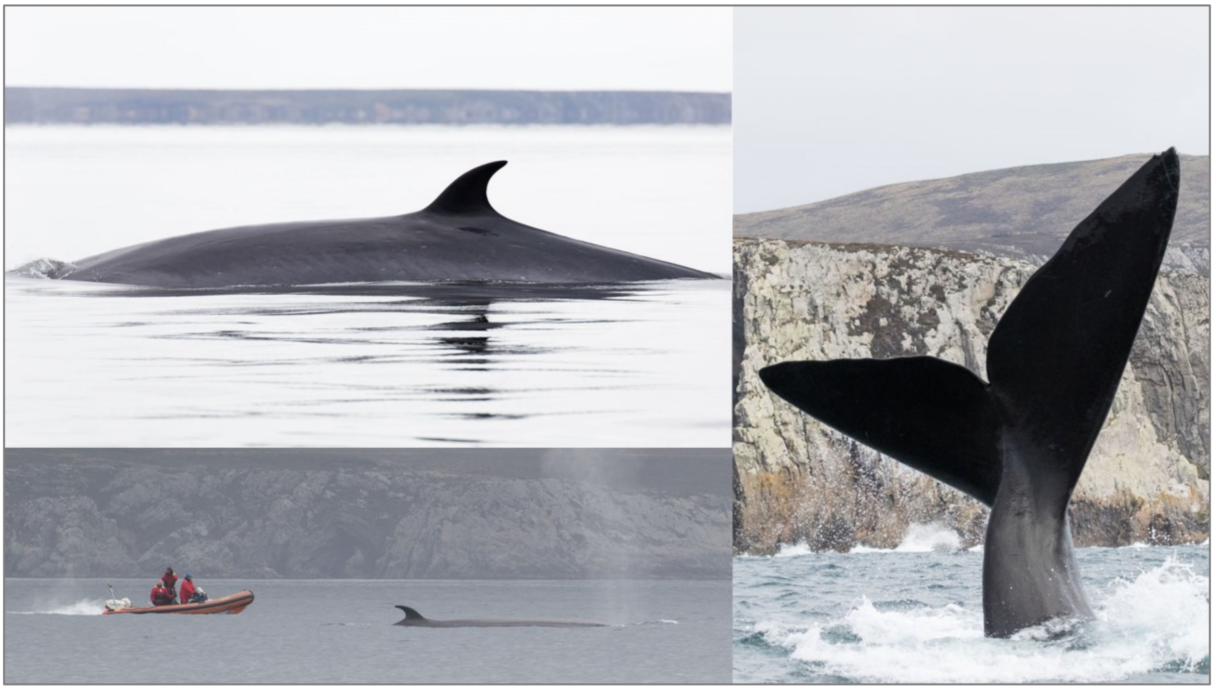
Conserving Falklands' whale populations: addressing data deficiencies for informed management



Technical Report for DPLUS082

Edited by Caroline R. Weir

Falklands Conservation
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Acronyms

BAS	British Antarctic Survey
BP	Basepairs
BS	Berkeley Sound
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CMP	Conservation Management Plan
COVID-19	Coronavirus disease 2019
DFT	Discrete Fourier Transform
DPLUS082	Darwin Plus project 082: Conserving Falklands' whale populations: addressing data deficiencies for informed management
DV	Distinctiveness Value
DVR	Digital Voice Recorder
EtOH	Ethanol
FC	Falklands Conservation
FIG	Falkland Islands Government
HCl	Hydrochloric acid
IUCN	International Union on the Conservation of Nature
IWC	International Whaling Commission
KBA	Key Biodiversity Area
LF	Low Frequency
LFDCS	Low-Frequency Detection and Classification System
M-dist	Mahalanobis-distance
MF	Mid Frequency
MPS	Minimum Population Size
MtDNA	Mitochondrial DNA
NEFSC	NOAA Northeast Fisheries Science Center
PAM	Passive Acoustic Monitoring
PCR	Polymerase chain reaction
PQ	Photographic Quality
QGIS	Quantum Geographic Information System
RIB	Rigid-hulled Inflatable Boat
SNR	Signal-to-Noise Ratio
SR	Sampling rate
SRW	Southern right whale
ST500	SoundTrap ST500-STD acoustic recorders
TA	Target Area for photo-identification
TCD	Total Complete Days
WHOI	Woods Hole Oceanographic Institute

Non-Technical Summary

Chapter 1: Introduction

- This report outlines the findings of a project led by Falklands Conservation in collaboration with multiple local and international project partners, and with funding from the UK Government through Darwin Plus (DPLUS082). The project was titled "*Conserving Falklands' whale populations: addressing data deficiencies for informed management*," and aimed to collect a range of data on sei whales (*Balaenoptera borealis*) and southern right whales (*Eubalaena australis*) to address several key knowledge gaps that were relevant to achieving evidence-based conservation management of whale populations. The report consists of seven standalone chapters that provide the results on different research components of the DPLUS082 project.
- The project fieldwork focussed on two coastal study sites within the Falkland Islands: (1) Falkland Sound; and (2) the north-east Falklands coastline including Berkeley Sound. These two sites were selected based on known whale occurrence and on logistical considerations for small boat work.
- All of the fieldwork described in this report was conducted with the appropriate research permits issued by Falkland Islands Government to cover non-invasive baleen whale work (R11/2017), biopsy sampling and suction-cup tagging (R23/2018), dead animal sampling (R40/2018), and the deposit of acoustic device anchors on the seabed (FEPA licence).

