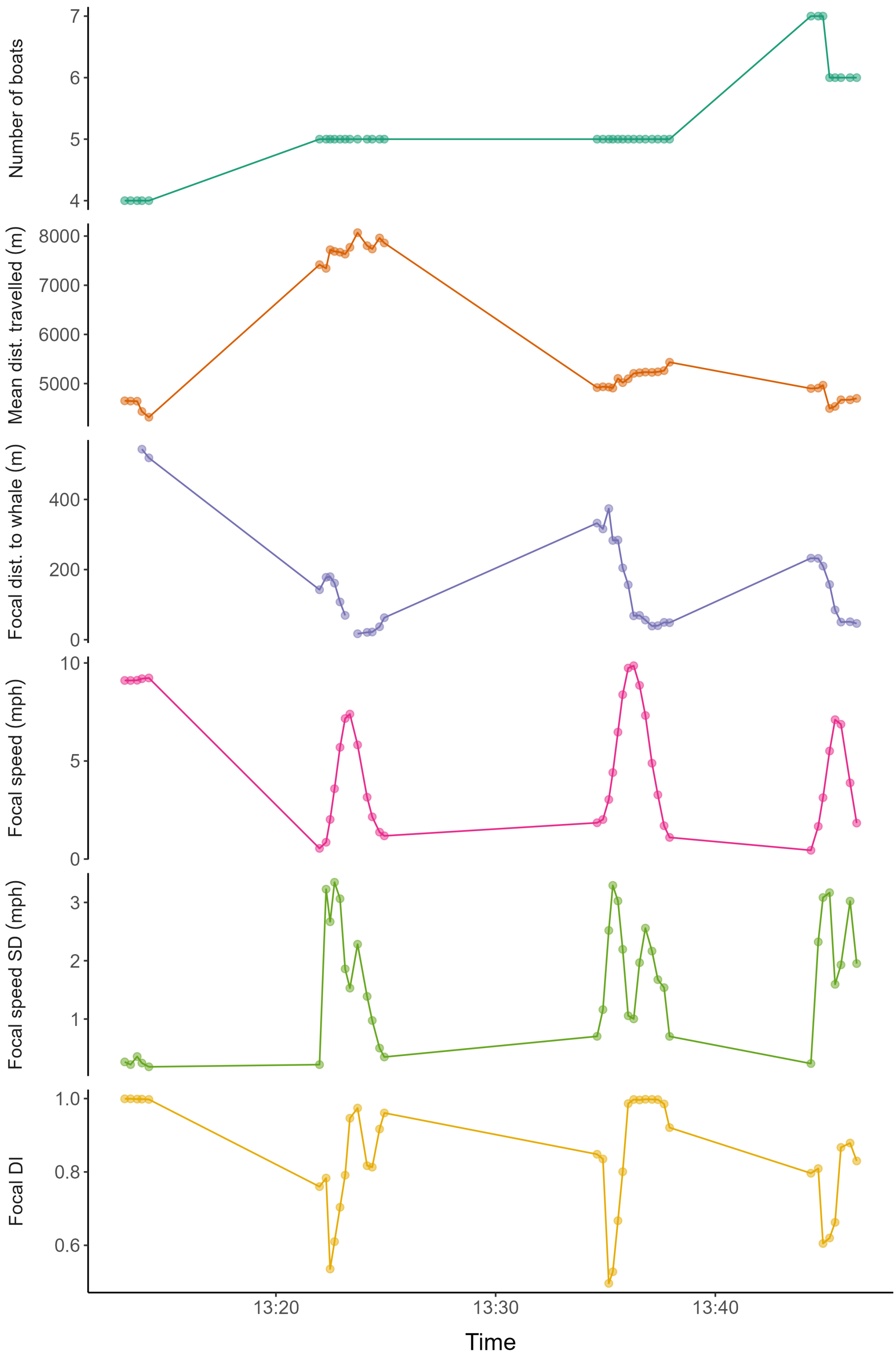

Figure 2.5: Variation in vessel behaviour within a single focal follow (#649, 31 August 2019). Number of boats and mean distance travelled refer to AIS-derived variables (within the preceding 30 minutes and 1,500 m of each surfacing). Distance to whale, focal speed, standard deviation of speed (speed SD) and DI (directness index) refer to the behaviour of the focal vessel (within 60 seconds preceding each surfacing). Each point corresponds to a whale surfacing. Note: the focal vessel values in this example would not be included in focal vessel GAMMs as other vessels are present.

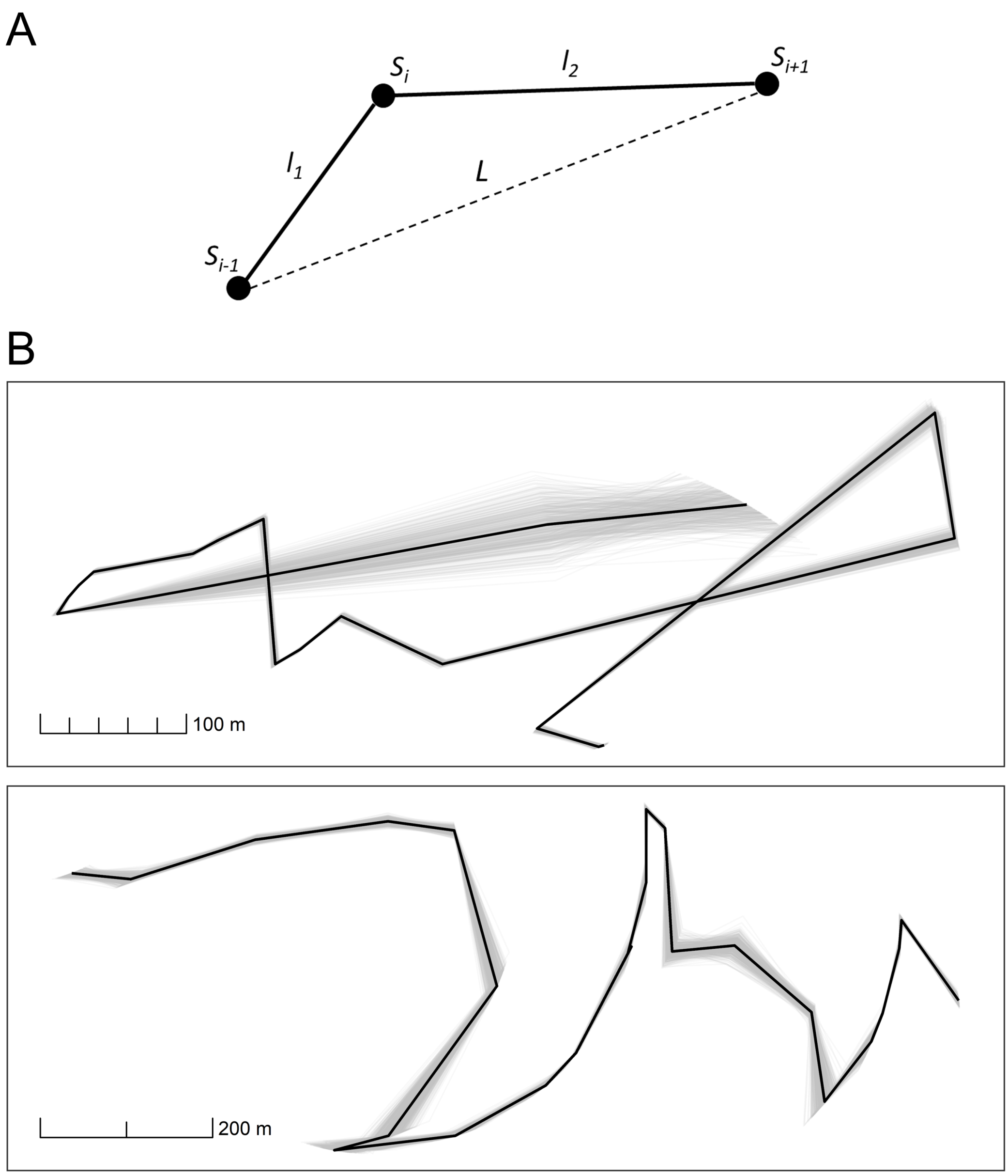

Figure 2.6: Example surface movement tracks derived from humpback whale focal follows. A) Example of a humpback whale movement track with three consecutive surfacings: S_{i-1}, S_i, S_{i+1}. The horizontal distances between each consecutive pair of surfacings are denoted by l_1 and l_2, and the net distance travelled between S_{i-1} and S_{i+1} is denoted by L. Directness index (DI) for S_i is calculated by $DI = \frac{L}{l_1+l_2}$. B) Bootstrap replicates for two separate movement tracks. The track from the original measured distance and azimuth values is denoted by the thick black line, and each bootstrap replicate ($n = 500$) is denoted by a thin grey line.

As SABs and SFEs are arguably visible at greater distances than other surfacing types, all tests or models of these response variables were filtered to distances <1000 m. To reduce the impact of measurement errors on whale movement metrics, swim speed and DI values were only calculated using positions derived from range finder azimuth readings if the measured distance was less than 120 m. For subsequent analyses, explanatory variables correspond to surfacing i for SAB, SFE, dive time, swim speed and DI, or mean values across a surfacing interval for number of breaths and mean IBI, unless stated otherwise.

Propagating measurement errors

Swim speed and DI were calculated from positional data derived from horizontal distance and azimuth measurements. To determine the impact of associated measurement errors on the outcomes of statistical tests and models for swim speed and DI, errors were propagated using a bootstrap resampling approach, with 500 replicates. For each replicate, distance and azimuth readings were randomly selected from a normal distribution of values, whose mean corresponds to the measured value and whose standard deviation is the derived standard error (SE) value. These values were then used to calculate positions (e.g., Figure 2.6B), swim speed and DI values to which the statistical test or model was then applied. The distribution of bootstrapped model/test outputs (effect sizes and p-values) was then visually inspected to determine the robustness of these model results to measurement errors. Model replicates were run using the *foreach* package for parallel processing on five cores (Daniel et al., 2022), reducing maximum total run time to <2 hours. To limit the complexity of this approach, errors were not propagated for distance as an explanatory variable or observer GPS positions, owing to small measurement errors.

2.3.4 Generalised additive mixed models

GAMMs were used to comprehensively assess the response of whale behaviour to variable vessel practices, implemented through the *mgcv* package (Wood, 2004, 2017). GAMMs allow for non-normal response variables and the testing of non-linear relationships, as well as random effects to reflect underlying variation. Boosted regression trees were also considered, which additionally allow implicit interactions between explanatory variables, but there is currently no R package to readily include random effects.

Two comprehensive GAMMs were fit for each whale response variable: AIS GAMMs related whale behaviour to the coarse-scale vessel environment through AIS-derived variables; and focal vessel GAMMs related whale behaviour to fine-scale vessel movement patterns through GPS-derived variables. All models were fitted using shrinkage cubic splines via the `bs="cs"` term (Wood and Augustin, 2002) with default smoothing values (10 knots), unless stated below. Smoothing parameters were estimated using restricted maximum likelihood (REML; Marra and Wood 2011), yielding more stable model fitting, with less under-smoothing, than alternatives such as generalised cross validation (Wood, 2011). GAMMs for each variable used different links and error structures – surface activity and feeding used a binomial family with a log link; dive time, IBI and swim speed used a Gaussian family with a log link; and number of breaths used a quasi-Poisson family with a log link. DI was arcsin-transformed prior to fitting Gaussian GAMMs with an identity link. Explanatory variables were transformed prior to model fitting to achieve normality, using square-root, cube-root, log and arcsin transformations. Model fit was evaluated through visual inspection of residual plots and diagnostic information produced using the `gam.check` function of mgcv.

A number of additional contextual variables were included as predictors in GAMMs for some or all response variables to account for other drivers of whale behaviour (Table 2.3). Factor variables were included as parametric terms, and numeric variables included as continuous smooths. Group type was categorised as *lone* (one animal) or *group* (more than one animal); sea state was categorised as *calm* (Beaufort sea state (BFS)$\leq$2) or *choppy* (BFS>2); and SFE and SAB were used as explanatory variables and categorised as present or absent across the set of surfacings for which the response variable was calculated (or across the entire follow for the response variable of dive time). In addition, the random effect of follow identity (ID) was included in all GAMMs to account for the high underlying heterogeneity across the data set, and for inter-individual differences in behaviour.

Following Currie et al. (2021), individual variable plots (labelled A) and the predictions of the response variable for individual predictors (labelled B) are presented for each term of the final GAMM. Absolute predictions of a response variable were produced using the `predict` function from the *stats* package (R Core Team, 2020), and responses to a single explanatory variable were predicted by fixing all other explanatory variables to their mean (numeric) or median (categorical) value. By including a dummy variable for follow ID in each GAMM, this random effect could be excluded from predictions.

Table 2.3: Explanatory variables used in generalised additive mixed models (GAMMs): AIS-derived vessel variables, GPS-derived focal vessel variables (including minutes since encounter start) and contextual variables (that were included in both AIS and focal vessel GAMMs). For each variable, the following information is provided: variable type (as included in the GAMM), unit (if applicable), values (for factor variables) and the response variables for which each predictor was used. IBI denotes inter-breath interval. For the response variable dive time, binary values of SAB and SFE reflect the presence of these activities in the entire follow; otherwise, values reflect presence within the set of surfacings from which the response variable was calculated. For the response variables of number of breaths and mean IBI per surfacing sequence, the value of distance to whale is the mean value from all surfacings in the sequence.

Variable	**Type**	**Unit**	**Values**	**Response variables**
		AIS variables		
Number of oak boats	integer			all
Number of RIBs	integer			all
Mean distance travelled by oak boats	continuous	metres		all
Mean distance travelled by RIBs	continuous	metres		all
		Focal vessel variables		
Distance to whale	continuous	metres		all
Vessel speed	continuous	mph		all
Vessel speed SD	continuous	mph		all
Vessel directness index (DI)	continuous			all
Minutes since encounter start	continuous	minutes		all
		Contextual variables		
Group size	factor		lone, group	all
Julian day	integer	all		
Year	factor		2018, 2019, 2020	all
Sea state	factor		calm, choppy	all
Surface feeding event (SFE)	factor		yes, no	dive time, breaths, IBI, speed, DI
Surface-active behaviour (SAB)	factor		yes, no	dive time, breaths, IBI, speed, DI
Previous dive time	continuous	seconds		breaths, IBI, speed, DI
IBI to previous surfacing		seconds		DI
IBI to next surfacing		seconds		speed, DI

AIS GAMMs

AIS-derived variables were related to each whale response variable to determine the influence of the broader vessel environment on whale behaviour. Owing to the coarse temporal resolution of AIS data, instantaneous vessel metrics could not be calculated. Therefore, variables must be derived from vessel positions over a period of time, as well as a specific distance radius around the whale. Informed by preliminary analyses (which compared the contribution of predictors calculated over different time frames and distance radii to the final GAMMs), explanatory variables were calculated using positions within the preceding 30 minutes and 1,500 m radius of a whale surfacing. If whale positions were not available due to missing azimuth measurements or missing GPS data, AIS variables were only calculated for a whale surfacing if the measured observer–whale distance was less than 300 m (which was considered valid owing to the coarse nature of AIS-derived variables). Two types of whale-watching vessels operate in Skjálfandi Bay: oak boats (including those used as a research platform) are larger and slower; and rigid inflatable boats (RIBs) are smaller, faster and more manoeuvrable. These two vessel types may alter whale behaviour in different ways and were therefore treated separately in this study. For each vessel type, I calculated i) the number of vessels present and ii) their mean distance travelled, preceding each whale surfacing (four variables, Table 2.3). For the number of oak and RIB boats, the number of knots was set to 3 in the GAMM. Mean distance travelled was calculated to approximate the magnitude of vessel movement in the vicinity of a whale.

Focal vessel GAMMs

GPS track-derived variables were related to each whale response variable to determine the influence of fine-scale variation in focal vessel movement on whale behaviour. To specifically focus on the interactions between the target whale and the focal vessel, AIS data were used to exclude all follows in which other vessels were present within a 1,500 m radius, both during the follow and in the preceding 30 minutes of all surfacings in the follow. The rate of surface activity was excluded as a response variable due to the low number of surfacings observed with SABs ($n = 11$). Explanatory variables describing vessel movement were calculated using GPS positions (at 5-second intervals) within the preceding 60 seconds of a whale surfacing, a time frame that was informed by preliminary analyses (comparing the contribution of predictors calculated over different time frames to the final GAMMs). Five vessel variables were derived: distance to whale (metres), speed (mph), standard deviation of speed (speed SD; mph) and DI (calculated using all 5-second positions). A speed unit of mph was chosen to match the IceWhale (2017) code of conduct. Speed SD and DI were included as proxies of vessel path predictability.

In addition, the number of minutes since the beginning of an encounter was included as an explanatory variable in focal vessel GAMMs since time elapsed during a whale-watching encounter is known to influence behavioural responses to vessel presence (Bejder et al., 1999; Schuler et al., 2019). In this study, an uninterrupted encounter often spanned multiple focal follows (e.g., due to missed surfacings). If there was one follow per animal per whale-watching trip, encounter minute for each surfacing was calculated as the time since the beginning of the focal follow. If there were multiple follows per animal per whale-watching trip, encounter minute for each surfacing in subsequent follows (after the first follow) was calculated as the time since the beginning of the first focal follow if the time gap between the end of the first follow and start of the next follow was less than 20 minutes. If the time gap between two follows of the same animal was greater than 20 minutes, the second follow was considered as a separate encounter and encounter minute for each surfacing was calculated as the time since the start of the second follow. Given the observed dive times of humpback whales in this study (nearly always <15 minutes), this was considered a valid approach. The time gap between two follows of the same whale during the same trip was >20 minutes on only eight occasions.

Temporal autocorrelation

Each surfacing within a focal follow (of a single whale) is not independent of other surfacings in the follow. Therefore, temporal autocorrelation may exist for all response variables. This is a common feature of behavioural data (and ecological data generally) and, if unaccounted for, would violate the independence assumption of GAMMs, potentially leading to an inflated Type 1 error rate (falsely rejecting the null hypothesis) and misleading inferences (Brown et al., 2011; Legendre et al., 2002). To explore temporal autocorrelation in each response variable, autocorrelation function (ACF) and partial ACF (PACF) plots were generated from the residuals of each full GAMM (prior to variable selection). ACF plots show the correlation between the time series and its own lagged values at different time lags, while PACF plots display the partial correlation at a given time lag after accounting for all smaller intervening time lags through regression. Both plots consist of calculated values (ACF or PACF) and a 95% confidence interval – if points lie outside of this envelope, there is significant (in this case temporal) autocorrelation. ACF and PACF plots were generated using `acf()` and `pacf()` functions, respectively, in R. To account for the block structure of the data, i.e., discrete focal follows (the random effect included in each GAMM), I calculated autocorrelation values separately for each follow by padding each follow in R with extra rows of *NA* values to ensure that no inter-follow comparisons were made. For the focal vessel GAMM for mean IBI, autocorrelation was not investigated due to the low number of observations per follow (only 15 out of 64 focal vessel follows had more than one IBI value); for GAMMs of dive time, number of breaths and mean IBI (AIS GAMM only), owing to the generally low number of observations per follow, ACF and PACF values up to lag 5 were plotted; and for the remaining variables, values up to lag 10 were plotted.

If significant autocorrelation was evident, I included an autoregressive structure with lag 1 (AR1) in the GAMM; this is a common approach to account for temporal autocorrelation in ecological data, including whale behaviour (e.g., Schuler et al. 2019; Sprogis et al. 2020a; Visser et al. 2011b). As part of this, I manually tested different values of the parameter Rho, which controls the strength of the autoregressive component; the magnitude of this value (between 0 and 1) indicates the strength of the autocorrelation between consecutive surfacings and the sign indicates its direction (Zuur et al., 2009). For GAMMs with different Rho values, I replotted ACF and PACF values; the model which resulted in the least visually apparent temporal autocorrelation was selected. To fit AR1 models, I used the `bam()` function from *mgcv* (instead of `gam`). For surface activity and surface feeding (i.e., binomial) response variables, fast REML computation (fREML) was used instead of REML and the `discrete=TRUE` command was used to enable incorporation of an AR1 model.

Model selection

Model selection followed Currie et al. (2021); Wood (2022). A fully saturated model was initially fit for each response variable and backwards stepwise selection across both additive and parametric terms was used to choose the most parsimonious model by minimising Akaike's information criterion (AIC) and maximising percentage deviance explained, supplemented by inspection of the REML score. Terms were considered for removal if they were linear terms with a coefficient value near to 0, or smooth terms with estimated degrees of freedom near to 0. If removing the term increased deviance explained (with no appreciable increase in AIC), or decreased AIC (with no appreciable decrease in deviance explained), it was removed; otherwise, it was retained in its original form. The final model fit was inspected by reviewing the residual plots.

Multicollinearity between explanatory variables was tested using Pearson's correlation and, if the magnitude r was greater than 0.7 between any two variables, the term with the least support for inclusion in the final model (low AIC and high deviance explained in the final model) was dropped. The `model.matrix` function was used to one-hot encode all categorical variables (convert different categories into separate variables, each with a value of 0 or 1) to examine collinearity. After model fitting, concurvity (more relevant to GAMs than collinearity) was also inspected using the `concurvity` function of *mgcv*. Concurvity occurs when a smooth term in a model can be approximated by another smooth term in the model, can inflate type 1 errors in statistical tests and hinders interpretation of the final model (Ramsay et al., 2003).

From the final model, the significance of explanatory variables was determined by inspection of p-values. Due to the increased risk of Type 1 errors (i.e., elevated false discovery rate) with multiple statistical testing (13 GAMMs), the threshold for statistical significance (α) was adjusted with a Bonferroni correction such that $\alpha = 0.004$.

2.3.5 Impact of speed compliance

The IceWhale code of conduct is adopted by all companies operating in Skjálfandi Bay, but there is evidence that compliance rates may be low (Martin, 2012). Whilst not collected to assess compliance, the focal follow data set can be used to compare whale behaviour when vessels are compliant or non-compliant with a recommended maximum speed of 6 mph in the approaching zone, 300–50 m from a whale (Table 1.1). Therefore, as an additional analysis, simple statistical tests were used to determine whether the distribution of values for each response variable was significantly different when vessels were above or below 6 mph in the 10 seconds preceding a surfacing. A Chi-squared test was applied to

SABs and SFEs (binary variables); a Kruskal–Wallis test (non-parametric ANOVA) was applied to the number of breaths per surfacing interval (count variables); and a parametric ANOVA was applied to dive time, swim speed and DI (continuous variables). The p-value threshold for significance was $\alpha = 0.007$ after Bonferroni correction (seven tests).

2.3.6 Summary graphic

Due to the large number of GAMMs (13) and other statistical tests (7), key results are highlighted in a summary graphic, denoting significant test results and the form of their relationships. Since the approaches here only elucidate statistical relationships and not mechanistic linkages, significant results in the graphic are colour-coded to denote my confidence that the statistical relationship between whale behaviour (dependent variable) and encounter (independent) variable is due to vessel practices influencing whale behaviour and not some other explanation. For example, a positive relationship between whale swim speed and vessel speed may be driven by vessels responding to increased whale swim speed to maintain a favourable distance for a high-quality encounter. Confidence is defined as follows, based on my knowledge and personal observations of the study system, instead of rigorous analysis:

- Low (red): low confidence that the statistical relationship reflects whales responding to vessels, or medium–high confidence that the relationship reflects vessels responding to whales
- Medium (orange): medium confidence that the statistical relationship reflects whales responding to vessels and medium–high confidence that the relationship does not reflect vessels responding to whales
- High (green): high confidence that the statistical relationship reflects whales responding to vessels and high confidence that the relationship does not reflect vessels responding to whales

2.4 Results

2.4.1 Focal follow data

During 633 hours of observation effort (across three survey seasons), 727 focal follows of humpback whales were conducted (Table 2.1), encompassing 9,562 surfacings. Mean follow duration was ten minutes (range: 9 seconds – 75 minutes) and the mean number of surfacings per follow was 13 (range: 2–161 surfacings). Of these, 300 follows had more than 10 surfacings. Whales were followed throughout Skjálfandi Bay, particularly in the centre of the bay (Figure 2.2). In total, 210 identifiable humpback whales were followed (151 in 2018, 64 in 2019 and 28 in 2020; Table 2.1 and Figure 2.7) and the majority (145) were followed three or fewer times. Surface feeding events (SFEs) and surface-active behaviours (SABs) were observed during 6% ($n = 563$) and 5.2% ($n = 491$) of surfacings, respectively. The mean duration of clear dives (IBI >30 seconds with clear arch or fluke behaviour) was 163 seconds (range = 30–933 seconds). Meanwhile, 691 surfacing intervals (surfacings between two clear dives, maximum inter-breath interval <30 seconds) were observed, with a mean of 2.8 breaths (median = 2, range = 1–20).

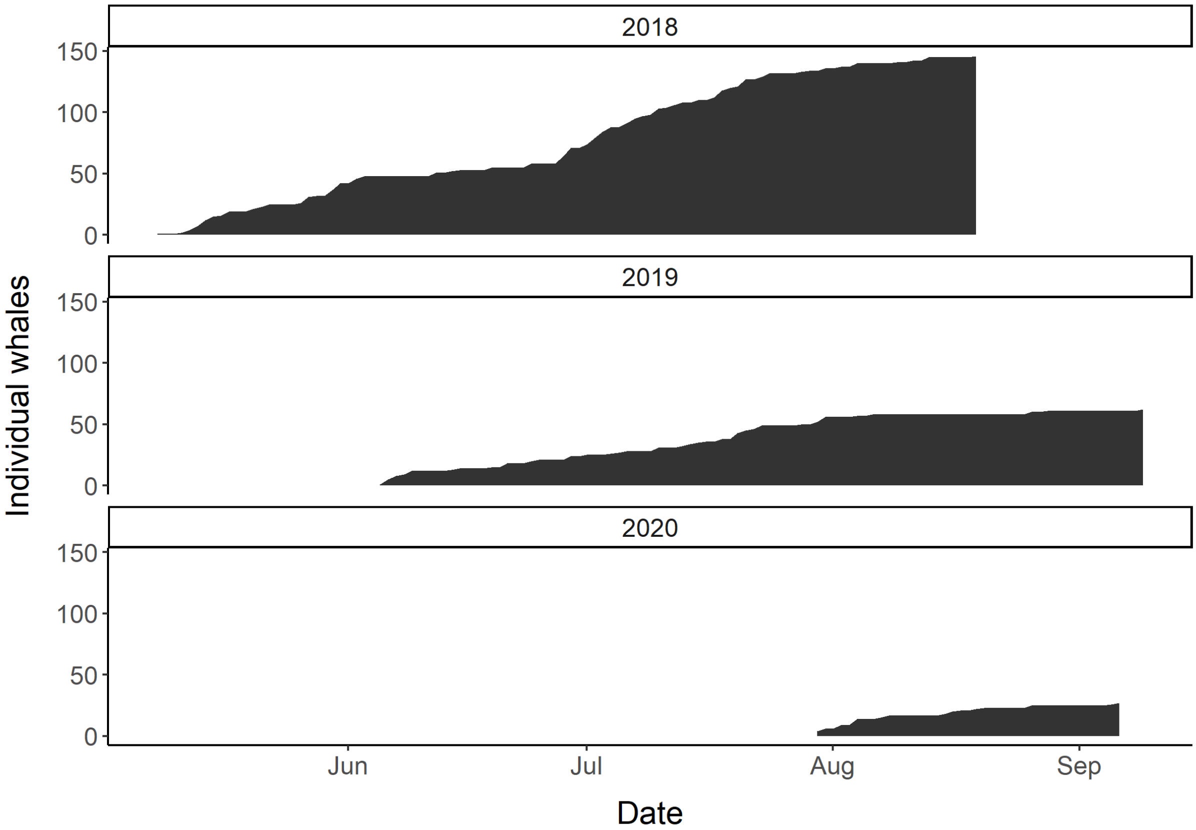

Figure 2.7: Discovery curve of identifiable humpback whales seen for each survey season.

Observer location was successfully recorded via GPS for 8,290 surfacings (87%) and whale position could be calculated using distance and azimuth measurements for 6,372 surfacings (67%). Of these, distance was derived from images for 90% of surfacings and from range finder readings for the remaining 10%; and azimuth was derived from images for 68% of surfacings and from range finder for the remaining 32%. Errors varied considerably between camera and range finder distances and azimuths: camera distance SEs increased with distance, up to ±10 m at 2,000 m true distance; range finder distance errors increased with distance and were up to ±1.6 m at 300 m; a single camera azimuth SE value of ±0.08° was used, not varying with distance; and a single range finder azimuth SE of ±1.42° was used, not varying with distance (Appendix A). After removing all distances greater than 1,000 m and excluding positions derived from range finder azimuths at distances greater than 120 m (owing to large errors), 4,771 surfacings were used to calculate movement metrics. Mean swim speed was 4.7 km/h ($n = 3937$, range = 0.2–18.9 km/h), and mean directness index (DI) was 0.92, with 75% of DI values greater than 0.9 ($n = 3108$, range = 0.07–1).

2.4.2 Generalised additive mixed models

A total of 13 GAMMs were fit: seven AIS GAMMs (Table 2.5) and six focal vessel GAMMs (Table 2.6). There were too few surface-active behaviours (SABs) in the focal vessel data set to fit a GAMM. Full graphic results (partial effects and predictions) are provided in Appendix C. For models of swim speed and directness index (DI), a bootstrap procedure was used to propagate measurement errors and visually determine whether model results were robust to such errors (Appendix D). Follow ID, included as a random effect, was the most significant variable, with the highest effective degrees of freedom (EDF) value, in all fitted GAMMs. For the GAMMs tested (excluding the focal vessel GAMM for mean IBI), all models other than the focal vessel GAMM for swim speed yielded significant temporal autocorrelation, apparent from the ACF/PACF plots (Appendix E). An AR1 autocorrelation structure reduced autocorrelation in each of these models, with the optimal Rho value ranging from -0.4 (focal vessel GAMM for dive time) to 0.3 (AIS GAMM for surface activity; Table 2.4). All graphical relationships were characterised by large confidence intervals (model estimates) or prediction intervals (model predictions), and response–predictor relationships varied in strength and complexity (Appendix C).

Table 2.4: AR1 structure included in AIS and focal vessel GAMMs for each response variable to reduce temporal autocorrelation. Numbers denote the Rho value specified in the AR1 model. Grey shading denotes that an AR1 structure was not considered; no surface activity focal vessel GAMM was fit and only 15 follows (out of 64) from the mean IBI focal vessel data set had more than one surfacing. A blank white cell denotes that there was no significant autocorrelation and an AR1 model was not included.

	AIS GAMM	**Focal vessel GAMM**
Surface activity	0.30	
Surface feeding	0.04	−0.10
Dive time	−0.10	−0.40
Breaths per surfacing interval	0.20	−0.30
Mean IBI per surfacing interval	0.40	
Swim speed	−0.05	
Directness index	0.18	0.10

Table 2.5: Results from AIS generalised additive mixed models (GAMMs). Cells shaded in light grey represent explanatory variables that were not considered for that model, and blank white cells indicate that the term was not included in the final model. Cell values are either parametric coefficient estimates, for factorial predictors, or the degree of smoothing, s(EDF)(effective degrees of freedom), for smooth predictors included in the final model. The (Bonferroni-corrected) significance of a model term is indicated by *, where: ***$p = 0 - 0.00008$; **$p = 0.00008 - 0.0008$; *$p = 0.0008 - 0.004$; .$p = 0.004 - 0.008$. SFE denotes surface feeding events, SAB denotes surface-active behaviours, IBI denotes inter-breath interval and DI denotes directness index.

	SAB	SFE	Dive time	Number of breaths	Mean IBI	Swim speed	DI
Intercept	−4.89***	−5.98***	5.05***	0.75***	2.60***	1.40***	1.27***
Year – 2019		1.19**	−0.20	0.02		0.06	
Year – 2020		−23.53	0.01	0.39**		−0.02	
Julian day		s(3.09)	s(5.59)***	s(4.84)**	s(0.34)	s(2.85)	
Sea state – choppy		−0.70		−0.11			−0.04
Group – alone	1.30*	−0.58		−0.07		0.05	0.02
Surface activity – present				0.87***			−0.05
Surface feeding – present			−0.70***		0.10	−0.16***	−0.06.
Dive time before				s(4.38)***			
IBI before							s(3.59)*
IBI after						s(2.62)***	
Number of oak boats		s(1.56)	s(1.86)*		s(1.36)*		
Number of RIB boats	s(1.07)*						s(0.66)
Oak boat mean dist. travelled	s(2.73)						
RIB boat mean dist. travelled	s(0.97)	s(1.06)					
Follow number (random)	s(119.73)***	s(166.60)***	s(285.46)***	s(54.96)*	s(90.35)***	s(259.98)***	
Rho (AR1)	0.3	0.04	−0.1	0.2	0.4	−0.05	0.18
Deviance explained (%)	50.9	55.1	76.8	72.1	57.1	26.5	16.8
n	6751	6751	1260	691	359	3937	3108

Table 2.6: Results from focal vessel (GPS) generalised additive mixed models (GAMMs). Cells shaded in light grey represent explanatory variables that were not considered for that model, and blank white cells indicate that the term was not included in the final model. Cell values are either parametric coefficient estimates, for factorial predictors, the degree of smoothing, s(EDF)(effective degrees of freedom), for smooth predictors included in the final model. The (Bonferroni-corrected) significance of a model term is indicated by *, where: ***$p = 0 - 0.00008$; **$p = 0.00008 - 0.0008$; *$p = 0.0008 - 0.004$; .$p = 0.004 - 0.008$. SFE denotes surface feeding events, SAB denotes surface-active behaviours, IBI denotes inter-breath interval and DI denotes directness index.

	SFE	**Dive time**	**Number of breaths**	**Mean IBI**	**Swim speed**	**DI**
Intercept	−3.19***	4.78***	0.64***	2.60***	1.56***	1.28***
Year – 2019	0.91	−0.66**	−0.11	−0.19		−0.07
Year – 2020	−4.07	−0.22	0.41	−0.10		−0.06
Julian day		s(3.97)				
Sea state – choppy	−1.12	0.33.				−0.10*
Group—alone	−1.36		−0.10			0.06
Surface activity – present			1.47***	0.13		−0.05
Surface feeding – present		−0.45			−0.16	−0.12
Dive time			s(3.37)***			
IBI before						s(0.65)*
IBI after					s(1.75)***	
Distance		s(0.48)		s(0.99)		
Vessel speed		s(1.54)*		s(1.09).	s(1.06)*	s(0.94)
Vessel speed SD			s(1.50).			
Vessel DI	s(0.75)				s(2.22)	s(2.06).
Encounter minute	s(1.05)*	s(2.22)	s(1.45)		s(2.15).	s(1.24)*
Follow number (random)	s(30.9)***	s(69.8)***	s(31.61)***	s(30.01)***	s(64.33)***	s(12.36)
Rho (AR1)	−0.1	−0.4	−0.3			0.1
Deviance explained (%)	54.4	78.7	80.3	91.6	39.2	14.2
n	859	270	144	64	737	586

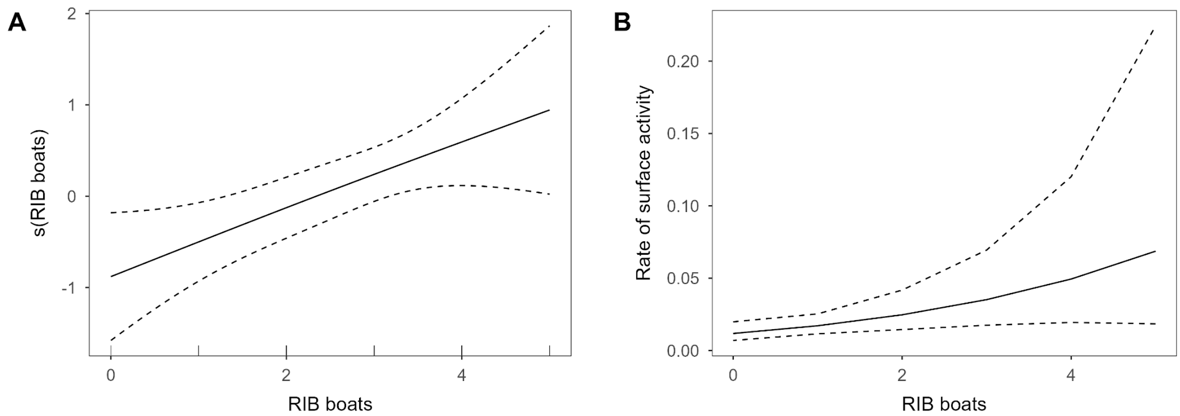

Figure 2.8: Results from the final AIS GAMM for surface-active behaviour (SAB), showing A) parameter estimates for the number of RIBs (within the preceding 30 minutes and 1,500 m of a surfacing) and B) model-predicted rate of SABs based on the number of RIBs. Solid black lines represent average model estimates or predicted values, and dashed lines represent the 95% confidence intervals of model estimates or fitted values. Vertical ticks (rugplot) on A) indicate the locations of observations.

AIS GAMMS

Four AIS-derived variables describing coarse-scale vessel presence and movement were considered: the number and mean distance travelled by oak and RIB boats separately within the preceding 30 minutes and 1,500 m. Deviance explained ranged from 16.8% (DI) to 76.8% (dive time). At least one AIS-derived variable was retained in the final GAMM for five response variables (excluding number of breaths and swim speed); of these, none were significant for surface feeding and DI (Table 2.5). The most frequently significant vessel variable was the number of oak boats (two variables – dive time and mean IBI), while the mean distance travelled variables were not significant for any response variable.

Surface-active behaviours (SAB)

The probability of observing SABs was related to three AIS-derived variables (i.e., retained in the final GAMM; Table 2.5). SABs exhibited a significant positive near-linear relationship with the number of RIBs ($EDF = 1.1,\ \chi^2 = 33.7,\ p = 0.004$; Figure 2.8). Meanwhile, broadly negative relationships with oak boat movement ($EDF = 2.7,\ \chi^2 = 20.4,\ p = 0.06$) and RIB movement ($EDF = 1.0,\ \chi^2 = 8.3,\ p = 0.02$) were not significant. The only significant contextual variable was group type ($p = 0.002$).

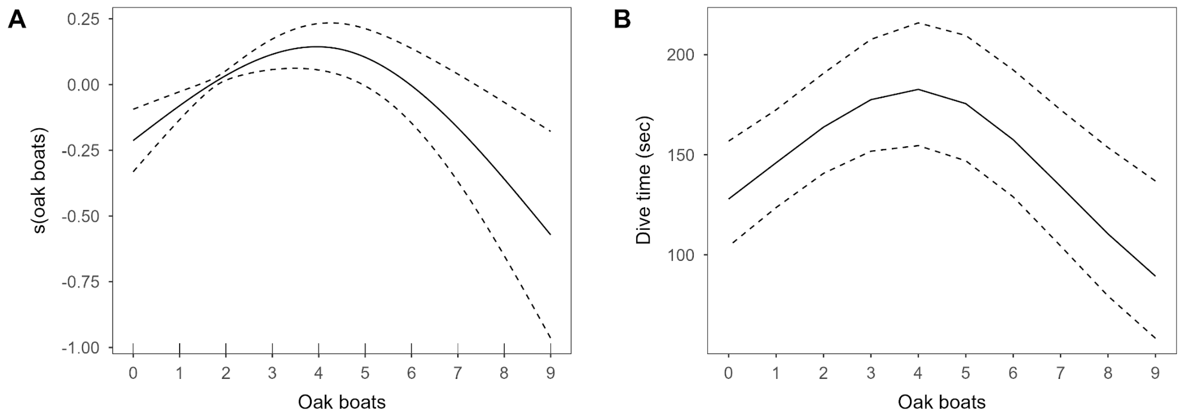

Figure 2.9: Results from the final AIS GAMM for dive time, showing A) parameter estimates for the number of oak boats (within the preceding 30 minutes and 1,500 m of a dive) and B) model-predicted dive time based on the number of oak boats. Solid black lines represent average model estimates or predicted values, and dashed lines represent the 95% confidence intervals of model estimates or fitted values. Vertical ticks (rugplot) on A) indicate the locations of observations.

Surface feeding events (SFE)

The per-surfacing probability of observing a surface feeding event (SFE) was not significantly related to any AIS-derived variable (Table 2.5), although the final model retained the number of oak boats ($EDF = 1.6,\ \chi^2 = 109.9,\ p = 0.01$), showing a broadly negative relationship, and RIB distance ($EDF = 1.1,\ \chi^2 = 237.4,\ p = 0.02$), showing a weakly positive relationship. Meanwhile, SFEs were significantly influenced by year ($2019\ p = 0.0002,\ 2020\ p = 1$).

Dive time

The duration of a clear dive was only significantly related to one AIS-derived variable, the number of oak boats (no other AIS variables retained in the final model; Table 2.5). Dive times increased from zero to four oak boats (to a maximum predicted value of 182 seconds) and decreased rapidly thereafter, with predicted values declining to 110 seconds at eight boats ($EDF = 1.9,\ F = 236.2,\ p = 0.001$; Figure 2.9). Dive times were also influenced non-significantly by year ($2019\ p = 0.01,\ 2020\ p = 0.92$), and significantly by Julian day and the presence of surface feeding in the follow (both $p < 0.00001$) in the final model.

Number of breaths per surfacing interval

No AIS-derived variables were retained in the final GAMM for the number of breaths per surfacing interval (Table 2.5). In contrast, all contextual variables other than the presence of surface feeding were retained in the final model: year ($2019\ p = 0.77,\ 2020\ p = 0.0008$), Julian day ($p = 0.0007$) and the presence of surface activity ($p < 0.00001$) were significant, while group type ($p = 0.19$) and sea state ($p = 0.03$) were not significant.

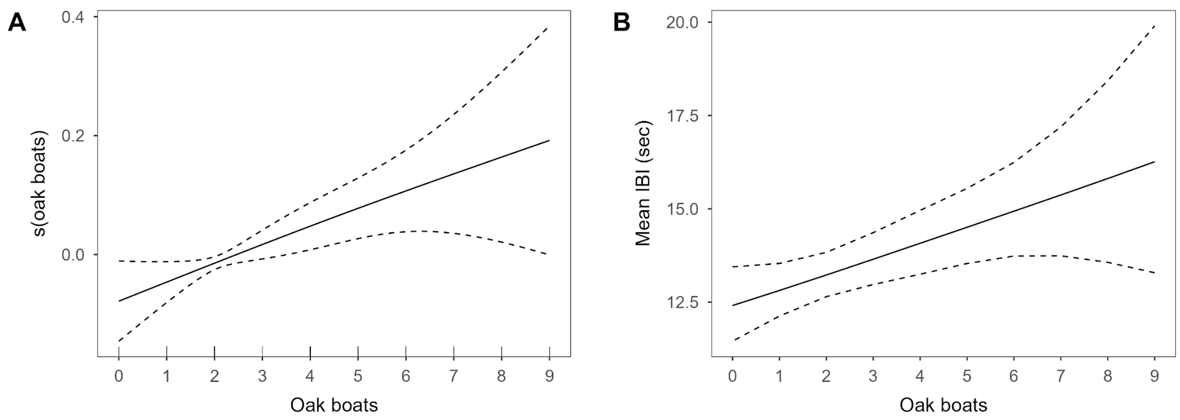

Figure 2.10: Results from the final AIS GAMM for mean inter-breath interval (IBI) per surfacing interval, showing A) parameter estimates for the number of oak boats (within the preceding 30 minutes and 1,500 m of a surfacing interval) and B) model-predicted IBI based on the number of oak boats. Solid black lines represent average model estimates or predicted values, and dashed lines represent the 95% confidence intervals of model estimates or fitted values. Vertical ticks (rugplot) on A) indicate the locations of observations.

Mean IBI per surfacing interval

The final AIS model for mean inter-breath interval (IBI) per surfacing interval only retained one AIS variable (Table 2.5). IBI exhibited a significant linear positive relationship with the number of oak boats ($EDF = 1.4,\ F = 17.1,\ p = 0.001$), with predicted values increasing from 12.8 seconds at one boat to 15.8 seconds at eight boats (Figure 2.10). The non-significant contextual predictors of SFE (presence in the follow; $p = 0.1$) and Julian day ($p = 0.27$) were also retained.

Swim speed

No vessel variables were retained in the final AIS GAMM for swim speed (Table 2.5). In contrast, five contextual variables were retained: SFE at either surfacing and IBI (both $p < 0.00001$) were significant, whereas year ($2019\ p = 0.08,\ 2020\ p = 0.73$), Julian day ($p = 0.02$) and group type ($p = 0.08$) were not significant.

Directness index (DI)

No vessel variable had a significant effect on directness index (DI) in the final AIS GAMM, although one variable was retained, with the number of RIBs showing a very weakly positive relationship ($EDF = 0.7,\ F = 1.9,\ p = 0.11$). This was supported by the measurement error bootstrap, with low EDF values and only 1% of replicates yielding $p < 0.004$ (Appendix D). Five contextual variables were also included: IBI before ($p = 0.003$) was significant, whereas sea state ($p = 0.02$), group type ($p = 0.28$), SAB ($p = 0.08$) and SFE ($p = 0.005$) were not significant.

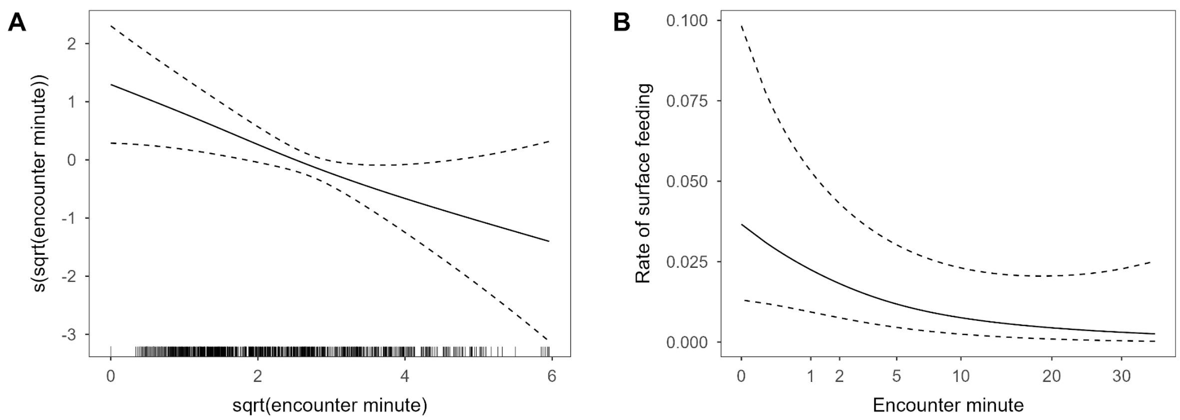

Figure 2.11: Results from the final focal vessel GAMM for surface feeding events (SFEs), showing A) parameter estimates for square-root-transformed encounter minute (defined as minutes since the start of an encounter) and B) model-predicted rate of SFEs based on encounter minute. Solid black lines represent average model estimates or predicted values, and dashed lines represent the 95% confidence intervals of model estimates or fitted values. Vertical ticks (rugplot) on A) indicate the locations of observations.

Focal vessel GAMMs

For follows in which only the focal vessel was present, each response variable (excluding SAB) was related to explanatory variables describing the fine-scale position and movement of the focal vessel (in the preceding 60 seconds), in addition to minutes since the beginning of an encounter. For every response variable other than whale DI, the focal vessel GAMM explained more deviance than its AIS counterpart, ranging from 14% (whale DI) to 91.6% (mean IBI). At least two focal vessel predictors were retained for each response variable and three were retained for dive time, swim speed and DI, although they were generally not significant after Bonferroni correction (Table 2.6). Vessel speed and encounter minute were the most frequently significant vessel variables (two response variables each), and encounter minute was retained in five out of six GAMMs.

Surface feeding event (SFE)

The final model for SFEs retained two vessel variables (Table 2.6): SFEs showed a weakly positive and non-significant relationship with vessel DI ($EDF = 0.8,\ \chi^2 = 2.5,\ p = 0.11$) and a significant negative linear relationship with encounter minute ($EDF = 1.1,\ \chi^2 = 13.2,\ p = 0.004$), with predicted SFE rates declining from 0.022 at 1 minute to 0.003 at 30 minutes (Figure 2.11). The rate of SFEs was also non-significantly influenced by year ($2019\ p = 0.31,\ 2020\ p = 0.63$), sea state ($p = 0.15$) and group type ($p = 0.03$).

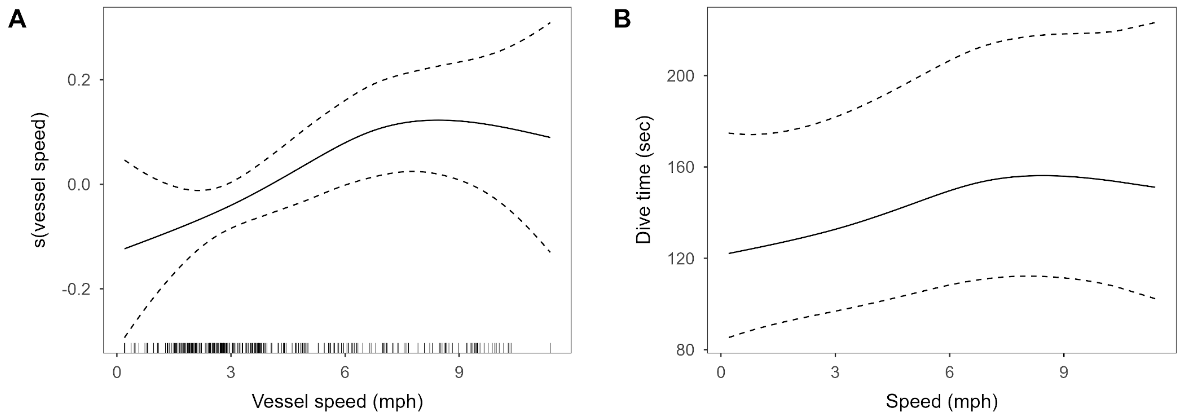

Figure 2.12: Results from the final focal vessel GAMM for dive time, showing A) parameter estimates for mean vessel speed (within the preceding 60 seconds of a dive) and B) model-predicted dive time based on vessel speed. Solid black lines represent average model estimates or predicted values, and dashed lines represent the 95% confidence intervals of model estimates or fitted values. Vertical ticks (rugplot) on A) indicate the locations of observations.

Dive time

Three vessel variables were retained in the final focal vessel GAMM for dive time (Table 2.6). The relationship with vessel speed was weakly positive but significant ($EDF = 1.5,\ F = 5.0,\ p = 0.003$), with higher dive times when vessel speed was greater than 6 mph (Figure 2.12), whereas a weak negative relationship with distance to whale ($EDF = 0.5,\ F = 0.4,\ p = 0.15$) was not significant. Encounter minute was also retained but non-significant ($EDF = 2.2,\ F = 7.0,\ p = 0.05$); dive times peaked at about 10 minutes into an encounter. The contextual variable year was significant ($2019\ p = 0.0005,\ 2020\ p = 0.47$), whereas Julian day ($p = 0.02$), sea state ($p = 0.008$) and SFE presence in the follow ($p = 0.02$) were not significant.

Breaths per surfacing interval

Two vessel variables were included in the final focal vessel GAMM for the number of breaths per surfacing interval and neither was significant (Table 2.6). Vessel speed SD had a weak positive linear and marginally non-significant relationship with breaths ($EDF = 1.5,\ F = 2.6,\ p = 0.005$), whilst values increase when encounters extend beyond 10 minutes ($EDF = 1.4,\ F = 1.3,\ p = 0.14$). Of four contextual variables included, previous dive time and SAB presence in follow (both $p < 0.00001$) were significant and year ($2019\ p = 0.42,\ 2020\ = 0.07$) and group type ($p = 0.43$) were not significant.

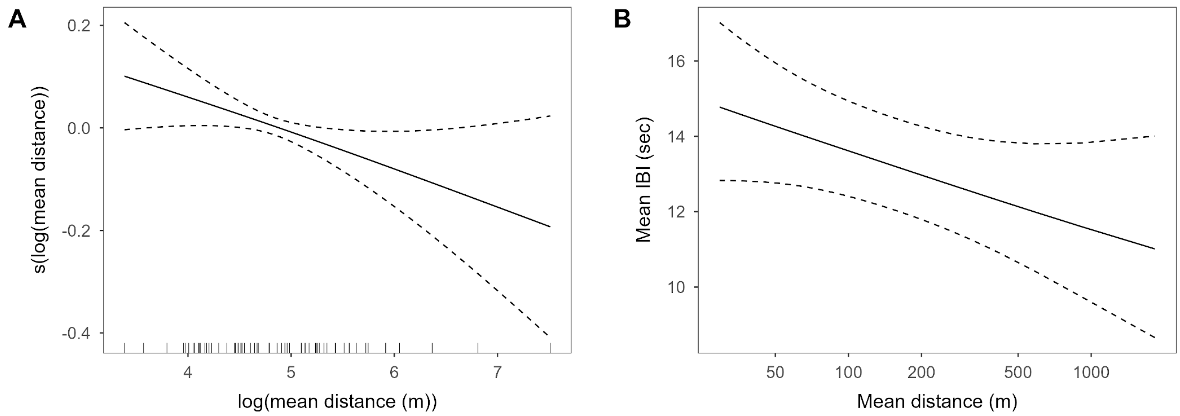

Figure 2.13: Results from the final focal vessel GAMM for mean inter-breath interval (IBI) per surfacing interval, showing A) parameter estimates for log-transformed mean distance to whale (within the preceding 60 seconds) and B) model-predicted IBI based on distance. Solid black lines represent average model estimates or predicted values, and dashed lines represent the 95% confidence intervals of model estimates or fitted values. Vertical ticks (rugplot) on A) indicate the locations of observations.

Mean IBI per surfacing interval

The final focal vessel GAMM for mean IBI per surfacing interval was fit to a small sample size ($n = 64$), such that the number of knots for each smoothing spline was limited to 3. Only two vessel variables were retained, neither significant (Table 2.6). IBI had a negative linear relationship with distance ($EDF = 1.0,\ F = 44.9,\ p = 0.02$; Figure 2.13) and a positive linear relationship with vessel speed ($EDF = 1.1,\ F = 55.8,\ p = 0.01$; Figure 2.14). Meanwhile, year ($2019\ p = 0.10,\ 2020\ p = 0.51$) and SAB presence in the follow ($p = 0.44$) were non-significant contextual predictors.

Swim speed

Three vessel variables were retained in the final focal vessel GAMM for swim speed (Table 2.6), although the significance and strength of relationships varied. Swim speed had a significant positive linear relationship with vessel speed ($EDF = 1.1,\ F = 2.9,\ p = 0.004$), which was supported by a narrow range of EDF values in the measurement error bootstrap and 52% of replicates yielding a significant result ($p < 0.004$; Appendix D). Furthermore, and swim speeds were higher at low vessel DI values ($EDF = 2.2,\ F = 1.8,\ p = 0.08$) and when encounters progressed beyond 20 minutes ($EDF = 2.2,\ F = 6.2,\ p = 0.03$; Appendix C), although the relationships were not significant; for both predictors, 0% of measurement error bootstrap replicates were significant.

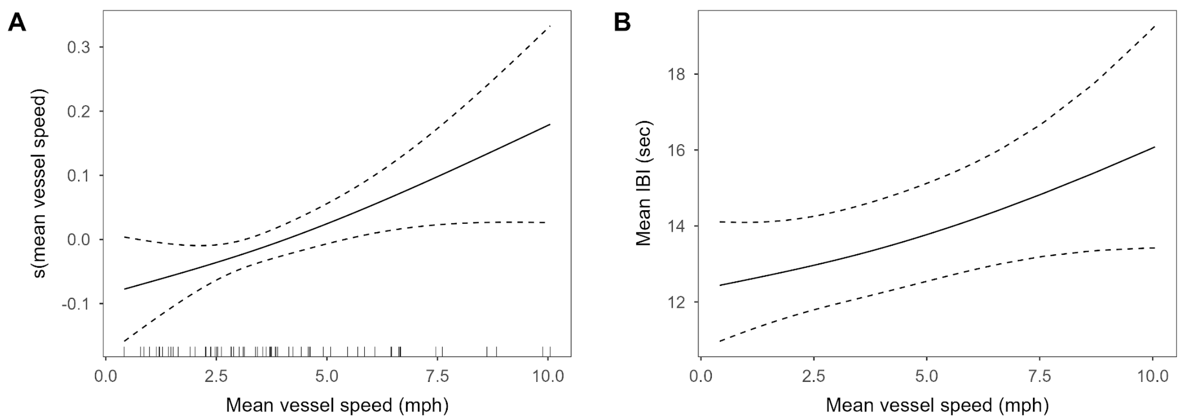

Figure 2.14: Results from the final focal vessel GAMM for mean inter-breath interval (IBI) per surfacing interval, showing A) parameter estimates for mean vessel speed (within the preceding 60 seconds) and B) model-predicted IBI based on vessel speed. Solid black lines represent average model estimates or predicted values, and dashed lines represent the 95% confidence intervals of model estimates or fitted values. Vertical ticks (rugplot) on A) indicate the locations of observations.

Directness index (DI)

Three vessel variables were included in the final focal vessel GAMM for whale DI (Table 2.6). The positive linear relationship between whale DI and encounter minute was significant ($EDF = 1.2,\ F = 1.0,\ p = 0.004$; Figure 2.15), and the measurement error bootstrap showed a narrow range of EDF values, although only 16.4% of replicates were significant ($p < 0.004$; Appendix D). Meanwhile, weak positive relationships with vessel speed ($EDF = 0.9,\ F = 0.3,\ p = 0.06$) and vessel DI ($EDF = 2.1,\ F = 1.1,\ p = 0.005$) were not significant, although 46.8% of bootstrap replicates for vessel DI were significant (despite a wide range of EDF values; Appendix D). In the final GAMM, whale DI was also influenced significantly by sea state ($p = 0.003$) and non-significantly by year ($2019\ p = 0.08,\ 2020\ p = 0.24$), group type ($p = 0.10$), preceding IBI ($p = 0.17$) and the presence of SABs ($p = 0.02$) or SFEs ($p = 0.30$) in any of the three surfacings.

2.4.3 Impact of speed compliance

Within the approaching zone, as defined by the IceWhale code of conduct (300–50 m), 4,711 surfacings were available with GPS data in the preceding 10 seconds. From these, vessel speed was greater than the recommended 6 mph speed limit preceding 23.4% of surfacings. Moreover, whale behaviour differed when vessel speed was above or below this 6 mph threshold (Figure 2.16). The rate of surface feeding was significantly higher with compliance (7.9% of surfacings) than non-compliance (3.6%; $\chi^2 = 7.9,\ p = 0.007$). Meanwhile, swim speed increased significantly from 4.48 km/h with compliance to 5.32 km/h with non-compliance (ANOVA $df = 1,\ F = 15.3,\ p < 0.0001$) and this result was robust to measurement errors (all bootstrap iterations significant, $p < 0.002$). Dive times were also higher when

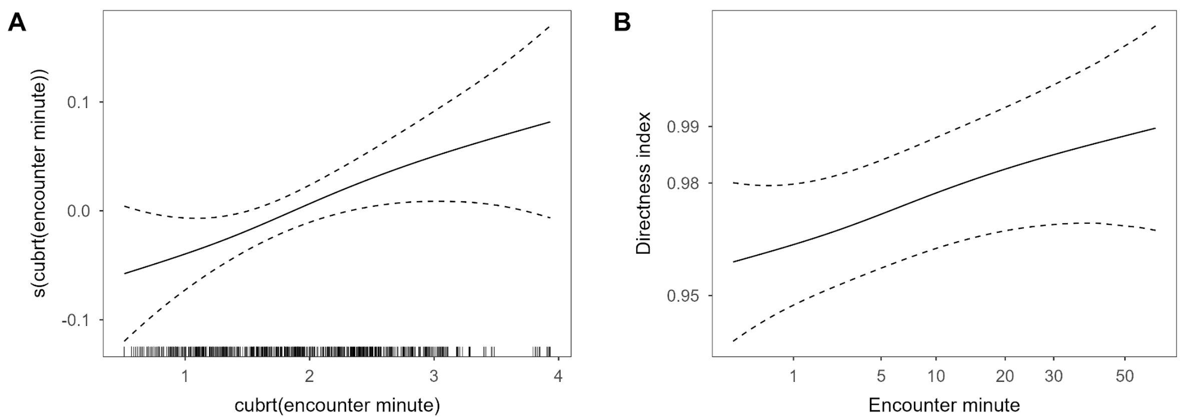

Figure 2.15: Results from the final focal vessel GAMM for arcsin-transformed whale directness index (DI), showing A) parameter estimates for square-root-transformed encounter minute (defined as minutes since the start of an encounter) and B) model-predicted whale DI based on encounter minute. Solid black lines represent average model estimates or predicted values, and dashed lines represent the 95% confidence intervals of model estimates or fitted values. Vertical ticks (rugplot) on A) indicate the locations of observations.

vessels were non-compliant (177 seconds, vs 126 seconds with compliance) but this was marginally non-significant (ANOVA $df = 1,\ F = 6.7,\ p = 0.009$). Meanwhile, the rate of surface activity, number of breaths and mean inter-breath interval per surfacing interval and directness index were not significantly different when the vessel was speed-compliant vs non-compliant.

2.4.4 Summary graphic

Key results from the GAMMs and compliance tests are summarised in Figure 2.17, with a category of approximate confidence that the observed relationships represent vessel encounter variables influencing whale behaviour. Of nine significant results (GAMM partial relationships or compliance tests, based on p-values and Bonferroni-corrected α levels), confidence is categorised as 'high' for three relationships: IBI and the number of oak boats; dive time and vessel speed; and whale DI and encounter minute. Three are categorised as medium confidence: dive time and the number of oak boats; SFE and encounter minute; and the effect of vessel speed compliance on SFE rate. The remaining significant relationships are categorised as low confidence (unlikely to represent vessels influencing whale behaviour): SAB and the number of RIBs; swim speed and vessel speed; and the effect of vessel speed compliance on swim speed.

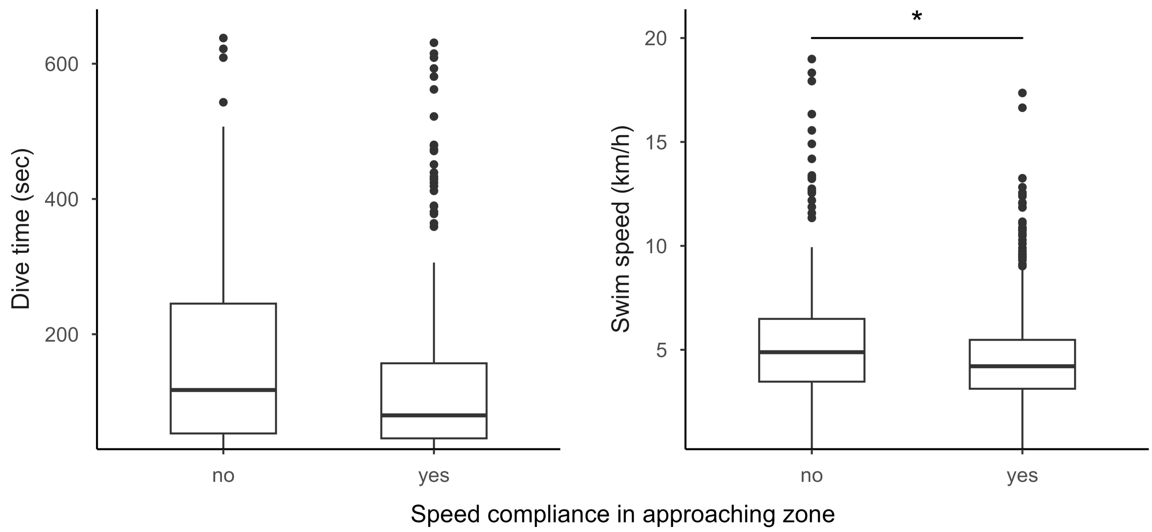

Figure 2.16: Boxplots of dive time and swim speed when the focal vessel was compliant or non-compliant with a 6 mph speed limit in the approaching zone (300–50 m distance from the whale) of the IceWhale code of conduct. Significant ANOVA tests for each variable ($p < 0.007$) are denoted by *.

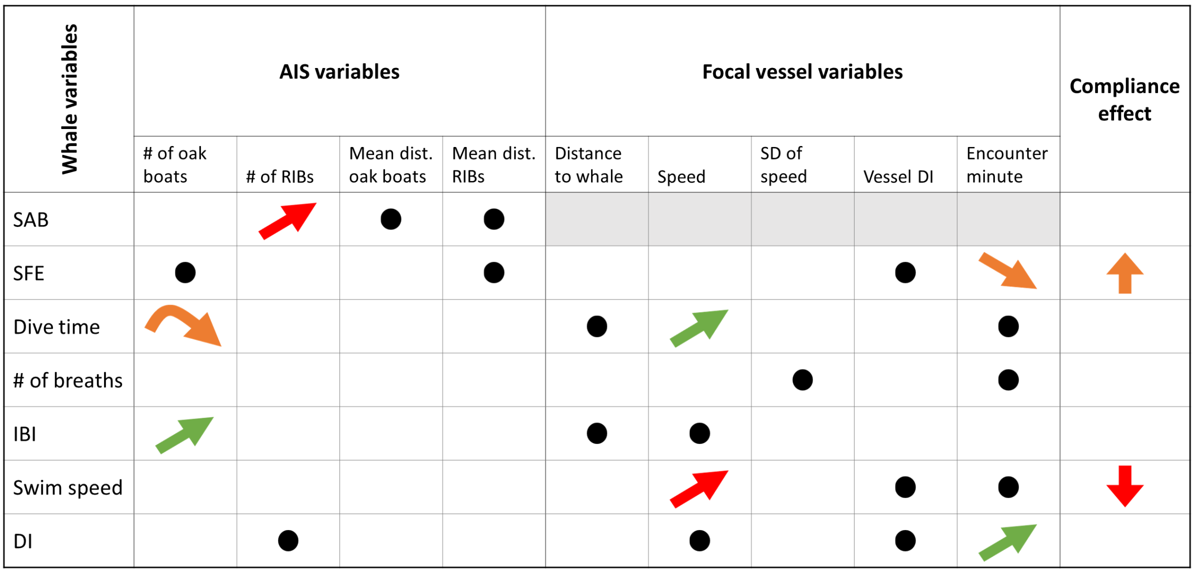

Figure 2.17: Summary of key results from Chapter 2. All significant explanatory variables in the GAMM ($p < 0.004$) and significant compliance effect tests ($p < 0.007$) are denoted by arrows. For GAMMs, the shape of the arrow reflects the shape of the relationship; for compliance effect tests, arrows denote that the response variable is either higher or lower when the vessel is compliant with maximum speed (6 mph) in the approaching zone (300–50 m from the whale) than when the vessel is non-compliant. The colour of the arrow denotes my confidence that the statistical result reflects whales responding to vessels. Red: low confidence (unlikely that the relationship represents vessel behaviour influencing whale behaviour); orange: medium confidence; green: high confidence (likely that the relationship represents vessel behaviour influencing whale behaviour).

2.5 Discussion

Behavioural observations from whale-watching vessels were combined with coarse- and fine-scale vessel positional data to model the influence of vessel practices on seven whale behavioural variables in Skjálfandi Bay, under a data-driven approach. Series of GAMMs and statistical tests highlight the complexity of whale–vessel interactions and the challenges of characterising whale-watching disturbance, particularly given the large number of dependent and explanatory variables included in the analyses. This approach was chosen to provide a comprehensive overview of whale–vessel behavioural interactions and, despite its complexity, several key results emerged. The number of vessels in an area was related to longer inter-breath intervals and changes in dive time. Focal vessel speed was associated with changes in respiration patterns, whereas the progress of an encounter (time since an encounter started) was related to decreasing surface feeding. Finally, non-compliance to speed recommendations in a national code of conduct was associated with changes in surface feeding and vertical movement. Observed relationships indicate potential avoidance strategies, feeding disruption and more cautious surface behaviours in response to a more extreme vessel environment and prolonged exposure. Whilst the underlying drivers and consequences of observed behavioural responses are currently unknown, these findings have implications for future targeted research and whale-watching policies in Iceland.

To maximise available information about vessel movement patterns, two positional data sets were used: AIS data, available for every whale-watching vessel, and GPS tracks, recorded during observation effort. Whilst the potential of AIS data for tracking whale-watching encounters is now being considered (Almunia et al., 2021; Nesdoly et al., 2022), building on the successes of monitoring global fishing activities (Taconet et al., 2019), this information has not been previously used to examine behavioural whale-watching impacts. In this study, AIS positions were used to both cautiously determine which focal follows were conducted in the absence of other vessels (including 30 minutes prior to the follow), and to estimate the number of RIB and oak boats and the magnitude of their respective movement. However, their utility for constructing explanatory variables was limited by coarse temporal resolution: positions were provided for each vessel every 2–4 minutes, and AIS-derived explanatory variables in the final GAMMs corresponded to the 30 minutes preceding a surfacing and a 1,500 m radius around its position. These metrics may not be relevant to short-term behavioural variability and, perhaps for this reason, AIS variables were less frequently included in the final GAMMs than focal vessel variables (although the number of significant relationships was comparable; Tables 2.5 and 2.6), and consistent differences between oak and RIB boats were not apparent. As such, it was challenging to disentangle the contribution of oak boats and RIBs to whale behaviour. Furthermore, using these AIS data to filter focal follows for the presence of other vessels may be imprecise, in particular because whale surfacing positions preceding a focal follow were unknown. Nevertheless, given that up to 10 vessels were observed around a single whale at one time (pers. obs.), which is not unusual in other whale-watching areas (Di Clemente et al., 2018; Kessler and Harcourt, 2013; Lachmuth et al., 2011; Schaffar et al., 2010), it was important to account for the broader vessel environment beyond our observation platform. In contrast, high-resolution GPS tracks (1-second resolution, downscaled to 5 seconds) enabled the characterisation of fine-scale variation in several facets of focal vessel behaviour without compromising the quality of whale behavioural data collection. These GPS tracks represent a more information-rich data set, involving less labour-intensive collection, than the visual estimates or manual theodolite measurements that are typically used to characterise vessel behaviour during encounters (Di Clemente et al., 2018; Currie et al., 2021; Fiori et al., 2019). In the future, establishing a system of recording fine-scale GPS tracks on all whale-watching vessels in an area would also circumvent the reliance on coarse-scale AIS positions.

Numerous whale–vessel relationships were retained in the final set of GAMMs from a large data set (727 follows, 210 whales); however, only seven relationships (including those with low confidence that vessel behaviour influenced whale behaviour) were significant (Figure 2.17). In part, this could be due to my approach in this study, using a large number of models (13) and other statistical tests (7). Bonferroni corrections were applied to α p-value levels to ensure that the false discovery rate (Type I error) was not elevated due to multiple statistical testing, but this is recognised as a conservative correction method, such that some genuine relationships are discarded as non-significant (Pike, 2011). Alternatively, there could be a genuinely low number of significant relationships between whale behaviour and vessel behaviour, across the variables tested. Given the frequent occurrence of non-significant results and low effect sizes in whale-watching impact assessments (Senigaglia et al., 2016), this is plausible. For the explanatory variables that were included in the final GAMMs but are not significant, the shape of their relationship with the response variable should not be interpreted as a key result, but the number of predictors retained in the model after variable selection could provide an indication of the complexity of the relationship between vessel and whale behaviour; 8/13 GAMMs had at least two vessel variables in the final model.

The significant relationships in this study should be interpreted with caution. Large confidence intervals in model outputs and even larger prediction intervals (Appendix C) suggest that relationships were not consistent across encounters. In fact, follow ID (random factor) was the most important explanatory variable in every GAMM (Tables 2.5 and 2.6), reflecting high inter- and intra-individual variability in behaviour, possibly related to personality (Díaz López, 2020). This ‘noise’ is congruent with other studies of whale-watching impacts (Di Clemente et al., 2018; Currie et al., 2021; Schuler et al., 2019) and cetacean behaviour generally (Baker and Herman, 1989; Williams et al., 2006b), underlining the need to frame whale-watching impacts in terms of average responses, not individual encounters. Furthermore, results require careful interpretation due to the inherent bi-directional nature of behavioural interactions between vessels and whales (Chion et al., 2013). GAMM outputs are statistical associations and do not specify the direction of a cause–effect relationship (Hastie and Tibshirani, 1990). The influence of whale behaviour on vessel practices is rarely included in analyses (although the use of behavioural transition probabilities does somewhat account for this; Di Clemente et al., 2018), but may exert effects over variable distances. For example, breaches can be seen from several kilometres away and are likely to attract a greater number of vessels, facilitated by regular radio communication between vessels in Skjálfandi Bay (pers. obs.). At close distances, vessels are likely to respond to immediate changes in whale behaviour to maintain optimal distance (Morete et al., 2007), which possibly explains the positive linear relationship between swim speed and vessel speed (and the generally positive relationship between whale DI and vessel DI) in this study (Appendix C). For this reason, in absence of supporting evidence from the literature, I used my personal observations and understanding of the study system to assign a category to each relationship, corresponding to my confidence that the statistical relationship represents vessel behaviour influencing whale behaviour (Figure 2.17). Since this is not an objective approach, this categorisation is imprecise and should be treated accordingly. Finally, results should not be used to infer the impact of whale-watching vessel presence on whale behaviour owing to a lack of control data (in the absence of vessels). Similarly, it is somewhat challenging to place these results in the context of the wider literature, because whale-watching impact studies primarily use vessel presence as the explanatory variable (Senigaglia et al., 2016). Nevertheless, the observed responses of dive times, respiration patterns, horizontal movement and surface feeding to variable vessel practices highlight the sensitivity of humpback whale behaviour to whale-watching vessels and may be used to guide future management in Iceland.

2.5.1 Surface activities

Two forms of surface activities were considered: surface-active behaviours (SABs) and surface feeding events (SFEs). Previous studies have linked whale-watching vessel presence to both an increase in humpback whale SABs (Baker and Herman, 1989; Di Clemente et al., 2018; Corbelli, 2006), interpreted as a negative response (Baker and Herman, 1989), and a decrease in surface feeding (Di Clemente et al., 2018), indicating disruption of foraging activity (Hazen et al., 2009). In this study, it was challenging to relate surface activities to vessel practices owing to their relative rarity: SFEs were observed in 6% of surfacings and SABs in 5.2%, with too few SABs to determine the impact of focal vessel behaviour (11 surfacings). From the remaining GAMMs, there were few clear relationships with vessel practices. A positive relationship between the rate of SABs and the number of RIBs in the area (congruent with observations in Alaska; Di Clemente et al., 2018) is difficult to interpret because vessels are attracted to surface-active whales (pers. obs.), resulting in a low confidence rating (Figure 2.17). Meanwhile, the per-surfacing rate of SFEs was significantly lower when vessels were not speed-compliant with the IceWhale code of conduct in the approaching zone (6 mph within 50–300 m) and as encounters extended (Figure 2.11). Confidence for both relationships was categorised as 'medium': vessels may travel more slowly when watching surface-feeding whales, although this was unclear from personal observations; while vessels may be attracted to whales engaged in surface feeding (a form of surface activity) and, if surface-feeding bouts are of limited duration, this could explain the association with encounter minute. Nevertheless, these relationships could represent disruption of foraging activity and both adherence to the code of conduct and limiting the duration of encounters could reduce potential disturbance (Christiansen et al., 2013a; Corbelli, 2006). In addition, whilst not significant, the inclusion of three other vessel variables in the final SFE GAMMs (number of oak boats, RIB mean distance travelled, focal vessel distance to whale) suggests that other aspects of the fine-scale and broader vessel environment could influence surface feeding.

2.5.2 Respiration patterns

Respiration patterns are often used as a proxy for energy expenditure (Christiansen et al., 2014; Currie et al., 2021) and have been assessed in several forms, including respiration rate per focal follow (Christiansen et al., 2014), respiration rate per surfacing interval (Currie et al., 2021; Stamation et al., 2010) and inter-breath interval (IBI; Corbelli, 2006). In this study, unbiased respiration rates could not be used as a response variable, owing to the short duration of many focal follows (mean = 6 minutes) and the high proportion of surfacing intervals (52%) with only one or two breaths. Therefore, the number of breaths and mean IBI (for intervals with >1 breath) per surfacing interval were used as two separate indices.

From both metrics combined, there was only one significant relationship across GAMMs and compliance tests, with IBI showing a positive linear association with the number of oak boats, which was assigned a high confidence rating as there was no clear mechanism for behaviours related to higher IBI attracting more vessels (Figures 2.10 and 2.17). Both variables retained two vessel predictors in the focal vessel GAMMs, but none were significant, although it is worth noting linear increases in IBI with increasing vessel speed (Figure 2.14) and proximity (Figure 2.13). Changes in IBI may, in part, reflect changes in swim speed (Dolphin, 1987; Whitehead et al., 1982). For example, increasing humpback whale IBI in the presence of more whale-watching vessels (Corbelli, 2006) and decreasing respiration rate during encounters (Stamation et al., 2010) were interpreted as the by-product of increasing swim speed as a horizontal avoidance response. Conversely, increases in swim speed for minke whales in Iceland (Christiansen et al., 2014) and humpback whales in Hawai'i (Currie et al., 2021) during whale-watching encounters were related to higher respiration rates, possible reflecting increased metabolic demand, although the relationship between respiration rate and energy expenditure in free-ranging cetaceans is unclear (Folkow and Blix, 1992; Isojunno et al., 2018). In this study, there was a negative relationship between swim speed and IBI (including dives), as shown by the results of AIS and focal vessel GAMMs (Appendix C). However, the number of vessels (oak boat or RIB) was not retained in the final model for swim speed, suggesting that the increase in IBI in response to the presence of more oak boats was not accompanied by clear changes in swim speed. Since whale-watching vessels may be perceived as a stressor by cetaceans (Frid and Dill, 2002; Wirsing et al., 2008), I hypothesise that increasing IBI during surfacing intervals, interpreted as more cautious surface behaviours, may reflect heightened perceived risk in a more extreme vessel environment.

2.5.3 Dive time

Dive time is a common response variable in whale-watching impact studies (Senigaglia et al., 2016) and humpback whales may perform dives as part of diverse activity states, including foraging and travelling (Friedlaender et al., 2013). I found that dive times were significantly related to two vessel variables (number of oak boats and focal vessel speed; Figure 2.17), with two additional non-significant variables retained in the focal vessel GAMM (distance to whale and encounter minute; Appendix C). From personal observations, I assigned a high confidence rating that the positive linear relationship between dive time and vessel speed could represent vessel behaviour influencing whale behaviour. It is plausible that vessels travel more quickly to approach whales that surfaced from a long dive preceding the focal dive (unit of response), and may therefore be more likely to perform another long dive afterwards, but this is a form of temporal autocorrelation and the AR1 structure should account for this. Other studies have generally found that dive times increase in the presence of whale-watching vessels (Schaffar et al., 2009; Senigaglia et al., 2016; Stamation et al., 2010), often interpreted as vertical avoidance. In contrast, the relationship with the number of oak boats was more complex, with dive times increasing from one to four vessels (to a maximum of 182 seconds), before declining steeply to eight vessels (110 seconds). The decrease in dive time at high vessel numbers could reflect a greater number of vessels approaching whales with lower dive times as they could be easier to observe (pers. obs.). Alternatively, the inverted U-shaped relationship could reflect the use of vertical avoidance (or another form of avoidance associated with extended dives) with increasing vessel numbers under lower traffic conditions, which is then not practised under high vessel traffic conditions. This is comparable with changes in killer whale evasive tactics with an increasing number of whale-watching vessels (Williams and Ashe, 2007) and the response may be driven by the declining effectiveness of the avoidance strategy (Williams and Ashe, 2007), or an increase in energy expenditure, which may explain shorter humpback whale dives during whale-watching encounters in Hawai'i (Currie et al., 2021). On balance, I assigned this relationship a medium confidence rating because it could be partially explained by whale behaviour influencing vessel behaviour under high-traffic conditions. Dive time was not influenced by the number of RIBs, perhaps because this vessel type was present in lower numbers around a whale (Appendix C). Moreover, the absence of a dive time response does not preclude other changes such as dive depth (Ovide, 2017).

2.5.4 Horizontal movement

In whale-watching impact assessments, movement patterns are often decomposed into swim speed and directness index (DI), as potential indicators of horizontal avoidance. Speed relates to energy expenditure (Christiansen et al., 2014), whereas DI can be considered a proxy for path predictability (Williams and Ashe, 2007). In this study, it was essential to account for the influence of measurement errors on these movement metrics, particularly given that distance errors increased with true distance and range finder azimuth measurements could be very large (Appendix A), possibly due to the nature of a moving vessel platform.

Generally, mysticete swim speed is higher in the presence of vessels in both feeding grounds (Christiansen et al., 2014; Corbelli, 2006; Schuler et al., 2019) and breeding grounds (Currie et al., 2021; Morete et al., 2007), which may be related to increasing travelling activity (Di Clemente et al., 2018). Results for DI are more variable, but changes are often significant (Amrein et al., 2020; Corbelli, 2006; Currie et al., 2021; Schaffar et al., 2009). Both increasing and decreasing DI in response to vessel presence have been interpreted as horizontal avoidance, either as a switch to travelling behaviour (higher DI) or by performing more sinuous movements (lower DI) with lower predictability (Christiansen et al., 2013b; Currie et al., 2021; Garcia-Cegarra et al., 2019). My results support limited evidence that both variables are also related to vessel behaviour, with swim speed GAMMs retaining three vessel variables in total and whale DI GAMMs retaining four (Tables 2.5 and 2.6). However, it is important to consider the effect of propagating measurement errors (e.g., the significant relationship between DI and vessel speed from the focal vessel GAMM was not robust to measurement errors). Furthermore, only two whale–vessel relationships were significant in the final horizontal movement GAMMs and, of these, a low confidence rating was assigned to the positive association between swim speed and vessel speed. This relationship could represent whales increasing swim speed in response to faster vessels (which could be considered a more extreme vessel environment), but in this study system it is likely (at least in part) to reflect vessels adjusting their speed according to whale swim speed in order to maintain suitable distance and provide high-quality encounters. For the same reason, higher swim speeds when vessels are non-compliant with the speed recommendations could be an artifact of vessels adjusting their speed to that of the target whale.

In contrast, the negative relationship between whale DI and encounter minute was assigned a high confidence rating; high DI values could correspond to generally more 'viewable' surface behaviours, which might lead to longer encounters, but this is unlikely to generate the observed statistical relationship as follow ID is included as a random variable. Encounter minute is rarely included as an explanatory variable, although Bejder et al. (1999) observed increased avoidance responses by Hector's dolphins after 70 minutes into an encounter, while Schuler et al. (2019) found a positive relationship between humpback whale respiration rate and time spent with vessels in Alaska, with no corresponding changes in swim speed. A decrease in DI with encounter progression could reflect horizontal avoidance with prolonged exposure to vessels, possibly as an anti-predator response (Christiansen et al., 2013a; Frid and Dill, 2002; Wirsing et al., 2008). Given that whales can be continuously exposed to whale-watching vessels for hours at a time, I encourage the consideration of encounter duration as a standard component of impact assessments.

2.5.5 Contextual non-vessel variables

Beyond vessel-related variables, several contextual variables were retained in both AIS and focal vessel GAMMs, accounting for the influence of the broader non-vessel environment on whale behaviour (Currie et al., 2021). For example, whales swimming alone generally exhibited fewer surface-active behaviours, while swim speeds were generally higher when animals were surface feeding. The potential impact of the COVID-19 pandemic is particularly interesting: whale-watching activity was considerably lower in 2020 than previous years in Skjálfandi Bay, altering the underwater soundscape and possibly contributing to elevated acoustic detections of humpback whales (Laute et al., 2022). I detected differences between survey years, with more breaths per surfacing interval and higher dive times in 2020 than 2018/19. However, data collection started later in the season in 2020, and GAMM results show that an increase in Julian day (later in the season) is related to higher dive times and more breaths per surfacing interval, limiting meaningful comparison between years.

Other contextual variables, beyond those included in this chapter, could influence whale behaviour and alter whale-watching impacts. For example, humpback whales (and other cetaceans) exhibit diel variation in activity states, including foraging (Friedlaender et al., 2009a, 2013) and vocalisations (Narganes Homfeldt et al., 2022). In Skjálfandi Bay, during summer, elevated acoustic detections at night (possibly representing elevated calling rates) could be driven by diurnal increases in vessel traffic (Laute et al., 2022). Furthermore, time of day can modulate cetacean behavioural responses to whale-watching activities (Lundquist et al., 2012): in Alaska, humpback whale foraging activity was positively associated with vessel presence and number of vessels in the morning, but not in the afternoon or evening (Di Clemente et al., 2018). In this chapter, observations were included across a range of times (08:30–23:00); therefore, time of day could be integrated into future analyses. Furthermore, demographic status, including sex and age class, can influence behavioural state and responses to disturbance, with possible ramifications for long-term fitness effects. Sex differences have been observed in southern resident orcas, with more frequent foraging disruption for females (versus males) in the presence of vessels (Holt et al., 2021) and different avoidance tactics (Williams et al., 2006b). Meanwhile, humpback whale groups with calves (compared to non-calf groups) exhibit different responses in: East Australia, with more frequent avoidance responses (Stamation et al., 2010); Peru, with decreases in swim speed possibly driven by the energetic constraints of calves (Garcia-Cegarra et al., 2019); and Hawai'i, with diurnal increases in group depth possibly driven by diel variation in vessel traffic (Pack et al., 2022). Individual demographic information is largely unavailable for Icelandic humpback whales – calves are very rarely sighted in coastal waters and individual life history is generally unknown – but this information could refine our future understanding of long-term whale-watching impacts.

2.5.6 Causes and implications of behavioural responses

It is challenging to determine the underlying causes and long-term consequences of short-term behavioural disturbance. Horizontal and vertical avoidance have previously been attributed to the perception of whale-watching vessels as a predatory threat by cetaceans (Christiansen et al., 2014; Higham et al., 2015; Schaffar et al., 2009), supporting the broader theory that human disturbance constitutes a predation risk stimulus (Beale and Monaghan, 2004; Frid and Dill, 2002). In this study, behavioural relationships suggest that the potential perceived risk may be greater when vessels are more numerous, at higher vessel speeds or during prolonged encounters (with more limited evidence of behavioural responses at closer vessel distances or when vessel movement is less predictable). Beyond physical presence, variability in underwater noise levels arising from different vessel practices may drive variability in whale behaviour. Vessel noise can disrupt cetacean communication and foraging behaviour (Blair et al., 2016; Dunlop, 2016, 2019; Fournet et al., 2018): in Skjálfandi Bay, calling rates are lower in the acoustic presence of vessels (Laute et al., 2022), and increasing dive depth and foraging disruption were related to elevated noise during close vessel passes (Ovide, 2017). Moreover, received noise levels and peak frequencies are likely to vary with speed, proximity (Erbe, 2002), vessel type (Arranz et al., 2021) and the number of vessels. Future research should combine fine-scale positional information with the vessel-specific acoustic signature of different vessel practices and manoeuvres to determine the contribution of vessel underwater noise to whale behaviour.

Short-term behavioural responses to sublethal disturbance may have long-term conservation implications (Christiansen and Lusseau, 2014; New et al., 2014; Nowacek et al., 2016). Under a population consequences of disturbance (PCoD) framework, foraging disruption and increases in energy expenditure may negatively impact individual fitness and vital rates at a population level (Costa, 1993; New et al., 2014, 2015; Pirotta et al., 2018), if disturbance persists (Bain et al., 2014; Lusseau and Bejder, 2007). These linkages have rarely been made for mysticetes, with Christiansen and Lusseau (2015) modelling non-significant impacts of behavioural disturbance on foetal growth in Icelandic minke whales. In this study, changes in dive times, respiration patterns and horizontal movements, in response to variable vessel practices, may reflect changes in foraging activity (Christiansen et al., 2013a) and energy expenditure (Williams and Noren, 2009). Without information regarding activity state (including control data), bio-energetics or cumulative exposure to vessels, it is not possible to determine the long-term impacts of vessel practices at a population level. However, humpback whales sighted in Skjálfandi Bay have also been observed in other whale-watching areas around Iceland (Happywhale, 2022), so cumulative exposure across a summer feeding season may be high (Weinrich and Corbelli, 2009).

The observed variation in behaviour and potentially high exposure may also have welfare consequences. Behaviour is used as a direct measure of welfare (Mellor et al., 2009) and the potential perception of vessels as a predatory threat may induce stress and compromise wellbeing. Whilst welfare is often discussed in a whale-watching context, it has not been explicitly assessed and I encourage the future application of existing frameworks to behavioural disturbance (Clegg et al., 2021; Clegg and Butterworth, 2017).

2.5.7 Future behavioural research

Data collection protocols could be improved in several ways. In 2018 and 2019, GPS data were collected using a Samsung Tablet and PocketGIS software, which regularly crashed, leading to prohibitive gaps in GPS tracks. In 2020, GPS position was recorded with a mobile phone and GPS Logger Pro, which was still accurate and far more reliable. Furthermore, protocols should be designed to avoid the use of the electronic laser range finder for azimuth measurement on moving vessel platforms. Measurement errors were not tested until the majority of data collection had taken place (instead relying on advertised equipment specifications) and dedicated calibration data taken from a stationary (or slow-moving) vessel did not reflect the magnitude of errors during focal follows, in varying sea states and vessel speeds. Future research may prioritise the use of images for azimuth calculation, facilitated by a smaller camera lens. In addition, the reliance on voice recordings and time-consuming manual transcriptions should be reduced to produce more standardised and streamlined field data. With only two observers, this is challenging because diverse information is recorded, including whale ID and confidence, all behavioural observations, range finder readings, missed surfacings and other contextual information. However, with an additional third observer, it may be possible to record information digitally. Deriving angles and distance by measuring image pixel distances and positions is also time-consuming.

Beyond improvements to data collection, the observed behavioural interactions between whales and vessels highlight residual knowledge gaps in Skjálfandi Bay, with consequences for management. First, a large set of control behavioural data should be collected to allow direct comparison of whale behaviour in the presence and absence of whale-watching vessels, and to determine the impact of variable practices relative to an undisturbed baseline (Christiansen et al., 2013b; Schuler et al., 2019). This may be possible from the less accessible western side of Skjálfandi Bay, where focal follows have previously been conducted (Laute et al., 2022), or narrower fjords in Iceland with humpback whale-watching activity, such as neighbouring Eyjafjörður.

Second, interactive effects between explanatory variables were not tested in this study but may exist. For example, Julian day was included as an explanatory variable in five AIS GAMMs, suggesting that whale behaviour varies by time of year, which could lead to seasonal variation in responses to different vessel practices. The interactive effects of vessel behaviour and other contextual variables on whale behaviour have been recognised in previous studies (Di Clemente et al., 2018; Currie et al., 2021; Stamation et al., 2010; Williams et al., 2006b) and could inform guidelines such as speed–distance restrictions or seasonal adjustments to vessel practices, but are rarely interrogated. To explicitly account for interactions, boosted regression trees (BRTs) may be considered as an alternative explanatory framework to GAMMs (Elith et al., 2008), but it is essential to include follow or whale ID as a random effect.

Third, the fine-scale behaviour of multiple vessels should be considered. This study separately assessed the potential impact of the coarse scale presence of multiple vessels and the fine-scale movement of a single vessel. Given that fine-scale models explained more behavioural variability (Tables 2.5 and 2.6), it is likely that important information was lost due to the coarse temporal resolution of AIS data. Recovering this information is important because the majority of whale-watching encounters involve multiple vessels (pers. obs.) and fine-scale movement was only recorded for oak boats and not RIBs, which are smaller, more manoeuvrable and likely to have a different acoustic signature. Collecting fine-scale information from each vessel requires a willingness from all companies to participate.

Fourth, interpreting GAMM results was hindered by a lack of robust *a priori* knowledge on whether statistical relationships were more likely to reflect whales influencing vessel behaviour or vice versa, instead relying on my imprecise categorisation. In reality, both are likely to influence each other, possibly involving feedback loops. In addition to control data, future research may account for this with agent-based models, in which both vessels and whales can be specified as 'agents of change' that interact with each other and the wider environment (Pirotta et al., 2014). For example, agent-based models have been implemented for the Gulf of St Lawrence, Canada, to understand how vessels respond to different whale species and estimate exposure levels (Chion et al., 2013, 2011).

2.6 Conclusion

Non-lethal behavioural disturbance of cetaceans from whale-watching activities has the potential to influence animal welfare and long-term fitness. Therefore, behavioural impact assessments are essential to monitor this disturbance and guide future sustainable management. I used a data-driven approach to investigate the interactions between humpback whale behaviour and whale-watching vessels in Skjálfandi Bay, Iceland, employing novel data sources and detailed indices for vessel behaviour in the absence of control observations. Whale–vessel interactions were complex, but responses indicate potential horizontal and vertical avoidance, as well as changes in foraging activity and respiration patterns, when vessels are faster, more numerous and encounters are prolonged. Moreover, speed compliance to a voluntary code of conduct may limit behavioural disturbance.

These results should encourage a review of existing whale-watching management (Currie et al., 2021; Garrod and Fennell, 2004). Specifically, GAMM relationships from this study may be used to determine whether numeric guidelines in the current IceWhale code of conduct are likely to be suitable, and to consider additional guidelines or management strategies. In particular, apparent threshold values of vessel practices that related to changes in whale behaviour could be used to inform a code of conduct. Whilst the observed responses to whale-watching vessels may not exert population-level effects, individual wellbeing may nevertheless be compromised and limiting any form of disturbance could improve population resilience to cumulative stressors. Moreover, the results of this chapter should inform more targeted investigation of specific vessel practices and, in a changing ocean, continued behavioural monitoring of whale-watching impacts is recommended to enable adaptive management

Chapter 3

Exploring the use of UAV-based blow sampling and steroid hormone measurement by LC–MS/MS to assess physiological stress in humpback whales

3.1 Abstract

Physiological monitoring is required to understand the consequences of disturbance for cetacean populations and may inform proactive species conservation. For example, the impact of whale-watching encounters and vessel traffic may not be behaviourally evident, but a chronic or repeated physiological stress response could still compromise individual health and fitness. For large whales, physiological monitoring is not routinely practiced due to the challenges of suitable sample collection, biomarker detection and biological interpretation. In this chapter, I explore the potential of blow sampling with an unoccupied aerial vehicle (UAV), paired with liquid chromatography–tandem mass spectrometry (LC–MS/MS) for steroid hormone detection, as a diagnostic tool for humpback whale physiology. First, I assessed the feasibility of sample collection, steroid hormone extraction and LC–MS/MS analysis to develop a standard protocol. Across three years of sampling (2018, 2019, 2021), 87 humpback whale blow samples were collected from vessel and land platforms around the coast of North Iceland, with little behavioural disturbance, and 68 were carried forward for LC–MS/MS analysis. In total, eight steroid hormones were detected, including the stress-related glucocorticoid cortisol and its inactive form cortisone, the androgen dehydroepiandrosterone sulphate (DHEAS), and the sex steroids testosterone and progesterone. Detection rates were generally low. For example, cortisol was detected in 10/68 samples (14%), testosterone in 15/63 samples (23%) and DHEAS in 48/63 samples (78%). To my knowledge, DHEAS has not been previously described in mysticetes. Second, I attempted to apply this method to an ecological question: do humpback whales exhibit a (chronic) physiological stress response to variable whale-watching activity and vessel traffic in North Iceland? In 2021, samples and ancillary information were collected in four areas around North Iceland with contrasting levels of whale-watching activity and vessel traffic. Unfortunately, due to low detection rates, I was unable to answer this question. This was further hindered by methodological inconsistencies and a lack of biological validation. Despite the pitfalls of my approach, these results highlight the potential of blow sampling in large whale conservation physiology, subject to improved sensitivity and validation.

3.2 Introduction

3.2.1 Physiological stress and large whales

Conservation physiology combines biological sampling techniques with sensitive and specific assays to monitor variation in physiological state in response to natural and anthropogenic pressures (Madliger et al., 2016). The field is rapidly growing in wildlife research (Madliger et al., 2018), and its importance for monitoring the health and status of free-ranging animals is increasingly realised (Semeniuk et al., 2010). For cetaceans, the population consequences of anthropogenic disturbances (New et al., 2014; Pirotta et al., 2018), such as vessel traffic or whale-watching activities, have primarily been assessed using behavioural metrics, yet a lack of behavioural response does not preclude physiological changes, with potential fitness and welfare consequences (Ditmer et al., 2015; Johnstone et al., 2012; Mercera et al., 2021; Walker et al., 2005b). In particular, disturbance may cause physiological 'stress' – defined as a systemic physiological response to a perceived stimulus, the stressor (Chrousos and Gold, 1992). Whilst stress functions as an adaptive response to acute change (Boonstra, 2005; Chrousos and Gold, 1992; McEwen, 1999), chronic or repeated acute stress can compromise individual health and reproduction (Cockrem, 2013; MacLeod et al., 2018; Martineau, 2007), including offspring fitness (MacLeod et al., 2021), and limit population resilience to future disturbance (Wright, 2012). Detecting a persistent stress response in individuals may predict these population-level consequences before they become apparent (Wikelski and Cooke, 2006; Wingfield et al., 1997).

In mammals, physiological stress responses are primarily effected through the hypothalamus–pituitary–adrenal (HPA) axis. Briefly (for more detail see Herman et al. 2016; Sapolsky et al. 2000), activation of the HPA axis triggers a hormonal cascade, including corticotrophin-releasing hormone (CRH) secretion from the hypothalamus, stimulating adrenocorticotropic hormone (ACTH) secretion from the posterior pituitary, which in turn triggers the production and release of glucocorticoids (GCs) from the adrenal. GCs control metabolism (including glucose homeostasis) and immune function throughout the body and, in most mammals, the dominant GC is cortisol. In the absence of a clear stressor, HPA axis activity exists at a basal level, with predictable circadian and ultradian (pulsatile) rhythms (Kalsbeek et al., 2012) and modulation in response to activity states such as feeding and exercise. In response to an acute stressor, HPA axis activity and circulating GC levels increase for a short period, acting on multiple organ systems to redirect energy resources and cope with the perceived challenge. This is a short-term response with high inter-individual variability (Cockrem, 2013), which is regulated by negative feedback loops; this includes cortisol inhibiting CRH and ACTH production in, and secretion from, the hypothalamus and pituitary, respectively, thereby reducing circulating cortisol levels. This negative feedback is designed to limit long-term exposure of tissues to the catabolic and immunosuppressive actions of GCs. When animals are subject to chronic or repeated stressors, persistent HPA axis activation may lead to dysregulation of its baseline activity, altering cortisol secretion which can have energetic and pathological consequences for target organs.

Due to their measurability and role in physiological stress, circulating levels of GCs are often measured as endocrine proxies for HPA axis activity and stress (Madliger et al., 2018), including in cetaceans (Champagne et al., 2012; Creel et al., 2002; Thomson and Geraci, 1986). This requires collection and storage of a biological sample whose biomarker concentrations reflect circulating levels at relevant timescales and respond predictably to a perceived stressor (Hunt et al., 2013; Touma and Palme, 2005). These biomarkers can be used to increase the sensitivity of disturbance monitoring and confer greater specificity to management techniques (Carey, 2005; Ellenberg et al., 2006, 2007; Madliger et al., 2016; Semeniuk et al., 2010). However, suitable sample collection represents a major logistical challenge for cetaceans, particularly large whales (de Mello and de Oliveira, 2016), leading to a lack of physiological monitoring (Hunt et al., 2013). Cetaceans spend most of their time underwater, often dive deep and can be far out to sea (Carwardine, 2019). Sample collection takes time, money or is intrusive (Hunt et al., 2013; Jahoda et al., 2003). Collecting blood samples from large whales is impractical and, as in other mammals, could invoke an acute stress response (Champagne et al., 2012; Desportes et al., 2007; Dickens and Romero, 2013), potentially confounding the result of a stress assessment. Other sample types such as faeces or blubber can provide information on chronic stress (Pallin et al., 2022; Rolland et al., 2012; Trana et al., 2016), nutritional status (Ayres et al., 2012) or life-history status (Dalle Luche et al., 2020; Hunt et al., 2006). For example, blubber cortisol concentrations were higher in stranded humpback whales than free-swimming animals (Mingramm et al., 2020), and North Atlantic right whale faecal GC levels were elevated in chronically entangled whales (Rolland et al., 2017). Steroid concentrations in these sample types also reflect circulating levels (Houser et al., 2021; Steinman and Robeck, 2021), but they are often challenging to collect or infrequently available (Hunt et al., 2013). Furthermore, these sample types do not allow detection of repeated acute stress responses, as biomarker levels do not respond rapidly to disturbance (Champagne et al., 2018; Steinman et al., 2020), or require invasive collection. As a result, direct physiological assessments of free-ranging large whales are limited, and physiological responses (or a lack thereof) may be erroneously inferred from behavioural evidence (Ditmer et al., 2015; Walker et al., 2005a).

3.2.2 Blow sampling

Cetacean exhaled breath condensate – known as blow – is a mixture of aerosolized airway surface liquid (ASL), seawater and atmospheric water (Martins et al., 2020). Blow has received recent attention as a potentially suitable biological matrix due to its presence in all large whales and non-invasive sample collection. In line with studies of human exhaled breath, a wide variety of bio-molecules have been detected in whale blow, including microbial and viral DNA (Apprill et al., 2017; Geoghegan et al., 2018; Pirotta et al., 2017; Vendl et al., 2020), whale DNA (Atkinson et al., 2021), RNA (Richard et al., 2022), proteins (Bergfelt et al., 2018; Thompson et al., 2019) and steroid hormones (Burgess et al., 2018;

Dunstan et al., 2012; Hudson et al., 2021; Hunt et al., 2014a). In captive cetaceans, concentrations of stress-related hormones in blow, such as cortisol, can reflect circulating levels (but not always; Mingramm et al., 2019a) and respond rapidly (20–30 minutes) to stressors (Thompson et al., 2014). Initial validation steps have been conducted in large whales, relating normalised levels of testosterone and progesterone to life history stage (Burgess et al., 2018); and relating absolute levels of progesterone, estradiol and cortisol to blubber steroid concentrations (Mingramm et al., 2019b). Whilst blow samples were initially collected from a vessel with a long pole (Hogg et al., 2009), unoccupied aerial vehicles (UAVs) now enable remote sampling, representing a faster, less obtrusive and safer alternative (Pirotta et al., 2017). Together, blow could represent a feasible sample type for dynamic physiological assessment of large whales (Hunt et al., 2014a).

Despite great promise, several barriers have prevented the application of blow sampling to ecological and conservation questions. First, ASL (the portion of interest) is highly dilute in exhaled breath (Effros et al., 2003), resulting in biomarker concentrations that are challenging to detect (Hogg et al., 2005; Trout, 2008). This is compounded by UAV-based collection due to reduced surface area on which a sample can be collected. Steroid hormones including cortisol, testosterone and progesterone have been detected in blow (including samples collected by UAV) using immunoassays, owing to their commercial availability and reported high sensitivity (Atkinson et al., 2021; Burgess et al., 2018; Taylor et al., 2015), but levels detected in the immunoassay studies remain near the limits of detectability of the kits. In studies of humans and other taxa, alternative methodological approaches have been adopted, owing to concerns of cross-reactivity and inconsistency at low detection limits (Cross and Hornshaw, 2016; Jewgenow et al., 2020). In addition, immunoassays are typically limited to one steroid hormone measurement at a time, limiting the depth of data that could be obtained from a single sample. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) represents a powerful alternative for steroid hormone measurement, promising high specificity and sensitivity (Dunstan et al., 2012; Hayden et al., 2017; Soldin and Soldin, 2009). The inherent nature of mass spectrometry also enables the inclusion of a large panel of steroids in a single assay (Chace, 2001; Seger and Salzmann, 2020; Shackleton, 2010), offering a cost-effective approach when many steroids are of interest. LC–MS/MS is increasingly used for steroid hormone analysis across clinical, veterinary and ecological studies (Cross and Hornshaw, 2016; Sheriff et al., 2011) but its application to blow steroids is limited to date. Validation on this sample type is laboratory dependent as LC–MS/MS methods are not commercially available for blow samples, thus requiring careful control for false positives (Trout, 2008). However, LC–MS has been successfully used to analyse steroid hormones in whale sample types including blood (Legacki et al., 2020), blubber (Boggs et al., 2017; Dalle Luche et al., 2020; Wittmaack et al., 2022) and even blow (Dunstan et al., 2012; Hogg et al., 2009). For example, Hogg et al. (2009) detected testosterone and progesterone in mysticete blow using LC–MS (without tandem MS); while Dalle Luche et al. (2019) validated an LC–MS/MS method for quantification of 11 steroid hormones, including cortisol, in humpback whale blubber, with high measurement accuracy.

Second, ASL dilution is highly variable, meaning that data interpretation requires some form of sample normalisation for inter-sample comparison. Generally, the amount of analyte in a biological sample, such as plasma, blubber or baleen in cetaceans (Hunt et al., 2013), is expressed as mass (or moles) of analyte per volume or mass of sample. These sample types are generally undiluted by environmental contaminants, such that differences in concentrations between samples can be used to make biological or ecological inferences. In contrast, ASL in blow is diluted by sea and atmospheric water, and this dilution is likely highly variable; in human exhaled breath condensate (without seawater contamination), ASL dilution was estimated between 1,000x and 50,000x (Effros et al., 2003) and it has not been possible to quantify dilution in blow (Burgess et al., 2018). Therefore, the interpretation of measured analyte amounts from blow samples requires some form of sample normalisation for inter-sample comparison. No 'gold standard' normaliser (i.e., a molecule with limited variation in circulating levels) has been found for exhaled breath in any taxon (Effros et al., 2003), although the potential utility of urea has been demonstrated in humans (Dwyer, 2004; Esther et al., 2009), and urea-normalised testosterone and progesterone levels in blow broadly reflect life history stage in North Atlantic right whales (Burgess et al., 2018). However, circulating levels of urea vary considerably in cetaceans, across a range of species (St. Aubin et al., 2013; Hall et al., 2007; Kjeld, 2001; Norman et al., 2012), and may not be suitable for sample normalisation (Hudson et al., 2021; Mingramm et al., 2019b). Cations such as sodium have been considered in human exhaled breath (Effros et al., 2003) but are unsuitable for blow samples that also contain seawater (Martins et al., 2020).

As an alternative to sample normalisers, hormone ratios may be used as biomarkers and circumvent the issue of variable dilution (e.g., suggested by Hunt et al. 2014a; Reckendorf et al. 2021). Provided that environmental contaminants (i.e., seawater) do not interact with hormones of interest, altering their actual or measured amounts (see Mingramm et al. 2019a), hormone ratios should remain insensitive to dilution. Moreover, ratios provide more information about physiological status than single steroid values (Guilliams and Edwards, 2010). In human clinical studies, the ratios of cortisol to cortisone and cortisol to dehydroepiandrosterone (DHEA) or DHEA sulphate (cortisol:DHEA(S)) have been related to acute and chronic stress, trauma and mental health (Cruess et al., 1999; Garner et al., 2011; Mocking et al., 2015; Qiao et al., 2017). These ratios act as proxies for the activity of 11β-hydroxysteroid dehydrogenase type 1, a key enzyme that reactivates inactive cortisone to cortisol (Tomlinson et al., 2004). For marine mammals, cortisol:DHEA has been related to health status in pinnipeds (Gundlach et al., 2018) and the ratio of faecal androgens to estrogens has been related to reproductive state in North Atlantic right whales (Rolland et al., 2005). Furthermore, a recent study found that serum cortisol:DHEAS increased in free-ranging narwhals (*Monodon monoceros*) following a capture–tagging procedure (Béland et al., 2023). However, the use of hormone ratios in ecological studies remains limited.

Third, biological interpretation of steroid hormones in whale blow is challenging due to a lack of physiological and biological validation (Touma and Palme, 2005). Little is known about the source and formation of exhaled breath condensate, in any taxon (Davis et al., 2012). In small captive odontocetes, variability in blow concentrations of cortisol, testosterone and progesterone appear to reflect circulating levels;

and cortisol levels increase in response to a perceived stressor (Champagne et al., 2018; Richard et al., 2017; Thompson et al., 2014). However, these validations have not been performed for mysticetes and even normalised hormone concentrations in blow may not reflect circulating levels or life history stage (Mingramm et al., 2019a,b). More generally, the responses of circulating cortisol and other GC levels to perceived stressors are not consistent across cetacean studies (St. Aubin et al., 2013; Lowe et al., 2021; Pallin et al., 2022; Romano et al., 2004; Teerlink et al., 2018); and the physiological role of hormones such as DHEAS is unknown in marine mammals.

3.2.3 Chapter aim

In this chapter, I explore the use of blow sampling to monitor the physiological response of humpback whales to vessel traffic and whale-watching activity in North Iceland. The presence and underwater noise of vessels (including whale-watching vessels) are a known source of disturbance for cetaceans, with negative impacts on behaviour (Christiansen et al., 2013a; Schuler et al., 2019; Williams et al., 2014) and acoustic communication (Cholewiak et al., 2018; Dunlop, 2019; Fournet et al., 2018). A growing body of evidence now suggests that physiological impacts may exist. Potential stress responses to vessel traffic have been inferred from elevated levels of faecal glucocorticoids in grey whales (Lemos et al., 2022) and North Atlantic right whales (Rolland et al., 2012), and blubber cortisol in humpback whales (Pallin et al., 2022), which may have population-level consequences (Pirotta et al., 2018). In contrast, Teerlink et al. (2018) found that blubber cortisol levels in humpback whales were not correlated with spatially variable whale-watching activity in Alaska. As vessel traffic continues to increase worldwide (Halpern et al., 2019; Tournadre, 2014), characterising this response will better inform future conservation strategies. Iceland was considered a suitable study area due to the reliable summer occurrence of humpback whales in several coastal areas, with spatially varying levels of whale-watching activity and vessel traffic.

I started by exploring the feasibility of blow sampling from humpback whales in Iceland to detect a panel of 14 steroid hormones implicated in stress and other physiological processes using LC–MS/MS. This involved unobtrusive collection with UAV, sample recovery from the collection device and steroid analysis via LC–MS/MS. From this, I attempted to develop a standard protocol and use this to determine the physiological stress response to vessel traffic, with the aim of using steroid hormone ratios as biomarkers. Following a description of methods and results for the two phases of this study (method development and its application), I discuss the steroid hormone contents of blow samples and the potential of blow sampling paired with LC–MS/MS for physiological assessment of large whales. Ultimately, I was not able realise this potential due to low detection rates and methodological inconsistencies, but I hope to share these pitfalls and outcomes to encourage the development of a robust method in the future.

3.3 Methods outline

[*Ethics statement:* sampling procedures in this chapter were approved by the University of Edinburgh School of GeoSciences Research Ethics and Integrity Committee, and permitted by the University of Iceland.]

Method development and application took place in two phases.

Phase 1: Explore the feasibility of UAV-based sample collection, combined with LC–MS/MS, to describe and quantify steroid hormones in humpback whale blow. The aim was to develop a method for steroid hormone quantification from these samples.

Phase 2: Apply the method developed in Phase 1 to a larger sample number to address an ecological question – do humpback whales exhibit physiological stress (elevated stress-related hormone levels) in response to variable whale-watching activity and vessel traffic in North Iceland? Samples were collected from areas of high and low vessel traffic and whale-watching activity.

Following a description of study sites and materials, and a summary of laboratory methods, I will provide the methods and results of Phase 1, followed by the methods and results of Phase 2. All plots were produced in R (R Core Team, 2020) using the *ggplot2* package (Wickham, 2022).

3.3.1 Study sites

Across the two phases of this study, humpback whale blow samples were collected from four areas with varying levels of whale-watching and vessel traffic (Figure 3.1): two areas with generally higher traffic (Skjálfandi Bay and Eyjafjörður) and two areas with generally lower traffic (Húnaflói and Ísafjarðardjúp). All four areas are part of the wider Icelandic feeding ground, with high rates of resighting between areas (Happywhale, 2022), and private recreational traffic is thought to be minimal at each site (Rasmussen, pers. comm.). Beyond whale-watching activity and vessel traffic (number of vessels), each area varies in terms of physical geography (e.g., spatial scale, bathymetry) and the composition of vessel traffic (e.g., vessel size, vessel movement patterns), although this not been characterised. Moreover, variability in other parameters that could influence whale endocrinology, including temperature, prey availability and ambient noise, is not known. Samples were collected from Eyjafjörður in 2018 and Skjálfandi Bay in 2019 as part of Phase 1 (method development), and from all four areas in 2021 as part of Phase 2 (ecological question). In 2021, we also attempted to collect samples from the Langanes Peninsula (in the far northeast), an area with very low vessel traffic, but inclement weather and a lack of coastal sightings prevented successful sampling.

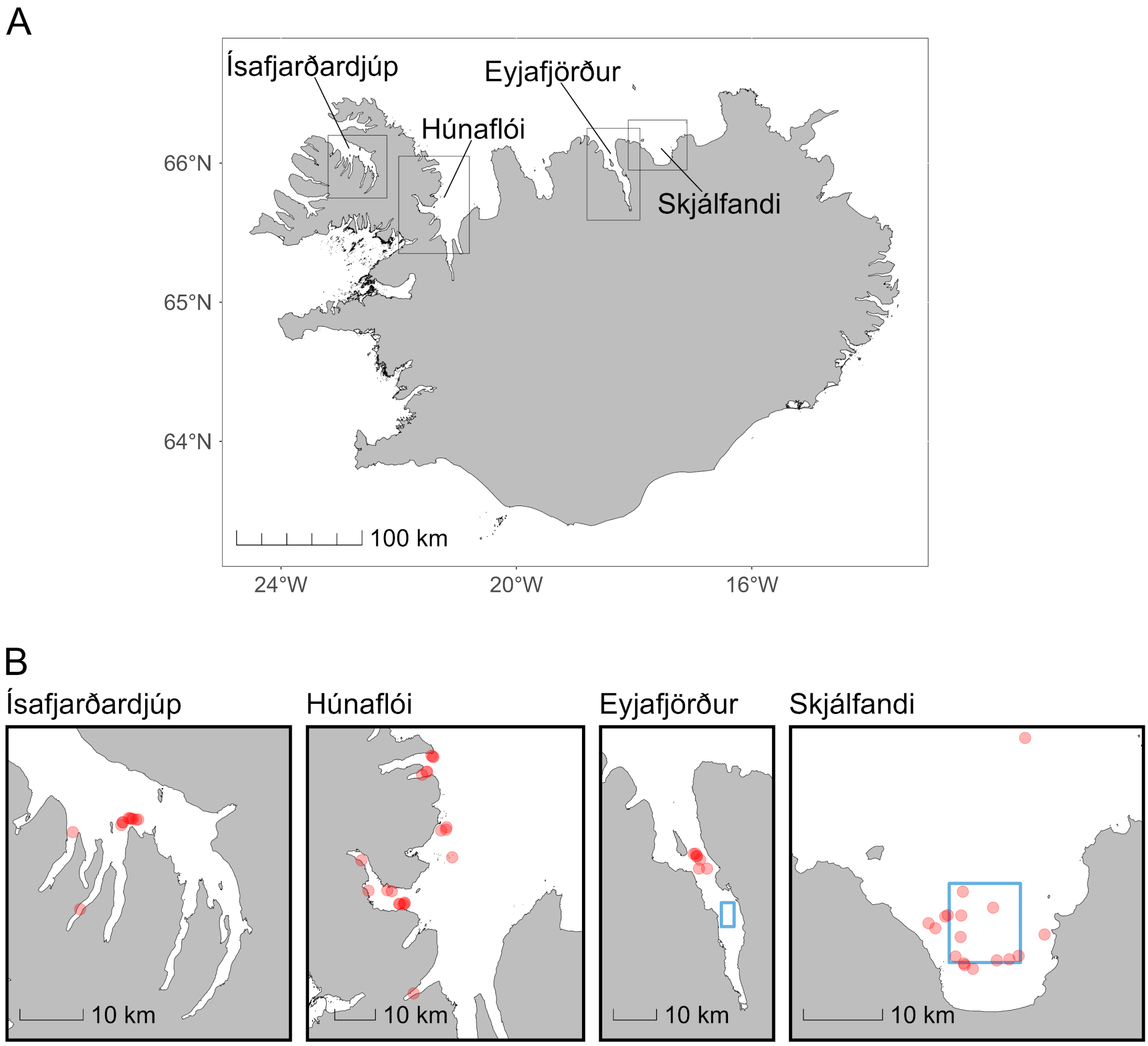

Figure 3.1: Map of the four blow sampling areas around North Iceland. A) The position of each area around Iceland: Skjálfandi Bay, Eyjafjörður, Ísafjarðardjúp and Húnaflói. B) Blow sampling locations in each area. Approximate sampling areas (blue boxes) are provided for Phase 1 samples collected in Eyjafjörður in 2018 ($n = 16$ samples) and Skjálfandi Bay in 2019 ($n = 16$); and precise sampling locations (red circles) are provided for each Phase 2 sample collected In 2021 ($n = 55$).

Skjálfandi Bay

Skjálfandi Bay is 25 km long and 10–20 km wide, located on the northeast coast of Iceland. A recognised feeding ground for various cetacean species (Rasmussen, 2009), the second largest whale-watching fleet in Iceland operates in the Bay, departing from Húsavík (Icelandic Tourist Board, 2020). Until 2019, four whale-watching companies operated up to 50 trips per day in the peak summer season (July–August), with larger 'oak boats' and smaller rigid inflatable boats (RIBs). The Bay is also popular with cruise ships, with 28 ship visits in 2019 (Cruise Iceland, 2022). Both activities declined due to the COVID-19 pandemic, with three whale-watching companies operating up to 30 trips per day and 21 cruise ship visits in 2021. Húsavík is also an important fishing harbour (Einarsson, 2009) and large cargo vessels traverse the Bay to serve the town.

Eyjafjörður

Eyjafjörður is 60 km long and 5–15 km wide, located on the north coast. This glacial fjord is home to a large whale-watching industry, based at several towns throughout the fjord (Akureyri, Hjalteyri, Hauganes and Dalvík), consisting of oak boats and RIBs (as in Skjálfandi Bay), as well as large motorised catamarans. As with Húsavík, the number of vessels and trips per day decreased in 2021 due to the COVID-19 pandemic. Akureyri, at the south of the fjord, is an important stop-over for cruise ships, with 155 visits in 2019 and 46 in 2021 (Cruise Iceland, 2022), and container vessels. There are also several fishing ports throughout the fjord (Einarsson, 2009), with small-scale fishing throughout summer.

Ísafjarðardjúp

Ísafjarðardjúp is a complex series of fjords and bays in northwest Iceland, approximately 40 km long and 10–15 km wide. Samples were collected in the inner part of the fjord system, away from Ísafjörður, a regionally important economic centre, fishing port and stop-over for cruise ships. Within the sampling area, vessel traffic is thought to be dominated by small-scale fisheries and a growing aquaculture industry. At least four passenger vessels operate out of Ísafjörður and primarily travel directly between Ísafjörður and Hornstrandir, traversing the mouth of the fjord, but they occasionally practice whale-watching in the study area.

Húnaflói

Húnaflói is a large bay in northwest Iceland, consisting of glacial fjords and more open water. Samples were primarily collected in the small fjord of Steingrímsfjörður. A single whale-watching vessel operates in the fjord from Hólmavík, with 1–2 trips per day in summer. Hólmavík and Drangsnes are small fishing ports, and small-scale fisheries operate through the sampling area. Large vessels (cruise and cargo ships) are not known to frequent the area.

3.3.2 Materials, consumables and reagents

Water (LC–MS grade), methanol (LC–MS grade) and propan-2-ol (HPLC and LC–MS grade) were from VWR, Lutterworth, UK. Ethanol (analytical reagent grade), formic acid (LC–MS grade) and ammonium hydroxide (35%) were from Fisher Scientific, Loughborough, UK.

Certified reference materials (1 mg/mL in methanol or acetonitrile) for androstenedione (A4), testosterone (T), 5α-dihdyrotestosterone (DHT), progesterone (P), cortisol (F), corticosterone (B), 17β-estradiol (E2), estrone (E1) and estriol (E3) were supplied by Cerilliant/Sigma–Aldrich, Dorset, UK. Cortisone (E) and 11-dehydrocorticosterone (A) were from Steraloids Inc, Newport, Rhode Island, USA. Dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS) and aldosterone (Aldo) were provided as powders from Sigma-Aldrich, UK. Reference standard solutions and powders were stored as directed by the manufacturers.

Stable isotope labelled internal standards were provided as certified reference materials (100 µg/mL in methanol or acetonitrile): 9,11,12,12-[$^2H_4$]-cortisol (d_4F), 2,2,4,6,6,17α,21,21,21-[$^2H_9$]-progesterone (d_9P), 2,3,4-[$^{13}C_3$]-testosterone ($^{13}C_3$-T), 2,3,4-[$^{13}C_3$]-androstenedione ($^{13}C_3$-A4) and 2,3,4-[$^{13}C_3$]-5α-dihydrotestosterone ($^{13}C_3$-DHT) were from IsoSciences/QMX laboratories, Thaxted, Essex, UK; 2,2,4,6,6,17α,21,21-[$^2H_8$]-corticosterone (d_8B) was from CK Isotopes, Unthank, Leicestershire, UK; 2,2,3,4,4-[$^2H_5$]-dehydroepiandrosterone (d_5DHEA), 2,2,3,4,4-[$^2H_5$]-dehydroepiandrosterone sulphate (d_5DHEAS), 2,3,4-[$^{13}C_3$]-17β-estradiol ($^{13}C_3$-E2), 2,3,4-[$^{13}C_3$]-estrone ($^{13}C_3$-E1) and 2,3,4-[$^{13}C_3$]-estriol ($^{13}C_3$-E3) were from Sigma-Aldrich, UK. 2,2,4,6,6,9,12,12-[$^2H_8$]-cortisone (d_8E) was provided as a powder from Sigma-Aldrich, UK.

2 mL 96-deep well plates and glass LC–MS vials were supplied by Waters, UK; plastic pipette tips by Gilson, USA; 3.5 mL glass vials by Scientific Laboratory Supplies, Alloa, UK; and 10 mm polystyrene Petri dishes by Breckland Scientific, Stafford, UK.

Stock solutions and calibration standards

Unless bought as certified reference materials, standard solutions were prepared by dissolving a suitable amount of steroid powder in methanol at a concentration of 1 mg/mL. Further serial dilutions of the steroids were carried out using methanol as appropriate. All solutions were stored in the dark, at $-20°C$ prior to use. Standards for calibration curves were prepared freshly on the day for each batch of samples analysed.

3.3.3 Laboratory methodology

Sample extraction and steroid LC–MS/MS analysis took place at the Edinburgh Clinical Research Facility Mass Spectrometry Core. Samples were processed in the laboratory in four batches: Batch 1 (2018 samples) and Batch 2 (2019 samples) in Phase 1; and Batch 3 (samples 2021_1–29) and Batch 4 (samples 2021_30–55) in Phase 2 (2021). Each batch used a slightly different methodology for extraction and analysis. Full details are provided for each phase; methological differences are summarised in Table 3.1; and chromatographic retention times, mass spectrometry parameters and internal standards for each steroid are provided in Appendix F.

Table 3.1: Summary of laboratory methodological differences between each batch of blow samples in Phase 1 (batches 1 and 2) and Phase 2 (batches 3 and 4). Pre-LC–MS/MS laboratory stages are listed: steroid extraction from Petri dishes using an ethanol–water (50:50 v/v) solution, transfer to vessels for LC–MS/MS injection (96-deep well plates, DWP, or glass LC–MS vials) and an extra centrifugation step for Batch 4. Reconstitution volumes refer to the water–methanol (70:30 v/v) solution in which samples were reconstituted following Petri dish extraction. Different LC column stationary phases (but of the same dimension) used to analyse different steroids within a batch are denoted by 'i', 'ii', etc. F – cortisol, E – cortisone, B – corticosterone, A – 11-dehydrocorticosterone, T – testosterone, A4 – androstenedione, DHT – dihydrotestosterone, DHEA – dehydroepiandrosterone, DHEAS – dehydroepiandrosterone sulphate, P – progesterone, E2 – 17β-estradiol, E1 – estrone, E3 – estriol, Aldo – aldosterone.

Phase	Batch	Samples	Steroids in method	Pre-LC–MS/MS analysis	Reconstitution volume (μL)	LC column	LC–MS/MS instrument
1	1	2018_1–5	F	ethanol–water extraction Transfer to 96-DWP	200	C18	Nexera MP QTrap6500+
1	2	2019_1–9	**i.** F, E, B, A, T, A4, DHT, P, E1, E2 **ii.** DHEAS, DHEA, E3, Aldo	ethanol–water extraction Transfer to 96-DWP	200	**i.** C18 **ii.** Biphenyl	Nexera MP QTrap6500+
2	3	2021_1–29	F, E, B, A, DHEAS, DHEA, T, A4, DHT, P, E1, E2, E3, Aldo	ethanol–water extraction Transfer to LC–MS vials	80	C18	Acquity I-Class QTrap6500+
2	4	2021_30–55	F, E, B, A, DHEAS, DHEA, T, A4, DHT, P, E1, E2, E3, Aldo	ethanol–water extraction Transfer to LC–MS vials Centrifugation	80	C18	Acquity I-Class QTrap6500+

3.4 Phase 1: developing a method to analyse blow steroids

In Phase 1, I assessed the feasibility of UAV collection of blow samples in the field, paired with LC–MS/MS analysis of steroids in the laboratory, for quantifying the steroid hormone contents for stress assessments. The final LC–MS/MS method screened for a panel of 14 steroid hormones to explore the use of hormone ratios to circumvent the issue of variable sample dilution.

3.4.1 Phase 1 methods

Collection of Phase 1 blow samples

Phase 1 blow samples were collected from humpback whales in Eyjafjörður (2018) and Skjálfandi Bay (2019; Figure 3.1). Samples were collected with a DJI Phantom 4 quadcopter UAV with live video feed. This UAV model is thought to produce small but significant increases in ambient underwater sound levels when flying directly above, at altitudes lower than 30 m (Laute et al., 2023). Two sterilised 100 mm plastic Petri dishes were used as a collection device for each sample; Petri dishes have previously been used to collect samples from free-ranging whales and detect steroid hormones using immunoassays (Atkinson et al., 2021; Burgess et al., 2016). Petri dishes were attached to the UAV in a forward-facing direction with an intermediary plastic frame consisting of plastic rods (Figure 3.2). For each blow sampling session (day), a 1 mL seawater sample was also collected and stored in a single Petri dish.

Sample collection involved three researchers: a trained UAV operator; a sample processor to seal, label and store samples; and an observer who launched and caught the UAV and assisted the operator in locating whales. Collection was only attempted in wind speeds lower than 20 km/h, with no precipitation and visibility greater than 10 km, to ensure that the UAV was visible at all times and flown safely. In 2018, the UAV was deployed from land in Eyjafjörður. In 2019, the UAV was deployed from a 10 m sailing boat or a 4.5 m rigid inflatable boat (RIB) in Skjálfandi Bay, with all three researchers on board the vessel. When operating the vessel, the whale was approached to a minimum of 300 m distance and the engine was switched off at least five minutes prior to and during sample collection to minimise potential disturbance.

Target whales were chosen by a combination of availability and suitable surface behaviour. Animals that exhaled multiple times in a dive sequence (typically travelling or feeding at depth) were considered easier to sample than animals that surfaced only once (typically surface-feeding animals) and were preferentially selected. Individual humpback whales were recognised via photo-identification, using natural markings on the ventral side of the tail flukes and the dorsal fin (Calambokidis et al., 2001; Katona and Whitehead, 1981). Identification photographs were taken from land (2018) or the vessel (2019) with a DSLR camera (Canon 70D, 100–400 mm lens) before each sampling event and inspected in real time, to determine whether the animal had been previously sampled. When possible, each sample was collected from a different animal, aided by live video feed from the UAV.

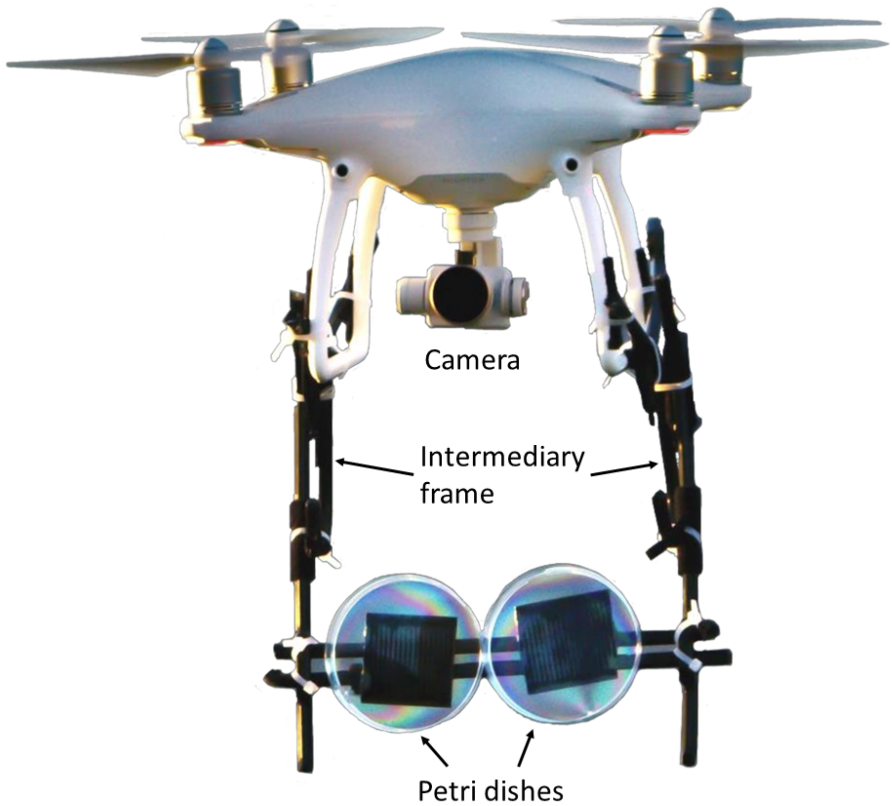

Figure 3.2: Blow sample collection system: commercially available unoccupied aerial vehicle (DJI Phantom 4), with inbuilt camera allowing first-person view operation; Petri dishes for sample collection; and an intermediary frame.

Following selection, the surface behaviour of the target animal was monitored at least 10 minutes before, during and five minutes after sample collection to detect possible disturbance due to the presence of the research vessel or blow sampling attempt. Notable changes in surface activity and swim speed were recorded. Adopting a precautionary approach, if potential visible signs of stress were observed (i.e., a change in surface activity or increase in swim speed), sampling effort for that animal was terminated and not re-attempted for at least seven days (Hodgson and Koh, 2016).

Upon selecting a target animal, two Petri dishes were attached to the intermediary frame with Velcro fasteners, their lids removed and the UAV launched by hand. The UAV was operated in first-person view and the whale's position was monitored by the operator and observers. In accordance with UAV regulations set by the Iceland Transport Authority[1], at least one researcher watched the UAV at all times. Upon sighting the animal at the surface, the UAV was positioned behind the path of the whale to minimise disturbance, before flying over the whale at a height of 2–3 m to coincide with its surfacing and pass through its exhale to capture the blow. The UAV was then flown back to the operator and the Petri dishes were capped with their lids; immediately sealed with Parafilm; and labelled with details of the whale, date, timing and location. Once sealed, the inner surfaces of both Petri dishes were examined together for presence, number and size of droplets, and classified as follows: very poor samples had few or no visible droplets; poor samples had many small or few (<5) large droplets per dish; moderate samples had at 5–20 large droplets; good samples had many (>20) large droplets; and very good samples had liquid covering at least 40% of the Petri dish. Of note, considerable evaporation could have taken place during the interval between sample collection (flying through exhaled breath) and flying the UAV back (at speeds up to >40 km/h) to the operator, which could take several minutes. For this reason, I did not attempt to measure sample volume. Samples were stored in a cooler box (Titan Deep Freeze) with frozen coolant blocks at 5°C in the field (temperature not monitored) for 1–24 hours; then at -20°C prior to laboratory analysis, interspersed by a single period of 15 hours at 0–5°C during international transport from Iceland to the UK.

Steroid analysis of Phase 1 blow samples

Samples were prepared for steroid analysis which required extraction from Petri dishes and calibration standard preparation. Analysis of the extracted samples was performed by LC–MS/MS, alongside calibration standards, followed by data evaluation of resulting chromatographic peaks.

LC–MS/MS was performed using a Nexera MP uHPLC system (Shimadzu, Milton Keynes, UK), interfaced to a QTrap 6500+ (AB Sciex, Warrington, UK) mass spectrometer for detection and quantitation of steroids. Instrument control and data acquisition were achieved using Analyst® 1.6.3 software (AB Sciex).

1. Icelandic Transport Authority, information material on drone operation: https://www.icetra.is/aviation/drones/information-material-on-drone-operation/

Chromatographic separation was achieved with gradient elution, using two different chromatographic columns with different chemistries and mobile phase systems, depending on batch and target steroids. All columns were fitted with a KrudKatcher Ultra In-Line Filter (0.5 µm porosity; Phenomenex, Macclesfield, UK) to minimise blockage and backpressure build-up.

The mass spectrometer was operated in positive ion electrospray ionisation (ESI) mode using a TurboIonSpray source and data were collected in unit resolution (0.7 m/z full width at half maximum). The TurboIonSpray source was operated at 600°C with an IonSpray voltage of 5.5 kV, a Curtain Gas of 30 psi, nitrogen nebuliser ion source gas 1 (GS1) and heater ion source gas 2 (GS2) of 40 psi and 60 psi, respectively.

Chromatographic data were integrated using the *Quantitate* section of Analyst® software. To address the unknown volume and dilution of blow samples, steroid quantities were expressed as mass per sample and not concentration.

Cortisol extraction from Petri dish surface

Following the collection of blow samples in 2018, the primary aim was to assess the feasibility of cortisol extraction from blow. Due to the hydrophobic nature of cortisol, a wash solution is necessary for sample extraction from plastic Petri dishes. Burgess et al. (2016) previously developed a protocol for the extraction of several steroids (including cortisol) in North Atlantic right whale blow from Petri dishes (with a view to sample collection using a long pole; Burgess et al., 2018), but this study employed immunoassays for steroid quantification. Moreover, I used Petri dishes with a surface area ∼10% of those used by Burgess et al. (2016), owing to practical limitations of UAV flight stability. Therefore, it was necessary to develop my own extraction methodology in the laboratory. I tested different ethanol–water wash solutions for percentage recovery of cortisol and relative standard deviation (RSD, %) using 'mock samples' of known concentration of steroid.

First, 250 ng/mL cortisol solution was constituted from water and a standard solution of 1 mg/mL cortisol in methanol. Nine mock blow samples in Petri dishes were then prepared: 200 µL of the solution (50 ng of cortisol) was aliquoted to a 10 mm Petri dish; the lid was placed on top; and the dish was incubated at room temperature for 30 minutes. An aliquot of isotopically labelled internal standard (d_4-cortisol, 25 ng) was added to each dish and incubated for five minutes at room temperature. Extraction solutions at three different ratios of ethanol:water (50:50, 70:30, 90:10 v/v) were then tested in triplicate for sample extraction efficiency. Each ethanolic solution was added to three dishes (3 mL per dish), which were then gently agitated for 30 seconds using a plate shaker (Fisher Scientific, UK). The content of each dish was transferred to a 3.5 mL glass vial (one vial per dish) using plastic tips of a 200 µL Thermofisher pipette. The solution was dried down under a stream of heated oxygen-free nitrogen (OFN, 40°C) and reconstituted in water–methanol (70:30 v/v; 200 µL).

Three unextracted 50 ng cortisol standards were prepared by aliquoting 200 µL cortisol solution and internal standard solution (25 ng) directly into 3.5 mL glass vials, drying down under OFN at 40°C as above, and reconstituting in water–methanol (70:30 v/v; 200 µL).

The nine 50 ng mock blow samples and three unextracted standards were transferred to individual wells of a 2 mL 96 deep-well polypropylene plate (Waters, UK) and sealed with a plate seal (Waters, UK) for subsequent LC–MS/MS analysis as a single batch of samples. Finally, 10 µL was injected onto the LC–MS/MS instrument for steroid analysis.

Percentage recovery of cortisol for each dish was calculated as:

$$Recovery\ (\%) = \frac{F_{ext,i}}{\overline{F_{QC}}};$$

where $F_{ext,i}$ is the standard peak area ratio of the cortisol:d_4-cortisol signal in extracted mock sample i and $\overline{F_{QC}}$ is the mean peak area ratio of the three unextracted quality controls. The peak area ratio is calculated by dividing the cortisol peak area by the d_4-cortisol peak area. A solution of 50:50 (v/v) ethanol–water yielded the highest mean recovery of cortisol (100%) and the lowest RSD (3.7%).

Three volumes of this 50:50 solution – 3, 5 and 7 mL – were then tested with the same process. A lower 3 mL volume yielded high mean recovery of cortisol (101%) and low RSD (7%), comparable with higher volumes. Thus, 3 mL of 50:50 ethanol–water was subsequently used to extract steroids from sample Petri dishes.

Cortisol analysis in 2018 blow samples

After some 2018 blow samples were used for method optimisation, the remaining samples (Batch 1) were extracted, as above, and analysed for the presence and amount of cortisol. Each sample consisted of two dishes, with 20 µL of d_4-cortisol solution (1 ng) added to each dish and the contents of both dishes transferred into a single vial. A calibration curve was derived from a set of eight standards (0.005–1 ng), plus a zero standard, extracted from Petri dishes using the same procedure.

Chromatographic separation of 10 µL of injected sample was achieved using a Kinetex C18 column (150 x 2.1 mm; 2.6 µm; Phenomenex, UK) with a mobile phase of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B), at a flow rate of 0.5 mL/min. The run started at 55% mobile phase B, holding to 4 minutes, rising to 100% at 10 minutes, holding until 12 minutes, falling to 55% at 12.1 minutes and ending at 16 minutes. d_4-cortisol (d_4F) was used as an internal standard (IS).

In the samples, detecting a clear chromatographic peak was often challenging, due to low peak areas (<10,000) that did not exceed signal-to-noise ratios (SNR) >2. To better describe the peaks detected in the samples, peaks were classified as follows: samples with 'no' peak had no visible peak at the expected retention time; samples with a 'possible' peak had a visible peak above the background signal but with a low SNR; samples with a 'probable peak' had a clearly defined peak and SNR>2; and samples with a 'definite' peak had a clearly defined peak and SNR>2.5 (Figure 3.3). The categories 'probable' and 'definite' constituted a clear peak and were accepted as cortisol. Quantification (assigning an amount to the peak) was achieved using a calibration curve ($1/x^2$ weighting), consisting of a zero standard and eight standards of increasing amounts. Standards were included in a calibration curve as long as the SNR>2.5:1 and the accuracy was <20% from the known amount. The lower limit of quantification (LLOQ) was calculated to be equal to the lowest non-zero standard amount in the calibration curve.

Exploring other steroids in 2019 blow samples

Samples collected in 2019 (Batch 2) were used to explore the presence of 14 steroid hormones in the steroidogenesis pathway, including cortisol, in humpback whale blow. These steroids constitute part of a typical panel of steroids that can be classified into five main groups – glucocorticoids, progestogens, androgens, estrogens and mineralcorticoids – that are analysed in this laboratory for clinical studies. The glucocorticoids cortisol (F), cortisone (E), corticosterone (B) and 11-dehydrocorticosterone (A) are commonly implicated in stress in mammals. Sex steroids were also included: androgens testosterone (T), androstenedione (A4), 5α-dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA) and its sulphate conjugate dehydroepiandrosterone sulphate (DHEAS) are implicated in male sexual development and ageing; the progestogen progesterone (P) regulates pregnancy and the estrous; while the estrogens 17β-estradiol (E2), estrone (E1) and estriol (E3) are implicated in female sexual development and pregnancy. Finally, the mineralcorticoid aldosterone (Aldo) plays an important role in mammalian sodium balance and osmoregulation (and is therefore particularly interesting in marine mammals; Ortiz 2001), and has been implicated in acute stress responses in cetaceans (Romano et al., 2004; Steinman et al., 2020). As with the 2018 samples, the limits of quantification were determined by including a set of calibration standards, with the same amounts of each steroid (0.005–1 ng, eight standards) and a zero standard.

Chromatographic separation of the panel of steroids was achieved using two different column chemistries. A Kinetex C18 column, with the same mobile phases and LC schedule as before, was used for the following steroids: F, E, B, A, T, A4, DHT, P, E1, E2 (e.g., Figure 3.4).

A separate chromatographic method was developed on a Kinetex biphenyl column (150 x 2.1mm; 1.7µm; Phenomenex, UK) using mobile phases of 0.05 mM ammonium fluoride in water (mobile phase A) and methanol (mobile phase B) to separate DHEAS, DHEA, E3 and Aldo, at a flow rate of 0.3 mL/min. The gradient started at 65% mobile phase B, holding to 1 minute, rising to 80% at 4.5 minutes, holding to 5.5 minutes, rising to 100% at 6.5 minutes, holding to 8 minutes, falling to 65% at 8.2 minutes and ending at 10 minutes. The DHEAS peak exhibited considerable drift in retention time using both methods, but it was less extreme using the biphenyl column. Meanwhile, the initial C18 method did not include DHEA, E3 and Aldo. A solution of isotopically-labelled IS was added to each dish (20 µL, 1 ng) to control for the recovery and confirm retention time of each of the 14 steroids. A list of steroids, corresponding IS and batch-specific retention times for each steroid and IS can be found in Appendix F.

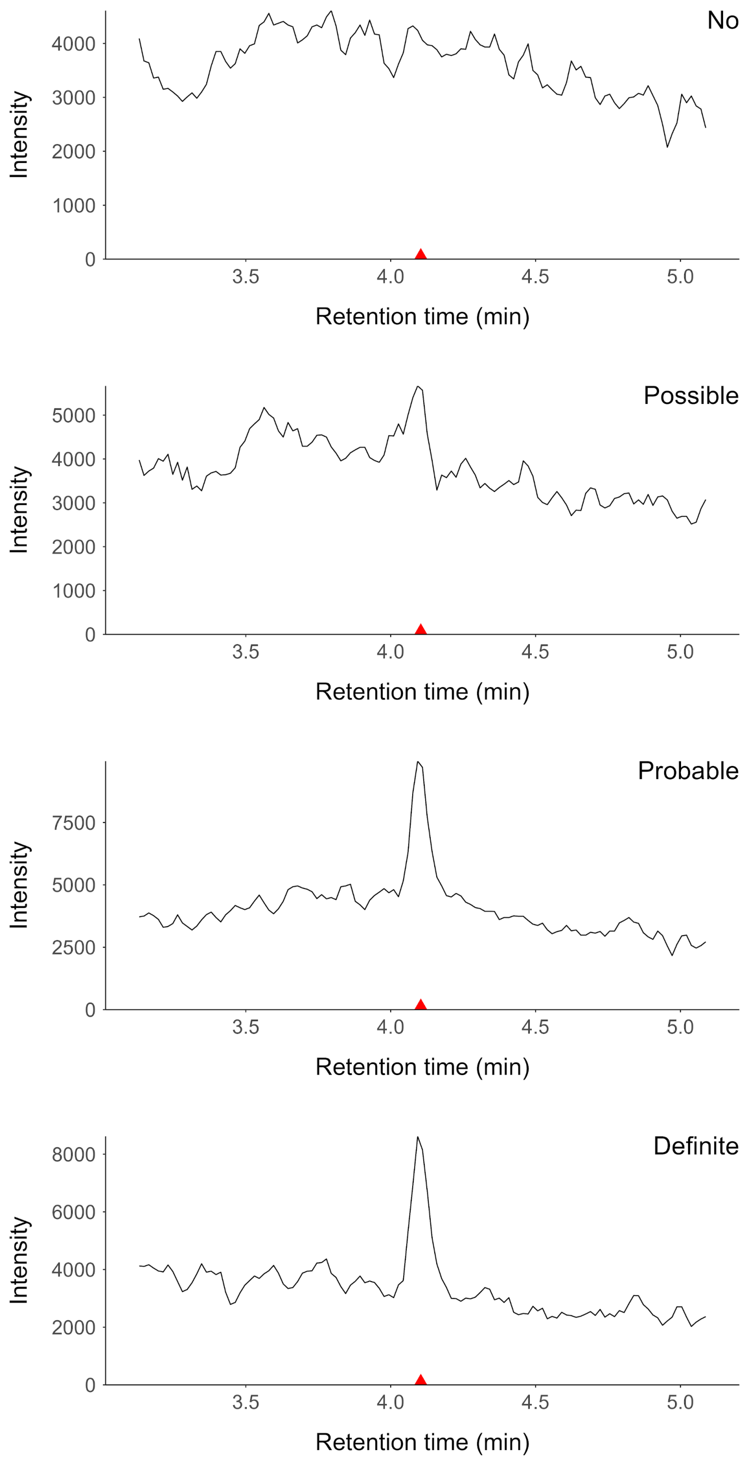

Figure 3.3: Example extracted ion chromatograms (from real blow samples) for the mass transition $m/z\ 363.1 \rightarrow 121$, corresponding to cortisol (retention time: 4.1 minutes on Kinetex C18 column), for each category of peak confidence: no peak (no visible peak), possible (visible peak, SNR<2), probable (clear peak, SNR>2) and definite (clear peak, SNR>2.5).

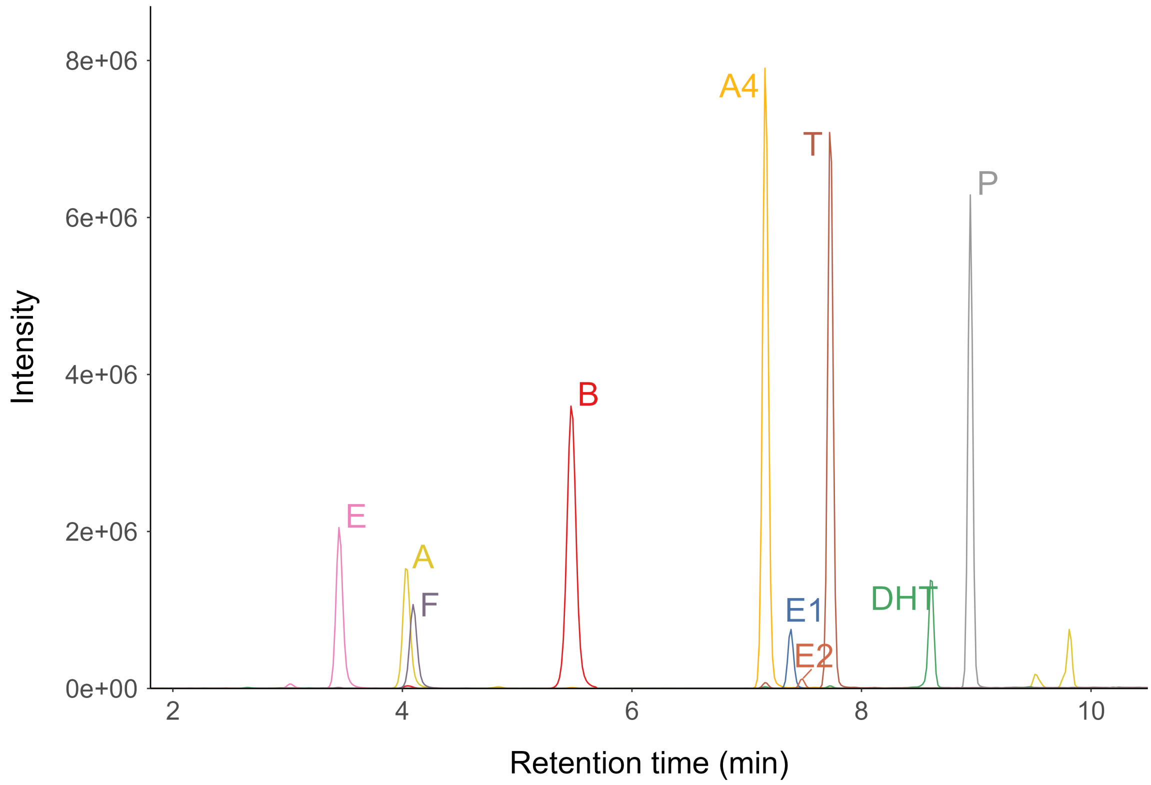

Figure 3.4: Extracted ion chromatogram from a mixture of key steroids at 5 ng/mL. Separation was performed on a Kinetex C18 column and analysed on a Nexera XP uPLC and QTrap6500+ mass spectrometer. Multiple reaction monitoring transitions in positive and negative ion electrospray ionisation mode, extracted for m/z $361.1 \rightarrow 121.2$ (E, cortisone), m/z $345.1 \rightarrow 121.2$ (A, 11-dehydrocorticosterone), m/z $363.1 \rightarrow 121.2$ (F, cortisol), m/z $347.1 \rightarrow 121.1$ (B, corticosterone), m/z $287.0 \rightarrow 97.0$ (A4, androstenedione), m/z $269.1 \rightarrow 144.9$ (E1, estrone), m/z $271.0 \rightarrow 182.9$ (E2, estradiol), m/z $289.1 \rightarrow 97.0$ (T, testosterone), m/z $291.3 \rightarrow 255.2$ (DHT, dihydrotestosterone), m/z $315.1 \rightarrow 97.1$ (P, progesterone).

As cortisol was the principal focus of this study, the same Petri dish extraction method was used to extract all steroids, i.e., a wash solution of 50:50 (v/v) ethanol–water (3 mL). Calibration standards were extracted from Petri dishes in the same way. Peak classification (no/possible/probable/definite), positive detection (probable or definite peaks) and quantitation (positive detection and calculated concentration >LLOQ) were performed as in Figure 3.3.

3.4.2 Phase 1 results

Blow sample collection

In total, 32 blow samples were collected from at least 13 whales. In 2018, 16 samples were collected from land during 12 hours of sampling effort (two sampling sessions), at an average of 810 m from the UAV operator (max = 2.2 km). Photo-identification from this land site was challenging due to low elevation and distance to target whales: only three sampled whales were positively identified, and five samples were collected from one whale (MN140). In 2019, 16 samples were collected from a research vessel during 14 hours of sampling effort. Distance to UAV operator was not recorded. Owing to closer distances (300–1,000 m), photo-identification was more feasible and samples were collected from ten whales, and a maximum of two samples per whale. One sample (2019_9) was collected by hand, instead of UAV, from a curious animal (MN60) that approached the stationary research vessel (4.5 m RIB) with the engine off and exhaled within 1 m of the researchers.

Out of the 32 samples, 12 were classified as very poor or poor, 11 were moderate and 9 were good or very good. No behavioural response was observed during blow sample collection. However, aerial images were also collected from several animals in 2019, using the same UAV model. One animal started splashing and swimming quickly at the surface during an aerial imagery attempt (the UAV was 20 m directly above the animal), which took place after successful blow sample collection (sample 2019_3).

Blow sample steroid analysis

Out of 32 samples, 14 were carried forward for LC–MS/MS analysis of steroids (five from 2018, Batch 1; nine from 2019, Batch 2; Appendix G), with the remaining samples used for methodological optimisation (nine samples from 2018) or other studies (six samples from 2019). Steroids were extracted from Petri dishes using the most suitable ethanol–water solution (50:50 v/v, 3 mL).

Out of five samples from 2018 (Batch 1), three presented a visible cortisol peak classified as ’probable’ or ’definite’, all from the same whale (MN140; Figure 3.5). Using a LLOQ of 5 pg, three samples were quantifiable, with a mean amount of 7.2 pg (range = 5.5–10 pg; Table 3.2). The internal standard (d_4-cortisol) produced a consistent peak area and retention time, and cortisol standards produced a consistent retention time. No peak was visible in two seawater controls, solvent blanks or the zero standard.

Nine blow samples and one seawater sample from 2019 were screened for 14 steroids, including cortisol. A peak of at least one steroid was clearly visible (probable/definite) in every sample, and seven steroids were detected in total (Table 3.2 and Figure 3.6). The number of steroids per sample with probable or definite peaks ranged from two to six (sample 2019_3; Figure 3.7). Retention times were generally consistent. Calibration curves mostly included the full range of standard amounts (5–1,000 pg), other than DHEAS (LLOQ = 25 pg).

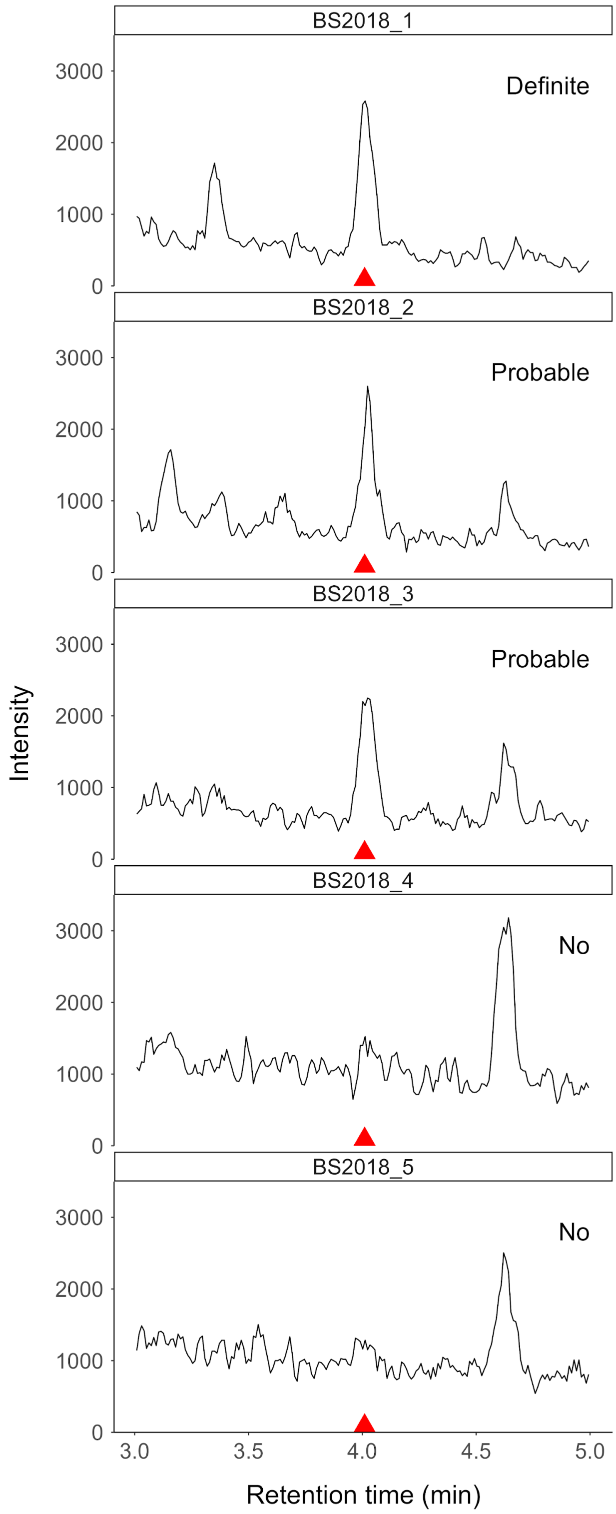

Figure 3.5: Extracted ion chromatograms for m/z $363 \rightarrow 121$, the mass transition for cortisol in this LC–MS/MS method, for five samples collected in 2018 processed using the final method (Batch 1). The retention time of cortisol (derived from cortisol standards) is 4.02 minutes, denoted by the red arrow and verified by the retention time of the d_4-cortisol peak (which has the same retention time).