Chapter 2: Distribution and behaviour

- Small boat surveys were carried out at Falkland Sound and North-east Falklands on 94 dates between January 2019 and June 2021, resulting in 632.7 hr and 9,215.9 km of survey effort. Effort was highest in 2019, and over the February to May period (the latter partly due to the 2021 season only covering that period). Due to logistical constraints, the North-east Falklands received higher, and more consistent, survey coverage than Falkland Sound.
- A total of 1,619 cetacean sightings was recorded during the small boat survey work carried out from 2019 to 2021, comprising a best estimate of 4,821 animals. Four species of baleen whale were recorded, predominantly comprising sei whales and southern right whales.
- Sei whales were recorded consistently between February and May, and southern right whales were recorded consistently between June and August. The arrival time, and duration of the core period, varied for both species between years, and sometimes extended into other months. Sei whale distribution was most widespread during March, when the species was sighted throughout most of the surveyed areas. The distribution of right whales favoured coastlines most exposed to the open ocean, particularly in the Volunteer Point to Cape Carysfort region.
- Adult-calf pairs of sei whales were recorded in the Falklands for the first time, indicating use of the region by lactating mothers and/or newly-weaned calves. Foraging appeared to be the primary driver of sei whale occurrence, evidenced by observations of surface feeding and defecations at the surface. The data contributed to the international recognition of nearshore Falklands' waters as a Key Biodiversity Area for sei whales.
- Southern right whales used nearshore Falklands' waters during winter for socialising and mating, with many surface-active groups encountered. Adults and juveniles were present; however, no neonate calves were confirmed. Consequently, Falklands' waters appear to comprise a newly-documented winter mating area for the south-west Atlantic population, and are of regional relevance for managing the species.

Chapter 3: Photo-identification

- Photo-identification refers to the collection of high-quality images of naturally-occurring markings on the bodies of animals, which can be used to recognise and catalogue individuals to document parameters such as their abundance and movements.
- Images of sei whales (*n* = 83,580) and southern right whales (*n* = 44,507) were taken throughout the small boat survey work. Cataloguing relied on dorsal fin marks and flank scarring for sei whales, and the pattern of rough skin ('callosities') on the heads of right whales.
- In combination, across 2019 and 2020 and at both sites, the minimum population size (MPS) generated for (non-calf) sei whales was 254 animals. A higher MPS was recorded in the north-east Falklands compared with Falkland Sound, reflecting uneven survey effort. The Falklands catalogue is now the largest available globally for sei whales, including well over 400 animals.
- Eight sei whale recaptures were recorded between sites (West Falkland, Falkland Sound, and north-east Falklands), including three recaptures between Falkland Sound and the north-east Falklands *within* the same year. These demonstrate the species' high mobility and wide use of Falklands' waters. Additionally, there were 11 recaptures that occurred *within* the same site but in different years, evidencing long-term fidelity of sei whales to the Falklands.
- A MPS of 246 southern right whales was generated using the left-side catalogue for 2017–2021. Ten inter-annual photographic recaptures were recorded, supporting some longer-term fidelity of a portion of the south-west Atlantic right whale population to the Falklands during winter. Over 93% of individuals were only photographically captured once, consistent with a continuous succession of different animals moving along the coast, and suggesting a more transitory presence of right whales compared to sei whales.

Chapter 4: Diet

- Throughout the small boat surveys, whale defecations were sampled whenever they were observed, in order to collect faecal material for prey species identification. Additionally, small skin samples were collected from both dead stranded and live whales in order to carry out stable isotope analysis to investigate their trophic foraging ecology (e.g. the level in the food chain at which they feed, and the geographic locations where they forage).
- Over the course of DPLUS082, a total of 51 faecal samples were collected from sei whales. Defecations by southern right whales were much rarer and were only observed on a small number of occasions amongst socialising aggregations, where it was not possible to safely approach for sampling. A total of 9 sei whale and 69 right whale skin samples were available for stable isotope analysis.
- The major groups identified in sei whale faecal samples included calanoid copepods (>90% of samples), decapod crustaceans of the family Munidiae (likely to be squat lobster, *Munida gregaria*; >80% of samples). and cyclopoid copepods (76% of samples). The latter are small in size and might have been incidental captures rather than targeted prey. No fish were recorded as prey. The dominant prey within the faecal samples varied by season and year, likely reflecting temporal variation in the availability of different prey species in Falklands' waters.
- Stable isotope analysis of the sei whale skin samples revealed very similar carbon values, suggesting that sei whales were utilising feeding grounds of similar latitude or water mass in the months before their arrival in the Falklands. The values were similar to prey isotope data recorded on the Patagonian Shelf. The nitrogen isotope values had a wider range, indicating variation between individuals in the targeted prey species.
- The southern right samples were inconclusive with regard to carbon isotopes, due to some analytical challenges; work is continuing to resolve those challenges. The samples had a wide range of nitrogen isotope vales which spanned at least two trophic levels. That suggests that right whales had targeted different feeding grounds or prey types in the months prior to their arrival in the Falklands, rather than the Islands comprising a preferred wintering destination for one feeding group.