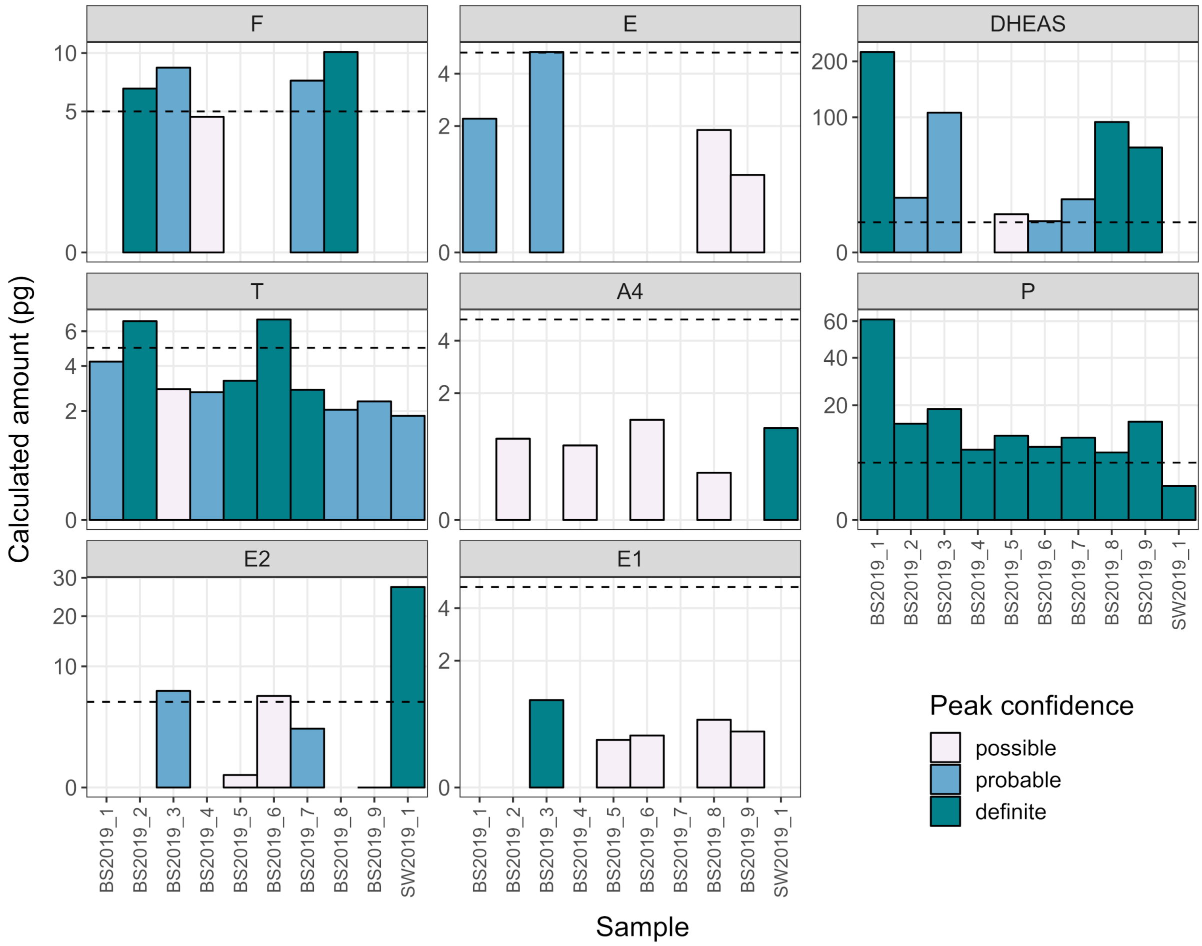

Figure 3.6: Calculated amount (pg per sample) of eight detected steroids from blow samples and one seawater sample from Phase 1, Batch 2 (blow samples 2019_1–9). The detected steroids were cortisol (F), cortisone (E), dehydroepiandrosterone sulphate (DHEAS), testosterone (T), androstenedione (A4), progesterone (P), estradiol (E2) and estrone (E1). The lower limit of quantification (LLOQ) for each steroid and batch is denoted by the horizontal black dashed line. Calculated amounts are only presented for visible peaks. DHEAS was separated using a biphenyl column and all remaining steroids were separated with a C18 column. BS – blow sample; SW – seawater.

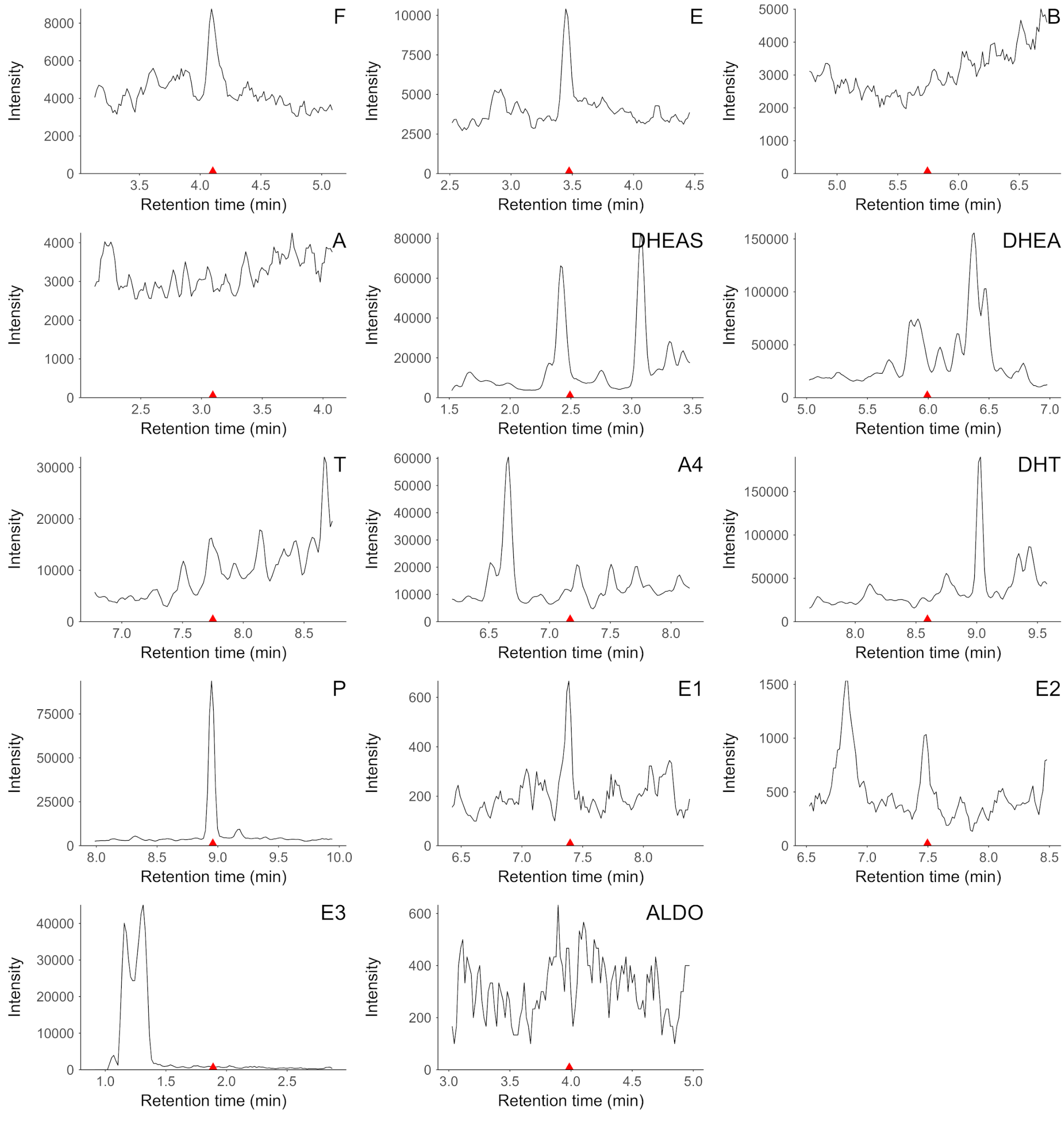

Figure 3.7: Chromatograms of the panel of 14 steroids from blow sample 2019_3 (Batch 2). The red arrow denotes expected retention time of the relevant steroid. In this sample, peaks were detected for cortisol (F, probable), cortisone (E, probable), dehydroepiandrosterone sulphate (DHEAS, probable), testosterone (T, possible), progesterone (P, definite), estrone (E1, probable) and estradiol (E2, probable). Corticosterone (B), 11-dehydrocorticosterone (A), dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), estriol (E3) and aldosterone (Aldo) had no visible peak.

Of the four glucocorticoid steroids in this method, two were detected in 2019 blow samples: cortisol and cortisone (Table 3.2 and Figure 3.6). Cortisol peaks were classified as 'probable' in two samples and 'definite' in two samples, while four samples had no visible peak. There were no visible peaks in seawater, blanks or the zero standard. All four clear peaks (from different whales) had a calculated amount greater than the method LLOQ of 5 pg (mean = 8.2 pg, range = 6.8–10 pg). Meanwhile, cortisone peaks were classified as 'probable' in two samples, and one sample had a calculated amount at the LLOQ (5.0 pg). There were no visible peaks of cortisone in seawater, blanks or the zero standard. Corticosterone and 11-dehydrocorticosterone were not detected in any blow sample or seawater sample.

Five androgens were included in the method: DHEAS, testosterone and androstenedione peaks were detected (Table 3.2 and Figure 3.6), whereas DHEA and DHT were never detected. Detecting DHEAS peaks was challenging due to drifting retention times across both standards and samples (1.72–2.47 minutes). However, this precisely matched the shift in d_5DHEAS IS peak for each sample, following the requirement that the IS and analyte peaks co-elute. In total, DHEAS peaks were classified as 'probable' in four samples and 'definite' in three samples, while one sample had no visible peak. The two samples with the weakest DHEAS signal were from the same whale (MN43). A small peak was visible in the zero standard (which was incorporated into the calibration curve) and no peak was visible in seawater samples. All seven clear peaks had a calculated amount greater than the LLOQ of 25 pg, with the highest values of any steroid (mean = 73.6 pg, range = 5.4–227 pg). Testosterone peaks were classified as 'probable' in four samples and 'definite' in four samples, with the remaining sample classified as 'possible'. There was also a probable peak in the seawater sample, a possible peak in the zero standard and no peaks in blanks. Two samples with clear peaks had a calculated amount greater than the LLOQ of 5 pg (mean = 6.8 pg, range = 6.7–6.8 pg). No clear peaks corresponding to androstenedione were present in blow samples, although there was a small but definite peak (below the LLOQ of 5 pg) in a seawater sample. DHEA peaks were not possible to discern due to a number of dominant peaks in each chromatogram.

Progesterone peaks were classified as 'definite' in all blow samples and the seawater sample (Table 3.2 and Figure 3.6). All blow samples had a calculated amount greater than the LLOQ of 5 pg (mean = 16.9 pg, range = 6.9–61.1 pg), whereas the seawater sample was lower than the LLOQ. The zero standard had a clear peak, which was incorporated into the calibration peak, whereas no peaks were visible in the blanks.

From the three estrogens, estradiol and estrone were detected whereas there were no clear estriol peaks (Table 3.2 and Figure 3.6). Estradiol peaks were classified as 'probable' in two samples and no peak was detected in four samples. No peaks were visible in blanks or the zero standard, but there was a large, definite peak in the seawater sample. One blow sample had a calculated amount greater than the LLOQ of 5 pg (sample 2019_3 = 5.7 pg), whereas the seawater sample was 27.4 pg. Meanwhile, an estrone peak was classified as 'definite' in only one sample, with a calculated amount lower than the LLOQ of 5 pg. No peak was detected in four blow samples, the seawater sample, zero standard and all blanks.

Table 3.2: Calculated amounts (pg per sample) for all detectable steroids in each blow sample across Phase 1 (batches 1 and 2) and Phase 2 (batches 3 and 4), related to contemporaneous calibration curve. Amounts are presented for peaks corresponding to each steroid that were classified as 'probable' (denoted by *) or 'definite', and amounts that were below the LLOQ are placed in brackets. F – cortisol, E – cortisone, DHEAS – dehydroepiandrosterone sulphate, T – testosterone, A4 – androstenedeione, P – progesterone, E1 – estrone, E2 – estradiol.

Phase	Batch	Sample	F	E	DHEAS	T	A4	P	E2	E1
1	1	2018_1	10.0							
1	1	2018_2	6.2*							
1	1	2018_3	5.5*	Not included in method						
1	1	2018_4								
1	1	2018_5								
1	2	2019_1		2.2	220.0	(4.2)*		61.0		
1	2	2019_2	6.8		16.4*	6.7		10.4		
1	2	2019_3	8.6*	5.0*	107.0*			18.7	6.4*	(0.9)
1	2	2019_4				(2.8)*		7.5		
1	2	2019_5				(3.3)		10.8		
1	2	2019_6			5.4*	6.8		8.2		
1	2	2019_7	7.2*		10.5*	(2.9)		10.3	(2.4)*	
1	2	2019_8	10.0		93.0	(2.0)*		6.9		
1	2	2019_9			60.0	(2.4)*	(1.1)	14.7		
2	3	2021_1						(0.3)*		
2	3	2021_2		(0.4)*				(0.9)		
2	3	2021_3						(0.5)*		
2	3	2021_4			(2.1)			(0.4)		
2	3	2021_5			(0.5)*			(0.5)		
2	3	2021_6						(0.4)		
2	3	2021_7		(0.4)*			0.6*	(0.7)		
2	3	2021_9					0.6*	(0.9)		
2	3	2021_10						(0.3)*		
2	3	2021_11						(0.7)		
2	3	2021_12			(0.1)*			1.4	(2.0)	
2	3	2021_13		(0.7)*	(3.2)*			1.6		
2	3	2021_14						(0.5)*		
2	3	2021_15			(4.7)			2.1	(0.8)	
2	3	2021_16		2.1*				2.3		
2	3	2021_17			(7.1)*			1.4		
2	3	2021_19		1.1*	35.3			(0.5)		
2	3	2021_20						1.4		
2	3	2021_21						(0.7)		

Table 3.2 continued

Phase	Batch	Sample	F	E	DHEAS	T	A4	P	E2	E1
2	3	2021_22	30.1	4.6	175.0		0.5*	4.9		35.6
2	3	2021_23		1.4*	423.0			2.6		
2	3	2021_24		1.1				(0.8)		
2	3	2021_26				0.8*		(0.6)*		
2	3	2021_27		1.5*			0.8*	2.1		
2	3	2021_29						(0.6)		
2	4	2021_18			19.2			1.3		
2	4	2021_25		0.7*	14.8		(0.4)*	1.1*	3.8	
2	4	2021_28			20.5*			1.7		
2	4	2021_30			40.7			2.4		
2	4	2021_31			(3.4)*			4.2		
2	4	2021_32					(0.4)*	(0.4)*		
2	4	2021_33			12.0			(0.7)		
2	4	2021_34			36.9		(0.4)*	15.8		
2	4	2021_35	(0.72)*					1.3		
2	4	2021_36						(0.4)		
2	4	2021_37			(0.9)			(0.2)		
2	4	2021_38					0.4*	1.7		
2	4	2021_39					(0.2)*			
2	4	2021_40						(0.6)*		
2	4	2021_41			(1.3)					
2	4	2021_42						(0.2)*		
2	4	2021_43						(0.6)*		
2	4	2021_44					1.7	(0.8)		
2	4	2021_45						1.4		
2	4	2021_46			(3.7)*			34.7		
2	4	2021_47			18.7	0.7*		1.8		
2	4	2021_48				1.1*		1.5		
2	4	2021_49			106.0	1.2	0.7*	(0.8)*		
2	4	2021_50				1.0*		1.2		
2	4	2021_51						(0.6)*		
2	4	2021_52				1.1*				
2	4	2021_53								
2	4	2021_54	(0.72)*							
2	4	2021_55				0.7*		1.5		

3.5 Phase 2: applying the method to an ecological question

In Phase 2, I aimed to use the method developed in Phase 1 to determine whether humpback whales exhibited physiological stress in response to elevated whale-watching activity and vessel traffic. Therefore, stress-related hormones were of primary interest. In Phase 1, I detected cortisol (7/14 samples), its inactive form cortisone (2/9 samples) and DHEAS (7/9 samples). Rates of steroid detection were low but blow sampling paired with LC–MS/MS demonstrated high potential to characterise humpback whale steroid hormone profiles. Moreover, given the high, variable dilution and small sample volumes, I concluded that steroid hormone ratios, determined via LC–MS/MS, were appropriate for inter-sample comparisons for this sample type.

The ratios cortisol:DHEAS and cortisol:cortisone were of particular interest. Cortisol:cortisone represents the ratio of the active to the inactive form of the stress-related hormone, and is used in mammalian studies to infer physiological stress at various timescales (López-Arjona et al., 2020; La Marca-Ghaemmaghami et al., 2013; Shimanoe et al., 2021). Meanwhile, cortisol:DHEAS has been related to stress and trauma in mammals (Fels et al., 2019; Gundlach et al., 2018; Murialdo et al., 2001; Qiao et al., 2017). To my knowledge, these ratios have been seldom applied to cetaceans applied to cetaceans for stress assessments. To better understand the steroid profile of each animal and blow samples generally, I continued to screen for the full panel of 14 steroid hormones.

3.5.1 Phase 2 methods

Due to the inconsistent detection of steroids in blow samples in Phase 1, changes were made to the sample collection protocol and LC–MS/MS method to maximise the sensitivity of the method. These changes were based on earlier experience in the field and laboratory, and were not rigorously tested beforehand due to constraints in time and resources. In this way, despite the targeted ecological question, this phase remains exploratory. These changes are outlined below.

Collection of Phase 2 blow samples

Samples were collected from all four sites in summer 2021: two areas with higher vessel traffic and whale-watching activity – Skjálfandi Bay and Eyjafjörður – and two areas with lower vessel traffic and whale-watching activity – Ísafjarðardjúp and Húnaflói (Figure 3.1). A combination of land and vessel platforms was used, depending on the distance between the target whales and accessible coastline, and access to a research vessel. In Skjálfandi Bay, samples were collected from a 4.5 m rigid inflatable boat (RIB) and a 13 m sailing vessel; in Eyjafjörður, samples were collected from land and a 5.5 m RIB; in Húnaflói, samples were collected from land and an 18 m whale-watching vessel; and in Ísafjarðardjúp, all samples were collected from land.

Sample collection was generally performed according to the protocol developed in Phase 1, using the same UAV and an intermediary frame altered to minimise vibrations (designed with TinkerCAD 3D printing software[2]). However, in 2021, we attempted to collect samples from multiple blows from the same whale during a single surfacing sequence, particularly if sample quality from the first blow appeared to be poor (deduced from UAV video feed). This was achieved by: flying through the blow during the first attempt; flying ahead of the whale; moving to the side and directly backwards (not over the whale) until the whale was clearly in view: and attempting to fly through and capture a second blow onto the same Petri dishes. As in Phase 1, behaviour was monitored before, during and after sample collection, and sample collection was terminated upon signs of disturbance. Sample storage followed the same protocol as Phase 1. A 1 mL seawater sample was collected during each sampling session (day). To improve our ability to identify sampled whales, which was poor in Phase 1, the UAV was also used to collect identification images (extracted from video footage) of the whale's dorsal fin and ventral tail flukes (by placing the UAV behind the whale).

For Phase 2, we additionally attempted to collect ancillary information on body condition and length using aerial imagery, in order to provide demographic context to steroid hormone profiles. This was performed with a separate UAV. However, blow samples and aerial images were often not both collected for the same whale, which prevented the global use of this information in blow sample analysis.

Steroid extraction from Phase 2 blow samples

The extraction procedure itself remained unchanged from Phase 1 (ethanol–water wash solution, 50:50 v/v, 3 mL). However, to increase steroid concentrations, I assessed the feasbility of reconstitution volumes of 70:30 (v/v) water–methanol lower than 200 µL. In a preliminary experiment, I repeated the dish extraction procedure with mock samples and reconstitution volumes at 80, 100, 150 and 200 µL, each in triplicate. I then assessed peak area responses for cortisol, testosterone and progesterone (20 ng of each per mock sample), and their respective corresponding internal standards d_4-cortisol, $^{13}C_3$-tetosterone and d_9-progesterone. I calculated the mean and residual standard deviation (RSD, %) of each peak area for each reconstitution volume. A volume of 80 µL consistently produced higher peak areas (twice as large as the 200 µL volume) and lower or comparable RSD values (F: 3.0%; d_4F: 2.4%; T: 1.8%; $^{13}C_3$-T: 0.2%; P: 8.3%; d_9P: 10.1%). Therefore, 80 µL was used for sample reconstitution from this point.

As in Phase 1, a set of calibration standards (including a zero standard) was used to quantitate steroids and determine the LLOQ. The calibration standards were constituted from a standard mix, containing the same 14 steroids, as in Phase 1, but with physiologically relevant concentration ranges, developed to correspond with human saliva steroid reference ranges. The proportion was consistent across the calibration range, with the highest standard containing the most DHEAS (10 ng); F, E, B, DHEA, P (1 ng); T, A4 (0.4 ng); DHT, Aldo, E2, E1, E3 (0.1 ng). For cortisol, ten non-zero standards ranged from 0.001 to 1 ng (two additional, lower standards compared with Phase 1).

2. TinkerCAD: https://www.tinkercad.com/

Samples and standards were processed in two batches (Batch 3 and Batch 4), with the intention of running each batch separately through the LC–MS/MS instrumentation (due to the large number of samples). Batch 3 contained calibration standards (extracted from Petri dishes), blow samples 2021_1–29 and two seawater samples. Batch 4 contained calibration standards, blow samples 2021_30–55 and four seawater samples. Initially, both batches were extracted from Petri dishes and reconstituted in the same way, and transferred to glass LC–MS vials instead of a polypropylene 96-deep well plate. For Batch 3, no further changes were made. However, large salt residues were visible in several reconstituted samples in Batch 4, likely due to differences in sample quality and repeated collection onto the same Petri dishes from the same animal. As the season progressed, more samples were collected from multiple blows and UAV positioning over the blowhole became more precise as the UAV operator's skills improved. Therefore, prior to LC–MS/MS analysis, all Batch 4 standards, samples and blanks were centrifuged at 2000 rpm for 20 minutes in glass vials. After the liquid was transferred to glass LC–MS vials, the salt residues were considerably reduced but not eliminated. Therefore, the suspended liquid of all blow and seawater samples (but not calibration standards) was re-transferred to a second LC–MS vial.

Steroid analysis of Phase 2 blow samples

Between Phase 1 and Phase 2, LC–MS/MS instrumentation was upgraded and laboratory protocols for steroid hormone assays were updated in the Mass Spectrometry Core laboratory. Therefore, different LC–MS parameters were used for the Phase 2 assay (Appendix F). LC–MS/MS was performed using an Acquity I-Class (Waters, Wilmslow, UK) system (which replaced the Nexera uPLC from Phase 1) interfaced to a QTrap 6500+ mass spectrometer. Chromatographic separation of 20 µL of injected sample was achieved using a Kinetex C18 column (150 x 2.1 mm; 2.6 µm) fitted with a KrudKatcher Ultra In-Line Filter (0.5 µm porosity). Using mobile phases of 0.05 mM ammonium fluoride in water (mobile phase A) and 0.05 mM ammonium fluoride in methanol (mobile phase B), at a flow rate of 0.3 mL/min, the run started at 50% mobile phase B, holding for four minutes, rising to 75% at 9 minutes and 100% at 10 minutes, holding until 12 minutes, falling to 50% at 12.1 minutes and ending at 16 minutes. The mass spectrometer was operated using the same parameters for IonSpray, curtain gas and ion source gases as Phase 1. Steroid-specific retention times and mass spectrometric parameters are given in Appendix F. I attempted to apply LC–MS/MS to samples and standards as extracted in two batches.

3.5.2 Phase 2 results

Blow sample collection

In total, 55 blow samples were collected across the four sites (Appendix G): 17 in Skjálfandi Bay, all from a vessel; 7 in Eyjafjörður (6 from a vessel, 1 from land); 11 in Ísafjarðardjúp, all from land; and 20 in Húnaflói (17 from land, 2 from a vessel). One sample (2021_13) was collected by hand in Skjálfandi Bay, from a curious whale (MN313) that made repeated approaches to the stationary research vessel (4.5 m RIB) with the engine off and exhaled within 1 m of the vessel. Samples were collected in July ($n = 13$ samples), August (19) and September (23), and up to 4.2 km from the observer (Appendix G).

Samples were collected from 29 known whales, multiple samples were collected from 14 whales (two samples each from eight whales, three samples each from six whales) and six samples were collected from whales with unknown identification (Appendix G). Unlike the 2018/19 blow sample attempts, many sampled whales in 2021 were deep feeding and easier to sample due to their surface behaviour, with a large number of breaths per surfacing sequence (generally six or more) and shorter distances travelled between dives. Deep feeding was observed from August 2021 onwards. Moreover, it was easier to collect multiple blows per surfacing sequence from deep-feeding whales. Whales were also observed travelling, resting and surface feeding. Surface feeding whales were the most difficult to sample, as they typically only breathed once per surfacing sequence.

Two discernible responses to blow sampling attempts involving different whales were observed. Both occurred in Skjálfandi Bay (sample 2021_3 on July 8th and sample 2021_5 on July 9th) and were visible as a clear tail swish, with splashing at the surface. From the first response, a 'trumpet' blow was also heard, as a loud and high-pitched exhale. Furthermore, on August 19th in Skjálfandi Bay, whale MN335 did not respond to a successful blow sampling attempt (sample 2021_32) but did respond negatively to a subsequent aerial imagery attempt, with clear tail swishing.

LC–MS/MS analysis

Out of 55 samples, 54 were carried forward for LC–MS/MS analysis; sample 2021_8 was damaged during transport from Iceland to the UK. As explained previously, due to excessive salt residues, Batch 4 samples were subject to centrifugation and an additional transfer steps to eliminate salt residues. This challenge was not encountered during Phase 1, likely due to the higher reconstitution volume.

Moreover, the LC–MS/MS analyses themselves presented challenges. During LC–MS Batch 3 (samples 2021_1–29), the LC system was blocked three times (during samples 2021_18, 25 and 28). Therefore, these samples were transferred to Batch 4 and the seawater samples from Batch 3 were excluded from analysis (to avoid further blockages). Subsequently, LC–MS Batch 4 (samples 2021_30–55, 18, 25 and 28; six seawater samples) stopped during a calibration standard. Upon inspection, the capillary was fuzzy and the mass spectrometer's curtain plate was coated in salt. The capillary was changed and the curtain plate was cleaned with water and methanol. Following a subsequent blockage at blow sample 2021_46, the KrudKatcher (a filter placed at the front of the liquid chromatography analytical column) was replaced and the remaining blow samples were transferred to another LC–MS vial to further eliminate salt residues. Eventually, all 54 samples were analysed by LC–MS/MS for the full run time of 16 minutes. Retention times were generally consistent across standards and samples for internal standards and steroids. The retention times of DHEAS and d_5DHEAS showed considerable drift but this was expected based on the results of Phase 1.

The LC–MS/MS outputs from the Phase 2 (2021) samples were considerably different to those from Phase 1 (2018/19) samples, in terms of the detection and strength of steroid peaks. Furthermore, there were differences in steroid signals between LC–MS batches 3 and 4. Therefore, I compared the performance of both batches with a practice run performed three days before Batch 3, consisting of a set of extracted calibration standards (Appendix H). Internal standard (IS) peak areas were lower for batches 3 and 4 than the practice batch, and were generally less consistent across calibration standards. Meanwhile, for the samples themselves, IS peak areas were more variable in Batch 4. In addition, Batch 4 appeared to have poorer sensitivity for both cortisol and cortisone than Batch 3, owing to higher LLOQ values (Figure 3.8) and lower steroid peak areas for each calibration standard (Appendix H).

Blow sample steroid hormone contents

The following steroids were detected in blow samples: cortisol, cortisone, testosterone, androstenedione, DHEAS, progesterone, estradiol and estrone (Table 3.2 and Figure 3.8). In total, 53 out of 54 blow samples had at least one discernible steroid peak (classified as 'probable' or 'definite'). However, some of these peaks (e.g., progesterone) were also visible in seawater samples. For the remaining steroids, no clear peaks were detected. The visible quality of each blow sample did not appear to reflect steroid contents determined by LC–MS/MS.

A cortisol peak was only classified as 'probable' in two blow samples and 'definite' in one sample from Phase 2 (Table 3.2 and Figure 3.8). For the majority of samples (45/54), no peak was detected for cortisol. There were also no visible peaks in seawater samples or blanks. Only one sample with a clear peak (probable or definite) had a calculated amount greater than the LLOQ (sample 2021_22, 30 pg). The LLOQ of Batch 4 (10 pg) was ten times poorer than Batch 3 (1 pg). Cortisol signals were less frequently detected in the Phase 2 (2021) blow samples than the Phase 1 (2018/19) blow samples.

A cortisone signal was more apparent in 2021 samples than cortisol, particularly Batch 3 (Table 3.2 and Figure 3.8). Cortisone peaks were classified as 'probable' in eight samples and 'definite' in two samples. The majority of samples still had no visible peak (31/54). There were no visible peaks in seawater. A peak was visible in one solvent blank from Batch 3 (the final run of the batch), although the peak height and area were lower than quantifiable sample peaks. A total of six clear sample peaks had a calculated amount greater than the LLOQ (Batch 1: LLOQ = 1 pg, mean = 2 pg, range = 1.5–4.6 pg), and the highest amount was 4.6 pg for sample 2021_22. There were apparent differences between batches: the LLOQ was higher (less sensitive) in Batch 4 (2.5 pg) than Batch 3 (1 pg) and no samples were quantifiable in Batch 4. In Batch 3, all clear peaks greater than the LLOQ were from samples collected between August 4th and August 9th, from Ísafjarðardjúp (5) and Húnaflói (1).

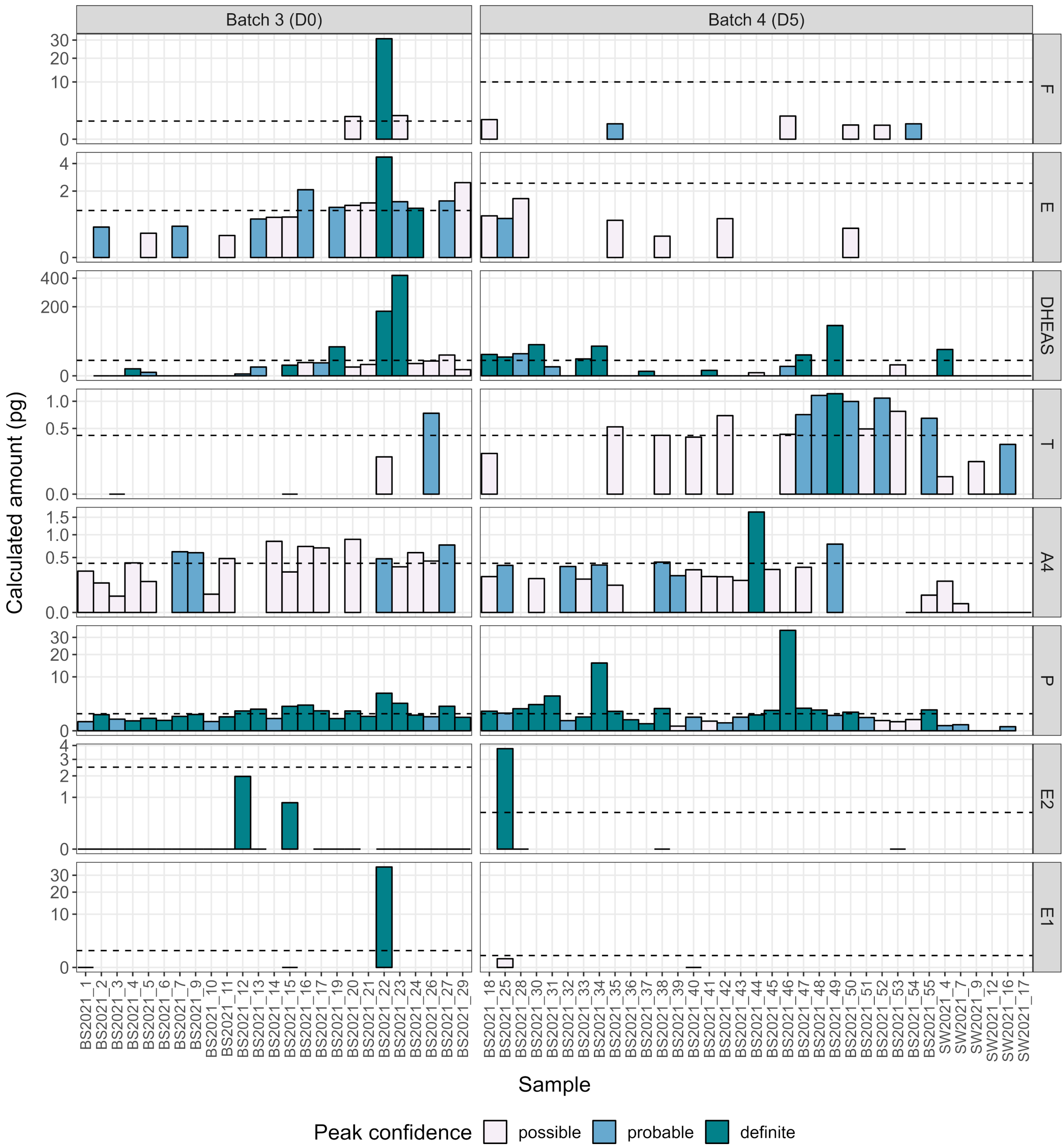

Figure 3.8: Calculated amount (pg per sample) of eight detected steroids from blow samples from Phase 2 – Batch 3 (blow samples 2021_1–29) and Batch 4 (blow samples 2021_30–55, 18, 25 and 28, and six seawater samples, run five days later). The detected steroids were cortisol (F), cortisone (E), dehydroepiandrosterone sulphate (DHEAS), testosterone (T), androstenedione (A4), progesterone (P), estradiol (E2) and estrone (E1). The lower limit of quantification (LLOQ) for each steroid and batch is denoted by the horizontal black dashed line. Calculated amounts are only presented for visible peaks. BS – blow sample, SW – seawater.

The majority of blow samples had a clear DHEAS signal, with peaks classified as ‘probable’ in 14 samples and ‘definite’ in 27 samples (Table 3.2 and Figure 3.8). Only two samples had no visible peak. The zero standard also had a clear peak (which was incorporated into the calibration curve) as did each seawater sample. However, only one seawater sample had a calculated amount greater than zero (2.0 pg) and this was five-fold lower than the LLOQ (10 pg). In contrast, 12 blow samples with clear peaks had a calculated amount greater than the LLOQ (mean = 80.2 pg), with values ranging from 12.0 pg (sample 2021_33) to 423 pg (sample 2021_23). Clear, quantifiable peaks were distributed across both batches and all four locations.

Peaks at the retention time of testosterone were classified as ‘probable’ in six samples and ‘definite’ in one sample, and the majority of samples had no visible peak (36/54; Table 3.2 and Figure 3.8). One seawater sample had a clear peak, although this was lower than the LLOQ (0.4 pg in both batches), and no blanks had a visible peak. All blow samples with a clear testosterone peak had a calculated amount greater than the LLOQ of 0.4 pg (mean = 0.97 pg): one sample from Batch 3 and five samples from Batch 4. In Batch 4, all quantifiable peaks were collected between September 11th and September 18th (the final sampling date): one from Húnaflói, one from Skjálfandi Bay and four from Eyjafjörður (three whales). Calculated amounts were similar, ranging from 0.7 pg (sample 2021_55) to 1.2 pg (sample 2021_49).

Eleven blow samples had a clear peak (ten probable and one definite) corresponding to androstenedione, while 17 samples had no visible peak. From seven samples with calculated amounts above the LLOQ of 0.4 pg across both batches, values ranged from 0.4 pg (sample 2021_38) to 1.7 pg (sample 2021_44; mean = 0.8 pg). There were no clear peaks in any seawater samples or blanks. Clear, quantifiable peaks were spread across both batches.

Progesterone showed the most consistent apparent signal across blow samples, with peaks classified as ‘probable’ in 12 samples and ‘definite’ in 37 samples (Table 3.2 and Figure 3.8). No sample had no visible peak. Three out of the six seawater samples had a ‘probable’ peak, but their calculated amounts were at least ten-fold lower than the LLOQ of 1 pg. In contrast, 22 blow samples had a calculated amount of progesterone greater than the LLOQ (mean = 3.96 pg), with amounts ranging from 1.1 pg (sample 2021_25) to 34.7 pg (sample 2021_46). Clear, quantifiable peaks were spread across both batches and all four locations.

Estradiol peaks were classified as ‘probable’ in three samples and ‘definite’ in 15 samples (Table 3.2 and Figure 3.8). The majority of samples (28) had no discernible peak. No seawater samples or blanks had a visible peak. However, there was a small peak in the zero standard, which was included in the calibration curve. From this, only one clear peak had a calculated amount greater than the LLOQ (2.5 pg in Batch 3, 0.5 pg in Batch 4), at a value of 3.8 pg (sample 2021_25). Finally, estrone peaks were classified as ‘probable’ in no samples ‘definite’ in sample 2021_22, with a calculated amount of 35.6 pg.

From these steroid contents, blow sample 2021_22 had a particularly high number of detected steroids and calculated amounts: 30.1 pg cortisol, 4.6 pg cortisone, 175 pg DHEAS, 4.9 pg progesterone, 0.4 pg androstenedione and 35.6 pg estrone. To rule out possible contamination by a standard mix of steroids and internal standards, I calculated hormone ratios of the sample and compared it with that of the standard mixture of steroids. The ratio of cortisol to cortisone is 1:1 in the standard mix, but is 6.7:1 in this sample; and the ratio of DHEAS to progesterone is 10:1 in the standard mix, but is 36:1 in the sample. Therefore, the steroid contents of sample 2021_22 are not due to contamination by the standard mix.

Impact of vessel traffic on blow steroid hormone ratios

I originally planned to use steroid ratios, in particular cortisol:cortisone and cortisol:DHEAS, to normalise steroid concentrations in an unknown volume of blow and investigate differences between areas of varying whale-watching activity and vessel traffic. However, cortisol, cortisone and DHEAS were not consistently detected in blow samples; as such, it was not possible to determine the impact of vessel traffic on steroid hormone ratios in the blow samples. There were apparent differences in steroid contents between samples, areas and time periods, but these could not be verified through quantitative analysis. For example, the testosterone signal appeared to be stronger in samples 2021_47–55 (which were collected in Skjálfandi Bay and Eyjafjörður in September), and the cortisone signal appeared to be stronger in samples 2021_19–24 (which were collected in Ísafjarðardjúp on August 4th). Due to the aforementioned challenges in running LC–MS/MS batches, and the inconsistent presence of steroid hormone peaks, it was not possible to determine the relevance, ecological or otherwise, of these stronger signals.

3.6 Discussion

To my knowledge, this study represents the first pairing of UAV-based collection and LC–MS to detect a panel of steroid hormones in cetacean blow. We collected 87 humpback whale blow samples across three years in the sub-Arctic waters of North Iceland, demonstrating the feasibility of UAV-based sampling from both vessel and land platforms, at a distance of up to 4.2 km from the UAV operator. From these samples, 68 were carried forward for LC–MS/MS analysis (14 in Phase 1, 54 in Phase 2). In Phase 1, samples were used to develop a method for extraction and steroid hormone detection. Across all sampling years, eight steroids were detected, including stress-related and sex hormones, although detection rates were low. In Phase 2, the method from Phase 1 was modified and applied to characterise differences in hormone profiles between areas with higher and lower whale-watching activity and vessel traffic in North Iceland. Ultimately, it was not possible to answer this ecological question: hormone detection rates remained too low and modifications to the method hindered comparison with Phase 1 samples. Furthermore, this method was applied without proper physiological and biological validation. Nevertheless, these results confirm that blow contains numerous steroid hormones that may provide key information about the physiological state of individuals. With future improvements and validation, blow sampling has the potential to enable dynamic physiological assessment of large whales. This work was achieved in collaboration with a clinical research facility for LC–MS analysis and a 3D printing studio to create the blow sampling frame, and I encourage such partnerships to expand the toolkit of conservation physiology.

3.6.1 Feasibility of collection

UAVs are increasingly used in cetacean research, with applications including photo-identification (Koski et al., 2015), photogrammetry (Christiansen et al., 2016a) and behavioural observation (Fiori et al., 2017; Torres et al., 2018). In line with previous research (Atkinson et al., 2021; Costa et al., 2023; Pirotta et al., 2017), our study supports the use of UAVs for feasible blow sampling, with 87 humpback whale samples collected over three seasons. Moreover, we have demonstrated that samples can be readily collected from land, incurring lower costs and carbon footprint. Previous studies have used a vessel platform to collect samples via a long pole or UAV (Atkinson et al., 2021; Burgess et al., 2018; Hogg et al., 2009), but this may not be necessary in coastal areas with reliable whale occurrence. Moreover, a land base appeared to increase the rate of successful sampling attempts, perhaps due to the higher elevation of the deployment/observation site (compared with a small vessel). Collecting samples from land did present a challenge in terms of identifying whales, but the use of the UAV camera to collect dorsal fin and fluke images considerably improved photo-identifcation success. A modified DJI Phantom 4 UAV was found to be a suitable in terms of speed, manoeuvrability and live video quality.

Generally, as the 2021 collection period progressed, sample quality improved in terms of droplet number and the procedure of collection that increased the number of blows per sample (Appendix G). This was likely driven by: i) the UAV operator's improving ability to collect samples (similar to Burgess et al., 2018; Costa et al., 2023); and ii) a general change in whale behaviour from surface feeding to deep feeding as summer and autumn progress in Icelandic waters (Rasmussen, pers. comm.). Deep-feeding whales take longer dives and more breaths per surfacing sequence (Goldbogen et al., 2008), which provides more time for the UAV operator to locate the animal and enables the collection of multiple blows per surfacing sequence. For this reason, collection attempts appeared to be more successful in Eyjafjörður and Ísafjarðardjúp, where sampling effort was concentrated later in the season and the majority of encountered whales were deep feeding, and less successful in Skjálfandi Bay, where sampling effort was concentrated earlier in the season and the majority of whales were surface feeding. Where appropriate, future blow sampling studies in feeding grounds should target deep-feeding animals. It is important to note that sample quality during visual inspection may not reflect the actual quality of the collected sample, as considerable evaporation can take place during UAV retrieval (i.e., up to several minutes of flying with open Petri dishes); a remotely controlled flip lid (as in Pirotta et al. 2017) could circumvent this issue. Furthermore, to quantify sample quality, future research could involve sample volumetric measurements (provided that the sample is immediately sealed following collection), although these measurements could not be used to derive biologically relevant analyte concentrations due to variable dilution (Burgess et al., 2018).

Blow samples were collected via UAV with little observed disturbance of target animals. In accordance with previous blow sampling efforts (Acevedo-Whitehouse et al., 2010; Apprill et al., 2017; Atkinson et al., 2021; Domínguez-Sánchez et al., 2018; Pirotta et al., 2017), this study found limited behavioural responses from target whales to UAVs: no discernible responses were detected in 2018–19 and two were detected in 2021. Both responses in 2021 consisted of tail swishing, followed by an increase in swim speed, and were interpreted as disturbance (Fiori et al., 2019; Pitman et al., 2015). However, the absence of behavioural evidence does not preclude a physiological response (Walker et al., 2005a). For example, brown bears exhibit a clear cardiac response to UAVs (increase in heart rate), but rarely a behavioural response (Ditmer et al., 2015). Further investigation of these 'hidden' responses in cetaceans is necessary to properly interpret biomarker levels from samples collected by UAV, particularly regarding repeat sample collection. Of note, whilst collecting aerial images of humpback whales with the same UAV model, from a height of 20 m, at least four whales exhibited a clear response (tail swishing, an increase in swim speed and early diving), including two animals that were previously blow sampled without a response. This supports a precautionary approach to UAV operation around cetaceans, in accordance with Hodgson and Koh (2016). The apparent differences may be related to differences in underwater noise transmission, with the UAV spending more time directly above the whale for aerial images than blow sampling (Laute et al., 2023).

3.6.2 Measuring steroids in blow samples by LC–MS/MS

A major aim of this study was to characterise the steroid hormone contents of whale blow, both to determine the impact of vessel traffic on whale physiology and to advance the field of blow sampling generally. Previous studies assessing hormones in whale blow have primarily used samples collected by hand, from restrained animals (Mingramm et al., 2019a; Thompson et al., 2014), or using a long pole from a boat (Burgess et al., 2016; Dunstan et al., 2012; Hogg et al., 2009; Hudson et al., 2021). As a collection device, UAVs provide a lower surface area for collection, thereby reducing the size of samples that are already small and ultra-dilute (Pirotta et al., 2017). Nevertheless, steroids have been detected in samples collected by UAV with immunoassays (Atkinson et al., 2021) and my results support the use of this safer, less obtrusive collection method (Johnston, 2019).

Whilst hormones in whale blow have been primarily detected and quantified with readily available immunoassays (e.g., Mingramm et al., 2019b; Thompson et al., 2014), this method limits the number of steroids that can be analysed per sample and, therefore, our ability to comprehensively monitor physiology and derive informative hormone ratios (Cross and Hornshaw, 2016). Given the potential for hormone ratios to circumvent the issue of variable dilution in blow samples, this is of particular concern. Therefore, I employed LC–MS/MS, which can be developed to enable parallel detection of a panel of steroids within the same method (Dalle Luche et al., 2020; Dunstan et al., 2012; Soldin and Soldin, 2009). LC–MS/MS has already been used to screen a panel of 11 steroids in humpback whale blubber (Dalle Luche et al., 2019), 10 steroids in grey whale blubber (Wittmaack et al., 2022) and 12 steroids in captive cetacean plasma (Legacki et al., 2020).

Analytical protocols in this study were adapted from previous studies. Sample extraction was modified from Burgess et al. (2016) and I used an LC–MS/MS method developed for clinical and other ecological studies (e.g., Ludwig et al., 2022). Steroid hormones are highly conserved across mammalian taxa (Lasley and Kirkpatrick, 1991), enabling the application of methods developed for other species to novel cetacean samples. More generally, collaboration with a clinical research group provided access to state-of-the-art equipment and technical expertise. I encourage stronger consideration of such interdisciplinary partnerships to augment the 'conservation physiology toolkit' (Hunt et al., 2013; Madliger et al., 2018; Romero, 2004).

I screened for 14 steroid hormones and detected eight steroids across the three years of sampling ($n = 68$ samples). These included steroid hormones that have previously been detected in blow, such as cortisol, progesterone and testosterone (Burgess et al., 2018; Dunstan et al., 2012; Mingramm et al., 2019b); and steroids that have been characterised in cetaceans but not detected in blow, such as cortisone and corticosterone (Dalle Luche et al., 2019), as well as the androgen DHEAS that has only recently been detected in cetaceans (Béland et al., 2023). Comparing the success of this method with other studies was challenging because detection rates and negative controls (such as seawater samples and solvent blanks) were often not included in the published literature. However, detection rates in this study were low and were not improved by modifications to the sample preparation component in Phase 2. A clear cortisol peak was only found in 14% of samples (10/68), in comparison with: 86% detection rate in North Atlantic right whale blow, collected with a long pole and analysed with immunoassays (Burgess et al., 2018); 88% detection in humpback whale blow, collected with a long pole and analysed via LC–MS (Dunstan et al., 2012); and 66% detection in beluga blow, collected with a long pole and analysed via immunoassays (Hudson et al., 2021). Testosterone and progesterone peaks were clearly visible in 23% (15/63) and 90% (58/63) of samples respectively, compared with 86% and 78% in Burgess et al. (2018), and progesterone showed a consistent signal in all processed seawater samples.

The low apparent success of steroid detection may be explained by the samples of unknown volume collected via UAV and the low visible quality of many samples, particularly those collected at the beginning of 2021. Future research may explore the use of larger sampling devices (Burgess et al., 2018) and nano-spray uPLC systems to improve assay sensitivity (Hayden et al., 2017). In addition, the influence of sample storage conditions on steroid hormone contents should be investigated. For endocrinological assessment, it is generally recommended to freeze biological samples as soon as possible after collection (Miki and Sudo, 1998; Reimers et al., 1983). However, this is often not practical in the field; in this study, samples were stored at 5°C for two periods of up to 24 hours (during field storage and international transport) due to field logistics and financial constraints. Generally, steroid hormone (including cortisol) levels across different biological sample types are resilient to different field storage conditions, including: mock blow samples on ice (4°C) for six hours (Burgess et al., 2016); centrifuged human saliva samples at 5°C for three months (Garde and Hansen, 2005); and sea lion faecal samples exposed to outdoor conditions for five days (Mashburn and Atkinson, 2004). However, results are variable across studies: Lynch et al. (2003) found significant increases in baboon faecal hormone metabolites (including GCs) when stored at 15–25°C for one week, while Toone et al. (2013) found significant declines in testosterone and estradiol (but not cortisol) in human saliva samples after storage at 5°C for seven days. As a result, the suitability of the specific storage conditions (for a relatively understudied sample type) should be assessed. The apparent high seawater content in blow samples may also have limited detection rates; a high-salt environment (including sodium chloride) can lead to ion suppression of neutral steroids during the ionisation phase of LC–MS (e.g., through formation of salt adducts; Wu et al. 2009). This suppression should have been investigated during Phase 1 of this study, although no obvious suppression of the internal standards was observed. Whilst the sample extraction procedure was deliberately simple (i.e., ethanol–water wash) to retain as much analyte as possible, methods to eliminate matrix components (including salt), such as supported liquid extraction (Cheng and Jiang, 2019; Ludwig et al., 2022), should be considered for future blow sample extraction. The influence of environmental contamination beyond seawater could also be investigated, but this is considered unlikely due to limited atmospheric exposure and researchers wearing face masks when sealing and handling samples.

Nevertheless, the detection of eight steroids demonstrates the potential of blow sampling paired with LC–MS/MS for physiological monitoring. I strongly encourage the continued development of this method, with a particular focus on steroid hormone ratios, which could be insensitive to sample dilution, to provide biologically relevant information (Hunt et al., 2014a; Reckendorf et al., 2021). Below, I discuss each steroid that was detected in whale blow and available information on their roles in cetacean and mammalian physiology, with a particular focus on hormone ratios.

3.6.3 Steroid hormones in whale blow

Two glucocorticoids (GCs) were detected in blow samples: cortisol and cortisone. In vertebrates, activation of the hypothalamus–pituitary–adrenal (HPA) axis in response to perceived stress stimulates the release and production of GCs, which in turn mediate an array of adaptive physiological responses (Cockrem, 2013; Sapolsky et al., 2000). Cortisol is one of the more dominant steroids in circulation in humans; is generally the dominant stress-related hormone in cetaceans (Atkinson and Dierauf, 2018; Thomson and Geraci, 1986); and was detected in 10/68 blow samples, which was a disappointing result. Across diverse taxa, including cetaceans (Champagne et al., 2018), circulating cortisol levels respond to acute stress in a time frame of minutes to hours (Cockrem, 2013; DeRango et al., 2019; Walker et al., 2005b). Studies in captive belugas show that blow cortisol levels can reflect this response (Thompson et al., 2014). Chronic stressors can lead to persistently elevated cortisol (and other GC) levels and negatively impact individual fitness (MacLeod et al., 2018). For example, in cetaceans: cortisol levels in striped dolphin blubber and serum are elevated in chronically stressed animals (Agusti et al., 2022); faecal GC and blubber cortisol concentrations in mysticetes increase during periods of high vessel traffic (Lemos et al., 2022; Pallin et al., 2022; Rolland et al., 2012); GC levels are related to the severity of entanglement in fishing gear (Lysiak et al., 2018); and cortisol in mysticete earplugs (which is accumulated over months and years) even relates to regional long-term whaling pressure (Trumble et al., 2018).

Cortisone is the inactive form of cortisol, and their inter-conversion is regulated by the enzymes 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) and 11β-HSD2. The mammalian response of cortisone to perceived stress is less well characterised than that of cortisol but changes appear to be far smaller in magnitude (Shimanoe et al., 2021; Trondrud et al., 2022). For this reason, the ratio of cortisol to cortisone is used as a proxy for acute physiological stress, primarily in clinical studies (La Marca-Ghaemmaghami et al., 2013; Shimanoe et al., 2021; Taylor et al., 2013), and its suitability as a biomarker in blow should be investigated in terms of biological relevance and insensitivity to dilution. Cortisone has been detected in cetacean blubber (Dalle Luche et al., 2019; Wittmaack et al., 2022) but, to my knowledge, not blow samples. I detected cortisone peaks in 12/63 blow samples, with a small peak in one solvent blank, but no peaks in any other blank or seawater samples.

The peak of another stress-related hormone, the androgen DHEAS, was clearly visible in 48/63 samples. In humans, DHEA is conjugated to sulphate to form DHEAS, largely in the adrenal gland. DHEAS is the most concentrated steroid in circulation in humans; declines with age; and is thought to buffer the effects of GCs by having anti-ageing, immune-enhancing and neuroprotective properties (Maninger et al., 2009; Mocking et al., 2015; Whitham et al., 2020) The ratio of cortisol to DHEA or DHEAS has been related to chronic stress, trauma and mental health in humans (Butcher et al., 2005; Khanfer et al., 2011; Mocking et al., 2015; Murialdo et al., 2001; Qiao et al., 2017). However, it has received little attention in non-human endocrinology (Whitham et al., 2020). Gundlach et al. (2018) found that cortisol:DHEA values were higher in diseased phocids, while cortisol:DHEA in pig saliva was lower in animals in improved housing conditions (Fels et al., 2019). DHEA variability has been explored in killer whales and bottlenose dolphins (Legacki et al., 2020; Robeck et al., 2017) and cortisol:DHEA reference values have been derived for four captive cetacean species (Miller et al., 2021), but not used to infer physiological stress. Finally, Béland et al. (2023) recently validated an immunoassay method to measure DHEAS in narwhal serum (to my knowledge, one of the first studies to detect DHEAS in cetaceans) and found that measured cortisol:DHEAS ratios increased from the beginning to the end of a capture–tagging procedure (mean time interval = 18.3 min, range = 12–28 min), although variable storage conditions could have influenced steroid measurements. DHEAS was the most concentrated steroid in this study (mean amount = 80.2 pg from Phase 2) and I strongly encourage investigation of the physiological role of DHEAS in cetaceans and the use of cortisol:DHEAS as a biomarker for stress (Whitham et al., 2020).

Beyond stress, several sex hormones were detected, including the androgens testosterone (15/63 samples) and androstenedione (11/63), progesterone (58/63) and the estrogen estrone (2/63). A clear peak corresponding to estradiol was detected in 22/63 samples, but the source of these peaks is unclear as 15 samples had a peak area lower than that detected in the zero calibration standard. In accordance with other mammals, humpback whale testosterone levels are higher in males (Hunt et al., 2019) and increase in the breeding season (Cates et al., 2019). Testosterone and androstenedione can also act as biomarkers of pregnancy in humpback whales (Dalle Luche et al., 2020), as can progesterone (Clark et al., 2016), a hormone typically associated with pregnancy and parturition in mammals. In North Atlantic right whale blow, urea-normalised progesterone levels were highest in pregnant females and testosterone was generally higher in mature animals than juveniles (Burgess et al., 2018). Furthermore, faecal androgen:estrogen ratios have been used to determine sex and reproductive status in North Atlantic right whales (Rolland et al., 2005). Whilst not related to physiological stress *per se*, these hormones provide important context for interpreting stress-related hormone levels – for example, cortisol concentrations often differ between sexes and life history stages (Tilbrook et al., 2000), including in baleen whales (Hunt et al., 2006, 2014b). However, in this study, the detection of a progesterone peak in all seawater samples and testosterone in one seawater sample may hinder the use of blow sampling to investigate sex hormones. These signals could reflect genuine hormone presence in seawater, which is generally attributed to sewage discharge and agricultural run-off, although detectable levels have also been documented in open-ocean and offshore environments (Atkinson et al., 2003; Liu et al., 2015). Alternatively, these peaks may represent analytical method interference (Burgess et al., 2016; Richard et al., 2017). Mingramm et al. (2019a) found that seawater produced false endocrine signals and likely amplified signals from blow samples when extracted from nylon collection material with ethanol, possibly due to seawater constituents interfering with the measurement assay (immunoassay) or facilitating extraction of chemical components from nylon fabric. However, Burgess et al. (2018) found no hormone (cortisol, progesterone, testosterone) or urea signal in seawater blanks extracted from Petri dishes using ethanol (comparable to the extraction method in this study) and measured by immunoassay. Therefore, the source of these seawater analyte signals requires further investigation in terms of the storage and extraction conditions that yield these peaks.

Unfortunately, some hormones included in the LC–MS/MS analysis were not detected. Peaks corresponding to aldosterone and corticosterone, which are potential stress indicators in cetaceans (Champagne et al., 2018; Lowe et al., 2021; Romano et al., 2004; Thomson and Geraci, 1986), were not detectable in any sample. This is unsurprising, given that aldosterone exists in lower circulating levels than cortisol (Thomson and Geraci, 1986) and is difficult to detect via LC–MS because it poorly ionises in a mass spectrometer; likewise, circulating levels of corticosterone are low in humans.

3.6.4 Applying blow steroid analysis to an ecological question

The ultimate goal of this study was to use blow sampling to quantitatively determine the physiological response of humpback whales to vessel traffic across North Iceland. Building on the findings of Phase 1, in Phase 2, I modified and applied blow sampling and steroid hormone measurement by LC–MS/MS to this ecological question. Several changes were made in an effort to increase the volume of blow collected and to concentrate the sample injected into the LC–MS, in order to improve sensitivity for these ultra-dilute samples. I hoped to use the ratios of stress-related hormones, such as cortisol:cortisone and cortisol:DHEAS, as biomarkers for stress that accounts for variable sample dilution.

From the blow samples collected in 2021 (Phase 2) across four areas, there were some apparent differences (Table 3.2 and Figure 3.8). For example: samples collected on August 4^{th} and 5^{th} from Ísafjarðardjúp, when high whale densities were observed (estimated 25 whales within 5–10 km^2), had a stronger apparent cortisone signal; samples collected after September 10^{th} had a stronger testosterone signal; and, compared with 2018/19 (Phase 1) samples, 2021 (Phase 2) blow samples had a weaker cortisol signal and were collected after a large decline in global vessel traffic and underwater noise due to the COVID-19 pandemic (the 'Anthropause'; Rutz et al. 2020), including in Iceland (Laute et al., 2022). However, I cannot quantitatively and/or confidently infer differences in steroid contents between samples, animals, areas and time points. Steroid detection rates were low: cortisol was our primary steroid of interest, and only one sample had a peak greater than the LLOQ; and cortisol was required to calculate ratios with two other stress-related steroids, cortisone and DHEAS. Additionally, methodological modifications between years and batches prevented meaningful comparison. Beyond deriving quantitative metrics, the physiological relevance of steroid hormone levels in blow remains largely unknown for mysticetes (Burgess et al., 2018; Mingramm et al., 2019a), preventing proper interpretation of the presence, absence and amount of steroids. Owing to these inconsistent protocols and the premature application of a frontier method, I was unable to answer my ecological question. Below, I discuss these shortcomings in an effort to promote the rigorous development of a method for conservation physiology.

Methodological consistency and optimisation

Between phases and even LC–MS batches, protocols were not entirely consistent, hindering comparability between samples (Madliger et al., 2021). Several changes were made at once without rigorous testing, so it was difficult to determine the impact of each alteration on LC–MS/MS outputs. First, changes were made to blow sample collection between phases 1 and 2 without testing their feasibility. In Phase 2 (2021), 22 samples were collected from multiple blows in an effort to increase sample volume, but this was not tested in Phase 1 and conferred no obvious improvement in steroid detection rates. This change should have been tested and optimised, with appropriate operator training (Hodgson and Koh, 2016), before applying the blow sampling method to an ecological question. In conservation physiology, method optimisation is often focused on analytical procedures rather than sample collection (Hunt et al., 2013).

Second, the sample extraction procedure and LC–MS parameters developed in Phase 1 were altered in Phase 2. A lower reconstitution volume was used to increase steroid concentration, which improved steroid peaks in a preliminary experiment but prevented comparison of steroid hormone contents between Phase 1 and Phase 2 samples. In addition, different LC column, mobile phases and MS parameters were used in Phase 2, due to updated LC equipment (the Shimadzu Nexera uHPLC was replaced with an Acquity I-Class system) and ongoing incremental improvements to the laboratory's analytical methods. For each improvement, the updated method was re-validated for human clinical samples (primarily saliva for the Phase 2 parameters) but not for blow samples. Blow represents a very different sample type to clinical samples such as serum, urine and saliva, in terms of both concentration and constitution. Again, since several changes were made at once, it was not possible to determine the causes of any changes in measured steroid profiles. Future methodological development should test the impact of each modification on all relevant sample types to facilitate genuine optimisation.

Third, this lack of testing and optimisation led to unforeseen challenges in Phase 2. Batches 3 and 4 stopped at several points, due to a blocked LC capillary, a saturated KrudKatcher or a coated curtain plate. This was likely driven, in part, by the high combined salt content of these blow samples. A maximum of 10 blow and seawater samples were analysed in a single batch in Phase 1, while this number was up to 35 in Phase 2. Furthermore, many samples were categorised as high quality or consisted of multiple blows, and had visible salt residues in the reconstituted solution, particularly in Batch 4. Therefore, Batch 4 samples were subject to additional extraction steps to eliminate salt residues (centrifugation and retransfer) that were not applied to Batch 3 and it was challenging to compare the steroid contents of the two batches. The comparability of samples with (likely) varying seawater content is further hindered by the possible ion suppression effects of salt (whose magnitude could vary with salt concentration) and the presence of steroid signals in seawater control samples. For example, (unquantified) apparent differences in cortisone and testosterone signals between Batch 3 and Batch 4 could be driven by variation in seawater content: higher salt content in Batch 4 blow samples, leading to ion suppression, could explain the lower cortisone signal; while the generally stronger testosterone signal in Batch 4 could be explained by higher seawater content which could produce false endocrine signals (hence the detection of testosterone in one seawater sample). This underscores the importance of exploring alternative or additional analyte extraction procedures, and future method development should incorporate mock blow samples with the approximate ion concentration of seawater to pre-empt such issues. In addition, methods such as supported liquid extraction (Cheng and Jiang, 2019; Ludwig et al., 2022) could eliminate salt residues and should be explored.

Physiological and biological validation

Prior to using biological samples for endocrine monitoring, biological and physiological validation should be performed (Palme, 2019; Touma and Palme, 2005; Whitham et al., 2020). Validation should be repeated for each novel study system, which is determined by the taxon, sample type, target steroids and ecological question (Touma and Palme, 2005). GC and other steroid hormone profiles are increasingly used to measure physiological stress for conservation purposes, but full validation is rarely presented in studies (Dantzer et al., 2014; Dickens and Romero, 2013). Strictly, physiological validation involves pharmacologically inducing changes in circulating steroid levels and evaluating whether these changes are reflected in measured concentrations (Touma and Palme, 2005). For example, an ACTH challenge stimulates an increase in cortisol (Dulude-de Broin et al., 2019). Biological validation, meanwhile, involves collecting samples before and after a known stressful event (e.g., capture) to evaluate the biological relevance of an established technique (Touma and Palme, 2005). Whilst physiological validation may not be strictly necessary (or possible for large whales), biological validation is essential to confidently interpret steroid hormone profiles (Palme, 2019).

Neither validation was performed within this study and there is little *a priori* information on the use of steroid hormones as reliable biomarkers for acute or chronic stress in baleen whales due to the challenges of studying large whale physiology at sea (Hunt et al., 2013). To my knowledge, physiological validation has not been achieved for blow samples, although the responses of circulating levels of steroids such as cortisol, aldosterone and corticosterone to ACTH challenge (acute stress response) have been documented in captive odontocetes (Champagne et al., 2018; St Aubin and Geraci, 1990; Thomson and Geraci, 1986). Furthermore, the mechanism of hormone transportation from blood into the respiratory tract is unknown, although passive diffusion is likely (Dellman and Eurell, 1998; Hogg et al., 2009). Limited biological validation has been performed: in captive beluga whales, cortisol levels in blow increased post-handling, in tandem with circulating levels, demonstrating an acute response (Thompson et al., 2014); and progesterone and testosterone levels varied with reproductive state and correlated with levels in plasma (Richard et al., 2017). Meanwhile, in captive bottlenose dolphin blow, the ratio of cortisol to progesterone increased during out-of-water examination (a possible stressor) but did not reflect cortisol variability in serum, possibly due to seawater in blow interacting with the nylon stocking collection material (Mingramm et al., 2019a). However, these results may not be representative of blow samples captured from mysticetes. Furthermore, there is inconsistent evidence for the response of circulating GCs to stress in cetaceans: cortisol responds to acute and chronic stressors in many circumstances, but not all (Atkinson et al., 2015; St. Aubin et al., 2013; Ortiz and Worthy, 2000; Romano et al., 2004). This mirrors the apparent complexity of the HPA response in other taxa (Busch and Hayward, 2009) and we need to better understand the scenarios in which specific hormones do and do not respond to stress. In particular, characterising the speed at which steroid hormone levels (in circulation and other biological samples) respond to stressors in mysticetes is crucial to determine the suitability of analytes and sample types to assess acute stress responses and chronic stress. Without this information for humpback whale blow, even with higher detection rates, it would have been difficult to interpret differences in measured analyte levels.

Proper validation of blow sampling for stress assessments also requires the determination of natural variability in circulating and blow steroid levels (Madliger and Love, 2014), such as diel oscillations. In mammals, the molecular clock associated with circadian rhythms is tightly coupled to glucocorticoid secretion by the HPA axis (Nader et al., 2010); combined with patterns in activity state, this drives cyclic diel variability in circulating glucocorticoid levels. Similar patterns have been found in captive cetaceans, including orcas, belugas and bottlenose dolphins (Houser et al., 2021; Schmitt et al., 2010; Steinman and Robeck, 2021). However, these patterns may differ in wild cetaceans (e.g., Hart et al. 2015) and have not been explored in mysticetes. Variability may also exist between demographic groups, including sex, age and life history stage (Reeder and Kramer, 2005), with serum cortisol concentrations in captive odontocetes generally higher in males and older animals (Houser et al., 2021; Miller et al., 2021; Steinman and Robeck, 2021), although sex and age differences in cortisol were not detected from humpback whale blubber samples across several studies (Mingramm et al., 2019b, 2020; Pallin et al., 2022). Within these general differences between groups and time points, considerable inter-individual variability is also likely to exist (Burgess et al., 2018; Houser et al., 2021; Mingramm et al., 2019b; Steinman and Robeck, 2021), such that the single-point data (one sample per animal) collected in this study are difficult to interpret (Lowe et al., 2021). Initial baseline data should be collected from a well-characterised population, such as North Atlantic right whales, for which demographics and environmental stressors are consistently monitored at individual and population levels (Corkeron et al., 2017; Frasier et al., 2007; Hamilton et al., 2007; Pace et al., 2021; Rolland et al., 2012). I encourage the continuation of initial efforts to characterise hormone variability in North Atlantic right whale blow (Burgess et al., 2018), and the comparison of multiple available sample types, such as blow and faeces (Burgess et al., 2017). The demographics of Icelandic humpback whales are poorly characterised, with limited information on sex or age in this study.

Whilst I was unable to determine the physiological stress response of humpback whales to whale-watching activity and vessel traffic, these stressors may be used for future biological validation. Vessels, including whale-watching vessels, are known to disturb humpback whales and other mysticetes by eliciting behavioural responses (Christiansen et al., 2013a; Currie et al., 2021; Schuler et al., 2019) and acoustically masking vocalisations (Cholewiak et al., 2018; Dunlop, 2019). Meanwhile, a growing body of evidence suggests that physiological impacts may exist for North Atlantic right whales (Rolland et al., 2012), grey whales (Lemos et al., 2022) and humpback whales (Pallin et al., 2022). However, the exact role of vessel traffic as a stressor remains uncertain (Ayres et al., 2012): humpback whale cortisol blubber did not respond to whale-watching vessel traffic in Alaska (Teerlink et al., 2018) and behavioural responses to whale-watching vessels vary between studies (Senigaglia et al., 2016). It would be difficult to use the study system in this chapter to infer physiological impacts of vessel traffic due to the large number of other differences (known and unknown) between each study site. Areas differ in terms of bathymetry and the composition of vessel traffic (vessel size, movement patterns, fishing activity etc.), and possible additional differences such as temperature and prey availability have been shown to influence glucocorticoid and other hormone levels (Ayres et al., 2012; Houser et al., 2011). Variability in local ecology may also drive different diel and seasonal patterns of HPA axis activity and other physiological processes, which must be characterised before performing an impact assessment.

3.7 Conclusion

The ultimate goal of this study – to answer an ecological question – was hampered by inconsistent methodologies, low detection rates and the absence of physiological and biological validation (Palme, 2019). Nevertheless, sharing these outcomes with the wider scientific community may help to advance the field of cetacean physiology and realise the potential of blow sampling in the future. As with most scientific disciplines, ecology and conservation are subject to publication bias (Begg, 1994; Lortie et al., 2007; Wood, 2020) – negative results are less frequently published and unsuccessful approaches are rarely shared. However, highlighting the aspects of an approach that were unsuccessful or required more consideration may prevent future studies from repeating the same mistakes (Pinfield et al., 2019). This is particularly important for 'frontier methods' such as blow sampling which remain relatively untested (Hunt et al., 2013; Madliger et al., 2018). Beyond academia, the challenges of applying such methods to answer conservation questions should be more openly communicated to conservation and policy-making communities (Cooke and O'Connor, 2010; Wood, 2020).

Despite its pitfalls, this study highlights the potential of blow sampling to form a key component of cetacean monitoring programmes (Hunt et al., 2013; Madliger et al., 2018). Whale blow was readily collected from vessel- and land-based platforms via UAV at relatively low cost, and samples contained a suite of steroid hormones related to stress and life history in mammals. Presently, there is still no established method for dynamic physiological assessment of large whales at sea, despite an increasing number, diversity and unpredictability of anthropogenic stressors (Gulland and Hall, 2007; Halpern et al., 2015; IUCN, 2020). As marine top predators, cetaceans are sentinels for ecosystem health (Fossi et al., 2012; Hazen et al., 2019; Moore, 2008), and monitoring animal physiology can detect and predict potential long-term impacts before they occur (Cooke et al., 2013; Wikelski and Cooke, 2006). Blow sampling has the potential to fill this monitoring gap: steroid hormones in blow may reflect short-term variability in circulating levels, including stress responses; collection via UAV enables unobtrusive and repeat sampling; and sensitive assays such as LC–MS/MS can detect a panel of steroid hormones at sub-nanogram levels. The use of steroid ratios is of particular interest and could simultaneously overcome the challenge of variable dilution and provide more information about physiological state, demonstrated by studies in humans and other mammals. Therefore, I encourage future efforts to improve and validate the blow sampling method and learn from my mistakes to advance marine mammal conservation.

Chapter 4

Spatiotemporal variability of humpback whale occurrence around North Iceland

4.1 Abstract

Characterising spatiotemporal patterns in species occurrence is essential to inform conservation status and predict species' responses to environmental change, allowing conservation measures to be preparatory and adaptive. For marine mammals, trends and relationships are frequently revealed through species distribution models (SDMs). SDMs are often fitted to expansive geographical areas to encompass large parts of a species' range, but trends derived from large-scale offshore models may not reflect local variability, particularly in oceanographically complex coastal areas.

In this chapter, I used two humpback whale sightings data sets to elucidate the association between the physical environment and offshore occurrence in North Iceland and determine whether trends in predicted offshore density reflect variability in coastal abundance. First, I applied an SDM to offshore sightings data, collected during shipboard line-transect surveys over a 28-year period (1987–2015), to determine the static and temporally dynamic environmental predictors of occurrence in North Iceland. Within this, I compared explanatory and predictive performance metrics of three model frameworks – generalised additive model (GAM), boosted regression tree (BRT) and a GAM–BRT ensemble – at two different cell sizes (5 km and 25 km). The final SDM was used to predict summer offshore density over a 14-year period (2006–2019), including novel years without survey effort. Second, I separately applied capture–recapture (CR) models to a long-term photo-identification data base from Skjálfandi Bay, collected on board whale-watching vessels, to estimate the number of whales visiting the Bay each summer during the same period (2006–2019). Three model frameworks were considered: Cormack–Jolly–Seber (CJS), Jolly–Seber–Schwarz–Arnason (JSSA) and multi-state open robust design (MSORD). Finally, I statistically related the SDM-derived and CR model-derived time series, accounting for the uncertainty of estimates with a bootstrap resampling approach, to determine whether trends in offshore density reflect coastal abundance.

The final SDM was a GAM fitted to a 25 km raster of sightings and environmental data. From this, key predictors of whale density included distance to coast; sea surface temperature; sea surface height and its spatial standard deviation; and seasonal variability in mixed layer depth (all $p < 0.005$). Predicted offshore density declined across 2006–2019 but these outputs should be treated cautiously, owing to large confidence intervals, spatial autocorrelation and limitations in assessing predictive performance. In contrast, coastal abundance estimates from a MSORD CR model robust increased significantly across the period ($p = 0.001$), with narrower confidence intervals. In line with limited evidence from previous research, a bootstrap approach revealed an apparent negative correlation between broad-scale offshore density and coastal abundance (negative Pearson's r in all bootstrap replicates). These contrasting trends suggest that coastal areas of North Iceland may be increasingly important for feeding humpback whales, with consequences for population exposure to whale-watching and other growing anthropogenic activities in the region.

4.2 Introduction

4.2.1 Population trends over space and time

Assessing spatiotemporal variation in cetacean occurrence is essential to inform marine conservation. Characterising the distribution of one or more species can be used to guide marine spatial planning (MSP) of anthropogenic activities; the designation of priority areas for conservation, such as Important Marine Mammal Areas (IMMAs[1]) or Ecologically and Biologically Significant marine Areas (EBSAs[2]); and the creation of effective marine protected areas (MPAs; Bailey and Thompson, 2009; Mouton et al., 2022; Sahri et al., 2021). For example, humpback whale distribution was used to guide changes to shipping routes in California (Dransfield et al., 2014), while the identification of critical habitats informed the designation of special areas of conservation (SACs) for harbour porpoise (*Phocoena phocoena*) in west Scotland (Embling et al., 2010) and an MPA for northern bottlenose whales (*Hyperoodon ampullatus*; and other cetaceans) in The Gully, Canada (Hooker et al., 1999).

The distribution of cetacean populations is not static in time. Physical ocean systems are dynamic and shifting due to anthropogenic climate change (IPCC, 2021; Suryan et al., 2021), and ecosystems are responding with increasing speed and frequency, often exceeding predictions (O'Neill et al., 2017; Tittensor et al., 2021). Therefore, as climate change accelerates, identifying species range shifts over time is required to determine population status (Mace et al., 2008), solve conservation problems (Dransfield et al., 2014) and lay the foundation for dynamic marine management (Maxwell et al., 2015; Silber et al., 2016). This is particularly important for mobile marine animals such as cetaceans that are likely to respond to changing prey availability (Chavez-Rosales et al., 2022; Víkingsson et al., 2015), such that static protective measures may become ineffective over time (Hartel et al., 2015). Moreover, as important marine top predators, cetaceans may serve as sentinels of wider ecosystem variability (Cartwright et al., 2019; Moore, 2008; Tulloch et al., 2019).

1. Important Marine Mammal Areas: https://www.marinemammalhabitat.org/
2. Ecologically or Biologically Significant marine Areas: https://www.cbd.int/ebsa/

The relationship between cetaceans and their physical and biotic environment is complex, but understanding these interactions can help to identify particular stressors in the environment (Kebke et al., 2022; Meyer-Gutbrod et al., 2021). For example, fluctuations in humpback whale abundance in Hawai'i were attributed to a basin-scale marine heatwave across North Pacific feeding grounds (Cartwright et al., 2019), while declining reproductive success in the Gulf of St Lawrence was related to fluctuating prey stocks (Kershaw et al., 2021). Furthermore, identifying key environmental correlates of occurrence can enable forecasts of upcoming changes in distribution (Becker et al., 2019; Torres et al., 2013), enabling proactive conservation for the most likely future (Barlow and Torres, 2021).

4.2.2 Species distribution models

Species distribution models (SDMs) are frequently used to explain and predict species habitat suitability, density and abundance over space and time (Elith and Leathwick, 2009). Applied to cetaceans, this commonly involves relating environmental variables to data from dedicated surveys (presence–absence data, typically line transect surveys) or opportunistic sighting records (presence-only data; Correia et al., 2021; El-Gabbas et al., 2021b). The resulting model can be used to both determine environmental drivers of occurrence and predict distribution across the study area. Predictions may represent long-term averages or time-specific values (Mannocci et al., 2017). Temporally resolved outputs can be derived from SDMs that match species information to contemporaneous environmental conditions (El-Gabbas et al., 2021a; Redfern et al., 2017), and can be used to deduce changes over time, either within the time frame of surveys (Chavez-Rosales et al., 2022) or, increasingly, as a prediction for the un-surveyed past, present and future (Barlow and Torres, 2021; Torres et al., 2013). For example, Becker et al. (2019) successfully predicted density across the California Current Ecosystem in a novel warm year (not used to construct the SDM) for eight cetacean species with diverse habitat associations.