Chapter 5: Genetics

- The population biology of sei whales and southern right whales was investigated, using mitochondrial DNA to assess maternally inherited patterns of diversity and population connectivity, and biparentally inherited microsatellites to investigate genetic diversity.
- Skin samples for genetic work were collected throughout DPLUS082, via biopsy sampling of live whales at sea and tissue sampling of dead stranded animals. Whale DNA was also extracted from some faecal samples. However, given the logistical limitations with shipping frozen samples to the UK for analysis, not all samples collected during DPLUS082 were available for inclusion in this report. A total of 39 sei whale samples (skin and faecal) and 87 right whale samples (skin only) were analysed.
- The samples collected from both sei whales and southern right whales were biased towards males. The 10 analysed skin samples from sei whales, resulted in three females and seven males (however, a previous sex ratio originating from a larger sample size in the Falklands was more even, and this may therefore be a result of small sample size). The bias was more marked in right whales, with 82 unique skin samples assigned to 65 males and 17 females. It is unclear whether females are genuinely scarcer, or whether this simply reflects higher availability of males for sampling due to their behaviour.
- The results indicated that sei whales in the Falkland Islands had high genetic diversity. When compared with other geographic regions, Falklands' whales exhibited genetic overlap with sei whales sampled in Chile, suggesting that there may be some interchange of individuals across the Atlantic–Pacific boundary. There was high differentiation from North Atlantic sei whales, supporting a separate South Atlantic stock without significant exchange across the equator.
- Southern right whales sampled in the Falklands exhibited similarly high genetic diversity to other South Atlantic wintering grounds. Falklands' whales had most genetic affinity with right whales from the Brazilian and Argentine calving grounds (and all were differentiated from South African animals), supporting linkage of these regions within the south-west Atlantic and suggesting that the Islands may be visited seasonally by a mixture of whales from both of those established calving areas.

Chapter 6: Suction-cup tagging

- Bio-logging tags can contain a wide range of sensors that provide information on how whales are behaving. They are particularly useful for documenting the subsurface behaviour of whales, recording dive patterns, movements, and prey species (via incorporated video cameras). Such tags can be attached to the skin of whales using suction cups, meaning that they are a minimally invasive technique that does not penetrate the skin. Sei whales in the Falklands have been noted to undertake relatively long subsurface foraging dives, and suction cup tag deployments were undertaken to clarify how the whales were using the environment during such foraging dives.
- A tag specialist visited the Falklands during March 2019, and tagging attempts were made during boat surveys. Suction cup tags were deployed on two sei whale individuals, both of which were surface-feeding at the entrance to Berkeley Sound. The tags stayed on the animals for ~12 hr.
- The two whales exhibited different behaviour; one remained in a relatively small area at the mouth of Berkeley Sound for the entire deployment, while the other made a sustained directional movement for over 40 km northwards after it ceased feeding off Berkeley Sound. Both animals performed combinations of skimming and surface lunges while feeding, with one animal also exhibiting numerous subsurface lunges. Totals of 56 and 91 feeding events per hour respectively were recorded for the two animals.
- Sei whales were found to be ecologically-flexible, and unique amongst baleen whales, having the ability to both skim feed and lunge feed. This likely allows them to exploit a variety of prey species and at different prey densities in the water column.

- The tags generated data on swim speeds, dive times and behaviour that can be used to inform the conservation and management of sei whales in the Falklands, for example providing insights into prey capture, overlap with human activities, and vessel collision risk.

Chapter 7: Acoustics

- A Passive Acoustic Monitoring (PAM) study was implemented using three SoundTrap acoustic devices deployed on the seabed at 7 km separation down the centre of Berkeley Sound. Each device was programmed to record continuously at frequency ranges up to 12 kHz, focussing on the low frequencies (<500 Hz) used most commonly by baleen whales. Each deployment lasted 4–6 months, after which batteries were changed and data downloaded.
- Two years of almost continuous PAM effort was achieved between December 2018 and December 2020, resulting in a combined total of >49,000 hours of data.
- Sei whale calls were detected primarily between December and May, while southern right whale calls were primarily between June and August. However, the onset and duration of the core season for both species varied between years, and small numbers of detections were recorded year-round. Both species were most consistently detected on the device located at the mouth of Berkeley Sound, and least at the innermost site.
- Several types of sei whale call were recorded, including well-documented downsweeps and a variety of novel call types that had not been previously reported for the species. Additionally, sei whale song was detected from February through May. This was the first documentation of singing by sei whales anywhere globally, and supports reproductive (i.e. male advertisement) behaviour in the Falklands.
- Southern right whale upsweep calls and a diversity of tonal calls were recorded, along with gunshot song. Gunshot songs are male-specific and associated with reproductive activity, adding to the body of evidence that the Falklands comprises a mating ground for right whales.
- PAM proved to be an invaluable technique for providing fine-scale temporal data on whale occurrence. The study provided new insights on reproductive behaviour by both species in the Falklands. It also provided an indication of potential noise disturbance affecting whales in Berkeley Sound, which merits further investigation in a management context.

Chapter 1: Introduction

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1.1 Project overview

In 2018, Falklands Conservation (FC) was successful with a project grant submitted to Darwin Plus (DPLUS082) titled "*Conserving Falklands' whale populations: addressing data deficiencies for informed management*." The project aimed to collect a range of data on sei whales (*Balaenoptera borealis*) and southern right whales (*Eubalaena australis*) in the Falkland Islands between 2018 and 2020, to address several key knowledge gaps that were relevant to achieving evidence-based conservation management of whale populations. This included:

- Increasing knowledge of the **spatial distribution** of, and preferred habitats used by, whales in the nearshore waters around the Islands (**Chapter 2**). RELEVANCE: identifying key sites for whales that could be incorporated into marine management, and assessing their overlap with, and mitigation of, human activities;
- Addressing gaps regarding the **temporal occurrence** of baleen whale species in the Falklands (**Chapter 2 and Chapter 7**). RELEVANCE: assessing their overlap with, and mitigation of, human activities;
- Improving understanding of the **number of whales** using Falklands' waters (**Chapter 3**). RELEVANCE: population size and trend data underpin management status assessments;
- Learning more about the **movements** of individual whales within the Falklands (**Chapter 3 and Chapter 6**). RELEVANCE: understanding how whales utilise Falklands' habitats; identifying areas of high use; assessing exposure to human activities;
- Investigating **whale diet** in the Falklands and across the wider region (**Chapter 4**). RELEVANCE: the distribution and abundance of prey species is likely to be a key driver of spatial and temporal (including inter-annual) sei whale occurrence;
- Investigating the **genetic diversity** of whales in the Falklands to assess population identity and diversity (**Chapter 5**). RELEVANCE: understanding local and regional population structure; assessing population resilience; identifying links with breeding grounds;
- Collecting novel information on sei whale **foraging behaviour** in the Falklands (**Chapter 6**). RELEVANCE: knowledge of how sei whales behave on the Falklands feeding ground is important to understanding more about their occurrence and potential exposure to human impacts (e.g. vessel strike);
- Acquiring information on the **sex ratios of baleen whale** individuals (**Chapter 5**). RELEVANCE: informs population assessments and knowledge of reproductive behaviour; understanding whether the Falklands are used by particular components of whale populations (e.g. mothers with calves).

The resulting data will address current knowledge gaps to inform ongoing work on Marine Spatial Planning and Marine Management Areas in the Falklands, with particular relevance to managing existing and future human activities that may potentially impact on whales including: continued growth in marine ecotourism (whale watching); use of nearshore waters by the fishing industry for transhipments, bunkering and anchoring; geophysical exploration and development; coastal construction (e.g. harbour development and dredging); and the potential development of marine aquaculture (e.g. salmon farming).

1.2 Study area

1.2.1 The Falkland Islands

The Falkland Islands are located approximately 500 km east of the southern Patagonian coast of South America, at latitudes of 51°S to 53°S and longitudes of between 57°W and 62°W (Figure 1.1). The Islands are situated in shallow (<200 m depth) waters that form an eastwards extension of the Patagonian continental shelf. The two main islands of East and West Falkland are divided by a channel of water named Falkland Sound (Figure 1.1), and their coastlines are indented by a number of large bays and inlets. Falkland Islands Government (FIG) declared the Falkland Islands Interim Conservation and Management Zone (FICZ) in October 1986, comprising an area of 300 km radius centred on Falkland Sound (Figure 1.1). In 1990 the Falkland Islands Outer Conservation Zone (FOCZ) was declared in the area between the FICZ and the 200 nautical mile economic zone boundary (Figure 1.1).

The Falklands are situated approximately 500 km north of the Antarctic Convergence, and the surrounding waters are cold temperate in temperature. Satellite imagery (see **Annex 1**), indicates average monthly sea surface temperature (SST) in the waters around the Islands ranging from 10–12°C in January (peak austral summer) to 4–6°C in July (peak austral winter).