SDMs can be constructed using a variety of spatiotemporal resolutions, environmental variables and model frameworks, depending on the study system, research question and data availability (Elith and Leathwick, 2009; Mannocci et al., 2017; Robinson et al., 2017). Frameworks range from parametric or semi-parametric models, such as generalised additive models (GAMs) and generalised linear models (GLMs), to machine-learning approaches, such as boosted regression trees (BRTs) and random forests (RFs). Each approach has strengths and drawbacks in terms of flexibility and performance (Virgili et al., 2017). Derville et al. (2019) compared the suitability of several SDMs for humpback whales in New Caledonia: while BRTs exhibited superior explanatory power (goodness of fit), GAMs had substantially higher predictive ability (performance on a novel data set), possibly because the more flexible BRT framework tends to overfit data, such that the model is more complex than the real relationships between response and explanatory variables (Anderson et al., 2011). Meanwhile, Becker et al. (2019) determined that density GAMs (which use the number of animals as a response variable) had higher predictive ability than habitat suitability GAMs and BRTs (which use presence–absence a response variable) for seven cetacean species in California. As an alternative to using a single framework to model distribution, SDMs may combine the strengths of multiple frameworks through an ensemble approach (El-Gabbas et al., 2021b,a). For example, applied to blue whale satellite tracking data in California, ensemble predictions that combined GAM and BRT outputs were more accurate than the predictions of either model alone (Abrahms et al., 2019).

4.2.3 Relating large-scale SDMs to local dynamics

To provide relevance to cetacean species with large distributions, sighting surveys and the resulting SDMs often cover large ocean areas (up to millions of kilometres, e.g., Becker et al., 2022). Environmental variables can be derived from observational data (*in situ* or remotely sensed) or predictions from general circulation models (GCM), such as Regional Ocean Modeling System (ROMS; Haidvogel et al., 2008) and HYbrid Coordinate Ocean Model (HYCOM; Chassignet et al., 2007). At regional to basin scales, both data types are often limited to coarse spatial resolutions (0.5–1°), which in turn restricts the resolution of model outputs (Bassoi et al., 2020). Therefore, large-scale SDMs may not be applicable to a population's entire range, which for cetaceans frequently includes coastal areas where the oceanography can be highly complex (Lowen et al., 2016). For example, Dalla Rosa et al. (2012) concluded that even a relatively small 4 km cell size provided insufficient resolution to model the relationship between humpback whale sightings and environmental variables close to shore. Given that cetaceans are subject to elevated and increasing anthropogenic activity and cumulative stressors in coastal waters (Avila et al., 2018; Halpern et al., 2019; Huntington, 2009), the ability (or lack thereof) of regional SDMs to reflect coastal changes in occurrence is important for species conservation.

Platforms of opportunity, such as whale-watching vessels, enable regular, affordable longitudinal monitoring of cetaceans in coastal waters in local coastal areas (Basran et al., 2019; Klotz et al., 2017; Stack and Currie, 2022; Palazzo et al., 2004). For species with naturally identifying marks, such as humpback whales (Franklin et al., 2020), sighting histories for individual animals can be used to estimate local abundance and other population parameters (e.g., survival and emigration) through capture–recapture (CR) methods (Bertulli et al., 2018; Robbins and Pace, 2018). Metrics such as abundance are easy to interpret and, whilst the lack of spatial coverage may hinder determination of species–environment associations (Grove et al., 2023), these time series can elucidate local fluctuations in population dynamics (Félix et al., 2011; Hammond et al., 2021). Across a species' range, a combination of large-scale surveys (to determine distribution and environmental drivers) and regular coastal monitoring (to characterise changes over time) may be used to better understand population responses to a changing ocean.

4.2.4 Icelandic humpback whales

In this chapter, I explore changes in offshore density and coastal abundance of humpback whales around North Iceland. An important foraging ground for humpback whales, particularly around the northern shelf (Paxton et al., 2009), Icelandic waters have experienced major changes in physical oceanography and biological communities during the last few decades, in line with basin-scale variability across the wider North Atlantic (Beaugrand et al., 2015). In the mid-1990s, a regime shift was attributed to coupled ocean–atmosphere processes (Alheit et al., 2019), including: a declining sub-polar gyre (SPG) that retracted westwards (Hátún et al., 2005); decreasing Atlantic Meridional Overturning Circulation (AMOC; Marzocchi et al., 2015); and prolonged positive North Atlantic Oscillation (NAO; Robson et al., 2012). Since then, North Iceland has been subject to progressive 'Atlantification', with warmer, more saline Atlantic-derived waters entering the region (Valdimarsson et al., 2012). These changes have altered regional primary productivity (McGinty et al., 2016), driven changes in zooplankton species composition (Gislason et al., 2021; Silva et al., 2014) and shifted the distribution of commercially and ecologically important fish species (Carscadden et al., 2013; García-Vernet et al., 2021; Olafsdottir et al., 2019; Vilhjalmsson, 2007).

The abundance of humpback whales (and other cetacean species) has also changed during this period. In offshore Icelandic waters, shipboard surveys conducted as part of the international North Atlantic Sightings Surveys (NASS) revealed a sharp increase in abundance (17% annually) from 1,722 (95% confidence interval 1,061–2,795) in 1987 to 13,965 (95% CI 7,993–24,793) in 2001 (Pike et al., 2005), which was also supported by aerial surveys in shelf waters (Pike et al., 2009). Abundance appears to have subsequently levelled off or even declined, with estimates of 14,553 (95% CI 5,819–27,906) in 2007 (Pike et al., 2020a) and 6,643 (95% CI 3,543–12,456) in 2015 (Pike et al., 2019). Drivers behind these changes are still uncertain (Víkingsson et al., 2015) but increasing abundance may, in part, be attributed to population recovery from historic commercial whaling (Smith and Reeves, 2002). In terms of environmental associations, humpback whales in the region are associated with lower sea surface temperatures and shallower depths (Paxton et al., 2009). In other feeding grounds, distance to isobaths (depth contours), slope, chlorophyll-a concentration and latitude are also important (Bassoi et al., 2020; Becker et al., 2022; Chavez-Rosales et al., 2022; Dalla Rosa et al., 2012; El-Gabbas et al., 2021b; Stephenson et al., 2020; Zerbini et al., 2016), although the importance of these variables varies and even contrasts across studies (Meynecke et al., 2021).

Changes in offshore humpback whale abundance may not reflect coastal variability. Regular monitoring of humpback whales in Skjálfandi Bay (North Iceland) since 2006, using whale-watching vessels as a platform of opportunity, has revealed increasing sighting rates (Klotz et al., 2017; Malinauskaite et al., 2022) and abundance (Bertulli et al., 2018) during the last 15 years. These changes are of management concern because increasing occurrence in coastal waters around Iceland may augment population exposure to potentially threatening human activities, including fisheries (Basran et al., 2019), whale-watching and future coastal infrastructure development to serve growing trans-Arctic shipping traffic (Kokorsch and Stein, 2022; Tillman et al., 2019).

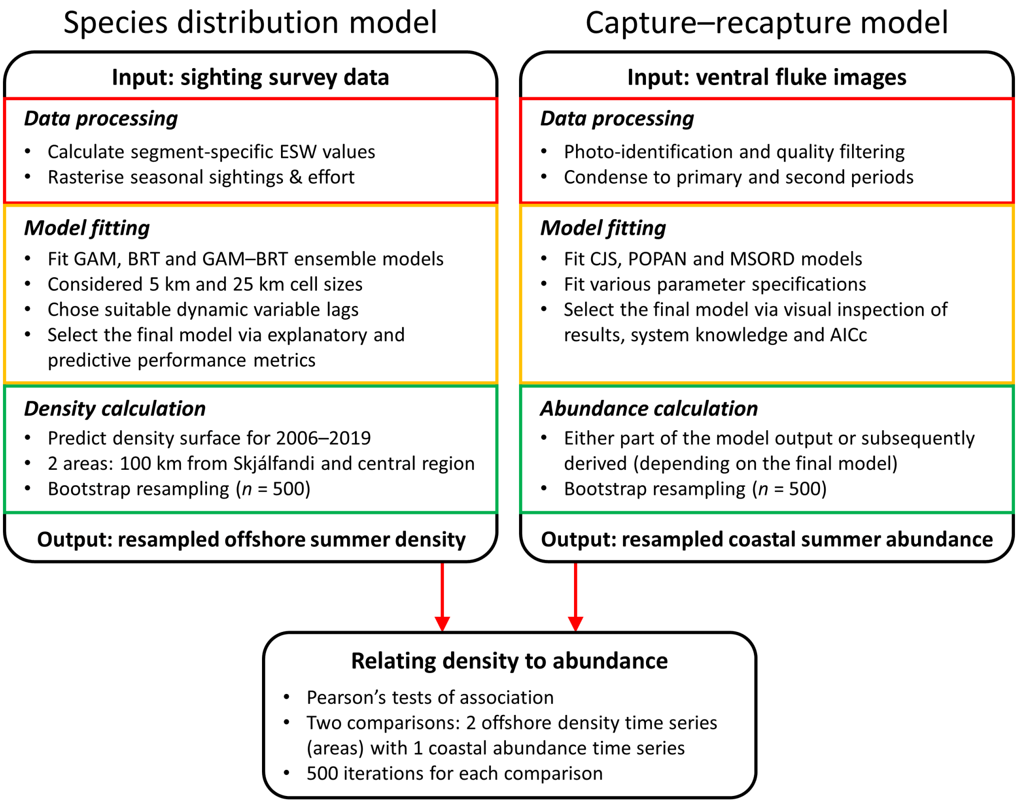

Figure 4.1: Overview of data processing and modelling approaches used Chapter 4.

4.2.5 Chapter aim

I aimed to characterise the association between offshore humpback whale occurrence and physical environmental variables in North Iceland, and to determine whether temporal variability in offshore density reflects coastal abundance trends. To achieve this, I used two distinct approaches, which I then bring together to interpret results (Figure 4.1). First, I applied an SDM to offshore sightings data, collected over a 28-year period (1987–2015), to elucidate important environmental predictors of occurrence and predict offshore density over a 14-year period (2006–2019). Second, I separately applied CR models to estimate the number of whales that visit Skjálfandi Bay each summer, using photo-identification data collected from whale-watching vessels over the same time period (2006–2019). In light of preliminary evidence, I hypothesise that temporal trends in coastal abundance may not reflect wider variability in the offshore zone.

4.3 Methods

The workflow of data collection, processing and analysis is outlined in Figure 4.1. Data processing and analyses were performed using R v4.1 (R Core Team, 2020) and plots were produced using the *ggplot* package (Wickham, 2022).

4.3.1 Study area

This study encompasses coastal and offshore waters of North Iceland (Figure 4.2), which are characterised by dynamic and spatially variable oceanography. Around Iceland, transverse topographic ridges and energetic atmospheric circulation create boundaries between water masses and generate persistent, productive frontal regions (Astthorsson and Vilhjálmsson, 2002). In North Iceland, a wide continental shelf extends 100–170 km offshore and the region is influenced by both Arctic- and Atlantic-derived water bodies (Figure 1.2), which differ in terms of temperature, salinity, surface topography and stratification (Astthorsson and Vilhjálmsson, 2002; Stefánsson, 1962). Oceanographic boundaries fluctuate between years (Valdimarsson et al., 2012) and hydrographic conditions are more variable in coastal waters due to the timing and magnitude of freshwater input and variable weather conditions (Gislason et al., 1994).

4.3.2 SDM data

North Icelandic Sightings Surveys

Humpback whale sightings data were collected during the Icelandic portion of vessel-based NASS, in the summers of 1987, 1989, 1995, 2001, 2007 and 2015 (Figure 4.2). NASS constitute a large-scale multi-national effort to survey cetaceans across in the Northeast Atlantic (Desportes et al., 2019; Pike, 2009). For this study, only survey effort around North Iceland was considered (65.5–69° N, 10–30° W), encompassing the core area of humpback whale sightings and excluding 1989, which did not sample this portion.

Surveys followed conventional line transect methods, with the survey design and observation protocols evolving between surveys. Full protocols have previously been published (Desportes et al., 2019; Pike et al., 2019, 2020a; Sigurjónsson et al., 1989, 1996; Víkingsson et al., 2009) and are briefly described here. Surveys primarily took place in July each year, with all survey effort between June 10th and August 14th (Table 4.1). Each survey had different line transects and varied in spatial extent, both within and outside Icelandic shelf waters (Figure 4.2). Surveys were generally performed in passing mode at a cruising speed of 8–12 knots, with delayed closure on sightings if species identification or group size were uncertain. Observations generally took place from two platforms on each vessel. The communication between platforms varied between survey years, with full communication in 1987 and 1995 (Sigurjónsson et al., 1989, 1996); one-way independence between primary and tracker platforms

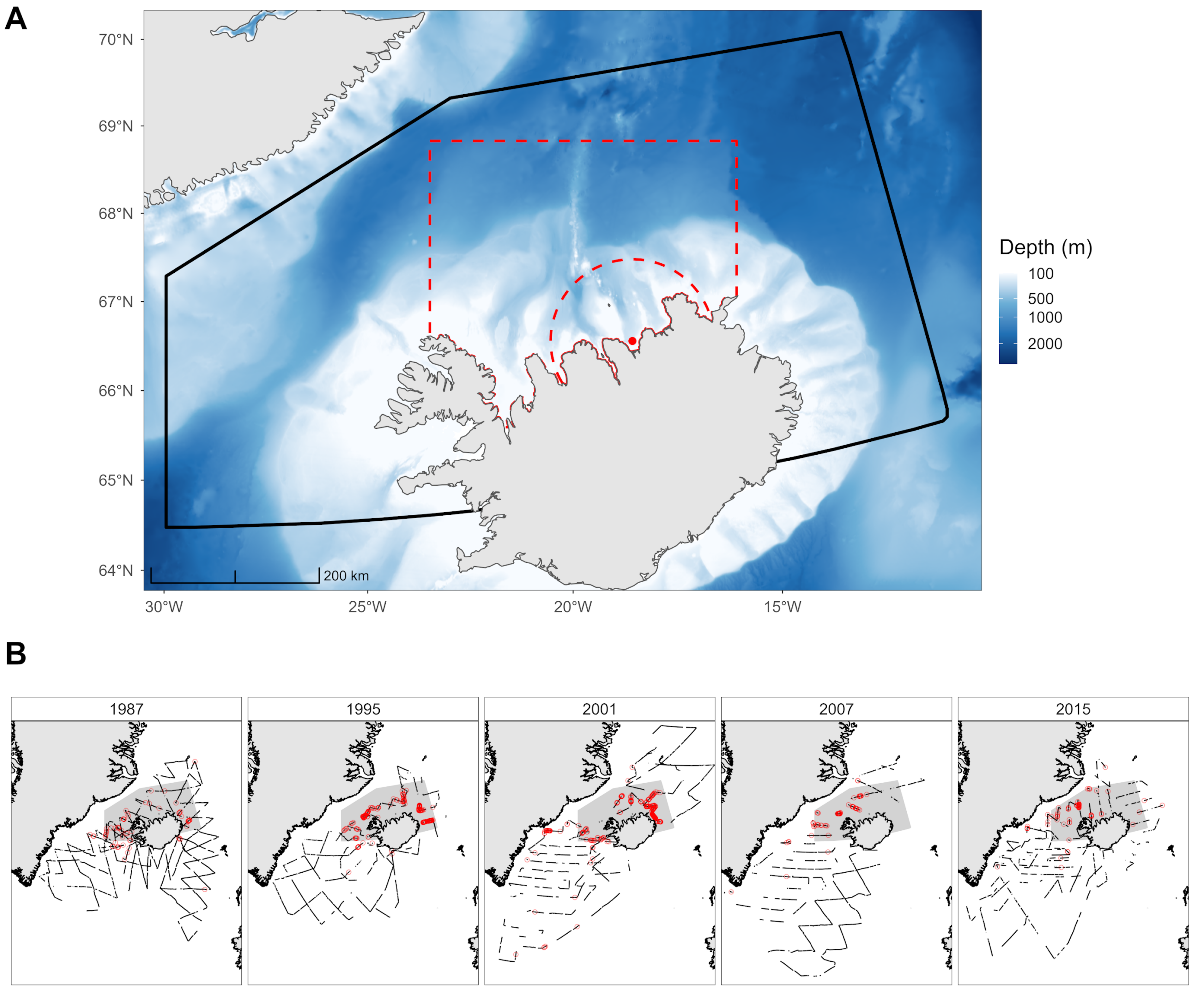

Figure 4.2: Study region and the Icelandic North Atlantic Sightings Surveys. A) The study area of North Iceland (black line), with Skjálfandi Bay (red circle) and two areas for density calculation (red dashed lines): 100 km from Skjálfandi Bay (circular) and the central portion of the study area (rectangular). B) Icelandic North Atlantic Sightings Surveys (black lines) with humpback whale sightings (red circles) and the study area of North Iceland (grey shading).

in 2001 and 2007 (Buckland and Turnock survey method; Buckland and Turnock, 1992; Pike et al., 2020a); and fully independent symmetrical primary platforms in 2015, with duplicates identified post-survey (Pike et al., 2020b). For this study, only sightings from the primary platform were retained and mark–recapture distance sampling was not performed.

Table 4.1: Summary of survey effort for each vessel-based NASS survey year in the study area. Effort values are the number of kilometres travelled along the transect whilst on effort. Whales sighted refer to the number of sightings multiplied by the group size of each sighting, not the number of identifiable whales seen. The 1989 survey was excluded from this study.

Year	Start date	End date	Effort (km)	Whales sighted
1987	24-Jun	28-Jul	4537	79
1995	01-Jul	28-Jul	2415	109
2001	26-Jun	30-Jul	2154	308
2007	02-Jul	22-Jul	1058	105
2015	10-Jun	10-Aug	2346	104

During observation, standard parameters used for estimation of abundance of large whales were recorded (Buckland et al., 2001). The length of each effort transect was recorded along with environmental conditions, coordinates, group size, horizontal distance from the vessel (measured by reticular binoculars or distance sticks; Lerczak and Hobbs, 1998) and angle from the track line for each sighting. Only effort and sightings in Beaufort sea states (BFSS) $\leq$5 were included. Each sighting was assigned a confidence rating by observers and only sightings with high or medium confidence were retained.

Sightings data processing

To convert effort segment lengths into area searched, effective strip width (ESW) was calculated by distance sampling methods, using detection functions fit in the R package *mrds* (version 2.2.5, Laake, 1999). Perpendicular distances were truncated to 6,000 m to remove outliers and reduce bias, and no observer bias in distance estimation was assumed. Hazard rate and half-normal key functions were initially tested and the most suitable function was selected by minimising Akaike's Information Criterion (AIC), comparing Cramér-von Mises goodness-of-fit tests and visual inspection of residuals (Buckland et al., 2015). To improve precision and reduce bias, BFSS and height of the primary observation platform were included as potential covariates via forwards selection ($\Delta AIC < 2$). BFSS was rounded to the nearest integer and states 0 and 1 were combined. Platform height was grouped into two categories, 9–10 m and 15 m. Group size cannot be included as a covariate for predictive models (Paxton et al., 2009). Covariates were assumed to affect the scale rather than the shape of the detection function (Thomas et al., 2010).

Using the final detection function to derive segment-specific ESW values (in km), segment area was then calculated as

$$Area = 2 \times ESW \times Length,$$

where $Area$ is the area searched, in km^2, and $Length$ is the transect length, in km.

Following this, the sightings and effort data were rasterised onto a planar grid in Albers equal-area conic (AEAC) projection, using packages *raster* (Hijmans, 2022) and *sf* (Pebesma, 2018). For each cell and survey year, the surveyed area and number of humpback whales sighted were calculated. This allows incorporation of short effort segments that result from operating in delayed closing mode. Two cell sizes were considered: 5 km and 25 km. Humpback whales are known to respond to the physical environment at multiple spatial scales (Becker et al., 2014; Hazen et al., 2009; Meynecke et al., 2021) and there was uncertainty about which cell size was most suitable (Baines and Weir, 2020; Jaquet and Gendron, 2002). A 5 km grid allows investigation of finer-scale relationships but may be unrealistically small, particularly if the width searched ($2 * ESW$) is similar to, or greater than, the cell length (Baines and Weir, 2020). Moreover, these cell sizes match the spatial resolution of available environmental data (see *Environmental data*). Surveyed cells were only retained if aggregate effort per survey season was greater than 1 km^2, for 5 km cells, or 5 km^2, for 25 km cells, in order to reduce spuriously high densities resulting from under-surveyed areas.

For each raster cell and grid, density can be expressed as animals/km^2. Of note, I assumed perfect detection of animals on the trackline (i.e., $g(0) = 1$). Given that $g(0)$ for humpback whales from vessel-based surveys has been estimated at 0.6–0.78 from Icelandic and Faroese NASS (Pike et al., 2010), and 0.65 (BFSS 5) to 0.92 (BFSS 1) in California (Barlow, 2015), the density values in this study should not be used for abundance estimation (Barlow, 2015).

Environmental data

Predictor variables for the SDM were chosen based on their potential to affect humpback whale distribution through their influence on prey availability (Table 4.2). All variables were reprojected to 5 km and 25 km AEAC grids. Static bathymetric variables were obtained from a GEBCO 25 arc-second depth raster (GEBCO Compilation Group, 2021), using the *raster* package. Slope, spatial standard deviation of slope (calculated over a 3 x 3 pixel box centred on each cell), aspect northing and easting were derived from the original depth raster using the `terrain` function, and distances to the coast, 100 m and 400 m depth contours were also obtained. Bathymetric variables are significant predictors of humpback whale occurrence in feeding grounds (Dalla Rosa et al., 2012; El-Gabbas et al., 2021b; Zerbini et al., 2016), including Iceland (Paxton et al., 2009), and the 400 m depth contour is considered to mark the Icelandic shelf area (Astthorsson and Vilhjálmsson, 2002). Although latitude and longitude can be included to account for spatial autocorrelation and improve performance (Becker et al., 2019), these variables were not considered due to the spatial heterogeneity in survey effort across years (Becker et al., 2022). Humpback whales are also highly mobile, such that ecological processes are unlikely to prevent species range shifts (Cañadas and Hammond, 2008; Tynan et al., 2005).

Contemporaneous dynamic predictors are essential to model inter-annual variation in density (Mannocci et al., 2017), and were derived from VIKING20X, an Atlantic Ocean GCM that provides hindcast simulations of oceanographic variability, with spatial resolution sufficient to capture mesoscale processes (Biastoch et al., 2021). The ability of VIKING20X to simulate oceanographic features has been previously demonstrated (Biastoch et al., 2021; Rühs et al., 2020). Five monthly outputs of this model were used: sea surface temperature (SST), sea surface height (SSH), mixed layer depth (MXL), current speed (9 m depth) and salinity (SAL, 9 m depth). MXL is defined as the depth at which water density is 0.01 kg/m^3 greater than at the surface. Two spatial resolutions were available in WGS84 projection: 0.05° and 0.25° cell sizes were reprojected to 5 km and 25 km AEAC grids, respectively. SST, SSH, MXL and salinity have all been implicated in humpback whale occurrence and distribution in feeding grounds (Becker et al., 2022, 2020b; Dalla Rosa et al., 2012; Dransfield et al., 2014; El-Gabbas et al., 2021a; Ramp et al., 2015), and can be used to identify variations in upwelling and water column stratification (Falk-Petersen et al., 2015; McClain and Firestone, 1993), which influence primary productivity and whale occurrence (Barlow et al., 2021). In addition, the spatial standard deviation of SST (sd.SST) and SSH (sd.SSH) were calculated for a 3 x 3 pixel box centred on each cell. High values of both variables indicate the presence of frontal zones that stimulate surface productivity (Popova and Srokosz, 2009); trap aggregations of plankton and fish (Genin et al., 2005); and have been implicated in humpback whale occurrence (Becker et al., 2020b; Dalla Rosa et al., 2012; Doniol-Valcroze et al., 2007). Finally, I calculated the absolute change in MXL (range.MXL) and SST (range.SST) between March and July, as an indication of the seasonal dynamism of the system. MXL alternates between high winter values and low summer values, and progressive shoaling of the mixed layer throughout spring and summer can influence primary productivity through nutrient and light availability (Joubert et al., 2014; Llort et al., 2019; Sathyendranath et al., 1995; Sverdrup, 1953), in conjunction with changes in water temperature. Biological variables such as chlorophyll concentration were not included, despite being significant predictors of baleen whale distribution (Dalla Rosa et al., 2012; Duengen et al., 2022), as data were not available for the entire study area and time period (McGinty et al., 2016).

Table 4.2: Names, abbreviations, sources and spatiotemporal resolutions of static and contemporaneous environmental variables used as predictors in species distribution models.

Variable	Unit	Code	Source	Temporal resolution	Original spatial resolution (°)
Depth	m	depth	GEBCO	static	0.004
Seabed slope	deg.	slope	GEBCO	static	0.004
SD(slope)	deg.	sd.slope	GEBCO	static	0.004
Aspect easting		east	GEBCO	static	0.004
Aspect northing		north	GEBCO	static	0.004
Distance to coast	km	dist.coast	GEBCO	static	0.004
Distance to 100 m contour	km	dist.100m	GEBCO	static	0.004
Distance to 400 m contour	km	dist.400m	GEBCO	static	0.004
Sea surface temperature	°C	SST	VIKING20X	month	0.05/0.25
Salinity (9 m)	ppt	SAL	VIKING20X	month	0.05/0.25
Mixed layer depth (9 m)	m	MXL	VIKING20X	month	0.05/0.25
Sea surface height	m	SSH	VIKING20X	month	0.05/0.25
Current speed (9 m)	m/s	speed	VIKING20X	month	0.05/0.25
Spatial standard deviation of SST	°C	sd.SST	VIKING20X	month	0.05/0.25
Spatial standard deviation of SSH	m	sd.SSH	VIKING20X	month	0.05/0.25
Seasonal (March–July) range of SST	°C	range.SST	VIKING20X	month	0.05/0.25
Seasonal (March–July) range of MXL	m	range.MXL	VIKING20X	month	0.05/0.25

4.3.3 SDM modelling approaches

Cetacean SDMs can be created from a variety of data types and modelling approaches. Here, I used SDMs to relate the number of whales sighted each survey year, per cell, to physical predictors, with $log(area\ searched)$ included as an offset. Model predictions are summer density values (animals/km^2). Due to small group sizes (mean = 1.6 animals), separate models were not fit for encounter rate and group size (Becker et al., 2016). I considered two distinct model frameworks: GAMs and BRTs. Both frameworks model nonlinear covariate responses, but GAMs use flexible smoothing functions while BRTs use binary splits to relate a response variable to multiple predictors. In addition, I fit a GAM–BRT ensemble to combine their complementary strengths. For all three approaches, I fit separate models to the 5 km and 25 km grids.

Prior to model fitting, each predictor was transformed to an approximately normal distribution. Collinearity between variables was then assessed with Pearson's correlation. If the correlation coefficient was greater than 0.7, models were tested with each variable and the variable which yielded the best model fit (low AIC in GAMs, high cross-validated deviance explained in BRTs) was selected. Although BRTs (Elith et al., 2008) and even GAMs (Wood, 2008) may be robust to multicollinearity, excluding correlated variables can facilitate interpretation of model results (Elith et al., 2008).

Following this, for each dynamic predictor with monthly values (i.e., excluding range.SST and range.MXL), time lags were considered. Oceanographic conditions in spring may influence whale occurrence in summer due to a temporal lag between suitable physical conditions, primary production and a response at higher trophic levels (Hayward and Venrick, 1998; Houghton et al., 2020; Ramp et al., 2015; Riekkola et al., 2019; Visser et al., 2011a). This lag between suitable physical conditions and whale occurrence can be several months (Croll et al., 2005; Dey et al., 2021; Ramp et al., 2015). Therefore, monthly values from March to July were considered. Preliminary analyses revealed that the optimal combination of variable months (in terms of goodness of fit) can only be determined by fitting all combinations of monthly values. Therefore, to limit the number of models that need to be fit (m^v, where m is the number of months and v is the number of variables), I only considered values for March, May and July and ran 243 (3^7) combinations through a loop function for each model, using the *foreach* package for parallel processing on five cores (Daniel et al., 2022). The final combination was selected via goodness of fit (AIC for GAMs, deviance explained for BRTs).

Generalised additive models

A GAM framework (Hastie and Tibshirani, 1990) allows non-linear responses to multiple predictors but the shape of the response–predictor relationship is still constrained, which can provide a favourable balance between complexity and ecologically intelligible SDM outputs (Becker et al., 2020a,b; Derville et al., 2018). GAMs were implemented through the *mgcv* package (Wood, 2004, 2017) and environmental predictors were included as smooth functions. Smoothing parameters were selected via restricted maximum likelihood (REML; Marra and Wood, 2011). Thin-plate regression splines were used for each potential predictor and automatic term selection through null space penalisation (Marra and

Wood, 2011) was employed via the `select=TRUE` term. Specific interactive terms were not expected *a priori* and so were not included. To limit overfitting, $gamma = 1.4$ was used (Kim and Gu, 2004; Wood, 2017). I considered four error structures with log links: Poisson; negative binomial ($\theta = 3$) and Tweedie ($p = 1.25$), which account for overdispersion; and zero-inflated Poisson, which accounts for overdispersion due to zero-inflation (Miller et al., 2013). The optimal error structure was selected by minimising AIC and inspecting model residuals, and the optimal combination of dynamic variable months was then selected by minimising AIC. From the final model, concurvity was inspected with the `concurvity` function (Wood, 2011).

Boosted regression trees

Machine learning approaches such as BRTs are increasingly used to model cetacean distribution (Derville et al., 2018; Stephenson et al., 2020; Torres et al., 2013). BRTs fit numerous regression trees in a forward, stagewise fashion (boosting) to produce a single model (Elith et al., 2008). The shape of the response–predictor relationship is not constrained and interactive effects between predictors are explicitly modelled. BRTs often have higher explanatory performance than equivalent GAMs but a tendency to over-fit can limit predictive performance in novel situations (Becker et al., 2020a; Derville et al., 2018; Meynecke et al., 2021).

BRTs with a Poisson link were implemented through the *dismo* package (Hijmans et al., 2022). Models can be tuned by several parameters. I opted for a low tree complexity (tc) of 2, allowing simple two-way interactions between predictors; a learning rate of 0.01; a bag fraction of 0.75; and a maximum of 5,000 trees. The optimal combination of dynamic variable months was selected by maximising cross-validated deviance explained (Leathwick et al., 2006). Because BRT results are different each time they are run, the ten lag combinations with highest deviance explained were refitted ten times each, and the month combination with the highest mean deviance explained was selected. From this model, variables with relative contribution <3% were removed (Elith et al., 2008) and the final model was fitted.

Ensemble predictions

Ensemble SDMs combine the predictions and benefits of multiple model frameworks (Araújo and New, 2007) and are now used to predict cetacean distribution, particularly for presence-only data (Claro et al., 2020; Mouton et al., 2022). While ensemble SDMs can incorporate many models, for simplicity, I only utilised the GAM and BRT frameworks, combining model outputs into a single GAM–BRT prediction. For each cell i, ensemble density ($D_{ens,i}$) was calculated as the mean of GAM and BRT predicted densities, weighted by the inverse of their cross-validated root mean square error (CV-RMSE), standardised to sum to 1:

$$D_{ens,i} = D_{GAM,i} * W_{GAM} + D_{BRT,i} * W_{BRT};$$

where $D_{GAM,i}$ and $D_{BRT,i}$ are the predicted cell densities, and W_{GAM} and W_{BRT} are weights from GAM and BRT models respectively. CV-RMSE for each framework was calculated through a 10-fold cross-validation process. For each iteration, 25% of survey days were withheld as an evaluation subset and the remaining 75% were used to train the model (Baines and Weir, 2020; Derville et al., 2018). RMSE was then calculated between the evaluation observations and predictions.

Model performance

Model performance can be assessed as either explanatory performance (goodness of fit) or predictive performance (performance on a novel data set; Oppel et al., 2012). A model with high explanatory power does not necessarily have good predictive ability (Becker et al., 2020a).

I used two types of measure to evaluate model performance: RMSE and the ratio of observed to predicted densities (obs:pred). Both metrics have been used to assess cetacean SDMs (Baines and Weir, 2020; Becker et al., 2020a, 2022). Because I aimed to calculate densities and not assess presence/absence, discrimination-based metrics such as area under the receiver operating characteristic curve or true skill statistic were not used. For explanatory performance, I calculated RMSE and obs:pred ratios for predictions of the full model to the entire data set as well as two years with contrasting sighting patterns and high survey effort (Figure 4.2): 1995 as a year with high sighting rates and 2015 as a year with low sighting rates. I assessed predictive performance by splitting the data set in two ways. The first was a 10-fold cross-validation procedure (see *Ensemble predictions* section). The second involved removing 1995 and 2015 in turn from the training data set and using the resulting fitted model to predict density for each of these years. The final cell size and model framework were selected by comparing these metrics, with priority given to predictive performance measures. Of note, these evaluation data sets are not independent of the training data sets used in the full model (Elith and Leathwick, 2009; Lee-Yaw et al., 2022). Aerial transect surveys have been conducted in an overlapping area as part of NASS (Desportes et al., 2019) but data were not available for this study (Paxton et al., 2009).

In addition, spatial autocorrelation in the final model was examined through semi-variogram analysis, using the packages *gstat* (Pebesma, 2004) and *spatstat* (Baddeley and Turner, 2005). Specifically, I compared the empirical variogram of deviance residuals with the Monte Carlo envelope of empirical variograms, derived from 500 random permutations of the residuals on the spatial locations (Diggle and Ribeiro, 2007; Zuur et al., 2010). Accounting for spatial autocorrelation is challenging for models that are used to predict responses in unsurveyed years (Dormann et al., 2007), but examination is nevertheless important to assess model fit.

Density calculation

The final SDM was used to predict summer density (to the same cell size) over the entire study area for each year from 2006 to 2019, which includes 12 novel years that were not surveyed during NASS. I then calculated mean summer density over two areas: 1) within a 100 km radius of Skjálfandi Bay (14,442 km^2); and 2) the wider central region, spanning approximately 65.4–68.6°N and 13.7–22.8°W (94,950 km^2; Figure 4.2). To obtain 95% confidence intervals (CIs) for density predictions, model fitting and subsequent density estimation were repeated 500 times using a bootstrap procedure, resampling the original sighting–effort rasters with replacement. The result was 500 density estimates for each year and area. To detect a significant temporal trend, the final annual density estimates for each area were linearly regressed against time (year), weighted by the inverse of the variance of each estimate (derived from resampled values) to account for varying precision (Grove et al., 2023; Kutner et al., 2005).

4.3.4 Capture–recapture data

In contrast to the SDM approach, I used CR models to estimate summer abundance at a single coastal site, Skjálfandi Bay. Since 2006, trained observers have used whale-watching vessels departing from Húsavík as opportunistic platforms for photo-identification (Bertulli et al., 2018; Grove et al., 2020; Klotz et al., 2017). Trips took place between March and November, average trip duration was approximately three hours and surveys took place between the hours of 08:00 and 23:00. Between one and three observers recorded effort and cetacean presence, and photographed the ventral side of the tail flukes of each encountered humpback whale using DSLR cameras with zoom lenses (between 55 and 400 mm). These images and visible markings were then used for photo-identification (Franklin et al., 2020; Katona and Whitehead, 1981), as in Bertulli et al. (2018); Klotz et al. (2017). For the purpose of this study, I only considered sightings between June and August (three months), encompassing the summer season (Table 4.3). Each animal was recorded as present or absent for each summer (or secondary period, see *Multi-state open robust design*), resulting in a database of annual sighting histories in Skjálfandi Bay.

4.3.5 Capture–recapture modelling approaches

CR models were used to estimate summer abundance from these sighting histories (see: King, 2014; McCrea and Morgan, 2014; Seber and Schofield, 2019). Abundance was defined as the total number of animals that visited the Skjálfandi Bay each summer, and not the number of animals present in the Bay at any one time. As such, it included an unknown proportion of summer-feeding Icelandic humpback whales. All CR models were fitted using MARK software (White and Burnham, 1999), accessed through R using the *RMark* package (Laake and Rexstad, 2012).

Various frameworks are available to estimate population parameters, including abundance, from CR data. In general, these frameworks involve a trade-off between modelling assumptions and the complexity of the model: more complex models with fewer assumptions can account for features such as transience, temporary emigration and capture heterogeneity (Boys et al., 2019; Jeyam et al., 2018; Madon et al., 2013), but these models are also more data-hungry, which can lead to identifiability issues

Table 4.3: Seasonal survey effort, the number of identifiable whales and the number of inter-seasonal re-sights (from all previous seasons within the period) for each three-month summer season (June–August) of photo-identification surveys in Skjálfandi Bay between 2006 and 2019. Effort refers to the number of days per season in which surveys were conducted.

Year	Effort (days)	Whales	Re-sightings
2006	71	25	
2007	81	32	7
2008	76	23	3
2009	74	32	6
2010	74	21	6
2011	68	32	1
2012	69	48	9
2013	79	62	16
2014	49	88	16
2015	67	53	16
2016	87	91	23
2017	80	137	45
2018	82	136	62
2019	86	83	33

with information-poor data sets (Forster, 2000; Grove et al., 2023; Lebreton et al., 1992). I considered three CR frameworks: Cormack–Jolly–Seber (Lebreton et al., 1992), Jolly–Seber–Schwarz–Arnason (Jolly, 1965; Schwarz and Arnason, 1996; Seber, 1965) and multi-state open robust design (Kendall and Bjorkland, 2001; Schwarz and Stobo, 1997). All models assume that each animal has an independent fate and that identifying marks are retained and recorded correctly. Parameters were linked to covariates with an inverse logit function and values were estimated from observed data using a maximum likelihood estimation (MLE) approach.

Each framework can be modified to account for factors that may bias abundance estimates, related to survival and capture heterogeneity, and I tested for three of these features prior to model fitting: transience, trap dependence and individual detection heterogeneity (IDH), defined as each animal having a unique sighting probability (Cubaynes et al., 2010; Gimenez and Choquet, 2010). To test for transience and trap dependence, I used Chi-squared goodness-of-fit testing, implemented through the *R2ucare* package (Choquet et al., 2009; Gimenez et al., 2018). A non-significant `TEST 3.SR` ($Z = 18.0,\ p = 0.15$) indicated a lack of transience and a non-significant `TEST 2.CT` ($Z = 6.0,\ p = 0.86$) indicated a lack of trap dependence. To test for IDH, I applied tests of positive association between previous and future encounters using Goodman–Kruskal's gamma (Jeyam et al., 2018). The global test was non-significant ($\gamma = 0.26,\ p = 0.21$), indicating a lack of IDH. As a result, fitted CR models did not account for transience, trap dependence or IDH.

Below, I outline the three frameworks used to model abundance. For each framework, the final combination of parameters was selected with AIC corrected for small sample size (AICc; Burnham and Anderson, 2004), supplemented by inspection of parameter values. Following this, the final model was selected with visual inspection of parameter values and associated CIs, combined with knowledge of the biological system. For each model, 95% CIs for abundance estimates were derived from a stratified bootstrap approach (500 replicates; Grove et al., 2023; King and McCrea, 2019; Morgan, 2008). For each bootstrap replicate, the number of animals first sighted in a given year was set equal to the observed value. From the selected models, the result was 500 abundance estimates for each year. To detect a significant temporal trend, the final seasonal abundance estimates (derived from the original data set) were linearly regressed against time (year). The regression was weighted by the inverse of the variance of each abundance estimate to account for varying precision (Grove et al., 2023; Kutner et al., 2005).

Cormack–Jolly–Seber

The CJS framework models capture histories as a function of apparent survival rate, ϕ_i, and per-animal detection probability, p_i, at each occasion (season) i. To estimate annual abundance, I followed Cubaynes et al. (2010); Grove et al. (2023) and used a Horvitz–Thompson estimator (Horvitz and Thompson, 1952; McDonald and Amstrup, 2001) such that:

$$\widehat{N}_i = \frac{n_i}{\hat{p}_i};$$

where $\widehat{N}_i$ denotes the estimated annual total abundance, $\hat{p}_i$ the estimated detection probability and n_i the number of sighted animals at occasion i. Using this framework, abundance is not estimated for the first occasion (2006). Survival was specified as constant or time-dependent. Because detection probability is likely to vary between seasons (years) as a function of survey effort, detection was modelled with seasonal effort, defined as the number of days during which surveys were conducted, as a covariate (Marucco et al., 2009). Time-varying detection was also considered but this specification led to identifiability issues (particularly in 2010) and was therefore removed. In total, four CJS models were fitted from all combinations of sub-models: two models for survival (constant and time-varying) and two for detection (constant and effort-varying).

Jolly–Seber–Schwarz–Arnason

The JSSA framework may be regarded as an extension of CJS, modelling the system as a super-population from which animals enter and leave the study area. JSSA has an additional parameter, probability of entry, $pent_i$, and provides an implicit estimate of the super-population abundance, N_{tot}. From this, seasonal abundances can be derived using estimates of $pent$ and survival. Survival and $pent$ were specified as constant or time-varying, while detection was constant or linked to survey effort, leading to a total of eight models. As with CJS, time-varying detection resulted in identifiability issues and this specification was removed.

Multi-state open robust design

For data sets that contain sufficient re-sighting information, robust design frameworks are used in cetacean research to model abundance, survival and residence patterns, (e.g., Boys et al., 2019; Kuit et al., 2021; Somerford et al., 2022). Unlike CJS and JSSA, which assume permanent emigration (Lebreton et al., 1992), robust design models are structured into primary and secondary periods, permitting the specification of additional parameters related to temporary emigration, by modelling animals present in the study area as observable and temporary emigrants as unobservable. This is particularly important for highly mobile species such as humpback whales and, given that Skjálfandi Bay represents a small (and unknown) proportion of the wider Icelandic humpback whale feeding grounds (Carwardine, 2019; Paxton et al., 2009), I anticipate temporary emigration in this system.

I specified a robust design model with each season as a primary period (i) and three secondary periods (j) per season, corresponding to calendar months (June, July, August). One month is considered a suitable length to incorporate sufficient survey effort for each secondary period. Different robust design models vary in their assumption of closure between secondary periods and, within the three-month primary periods, I anticipate this assumption to be violated. Therefore, a multi-state open robust design (MSORD) framework was selected, permitting individuals to enter and leave the system within a season, accounting for temporary emigration and transience (Kendall and Bjorkland, 2001). MSORD models are more data-hungry than the other frameworks considered here and estimate the following parameters: apparent survival between primary periods (S_i); detection probability in each secondary period (p_j); probability of entry for each secondary period ($pent_j$); persistence in the population across secondary periods (φ_j); and movement between strata between primary periods (Ψ_i). Using the two-state specification to model observable and unobservable states, I estimated transition probabilities $\Psi_i^{P\text{-}E}$, movement out of the study area, and $\Psi_i^{E\text{-}P}$, movement into the study area (where state P is present and state E is emigrant). The probability that a whale remains in the same state is derived by subtraction (e.g., $\Psi_i^{P\text{-}P} = 1 - \Psi_i^{P\text{-}E}$).

Survival was specified as constant or season-varying. Detection was modelled as constant, varying across seasons, varying across months, or with survey effort (number of days) per secondary period. Probability of entry and persistence were modelled as constant, varying across seasons or varying across months. Temporary emigration was allowed to be absent ($\Psi_i^{E\text{-}P} = \Psi_i^{P\text{-}E} = 0$); even flow ($\Psi_i^{E\text{-}P} = \Psi_i^{P\text{-}E}$); or random ($\Psi_i^{P\text{-}E} = \Psi_i^{E\text{-}E}$), such that the probability of being a temporary emigrant is independent of previous availability. Even-flow and random Ψ were specified as constant or time-varying. Markovian temporary emigration ($\Psi_i^{P\text{-}E} \neq \Psi_i^{E\text{-}E}$) was also considered but excluded due to identifiability issues. Using all combinations of parameter specifications, a total of 144 MSORD models were fitted.

4.3.6 Relating offshore density to coastal abundance

In total, two offshore density time series (100 km around Skjálfandi Bay and the wider central region) were produced using the final SDM and one coastal abundance time series for Skjálfandi Bay was produced using the final CR model, for each summer from 2006 to 2019. Each time series consisted of values derived from the original sightings data sets, plus 500 bootstrap replicates derived from resampling of the sightings data set. I applied Pearson's tests of association to determine the linear correlation between the coastal abundance time series and each offshore density time series, for the final set of values and all 500 bootstrap replicates. For each comparison, I examined the distribution of Pearson's r values from all replicates combined to determine whether the relationship was robust to uncertainty in density and abundance estimates.

4.4 Results

4.4.1 Species distribution model

Sightings data

Across five NASS survey years, there were 709 sightings of individual humpback whales within the area of interest during 12,510 km of survey effort (Table 4.1). Effort per survey ranged from 1,058 km in 2007 to 4,537 km in 1987 and the number of whales seen per survey ranged from 79 in 1987 to 308 in 2001 (Table 4.1), with a mean group size of 1.6 animals and a maximum of 20. Sightings were primarily to the east and northwest of Iceland (Figure 4.2).

To derive effective strip width (ESW) values, the final detection function used a hazard rate key function and retained Beaufort sea state but not platform height as a covariate. Resulting ESW values ranged from 1,153 m (BFSS 5) to 2,178 m (BFSS 2).

Model selection

I considered three model frameworks (GAM, BRT, ensemble), two cell sizes (5 km, 25 km) for each framework and dynamic variable values (excluding range.SST and range.MXL) from three months (March, May, July) in each model. For both the 5 km and 25 km GAMs, the Tweedie error distribution best described the data. Values from all three months were included across the models, with May values most commonly selected, no July values in the 25 km GAM and no March values in the 25 km BRT.

Model frameworks and cell sizes varied in terms of explanatory and predictive performance (Table 4.4). Models trained with (and predicted to) a 25 km cell grid yielded lower RMSE and obs:pred ratios closer to 1 than models applied to a 5 km cell grid, for all frameworks and both explanatory and predictive metrics. Out of the 25 km models, the BRT had the best explanatory performance for all metrics other than obs:pred for 2015 (Table 4.4). In contrast, the BRT had the lowest predictive performance (other than cross-validated RMSE). The ensemble model had slightly lower predictive RMSE than the GAM from cross-validation and removing 1995 or 2015 from the model. However, the GAM produced considerably better obs:pred ratios for all three test types. Because the aim of this study is to track temporal changes in density over an area, the 25 km GAM was selected as the final model. Nevertheless, predicted densities from this model should be interpreted with caution. Visual inspection of model predictions for 1995 and 2015 using either the global model (trained with all survey years) or a model trained with the year removed revealed differences in predicted distribution (Figure 4.3). For 1995, there was an eastward shift in highest density values for the model fitted without 1995 data, compared to the global model. For 2015, density distributions from the two models were more similar.

Table 4.4: Explanatory and predictive performance evaluation metrics for generalised additive model (GAM), boosted regression tree (BRT) and ensemble models fitted and predicted at two cell sizes, 5 km and 25 km. RMSE denotes root mean square error and obs:pred denotes the ratio of observed to predicted density. CV denotes 10-fold cross-validation, whereas years (1995, 2015) denote metrics for which that year was removed from model fitting, and the resulting model was used to predict density for that year. For each metric, the value (model) corresponding to the best performance is in bold.

		5 km cell			***25 km cell***		
	Metric	BRT	GAM	Ensemble	BRT	GAM	Ensemble
Explanatory	RMSE total	0.20	0.36	0.27	**0.10**	0.19	0.13
	obs:pred total	1.10	1.20	1.15	**1.04**	1.13	1.08
	RMSE 1995	0.35	0.72	0.50	**0.11**	0.34	0.20
	obs:pred 1995	1.35	1.55	1.44	**1.09**	1.30	1.18
	RMSE 2015	0.10	0.13	0.11	**0.07**	0.08	**0.07**
	obs:pred 2015	0.68	0.71	0.69	0.79	**0.94**	0.85
Predictive	RMSE CV	0.67	0.68	0.68	**0.19**	0.20	**0.19**
	obs:pred CV	2.38	1.62	1.88	1.56	**1.19**	1.33
	RMSE 1995	0.81	0.81	0.74	0.41	0.41	**0.32**
	obs:pred 1995	5.41	2.38	3.04	3.04	**1.21**	1.29
	RMSE 2015	0.15	0.15	0.14	0.10	0.10	**0.09**
	obs:pred 2015	0.69	0.60	0.59	0.62	**0.71**	0.45

Model output

From the 25 km GAM, the percentage of deviance explained was 47.5%. Eleven explanatory predictors were retained, ten of which were significant (Table 4.5). Static variables were dist.coast ($p < 0.001$), dist.400 ($p = 0.05$), slope ($p = 0.06$) and aspect northing ($p = 0.004$). Dynamics variables were: SST ($p < 0.001$), its spatial standard deviation (sd.SST; $p < 0.001$) and its seasonal range (range.SST; $p = 0.03$); SSH ($p < 0.001$) and its spatial standard deviation (sd.SSH; $p < 0.001$); and MXL ($p = 0.05$) and its seasonal range (range.MXL; $p = 0.002$; Table 4.5). The variables depth, dist.100, sd.slope, salinity and current speed were excluded due to collinearity, and aspect easting was excluded as a

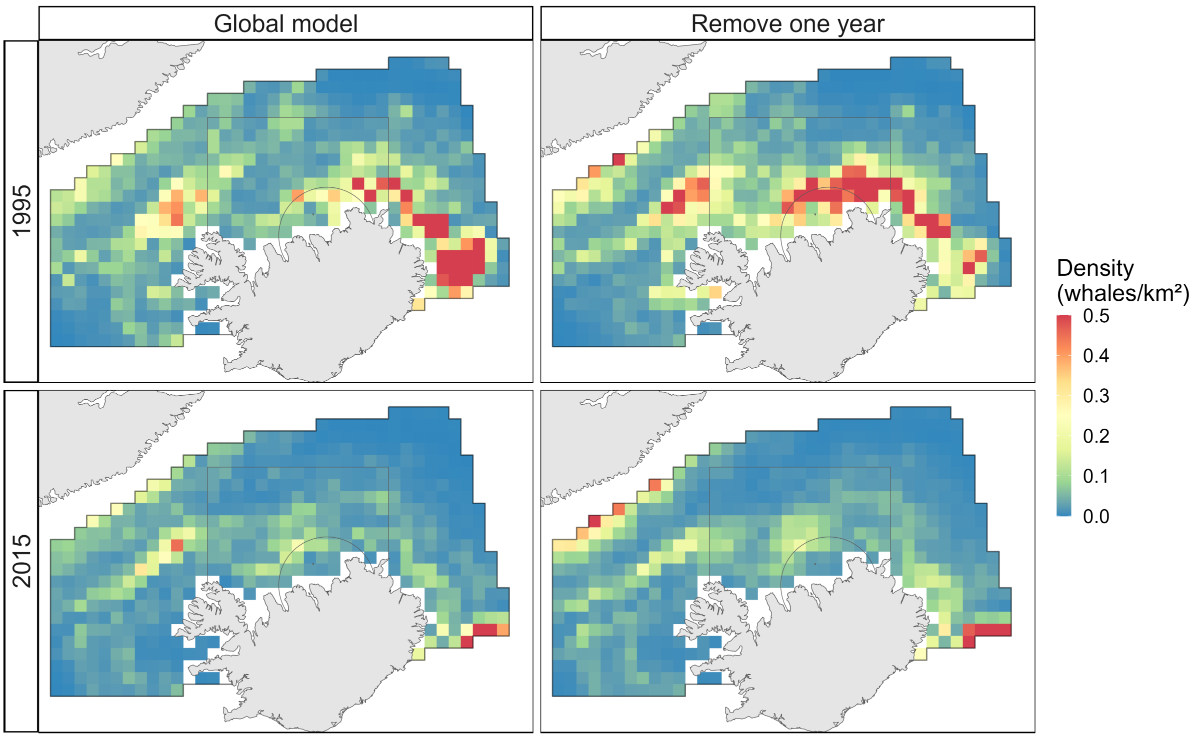

Figure 4.3: The predictions of generalised additive models (GAMs), fitted using a 25 km cell size, for 1995 and 2015 using either the global model (all years included in model training) or a model with the target year excluded.

non-significant term. For dynamic variables with monthly values, the best-fitting combination of months (selected in the final model) was March SST, May sd.SST, May SSH, March sd.SSH and May MXL. This combination had an AIC value 5.0 lower than the next best-fitting combination (Table 4.6). Semi-variogram analysis revealed some residual spatial auto-correlation, with 2 out of 15 points lying outside the Monte Carlo envelope (Appendix I).

Table 4.5: Results for the final generalised additive model (GAM) at 25 km resolution. EDF denotes effective degrees of freedom. A cube-root transformation is denoted by 'cubert'. dist.coast – distance to coast, dist.400 – distance to 400 m contour, slope – seabed slope, northing – aspect northing, SST – sea surface temperature, sd.SST – spatial standard deviation of SST, range.SST – March–July seasonal range of SST, SSH – sea surface height, sd.SSH – spatial standard deviation of SSH, MXL – mixed layer depth, range.MXL – March–July seasonal range of MXL.

Variable	**EDF**	F**-statistic**	p**-value**
s(dist.coast)	5.95	7.26	<0.001
s(sqrt.dist.400)	1.67	0.47	0.045
s(log(slope))	0.69	0.26	0.058
s(north)	3.39	1.41	0.004
s(March SST)	6.77	4.72	<0.001
s(cubert(May sd.SST))	0.93	1.51	<0.001
s(range.SST)	0.77	0.4	0.025
s(May SSH)	3.24	3.31	<0.001
s(log(March sd.SSH))	3.12	2.43	<0.001
s(log(May MXL))	0.7	0.28	0.046
s(cubert(range.MXL))	1.9	1.01	0.002

From the 25 km GAM, there were contrasting relationships and functional forms for different predictors (Figure 4.4). Humpback whales were strongly associated with bathymetry: densities were higher within 40–70 km of the coast, with a gradual decline as distance increased; and values also increased with increasing seabed slope. Meanwhile, SST and SSH were the most important dynamic predictors. Densities were higher when March SST was below 0°C or above 6°C, and at May SSH values lower than -0.8 m. Spatial standard deviations of both SSH and SST also influenced occurrence, with higher density at low values of March sd.SSH and a positive relationship with May sd.SST. Meanwhile, there was a weak negative relationship with May MXL. Finally, densities were higher when the March–July range in both MXL (complex relationship) and SST (linear relationship) was lower.

From seasonal 25 km GAM predictions, there was considerable inter-annual variation in summer density (animals/km^2) for the two areas of interest (100 km around Skjálfandi Bay and the wider central area) during the period 2006–2019. In the smaller 100 km region, density ranged from 0.009 animals/km^2 (95% CI 0.001–0.022) in 2019 to 0.21 animals/km^2 (95% CI 0.04–1.602) in 2010. In the larger central region, density ranged from 0.007 animals/km^2 (95% CI 0.001–0.023) in 2017 to 0.07 animals/km^2 (95%

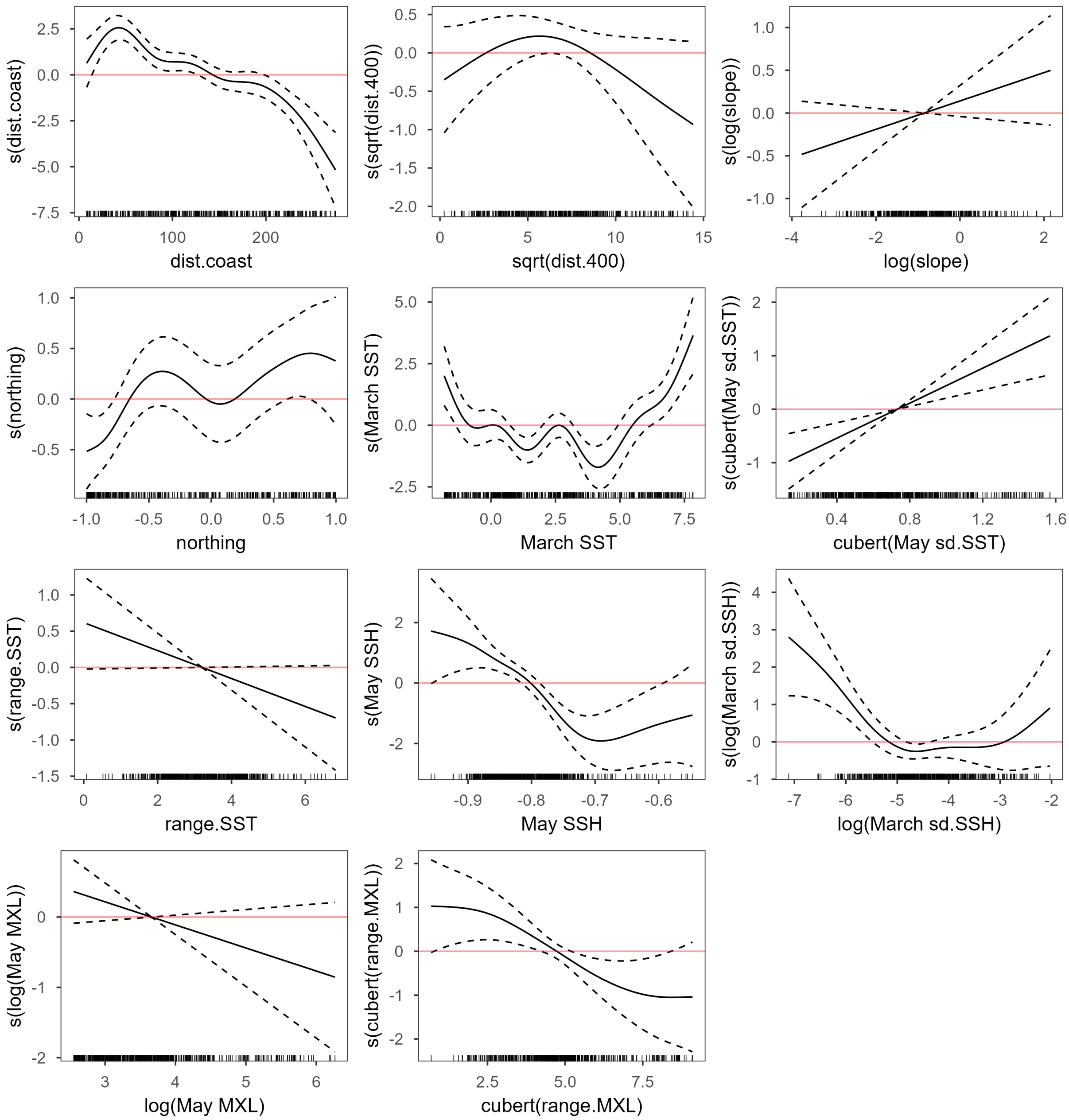

Figure 4.4: Model terms for the best-fitting 25 km generalised additive model (GAM). Estimated smooth functions (solid black lines) with 95% confidence intervals (dashed lines) are shown for each transformed explanatory variable. The x-axis displays the variable range with rug plots indicating sampled values. The y-axis displays the partial effect of a smooth function on density, with zero effect denoted by the red line. A cube-root transformation is denoted by ‘cubert’. dist.coast – distance to coast, dist.400 – distance to 400 m contour, slope – seabed slope, northing – aspect northing, SST – sea surface temperature, sd.SST – spatial standard deviation of SST, range.SST – March–July seasonal range of SST, SSH – sea surface height, sd.SSH – spatial standard deviation of SSH, MXL – mixed layer depth, range.MXL – March–July seasonal range of MXL.

Table 4.6: Generalised additive model (GAM) fit (Akaike's information criterion, AIC) of the top 10 combinations (out of 243) of calendar months (March, May or July) for each dynamic variable with monthly values, at 25 km cell size. A cube-root transformation is denoted by 'cubert'.

log(MXL)	SSH	SST	log(sd.SSH)	cubert(sd.SSH)	AIC	ΔAIC
May	May	March	March	May	1451.7	0.0
May	May	March	March	March	1456.7	5.0
May	May	March	March	July	1460.1	8.4
May	July	March	March	May	1460.5	8.8
May	July	March	March	March	1461.0	9.2
March	May	March	March	May	1461.1	9.4
July	May	March	March	May	1461.1	9.4
May	May	May	March	May	1462.2	10.5
March	May	March	March	March	1463.6	11.8
July	May	March	March	March	1463.6	11.8

CI 0.014–0.331) in 2010. Moreover, it appears that density generally declined with time in both regions, although confidence intervals are large and overlapping (Figure 4.5). This is supported by significant weighted linear regression of density against year for the 100 km region ($F = 5.36,\ R^2 = 0.25,\ p = 0.04$) and the central region ($F = 13.5,\ R^2 = 0.49,\ p = 0.003$). From visual inspection, density appears to have decreased east of Iceland and possibly increased in the area near Greenland.

4.4.2 Capture–recapture model

Sightings data

For each three-month summer season (June–August) between 2006 and 2019, humpback whale photo-identification surveys were conducted on 1,043 days in Skjálfandi Bay (Table 4.3), with seasonal effort ranging from 49 days in 2014 to 87 days in 2016. From a total of 693 identifiable whales, the number of whales seen per period ranged from 21 in 2010 to 137 in 2017, and the number of inter-seasonal re-sights ranged from one in 2010 to 62 in 2018 (Table 4.3). The majority of whales (517, 75%) were sighted in only one year, with whales sighted in up to seven different years (one whale). The number of whales that were re-sighted between secondary periods (months) within each primary period (season) ranged from zero in 2010 to 32 in 2012 (67% of all whales sighted in the season).

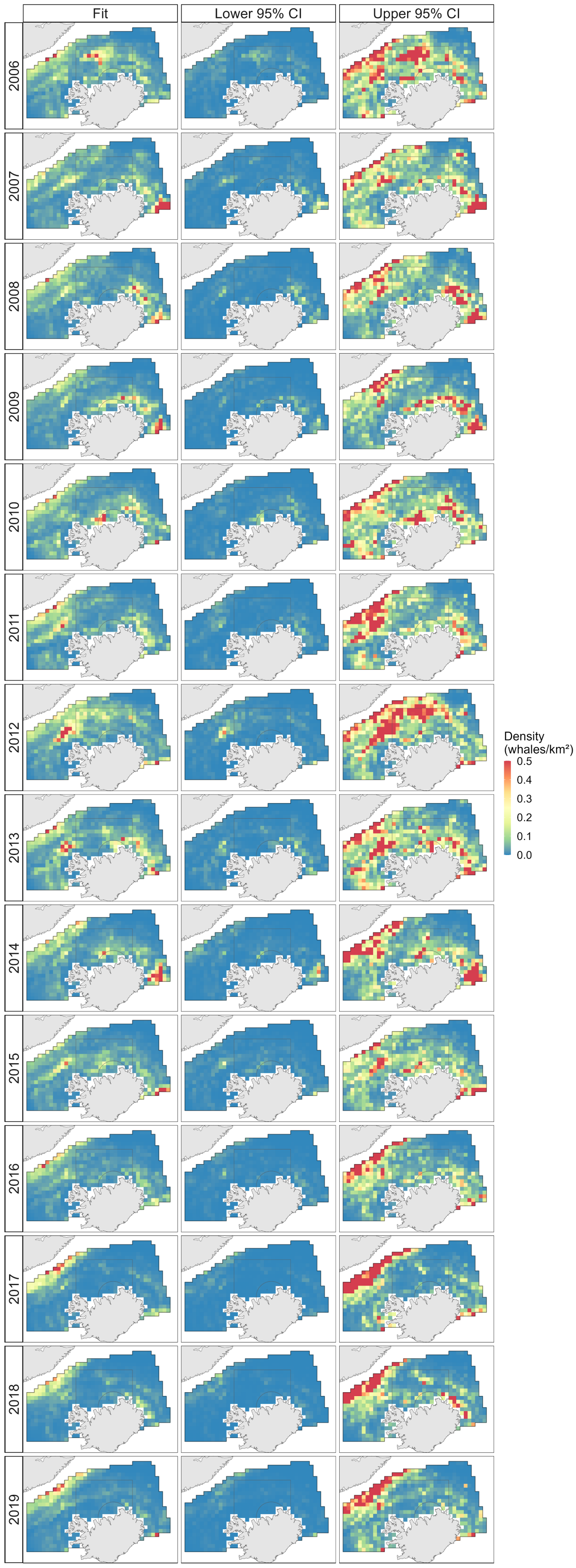

Figure 4.5: Predicted summer densities and 95% confidence intervals for 2006–2019, from the 25 km generalised additive model (GAM) constructed using data from 1987, 1995, 2001, 2007 and 2015. The two areas of interest are denoted by grey lines: 100 km around Skjálfandi Bay (circular) and the larger central area (rectangular).

Model selection

Three model frameworks were considered: CJS, JSSA and MSORD (Appendix J). The MSORD framework was chosen because the final MSORD model incorporated random temporary emigration, which cannot be accounted for in CJS and JSSA frameworks, and model outputs resulted in narrower bootstrapped CIs for abundance estimates (Figure 4.6). Moreover, CJS and JSSA models yielded abundance estimates for 2014 that were higher than expected, perhaps because detection was linked to seasonal survey effort and effort was lower in 2014 (49 days). Despite this, all model frameworks yielded comparable abundance trends (Figure 4.6).

The best-fitting MSORD consisted of constant survival (S), random and season-varying temporary emigration (Ψ), season-varying probability of entry ($pent$), month-varying persistence (φ) and constant detection (p). This model had an AICc value 2.1 lower than the second best-fitting model (in which detection was related to seasonal effort, rather than constant; Appendix J). From the best-fitting model, the estimated number of whales visiting Skjálfandi Bay each year ranged from 30 animals (95% CI 22–40) in 2010 to 183 animals (95% CI 155–203) in 2018. Abundance appeared to increase across the period, with a peak in 2017–2018, and this was supported by significant weighted linear regression ($F = 18.4,\ R^2 = 0.57,\ p = 0.001$).

4.4.3 Relating offshore density to coastal abundance

Offshore densities for two areas, derived from an SDM, and coastal abundance, derived from CR models, showed contrasting trends (Figures 4.7 and 4.8). Density from the final 25 km GAM significantly decreased in both the smaller and larger areas, although confidence intervals are large (particularly during 2006–2015) and overlapping. In contrast, the coastal time series showed the opposite trend of significantly increasing abundance, with narrow associated CIs. Pearson's tests of association revealed a non-significant negative linear relationship between seasonal coastal abundance and offshore density in the smaller 100 km radius around Skjálfandi Bay ($r = -0.39,\ t_{12} = -1.48,\ p = 0.16$), but a highly significant negative relationship with offshore density in the larger central area ($r = -0.70,\ t_{12} = -3.39,\ p = 0.005$). These results are supported by bootstrapped estimates. Correlation coefficients were negative for all replicates of both comparisons ($r < 0$; Figure 4.8).

4.5 Discussion

Species distribution models play an important role in marine conservation, elucidating species–environment relationships and predicting species occurrence or density over space and time. However, SDMs fitted to large geographic areas may not capture local variability, particularly in coastal areas exposed to elevated levels of anthropogenic activity (Avila et al., 2018), limiting our ability to inform effective marine management (Edgar et al., 2016). I used a broad-scale SDM to determine that humpback whale density in North Iceland is influenced by various static and temporally dynamic physical environmental variables, highlighting regional sensitivity to climate-driven oceanographic change. Tentative density

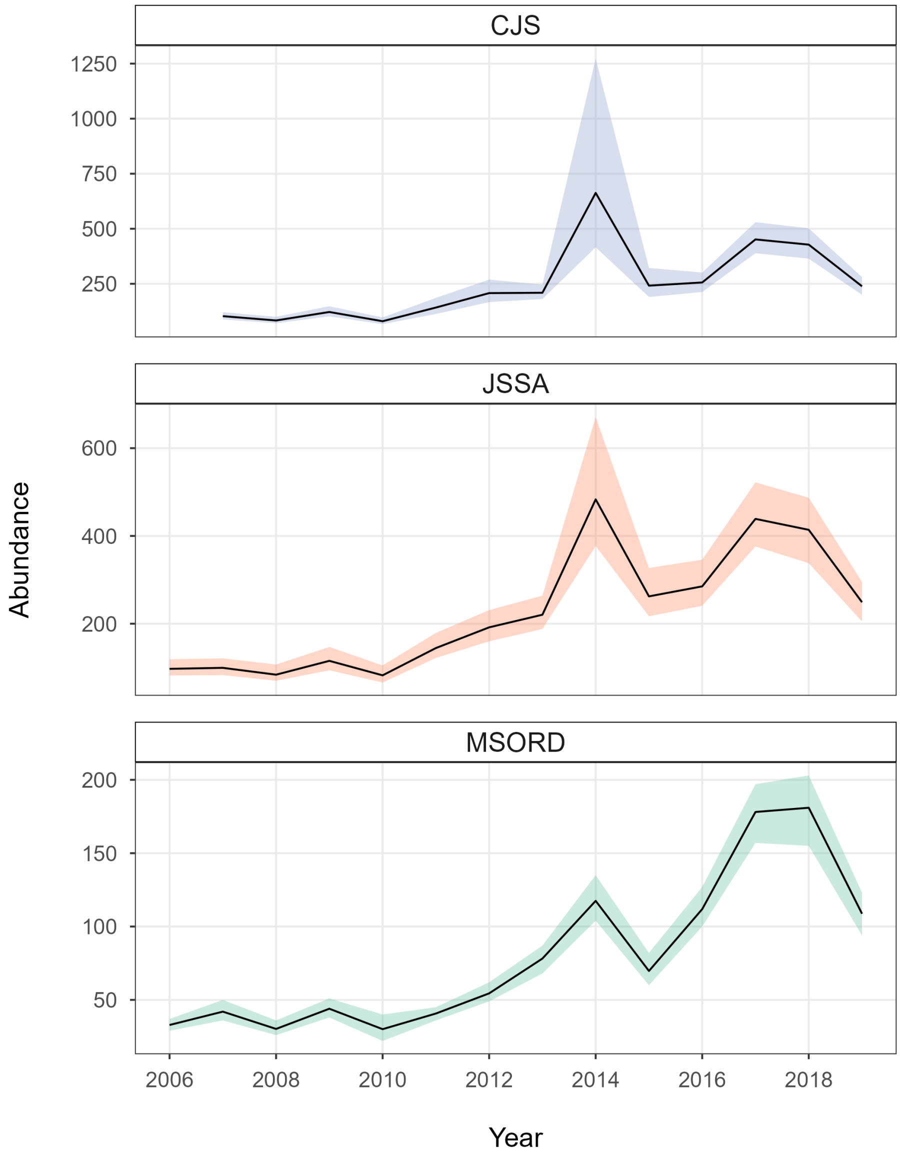

Figure 4.6: Maximum likelihood estimates of the number of humpback whales visiting Skjálfandi Bay (termed abundance) each June–August summer season (black lines) with 95% confidence intervals (shaded areas) for each model framework: Cormack–Jolly–Seber, CJS; Jolly–Seber–Schwarz–Arnason, JSSA; multi-state open robust design, MSORD.

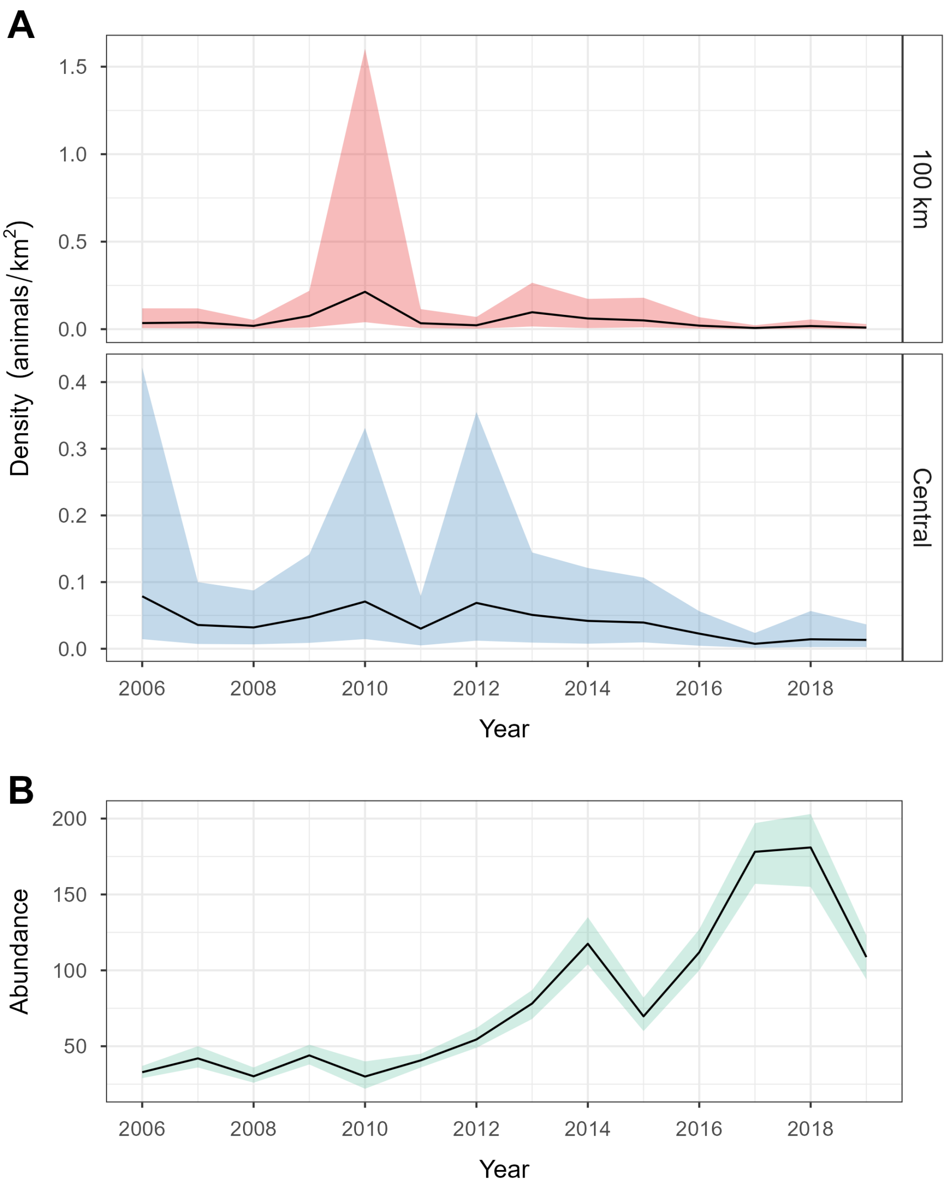

Figure 4.7: Summer offshore density estimates in North Iceland and coastal abundance estimates in Skjálfandi Bay. A) Seasonal density estimates for two areas — 100 km around Skjálfandi Bay and the larger central region — derived from species distribution model predictions (25 km generalised additive model). B) The number of humpback whales visiting Skjálfandi Bay (termed abundance) during each three-month summer season (June–August), derived from the final multi-state open robust design capture–recapture model.

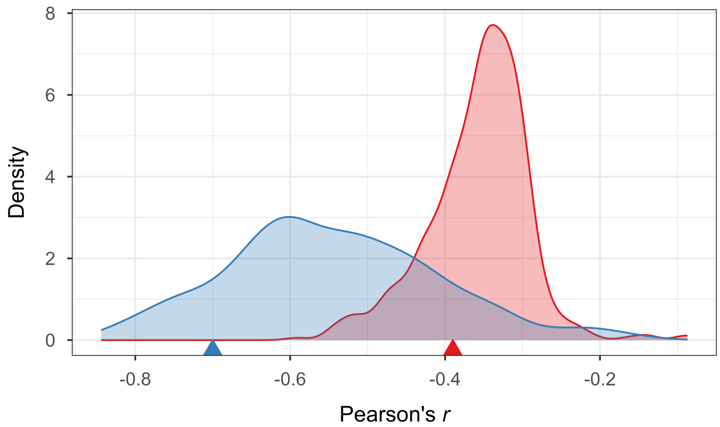

Figure 4.8: Density distribution of Pearson's correlation coefficient (r) between summer coastal abundance and offshore density estimates in either the smaller 100 km radius around Skjálfandi Bay (red) or the larger central region (blue), from 500 bootstrap replicates. Results from the final time series (not resampled) are denoted by triangular markers.

predictions from the final GAM suggested that regional offshore habitat suitability declined during the period 2006–2019. In contrast, local abundance at a coastal whale-watching hotspot, Skjálfandi Bay, increased during the same period and had a negative linear relationship with predicted offshore density. A combination of large-scale offshore surveys and regular coastal monitoring provided a more complete picture of temporal variability in occurrence across the Iceland feeding ground than could be provided by either approach alone. I discuss the robustness of these time series and their relationship; the potentially increasing importance of Skjálfandi Bay for Icelandic humpback whales; and implications for future research and regional management.

4.5.1 SDM performance

As with most cetacean sighting surveys, the spatial coverage of Icelandic NASS surveys varied across survey years, inhibiting spatial comparisons between time points (Becker et al., 2022; Chavez-Rosales et al., 2022). Fitting an SDM to these data to predict density over space and time enabled both determination of environmental correlates and tentative time series analysis. Despite extensive guidance on how to perform an SDM (Elith and Leathwick, 2009; Mannocci et al., 2017; Robinson et al., 2011), uncertainty remains for each study system. Therefore, testing multiple approaches is often sensible (Baines and Weir, 2020; Derville et al., 2018; Houghton et al., 2020). Hence, I considered two cell sizes (5 and 25 km), three model frameworks (GAM, BRT, ensemble) and dynamic variable values from three months (March, May, July) to fit the most suitable SDM.

Cell sizes were dictated by biological realism and availability of environmental data. Models fit to a coarser 25 km cell size outperformed a finer 5 km cell size in both explanatory and predictive power (Table 4.4). This is unsurprising, given that dynamic variable values were temporally coarse (monthly) and may poorly reflect fine-scale oceanographic features. A 5 km cell size may be unsuitably small for the sightings data, with an effective strip width of up to 2,178 m, such that sighted whales and the observation vessel may have frequently been in different cells (Baines and Weir, 2020).

Using a 25 km grid, the relative performance of different frameworks was dependent on the metric used (Table 4.4), highlighting the importance of selecting performance statistics that are best suited to the model and research question (Lee-Yaw et al., 2022). Whilst the BRT outperformed the GAM and ensemble in terms of explanatory power, this framework also exhibited the weakest predictive performance, determined using 10-fold cross-validation or by using 1995 and 2015 separately as evaluation data sets. This supports existing evidence (including other humpback whale SDMs) that the unrestricted form of response–predictor relationships in tree-based methods can lead to complex fits that are not ecologically intelligible or suitable for novel situations (Becker et al., 2020a; Derville et al., 2018; Oppel et al., 2012). Meanwhile, GAM and ensemble frameworks exhibited superior predictive ability measured by obs:pred ratios and RMSE values respectively, suggesting that combining the outputs of multiple models does not always improve performance (Hao et al., 2020). Given that the SDM was subsequently used to calculate mean density over a large area, obs:pred ratios were prioritised and a GAM was selected. However, for all frameworks, high RMSE values suggest that a large proportion of variability in observed densities was unexplained.

Despite the consideration of numerous factors in model fitting and selection, this SDM is an initial attempt to predict humpback whale density in North Iceland, and the process should be refined to reduce biases, improve accuracy and better inform conservation decisions. First, I assumed perfect detection of humpback whales along the survey track line (i.e., $g(0) = 1$) because survey-specific $g(0)$ values were not available for each year. Humpback whale $g(0)$ values for individual NASS surveys have ranged from 0.6 to 0.78 (Pike et al., 2009, 2010, 2019), so the density values presented here are likely an under-estimate of true density and abundance (Pike et al., 2020a). Extrapolation of $g(0)$ estimates from surveys with mark–recapture distance sampling could be considered for future estimation of sighting probabilities. Second, 95% CIs for the final density output of the SDM could be refined by incorporating other sources of uncertainty, such as ESW calculation and environmental covariate predictions, through resampling methods (Becker et al., 2022; Miller et al., 2022).

Third, a truly independent sightings data set was not used to assess SDM predictive performance. Whilst this is rarely performed in practice (Guisan et al., 2006; Redfern et al., 2017), it limits our understanding of the final GAM's transferability to novel situations, particularly outside of the covariate space (Lee-Yaw et al., 2022; Smith et al., 2021). Given that density surfaces were predicted for unsurveyed years, this is a considerable limitation of the study and my SDM predictions may be treated as hypotheses to test with independent data (Elith and Burgman, 2002; Lee-Yaw et al., 2022). Future validation could involve aerial NASS surveys conducted in an overlapping (albeit more coastal) area

(Pike et al., 2020b), but these still do not cover the temporal range of predictions (each year between 2006 and 2019). Nevertheless, predictive performance was somewhat validated in this study through temporal cross-validation and predicting densities in two years with contrasting sighting rates and distributions (1995 and 2015). The outputs of these models appear to reflect these differences (Figure 4.3).

Fourth, limited spatial auto-correlation or dependence is apparent in the final GAM residuals (Appendix I), suggesting the existence of spatially explicit variables that were not included in the model but explain distribution over space and time (Besag, 1974; Legendre and Legendre, 1998). Whilst not necessarily generating biases (Diniz-Filho et al., 2003), SDM outputs are likely to reflect this autocorrelation (Redfern et al., 2013). This can be partially resolved, for example, by including spatial interaction terms or spatial eigenvector filtering (Becker et al., 2019; Chavez-Rosales et al., 2022; Corkeron et al., 2011). However, these approaches were not used because spatial dependence may vary over time and density predictions were largely for un-surveyed years. Spatial variables (i.e., latitude, longitude) may limit our ability to model range shifts and likely act as a proxy for other important predictors, including social factors (Montoya et al., 2009), hindering our understanding of species–environment relationships (Becker et al., 2016; Wood, 2017). Nevertheless, spatial auto-correlation does restrict the transferability of habitat models (Dormann et al., 2007) and the GAM density predictions should be interpreted with caution.

4.5.2 Environmental predictors of offshore occurrence

In line with other feeding grounds, humpback whale density in North Iceland is strongly influenced by the static and dynamic physical environment (e.g., Dalla Rosa et al., 2012; Friedlaender et al., 2006; Zerbini et al., 2016), likely as a function of prey distribution (Barlow et al., 2020). In this study, without explicit spatial or temporal predictors (latitude, longitude and year), the deviance of whale density explained by the physical environment was 47.5%. This is generally in line with (or higher than) comparable feeding ground SDMs for humpback whales, including studies that do include spatiotemporal predictors — e.g., 31.9% in the western North Atlantic (Chavez-Rosales et al., 2019), 33% in Antarctica (Bassoi et al., 2020), 39.2% in British Columbia (all years combined; Dalla Rosa et al., 2012), 40.6% in California (50.1% including latitude and longitude; Becker et al., 2019) and 68.3% in the Bering Sea (Zerbini et al., 2016). Meanwhile, GAMs applied to Iceland NASS sightings from 1995 and 2001 separately explained 32.4% and 31.7% of deviance, respectively, using SST and depth as predictors (Paxton et al., 2009). Of note, response–predictor relationships may not fully reflect foraging habitat suitability because humpback whales are mobile and migratory marine predators (Dalla Rosa et al., 2008; Kennedy et al., 2014), and training data may have included sightings of both foraging and non-foraging (i.e., transiting) animals.

Spatiotemporal distribution was influenced by four static and seven dynamic variables (Table 4.2 and Figure 4.4). First, bathymetry was a strong predictor of density. Whales were mainly sighted in Icelandic shelf waters, close to the coast (<100 km), and in areas of higher seabed slope. Support for these relationships is mixed in other feeding ground studies (Meynecke et al., 2021), although humpback whales show a general preference for shelf waters (Becker et al., 2019; Chavez-Rosales et al., 2019;

Dalla Rosa et al., 2012; Paxton et al., 2009) and steep slopes can generate areas of productive upwelling, including in Arctic regions (Falk-Petersen et al., 2015). Detecting a significant relationship between bottom topography and habitat suitability may be limited by the coarse spatial resolution of the 25 km GAM.

In addition, there was a strong relationship between whale density and several dynamic predictors, including SST, SSH and MXL (Figure 4.4). For predictors with monthly values, I considered three months: July (the principal month of sightings data), May and March. Interestingly, July was not selected for any predictor in the final GAM, suggesting that suitable ecological conditions during mid-summer are dictated, at least in part, by suitable physical conditions in spring and early summer. This is likely explained by temporal lags in primary production and zooplankton responses in this ecological system (Hayward and Venrick, 1998), given that summer biomass in Icelandic waters is driven by bottom-up processes, with a single strong phytoplankton bloom around North Iceland (McGinty et al., 2016). The lag between physical conditions and baleen whale occurrence apparently varies between study systems, with reported values including one month (Dalla Rosa et al., 2012; Visser et al., 2011a), two months (Houghton et al., 2020; Riekkola et al., 2019) and more than three months (Croll et al., 2005; Dey et al., 2021). This highlights the importance of careful consideration of candidate SDM predictors (Redfern et al., 2006).

Around North Iceland, humpback whales showed a weaker preference for waters with March SST values <2°C and a strong preference for values >6°C, matching generally higher modelled densities in the northwest and southeast of the region. Temperature is an important predictor of distribution across feeding grounds, but the specific preferences vary and include higher suitability at temperatures <3°C in the Southern Ocean (Bassoi et al., 2020; El-Gabbas et al., 2021a); 7–8°C in the Bering Sea (Zerbini et al., 2016); 8–12°C in British Columbia (Dalla Rosa et al., 2012); and <15°C off California (Becker et al., 2019). Moreover, this result generally aligns with a preference of 6–8°C from 1995 and 2001 Iceland NASS data, determined by Paxton et al. (2009). The bimodal relationship may indicate that humpback whales are able to exploit multiple niches, with existing evidence of prey switching in response to fine-scale availability (Witteveen et al., 2015) and changing oceanographic conditions (Fleming et al., 2016). This dietary flexibility could limit our ability to predict habitat suitability as ocean climate continues to change (Silber et al., 2017). Beyond temperature, densities were also higher at lower SSH values and when the mixed layer was shallower than 50 m. SSH may act as a proxy for mesoscale eddies which can enhance prey biomass (Johnston et al., 2005; Oschlies and Garçon, 1998; Robinson, 2010); while MXL determines the balance between light and nutrient availability for proliferating phytoplankton (Sverdrup, 1953), and a shallower mixed layer was associated with coastal and Arctic-derived waters around Iceland (Appendix K). Both variables have previously been implicated in humpback whale distribution (Chavez-Rosales et al., 2022; El-Gabbas et al., 2021b).

SDMs typically relate whale occurrence to absolute values of dynamic predictors. However, spatial gradients (e.g., thermal fronts) and seasonal dynamism (e.g., shoaling of the mixed layer) in the physical environment also influence productivity (Doniol-Valcroze et al., 2007; Llort et al., 2019; Sathyendranath et al., 1995), and both were implicated in humpback whale distribution around Iceland. Interestingly, density exhibited contrasting relationships with spatial standard deviations of SST (linear positive) and SSH (generally negative), despite higher values of both variables indicating frontal regions that may stimulate primary productivity and concentrate prey (Genin et al., 2005; Olson and Backus, 1985; Sharples and Simpson, 2019). Complex relationships and spatial lags between baleen whale occurrence and frontal zones have been found in other studies (Becker et al., 2019; Dalla Rosa et al., 2012; Doniol-Valcroze et al., 2007; Meynecke et al., 2021), which may be attributed to i) surface manifestations of frontal regions not reflecting gradients at depth; and ii) aggregation of passive dispersing prey species taking time (Olson and Backus, 1985). Meanwhile, the seasonal (March–July) ranges of SST and MXL were negatively related to density, suggesting that environments with greater stability (i.e., lower seasonal change) throughout spring and summer provide more suitable summer feeding habitat. Summer shoaling of the mixed layer is of particular interest, dictating changes in the light–nutrient balance throughout a season and, therefore, primary production (Llort et al., 2019).

4.5.3 Temporal trends in offshore density

The multi-faceted relationships between density and dynamic predictors highlight the potential sensitivity of Icelandic humpback whales to oceanographic change. Moreover, maps of predicted offshore density (Figure 4.5) and dynamic predictors (Appendix K) for each year between 2006 and 2019 suggest that recent changes in the physical environment may have already driven changes in distribution and occurrence. Density hindcasts should be interpreted with caution due to aforementioned caveats in the modelling process.

Predicted densities were highest in the southeast and northwest of the study area (Figure 4.5), matching the general distribution of sightings and the predictions of density GAMs fitted by Paxton et al. (2009). However, there was considerable inter-annual variability in distribution, albeit with large CIs for all years. For example, 2010 predictions were characterised by high densities in the central portion, closer to the coast, while 2017 had low predicted densities in the east. Across the period, there was a general decline in predicted density across eastern, central and southwestern portions; and a significant decreasing trend for densities in the two areas of interest – 100 km around Skjálfandi Bay and the wider central area. In contrast, predicted densities showed no visual change, or even increased slightly, in the northwest, closer to Greenland. These predictions align with (uncorrected) abundance estimates for humpback whales in Icelandic and Faroese waters of 14,553 (95% CI 5,819–27,906) in 2007 (Pike et al., 2020a) and 6,643 (95% CI 3,543–12,456) in 2015 (Pike et al., 2019), a decrease of more than 50%. However, due to large and overlapping CIs (similar to our density estimates), the decline was not significant (Pike et al., 2019).

The period 2006–2019 falls within a 'warm' period in Icelandic waters, with increasing influence of Atlantic-derived water, which started in the mid-1990s (Häkkinen and Rhines, 2004; Hátún et al., 2009) and persisted to the present day (Gislason et al., 2021). This 'Atlantification' involved changes in physical parameters that were important predictors in the final GAM, which are evident in the outputs of the VIKING20X oceanographic hindcast used to fit the model (Appendix K). It appears that changes in predicted density were not uniformly related to changes in all physical predictors: based on the final

GAM, some changes in predictors would be expected to increase density (based on the final GAM), while others would be expected to decrease density (Appendix K). For example, in the central area of interest, range.SST decreased across the period, to values that were associated with higher densities in the GAM. In contrast, range.MXL, and to a lesser extent MXL and May sd.SSH, increased across the period, to values that were associated with lower densities in the GAM. In the areas of interest, March SST also appeared to increase to values associated with lower densities ($\sim$3–4°C), but the response–predictor relationship was complex in the final GAM. Overall, density decreased in the central area, but these contrasting relationships highlight the challenges of predicting species occurrence during a period of environmental change (Robinson et al., 2017). Nevertheless, the sensitivity of humpback whales to environmental change over time is apparent and supported by previous studies (Davis et al., 2020; Meynecke et al., 2021, 2020; Víkingsson et al., 2015).

It is also possible that observed temporal changes in offshore density were indirectly driven by physical changes that were not encompassed by the selected environmental variables in this chapter or took place outside of the study area. In particular, East Greenland has experienced a regime shift since the 2000s: declining drift-ice export out of the Fram Strait (leading to declining coastal sea ice extent) and increasing water temperature have increased oceanic productivity and coincided with both a decline in local catches of Arctic marine mammals such as narwhals and the apparent sudden appearance of boreal cetaceans, including humpback whales, in addition to a potential influx of capelin (Heide-Jørgensen et al., 2023). An aerial line transect survey in summer 2015 produced a humpback whale abundance estimate of 4,223 (95% CI: 1,845–9,666) – possibly one fifth of the entire North Atlantic population – in an area with historically persistent sea ice coverage and few humpback whale sightings (Hansen et al., 2018; Heide-Jørgensen et al., 2007). This aligns with observations from other areas in the Arctic (such as the Bering Sea, Svalbard and eastern Canada) of sub-Arctic cetaceans expanding their range into historically inaccessible areas as sea ice retreats (Higdon and Ferguson, 2011; Moore, 2016; Posdaljian et al., 2022; Storrie et al., 2018). Therefore, the declining offshore density in Iceland could be attributed to the movement of animals towards East Greenland, consistent with the predicted increase in density in the western portion of the study area. Any feeding ground expansion could increase the amount of suitable foraging habitat available to North Atlantic humpback whales, facilitated by the species' dietary flexibility (Fleming et al., 2016). To better understand this potential interaction of habitat suitability and whale density between adjacent feeding grounds, the geographical scope of this SDM could be expanded in the future.

4.5.4 Relating offshore density to coastal abundance

In addition to offshore density, I modelled the number of humpback whales visiting Skjálfandi Bay, a small coastal feeding ground, each summer between 2006 and 2019. Fortunately, the sightings data set contained enough information to fit an MSORD, relaxing the assumption of closure between secondary period (months). The final model included random temporary emigration, in which the rate of emigration is independent of previous availability. This aligns with our understanding of Skjálfandi Bay, as a small part of the wider Icelandic feeding ground in which animals are likely to enter and leave across seasons

(Pike et al., 2019, 2020a; Rasmussen, 2009). Temporary emigration is a common feature of cetacean feeding grounds, and failing to account for this can bias abundance estimates (Boys et al., 2019; Robbins and Pace, 2018). Nevertheless, MSORD abundance trends were supported by estimates from simpler CJS and JSSA frameworks.

Previous studies have documented the increase in sighting rates of humpback whales in Skjálfandi Bay up to 2018 (Klotz et al., 2017; Malinauskaite et al., 2022), and they are now the most commonly sighted species during whale-watching trips. I extended a 2006–2013 abundance time series from Bertulli et al. (2018) and demonstrated that the observed changes reflect a genuine increase in abundance, with narrow 95% CIs and a significant linear trend for the three-month summer season (Figure 4.7B). In particular, there was a sudden increase in modelled abundance from 2016 onwards. The general increase in abundance mirrors similar trends reported anecdotally in other coastal areas around North Iceland, including Eyjafjörður, Ísafjarðardjúp and Steingrímsfjörður (Rasmussen, pers. comm.). The environmental drivers of these trends are unknown, in part owing to a lack of high-resolution environmental data suitable for complex coastal ecosystems (Malinauskaite et al., 2022). However, sea surface temperature is generally increasing in Skjálfandi Bay (Malinauskaite et al., 2022), as in offshore areas, and changes in local sighting rates of other cetacean species have been observed. For example, minke whale sightings in the bay have decreased from the 1990s to 2018 (Lechwar et al., 2023), in line with declining species abundance across the wider Icelandic shelf area (Pike et al., 2020b), and blue whale sightings have fluctuated across the same period (Malinauskaite et al., 2022). As a local foraging ground, the observed changes in the cetacean community in Skjálfandi Bay could be driven by changes in prey availability as a result of physical oceanographic shifts. Increasing local abundance could additionally be driven by North Atlantic population growth (or at least the population segment that visits Iceland), but there is little information regarding recent North Atlantic abundance trends (Grove et al., 2023) and this is unlikely to explain the sudden increase in abundance from 2016 onwards.

Comparing SDM and CR model predictions, offshore density (in either the larger central area or smaller 100 km radius) and Skjálfandi Bay abundance showed contrasting trends (Figure 4.7), supported by a negative linear relationship in all bootstrap replicates for both comparisons. A mechanistic link between these time series cannot be determined from this study but it is possible that the increase in coastal abundance was driven by declining offshore habitat suitability and/or increasing coastal habitat suitability (Weinrich et al., 1997), resulting in the overall coastward movement of Icelandic humpback whales. Alternatively, the apparent relationship could reflect two unrelated processes; for example, decreasing offshore density could reflect the movement of animals towards East Greenland (out of the study area) instead of Icelandic coastal areas, while increasing abundance in Skjálfandi Bay could reflect the movement of animals from another coastal area. Within this, humpback whales may respond to different environmental cues in coastal and offshore environments. Coarse-scale offshore SDMs and fine-scale coastal SDMs for humpback whales show distinct relationships with the physical environment, including bathymetry and temperature (Bassoi et al., 2020; Dalla Rosa et al., 2012; Friedlaender et al., 2006; Keen et al., 2017). This is unsurprising, given that cetaceans respond to different cues at different scales and coastal areas, including Iceland, are generally characterised by higher freshwater input, greater tidal influence and more complex underwater topography (Astthorsson and Vilhjálmsson, 2002).

4.5.5 Conservation importance

The results presented here may have considerable implications for marine management in offshore and coastal Icelandic waters. First, humpback whales are sensitive to climate-driven oceanographic changes around Iceland. Factors such as temperature, sea surface height and mixed layer depth are changing at regional and global scales (Gislason et al., 2021; IPCC, 2021; McGinty et al., 2016), and humpback whale distribution is likely to shift in response (Becker et al., 2019; Chavez-Rosales et al., 2022). By understanding and forecasting this change, we can determine the dynamic spatial overlap between species distribution and offshore industries (Breen et al., 2016; Dransfield et al., 2014), which are likely to increase in Icelandic waters. For example, melting sea ice in the high Arctic has opened an economic 'Polar Silk Road' for cargo vessels across the Arctic Ocean, with forecast increases in shipping traffic around Iceland (Tillman et al., 2019). Ship strike and anthropogenic noise are population-level threats to humpback whales (Dunlop, 2019; Jensen and Silber, 2003; Williams and O'Hara, 2010) and determining the co-occurrence between core foraging areas and shipping routes in space and time is essential to mitigate these impacts (Koubrak et al., 2021). Owing to the generally low explanatory power of cetacean SDMs (Baines and Weir, 2020; Becker et al., 2019; Chavez-Rosales et al., 2019), it is important to convey predictive uncertainty in support of a precautionary approach to management (Brodie et al., 2022).

Second, the contrasting trends of offshore density and coastal abundance may augment population exposure to coastal anthropogenic stressors. The growing number of humpback whales that visit Skjálfandi Bay, paired with the apparent decline in offshore density (and regional abundance; Pike et al., 2019, 2020a), suggests that coastal areas may be increasingly important foraging grounds for the Icelandic population segment. The footprint of human activity is often more extreme in coastal environments, and is continuing to grow (Halpern et al., 2015, 2008). Around Iceland, whale-watching activities are expanding geographically in the North (Icelandic Tourist Board, 2020), primarily targeting humpback whales, and there are plans to build or develop ports around Iceland to serve the Polar Silk Road, such as Finnafjörður in the northeast (Kokorsch and Stein, 2022; Panahi et al., 2021). Therefore, the findings of this study should encourage a review of protective measures for humpback whales within the Icelandic exclusive economic zone to mitigate the potential future impacts of increasing spatial overlap with anthropogenic stressors.

4.6 Conclusion

Broad-scale species distribution models are essential to understand the responses of cetaceans to accelerating environmental change, but may not reflect local variability, particularly in coastal areas. An SDM revealed that humpback whales are sensitive to spatiotemporal variability in the physical environment in offshore waters around North Iceland, and that densities have decreased in line with the oceanographic changes in this sub-Arctic region. However, SDM-derived densities did not reflect an increasing abundance trend in a coastal whale-watching hotspot. Conversely, the local abundance time series was less suitable for elucidating environmental predictors and temporal variability could not be extrapolated to the wider feeding ground. Taken together, these results highlight the importance of multiple, complementary methods, from regular coastal observation to intermittent offshore surveys (Becker et al., 2019; Gabriele et al., 2017), to elucidate temporal changes in the abundance and distribution of wide-ranging species such as humpback whales (Hammond et al., 2021). As climate change accelerates and anthropogenic activities continue to intensify around Iceland and throughout the global ocean, this information is essential to determine population-level exposure to cumulative stressors and inform precautionary, evidence-based conservation measures.

Chapter 5

Using ecological evidence to inform whale-watching guidelines in Iceland

5.1 Abstract

In order to mitigate the potential impacts of whale-watching on whales, management must be informed by an understanding of cetacean ecology and the interactions between whales and whale-watching vessels. Where evidence is lacking, a precautionary approach is essential. Throughout this thesis, I have studied the interactions between humpback whales and whale-watching vessels in Iceland, and explored changes in occurrence and habitat use in the region. In Chapter 5, I use the results to challenge the suitability of Iceland's existing voluntary code of conduct (the only policy in place to mitigate impacts) in order to inform more effective recommendations for sustainable whale-watching activities. As part of this, I systematically review whale-watching policies from other areas and consider the role of governance in encouraging compliance and local support.

Iceland's code of conduct is generally comprehensive and appears to limit behavioural disturbance to an extent. However, based on the results of Chapter 2 and the precedent of other codes worldwide, I encourage IceWhale to consider changes to existing guidelines, including: decreasing the maximum encounter duration from 30 to 20 minutes; reducing the maximum approach speed; limiting the number of vessels per encounter (e.g., a maximum of four vessels); and considering changes to approach distance. Data used in Chapter 4 indicate that per-animal exposure may be elevated in Skjálfandi Bay in late August and early September, such that seasonal restrictions of whale-watching activities, including a modified code of conduct, could limit disturbance. Spatial management (e.g., the creation of marine protected areas) may also be effective but this requires an improved understanding of cetacean spatial ecology in Iceland. Finally, I support calls to pursue an adaptive approach to whale-watching management: coastal waters may be increasingly important for Icelandic humpback whales, resulting in elevated exposure to anthropogenic stressors, but our ability to predict future changes is limited, particularly as climate change accelerates. Effective management should include regular assessment of whale-watching impacts, building upon the findings of Chapter 3 to include robust physiological monitoring. To ensure that management is fair and effective, resulting policies should be implemented through an inclusive governance framework that centres local communities, education and strategies to ensure compliance. By adopting these recommendations, the Icelandic whale-watching industry can mitigate its potential negative impacts on humpback whales, thereby improving the resilience of both whales and coastal communities to future change.

5.2 Introduction

Vessel-based whale-watching activities form part of a social–ecological system, in which humans interact with cetacean populations (Folke et al., 2011; Malinauskaite et al., 2022). Whale-watching provides economic, recreational, educational and cultural ecosystem services to human coastal communities (García-Cegarra and Pacheco, 2017; Malinauskaite, 2021; Malinauskaite et al., 2021; Muloin, 1998). In turn, the industry can justify the development of marine protected areas (MPAs; Jefferies, 2016), fund ongoing conservation initiatives (Chalcobsky et al., 2017; Prince of Whales, 2022) and spread pro-environmental messages (Cárdenas et al., 2021; Lopez and Pearson, 2017). However, whale-watching vessels are also a source of short-term behavioural disturbance for cetaceans (Parsons, 2012), which may translate into long-term population-level impacts (Bain et al., 2014; Lusseau et al., 2006), with consequences for cetacean conservation and welfare. Furthermore, vessel-based whale-watching combines with other regional anthropogenic stressors (particularly in coastal waters) and climate change to exert cumulative effects on cetacean populations (National Academies, 2017; New et al., 2015). As the footprint of human activity grows across the global ocean, mitigating negative impacts from whale-watching activities – even minor disturbance – can help to increase the resilience of populations (Fumagalli et al., 2021).

5.2.1 Managing whale-watching

Motivated by the objectives of conservation, sustainability and preserving animal welfare (Jefferies, 2016; Mazzoldi et al., 2019; Papastavrou et al., 2017), whale-watching activities are managed in myriad ways in terms of policies (rules or guidelines) and governance (how policies are developed, implemented and enforced; Carlson, 2012; Jefferies, 2016). The variety of management schemes is driven by the varying social, cultural, economic and ecological environments in which the industry operates (Higham and Bejder, 2008). Regulations can be informed by assessments of local cetacean populations and whale-watching impacts (Bejder et al., 2006; Lusseau and Higham, 2004), or general information and existing policies from other regions, if local systems are understudied (Carlson, 2012; IWC, 2019b). In the absence of evidence of whale-watching impacts, management schemes may take a precautionary approach, introducing regulatory measures in advance of formal scientific proof (Hoyt, 2011; Notarbartolo-di Sciara et al., 2009).

Most commonly, practices are regulated through a code of conduct, specifying the permitted behaviour of individual whale-watching vessels (Carlson, 2012). These codes can be legally binding regulations at local to national levels, or voluntary guidelines at local to global levels, and vary in their detail and specificity (Parsons, 2012). Codes target various aspects of vessel behaviour – most commonly approach distance, speed, encounter duration and number of vessels (Garrod and Fennell, 2004) – and can constitute numeric or descriptive regulations. There may be special provisions for specific behavioural states (e.g., foraging, resting, nursing), different life history stages (e.g., mother–calf pairs) and endangered or threatened species. Mandatory regulations are often accompanied by licensing schemes to regulate both the number of vessels (and therefore encounters) and the competence and training of captains and guides (Carlson, 2012). Alternatively, for voluntary codes, certification schemes can be used to confirm or advertise that vessels adhere to a particular code and follow sustainable whale-watching practices, lending both ethical and financial incentives to tour operators (ACCOBAMS, 2021; Lissner and Mayer, 2020).

In addition, spatiotemporal management can be used to regulate whale-watching activities, owing to the heterogeneity in whale occurrence, behaviour and whale-watching activity in space and time (Meynecke et al., 2021). MPAs are commonly used to mitigate local stressors for marine mammals (Hoyt, 2011), including vessel-based whale-watching. MPAs may be ‘no-take’, preventing all whale-watching activity (BC Parks, 2007; Guerra and Dawson, 2016), or have different regulations for vessel practices (Government of Canada, 2002; Notarbartolo-di Sciara et al., 2009). This spatial management may include a temporal component, with seasonal closures to whale-watching activities (Washington Department of Fish and Wildlife, 2021) or time-of-day restrictions (Carlson, 2012). If appropriate legal instruments are combined with regular monitoring of whale populations and whale-watching impacts, management can be adaptive, reacting to changes in the social–ecological system (Carlson, 2012; US National Park Service, 2022).

5.2.2 Effectiveness of whale-watching management

Whilst codes of conduct and spatiotemporal management are widely implemented to regulate whale-watching activities, their effectiveness varies, often limited by non-compliance and a lack of scientific basis. Adherence to voluntary regulations is largely based on ethical obligation and peer pressure (Garrod and Fennell, 2004), with no formal repercussions of violating guidelines. For commercial whale-watching operators, high rates of non-compliance to voluntary codes have been observed in areas around the world, including western Canada (Moore et al., 2021; Rani, 2022), Hawai’i (Wiener et al., 2010), northeast USA (Wiley et al., 2008), New Zealand (Duprey et al., 2008) and Zanzibar (Christiansen et al., 2010); although some studies have also found high compliance rates (Fraser et al., 2020; Hoarau et al., 2020). Without regular enforcement, mandatory regulations are also subject to violations (Amrein et al., 2020; Freitas et al., 2021; Sitar et al., 2016; Fiori et al., 2019), which may be driven by changing socio-ecological conditions (Chalcobsky et al., 2017). Infringements of both voluntary and mandatory codes can lead to greater behavioural disturbance (Amrein et al., 2020; Koroza and Evans, 2022; Fiori et al., 2019) and compliance can actually lead to encounters of higher perceived quality and, therefore, improved tourist experiences (Hoarau et al., 2020; Sitar et al., 2016). Beyond compliance, management effectiveness can be limited if regulations are not informed by up-to-date information concerning cetacean ecology and responses to disturbance (Fumagalli et al., 2021; Visser et al., 2011b). Regulations developed in the absence of evidence may not properly mitigate whale-watching disturbance (Bejder et al., 2006), whilst changes in distribution and habitat use over time may render spatiotemporal restrictions ineffective (Hartel et al., 2015).

The conservation benefits of whale-watching management can be improved by incorporating ecological and social information into inclusive decision-making and monitoring processes (Bejder et al., 2022; IWC, 2019b). Studies assessing the response of cetaceans to disturbance provide policy recommendations at local to regional levels, using clear evidence and adopting a precautionary approach (Currie et al., 2021; NOAA, 2021; Williams et al., 2009). Meanwhile, tourist education and operator training are increasingly realised as key to maintaining compliance and managing expectations (Hooper et al., 2021; Johnson and McInnis, 2014). Finally, a multi-stakeholder approach to policy development, governance and monitoring results in more effective and equitable management, combining multiple knowledge sources and perspectives (Fumagalli et al., 2021; Sironi et al., 2005). To facilitate the development of responsible management, the International Whaling Commission (IWC) has held several workshops and created an online 'Whale-Watching Handbook', with guidelines and case studies (IWC, 2018, 2019b).

5.2.3 Chapter aim

In this final chapter, I aim to provide policy recommendations for effective whale-watching management in Iceland, with a particular focus on Skjálfandi Bay, northeast Iceland (Figure 1.4). Following the rapid growth of the industry in Iceland (Icelandic Tourist Board, 2020), Skjálfandi Bay is now one of five whale-watching areas around the country, which vary in terms of fleet size and target species (see *Húsavík and Skjálfandi Bay*; Rasmussen, 2014). From a conservation perspective, whale-watching activities are largely unregulated in Iceland, with only a voluntary code of conduct (Table 1.1) developed by IceWhale (2017), the non-profit Icelandic Whale Watching Association formed by Icelandic whale-watching operators. The code was developed and signed in 2015, and is largely based on information from other areas (IceWhale, 2017), owing to the lack of targeted impact studies conducted in the country (with an exception of minke whale-watching in Faxaflói; Christiansen and Lusseau, 2015). However, there is no regular monitoring of vessel practices and whale-watching impacts, preventing adaptive management. Moreover, there is evidence of low compliance rates to previous codes of conduct in Skjálfandi Bay, in terms of approach distance and speed (see *Whale-watching research and management in Iceland*; Martin, 2012), although the reasons for this are unknown. Therefore, there is arguably a need to review and update existing policy and governance, using multiple lines of evidence.

My thesis aimed to use ecological information to inform whale-watching policies in Iceland by examining the interaction between humpback whales and whale-watching activities. In Chapter 5, I begin by summarising the behavioural interactions between whales and whale-watching vessels (Chapter 2); attempts to determine the physiological response of whales to vessels (Chapter 3); and potential changes in humpback whale distribution and local abundance around North Iceland (Chapter 4). I then integrate these results with the wider scientific literature, existing policies around the world and evidence of their success to make specific and more general management recommendations. Finally, I discuss practical considerations for implementing these recommendations, including governance strategies and education, to encourage the development of responsible and sustainable whale-watching in Iceland.

5.3 Summary of thesis results

5.3.1 Chapter 2: behavioural responses to variable vessel practices

Whale-watching codes of conduct worldwide are informed by visual observation of the behavioural responses of whales to whale-watching vessels (Amrein et al., 2020; Currie et al., 2021; Williams et al., 2006b). Therefore, in Chapter 2 I determined the influence of whale-watching vessel presence, positioning and movement on humpback whale behaviour in Skjálfandi Bay. Seven facets of whale behaviour were related to different aspects of vessel behaviour, including distance to whale, speed and its variation, directness index and number of vessels in the area, as well as encounter duration. Relationships between vessel and whale behaviour were complex, with large confidence intervals (Appendix C), and statistical associations do not equate to vessel practices influencing whale behaviour. Moreover, the large number of relationships investigated in a single study may have limited the ability to detect significant whale–vessel associations. Nevertheless, some clear results emerged with direct relevance to the code of conduct (Box 5.1), and other, non-significant results lend support to the consideration of other changes to the code through future research.

Vessel speed

Vessel speed was significantly associated with dive time, with durations increasing linearly from 0–1 mph (122.1–124.7 seconds) up to 6 mph (149.8 seconds; Figure 2.12). This could reflect a switch to vertical avoidance, possibly including changes in dive depth (Ovide, 2017), or some other form of avoidance associated with increasing dive times. Increasing vessel speed was also associated with increasing IBI values (Figure 2.14) and whale DI values (more linear swimming path); these relationships were not significant but vessel speed was nevertheless retained in the final GAMMs. This suggests that whales may respond weakly to increasing swim speed by performing horizontal avoidance (DI) or adopting more cautious surface behaviours (IBI), but this requires more targeted investigation. Meanwhile, when vessels are non-compliant with the IceWhale speed guideline (6 mph) in the approaching zone (300–50 m from a whale), whales exhibited lower rates of surface feeding, indicating potential disruption of foraging behaviour, and longer dive times (Figure 2.16).

Encounter progression

The time elapsed since the beginning of an encounter was significantly related to the rate of surface feeding and whale directness index. Predicted per-surfacing rates of surface feeding decreased from 0.022 at 1 minute to 0.003 at 30 minutes (Figure 2.11), whilst predicted DI values increased from 0.965 at 1 minute to 0.987 at 30 minutes (Figure 2.15). There are no clear threshold exposure times above which behavioural responses shift, although SFE rates remain low above 10–15 minutes. Encounter minute was also retained in the final GAMMs for dive time, number of breaths per surfacing interval and swim speed (with increases in swim speed above 20 minutes; Appendix C). These findings are concerning given that this study was limited to exposure times of up to 50 minutes involving a single vessel but, from personal observations, individual whales can be constantly exposed to whale-watching vessels for several hours, particularly when there are few whales in the bay.

Number of vessels

Two types of vessels operate in Skjálfandi Bay – faster, smaller rigid inflatable boats (RIBs) and larger, slower oak boats. Whilst the movement of both vessel types was not associated with any variable (perhaps due to the use of coarse-scale AIS positional data), and there was low confidence that the number of RIBs influenced surface activity, the number of oak boats was significantly related to both dive time and IBI. Mean IBI increased with the number of oak boats (Figure 2.10), suggesting more cautious surface behaviour in a more extreme vessel environment. In contrast, dive times increased to a threshold of four oak boats (up to 182 seconds) before decreasing rapidly to a predicted value of 110 seconds at eight boats (Figure 2.9). This could suggest that a vertical avoidance tactic is abandoned (or changes) above a threshold number of vessels (Williams and Ashe, 2007).

Other vessel variables

Other vessel variables were included in the final GAMMs but did not yield significant relationships with whale behaviour. For example, vessel distance to whale was retained in dive time and IBI models. Meanwhile, at least one proxy for vessel movement predictability – standard deviation of speed and vessel DI – was retained models for SFE, number of breaths, swim speed and DI. Therefore, these explanatory variables improved the fit of the models to response variables, suggesting that they may influence whale behaviour in some way, but their partial relationships were not significant.

Taken together, the observed variability in vessel practices during whale-watching encounters influenced humpback whale behaviour. In response to higher speeds, prolonged encounters and the presence of more vessels in the area, whale responses included vertical and horizontal avoidance, foraging disruption and more cautious surface behaviours. Other vessel variables also contributed to explaining variability in whale behaviour, highlighting that whale–vessel interactions are complex and multi-faceted. If exposure is sufficiently high, these short-term behavioural changes could negatively impact energy reserves and ultimately vital rates (Bain et al., 2014; Christiansen and Lusseau, 2014). Therefore, current activities should be more carefully managed to limit behavioural disturbance.

Chapter 2: key results

Whale behaviour is related to vessel behaviour in the following ways.

- **Vessel speed:**
  - increase in dive time up to 6 mph
  - non-compliance in the approaching zone: less surface feeding
- **Encounter progress:**
  - low rates of surface feeding beyond 10–15 minutes
  - linear increase in whale DI with extending encounters
- **Number of vessels:**
  - increasing dive times from 1 to 4 oak boats, decreasing dive times above 4 oak boats
  - increasing IBI when more oak boats were present
- Other vessel variables (e.g., distance to whale, SD of speed, vessel DI) explained variability in whale behaviour but yielded non-significant partial relationships

Box 5.1: Key results from Chapter 2.

5.3.2 Chapter 3: steroid hormone analysis of humpback whale blow

To provide a more complete picture of the consequences of disturbance, impact assessments should include physiological monitoring in addition to behavioural observation (Johnstone et al., 2012; Mercera et al., 2021; Walker et al., 2005b). However, dynamic physiological assessment of large whales remains a major challenge in conservation physiology (Hunt et al., 2013). In Chapter 3, I explored the potential of a frontier method to assess physiological stress in humpback whales: UAV-based blow sample collection and steroid analysis using LC–MS/MS. The research was split into two phases. In Phase 1, I developed a method for sample collection and the analysis of a panel of 14 steroids, including stress-related hormones as well as other steroids to provide broader physiological context. In Phase 2, I attempted to apply the method developed in Phase 1 to a relevant ecological question: do humpback whales exhibit a physiological stress response to variable whale-watching activity and vessel traffic in North Iceland?

The results of this chapter highlight the potential of blow sampling for physiological monitoring (Box 5.2). Across three sampling seasons, 87 samples were collected from both land and vessel platforms, from at least 42 whales. In addition to blow sample collection, the UAV was also critical for photo-identification when operating from a distant land base. From 68 samples taken forward for LC–MS/MS analysis, eight steroid hormones were detected: the stress-related hormones cortisol, cortisone and DHEAS, as well as testosterone, progesterone, androstenedione, estradiol and estrone (Table 3.2). The simultaneous detection of multiple steroids is of particular interest because hormone ratios could be used as proxies for physiological stress, overcoming the barrier of variable sample dilution. Unfortunately, steroid detection rates in Phase 2 were too low to determine physiological responses to vessel traffic or whale-watching activities. In addition to improved sensitivity, the interpretation of steroid hormone levels in blow also requires biological (and ideally physiological) validation. Nevertheless, based on these results I encourage the continued development of a robust blow sampling method to assess the hidden responses of free-swimming whales to anthropogenic activities, providing key information for evidence-based management.

Chapter 3: key results

- 87 blow samples collected from at least 42 humpback whales
- UAV deployed from land and vessel platforms
- Eight steroid hormones detected, including cortisol, cortisone and DHEAS
- Low detection rates, varying across sampling seasons and LC–MS batches
- UAV-based blow sample collection and LC–MS/MS steroid analysis have high potential for physiological monitoring but require biological validation and improved sensitivity

Box 5.2: Key results from Chapter 3.

5.3.3 Chapter 4: temporal changes in humpback whale occurrence

Beyond impact assessments, characterising spatiotemporal patterns in species occurrence and abundance is essential to inform the management of regional anthropogenic activities such as whale-watching (Hartel et al., 2015). Moreover, elucidating the relationship between species occurrence and oceanographic conditions can enable predictions of future responses to accelerating environmental change. Therefore, in Chapter 4 I examined the relationship between humpback whale occurrence in offshore North Iceland and the physical environment; and determined temporal changes in offshore density and local, coastal abundance. To achieve this, I used two distinct data sets and approaches: a species distribution model (SDM) was applied to offshore sightings data to determine key environmental correlates and predict summer offshore density each year from 2006 to 2019; and a capture–recapture (CR) model was applied to photo-identification sightings from Skjálfandi Bay to predict summer abundance each year from 2006 and 2019.

The SDM highlighted that humpback whales may be sensitive to environmental change in Icelandic waters (Box 5.3). The primary physical predictors of occurrence included static variables, namely bathymetry, and dynamic variables, including sea surface temperature, sea surface height and mixed layer depth (Table 4.5 and Figure 4.4). These variables may influence whale distribution through their effects on prey availability. Physical conditions in spring (March or May) had higher explanatory power than those in summer (July), at the time of sightings, suggesting a delay between suitable physical conditions and responses at higher trophic levels. From this SDM, offshore density in North Iceland generally decreased between 2006 and 2019, although confidence intervals were overlapping. In contrast, the number of whales using Skjálfandi Bay each summer increased over the same period (Figure 4.7). There was a significant negative relationship between offshore density and coastal abundance over time. Whilst the causality of this relationship is unknown, coastal areas such as Skjálfandi Bay may be increasingly important for foraging humpback whales around Iceland. This may increase population exposure to both whale-watching activities and other coastal stressors, such as vessel traffic and planned port construction projects around Iceland (Kokorsch and Stein, 2022). Furthermore, the apparent sensitivity of humpback whales to variation in the physical environment, paired with forecast oceanographic change, suggests that distribution may undergo major changes in the future. This could have consequences for both whale-watching impacts and the availability of humpback whales for whale-watching, which will determine the future resilience of the Icelandic industry (Lambert et al., 2010; Richards et al., 2021). In light of this and residual uncertainty in predicting future changes, I call for urgency in developing a comprehensive whale-watching management strategy.

Chapter 4: key results

- Key environmental correlates of occurrence: bathymetry, sea surface temperature, sea surface height, mixed layer depth
- Decreasing offshore density and 'Atlantification' of North Icelandic waters from 2006 to 2019
- Increasing number of whales using Skjálfandi Bay from 2006 to 2019
- Significant, contrasting relationships between offshore density and coastal abundance
- Skjálfandi Bay may be increasingly important for Icelandic humpback whales

Box 5.3: Key results from Chapter 4.

5.4 Policy recommendations

By informing relevant policies, ecological information can be translated into meaningful conservation action, balancing environmental protection with other societal priorities (Alm, 2002; Bejder et al., 2022; Cooke and O'Connor, 2010; Madliger et al., 2016). To ensure that scientific results are properly communicated to policymakers and other stakeholders, whale-watching impact assessments should include specific and general management recommendations within their reporting (Chapman et al., 2015; Lusseau et al., 2006; Amrein et al., 2020).

An important consideration is the specific aim of the management strategy. Applied to whales, whale-watching policies may be designed to prioritise conservation of the population and/or the welfare of individual animals. Conservation goals concern the sustainable use of natural resources (IUCN, 1980; Jefferies, 2016) and require consideration of population-level impacts on vital rates and population dynamics, for example following the population consequences of disturbance (PCoD) model (Christiansen and Lusseau, 2015; Pirotta et al., 2018). Meanwhile, welfare goals are typically concerned with wellbeing at an individual level (Clegg and Butterworth, 2017). However, the importance of considering "feelings and fitness" together, simultaneously advancing conservation and welfare objectives, is increasingly realised, resulting in the nascent field of conservation welfare (Beausoleil et al., 2018; Clegg et al., 2021). Therefore, I will make policy recommendations with a view to achieving both goals.

I consider four aspects of whale-watching policy with existing precedent: code of conduct, temporal management, spatial management and adaptive management (Figure 5.1). For each policy type, I discuss: relevant ecological and impact information from this thesis and other published literature; existing policies worldwide, and their successes and challenges; and my recommendations. Systematic literature searches and graphics are used to convey important information beyond this thesis. Recommendations are provided for whale-watching in Iceland, based on the industry primarily targeting humpback whales in an important foraging ground. Disturbance can negatively impact foraging success, which may alter energy reserves (Costa, 1993), leading to a potential trade-off with breeding success to minimise risk to survival probability (Christiansen and Lusseau, 2014; Frid and Dill, 2002; Stearns, 1992).

Owing to residual knowledge gaps, I attempt to practise the precautionary principle (IWC, 2014), in order to make the best use of limited conservation resources (Walls, 2018). A precautionary approach shifts the burden of proof from demonstrating impacts before management action, to demonstrating no significant impacts before regulations are changed or removed, thereby ensuring that exploitation does not proceed faster than our understanding of its impacts (Bejder et al., 2006; Food and Argiculture Organization, 1995; Guerra and Dawson, 2016; Hoyt, 2011; Notarbartolo-di Sciara et al., 2009).

5.4.1 Code of conduct

Codes of conduct for the behaviour of whale-watching vessels around cetaceans, implemented as voluntary guidelines or mandatory regulations, are the most common tool for managing whale-watching impacts and are broadly similar across different regions (Carlson, 2012; Garrod and Fennell, 2004). Codes are primarily informed from behavioural response assessments (Currie et al., 2021; Garrod and Fennell, 2004; Senigaglia et al., 2016) and, in many areas (including Iceland), are the only tool in place to specifically limit whale-watching disturbance. If embraced by local communities, codes can empower and encourage operators to make long-term commitments to sustainability and promote important stewardship messages (Gjerdalen and Williams, 2000).

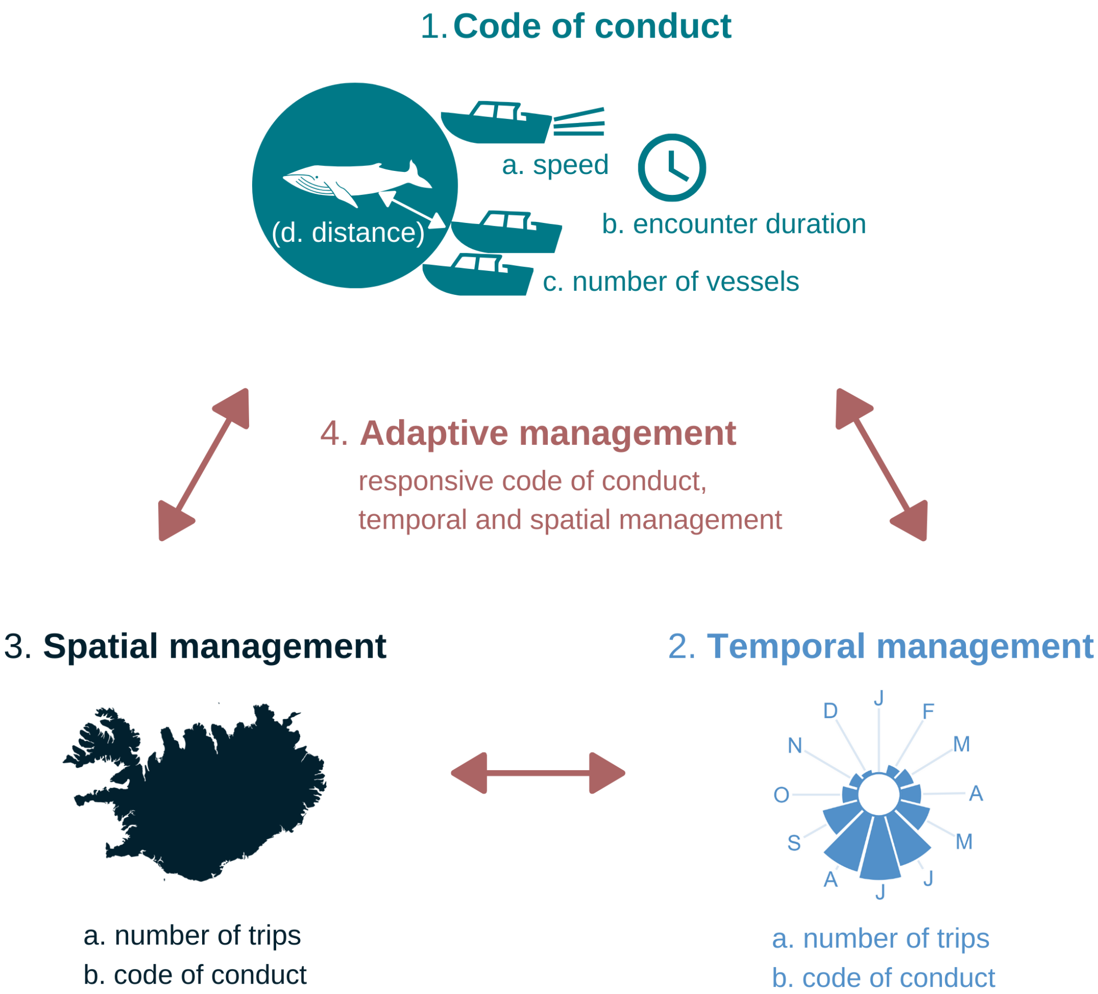

Figure 5.1: Summary of policy recommendations for whale-watching in Iceland.

Literature search

To more clearly guide code of conduct recommendations, I performed a systematic literature search of existing codes around the world to determine how current Icelandic guidelines compared with other guidelines and regulations. Guidelines are often numeric (e.g., minimum approach distance, speed–distance restrictions, maximum encounter duration), enabling simple, visual comparisons. In this way, I can assess whether there is a precedent for any recommended changes. The review was performed using a combination of two sources. First, I referred to the IWC's online Whale Watching Handbook[1], which includes a compendium of guidelines (including Carlson, 2012 and updated guidelines). Second, as a supplementary tool, I performed Google searches with the following terms: "whale watching code of conduct", "whale watching guidelines", "whale watching regulations".

For each code, I checked multiple sources to identify the most recent set of regulations. This review was only performed for policies relevant to vessel behaviour during whale-watching encounters (i.e., a code of conduct) and not other types of management (e.g., spatiotemporal restrictions). Codes pertaining only to small cetaceans were not included. I considered national and regional codes, and did not include local codes that only applied to a single port (unless this constituted the entire national whale-watching fleet). I also considered codes for Arctic and Antarctic regions, as well as international codes that were adopted by multiple countries (e.g., World Cetacean Alliance[2]).

A total of 49 codes were included. For each code, I listed all regulations and recorded the presence, form and numeric value of the following guideline categories in a table: minimum approach distance, maximum speed (with or without a distance radius), maximum encounter duration and maximum number of vessels (with or without a distance radius) per encounter. I also noted whether guidance had been provided for interpreting whale behaviour. As common numeric guidelines, the code-specific values for approach distance, encounter duration, speed–distance restrictions and number of vessels were plotted to compare IceWhale values with other codes (Figure 5.2).

Minimum approach distance

Approach distance has been related to changes in whale behaviour in several studies, although humpback whale behavioural responses differ: in Australia, whales exhibited horizontal avoidance at distances <100 m (Stamation et al., 2010); in New Caledonia, whales adopted a less linear path during closer vessel approaches (Schaffar et al., 2013); and swim speed had a complex relationship with distance in Hawai'i (Currie et al., 2021). However, in Chapter 2, despite distance to whale improving the fit of dive time and IBI models, this predictor was not significantly associated with any facet of whale behaviour (in line with Corbelli 2006).

1. International Whaling Commission Whale Watching Handbook: https://wwhandbook.iwc.int/en/
2. World Cetacean Alliance, global guidelines: https://worldcetaceanalliance.org/global-guidelines/

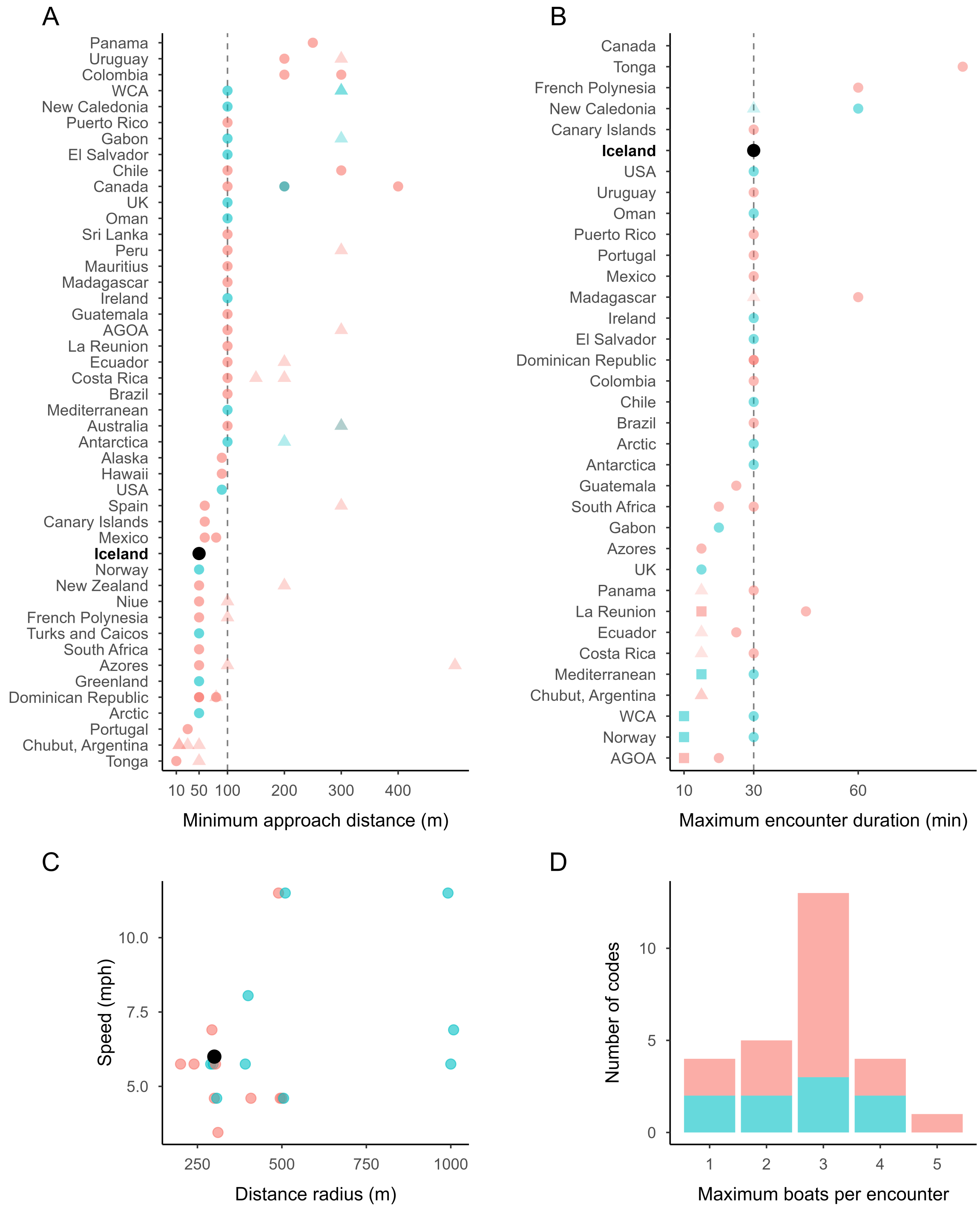

Figure 5.2: Numeric vessel guidelines or regulations from whale-watching codes of conduct around the world. In each panel, values are from all codes from the literature review with numeric values. A) Minimum approach distance to whales. B) Maximum encounter duration (with or without a distance radius). C) Speed restrictions within a distance radius. D) A histogram of the permitted number of vessels per group of whales. Pink denotes mandatory codes and blue denotes voluntary codes. In panels A–C, the black circle denotes the value for Iceland (IceWhale Code of Conduct for Responsible Whale Watching). In panels A and B, circles denote baseline values (under normal circumstances), light triangles denote special circumstances (e.g., mother–calf pairs, feeding/resting/socialising animals, endangered species) and the dashed line denotes the most common value. In panel B, squares denote encounter durations when multiple vessels (or a specified number >1) are present

From the literature search, approach distance was the most common numeric regulation, specified by 48 out of 49 codes (31 mandatory, 17 voluntary). Values ranged from 10 m to 500 m and the most common value was 100 m (23 codes, with 3 additional codes specifying 100 yards/90 m; Figure 5.2A). IceWhale's recommended approach distance of 50 m is in line with ten other codes. Meanwhile, 17 codes specified different distances in special circumstances, for example when animals were feeding or resting. Despite its near-ubiquitous use, there is evidence of low compliance rates to approach distances around the world. In Skjálfandi Bay, the data used in Chapter 2 suggest that vessels regularly approach to within 50 m. Meanwhile, Martin (2012) found that vessels were not idling within 50 m (suggesting approaches closer than 50 m) during 67% of encounters between 2009 and 2011 in the Bay, violating the code of conduct that was in place at the time. In Argentina, non-compliance with a 100 m approach distance to southern right whales led to a change in policy, with updated distances reduced to 15–50 m (Sironi et al., 2005). However, compliance rates with 100 m approach distances to humpback whales were generally high in British Columbia (Fraser et al., 2020) and La Reunion (Hoarau et al., 2020).

Whilst there is no clear evidence from Chapter 2 that approach distances should be increased to minimise disturbance, GAMM results should encourage the more targeted investigation of behavioural responses to this predictor. In particular, future research could seek to determine whether the IceWhale minimum approach distance recommendation should be increased form 50 m to 100 m, in line with a large number of codes worldwide (Figure 5.2A), whilst maintaining (or improving) compliance.

Vessel speed

Chapter 2 revealed that higher vessel speeds were associated with possible vertical avoidance (increasing dive time), with a decrease in dive time as the vessel slowed below 6 mph, while non-compliance to the 6 mph speed restriction in the approaching zone was related to fewer SFEs, suggesting that adherence to the current guideline does limit behavioural disturbance. In the scientific literature, the relationship between vessel speed and whale behaviour is poorly characterised, although Koroza and Evans (2022) found that bottlenose dolphins in Wales responded more strongly to vessel types that travel more quickly (e.g., speed boats). Furthermore, vessel speed influences underwater noise (Erbe, 2002), underwater vessel noise negatively impacts calling behaviour in Skjálfandi Bay (Laute et al., 2022) and whales can likely hear vessels from large distances (Richardson and Würsig, 1997; Sprogis et al., 2020b).

From the literature search, 35 out of 49 codes provided some form of speed guidance. Of these, 27 specified numeric guidelines and 21 provided speed–distance restrictions (11 mandatory, 10 voluntary; Figure 5.2C). In the vicinity of cetaceans, the most common maximum speeds were 4.5 mph (4 knots, 9 codes) and 5.75 mph (5 knots, 9 codes). Considering speed–distance restrictions, 11 out of 21 codes specified regulations that can be considered more cautious than IceWhale, with either lower speeds within the same or greater distances, or the same or lower speeds within greater distances (Figure 5.2C). Beyond numeric guidelines, unlike IceWhale other codes commonly specified travelling at no-wake speed or no faster than the speed of the slowest animal in a group. For example, both the Agoa

Marine Mammal Sanctuary (French Caribbean) and ACCOBAMS[3] (Mediterranean) guidelines specify maximum speeds of 5 knots, or the speed of the slowest animal (whichever value is lower), within a ‘caution zone’ (300-100 m). There is little information on levels of compliance to these speed guidelines, although Chapter 2 suggests regular non-compliance in Skjálfandi Bay, with measured vessel speed greater than 6 mph in the approaching zone preceding 23.4% of surfacings.

Despite the impact of speed on whale behaviour being understudied, I recommend a review of Ice-Whale’s speed–distance guidelines. The 6 mph speed recommendation, when followed, does appear to limit behavioural disturbance. However, the relationship between vessel speed and dive time suggests that disturbance could be further limited by reducing vessel speed below 6 mph, which would align with other codes worldwide, although there was no clear threshold below which dive time did not change. Furthermore, I recommend changing the existing guideline to specify that vessels do not travel faster than the slowest animal in the approaching zone.

Encounter duration

Key findings of Chapter 2 were the possible responses of horizontal avoidance (increasing DI) and disruption of surface feeding (decreasing SFE) as an encounter progresses. There were no clear thresholds within these significant relationships, although the rate of surface feeding remained low beyond 10–15 minutes and, non-significantly, swim speed increased beyond 20 minutes. Surprisingly, encounter duration is rarely included in impact assessments, although Schuler et al. (2019) elucidated a positive relationship between humpback whale respiration rate and time spent with whale-watching vessels in Alaska.

Despite this lack of information, encounter duration is commonly included in codes of conduct to limit cumulative exposure, with 35 out of 49 codes providing numeric guidance or regulations (Figure 5.2B). Values ranged from 5 to 90 minutes and the most common value was 30 minutes (23 codes), in line with IceWhale guidance in the approaching zone. Six codes specified shorter baseline durations and several codes additionally specified different durations depending on life history and behavioural state (six codes), or the number of vessels in the area (six codes). Information on compliance rates is limited (and not available for Iceland), although Moore et al. (2021) revealed low compliance to a 30-minute encounter limit in Canada and Freitas et al. (2021) demonstrated that the maximum usage time for restricted use zones to protect Brazilian Guiana dolphins (*Sotalia guianensis*) was not respected.

Based on limited information, I recommend a precautionary review of the maximum encounter duration. There is a precedent within existing codes of conduct to potentially limit encounters to 20 minutes, which could limit the magnitude of behavioural disturbance in line with Chapter 2. Amending this guideline could also limit the number of vessels around each whale, which could further reduce disturbance. However, further information on cumulative exposure to vessels is necessary to better inform this

3. ACCOBAMS: Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area. https://accobams.org/

guideline and possible modifications to encounter duration when multiple vessels are present. From personal observations, owing to the large number of vessels operating in the Bay, whales may spend long periods of time continuously engaged in whale-watching encounters, regardless of the time spent with a particular vessel.

Number of vessels

In whale-watching areas around the world, individual whales are often surrounded by large numbers of vessels during whale-watching encounters (Lachmuth et al., 2011; Sitar et al., 2017), including 12 vessels around humpback whales in Southeast Alaska (Kessler and Harcourt, 2013), 15 in New Caledonia (Schaffar et al., 2010) and 14 in Alaska (Di Clemente et al., 2018). In Skjálfandi Bay, up to 17 vessels operate in the area (as of 2022) and up to ten vessels were observed around a single whale at once during the study period (pers. obs.). Chapter 2 revealed an apparent change in vertical avoidance strategy (dive time) when the number of oak boats increased above four, and more cautious surfacing behaviours (higher IBI) when more vessels were in the area. The importance of vessel number is supported by other studies. For example, humpback whales performed more surface-active behaviours as vessel number increased in Alaska (Di Clemente et al., 2018) and Newfoundland (Corbelli, 2006) feeding grounds, while Williams and Ashe (2007) determined that orcas abandoned a horizontal avoidance strategy when more than three vessels were present.

The IceWhale code provides no numeric guidance on the number of vessels, instead offering recommendations to “limit the number of vessels” and “take turns if there are more boats in the area”. In contrast, from the literature review, 29 codes provided numeric guidance (19 mandatory and 10 voluntary). The most common number of vessels permitted within an approaching zone (various distances) was three (13 codes; Figure 5.2D), whilst 12 codes specified a maximum of 1–2 vessels. It is also common to provide guidance on the relative positioning of multiple vessels: guidelines for Agoa, Gabon, Oman and French Polynesia suggest that all vessels are positioned on the same side of the whale (Carlson, 2012). Compliance rates with vessel number guidelines vary considerably between codes and areas (Guerra and Dawson, 2016; Hoarau et al., 2020; Sitar et al., 2016), and there is evidence that non-compliance leads to behavioural disturbance (Amrein et al., 2020).

Due to the apparent response of whales to an increasing number of vessels, I recommend amending the IceWhale code to include clear, numeric guidance on vessel number, in accordance with other codes worldwide. For example, a maximum of four vessels would align with the results of Chapter 2 and would be less strict than the majority of codes. To facilitate this, the number of vessels engaged in whale-watching at any time could be limited by restricting the minimum number of passengers per whale-watching trip (i.e., preventing many trips with fewer passengers). Guidance on vessel positioning should also be considered.

Other guidelines

Chapter 2 did not provide information relevant to other common guidelines, such as approach angle, gear changes, communication between vessels and departure behaviour. Nevertheless, the IceWhale code already provides guidance for these practices in a more detailed "Operators Manual"[4], which is generally in line with other codes. Furthermore, the code recommends keeping a constant course (no sudden changes in speed or direction around a whale), which is supported by the results of Chapter 2, with both vessel speed variability and vessel DI (path predictability) retained in the final set of GAMMs. Due to non-significant partial relationships and the complexity of these variables, it is not possible to provide clear numeric guidance.

5.4.2 Temporal management

Whilst codes of conduct are generally invariant across time, seasonal or diel (time-of-day) restrictions of whale-watching activity can serve to minimise cumulative disturbance (Carlson, 2012). Furthermore, whales exhibit periodicity in occurrence and behaviour at multiple timescales. Humpback whales have distinct breeding and feeding seasons and undergo annual migrations between high-latitude feeding grounds and low-latitude breeding grounds (Carwardine, 2019; Mackintosh, 1946). Meanwhile, prey availability can drive reliable intra-seasonal shifts in habitat use and foraging behaviour (Keen et al., 2017; Nichols et al., 2022); and humpback whales exhibit diel variation in foraging strategy and dive patterns (Friedlaender et al., 2009a, 2013; Nichols et al., 2022; Derville et al., 2020). Temporal variation in humpback whale occurrence and behaviour is also apparent in Skjálfandi Bay. In the absence of visual survey effort in winter months, sighting rates are generally low in early spring and higher in summer months (using data from 2012; Klotz et al., 2017) and possibly autumn (Rasmussen, pers. comm.). Meanwhile, singing activity has been recorded during winter, but not other seasons (Magnúsdóttir et al., 2014), and summer vocalisation rates are higher at night (Laute et al., 2022). In addition, the results of Chapter 2 suggest that the rate of surface feeding was higher in early summer (May–July) than late summer (August–September; Appendix C).

Beyond whale occurrence and behaviour, temporal management of whale-watching should also consider seasonal variability in whale-watching activity, as per-animal exposure is likely to be greater when there are fewer whales and more trips (Fumagalli et al., 2021; Guerra, 2019). Under these conditions, whales may spend more time in the presence of a lager number of vessels, and both encounter duration and boat number influenced behaviour in Chapter 2. The number of whale-watching trips per day is dependent on the occurrence of whales but is also likely to respond to seasonal tourist demand and weather conditions (Malinauskaite et al., 2022). I used photo-identification data processed during Chapter 4 (2016–2021) and published whale-watching schedules from each company (2022) to visually compare seasonal variation in the number of whales seen and the scheduled number of whale-watching trips per day (Figure 5.3, further details in legend). Fewer whales were seen in March–April and August–

4. IceWhale's code of conduct for responsible whale watching - operators manual: https://s3-eu-west-1.amazonaws.com/wwhandbook/guideline-documents/Iceland_IceWhale-CoC-OperatorsManual.pdf

September, and more whales were seen in June–July. Meanwhile, the daily number of whale-watching trips peaked in July–August, with fewer trips in spring and autumn. In August, whilst the number of whales seen per day decreased sharply throughout the month, the number of whale-watching trips remained high. As a result, both cumulative exposure and the number of vessels per encounter may be greater (Christiansen et al., 2010) at the end of summer, possibly leading to elevated levels of disturbance.

Temporal management may be considered to limit seasonal whale-watching exposure in Skjálfandi Bay. Existing policies highlight a range of approaches to regulating whale-watching activities at seasonal and diel timescales. For example, time-of-day limitations have been enforced in Samaná Bay, Dominican Republic, where whale-watching trips must end by 16:00 (Carlson, 2012); and Mauritius, where dolphin-watching can only take place between 06:00 and 12:00 (Government of Mauritius, 2012). In Chubut Province, Argentina, it is prohibited to approach southern right whale mother–calf pairs before August 31st each year (although compliance is low; Chalcobsky et al., 2017). Washington State, USA, has imposed a more extreme form of temporal management for commercial whale-watching of endangered Southern Resident killer whales (Carretta et al., 2022) to limit vessel noise and disturbance: licensed vessels are only able to make approaches closer than 0.5 miles between July and September (core feeding season), during two two-hour periods (10:00–12:00, 15:00–17:00) each day (Washington Department of Fish and Wildlife, 2021). Other whale-watching areas around the world have expressed concern about chronic exposure to vessels during periods when there are few whales (Fumagalli et al., 2021; Guerra, 2019), and have proposed time-of-day closures of critical habitats (NOAA, 2021), but regulations are yet to be introduced.

Based on the precedent of existing management strategies, I encourage the consideration of temporal management of whale-watching activities in Skjálfandi Bay to minimise cumulative exposure in late summer. Limiting the whale-watching season is extreme and not recommended, given the large number of target species and the 'least concern' conservation status of humpback whales (Cooke, 2018b). Seasonal restrictions on the number of vessels or trips per day or permitted whale-watching hours could limit exposure, but may have negative economic consequences for whale-watching operators. Finally, the code of conduct may be altered at certain times of year. For example, as shown in Chapter 2, restricting the number of vessels or decreasing maximum encounter duration may limit disturbance.

5.4.3 Spatial management

In addition to temporal management, whale-watching activities can be managed in space to protect core and critical habitats (Lusseau and Higham, 2004). The distribution of humpback whales, like other cetaceans, is driven by physical, biological and social factors (Becker et al., 2014; Ersts and Rosenbaum, 2003; Herr et al., 2016). In feeding grounds, humpback whale distribution is largely determined by prey availability and, in turn, physical proxies (Dalla Rosa et al., 2012; Friedlaender et al., 2009b), as demonstrated for offshore North Iceland in Chapter 4, with higher density to the east and northwest

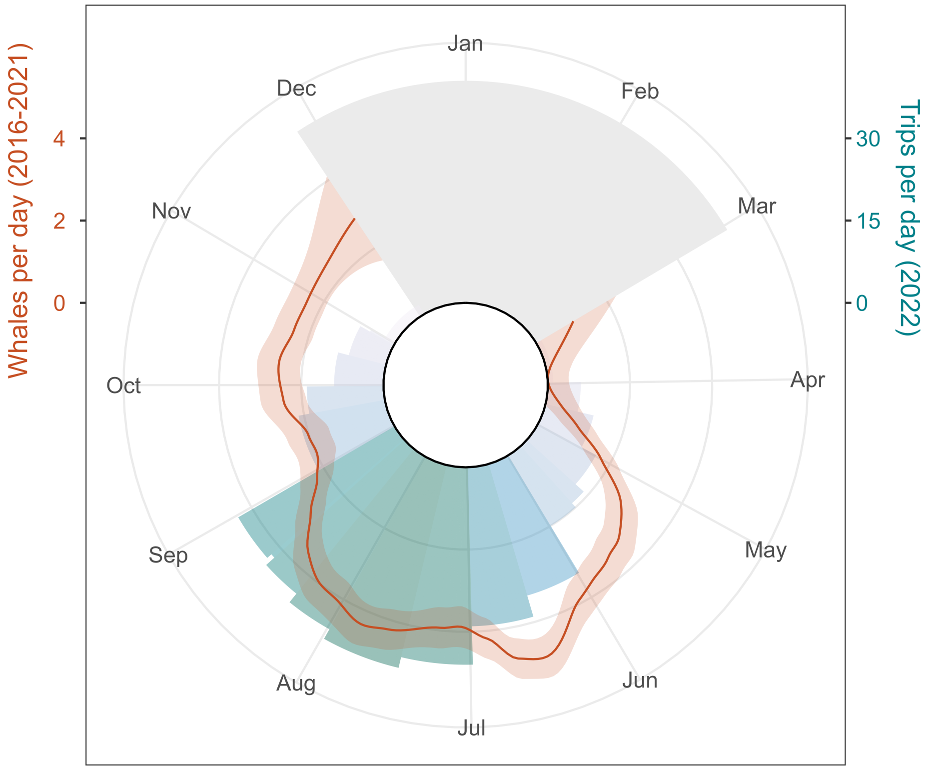

Figure 5.3: Seasonal humpback whale occurrence and whale-watching intensity in Skjálfandi Bay. The orange line denotes the mean number of identifiable whales seen per day (loess smooth from daily values, span = 0.3), averaged across 2016–2021 (confidence intervals denotes by the light orange ribbon), derived from photo-identification data from Chapter 4. Bars denote the scheduled number of whale-watching trips per day in 2022, determined from published schedules for all three whale-watching companies (North Sailing: https://www.northsailing.is/; Gentle Giants: https://www.gentlegiants.is/; Húsavík Adventures: https://husavikadventures.is/; Accessed 1 January 2023). This does not represent the actual number of trips, which was often limited by adverse weather (Malinauskaite et al., 2022). The whale-watching season lasts from March to November, and there are no available occurrence data for December–February (grey shaded area).

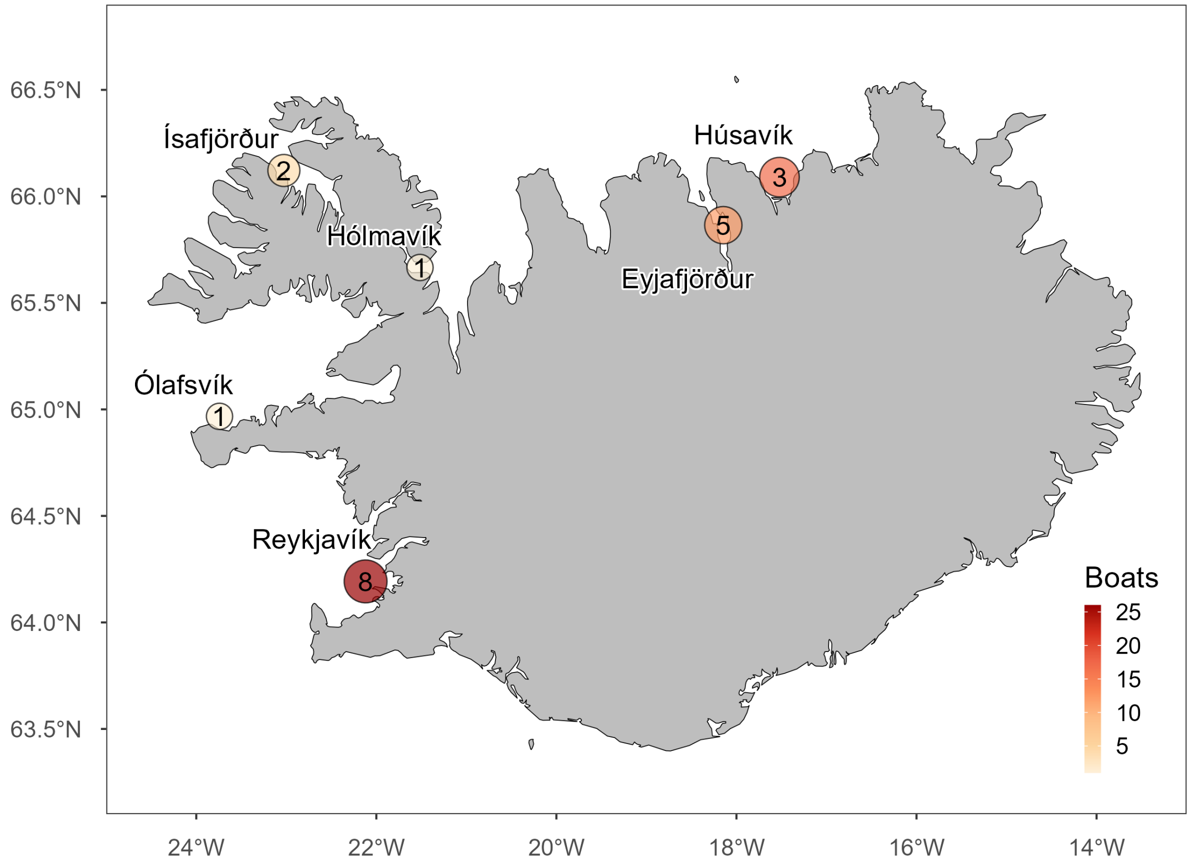

Figure 5.4: Map of whale-watching fleets across Iceland. Names represent the whale-watching port in each area, except for Eyjafjörður, which has several separate ports with whale-watching vessels. The number of companies is denoted by the number, and the number of vessels is denoted by the size and colour of the circle. All data were compiled from company websites, which were identified using a Google search of "whale watching companies Iceland" in January 2023.

of the study area. Habitat selection takes place at various spatial scales, from basin-scale (Chavez-Rosales et al., 2019) to local (Hazen et al., 2009; Keen et al., 2017). In addition to whale populations, the presence, magnitude and form of vessel-based whale-watching activities are spatially structured, driven by whale distribution and socio-economic factors (Cisneros-Montemayor et al., 2010).

Around Iceland, there is limited information on the spatial distribution of humpback whales in coastal areas, as transect surveys have primarily been conducted in offshore waters (as in Chapter 4). Skjálfandi Bay is considered to be an important local foraging area (Klotz et al., 2017) but the importance of adjacent coastal areas is unknown. Meanwhile, there are six key whale-watching fleets: Reykjavík (operating in Faxaflói), Ólafsvík (operating in Breiðafjörður), Ísafjörður (operating in Ísafjarðardjúp), Hólmavík (operating in Steingrímsfjörður), Eyjafjörður (operating out of several ports) and Húsavík (operating in Skjálfandi Bay). These fleets vary in terms of the number of companies and the maximum number of whale-watching vessels (Figure 5.4).

Spatial management, usually in the form of MPAs, is increasingly used to limit various anthropogenic impacts on whales (Gormley et al., 2012; Hoyt, 2011; Rockwood et al., 2020). MPA design may be informed by comprehensive ecological evidence (Guerra and Dawson, 2016), or employed as a precautionary measure in the absence of further scientific information (Guerra and Dawson, 2016; Lauck et al., 1998; Notarbartolo-di Sciara et al., 2009). In particular, limiting disturbance in energetically important areas has the potential to provide disproportionate benefits for the whole population (Bejder et al., 2006; Williams et al., 2006a). Whilst MPAs may target critical habitats for particular taxa, they nevertheless enable an ecosystem-based approach (Cook et al., 2019; Pauly et al., 2002).