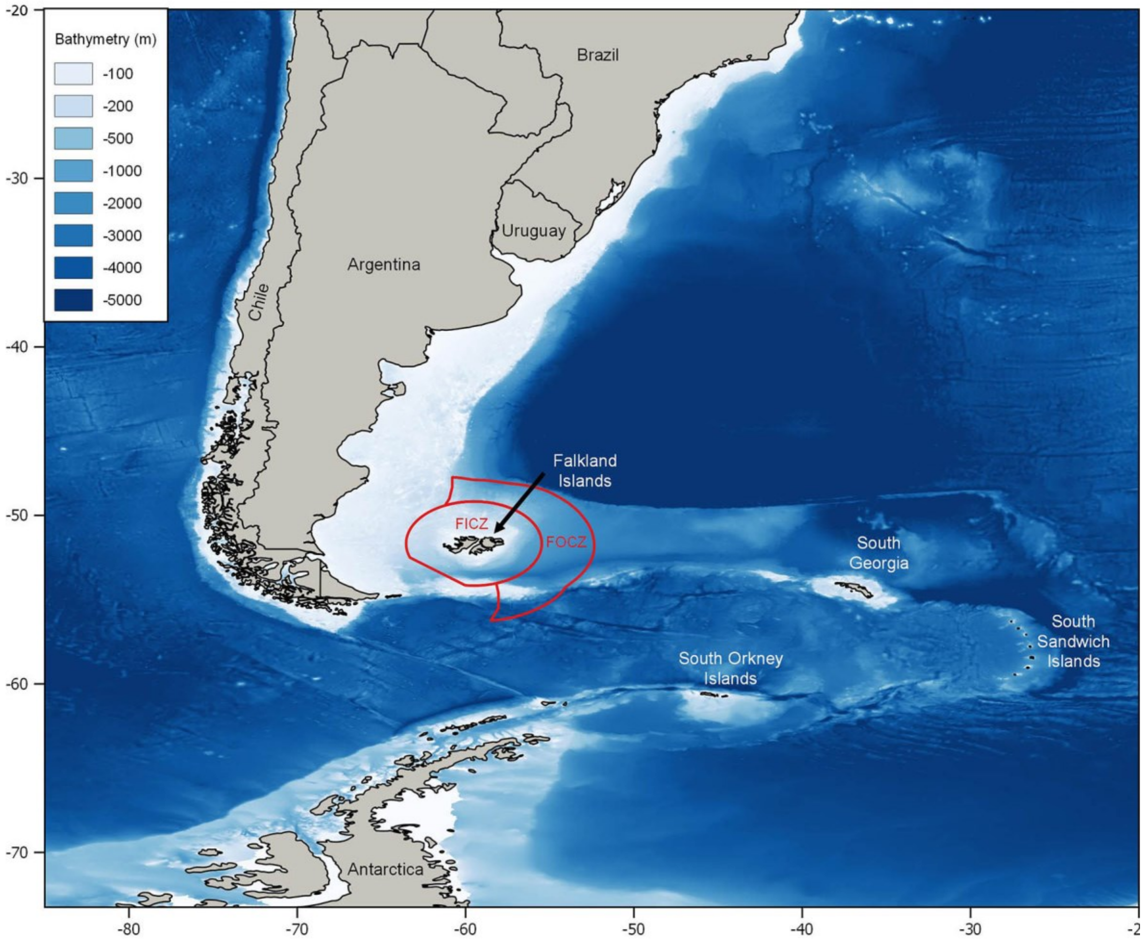

Figure 1.1. *Geographic position of the Falkland Islands off South America, showing bathymetry and the locations of the Falkland Islands Interim Conservation and Management Zone (FICZ) and Falkland Islands Outer Conservation Zone (FOCZ).*

1.2.2 Focal study sites

The project focussed on two study sites within the Falklands (Figure 1.2): (1) Falkland Sound (FS), limited to the waters between the Tyssen Islands and White Rock Point that could be surveyed from a boat launch at New Haven; and (2) north-east Falklands (NEF), comprising the area between Cape Pembroke and Cape Carysfort (including Berkeley Sound) that was surveyed during boat launches from Stanley. Both study areas were situated in coastal waters of <60 m water depth (Figures 1.3 and 1.4)

Several factors led to the selection of these sites, including:

- The identification by Taylor et al. (2016) of six small marine sites in the Falkland Islands that were priorities for research due to their potential to qualify as Key Biodiversity Areas (KBAs) for sei whales[1] (Figure 1.5); these sites included a small area in FS and most of Berkeley Sound;
- Logistical constraints: NEF is located in close proximity to Stanley which optimises survey opportunities around short weather windows, while FS is the only other site proposed by Taylor et al. (2016) that is readily accessible by road in East Falkland;
- Known whale occurrence: interview surveys (Frans and Augé; 2016) and earlier targeted whale work in Berkeley Sound (Weir, 2017), provided support for whale presence at both sites; and
- The presence of anthropogenic activities at both sites (i.e. ferry route in FS, transhipment and mooring area in Berkeley Sound) which made them important areas for assessing overlap and interactions with whales.