Applied to whale-watching activities, spatial management is usually applied to resident, isolated or endangered populations, and can take several forms. For example, in the Saguenay–Gulf of St Lawrence Marine Park, a critical baleen whale foraging area in eastern Canada, whale-watching activities are allowed but a stricter code of conduct is enforced and mobile “observation zones” are used to minimise per-animal exposure (Government of Canada, 2002). In Francisco Coloane Marine Park, Chile, a humpback whale foraging ground, a speed limit of 4 knots is enforced to limit disturbance (Ruiz Troemel et al., 2014). In response to unsustainable dolphin-watching activities in Doubtful Sound, New Zealand, areas of core resting habitat for bottlenose dolphins have been designated as no-vessel zones to provide a safe haven, and additional zones designated for licensed operators, which has limited vessel exposure (Fumagalli et al., 2021; Lusseau and Higham, 2004). Spatial restrictions are generally mandatory but can also be voluntary. For example, within Robson Bight Ecological Reserve, Canada, a critical habitat for Northern Resident killer whales, all whale-watching activities are strongly discouraged (BC Parks, 2007), which appears to result in higher rates of cetacean habitat use within the reserve (Rani, 2022). However, reserve boundary compliance was low for private and charter pleasure vessels, resulting in behavioural disturbance (Rani, 2022; Jelinski et al., 2002).

Owing to the lack of spatial information about occurrence, abundance and habitat use for humpback whales around coastal Iceland, I cannot make specific recommendations for spatial management. However, given its high potential to limit cumulative disturbance from whale-watching and other anthropogenic activities, combined with management precedents from other regions, I strongly encourage research efforts to improve our understanding of cetacean spatial ecology in Icelandic waters. The future designation of protected areas could be integrated into marine spatial plans that are being developed for the Westfjords and Eastfjords of Iceland (Landsskipulagsstefna, 2018), and would strongly align with Iceland’s National Biodiversity Strategy and Action Plan as a Party to the CBD (Ministry for the Environment and Natural Resources, 2013).

5.4.4 Adaptive management

Typically, marine management strategies are fixed in space and time (Carlson, 2012), with legal instruments that are difficult and slow to change (Chalcobsky et al., 2017). This may not be appropriate for dynamic marine systems in which seasonal and spatial patterns are neither consistent over time nor predictable (Robinson et al., 2011). The impact of anthropogenic activities on the global ocean is increasing (Halpern et al., 2019), with resulting shifts in cetacean population dynamics and habitat use (Becker et al., 2019; Cartwright et al., 2019; McComb-Turbitt et al., 2021). Moreover, our ability to predict these responses is limited: for example, humpback whale distribution models generally have low explanatory power (Becker et al., 2010; Dalla Rosa et al., 2012; Meynecke et al., 2021) and forecasts of future environmental change are often inaccurate (Malinauskaite et al., 2022).

The results of Chapter 4 exemplified these changes and challenges. Contrasting trends of increasing abundance in Skjálfandi Bay and decreasing offshore density between 2006 and 2019 suggest that coastal waters are increasingly important for Icelandic humpback whales. However, both time series exhibited large inter-annual fluctuations and there was low confidence in offshore density predictions, with the final SDM explaining only 47.5% of deviance. Nevertheless, humpback whales appear to be sensitive to climate change in Icelandic waters: offshore occurrence was related to sea surface temperature, mixed layer depth and sea surface height; and changes in these variables apparently drove declining predicted offshore density. Therefore, current and anticipated future oceanographic change may increase the exposure of Icelandic humpback whales to coastal stressors such as whale-watching activities, but the magnitude of this change is uncertain.

As a result, fixed whale-watching regulations may become ineffective as these unforeseen environmental and population changes take place. For example, in the Bay of Islands, New Zealand, the fine-scale distribution of bottlenose dolphins shifted over time, such that vessel exclusion zones no longer protected critical habitats (Hartel et al., 2015). Meanwhile, in Argentina, collective discussions involving tour operators concluded that existing whale-watching policies were outdated due to changes in whale distribution and whale-watching intensity, leading to low compliance rates (Chalcobsky et al., 2017; Sironi et al., 2005).

To circumvent these issues, management frameworks may be adaptive, i.e., designed in such a way that policies can respond rapidly to changing conditions in the social–ecological system, in order to proactively mitigate potential disturbance (Tyne et al., 2014). To facilitate iterative, learning-based decision-making (Fuentes et al., 2016), adaptive policies should be accompanied by regular monitoring of the target population and whale-watching impacts (McComb-Turbitt et al., 2021). By enabling contemporary information on whale populations and whale-watching activities to flexibly inform policies in advance of negative impacts, effective adaptive management enshrines the precautionary principle (Fumagalli et al., 2021; Higham et al., 2008). Applied to whale-watching activities (or vessel traffic more generally), its current usage is limited. For example, whale-watching regulations in Samaná Bay explicitly state that the maximum encounter duration may be changed, depending on the number of whales in the area (Carlson, 2012). To limit humpback whale exposure to vessel traffic, Glacier Bay National Park, Alaska, utilises “whale waters” – areas with speed restrictions which move according to the distribution and occurrence of humpback whales, which in turn are regularly monitored by the US National Parks Service (US National Park Service, 2022). Similarly, Dynamic Management Areas are used to minimise spatial overlap between North Atlantic right whales and large vessel traffic in the western North Atlantic, thereby minimising the risk of ship strike (NOAA, 2008). For areas without an existing strategy, its implementation is often recommended in ecological impact studies, e.g., Fumagalli et al. (2021); Hoarau et al. (2020).

I encourage the incorporation of an adaptive component into existing and future whale-watching policies in Iceland. For example, seasonal changes to the code of conduct may depend on the number of whales in the area or observed responses to whale-watching vessels, which must be regularly monitored, to limit per-animal cumulative disturbance. To provide transparency to these flexible policies, clear limits of acceptable change (LAC, e.g., an increase in local abundance or a shift in behavioural response) should be developed (Higham et al., 2008). Beyond whale-watching, the results of Chapter 4 should be integrated into existing marine spatial planning law in Iceland (Landsskipulagsstefna, 2018), enabling the adaptive management of other coastal stressors such as shipping traffic and infrastructure development to mitigate lethal and sub-lethal impacts.

5.5 Discussion

Effective cetacean conservation requires up-to-date ecological information on the interactions between cetaceans and anthropogenic activity, or a precautionary approach in the absence of comprehensive evidence (IWC, 2014). I integrated the primary results of this thesis with published research, apparent knowledge gaps and existing policies worldwide to make specific and general recommendations for whale-watching management in Iceland (Figure 5.1). Based on the behavioural response of whales to vessel practices, I recommend specific changes to the IceWhale code of conduct, including more cautious guidance for vessel speed, encounter duration and number of vessels (and a suggestion to further investigate approach distances). Combining evidence of disturbance with information on seasonal variation in sightings and whale-watching activity, I recommend some form of temporal regulation, which may include changes to the code of conduct in late summer to reduce per-animal exposure. Pending a better understanding of cetacean spatial ecology in Icelandic coastal waters, I suggest consideration of spatial management to limit disturbance in critical habitats from whale-watching and other anthropogenic activities. Finally, given the changing distribution of humpback whales and our limited ability to predict future shifts, I support recent calls from other whale-watching areas to implement an adaptive management strategy. These recommendations are made with an understanding that long-term impacts remain poorly understood (New et al., 2015) and that whale-watching disturbance is only one component of the cumulative effects of anthropogenic activity on cetaceans (National Academies, 2017). Other threats, such as climate change, are likely more severe (Meynecke et al., 2020), but mitigating impacts from any stressor will improve the current and future resilience of populations (Fumagalli et al., 2021; Pirotta et al., 2022).

Whilst my recommendations considered the precedent of existing policies worldwide and their success, they are largely based on ecological information. However, as part of a social–ecological system, whale-watching management is more complex than cetacean ecology, with its form, compliance and effectiveness influenced by human social, political and economic factors (Higham and Bejder, 2008; Malinauskaite et al., 2021; Walls, 2018). I proposed several changes that represent stricter control of whale-watching activities, but high levels of non-compliance appear to exist in Skjálfandi Bay (Chapter 2; Martin, 2012). This is typical of whale-watching areas and policies around the world, including policy suggestions given here (Amrein et al., 2020; Moore et al., 2021; Sironi et al., 2005). As a result, implementing these policies in Iceland requires mechanisms to encourage reasonable compliance (Duprey et al., 2008) and support from local industries (Cooney, 2004). Within this, policies should balance the potential negative impacts of whale-watching on cetaceans with the need for a profitable, thriving industry that supports local livelihoods (Chalcobsky et al., 2017; Higham and Bejder, 2008). Below, I discuss governance and education as key considerations for effective, sustainable and equitable management.

5.5.1 Governance and regulation

The practicalities of developing, enacting and regulating policies are determined by the form of governance. Suitable governance is essential for rational ocean management (Jefferies, 2016) and can influence compliance with, and local support for, policies (Garrod and Fennell, 2004). I discuss three considerations for whale-watching governance and regulation in Iceland. First, management can take the form of voluntary guidelines, such as the IceWhale code of conduct, or mandatory regulations. Voluntary cooperation relies on all users agreeing to manage their behaviour in a way that mitigates potential negative impacts (Baranzini and Thalmann, 2004), placing implicit trust in local communities and businesses to assume responsibility for their own environmental actions (Gjerdalen and Williams, 2000). However, due to low rates of compliance to voluntary codes, the introduction of mandatory regulations is often recommended (NOAA, 2021; Parsons, 2012). Therefore, data from Skjálfandi Bay support the consideration of whale-watching legislation if voluntary compliance is not improved through other means (see below). Furthermore, adaptive management typically relies on mandatory regulations (Tyne et al., 2014).

Second, it is important to decide who contributes to the development and regulation of a set of policies, both to maintain compliance and to ensure an inclusive approach to whale-watching management that safeguards livelihoods (IWC, 2014). As a local stressor, whale-watching impacts should be managed by local solutions (IWC, 2018; Jupiter et al., 2014). Co-management by multiple stakeholders, including local communities, business, non-governmental organisations and government, ensures that relevant competing interests are integrated into a single management framework (IWC, 2021) and facilitates adaptive management (Lundquist, 2014). Authentic collaborations with local communities also enable the incorporation of diverse information sources, including scientific evidence but also local ecological knowledge (Carter and Nielsen, 2011; Gerhardinger et al., 2009). Applied to whale-watching, co-management has led to successful regulation in areas such as the Dominican Republic (Gleason, 2015) and Kaikōura, New Zealand (Fumagalli et al., 2021), although including the views of all stakeholders can be challenging (Neves-Graça, 2004). The IceWhale code of conduct was approved during a 2015 workshop of whale-watching operators, assisted by international experts and based on whale-watching research and policies around the world (IceWhale, 2017). Owing to the success of co-management, future policy development in Iceland should continue to involve tour operators and additionally include local communities, for whom whale-watching activities are economically and culturally important (Malinauskaite et al., 2022), and government. Icelandic policies may also be informed by guidance from NAMMCO, which provides evidence-based recommendations to its national parties (NAMMCO, 2015), and the IWC, which hosts workshops to promote capacity building and information sharing in whale-watching management (IWC, 2014).

Third, governance strategies should have instruments for monitoring or enforcing policies (Baranzini and Thalmann, 2004; Keane et al., 2011). Without these systems, compliance rates can be low for mandatory regulations (Amrein et al., 2020; Sitar et al., 2016; Steckenreuter et al., 2012) and voluntary codes (Moore et al., 2021; Rani, 2022; Wiley et al., 2008). Around the world, several strategies are employed to maintain compliance. For example, licensing schemes are commonly used to control the number of vessels in space and time, enabling adaptive management (Tyne et al., 2014), and to ensure that captains and guides meet specified standards. Areas including South Africa (van Schalkwyk, 2008), the Azores (Oliveira et al., 2009), Portugal, Sri Lanka, Tonga and Argentina (Carlson, 2012) require commercial whale-watching vessels to obtain a permit. Alternatively, voluntary guidelines may be accompanied by a certification scheme, under which operators are expected to comply with a particular code (ACCOBAMS, 2021; Whale SENSE, 2021; World Cetacean Alliance, 2022). In addition, observers can be used to monitor vessel behaviour and their adherence to regulations, which can facilitate adaptive management. In the Salish Sea, vessels with the power to enforce regulations (Ferrara et al., 2017) or conduct monitoring (Seely et al., 2017) are linked to significantly higher rates of compliance. As a more cost-effective strategy, monitoring may take place on-board whale-watching vessels and may even involve tourists, who are often concerned about the sustainability of practices (Cárdenas et al., 2021; García-Cegarra and Pacheco, 2017; Villagra et al., 2021). Although IceWhale does not have a systematic monitoring strategy, anonymous observers have previously been placed on whale-watching vessels (Sigursteinn Másson, pers. comm.) and I encourage the sustained continuation of this scheme. Moreover, given that not all Icelandic operators subscribe to the code, a more targeted certification scheme could be developed.

5.5.2 Education and training

To further increase the effectiveness of whale-watching management, policies should be supplemented with appropriate operator training and education (IWC, 2018). A combination of certification and training can ensure more responsible vessel practices (IWC, 2019b), and qualified guides are often recommended or legally required to improve tourist experiences (ACCOBAMS, 2021; Carlson, 2012; Finkler and Higham, 2020; Notarbartolo-di Sciara et al., 2009). Furthermore, guidelines and regulations should be accompanied by information on the specific benefits of different policies to whale populations

(Duprey et al., 2008). Whale-watching captains often know and support guidelines but are noncompliant, which may reflect a lack of understanding about cumulative effects of violations (Hooper et al., 2021). Therefore, there are calls to shift codes of conduct from an ontological format, which simply bans irresponsible behaviours (Orams, 1996), to a teleological format, in which the background and aims of recommendations are provided (Ballantyne et al., 2009; Granquist and Nilsson, 2016). This applies to IceWhale's operators' manual, which is generally detailed and includes guidance for recognising signs of disturbance but does not discuss the consequences of disturbance.

Informative and well-advertised guidelines could also serve to educate whale-watching passengers and facilitate compliance. Tourists are concerned about environmental sustainability and minimising impacts to marine life (Draheim et al., 2010; Filby et al., 2015; Sitar et al., 2017), including in Iceland (Chauvat et al., 2021; Lissner and Mayer, 2020), and this can impact visitor experience and operator choice (Bentz et al., 2016; Lissner and Mayer, 2020). In this way, tourist education can encourage sustainable practices of industry operators (Filby et al., 2015) and confer economic benefits to companies certified by an ecolabel (Lissner and Mayer, 2020). Furthermore, accurate advertising and information in advance of whale-watching trips can manage tourist expectations (Kessler and Harcourt, 2010), reducing pressure on captains to violate regulations (Sironi et al., 2005). Beyond whale-watching impacts, the information provided during a trip can improve tourist knowledge and pro-conservation intentions (García-Cegarra and Pacheco, 2017), although the resulting impact on human behaviour is debated (Ballantyne et al., 2009; Lopez and Pearson, 2017; Orams, 1996; Sarti et al., 2022). Whilst education is a mandatory or recommended component of whale-watching tours in many areas (Carlson, 2012), education programmes are often lacking (Gleason, 2015; Hooper et al., 2021; Kessler and Harcourt, 2010), and operator advertising may not reflect whale-watching guidelines, resulting in unrealistic tourist expectations (Judge et al., 2020). In Iceland, I recommend the development of a set of guidelines for whale-watching education, ideally implemented through IceWhale, to ensure that all operators provide appropriate information before, during and after trips (Johnson and McInnis, 2014). There is extensive online guidance for designing effective education programmes (IWC, 2019b; Johnson and McInnis, 2014) and emerging technologies, such as virtual reality, can now be used to engage people in exciting and novel ways (Bejder et al., 2022).

5.5.3 Research suggestions

Whilst the results of this thesis have informed policy recommendations, there are several key knowledge and research gaps concerning population status, whale-watching impacts and the human dimension of whale-watching. These gaps are likely to limit the efficacy of whale-watching management in Iceland (Jefferies, 2016; Tyne et al., 2014). Therefore, I strongly encourage future management plans to embed continued and expanded research within their proposed activities, particularly given the requirement of regular monitoring for adaptive, learning-based management. Below, I provide five broad recommendations for future research but recognise the logistical and financial barriers to sustaining comprehensive, inter-disciplinary monitoring of cetacean populations.

First, baseline information about humpback whale occurrence, population dynamics and habitat use is lacking. Skjálfandi Bay is thought to be an important local foraging ground, with hundreds of whales visiting every summer (Chapter 4). However, owing to poor survey coverage in other parts of coastal Iceland (including whale-watching areas) and high uncertainty in regional abundance estimates (Pike et al., 2019, 2020a,b), its significance at a national scale is unknown, hindering the development of spatial management plans. Moreover, studies of abundance and habitat use are primarily focused on summer months, despite evidence of persistent occurrence throughout autumn and winter (Magnúsdóttir et al., 2014). To enable consistent long-term monitoring, visual surveys can be supplemented by long-term acoustic deployments (Laute et al., 2022) to determine critical habitats in space and time (Heenehan et al., 2017), and citizen science to improve spatial coverage (Stoller, 2020). At a finer scale, tagging data can be used to characterise habitat use (Friedlaender et al., 2013; Hazen et al., 2009; Nichols et al., 2022). In addition, there is little demographic information about Icelandic humpback whales at an individual or population level (in terms of sex, age or life history stage), which hindered the interpretation of physiological results in Chapter 3. This information could also augment behavioural analyses to determine which demographic groups are more or less sensitive to whale-watching disturbance. Genetic and endocrinological studies can be used to determine sex ratios and age distribution (Brown et al., 1995), using non-invasive sample types such as blow (Atkinson et al., 2021).

Second, our ability to predict future changes in abundance and distribution is limited for humpback whales (Meynecke et al., 2021) and other marine taxa (Robinson et al., 2011). In particular, climate change has resulted in entire ecosystem shifts that exceeded predictions (IPCC, 2021; O'Neill et al., 2017) and these changes are likely to continue. Chapter 4 revealed considerable shifts in habitat use in offshore North Iceland over a 14-year period, predicted by changes in various oceanographic parameters, but also highlighted the uncertainty about the magnitude and form of these changes in years with no survey effort. Improving our predictive capacity requires sustained baseline monitoring to understand the historical and contemporary response of cetaceans to environmental change (Becker et al., 2019); and accurate forecasts of physical oceanographic change, which are currently lacking at high spatial resolution (Malinauskaite et al., 2022). These predictions could provide an evidence base for adaptive management schemes, with an element of precaution at least equal to levels of uncertainty in predictive outputs (Richards et al., 2021). Moreover, this information would enable whale-watching communities that are economically dependent on the occurrence of cetaceans to improve their resilience to the most likely future (Meynecke et al., 2017).

Third, despite the contributions of Chapter 2, the short-term impact of whale-watching on Icelandic humpback whales remains understudied. A key component of impact assessments is the collection of control data (Christiansen et al., 2013a; Fumagalli et al., 2021), which were unfortunately unavailable for this thesis. Applied to behavioural whale-watching disturbance, this typically involves observation from land, to remove any sampling effects on whale behaviour. This is challenging in Skjálfandi Bay, with humpback whales typically found far (5–10 km) from accessible land. Narrower, more accessible fjords with whale-watching activities, such as neighbouring Eyjafjörður, could prove suitable for the collection of test and control data. Future behavioural monitoring could also build upon my assessments to answer more targeted questions, concerning the effectiveness of current and proposed guidelines (Currie et al., 2021), and the interactive effects of different components of vessel behaviour. In addition, these results should be used assess the implications of whale-watching disturbance for animal welfare (Clegg et al., 2021). Beyond behaviour, I was unable to determine the physiological response of whales to whale-watching activities, which may represent an important, hidden impact (Rolland et al., 2012; Walker et al., 2005a) and could act as an early warning system for population-level effects (Simmonds, 2018). Recent studies have demonstrated the feasibility of using blubber and faecal samples to investigate responses to vessel traffic for humpback and grey whales, with an apparent positive relationship between cortisol levels and vessel traffic (Lemos et al., 2022; Pallin et al., 2022). Whilst this could be applied to Icelandic humpback whales, I strongly encourage continued development of blow sampling (Atkinson et al., 2021; Burgess et al., 2018) for repeat, non-invasive sample collection, which may enable determination of short-term responses to whale-watching encounters.

Fourth, typical of research progress in other areas (New et al., 2015), long-term whale-watching impacts at a population level are unknown for humpback whales in Iceland. This information is essential to determine the true conservation benefits of whale-watching regulations (IUCN, 1980) and is an important objective of the IWC's Standing Working Group on Whale Watching (IWC, 2018). This requires an understanding of the links between short-term disturbance (largely determined through behavioural observation) and vital rates, for example using the PCoD framework (Pirotta et al., 2018; Christiansen and Lusseau, 2015). Elucidating these linkages requires the collection of control behavioural data to quantify changes in activity budgets; information on the relationships between activity states, bioenergetics, body condition and vital rates; and an estimate of per-animal exposure to whale-watching vessels (Lusseau, 2003; New et al., 2015; Pirotta et al., 2018; Christiansen et al., 2015). Derived information on long-term whale-watching impacts can be used to determine LAC, providing measurable threshold changes to trigger policy responses under an adaptive management strategy (Higham et al., 2008; Lundquist, 2014), and maximum sustainable tourism yield (Bejder et al., 2022). Moreover, the impacts of whale-watching can be integrated with those of other stressors to determine the cumulative effects of anthropogenic activities on populations, which is most relevant to species conservation (Rudd, 2014).

Fifth, whale-watching research should recognise the importance of the human component of this social–ecological system (Fumagalli et al., 2021; Malinauskaite, 2021). Policy recommendations can be based upon ecological information, but developing a rational management framework requires an understanding of the social, economic and political landscape of whale-watching activities in Iceland (Jefferies, 2016). Previous research has elucidated the ecosystem services that whales provide in Icelandic communities (Malinauskaite et al., 2021), characterised the pro-conservation views of Icelandic tourists (Chauvat et al., 2021; Granquist and Nilsson, 2016; Lissner and Mayer, 2020) and considered the impact of climate-driven changes in weather on whale-watching potential (Malinauskaite et al., 2022). From the perspective of minimising disturbance, future research should explore the factors that drive non-compliance to the code of conduct; the most effective strategies for educating captains, guides and tourists; and the potential for tourists to positively influence vessel practices. Furthermore, Chapter 2 highlighted the bi-directional relationship between whales and vessels, and modelling approaches such as agent-based models could be used to better understand their respective influences on each other (Chion et al., 2013, 2011).

This thesis focused on humpback whales. However, other whale species use Icelandic waters as foraging grounds and are targeted by whale-watching activities, including endangered blue whales (Cooke, 2018a) in Skjálfandi Bay (Malinauskaite et al., 2022), whose distribution, abundance and whale-watching impacts are more poorly characterised. Moreover, the lower regional abundance of blue whales (Pike et al., 2020b) may render this species more vulnerable to population-level impacts from local stressors. Therefore, I strongly encourage comparable assessments of other cetacean species in Iceland, with the aim of developing management plans that protect all relevant populations.

5.6 Conclusion

The primary aim of this thesis was to inform Icelandic whale-watching policy by characterising the response of humpback whales to whale-watching vessels and exploring changes in occurrence and distribution. Some objectives of this project, such as measuring the physiological stress response of humpback whales to whale-watching activities, were ultimately unsuccessful, highlighting the importance of careful planning in advance of scientific research. Nevertheless, I was able to produce several results and combine these with existing whale-watching policies to inform a set of management recommendations. As a next step, these recommendations need to be properly communicated to policy-makers to yield conservation benefit (Possingham et al., 2001). The dialogue between natural scientists and policy makers is often fractured and undermined by a lack of trust (Alm, 2002; Guisan et al., 2013), but I hope that this final chapter provides a platform for integrating my research into effective management.

In addition to providing new information, my results highlight residual knowledge gaps and the importance of sustained monitoring to better understand the interactions between whales and anthropogenic activities. Fortunately, this project was the product of authentic collaborations with local institutions which are well placed to continue this work, avoiding the pitfalls of parachute science (Parsons et al., 2017). In Skjálfandi Bay, long-term partnerships exist between local research groups and whale-watching operators, which was key to the success of this work. As Iceland rebuilds its whale-watching industry in the wake of COVID-19, I encourage the expansion of these relationships as part of a bold strategy for inclusive co-management and monitoring (Bejder et al., 2022; Fumagalli et al., 2021). This is essential for proactive conservation that bolsters the resilience of both whale populations and whale-watching activities to an increasingly uncertain future.

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Appendix A

Calculating whale positions from distance and azimuth measurements

Directly measuring whale location was not possible. However, if observer location is known, whale position can be calculated by measuring the horizontal distance and azimuth (compass bearing) between the observer and the whale. The latitude (Lat_w) and longitude (Lon_w) positions (angles, in radians) of the whale at each surfacing were calculated following Christiansen et al. (2013a).

$$Lat_W = arcsin\left(\sin(Lat_O) \times cos\left(\frac{D}{R_E}\right) + cos(Lat_O) \times sin\left(\frac{D}{R_E}\right) \times cos(Az)\right)$$

$$Lon_W = Lon_O + arctan2\left(cos\left(\frac{D}{R_E}\right) - sin(Lat_O) \times sin(Lat_W); sin(Az) \times sin\left(\frac{D}{R_E}\right) \times cos(Lat_O)\right)$$

Where Lat_o and Lon_o are observer latitude and longitude, in radians; Az is azimuth between observer and whale, in radians; D is horizontal geodesic distance between observer and whale, in kilometres; and R_E is the radius of the earth (6371 km).

Accurately measuring distance and bearing to a moving target at sea can be challenging, with the most suitable method varying with distance to target. Thus, to provide redundancy, two methods each were used to measure distance and azimuth, using either DSLR images (photogrammetry) or range finder measurements. Initially, only one method was used to calculate azimuth (electronic compass), following Christiansen et al. (2013a), and fieldwork was developed according to this. A second azimuth method (photogrammetry) was subsequently added since many range finder azimuths were missed or deemed inaccurate. Measurement and derivation of azimuth and distance values are detailed below, followed by the measurement of positional errors.

A.1 Azimuth measurements

For the majority of surfacings, azimuth was measured directly by observer B, using a range finder with an inbuilt electronic compass (Trupulse 360 R, Laser Technology Inc.[1]). To minimise magnetic interference, observer B avoided standing close to large metallic objects on each vessel. Range finder azimuth measurements taken from particular platform positions were subsequently removed from the data set due to the proximity and clear influence of large metallic objects on readings (which were determined after data collection effort had started).

The data collection procedure was designed such that azimuth readings were only derived from range finder measurements. However, these range finder readings were subsequently determined to be inaccurate and values were missing for a considerable proportion of surfacings in the final data set. Therefore, where possible, azimuths were also derived from photographs, captured by observer A (attempted on every surfacing to determine distance), incidentally containing both the whale and a recognisable, permanent feature in the landscape (Figure A.1). These included headlands, buildings and waterfalls. Image-derived azimuths were preferentially selected over range finder azimuths, and were calculated as:

$$Az_w = Az_f + Aov\left(\frac{px_w - px_f}{width}\right)$$

Where Az_w is the azimuth of the whale relative to the observer, in radians; Az_f is the azimuth of the feature relative to the observer, in radians; Aov is the angle of view of the camera, in radians; px_w is the x-axis position of the centre of the whale in the image, in pixels (from left to right); px_f is the x-axis position of the centre of the feature, in pixels; and $width$ is the width of the image, in pixels. Az_f was calculated using the known coordinates of the observer and the feature (known features were mapped out in QGIS; QGIS Development Team 2020; Figure A.2), with the `bearing` function of the *geosphere* package in R (Hijmans, 2021).

A.2 Distance measurements

As with azimuth, horizontal distance between the observer and the whale was measured using either a range finder or photogrammetric methods. At short distances (less than 300 m), observer B attempted to measure horizontal distance directly with the electronic range finder. These readings provide planar distance, which is about 3 cm shorter than geodesic distance at 300 m (considered negligible here).

When range finder readings were not available (primarily at larger distances), images containing the whale and either the horizon (sky–sea) or shoreline (land–sea), captured for every surfacing by observer A, were used to calculate geodesic distance. Images containing both the whale and the horizon could only be captured at distances greater than ~50 m. Distance was calculated based on the trigonometric

1. Laser Technology Inc.: https://lasertech.com/

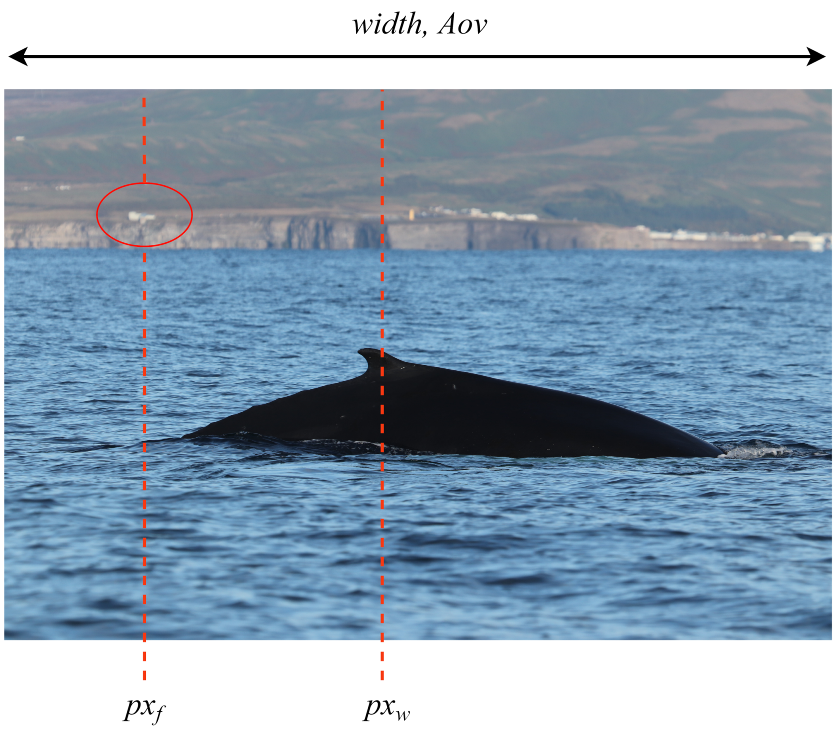

Figure A.1: An example of pixel measurements used to calculate azimuth from images. px_w is whale horizontal position, in pixels. px_f is feature horizontal position, in pixels (here a clifftop building).

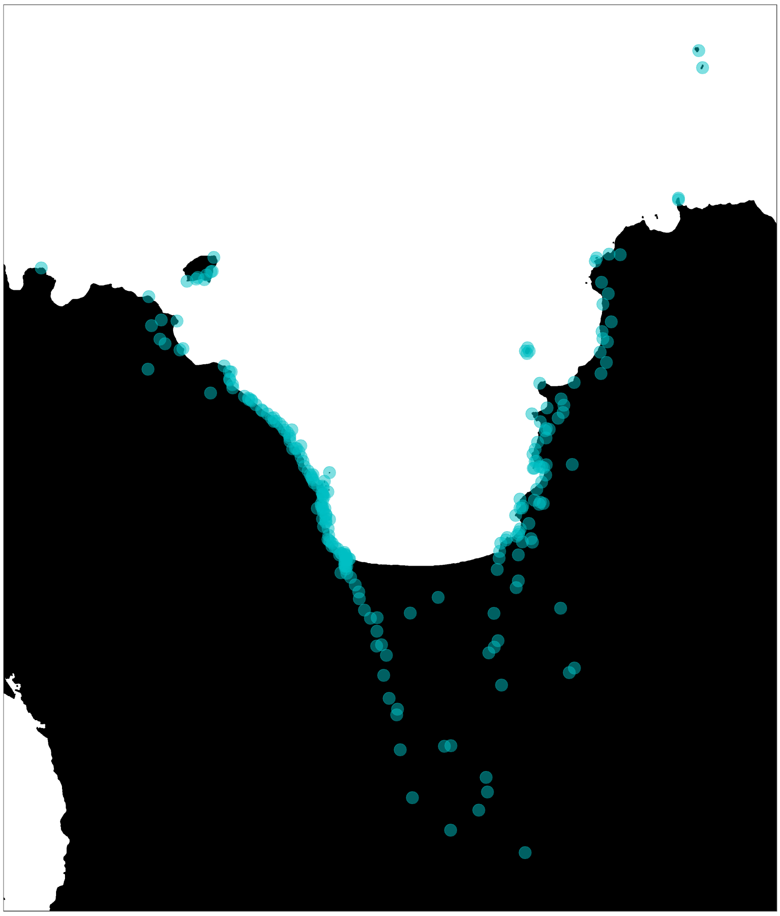

Figure A.2: Locations of each land feature for photogrammetric azimuth calculations.

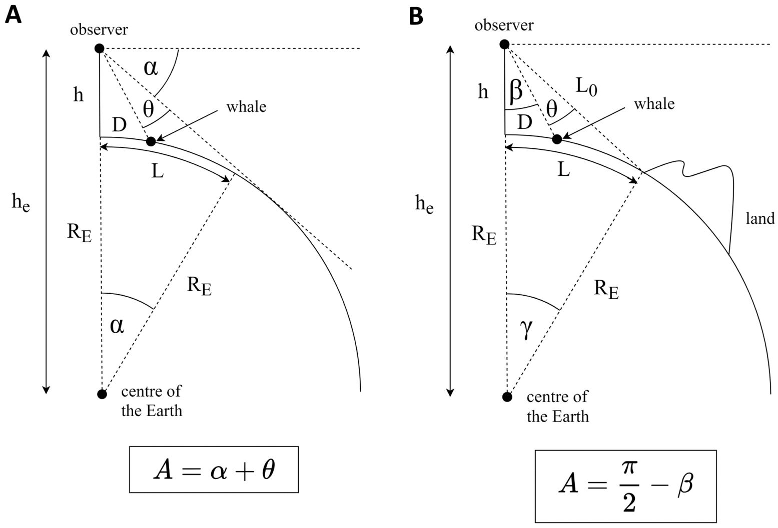

Figure A.3: Trigonometric calculations to calculate horizontal (geodesic) distance height and angle data.

relationship between the observer, the whale, the horizon and the centre of the Earth, with all angles in radians (Figure A.3, adapted from Kinzey and Gerrodette 2003). As precise heights were needed, some surfacings were removed from these calculations due to non-specific platform positions recorded (particularly at the beginning of 2018).

First, the vertical angle θ subtended between the whale and the horizon (as in Christiansen et al. 2013a; Gordon 2001) was calculated for each surfacing with an appropriate image.

$$tan(\theta) = \frac{\left(\frac{V}{H}\right) \times S}{f \times C}$$

Where V is the distance between the horizon (true or false) and the waterline of the whale in the image, in pixels, measured with the `straight` function of ImageJ (Schneider et al., 2012); H is image height, in pixels; S is the height of the image sensor of the camera, in mm; f is the focal length of the camera, in mm; and C is the crop factor of the camera model. Image metadata were extracted using ExifTool, accessed through the *exifr* package in R (Harvey, 2016).

From this, geodesic distance to the whale (D, in km) can be calculated, following Kinzey and Gerrodette (2003).

$$D = h_e \times sin(A) - \sqrt{R_E^2 - (h_e \times cos(A))^2}$$

Where R_E is the radius of the earth (6371 km); h is the eye height of the observer, in km; $h_e = R_E + h$; and A is the angle between the whale and the horizontal.

When a photo includes a true horizon, $A = \alpha + \theta$, where α is the angle above the horizon to the horizontal tangent. α is equal to $arctan\left(\sqrt{2R_E h\ +\ h^2}/R_E\right)$. However, for a false horizon (shoreline), where θ is not adjacent to α, we instead need to solve $A = \frac{\pi}{2} - \beta$, where β is the angle between the whale and the vertical, centred around the observer. β can be calculated in a series of three equations.

First, we calculate γ, the angle between observer and the false horizon, centred at the centre of the earth (known as central arc angle).

$$\gamma = \frac{L}{R_E}$$

Where L is the distance between the observer and the false horizon. This was calculated in R, using the Az reading. Second, we calculate L_0, the straight-line distance between the observer (at eye-height h) and the false horizon.

$$L_0 = \sqrt{R_E^2 + (R_E + h)^2 - 2R_E\ (R_E + h)Cos(\gamma)}$$

Third, we calculate angle β.

$$\beta = arccos\left(\frac{2hR_E + h^2 + L_0^2}{2(R_E + h)L_0}\right)$$

Distance D can then be calculated.

A.3 Positional errors

Methods

Each distance and azimuth measurement has associated errors relating to instrument precision (intrinsic error) and conditions of deployment (e.g., vessel movement). These will propagate through subsequent analyses, altering the outcomes of any modelling and statistical tests. Therefore, it is crucial to quantify these errors, either to confirm that measurements are accurate or to explicitly include these error values in analyses. Errors were determined partly following Christiansen et al. (2013a).

Two observers on board a whale-watching vessel (Sæborg, platform height = 3.55 m) conducted a mock focal follow of a small research vessel ('test whale') with known GPS position (recorded every second). Range finder readings and suitable photographs were taken at a variety of distances. Measurements were made in near-optimal conditions, with both vessels moving slowly, excellent visibility and Beaufort sea state 1.

From the data collected, distance and azimuth values were recorded or calculated using all four methods. The relationships between the measured values and true (GPS) values were investigated using linear models, fit using the `lm` function in R. Under perfect measurements, we would expect R^2 to be 1. Additionally, for range finder azimuth, a correction factor was derived from the intercept of the linear model. The electronic compass of the range finder is calibrated in an imprecise way (by simply pointing the range finder approximately North, East, South and West), and so a correction factor is required (which also accounts for magnetic declination). This correction factor was applied to all range finder azimuth measurements.

To determine the magnitude of errors, standard error (SE) values (SE of the absolute values of the difference in distance and bearing) were calculated for each measurement type, where simple error is measured value minus GPS value. However, measurement errors may increase with distance (particularly distance measurement errors). To investigate this, simple error was linearly regressed against GPS distance (Pearson's correlation). If the correlation was not significant, a single SE value was used. If the correlation was significant, following Christiansen et al. (2013a), a "while" loop function was used to estimate the cumulative SE for different subsets of the data, whereby each subsequent iteration specified a lower minimum distance threshold value. In other words, the loop started with far distances only and included closer values with each iteration. In this way, the cumulative SE throughout the entire range of distances could be calculated, resulting in a unique interpolated SE value for each measured whale distance or azimuth value. Only SE values estimated using three or more measurements were used for interpolation.

Initial inspection of this data set suggested that the derived range finder azimuth errors were unrealistically small. Unlike the other three measurement methods, the electronic compass within the range finder is likely to be affected by movement and so, on a moving platform at sea and measuring a moving target, often while the vessel is rocking, errors are likely to be larger. This was supported by personal observations at sea, where repeat azimuth readings for the same distant target could give quite different results. To explore these potential errors in a more realistic setting, 100 'real' range finder azimuth readings of target whales were compared with camera azimuth readings, which are considered to be highly accurate (provided accurate observer GPS positions). To ensure comparability, readings were only compared if the whale was more than 150 metres away (to minimise the impact of time delays between the two methods or GPS errors). The linear correlation was inspected and SE values were calculated as before. These derived errors were subsequently propagated in generalised additive mixed models and statistical tests.

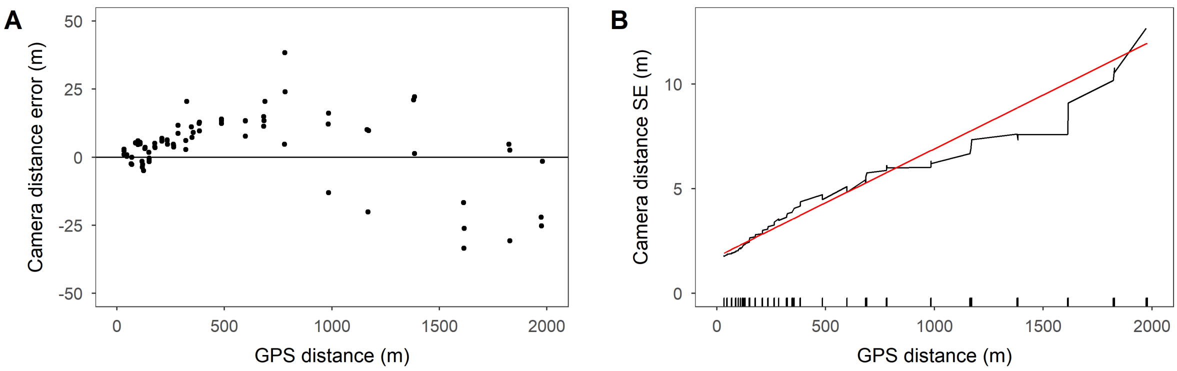

Figure A.4: Camera distance A) absolute and B) standard errors (SE). The red line denotes the linear relationship between GPS distance and SE.

Results

Camera distance

Errors for distances derived from images were generally small (Figure A.4A). From the mock focal follow, conducted from a whale-watching vessel, 104 distance measurements were derived from camera images. There was a near-perfect linear correlation between measured and true values ($R^2 > 0.999$). Within 300 m, error was no greater than 6 m, and no greater than 40 m within 1 km (Figure A.4B). From a linear model, absolute error increased with distance ($R^2 = 0.91,\ t = 29.35,\ p < 0.001$). The loop function produced standard error values of <6 m below 1000 m distance and <3 m below 200 m (Figure 3B).

Range finder distance

Range finder distance measurements generally had small errors, all less than 10 m (Figure A.5A). From the mock focal follow, 55 range finder distance readings were taken. There was a near-perfect linear correlation between measured and true values ($R^2 > 0.999$). From a linear model, absolute error increased with distance ($R^2 = 0.73,\ t = 11.97,\ p < 0.001$). The loop function produced standard error values of <2 m across all distances, and <1 m below 200 m (Figure A.5B).

Camera azimuth

Errors for azimuths derived from images were generally small (Figure A.6). From the mock focal follow, 72 distance measurements were derived from camera images. There was a near-perfect linear correlation between measured and true values ($R^2 > 0.999$). The total standard error was 0.08° and error did not decrease with distance. Rather, errors were far larger at closer distance (up to 1.9°), perhaps due to GPS errors and slight time mismatches between images and GPS positions. Errors were minimal (<0.1°) at distances greater than 800 m.

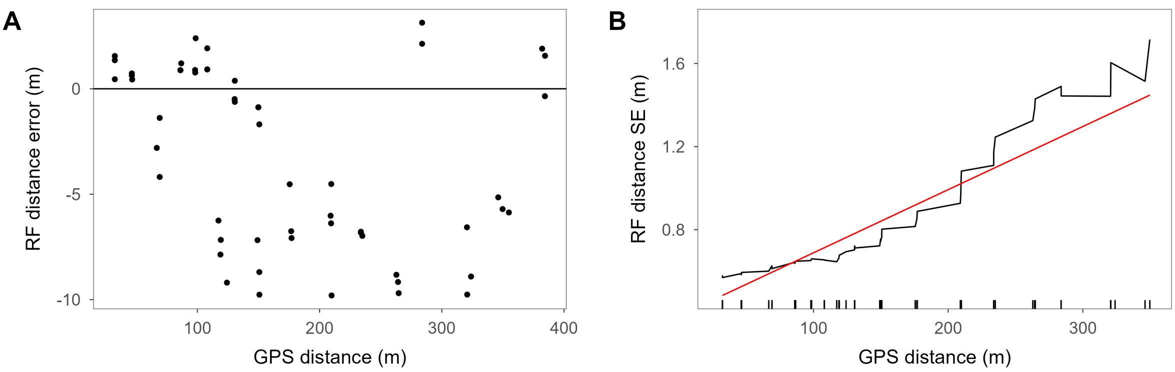

Figure A.5: Range finder distance A) absolute and B) standard errors. The red line denotes the linear relationship between GPS distance and SE.

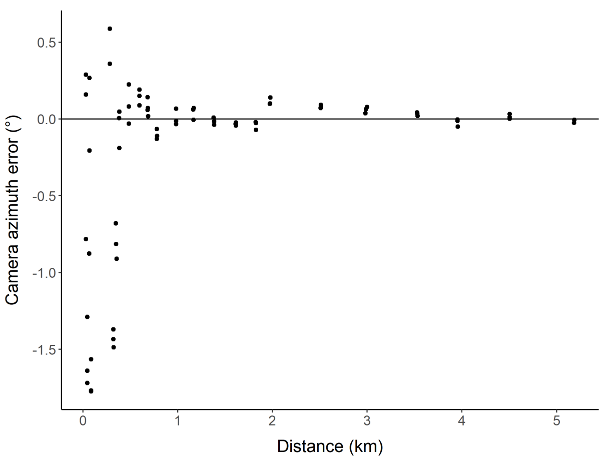

Figure A.6: Camera azimuth absolute errors.

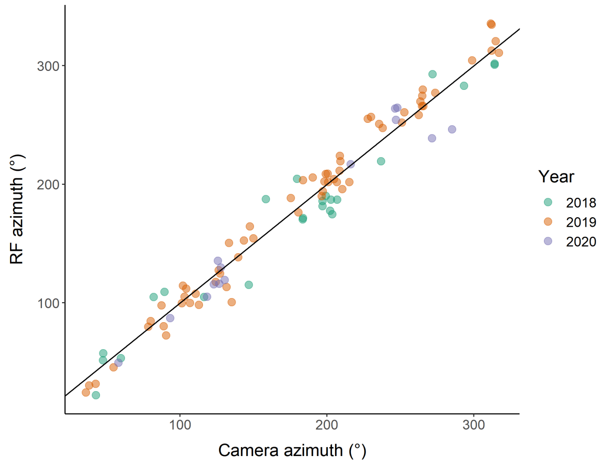

Figure A.7: Camera vs range finder azimuth readings for real whale measurements.

Range finder azimuth

Under the near-optimal conditions of the mock focal follow, 101 range finder azimuth readings showed a very good linear correlation with true values ($R^2 = 0.98$) and a total standard error of 0.28°, with error not decreasing with distance. From a linear model of range finder azimuths against known (GPS-derived) values, a correction factor (model intercept) of 14.04° was obtained, accounting for magnetic declination and imprecise instrument calibration.

However, these error estimates are not considered realistic, given the likely influence of vessel and whale movement on range finder precision and accuracy. Therefore, from 'real' focal follows, 100 range finder azimuth readings of target whales were compared to camera azimuth readings, which are highly accurate and used as 'true' azimuth values (Figure A.7). Errors were as high as 40° and did not vary with distance, and the total standard error was 1.42°.

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Appendix B

Surface behaviour ethogram

A detailed ethogram for focal follows (Table B.1) was derived from several behavioural studies and used to categorise humpback whale surface behaviours (Di Clemente et al., 2018; Corkeron, 1995; Pivorunas, 1979; Darling and Berube, 2001; Dunlop et al., 2008; Friedlaender et al., 2009a; Goldbogen et al., 2010; Kavanagh et al., 2017).

Code	**Name**	**Description**
blo	Blow	Visible plume of exhaled air above the surface
hdr	Headrise	Head rises vertically above the surface of the water
bre	Breach	Leap in which at least half of the body exits the water
pfs	Pectoral fin slap	Pectoral fin(s) are raised out of the water and forcibly slapped on the surface
tlo	Tail lob	The throwing of the entire fluke and peduncle out of the water in a lateral motion
tsl	Tail slap	Strikes the surface with its tail flukes (ventral or dorsal)
hdl	Head lunge	Energetic forward motion in which less than half of the body exits the water
pfw	Pectoral fin wave	Waves pectoral fin above the surface
twa	Tail wave	Waves tail above the surface
rol	Roll	Rolls on its long axis at the surface (not accompanied by pectoral fin slaps)
flu	Fluke	Submerges lifting its flukes above the surface
arc	Arch	Submerges strong back arch flukes remain below the surface
plf	Partial lunge-feed	Rises above surface with water draining from mouth and ventral pleats expanded
lfe	Lunge-feed	Rises above surface with mouth open clearly feeding

Table B.1: Ethogram used to categorise surface behaviours, including the code for each behaviour, its full name and a description.

In addition, several behaviours were observed *ad libitum* as they could not be reliably observed across all sampling conditions (e.g., distance to whale, sea state, ambient noise) to include in focal follow sampling:

- Logging (remains stationary on the surface, with no other behaviour visible)
- Rise (dorsal surface of the whale is visible briefly at the surface of the water, but a blow is not observed)
- Tail swish (tail rapidly through water in sideways movement)
- Trumpet blow (high-pitched, loud exhalation)
- Tractor blow (low-pitched, loud exhalation, with tractor-like sound)
- Surface travel (swims with part of its body not submerging below the surface)
- Bubbles (bubbles evident prior to whale surfacing)

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Appendix C

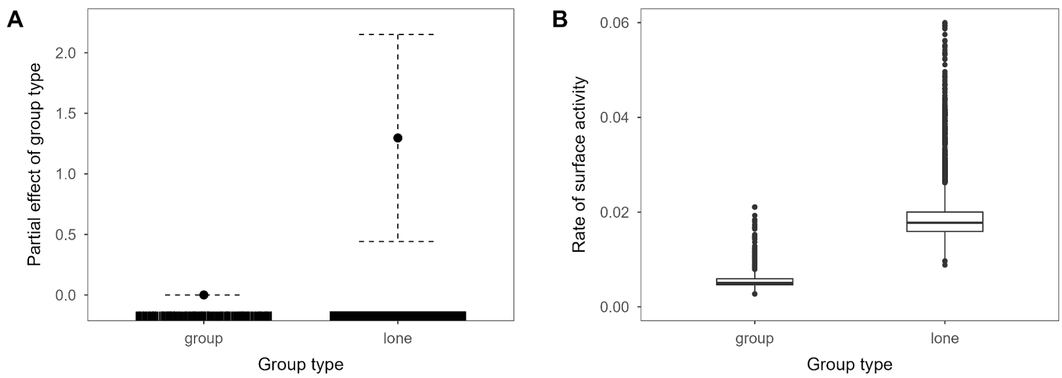
Generalised additive mixed model plots

Partial dependence plots are presented for each type of generalised additive mixed model (GAMM): AIS vessel variables and focal vessel variables. Following Currie et al. (2021), individual variable plots (labelled A) and the predictions of the response variable for individual predictors (labelled B) are presented for each term of the best fit GAMM. A value of zero on the y-axis indicates no effect of the covariate on the estimated response; values above zero indicate a positive relationship; and values below zero indicate a negative relationship. The x-axis for each variable plot contains small vertical ticks indicating the locations of observations (i.e., a rug-plot). To display the absolute predictions of the response variable (whale behaviour metric), the `predict` function from the *stats* package (R Core Team, 2020) was used for each of the best fit models. Specifically, to predict the response to a single explanatory variable, every other explanatory variable was fixed to either its mean (numeric) or median (categorical) value. The random effect of follow number was removed from predictions by including a dummy variable in the GAM formula.

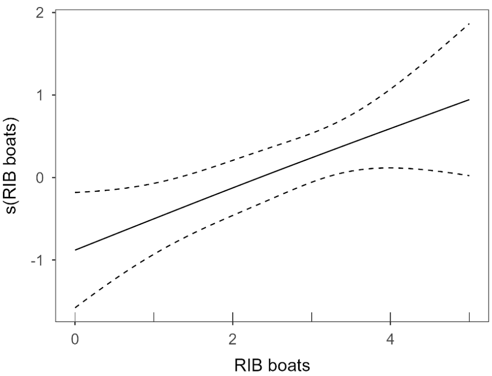
A square-root transformation is denoted by 'sqrt' and a cube-root transformation is denoted by 'cubert'.

C.1 AIS GAMMs

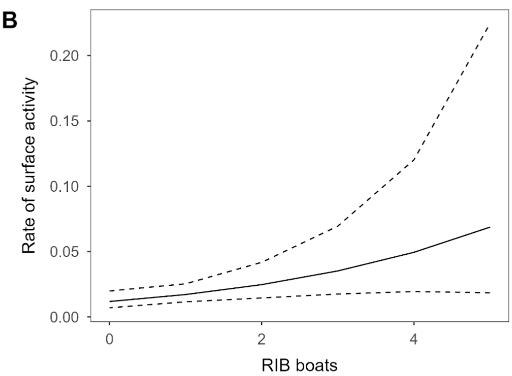
Surface-active behaviours (SAB)



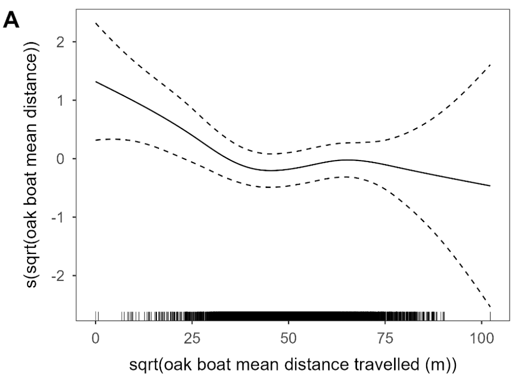
A



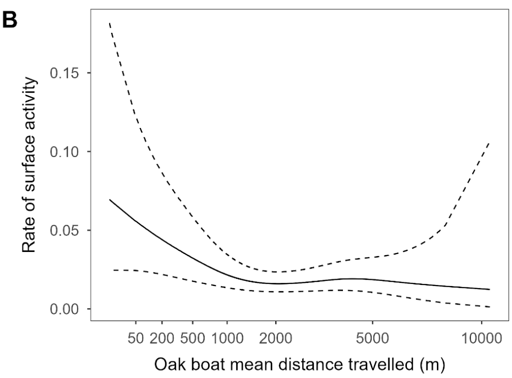
B



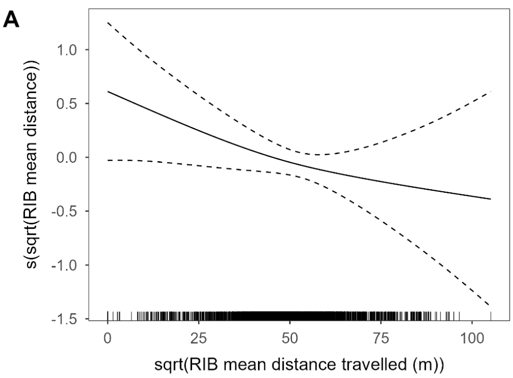
A



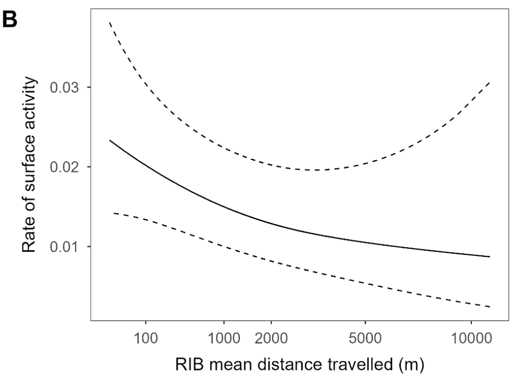
B



A

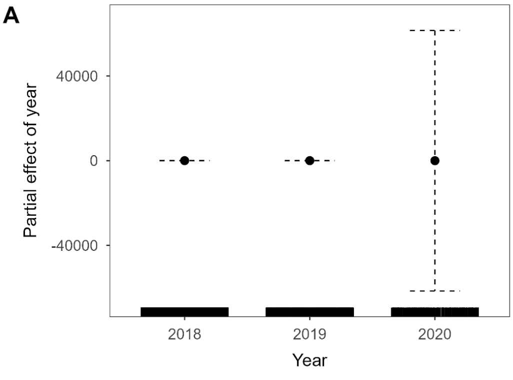


B

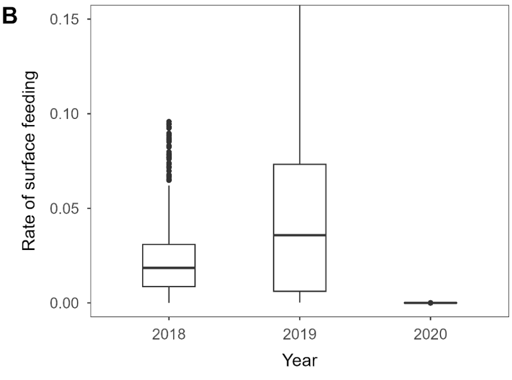


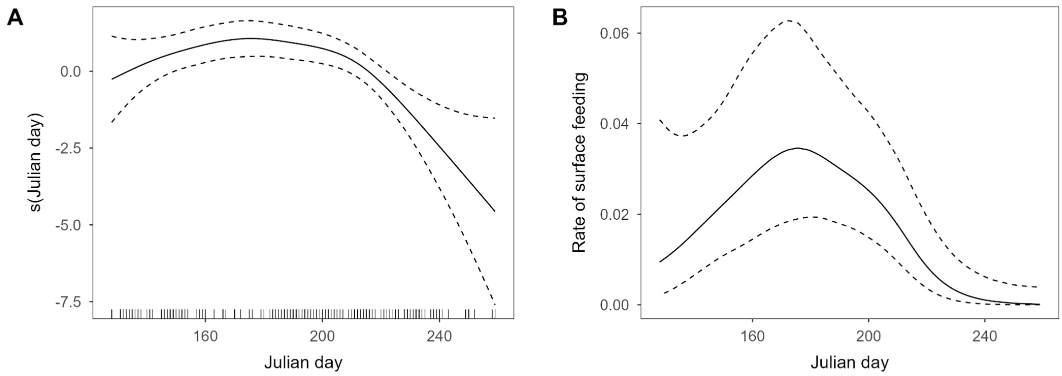
Surface feeding events (SFE)

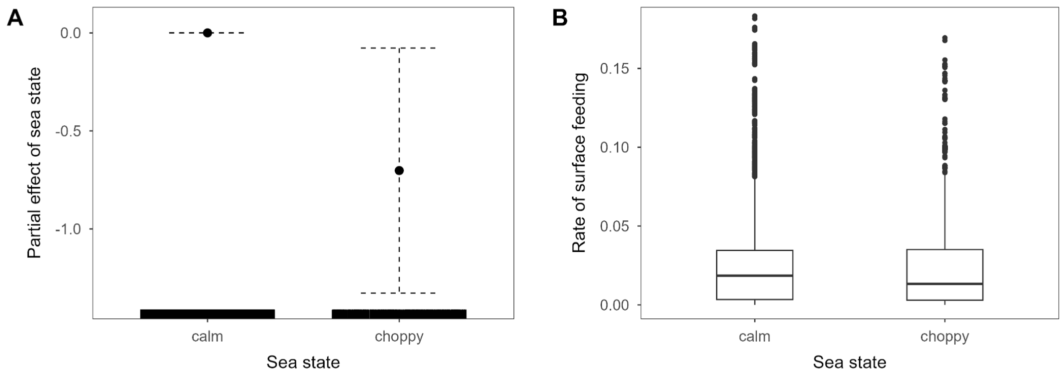
A

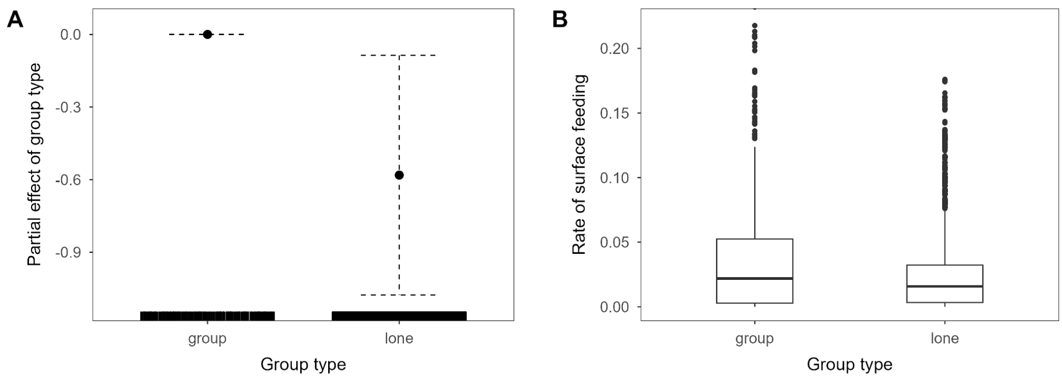


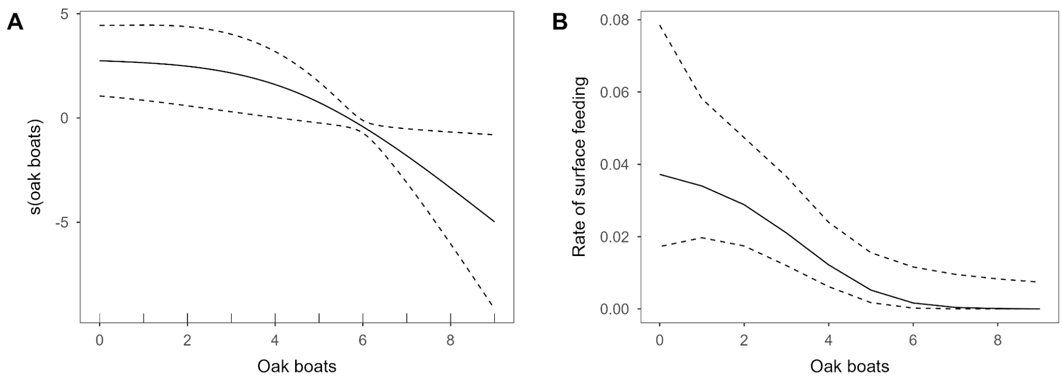
B

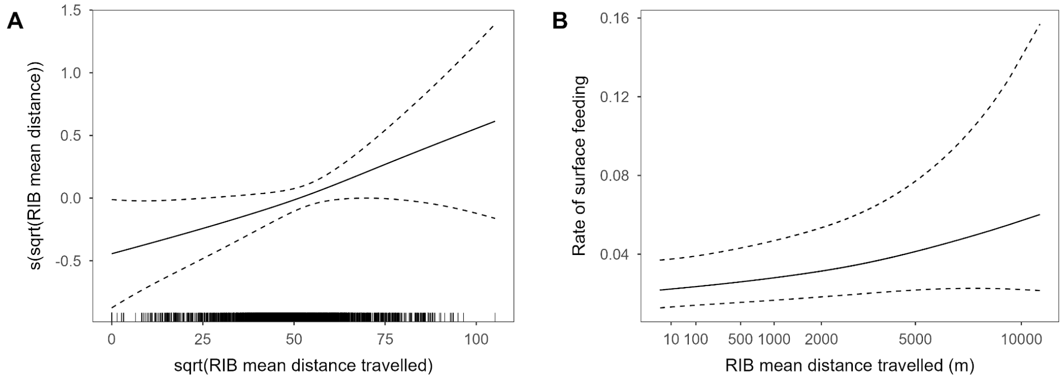



A
B
s(Julian day)
0.0
-2.5
-5.0
-7.5
160
200
240
Julian day
Rate of surface feeding
0.06
0.04
0.02
0.00
160
200
240
Julian day

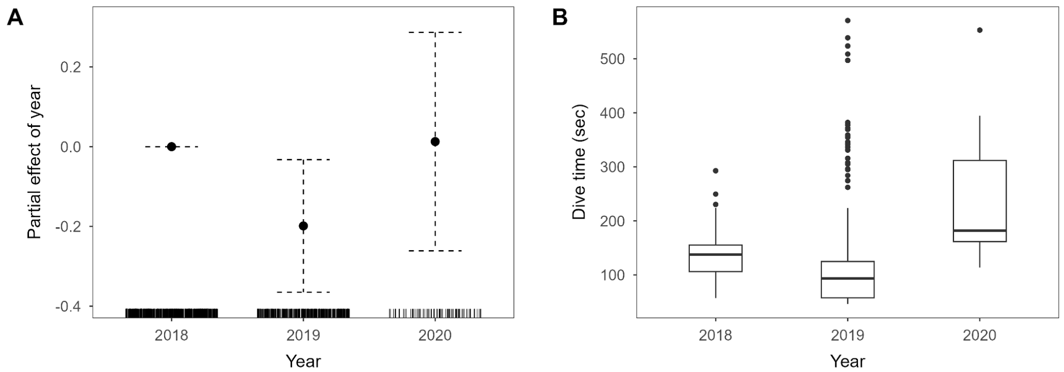

A
B
Partial effect of sea state
0.0
-0.5
-1.0
calm
choppy
Sea state
Rate of surface feeding
0.15
0.10
0.05
0.00
calm
choppy
Sea state

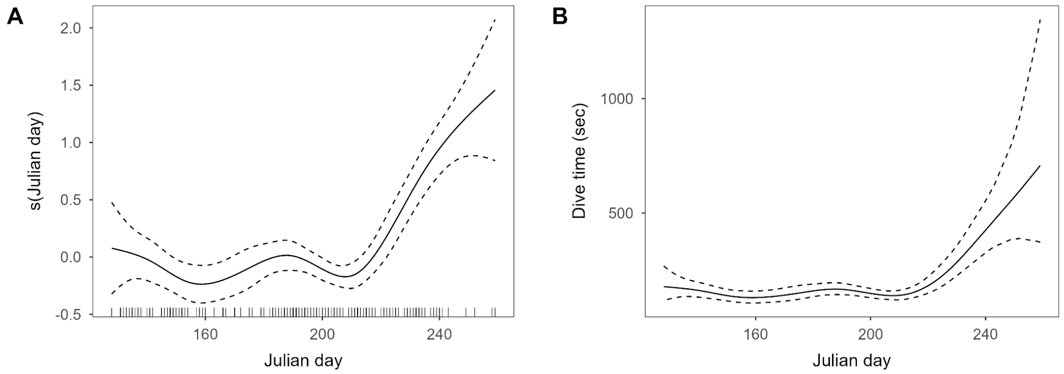

A
B
Partial effect of group type
0.0
-0.3
-0.6
-0.9
group
lone
Group type
Rate of surface feeding
0.20
0.15
0.10
0.05
0.00
group
lone
Group type

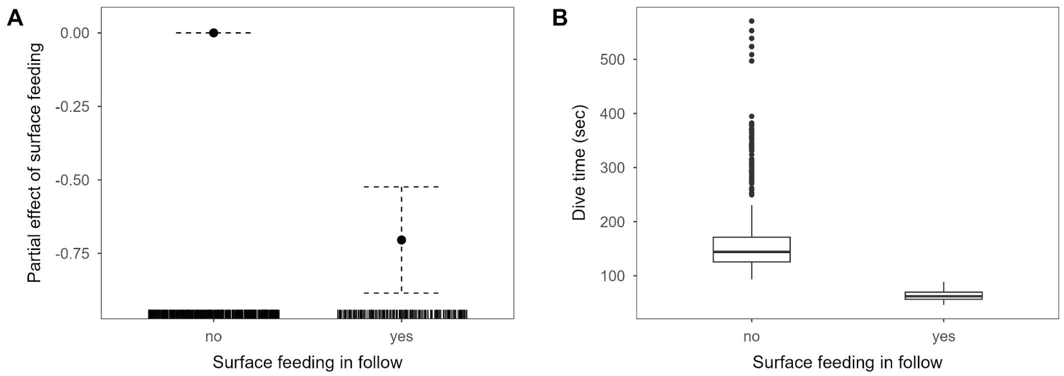

A
B
s(oak boats)
5
0
-5
0
2
4
6
8
Oak boats
Rate of surface feeding
0.08
0.06
0.04
0.02
0.00
0
2
4
6
8
Oak boats

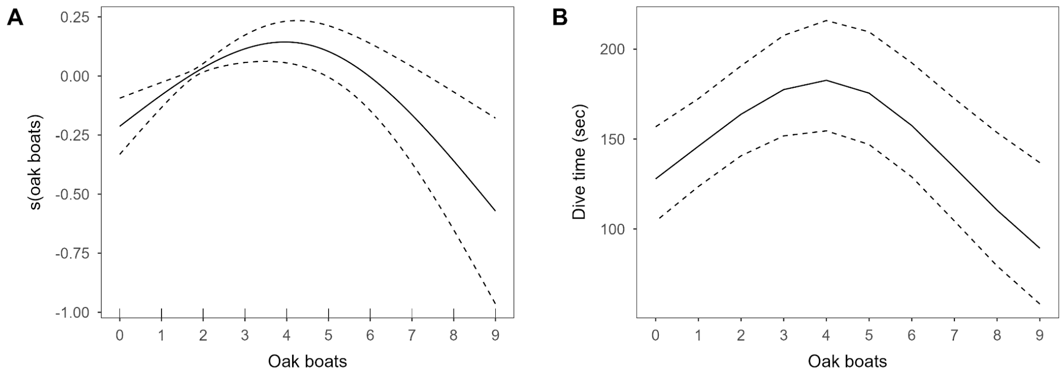

A
s(sqrt(RIB mean distance))
1.5
1.0
0.5
0.0
-0.5
0
25
50
75
100
sqrt(RIB mean distance travelled)
B
Rate of surface feeding
0.16
0.12
0.08
0.04
10 100
500 1000
2000
5000
10000
RIB mean distance travelled (m)

Dive time

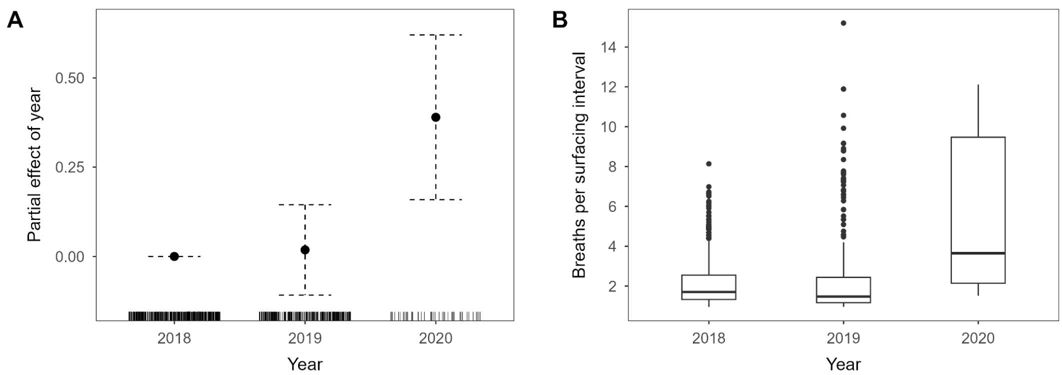

A
Partial effect of year
0.2
0.0
-0.2
-0.4
2018
2019
2020
Year
B
Dive time (sec)
500
400
300
200
100
2018
2019
2020
Year

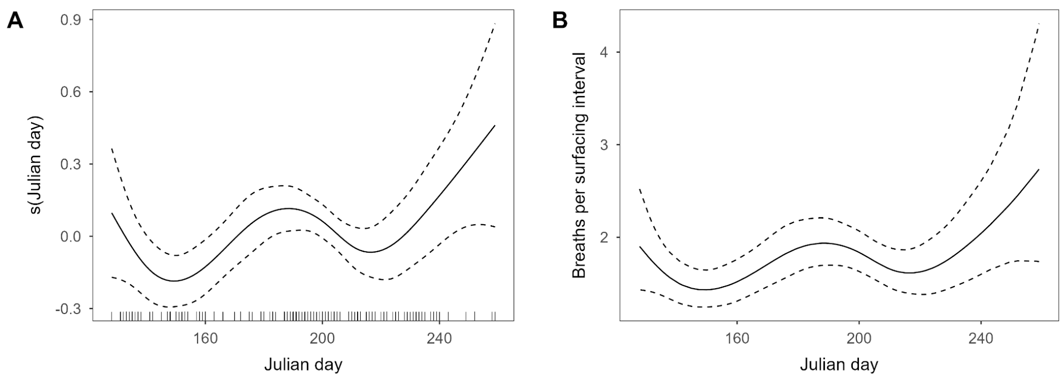

A
s(Julian day)
2.0
1.5
1.0
0.5
0.0
-0.5
160
200
240
Julian day
B
Dive time (sec)
1000
500
160
200
240
Julian day

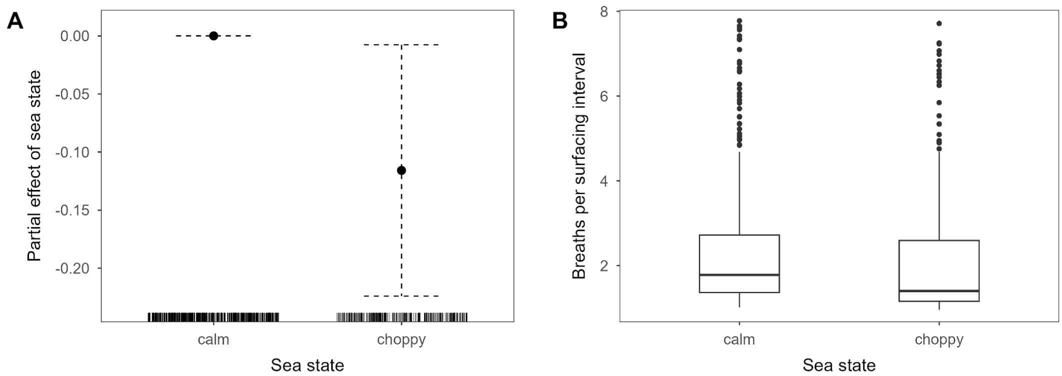

A
Partial effect of surface feeding
0.00
-0.25
-0.50
-0.75
no
yes
Surface feeding in follow
B
Dive time (sec)
500
400
300
200
100
no
yes
Surface feeding in follow

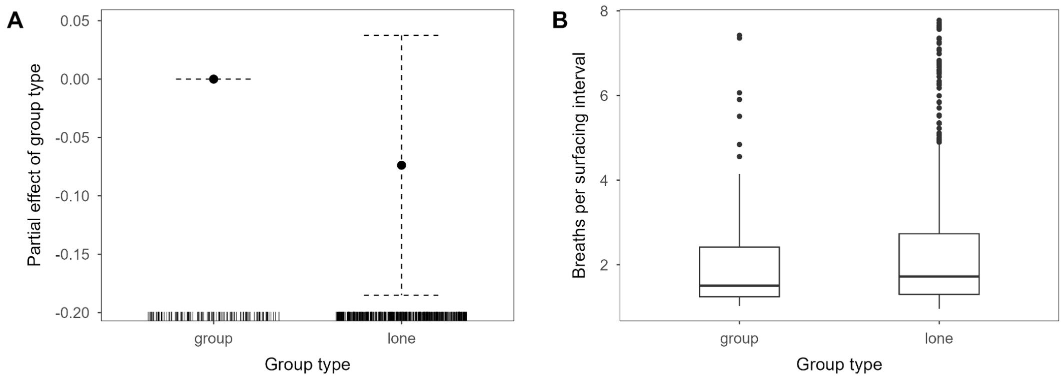


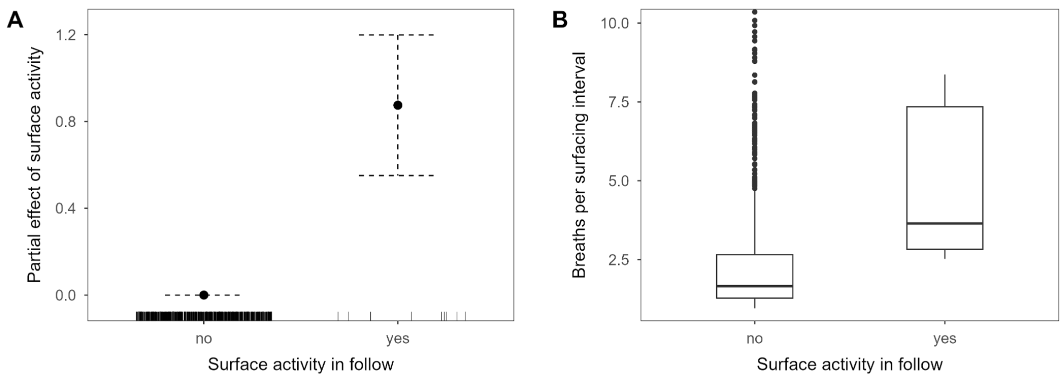
Breaths per surfacing interval

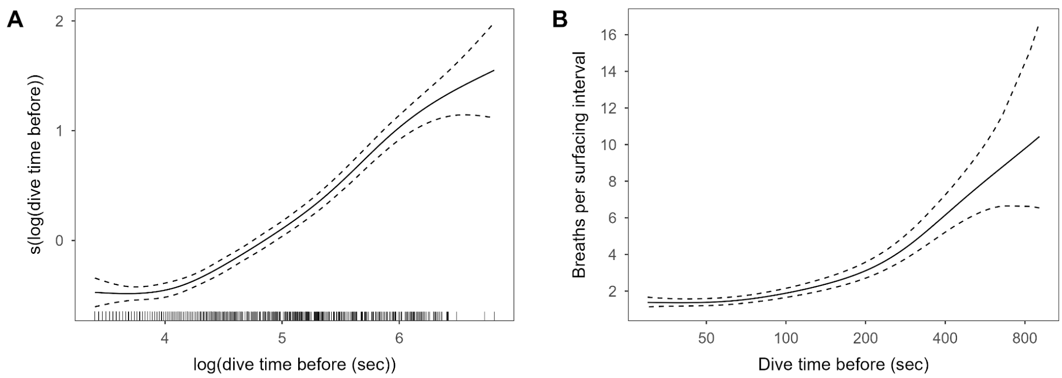




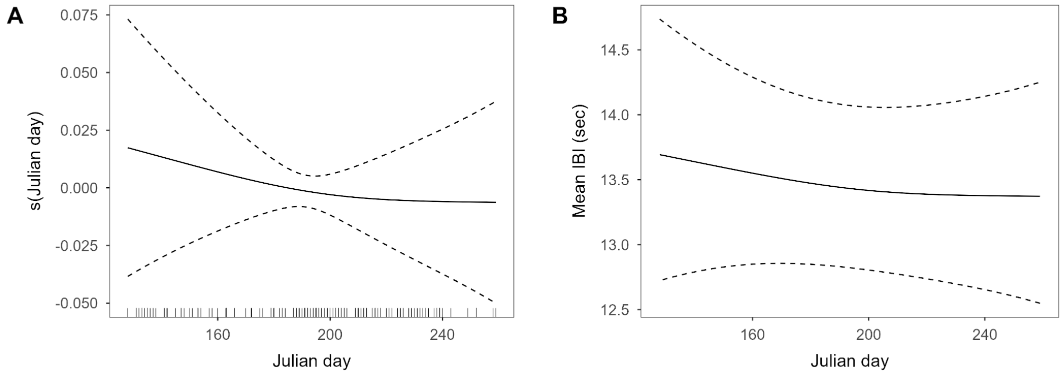


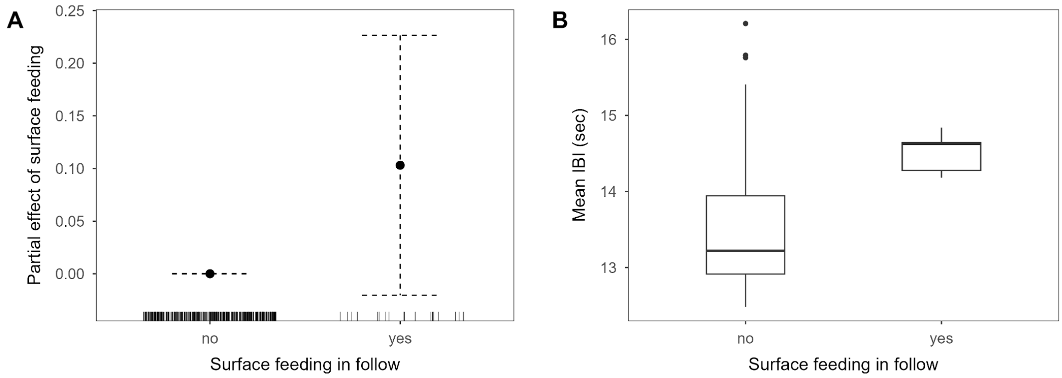


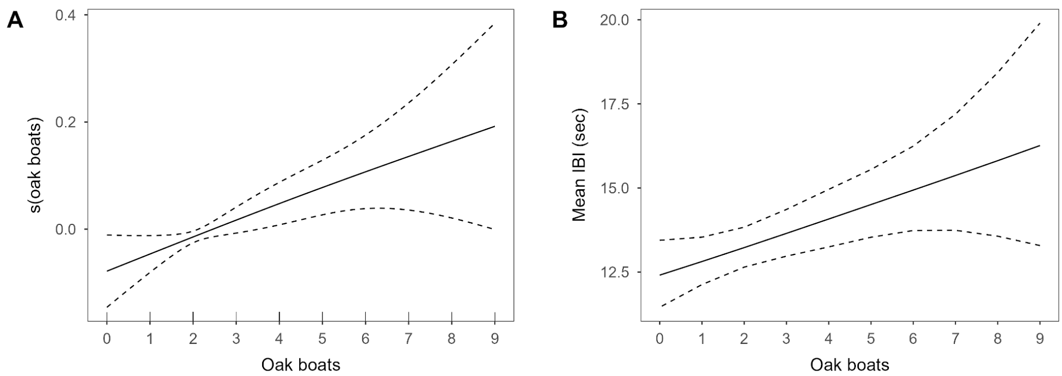




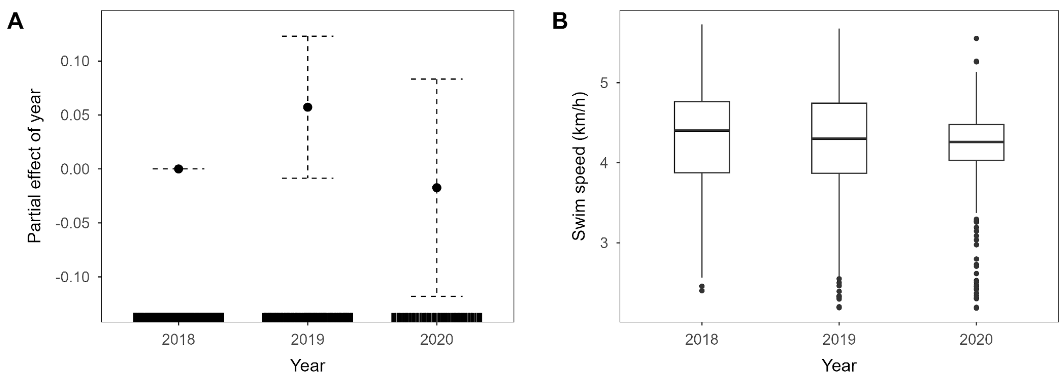
Mean inter-breath interval (IBI) per surfacing interval

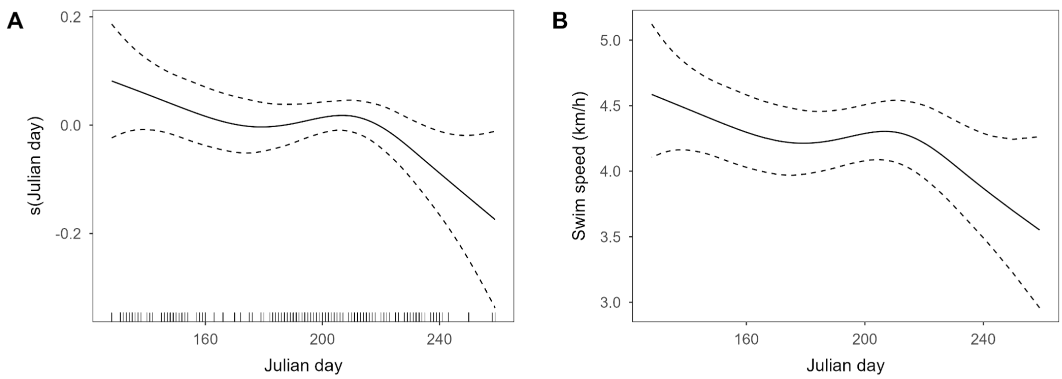


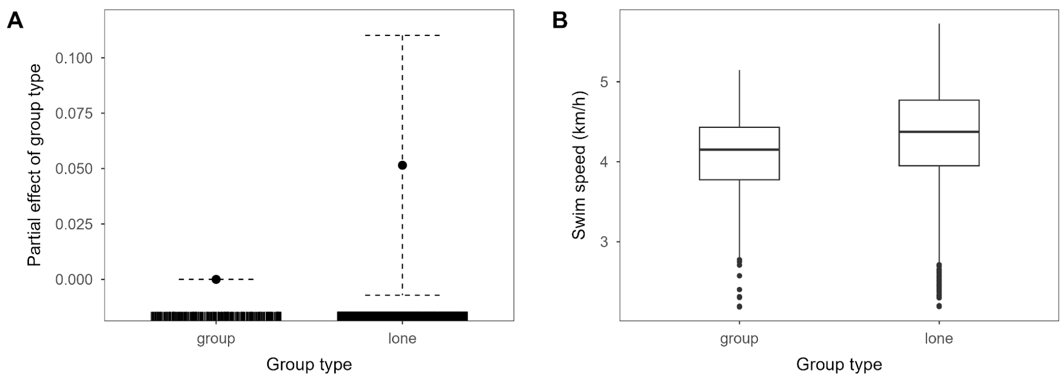

A
Partial effect of surface feeding
0.25
0.20
0.15
0.10
0.05
0.00
no
yes
Surface feeding in follow
B
Mean IBI (sec)
16
15
14
13
no
yes
Surface feeding in follow

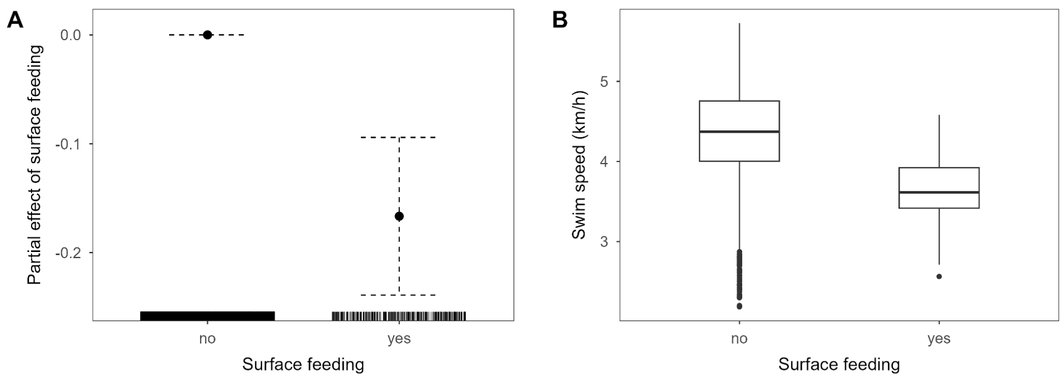

A
s(oak boats)
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9
Oak boats
B
Mean IBI (sec)
20.0
17.5
15.0
12.5
0 1 2 3 4 5 6 7 8 9
Oak boats

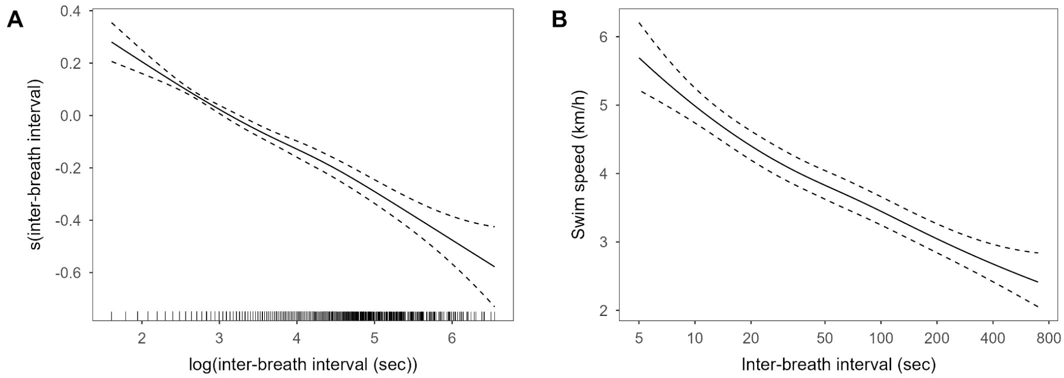
Swim speed


A
Partial effect of year
0.10
0.05
0.00
-0.05
-0.10
2018
2019
2020
Year
B
Swim speed (km/h)
5
4
3
2018
2019
2020
Year

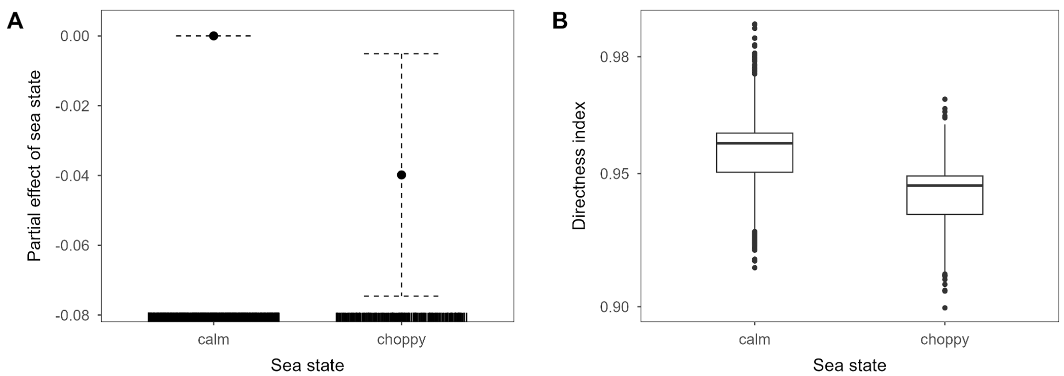

A
s(Julian day)
0.2
0.0
-0.2
160
200
240
Julian day
B
Swim speed (km/h)
5.0
4.5
4.0
3.5
3.0
160
200
240
Julian day

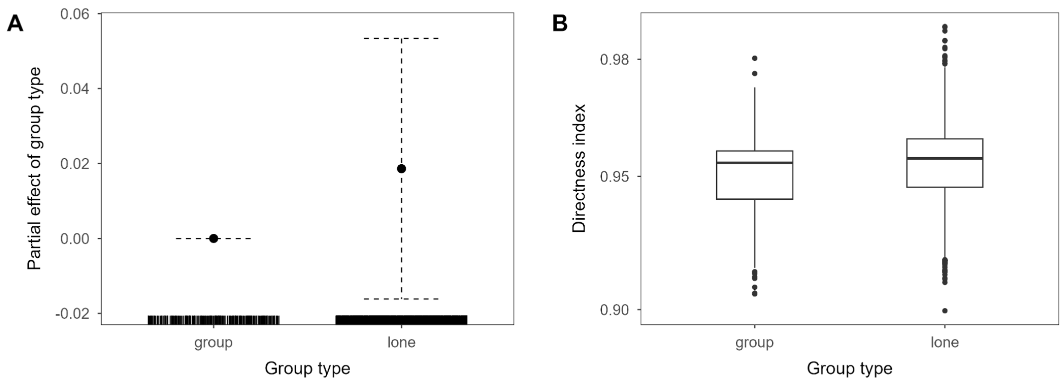


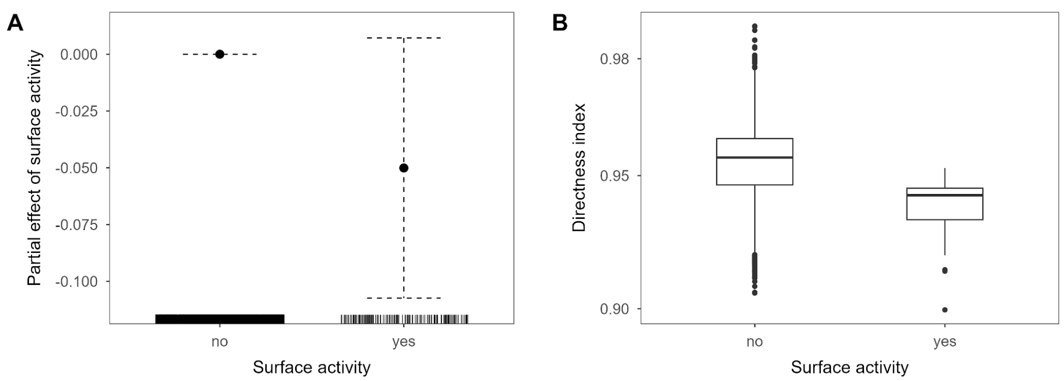


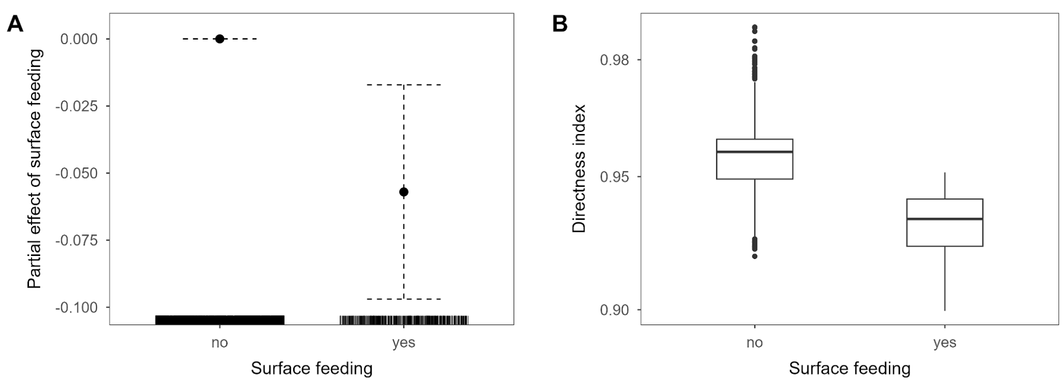


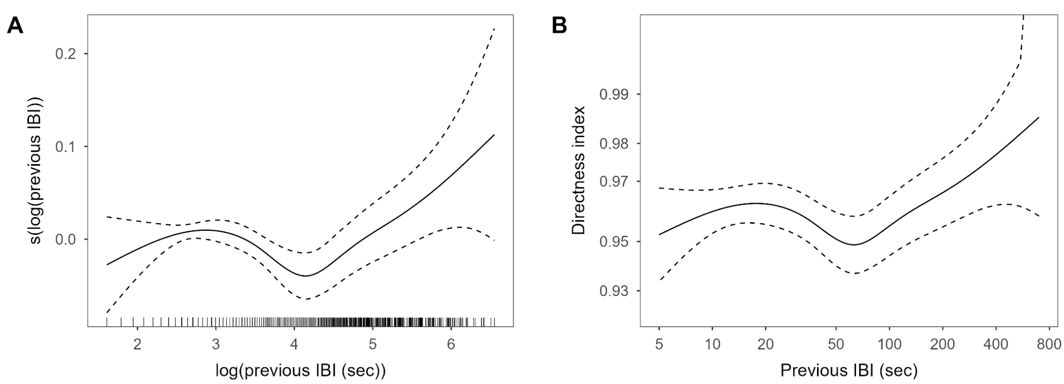
Directness index

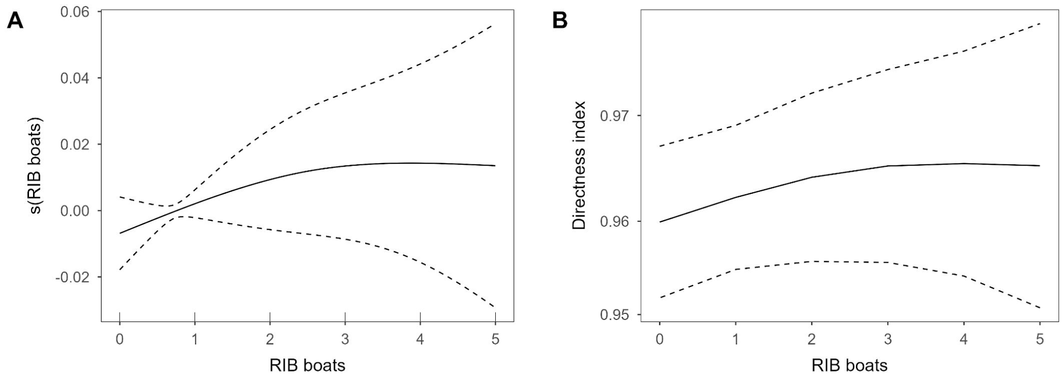



A
B
Partial effect of group type
0.06
0.04
0.02
0.00
-0.02
group
lone
Group type
Directness index
0.98
0.95
0.90
group
lone
Group type


A
B
Partial effect of surface activity
0.000
-0.025
-0.050
-0.075
-0.100
no
yes
Surface activity
Directness index
0.98
0.95
0.90
no
yes
Surface activity

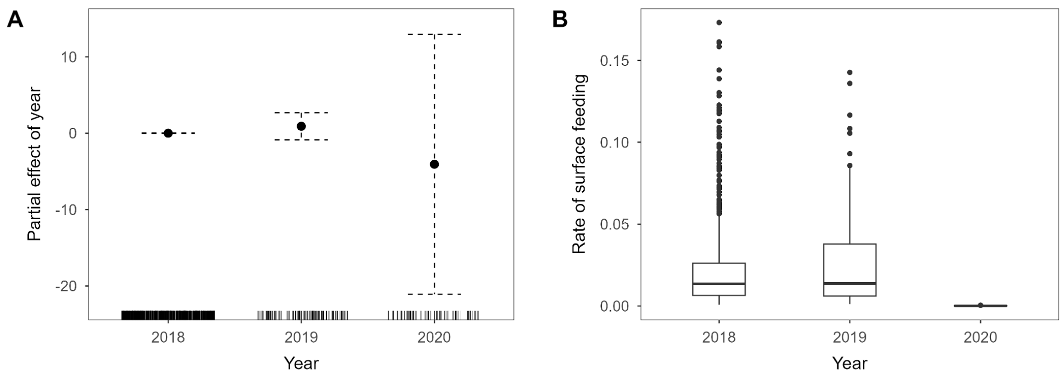

A
B
Partial effect of surface feeding
0.000
-0.025
-0.050
-0.075
-0.100
no
yes
Surface feeding
Directness index
0.98
0.95
0.90
no
yes
Surface feeding

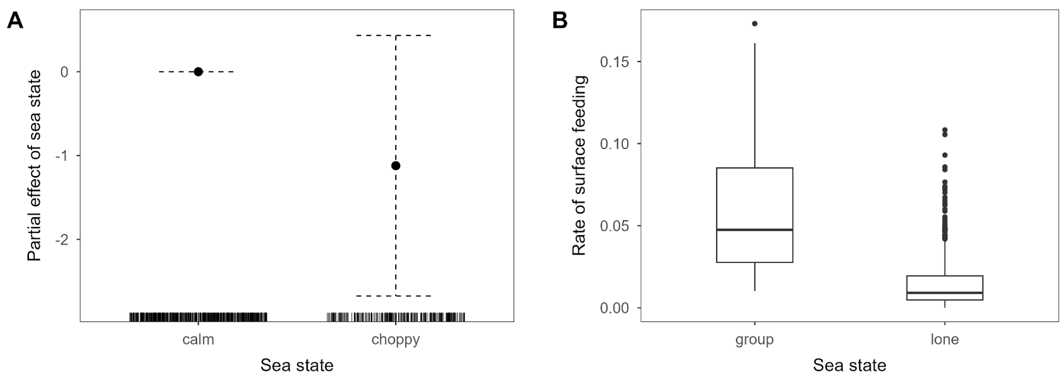

A
B
s(log(previous IBI))
0.2
0.1
0.0
2
3
4
5
6
log(previous IBI (sec))
Directness index
0.99
0.98
0.97
0.95
0.93
5
10
20
50
100
200
400
800
Previous IBI (sec)

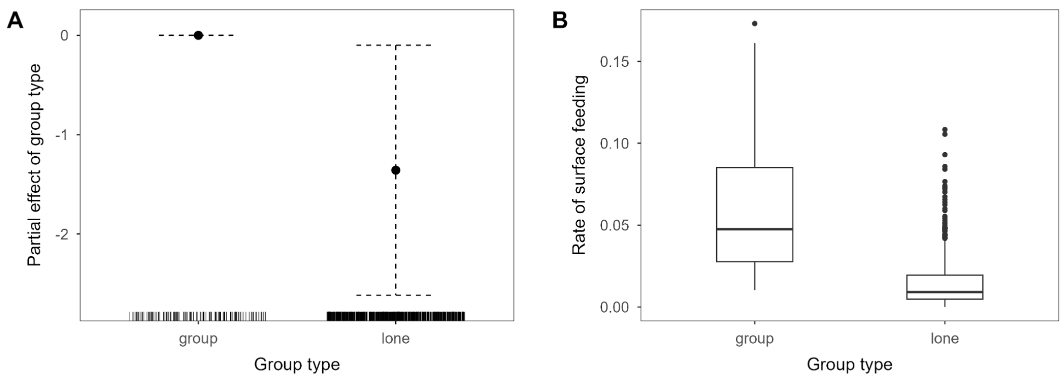


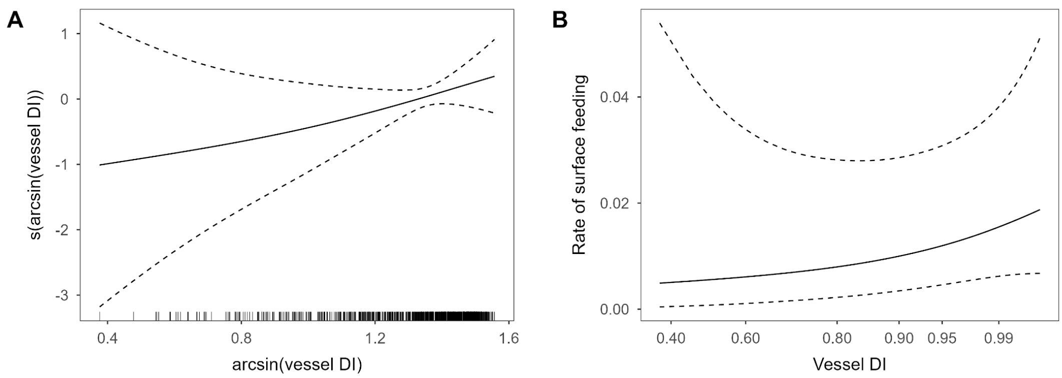
C.2 Focal vessel GAMMs

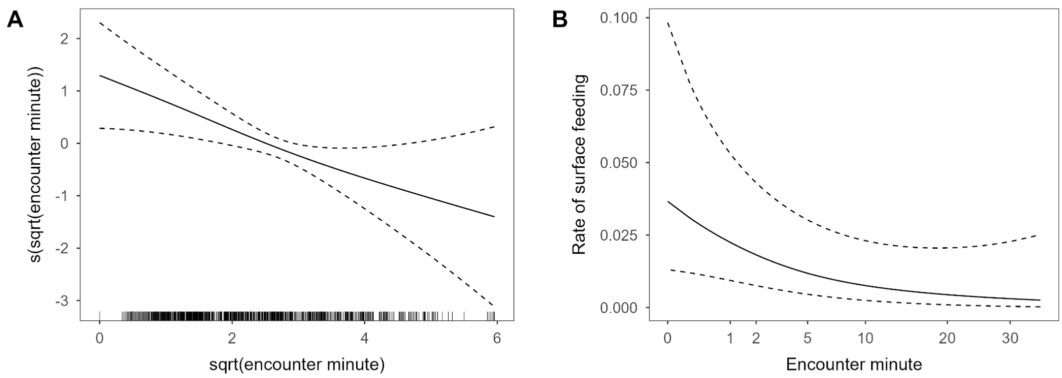
Surface feeding events



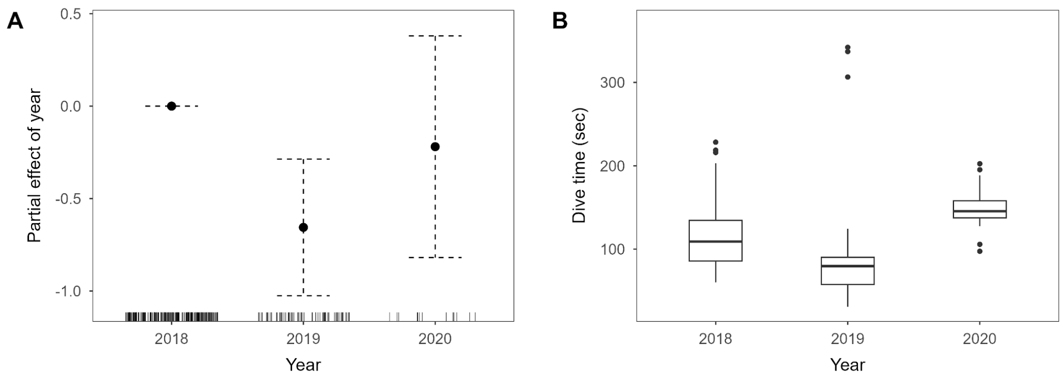


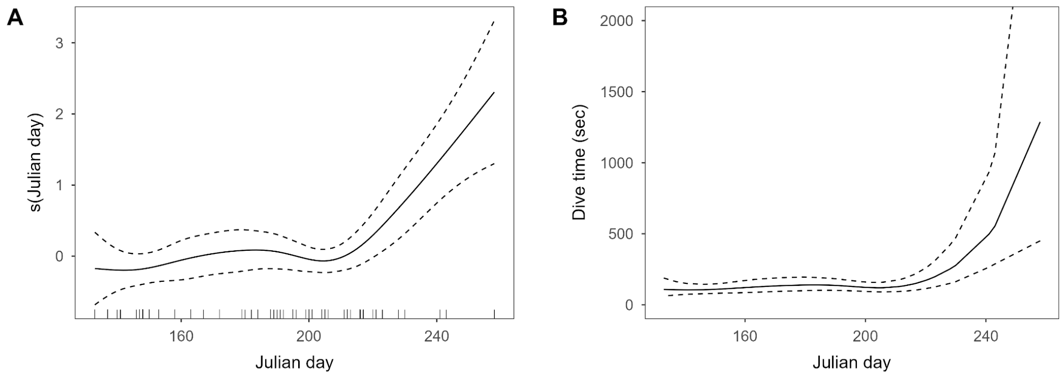

A
Partial effect of group type
0
-1
-2
group
lone
Group type
B
Rate of surface feeding
0.15
0.10
0.05
0.00
group
lone
Group type

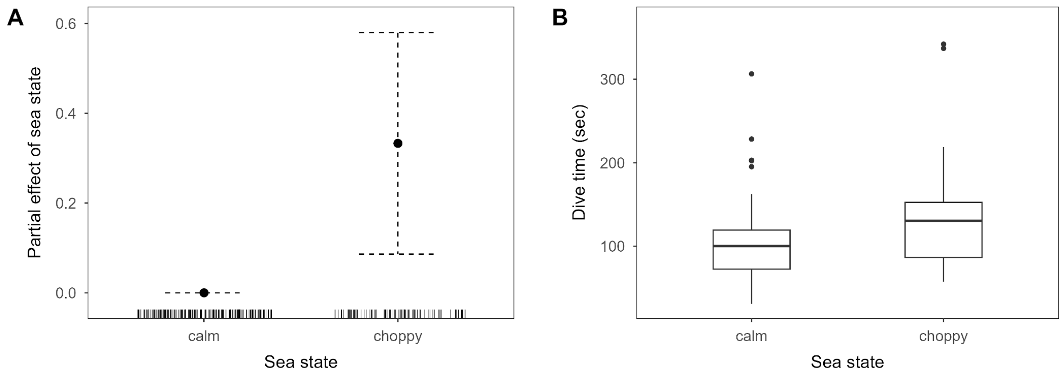

A
s(arcsin(vessel DI))
1
0
-1
-2
-3
0.4
0.8
1.2
1.6
arcsin(vessel DI)
B
Rate of surface feeding
0.04
0.02
0.00
0.40
0.60
0.80
0.90
0.95
0.99
Vessel DI

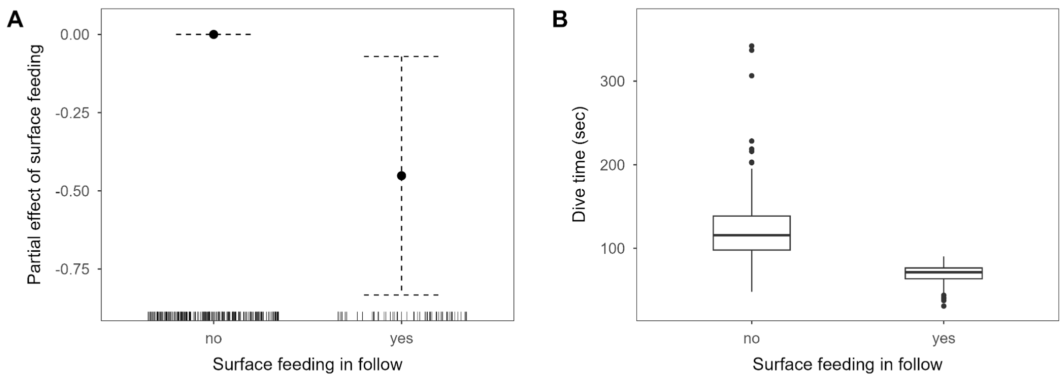

A
s(sqrt(encounter minute))
2
1
0
-1
-2
-3
0
2
4
6
sqrt(encounter minute)
B
Rate of surface feeding
0.100
0.075
0.050
0.025
0.000
0
1
2
5
10
20
30
Encounter minute

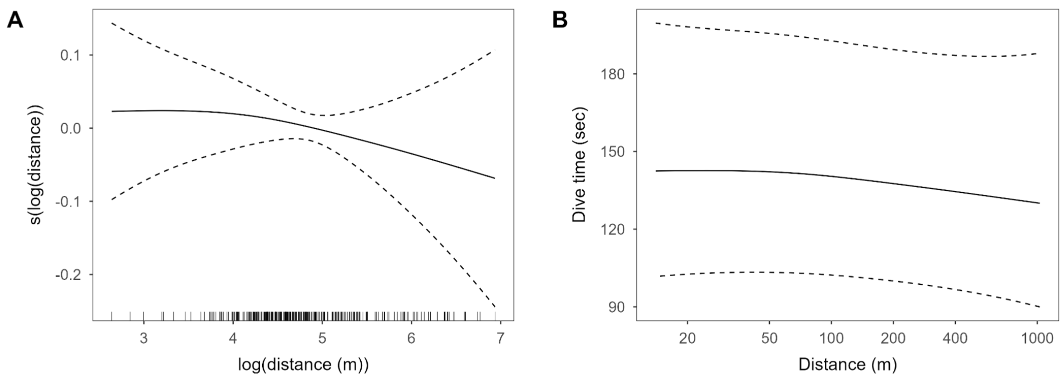
Dive time

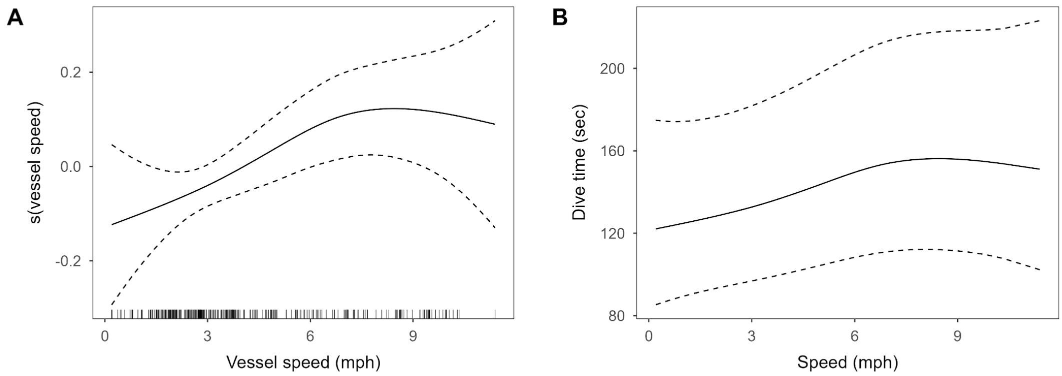

A
Partial effect of year
0.5
0.0
-0.5
-1.0
2018
2019
2020
Year
B
Dive time (sec)
300
200
100
2018
2019
2020
Year

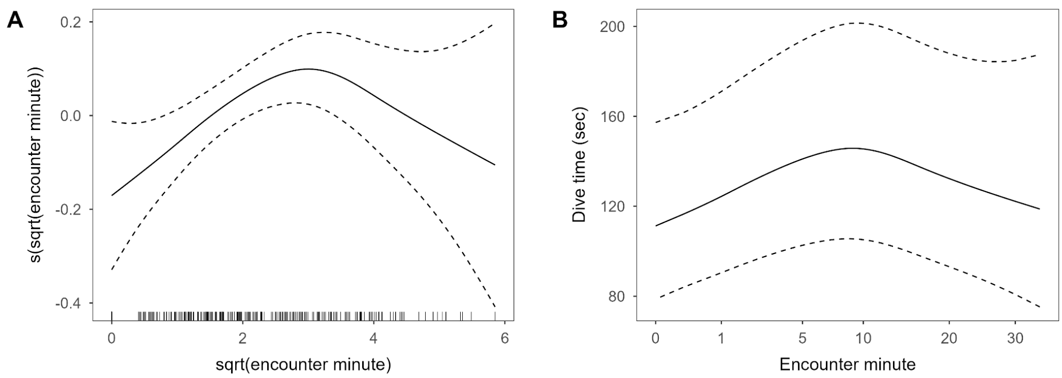

A
s(Julian day)
Julian day
B
Dive time (sec)
Julian day


A
Partial effect of sea state
calm
choppy
Sea state
B
Dive time (sec)
calm
choppy
Sea state

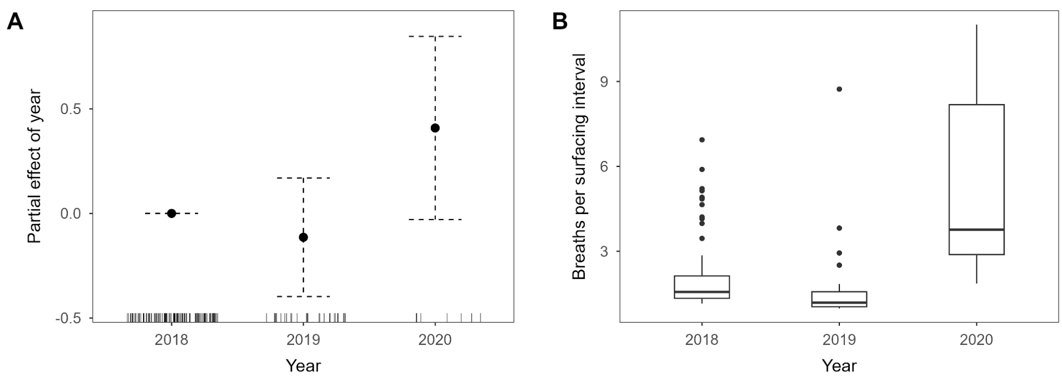

A
Partial effect of surface feeding
no
yes
Surface feeding in follow
B
Dive time (sec)
no
yes
Surface feeding in follow

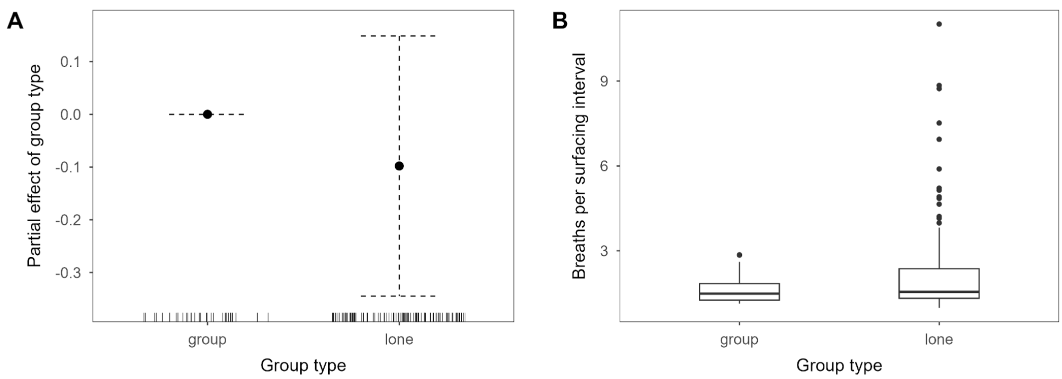

A
s(log(distance))
log(distance (m))
B
Dive time (sec)
Distance (m)

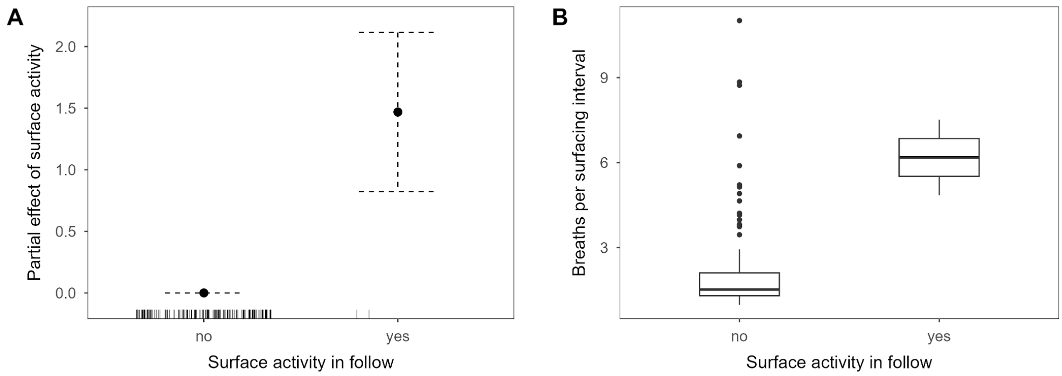


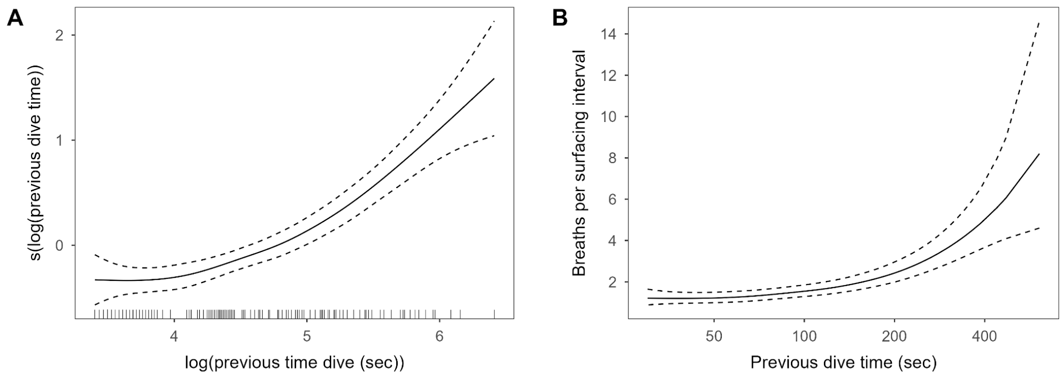


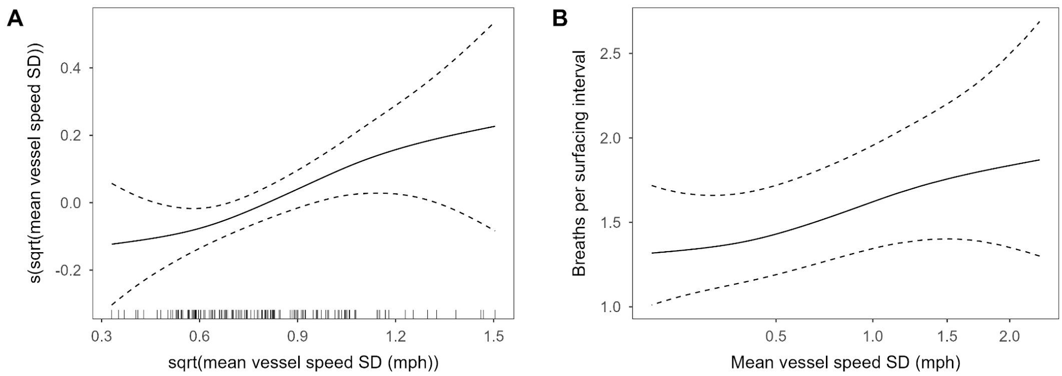
Breaths per surfacing interval

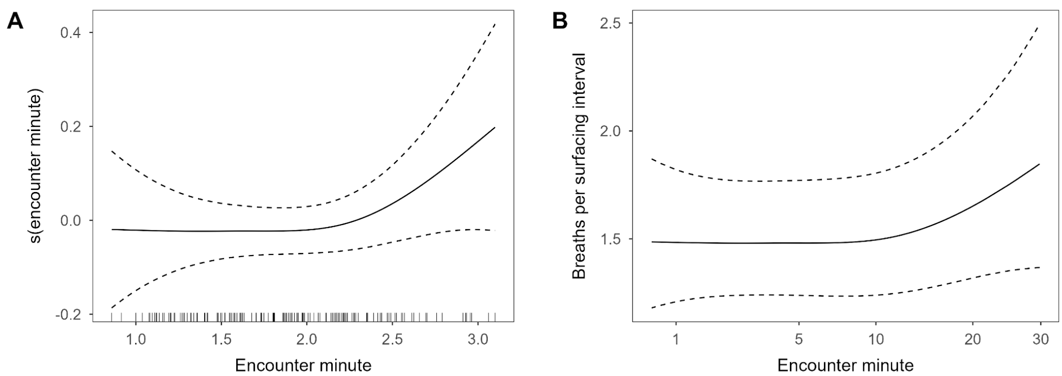




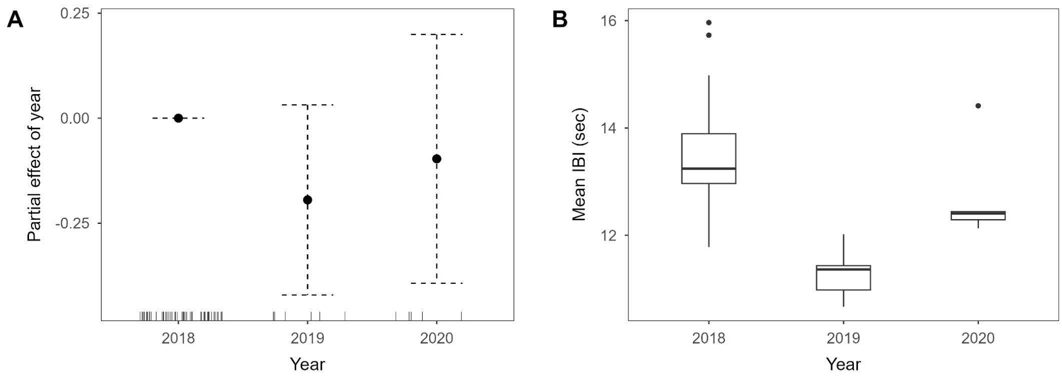

A
Partial effect of surface activity
2.0
1.5
1.0
0.5
0.0
no
yes
Surface activity in follow
B
Breaths per surfacing interval
9
6
3
no
yes
Surface activity in follow

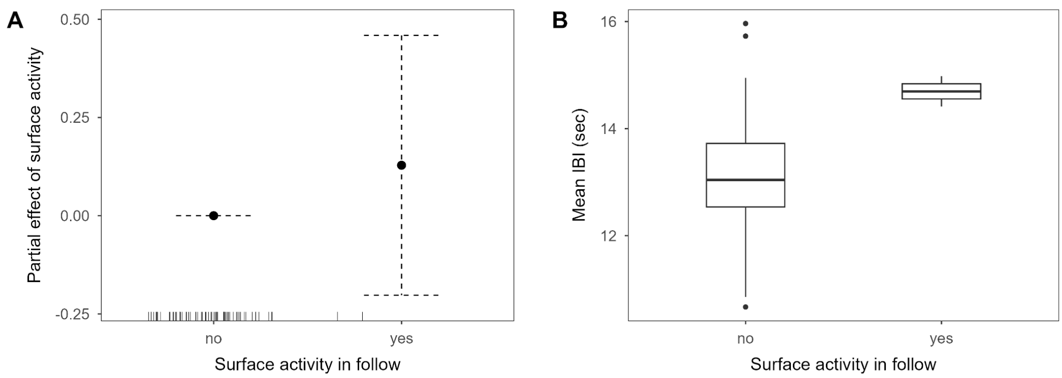

A
s(log(previous dive time))
2
1
0
4
5
6
log(previous time dive (sec))
B
Breaths per surfacing interval
14
12
10
8
6
4
2
50
100
200
400
Previous dive time (sec)

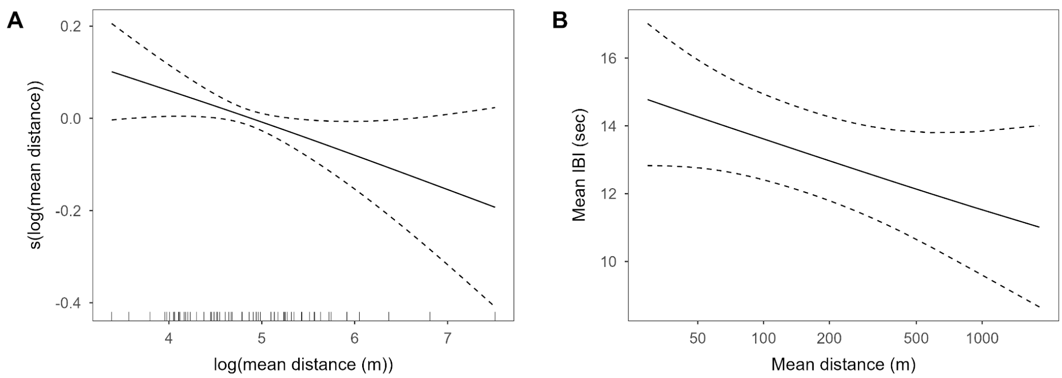

A
s(sqrt(mean vessel speed SD))
0.4
0.2
0.0
-0.2
0.3
0.6
0.9
1.2
1.5
sqrt(mean vessel speed SD (mph))
B
Breaths per surfacing interval
2.5
2.0
1.5
1.0
0.5
1.0
1.5
2.0
Mean vessel speed SD (mph)

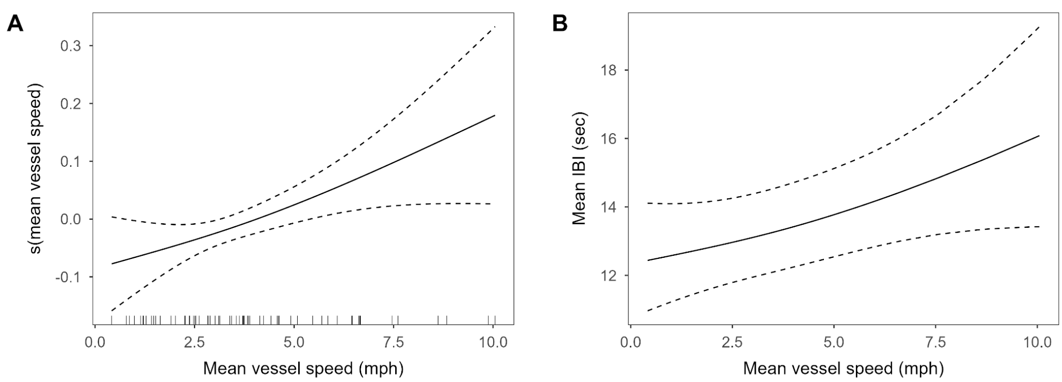

A
s(encounter minute)
0.4
0.2
0.0
-0.2
1.0
1.5
2.0
2.5
3.0
Encounter minute
B
Breaths per surfacing interval
2.5
2.0
1.5
1
5
10
20
30
Encounter minute

Mean inter-breath interval (IBI) per surfacing interval

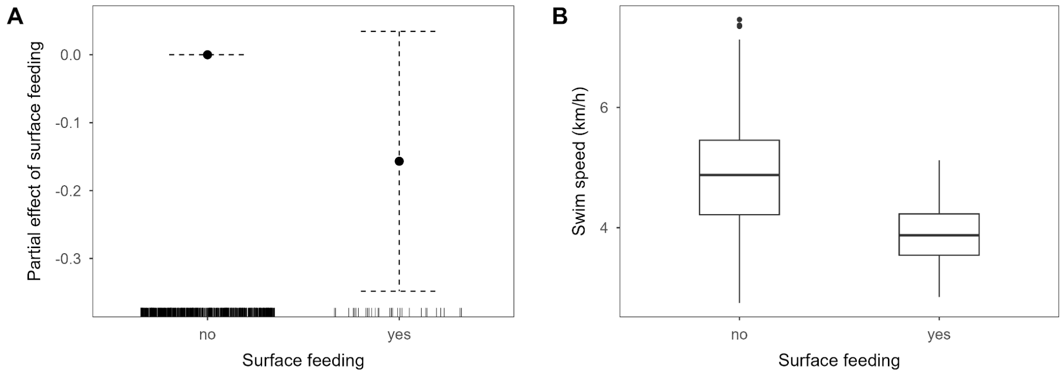


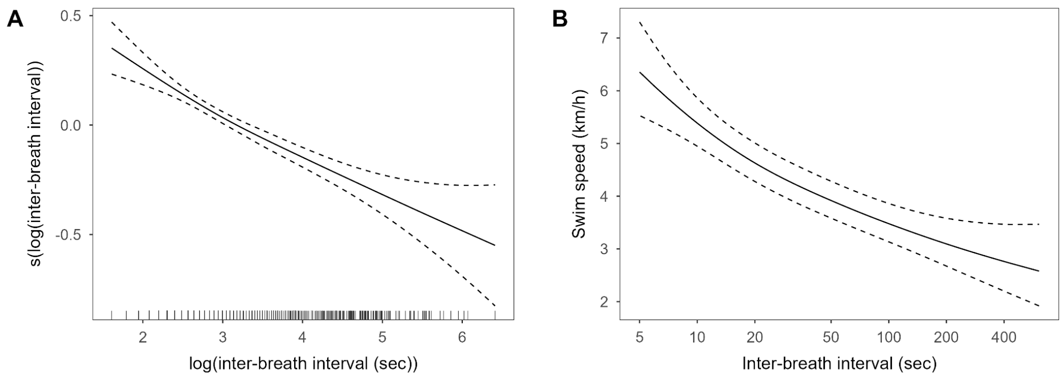


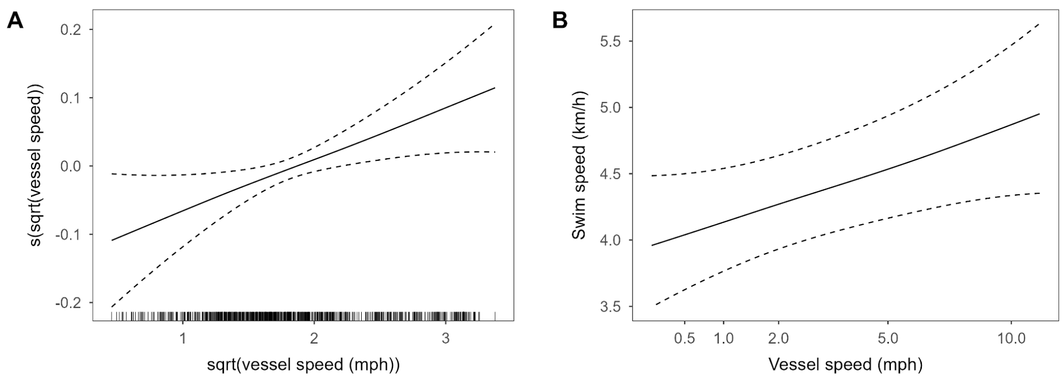


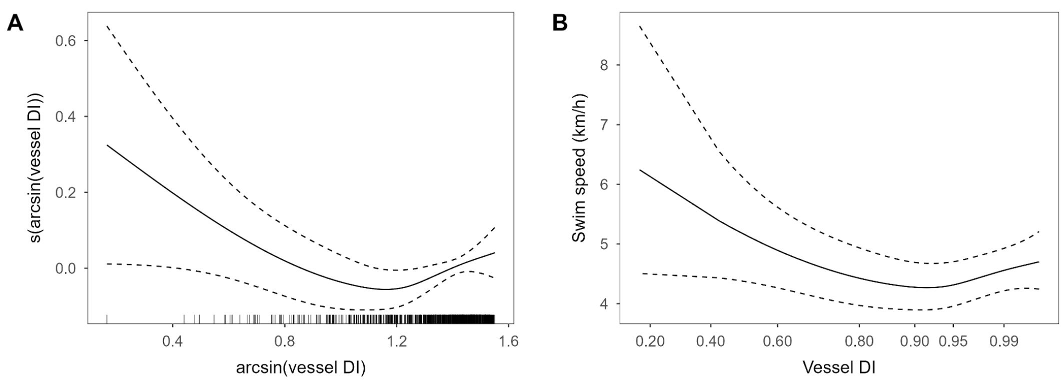


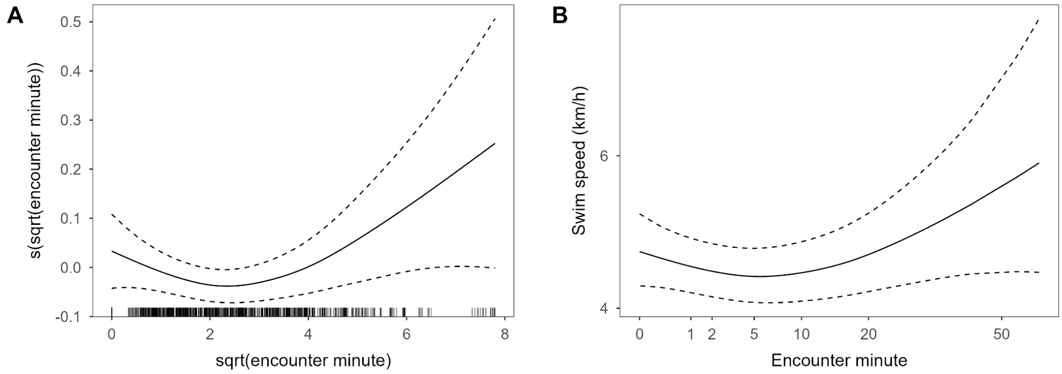
Swim speed


A
Partial effect of surface feeding
0.0
-0.1
-0.2
-0.3
no
yes
Surface feeding
B
Swim speed (km/h)
6
4
no
yes
Surface feeding

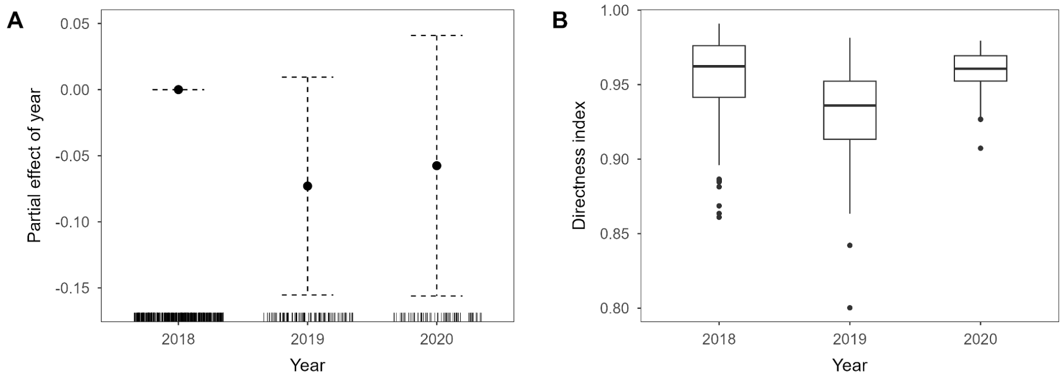

A
s(log(inter-breath interval))
0.5
0.0
-0.5
2
3
4
5
6
log(inter-breath interval (sec))
B
Swim speed (km/h)
7
6
5
4
3
2
5
10
20
50
100
200
400
Inter-breath interval (sec)

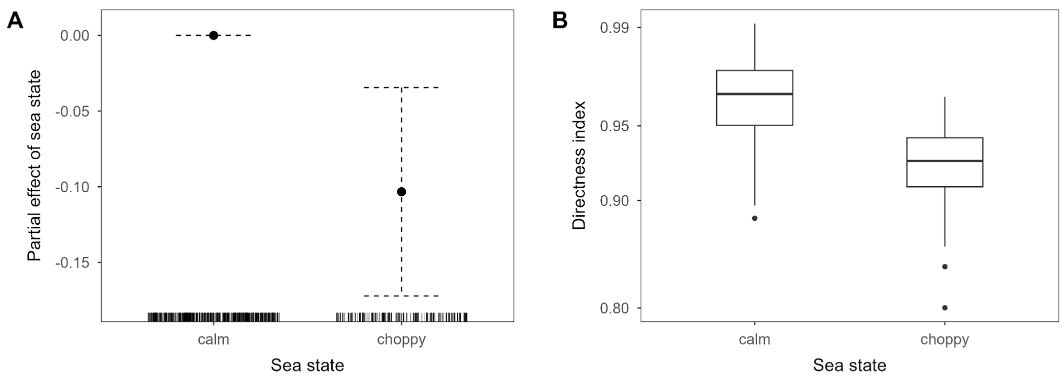

A
s(sqrt(vessel speed))
0.2
0.1
0.0
-0.1
-0.2
1
2
3
sqrt(vessel speed (mph))
B
Swim speed (km/h)
5.5
5.0
4.5
4.0
3.5
0.5
1.0
2.0
5.0
10.0
Vessel speed (mph)

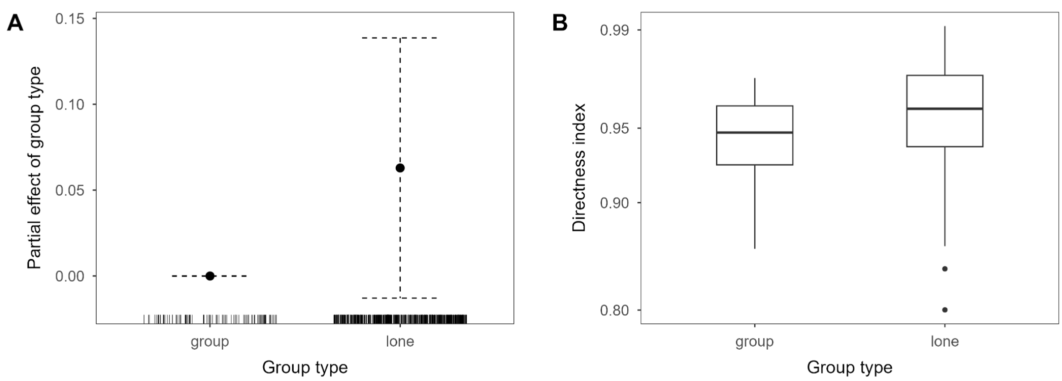

A
s(arcsin(vessel DI))
0.6
0.4
0.2
0.0
0.4
0.8
1.2
1.6
arcsin(vessel DI)
B
Swim speed (km/h)
8
7
6
5
4
0.20
0.40
0.60
0.80
0.90
0.95
0.99
Vessel DI

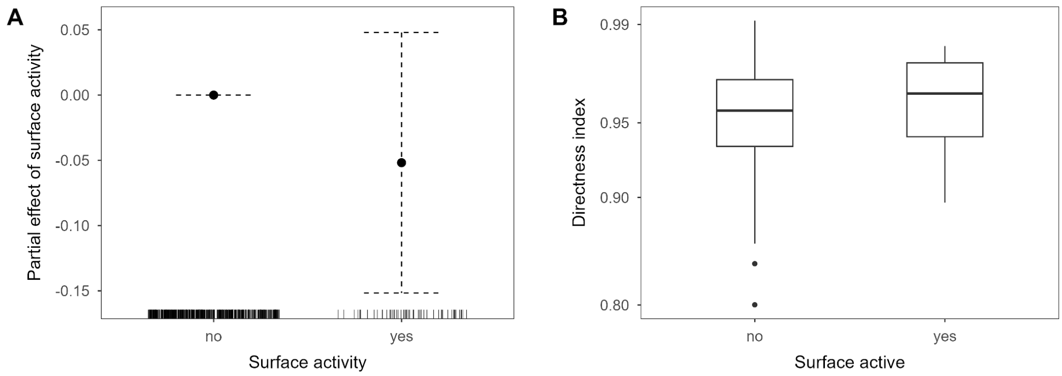


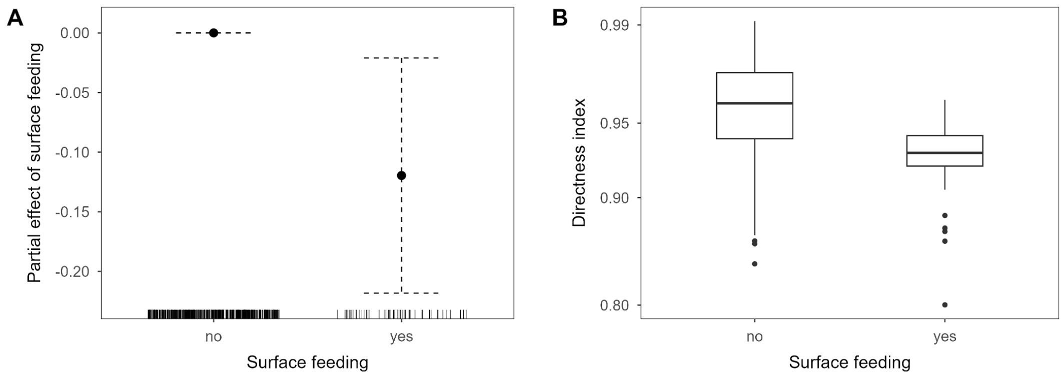
Directness index

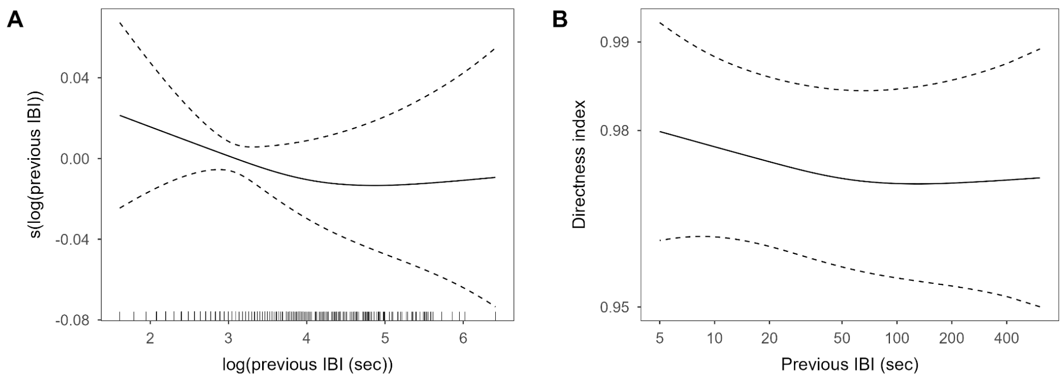


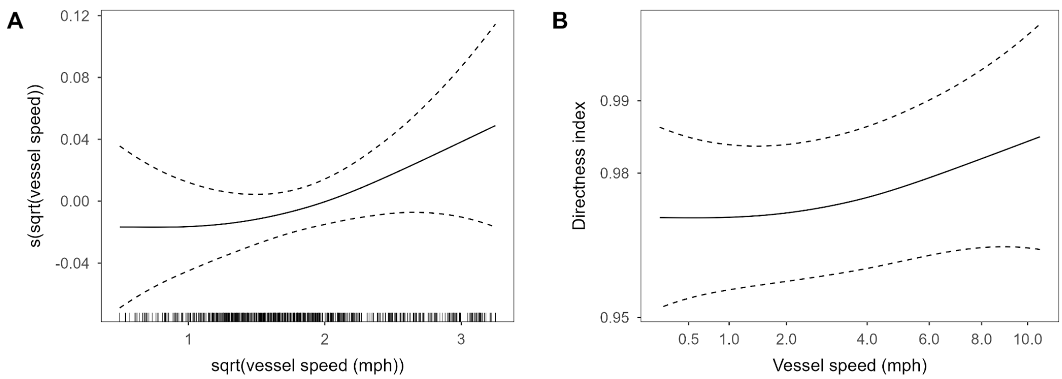


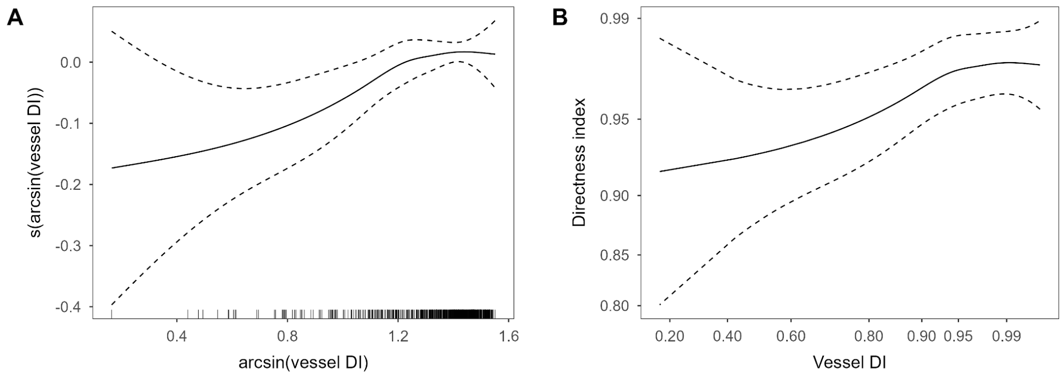


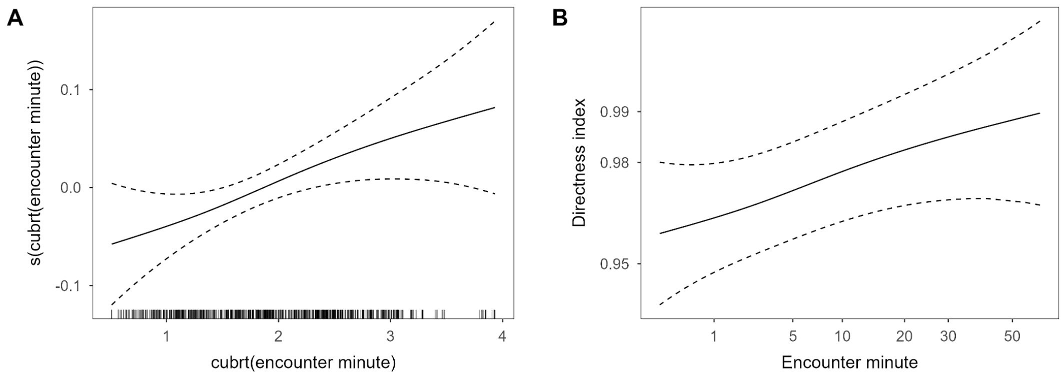

A
0.05
0.00
-0.05
-0.10
-0.15
Partial effect of surface activity
no
yes
Surface activity
B
0.99
0.95
0.90
0.80
Directness index
no
yes
Surface active


A
0.00
-0.05
-0.10
-0.15
-0.20
Partial effect of surface feeding
no
yes
Surface feeding
B
0.99
0.95
0.90
0.80
Directness index
no
yes
Surface feeding


A
0.04
0.00
-0.04
-0.08
s(log(previous IBI))
2
3
4
5
6
log(previous IBI (sec))
B
0.99
0.98
0.95
Directness index
5
10
20
50
100
200
400
Previous IBI (sec)


A
0.12
0.08
0.04
0.00
-0.04
s(sqrt(vessel speed))
1
2
3
sqrt(vessel speed (mph))
B
0.99
0.98
0.95
Directness index
0.5
1.0
2.0
4.0
6.0
8.0
10.0
Vessel speed (mph)


A
s(arcsin(vessel DI))
0.0
-0.1
-0.2
-0.3
-0.4
0.4
0.8
1.2
1.6
arcsin(vessel DI)
B
Directness index
0.99
0.95
0.90
0.85
0.80
0.20
0.40
0.60
0.80
0.90
0.95
0.99
Vessel DI


A
s(cubrt(encounter minute))
0.1
0.0
-0.1
1
2
3
4
cubrt(encounter minute)
B
Directness index
0.99
0.98
0.95
1
5
10
20
30
50
Encounter minute

References

Currie, J. J., McCordic, J. A., Olson, G. L., Machernis, A. F., and Stack, S. H. (2021). The impact of vessels on humpback whale behavior: the benefit of added whale watching guidelines. *Frontiers in Marine Science* 8, 601433. doi:10.3389/fmars.2021.601433

R Core Team (2020). R: A Language and Environment for Statistical Computing. https://www.r-project.org/ (Accessed: 2020-12-11)

Appendix D

Measurement error bootstrap GAMM results

Results of the bootstrapping procedure to propagate measurement errors through generalised additive mixed models (GAMMs) for swim speed and directness index (DI) are presented.

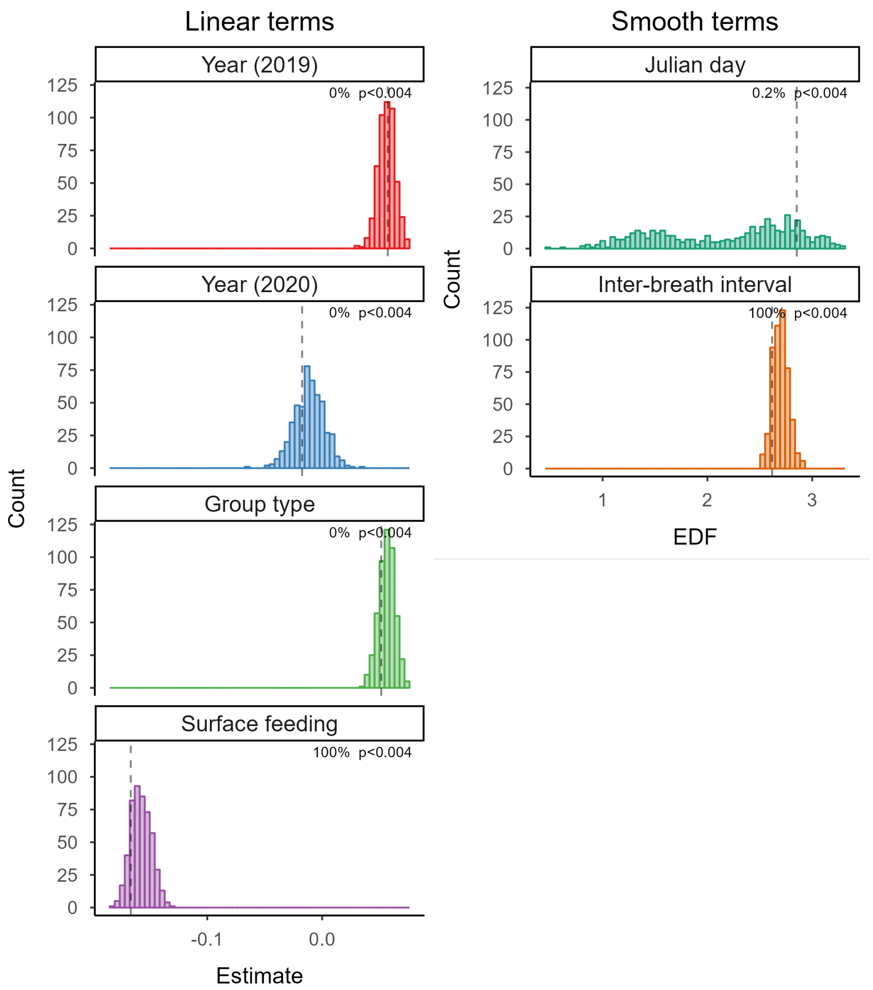
Each figure shows the results of a bootstrap procedure (500 replicates) to investigate the impact of measurement errors for horizontal distance and azimuth, derived from photographs or range finder readings, on the results of swim speed and directness index (DI) GAMMs. Whilst such measurement errors will influence explanatory variables in all GAMMs (distance between whale and focal vessel; all AIS variables), this impact is likely to be minimal compared to the influence on whale movement data. Moreover, distance errors are generally very small.

Each plot follows a standard format:

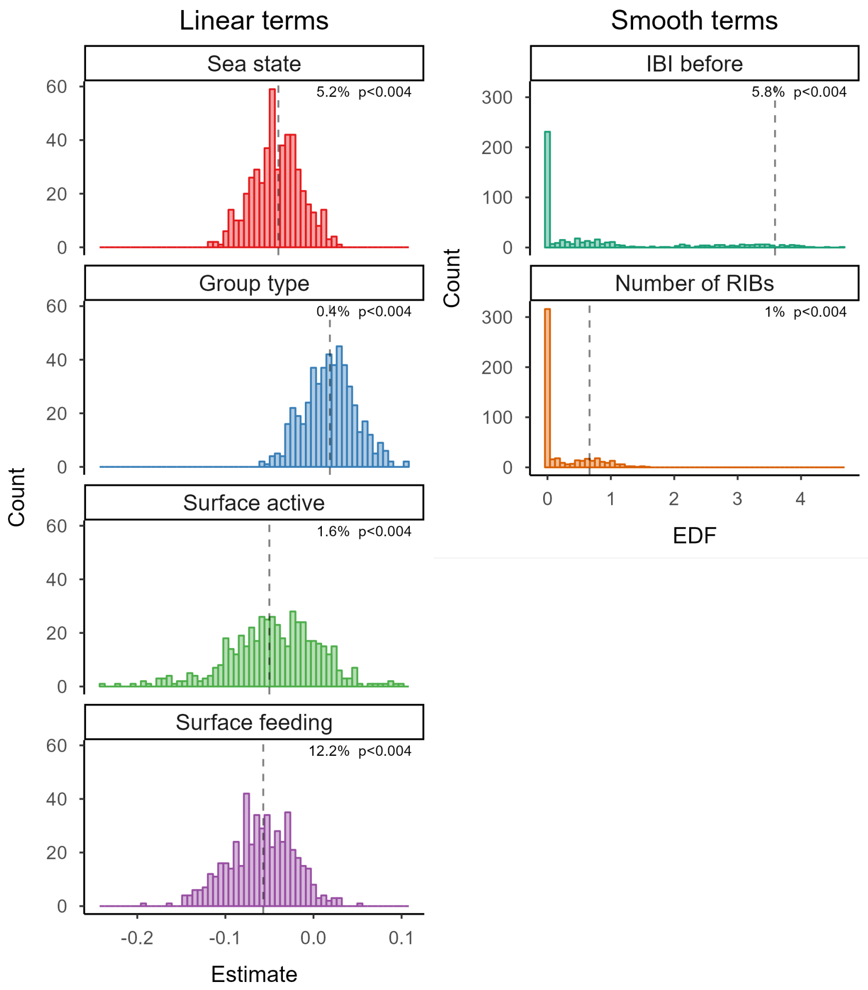
- Left side: the bootstrapped estimates for the slope of each linear term (histogram, coloured bars) with the effect from the original full model denoted by a grey line.
- Right side: the bootstrapped estimates of effective degrees of freedom (EDF) for each smooth term (histogram, coloured bars) with the EDF from the original full model denoted by a grey line.
- The percentage of replicates in which the term was significant in the GAMM is given in the top-right corner of each sub-plot.