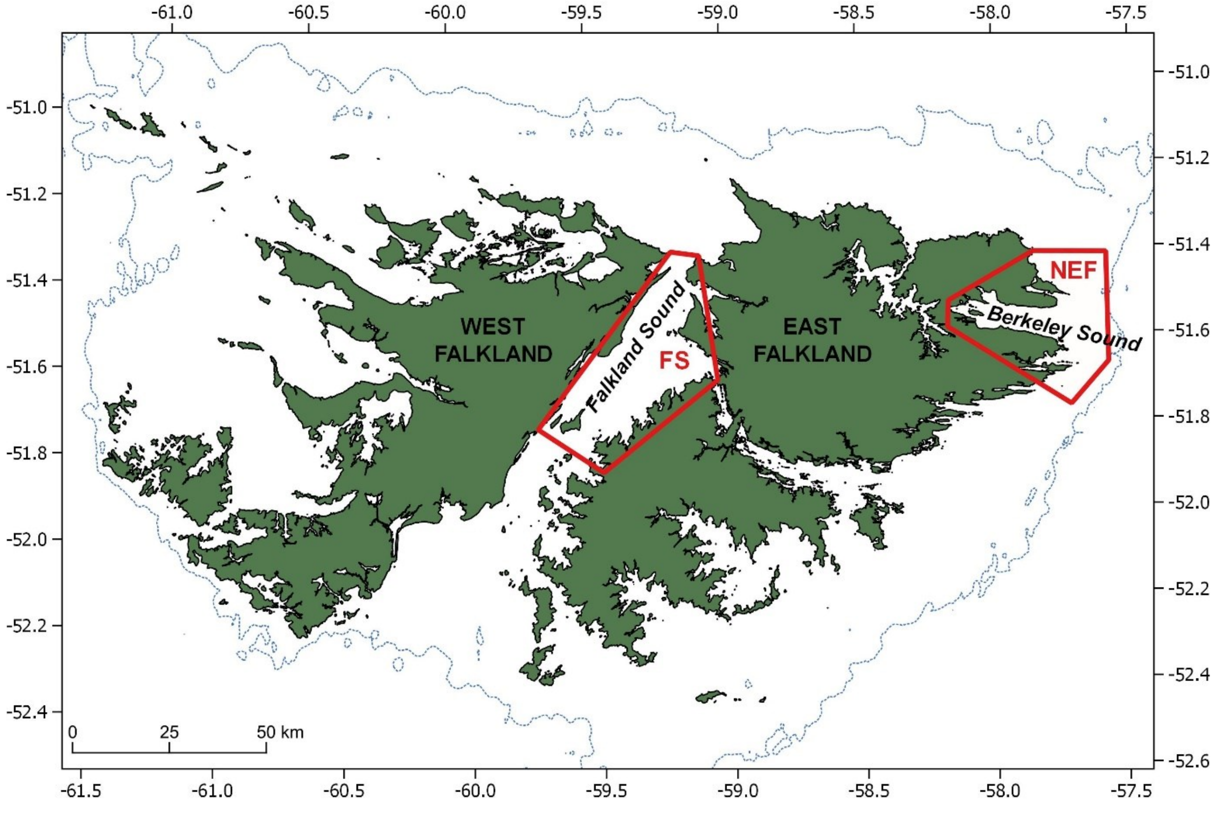

Figure 1.2. *The two primary study sites in the Falkland Islands: Falkland Sound (FS) and north-east Falklands (NEF).*

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[1] Berkeley Sound was also identified by Taylor et al. (2016) as a potential KBA for fin whales (*Balaenoptera physalus*).