AIS GAMMs

Swim speed

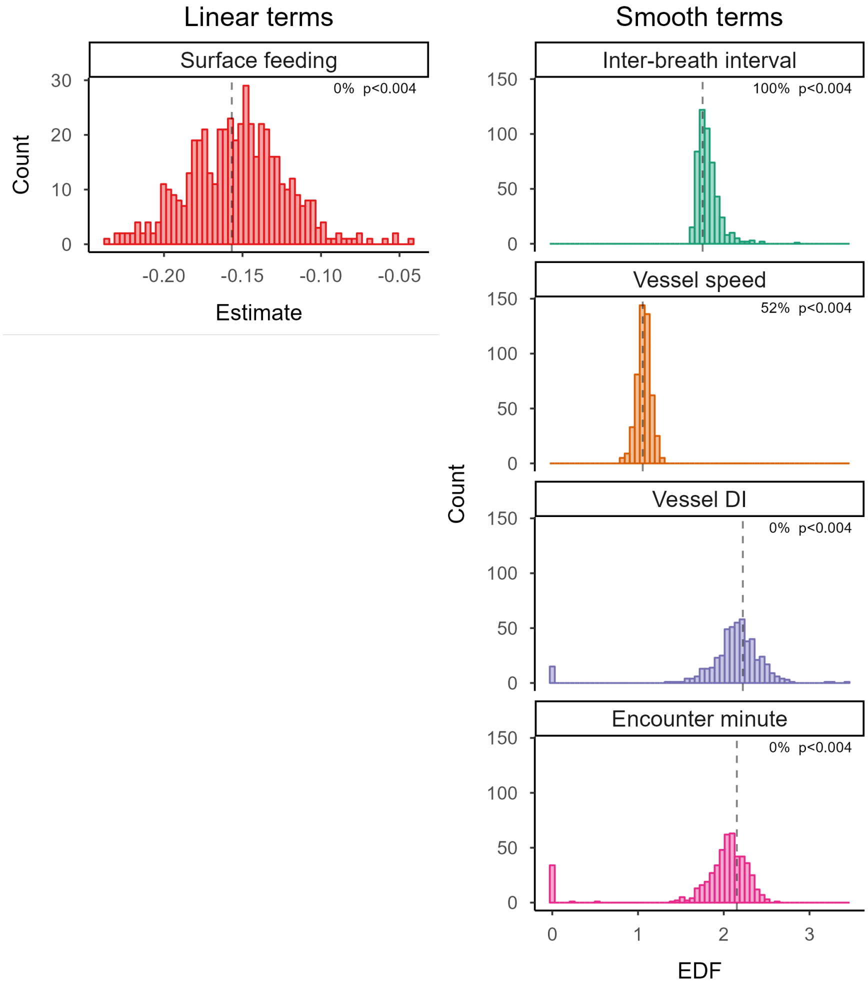


Directness index

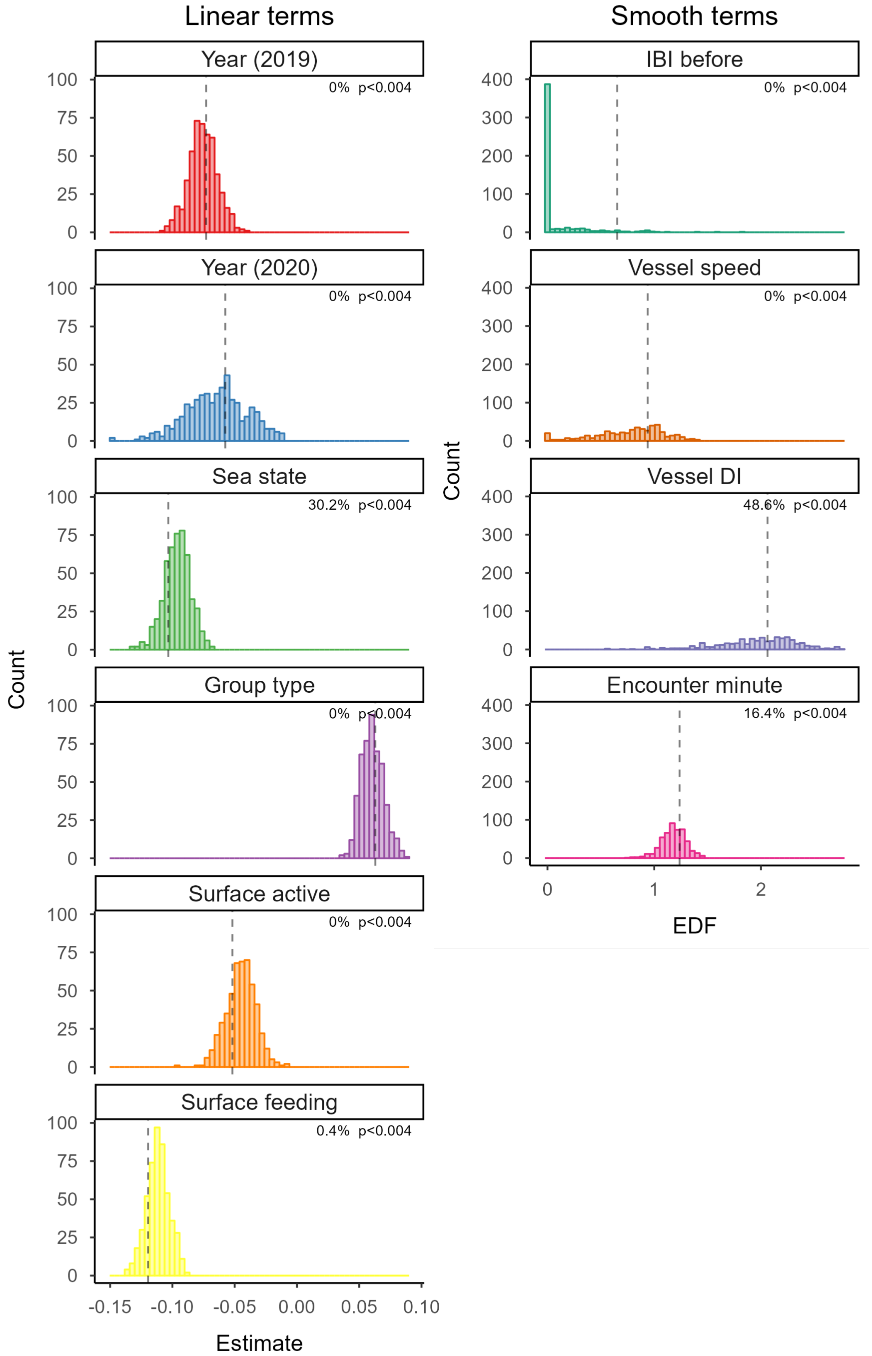


Focal vessel GAMMs

Swim speed



Directness index



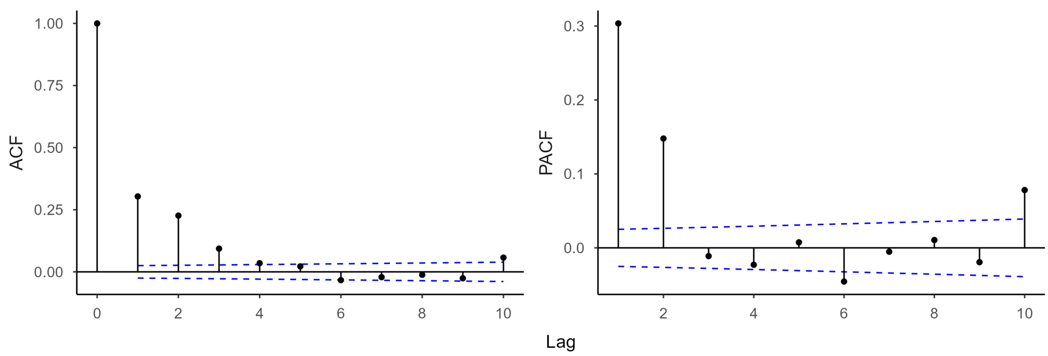
Appendix E

Temporal autocorrelation plots

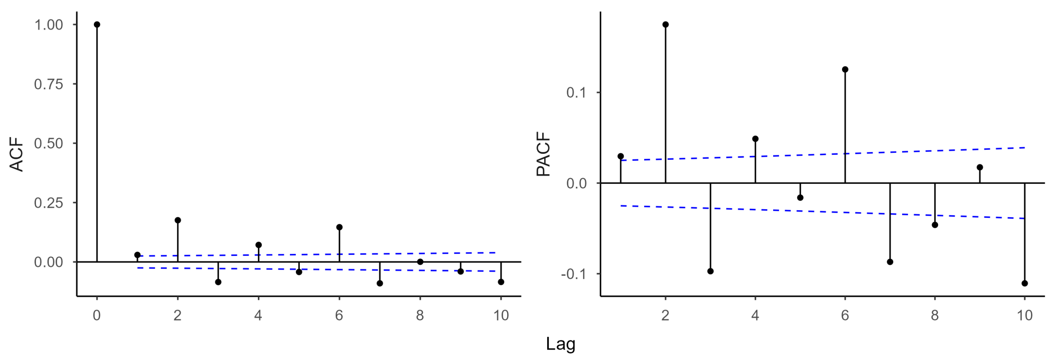
Plots of autocorrelation function (ACF) and partial autocorrelation (PACF) values are presented for each response variable for each type of generalised additive mixed model: AIS vessel variables and focal vessel variables. Each subplot consists of ACF/PACF values (black points) and a 95% confidence interval (blue dashed lines). Values are calculated from the residuals of the full GAMM prior to variable selection and without the addition of an autocorrelation (AR1) structure.

E.1 AIS GAMMs

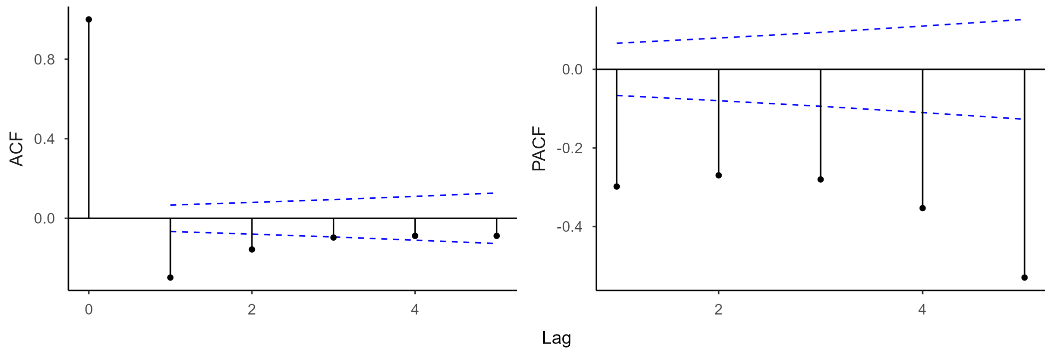
Surface-active behaviours (SAB)



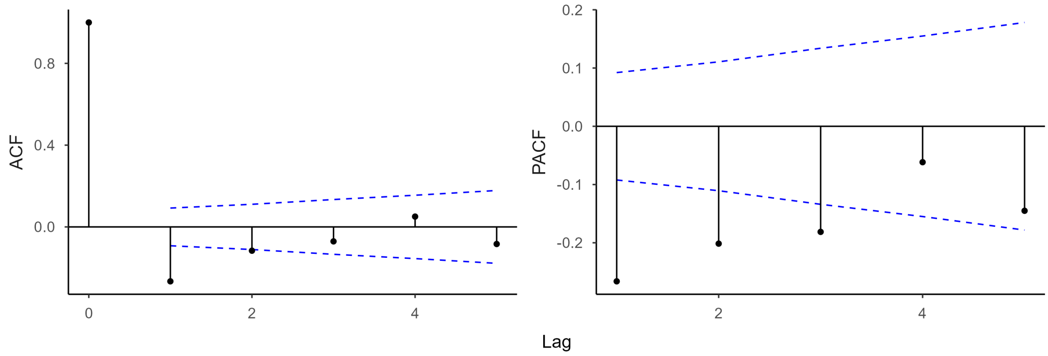
Surface feeding events (SFE)



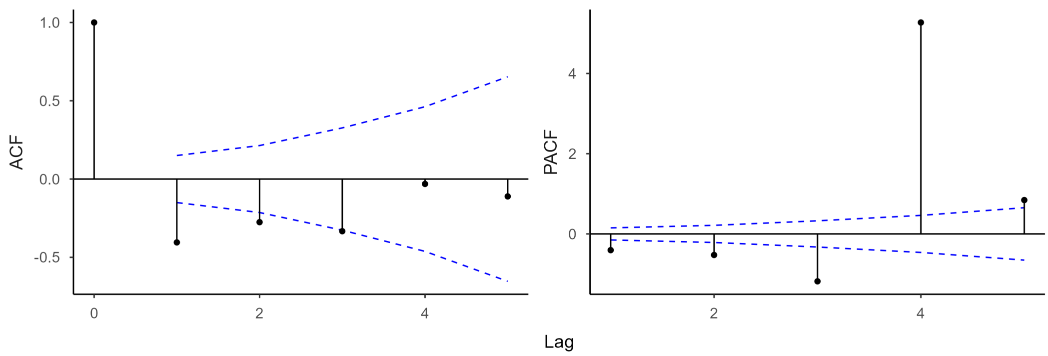
Dive time



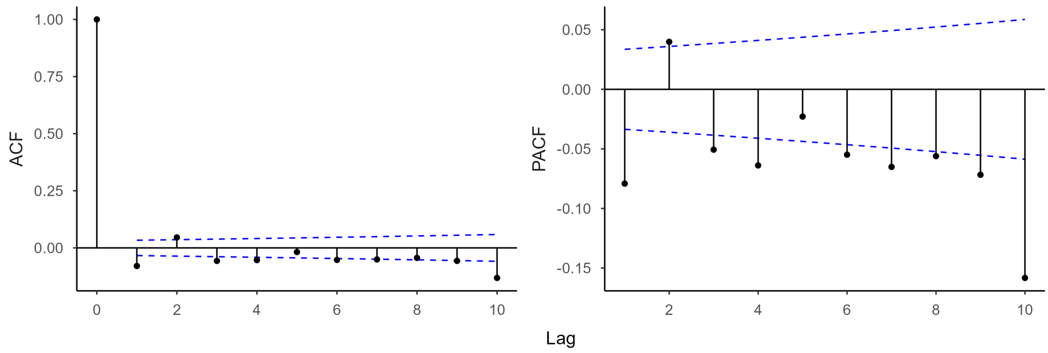
Breaths per surfacing interval



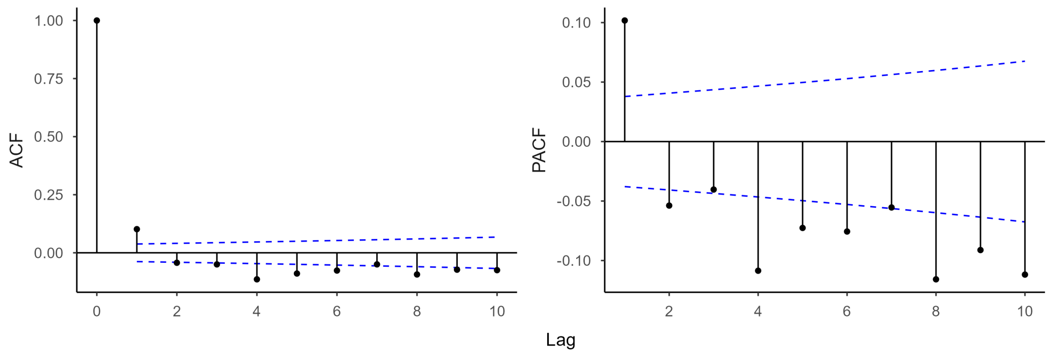
Mean inter-breath interval (IBI) per surfacing interval



Swim speed

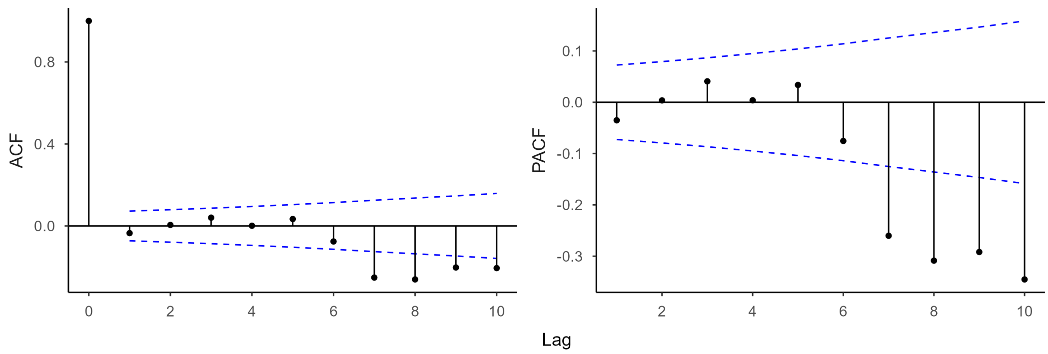


Directness index

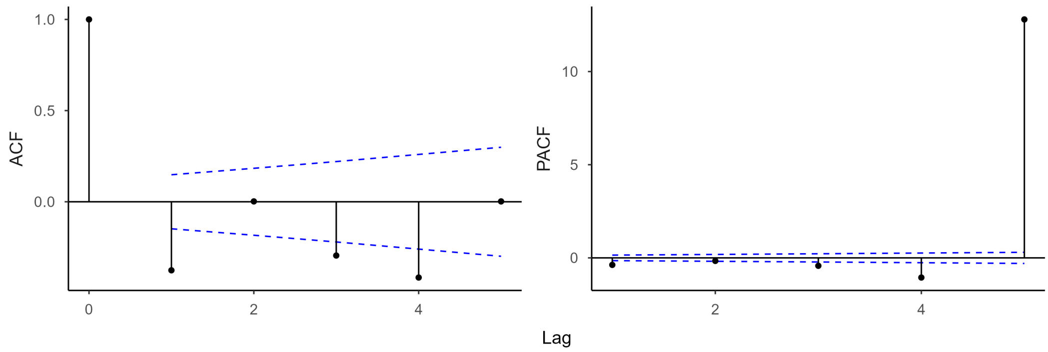


E.2 Focal vessel GAMMs

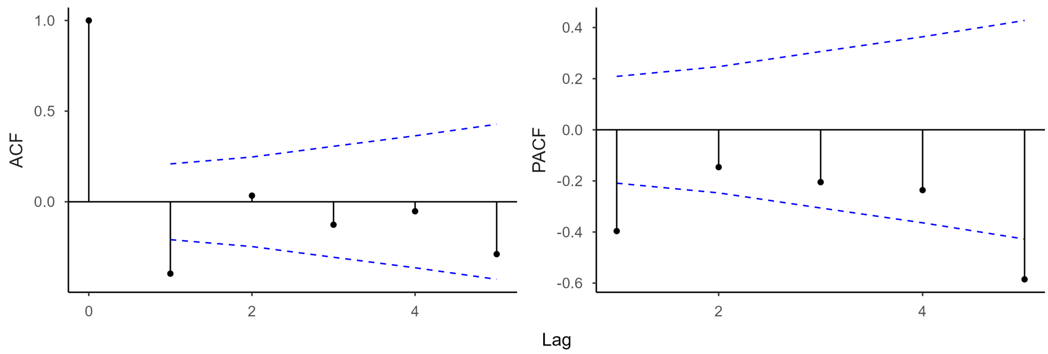
Surface feeding events



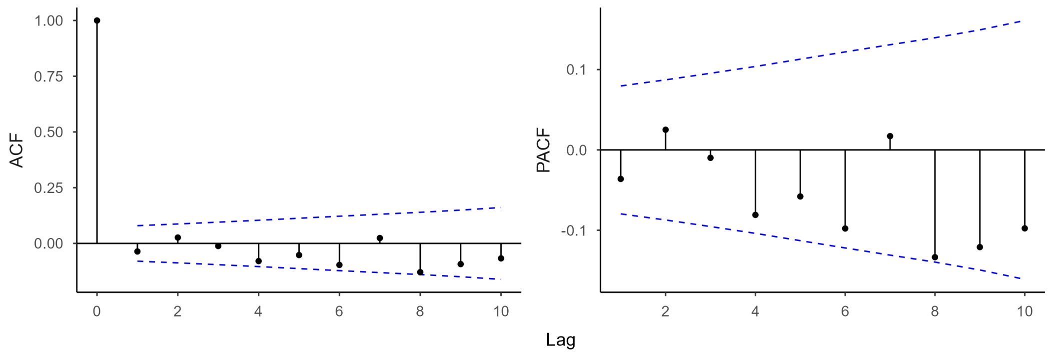
Dive time



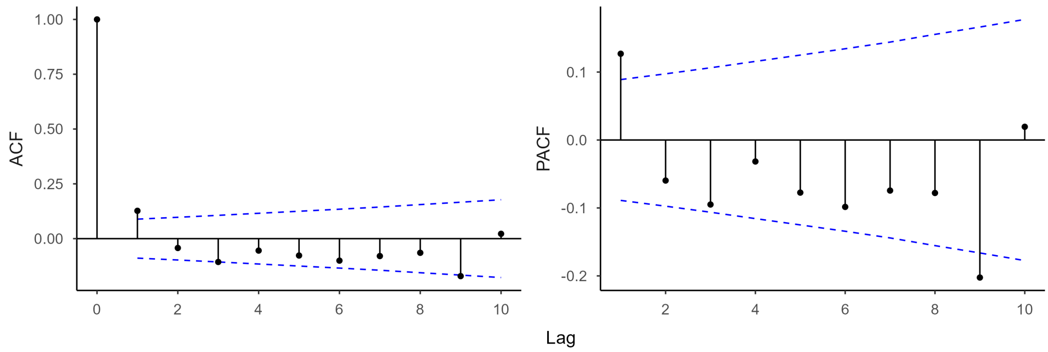
Breaths per surfacing interval



Swim speed



Directness index



Appendix F

Compound-specific LC–MS/MS parameters

Compound specific parameters for both HPLC and MS–MS were tuned as part of ongoing clinical studies at the Edinburgh Clinical Research Facility Mass Spectrometry Core, and were not modified for blow sample analysis. As such, there were differences between phases and batches. Batch-specific LC retention times (Table F.1), phase-specific MS parameters (Table F.2) and corresponding internal standards for each steroid (Table F.3) are provided.

Table F.1: Retention times for each analyte and internal standard for the 2018/19 and 2021 sample analyses. Range is given for DHEAS and d_5DHEAS, which have inconsistent retention times.

	Phase 1 (2018/19 samples) Nexera uPLC Qtrap 6500+		Phase 2 (2021 samples) Acquity I-Class Qtrap 6500+	
Steroid	**C18, Batch 1/2 Retention time (min)**	**Biphenyl, Batch 2 Retention time (min)**	**C18, Batch 3 Retention time (min)**	**C18, Batch 4 Retention time (min)**
Cortisol (F)	4.1		3.52	3.39
Cortisone (E)	3.45		2.98	2.89
Corticosterone (B)	5.48		5.39	5.12
11-dehydrocorticosterone (A)	4.04		3.56	3.44
Dehydroepiandrosterone (DHEA)		5.75	8.23	8.05
Dehydroepiandrosterone sulphate (DHEAS)		1.72–2.47	2.38	2.24
Testosterone (T)	7.74		7.72	7.54
Dihydrotestosterone (DHT)	8.62		8.19	8.02
Progesterone (P)	8.96		9.69	9.53
Aldosterone (Aldo)		4.01	2.6	2.54
Androstenedione (A4)	7.17		6.98	6.79
Estrone (E1)	7.39		7.15	6.94
Estradiol (E2)	7.49		7.27	7.06
Estriol (E3)		1.89	2.49	2.43
d_4-cortisol (d_4F)	4.07		3.5	3.37
d_8-cortisone (d_8E)	3.4		2.94	2.84
d_8-corticosterone (d_8B)			4.7	4.48
d_5-dehydroepiandrosterone (d_5DHEA)		6.28	8.19	8.02
d_5-dehydroepiandrosterone sulphate (d_5DHEAS)		1.71–2.47	2.36	2.23
$^{13}C_3$-testosterone ($^{13}C_3$-T)	7.72		7.72	7.54
$^{13}C_3$-dihydrotestosterone ($^{13}C_3$-DHT)	8.61		8.19	8.02
d_9-progesterone (d_9P)	8.91		9.61	9.46
d_8-aldosterone (d_8Aldo)		3.94	2.56	2.51
$^{13}C_3$-androstenedione ($^{13}C_3$-A4)	7.16		6.97	6.79
$^{13}C_3$-estrone ($^{13}C_3$-E1)	7.39		7.15	6.94
$^{13}C_3$-estradiol ($^{13}C_3$-E2)	7.49		7.27	7.06
$^{13}C_3$-estriol ($^{13}C_3$-E3)			2.49	2.43

Table F.2: Compound-specific mass spectrometric parameters for each phase. Q1 mass and Q3 mass are the target masses in the first and third quadrupoles respectively. Polarity of the mass spectrometer is either positive (+) or negative (–). DP – declustering potential, CE – collision energy, CXP – collision cell exit potential.

Steroid	Ion	Q1 mass (Da)	Q3 mass (Da)	Polarity	Phase 1 (2018/19 samples) DP (V)	Phase 1 CE (V)	Phase 1 CXP (V)	Phase 2 (2021 samples) DP (V)	Phase 2 CE (V)	Phase 2 CXP (V)
Cortisol (F)	$[M+H]^+$	363.1	121.2	+	76	31	8	76	31	8
Cortisone (E)	$[M+H]^+$	361.1	163.1	+	81	31	26	81	31	26
Corticosterone (B)	$[M+H]^+$	347.1	121.1	+	76	29	8	76	29	8
11-dehydrocorticosterone (A)	$[M+H]^+$	345.1	121.2	+	66	31	12	66	31	12
Testosterone (T)	$[M+H]^+$	289.1	97	+	101	29	12	101	29	12
Dihydrotestosterone (DHT)	$[M+H]^+$	291.3	255.2	+	116	21	30	116	21	30
Androstenedione (A4)	$[M+H]^+$	287	97	+	61	27	14	61	27	14
Dehydroepiandrosterone (DHEA)	$[M+H]^+$	289.1	253	+	121	15	46			
Dehydroepiandrosterone (DHEA)(-H_2O)	$[M+H-H_2O]^+$	271.1	253	+				106	17	12
Progesterone (P)	$[M+H]^+$	315.1	97.1	+	96	23	10	96	23	10
d_4-cortisol (d_4F)	$[M+H]^+$	367.3	121.1	+	80	29	16	76	31	8
d_8-cortisone (d_8E)	$[M+H]^+$	369.2	169	+	96	33	20	81	31	26
d_8-corticosterone (d_8B)	$[M+H]^+$	355.3	125.1	+				76	29	8
$^{13}C_3$-testosterone ($^{13}C_3$-T)	$[M+H]^+$	292.1	100	+	96	29	12	101	29	12
$^{13}C_3$-dihydrotestosterone ($^{13}C_3$-DHT)	$[M+H]^+$	294.2	258.3	+	61	21	12	116	21	30
$^{13}C_3$-Androstenedione ($^{13}C_3$-A4)	$[M+H]^+$	290.2	100.1	+	31	27	12	61	27	14
d_5-dehydroepiandrosterone (d_5DHEA)	$[M+H]^+$	294.1	258.2	+	141	11	34			
d_5-dehydroepiandrosterone (d_5DHEA)(-H_2O)	$[M+H]^+$	276.1	258.1	+				106	17	12
d_9-progesterone (d_9P)	$[M+H]^+$	324.1	100	+	151	31	15	96	23	10
Dehydroepiandrosterone sulphate (DHEAS)	$[M+H]^-$	367.1	96.9	–	-110	-38	-11	-110	-38	-11
Aldosterone (Aldo)	$[M+H]^-$	359.1	188.9	–	-70	-24	-21	-70	-24	-21
Estradiol (E2)	$[M+H]^-$	271	182.9	–	-110	-52	-19	-110	-52	-19
Estrone (E1)	$[M+H]^-$	269.1	144.9	–	-150	-48	-15	-150	-48	-15
Estriol (E3)	$[M+H]^-$	287.1	171	–	-155	-48	-29	-155	-54	-9
d_5-dehydroepiandrosterone sulphate (d_5DHEAS)	$[M+H]^-$	372.1	97.9	–	-40	-36	-7	-40	-36	-7
d_8-aldosterone (d_8Aldo)	$[M+H]^-$	367.2	193.9	–	-75	-26	-21	-75	-26	-21
$^{13}C_3$-estrone ($^{13}C_3$-E1)	$[M+H]^-$	274	185.9	–	-155	-54	-19	-155	-54	-9
$^{13}C_3$-estradiol ($^{13}C_3$-E2)	$[M+H]^-$	272	147.8	–	-165	-50	-17	-165	-50	-17
$^{13}C_3$-estriol ($^{13}C_3$-E3)	$[M+H]^-$	290.2	173.9	–				-155	-50	-25

Table F.3: Steroids and associated internal standards for the Phase 1 (2018/19 samples) and Phase 2 (2021 samples) LC–MS/MS analyses. Different internal standards in Phase 2 are in bold italic.

	Internal standard selected at time of data analysis	
Steroid	**Phase 1 (2018/19 samples)**	**Phase 2 (2021 samples)**
Cortisol (F)	d_4-cortisol (d_4F)	d_4-cortisol (d_4F)
Cortisone (E)	d_8-cortisone (d_8E)	d_8-cortisone (d_8E)
Corticosterone (B)	d_8-cortisone (d_8E)	***d_8-corticosterone (d_8B)***
11-dehydrocorticosterone (A)	d_8-cortisone (d_8E)	***d_8-corticosterone (d_8B)***
Dehydroepiandrosterone (DHEA)	d_5DHEA	d_5DHEA
Dehydroepiandrosterone sulphate (DHEAS)	d_5DHEAS	d_5DHEAS
Testosterone (T)	$^{13}C_3$-testosterone ($^{13}C_3$-T)	$^{13}C_3$-testosterone ($^{13}C_3$-T)
Dihydrotestosterone (DHT)	$^{13}C_3$-dihydrotestosterone ($^{13}C_3$-DHT)	$^{13}C_3$-dihydrotestosterone ($^{13}C_3$-DHT)
Progesterone (P)	d_9-progesterone (d_9P)	d_9-progesterone (d_9P)
Aldosterone (Aldo)	d_8-aldosterone (d_8Aldo)	d_8-aldosterone (d_8Aldo)
Androstenedione (A4)	$^{13}C_3$-androstenedione ($^{13}C_3$-A4)	$^{13}C_3$-androstenedione ($^{13}C_3$-A4)
Estrone (E1)	$^{13}C_3$-estrone ($^{13}C_3$-E1)	$^{13}C_3$-estrone ($^{13}C_3$-E1)
Estradiol (E2)	$^{13}C_3$-estradiol ($^{13}C_3$-E2)	$^{13}C_3$-estradiol ($^{13}C_3$-E2)
Estriol (E3)	$^{13}C_3$-estradiol ($^{13}C_3$-E2)	***$^{13}C_3$-estriol ($^{13}C_3$-E3)***

Appendix G

Blow samples used in Chapter 3

The following tables contain details of the blow samples used in Phase 1 and Phase 2 of this study. In Phase 1, out of 32 samples collected, 14 were used to test the developed protocol (only these samples are listed). In Phase 2, out of 55 blow samples, 54 were processed for steroid hormone detection and quantification (sample 2021_8 was damaged during transport from Iceland to the UK).

Phase 1 (2018/19)

Samples were collected in two areas: Eyjafjörður in 2018 and Skjálfandi Bay in 2019 (Table G.1). Samples were collected from either land or a vessel platform. Sample 2019_8 was collected by hand (instead of using a UAV) due to deliberate approaches to the vessel from a curious animal. Visible blow sample quality (in terms of the amount and visible liquid) was categorised as: none (0), very poor (VP), poor (P), moderate (M), good (G), very good (VG). The predominant behavioural state and any details of behavioural responses to the UAV were also noted. Distance to operator was not consistently recorded and so is not presented here.

Table G.1: Blow samples collected in Phase 1 (2018/19) that were analysed using LC–MS/MS. Whale identification (ID) is not provided when suitable identification images could not be obtained during sample collection. Visible quality was categorised as none (0), very poor (VP), poor (P), moderate (M), good (G) or very good (VG).

Sample #	Date	Time	Area	Platform	Whale ID	Visible quality	Behavioural state	Behavioural response?
2018_1	20/06/2018	21:07	Eyjafjörður	land	140	VP	feeding	no
2018_2	20/06/2018	21:23	Eyjafjörður	land	140	P-M	feeding	no
2018_3	22/06/2018	23:32	Eyjafjörður	land	140	M-G	feeding	no
2018_4	23/06/2018	19:18	Eyjafjörður	land	143	P-M	travelling	no
2018_5	23/06/2018	19:42	Eyjafjörður	land		P-M	travelling	no
2019_1	22/06/2019	20:43	Skjálfandi	vessel		P	feeding	no
2019_2	10/07/2019	23:00	Skjálfandi	vessel	41	VP-P	feeding	no
2019_3	10/07/2019	23:34	Skjálfandi	vessel	41	P	travelling	annoyance following later aerial imagery
2019_4	11/07/2019	00:15	Skjálfandi	vessel	43	M	feeding/travelling	no
2019_5	11/07/2019	00:30	Skjálfandi	vessel	43	VP-P	feeding	no
2019_6	12/07/2019	07:59	Skjálfandi	vessel	49	VP	resting	no
2019_7	12/07/2019	09:00	Skjálfandi	vessel	40	VP-P	feeding	no
2019_8	23/07/2019	08:58	Skjálfandi	vessel	1	M	feeding/resting	no
2019_9	27/07/2019	06:45	Skjálfandi	vessel	60	M	resting/curious	no

Phase 2 (2021)

Samples were collected in four areas: Skjálfandi Bay and Eyjafjörður, with higher vessel traffic and whale-watching activity; and Ísafjarðardjúp and Húnaflói, with lower vessel traffic and whale-watching activity (Table G.2). Samples were collected from either a land or vessel platform. Sample 2021_13 was collected by hand (instead of using a UAV) due to deliberate approaches to the vessel from a curious animal; and sample 2021_8 was damaged during transport from Iceland to the UK, and was not included in laboratory analysis. Visible sample quality was categorised as in Phase 1. Behavioural state and responses to the UAV were also recorded. Distance between the research platform (UAV operator) and the whale during blow sample collection could only be accurately provided for samples collected from land.

Table G.2: Blow samples collected in Phase 2 (2021). Whale identification (ID) is not provided when suitable identification images could not be obtained during sample collection. Visible quality was categorised as none (0), very poor (VP), poor (P), moderate (M), good (G) or very good (VG). Accurate values of distance to operator at the time of blow sample collection were only available for samples collected from land.

Sample #	Date	Time	Area	Platform	Distance (m)	Whale ID	Visible quality	Behavioural state	Behavioural response?	Number of blows
2021_1	07/07/2021	23:07	Skjálfandi	vessel		299	VP	feeding	no	1
2021_2	07/07/2021	23:53	Skjálfandi	vessel		300	VP	feeding	no	1
2021_3	08/07/2021	00:47	Skjálfandi	vessel		305	VP	feeding	annoyance (tail splash + trumpet blows)	1
2021_4	09/07/2021	07:29	Skjálfandi	vessel		208	0–VP	travelling	no	1
2021_5	09/07/2021	07:40	Skjálfandi	vessel		208	VP	travelling	annoyance (tail swish)	1
2021_6	12/07/2021	01:40	Skjálfandi	vessel	257	130	VP	feeding	no	1
2021_7	13/07/2021	14:52	Skjálfandi	vessel		301	VP	deep feeding/travelling	no	1
2021_8	19/07/2021	22:59	Skjálfandi	vessel	371	130	VP	feeding/travelling	no	1
2021_9	21/07/2021	22:18	Skjálfandi	vessel	560		P	unknown	no	1
2021_10	24/07/2021	05:55	Húnaflói	land		311	G	feeding	no	2
2021_11	30/07/2021	06:00	Skjálfandi	vessel		53	M	travelling	no	1
2021_12	30/07/2021	21:18	Skjálfandi	vessel		313	P	feeding	no	1
2021_13	31/07/2021	07:40	Skjálfandi	vessel	1	313	M	curious	no	3
2021_14	02/08/2021	18:23	Ísafjarðardjúp	land	589	331	G	feeding	no	2
2021_15	04/08/2021	13:05	Ísafjarðardjúp	land	1217		P–M	deep feeding	no	1
2021_16	04/08/2021	13:23	Ísafjarðardjúp	land	1441	317	M–G	deep feeding	no	1
2021_17	04/08/2021	13:36	Ísafjarðardjúp	land	1240	321	P	deep feeding	no	3
2021_18	04/08/2021	15:28	Ísafjarðardjúp	land	1243		M–G	deep feeding	no	1
2021_19	04/08/2021	15:50	Ísafjarðardjúp	land	1658		VP	deep feeding	no	1
2021_20	04/08/2021	16:00	Ísafjarðardjúp	land	1340		VP	deep feeding	no	1

Table G.2 continued

Sample #	Date	Time	Area	Platform	Distance (m)	Whale ID	Visible quality	Behavioural state	Behavioural response?	Number of blows
2021_21	04/08/2021	16:32	Ísafjarðardjúp	land	1629	323	P–M	deep feeding	no	1
2021_22	05/08/2021	10:16	Ísafjarðardjúp	land	1546		P–M	deep feeding	no	1
2021_23	05/08/2021	12:39	Ísafjarðardjúp	land	1590	321	P	travelling	no	1
2021_24	05/08/2021	19:14	Ísafjarðardjúp	land	956	330	VP–P	feeding	no	1
2021_25	08/08/2021	18:24	Húnaflói	land	267	332	G–VG	feeding	no	1
2021_26	09/08/2021	07:56	Húnaflói	land	1204	332	M–G	resting	no	2
2021_27	09/08/2021	12:11	Húnaflói	land	516	333	M	feeding	no	3
2021_28	10/08/2021	11:24	Húnaflói	land	891	53	M	feeding	no	2
2021_29	13/08/2021	12:03	Skjálfandi	vessel		305	VG	feeding	no	2
2021_30	13/08/2021	12:39	Skjálfandi	vessel		305	VP–P	feeding	no	2
2021_31	18/08/2021	05:42	Skjálfandi	vessel		334	M	resting	no	2
2021_32	19/08/2021	07:41	Skjálfandi	vessel	723	335	M	resting	annoyance following later aerial imagery	2
2021_33	05/09/2021	10:21	Húnaflói	land	1743	338	P–M	feeding	no	1
2021_34	05/09/2021	11:18	Húnaflói	land	1362	338	P–M	feeding	no	1
2021_35	05/09/2021	17:56	Húnaflói	land	838	338	M–G	feeding	no	1
2021_36	06/09/2021	12:01	Húnaflói	vessel		339	P–M	surface feeding	no	1
2021_37	06/09/2021	15:59	Húnaflói	vessel		340	VG	feeding	no	1
2021_38	09/09/2021	14:37	Húnaflói	land	1040	343	M–G	deep feeding	no	2
2021_39	09/09/2021	14:54	Húnaflói	land	1671	39	VG	deep feeding	no	2
2021_40	09/09/2021	16:39	Húnaflói	land	1587	39	VG	deep feeding	no	3
2021_41	09/09/2021	17:08	Húnaflói	land	1702	343	VG	deep feeding	no	3
2021_42	10/09/2021	17:20	Húnaflói	land	1052	339	VP	feeding	no	1
2021_43	10/09/2021	18:38	Húnaflói	land	1436	339	VP–P	feeding	no	1
2021_44	11/09/2021	07:26	Húnaflói	land	1995	39	VG	travelling	no	1

Table G.2 continued

Sample #	Date	Time	Area	Platform	Distance (m)	Whale ID	Visible quality	Behavioural state	Behavioural response?	Number of blows
2021_45	11/09/2021	11:11	Húnaflói	land	2666	65	G	surface feeding	no	2
2021_46	11/09/2021	11:37	Húnaflói	land	2520	344	G–VG	surface feeding	no	1
2021_47	11/09/2021	17:05	Húnaflói	land	1891	344	G–VG	surface feeding	no	3
2021_48	15/09/2021	16:02	Eyjafjörður	land	4203	345	P	deep feeding	no	2
2021_49	16/09/2021	16:09	Skjálfandi	vessel	100	346	M–G	deep feeding	no	1
2021_50	18/09/2021	11:54	Eyjafjörður	vessel	663	345	M–G	deep feeding	no	2
2021_51	18/09/2021	12:47	Eyjafjörður	vessel	1102	350	G–VG	deep feeding	no	1
2021_52	18/09/2021	13:40	Eyjafjörður	vessel	173	345	M–G	deep feeding	no	2
2021_53	18/09/2021	14:08	Eyjafjörður	vessel	321	350	VG	deep feeding	no	2
2021_54	18/09/2021	15:36	Eyjafjörður	vessel		345	VP	deep feeding	no	4
2021_55	18/09/2021	15:48	Eyjafjörður	vessel		350	VG	deep feeding	no	1

Appendix H

Comparing Phase 2 LC–MS/MS performance between batches

The LC–MS/MS outputs from the 2021 blow samples were considerably different to those from 2018/19, with a weaker (and generally absent) cortisol signal but a stronger cortisone signal. Moreover, results seemed to differ between the two 2021 sample batches that were analysed: Batch 3 (samples 2021_1–29) and Batch 4 (samples 2021_18, 25, 28, 30–55), run five days later. For example, Batch 3 had a stronger cortisone signal and a weaker testosterone signal than Batch 4. It is possible that these differences are driven by run performance, as well as other methodological or ecological differences. To investigate this, I visually compared the internal standard and analyte peak areas of Batch 3 and Batch 4. Furthermore, I included the results of a practice calibration curve that was run three days before Batch 3, using the same dish extraction and LC–MS method.

Internal standard peak consistency

First, I visualised the peak area of four internal standards (IS; d_4-cortisol, d_8-cortisone, $^{13}C_3$-testosterone and d_9-progesterone) for each calibration standard and batch (practice, Batch 3, Batch 4; Figure H.1). For each IS, peak areas were greater for the practice batch than either sample batch, and were quite consistent across standards. However, differences between sample batches 3 and 4 varied for each IS. For d_4-cortisol, Batch 4 peaks were slightly lower than Batch 3, but their consistencies were comparable. For d_8-cortisone, Batch 4 peak areas were approximately 50% lower than Batch 3, and consistencies were comparable. For $^{13}C_3$-testosterone, Batch 4 peak areas were generally higher but less consistent. For d_9-progesterone, Batch 4 peak areas were higher than Batch 3, with similar consistency.

Second, I visualised the relative peak area of the same four internal standard for all blow samples, for each batch, to determine signal consistency (Figure H.2). For all four internal standards, Batch 3 peak areas were far more consistent than Batch 4. For Batch 4, changes in the relative peak area between samples were generally consistent across IS compounds.

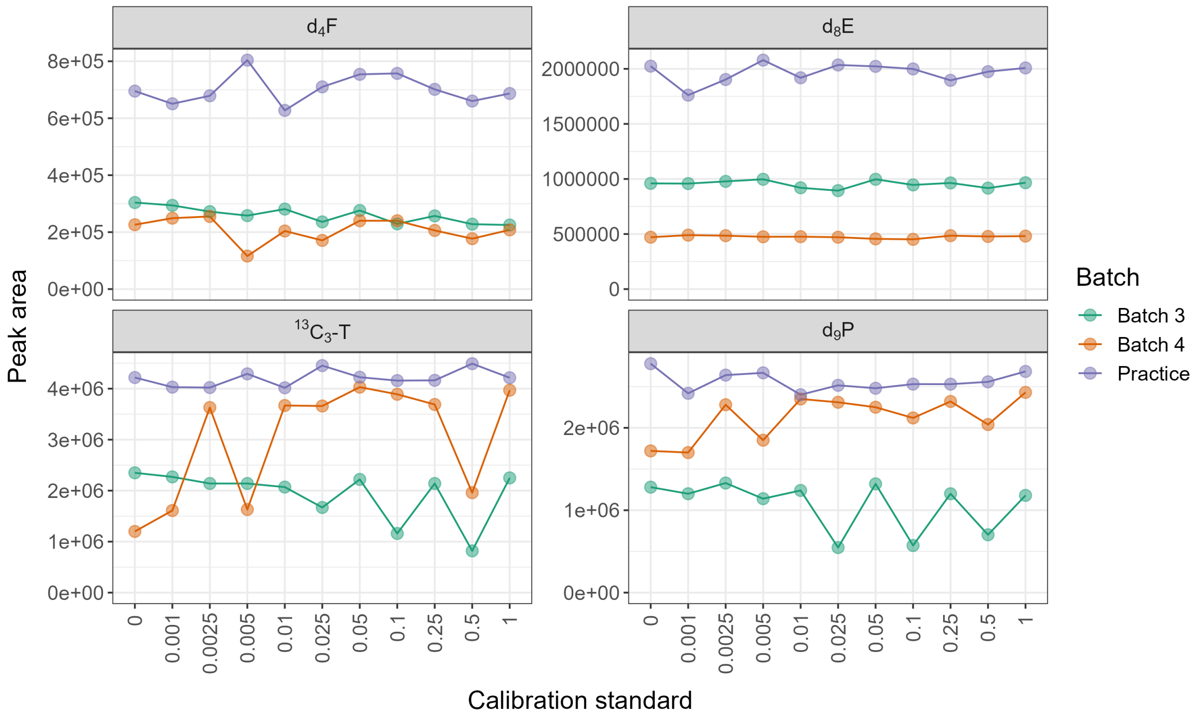


Figure H.1: Peak areas of four internal standards (d_4-cortisol, d_4F; d_8-cortisone, d_8E; $^{13}C_3$-testosterone, $^{13}C_3$-T; d_9-progesterone, d_9P) for each calibration standard in three batches: practice and sample batches 3 and 4. Calibration standard names refer to the amount of cortisol in each standard.

Analyte standard peak areas

To assess differences in assay sensitivity, I compared the lower limit of quantification (LLOQ) for the corresponding four analytes (cortisol, cortisone, testosterone and progesterone) and each batch (Figure H.3). LLOQ is defined as the lowest non-zero standard included in the calibration curve, determined by accuracy and the ratio of the qualitative to quantitative ions. Batch 4 had a lower LLOQ for both cortisol (0.001 ng compared with 0.01 ng) and cortisone (0.001 ng compared with 0.0025 ng). There were no differences in LLOQ for testosterone and progesterone.

Beyond the final LLOQ values, there were apparent differences in peak areas from calibration standards across batches (Figure H.4). For each analyte, peak areas from the practice batch were consistently higher than sample batches, particularly for cortisol and cortisone. For cortisol, sample batches 3 and 4 had comparable peak areas from the 0.01 standard and above, but Batch 4 peaks were far smaller at lower standards. For cortisone, Batch 3 peaks were consistently larger than Batch 4, and Batch 4 had no signal for the 0.001 standard. For testosterone and progesterone, Batch 4 peak areas were generally higher than Batch 3.

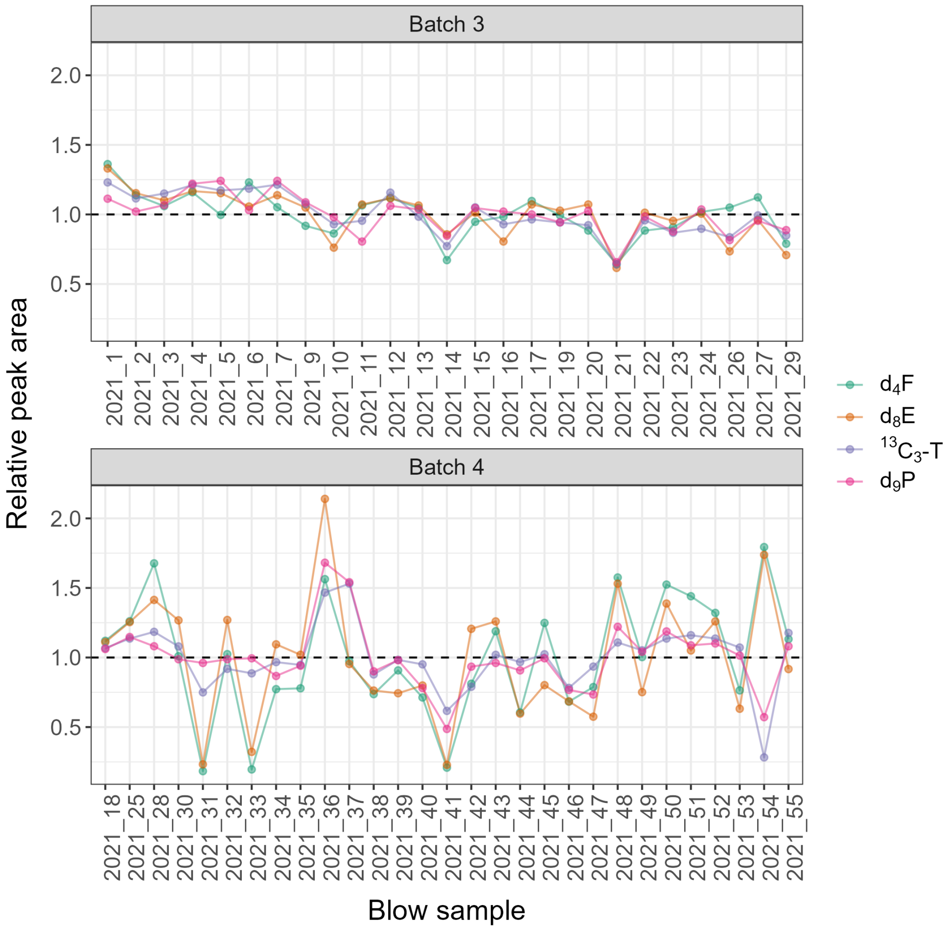


Figure H.2: Relative peak area for four internal standard compounds (d_4-cortisol, d_4F; d_8-cortisone, d_8E; $^{13}C_3$-testosterone, $^{13}C_3$-T; d_9-progesterone, d_9P), for batches 3 and 4. For each batch and compound, relative peak area for a sample was defined as the peak area divided by the mean peak area of all samples in the batch.

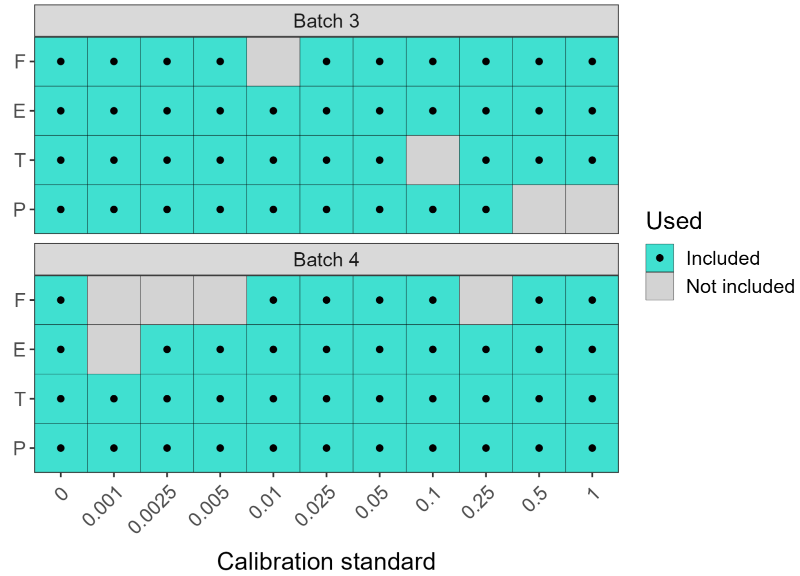


Figure H.3: Standards used in the calibration curve for four analytes in sample batches 3 and 4: cortisol (F), cortisone (E), testosterone (T) and progesterone (P). Calibration standard names refer to the amount of cortisol in each standard.

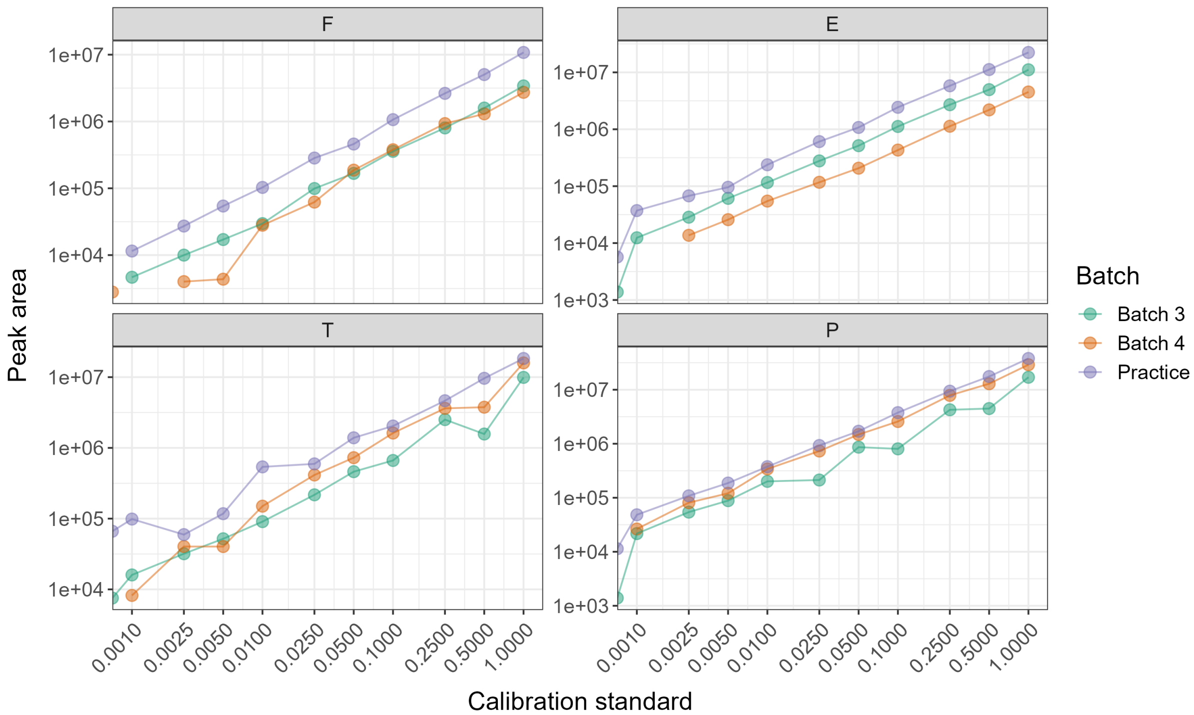


Figure H.4: Peak areas for each calibration standard in batches 3 and 4 for four analytes: corsitol (F), cortisone (E), testosterone (T) and progesterone (P). Calibration standard names refer to the amount of cortisol in each standard.

Appendix I

Spatial autocorrelation from the final 25 km GAM SDM

Here, I show results of a semi-variogram analysis to inspect spatial autocorrelation in the final 25-km generalised additive model (GAM; Figure I.1A) and the spatial pattern of deviance residuals from the final 25-km GAM for each survey year (Figure I.1B).

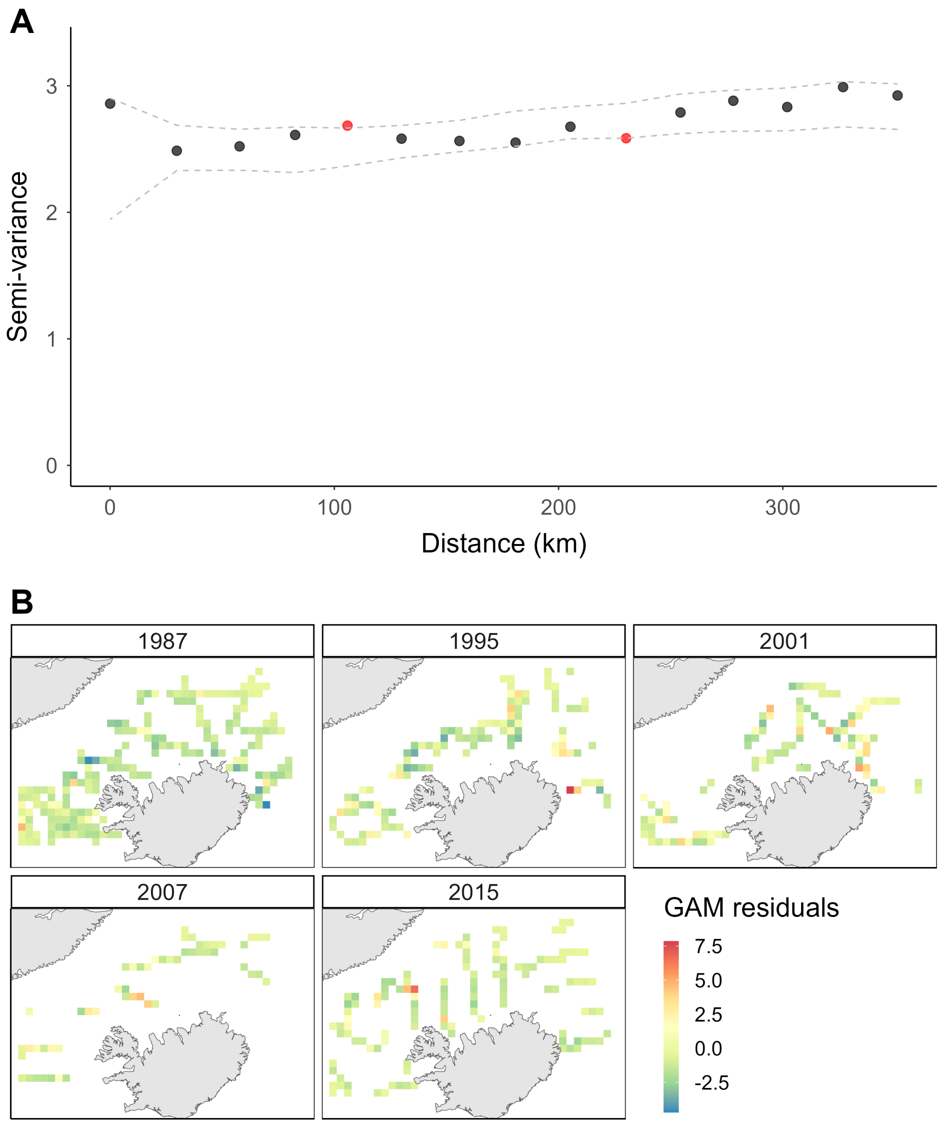


Figure I.1: Spatial autocorrelation of the deviance residuals from the final 25 km generalised additive model (GAM). A) Semi-variogram of the empirical residuals (dots) and Monte Carlo envelope (dashed lines). Red points indicate empirical values outside of the envelope. B) Deviance residuals from the final 25-km GAM for each survey year.

Appendix J

Results for capture–recapture models

Here, I present the model selection results for each capture–recapture (CR) model in Chapter 4: Cormack–Jolly–Seber (CJS), Jolly–Seber–Schwarz–Arnason (JSSA) and multi-state open robust design (MSORD). Selection of the final parameter specifications for each model type was achieved via AICc. For frameworks with >10 competing models, only the 10 best-fitting options are presented here.

Cormack–Jolly–Seber

Apparent survival (ϕ)	Detection (p)	Parameters	AICc	ΔAIC	Weight	Deviance
season	effort	16	1592.7	0.0	0.98	304.4
season	constant	15	1601.0	8.3	0.02	314.8
constant	effort	3	1606.9	14.2	0.00	345.2
constant	constant	2	1618.4	25.7	0.00	358.7

Jolly–Seber–Schwarz–Arnason

Apparent survival (ϕ)	Detection (p)	Probability of entry ($pent$)	Parameters	AICc	ΔAIC	Weight	Deviance
season	effort	season	31	1709.4	0.0	0.94	season
season	constant	season	30	1714.9	5.5	0.06	season
constant	effort	season	18	1724.3	14.9	0.00	season
constant	constant	season	17	1731.5	22.1	0.00	season
season	constant	constant	17	1760.0	50.6	0.00	constant
season	effort	constant	18	1760.2	50.8	0.00	constant
constant	effort	constant	5	1837.9	128.5	0.00	constant
constant	constant	constant	4	1840.1	130.7	0.00	constant

Multi-state open robust design

Apparent survival (S)	Temporary emigration (Ψ)	Probability of entry ($pent$)	Persistence (φ)	Detection (p)	Parameters	AICc	ΔAIC	Weight	Deviance
constant	random+season	season	month	constant	33	4683.5	0.0	0.73	4615.6
constant	random+season	season	month	effort	34	4685.6	2.1	0.26	4615.6
year	random+season	season	month	constant	46	4693.7	10.2	0.00	4598.0
year	random+season	season	month	effort	47	4695.8	12.3	0.00	4598.0
year	random	season	month	constant	33	4705.7	22.2	0.00	4637.8
year	random	season	month	effort	34	4707.0	23.5	0.00	4637.0
constant	random+season	season	month	constant	46	4709.3	25.8	0.00	4613.6
constant	random+season	season	month	effort	47	4710.0	26.4	0.00	4612.1
year	random+season	season	month	constant	59	4719.9	36.4	0.00	4595.8

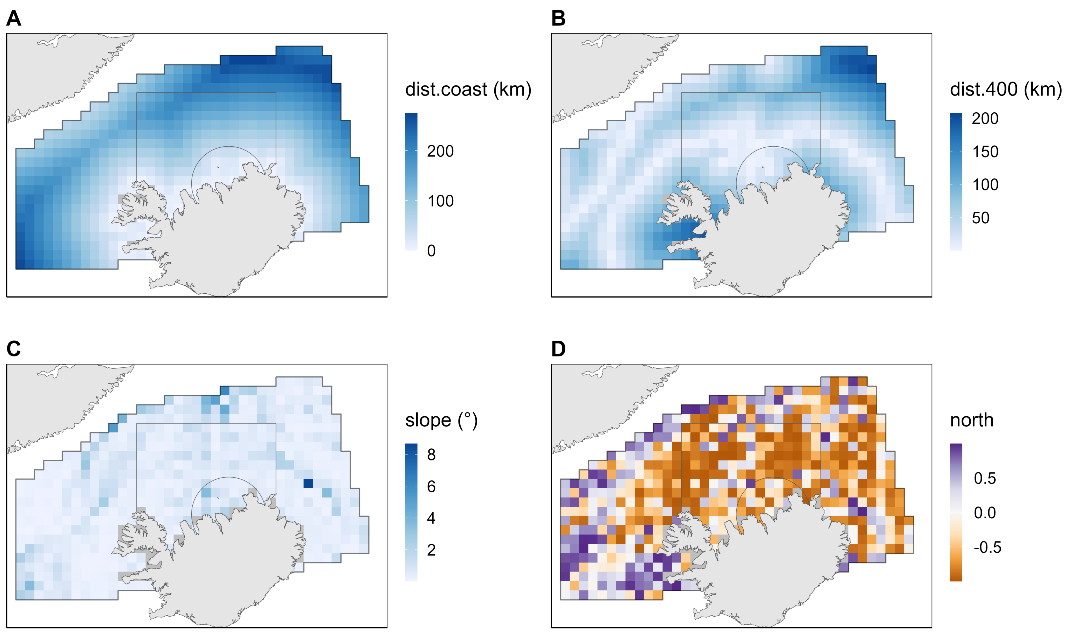
Appendix K

Environmental variable maps for 2006–2019

Maps are presented for static variables and each year of dynamic variables used to predict humpback whale summer density during 2006–2019 at 25 km resolution.

Static variables

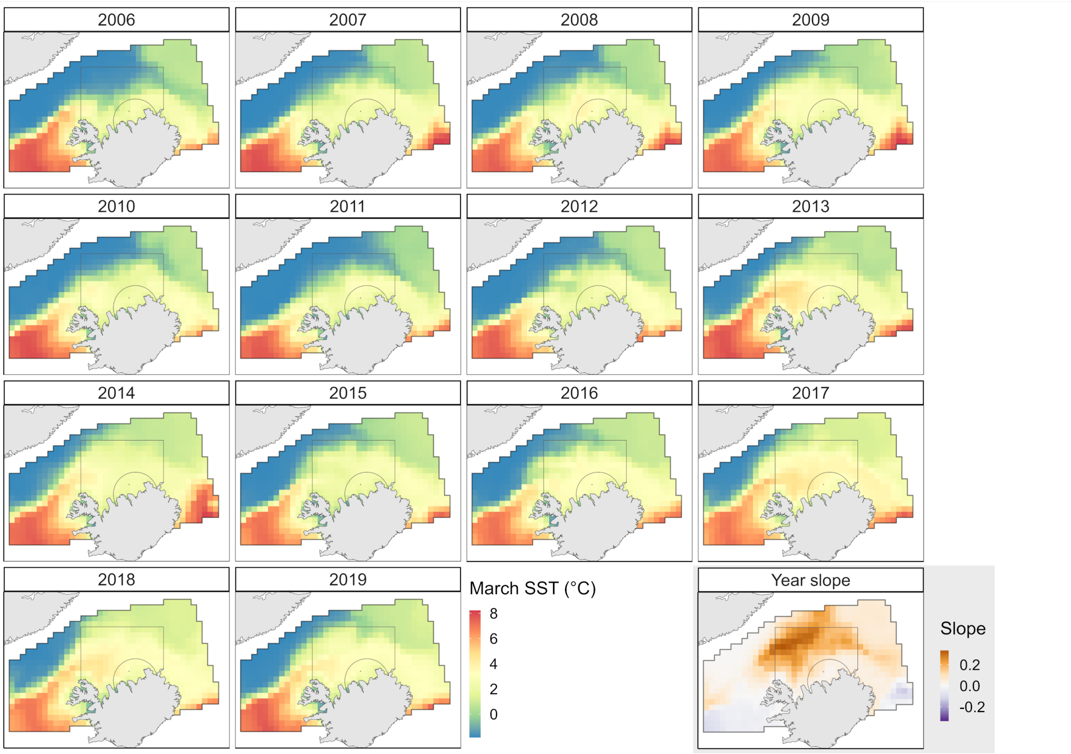
Absolute values are presented for the four retained static variables: distance to coast (dist.coast, A), distance to 400 m contour (dist.400, B), seabed slope (slope, C) and aspect northing (north, D).



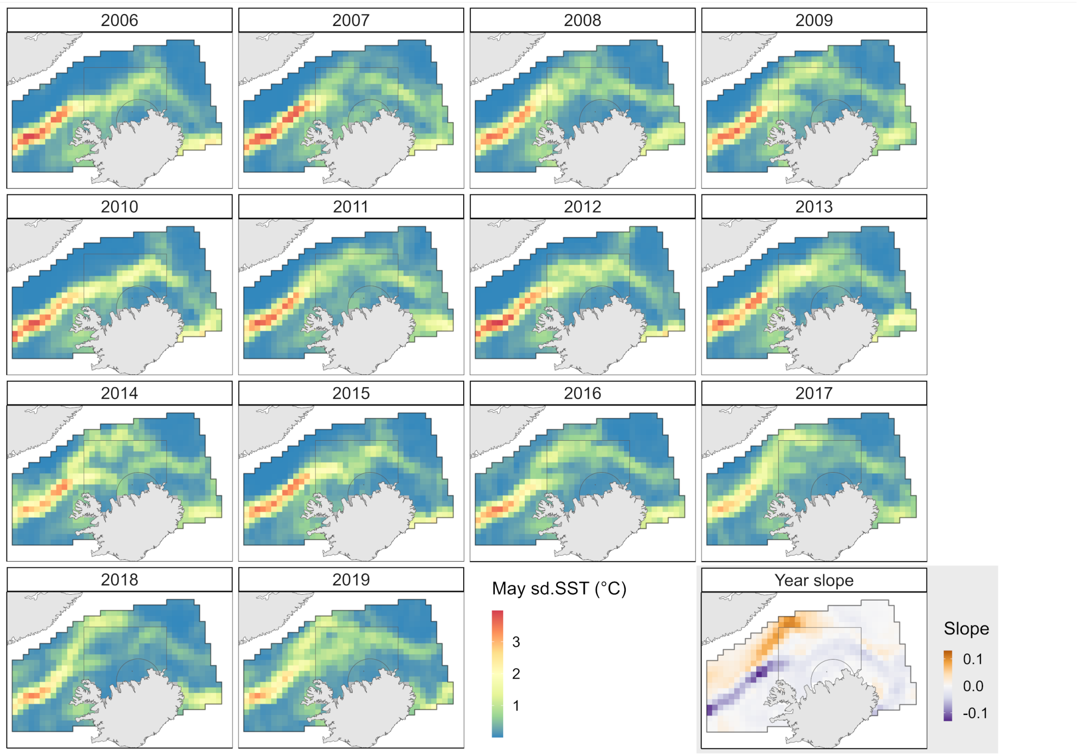
Dynamic variables

For each dynamic variable (for the selected month, if appropriate), I show the values for each year during 2006–2019 and the slope term from a linear regression of the variable against year for each cell.

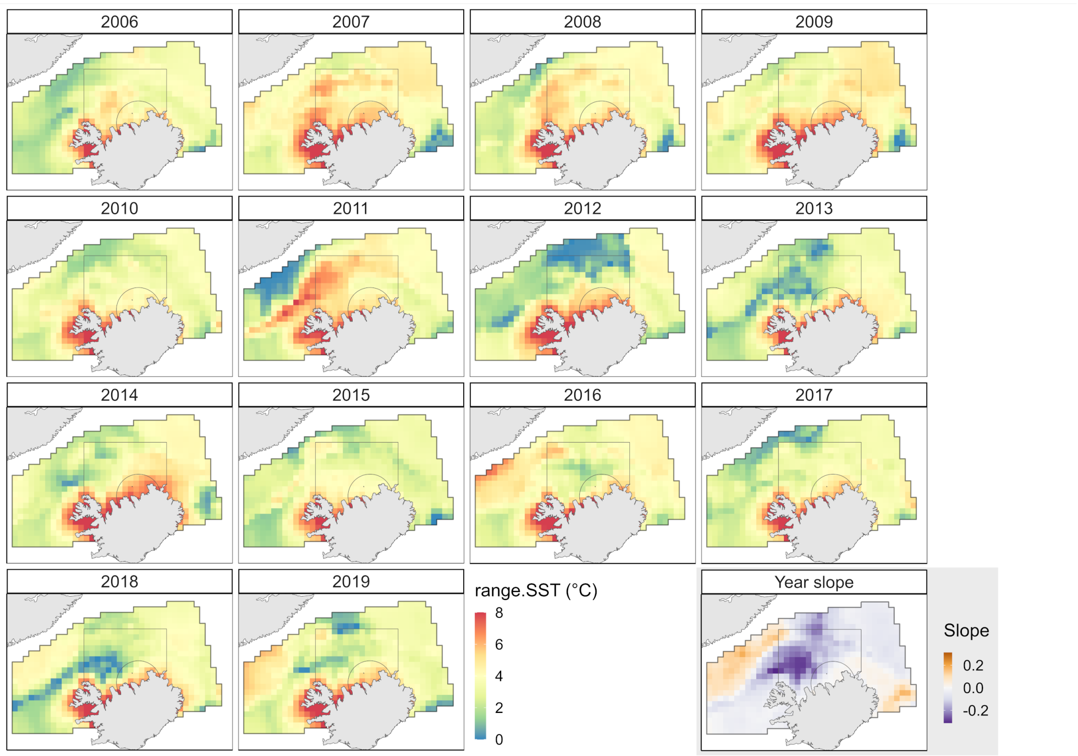
March sea surface temperature (SST)



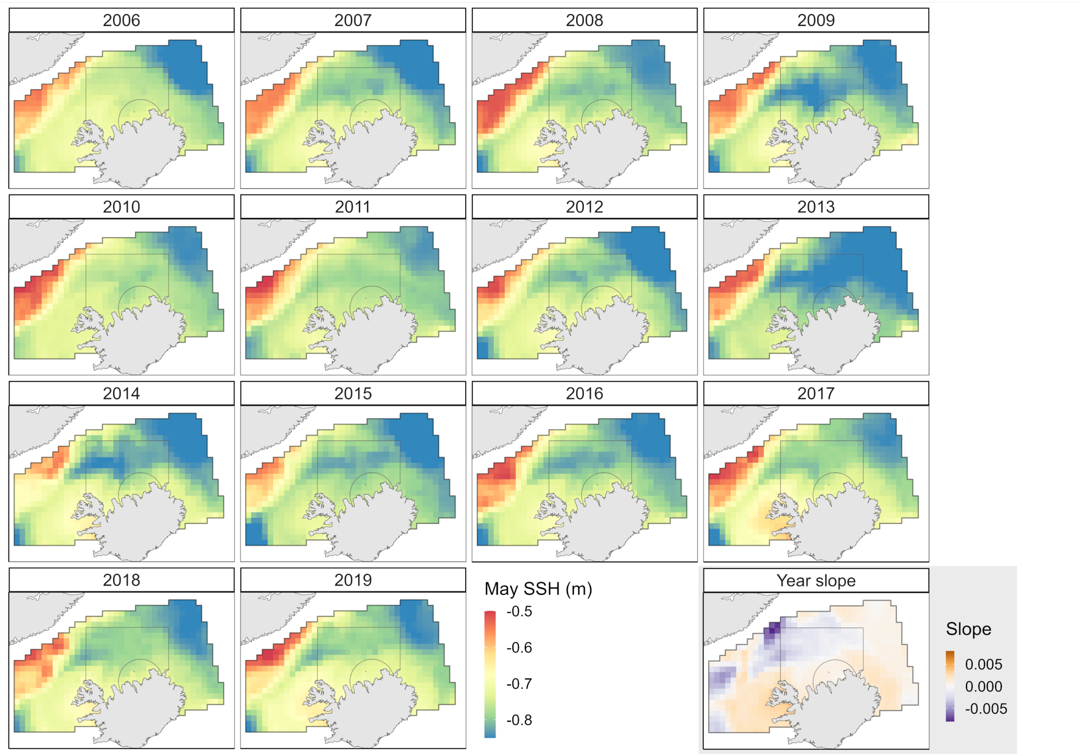
May standard deviation of sea surface temperature (sd.SST)



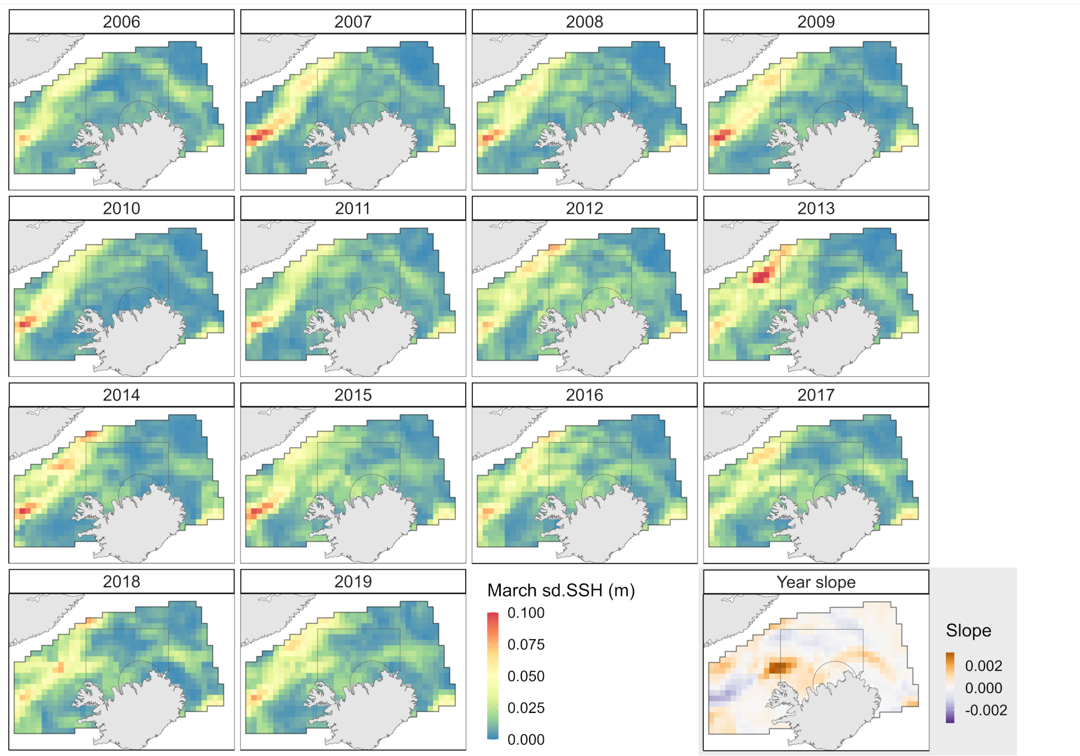
Sea surface temperature March-July range (range.SST)



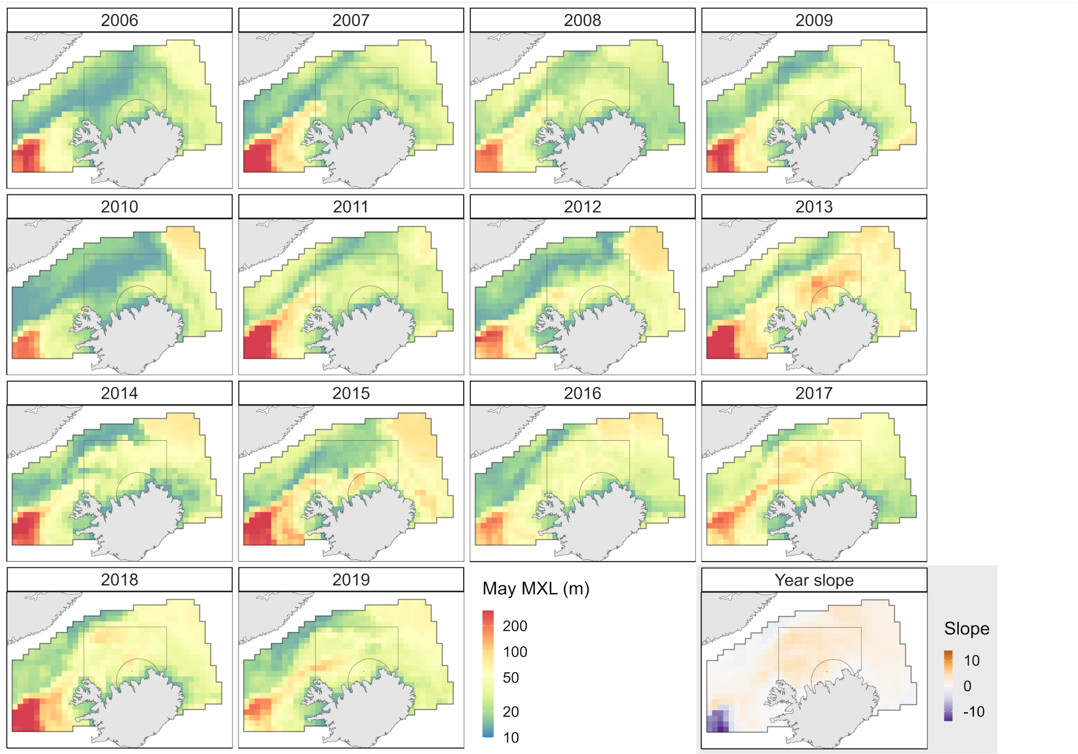
May sea surface height (SSH)



March standard deviation of sea surface height (sd.SSH)



May mixed layer depth (MXL)



Mixed layer depth March-July range (range.MXL)