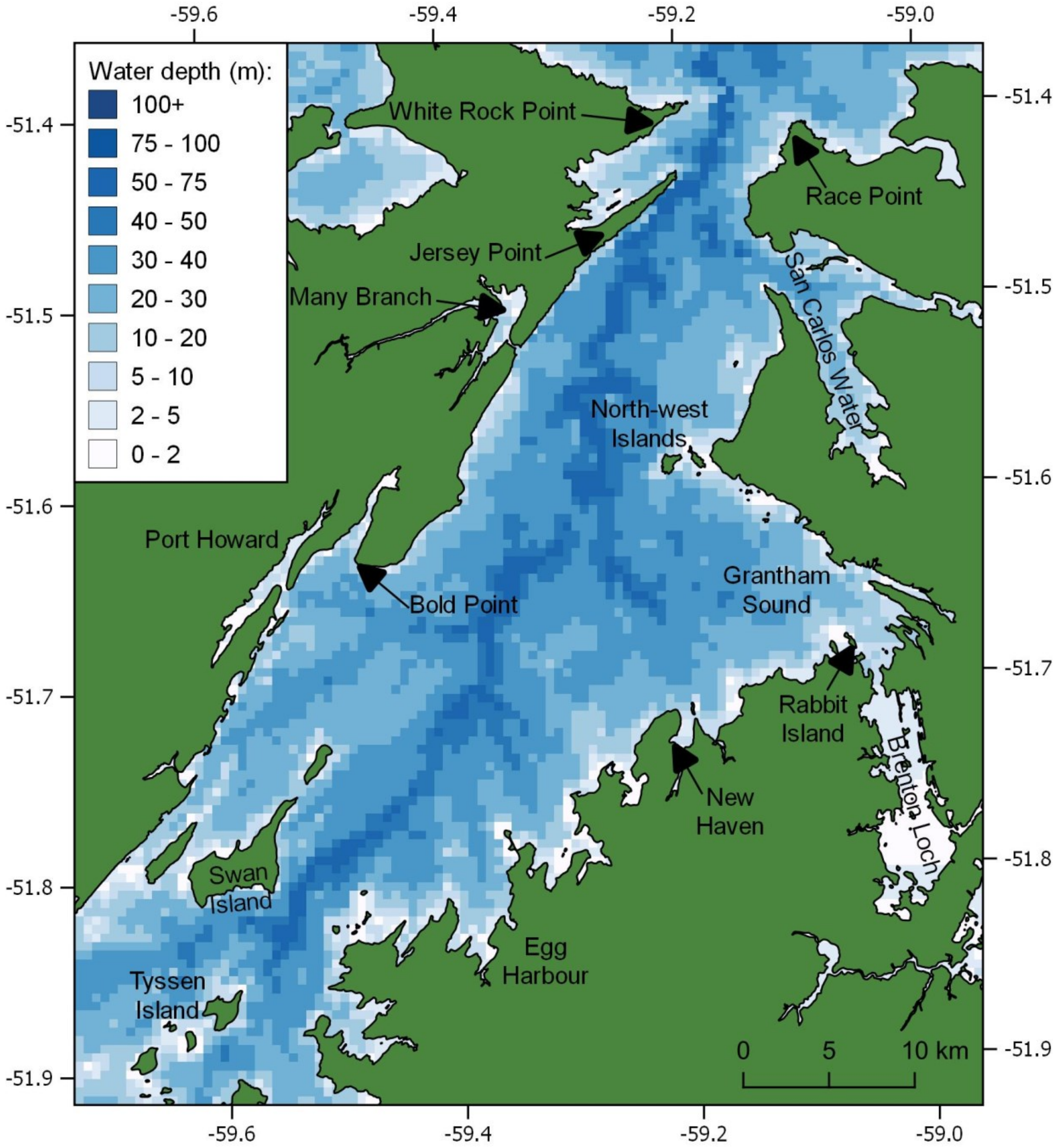

Figure 1.3. *Place names and water depths (presented as 500 m grid cells) at the Falkland Sound study area.*

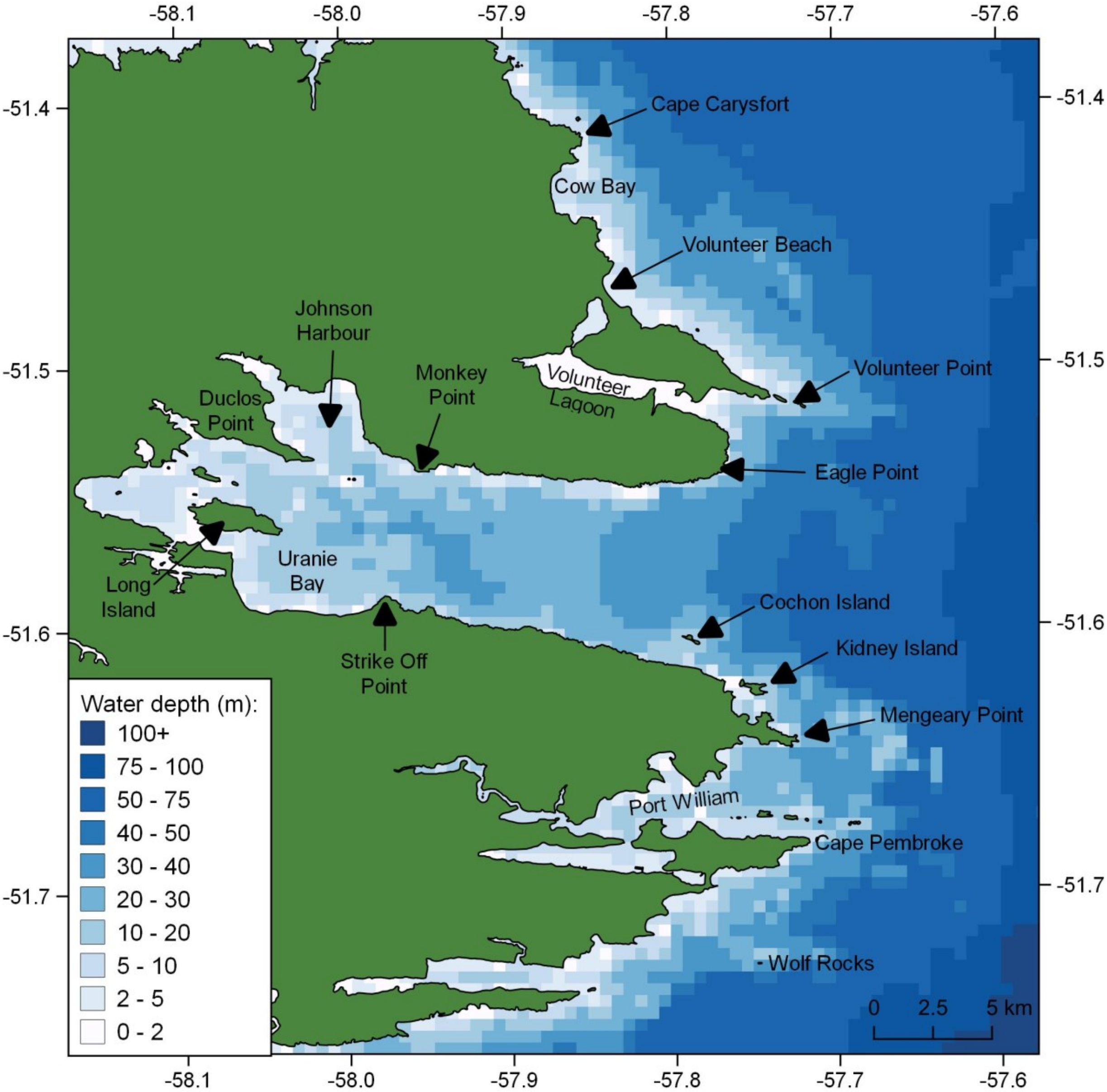

Figure 1.4. *Place names and water depths (presented as 500 m grid cells) at the North-east Falklands study area.*

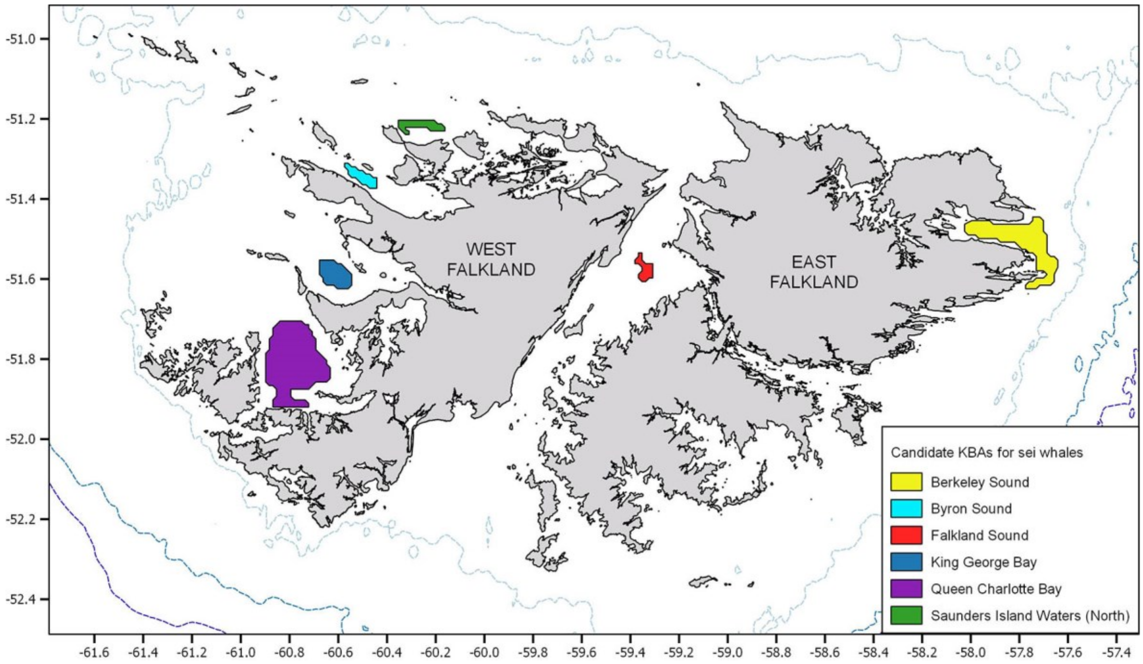

Figure 1.5. *Locations of six small marine areas highlighted by Taylor et al. (2016) as priorities for research due to their potential to qualify for sei whale Key Biodiversity Areas (KBAs). The 100 m, 200 m and 300 m depth isobaths are shown.*

1.3 Background information on whales in the Falkland Islands

Until recently, the occurrence of whales in the waters around the Falkland Islands was documented primarily from whaling carried out between the 1700s and the 1900s (e.g. Townsend, 1935; Horwood, 1987; Allison, 2016a). A shore station operated at New Island in the Falklands between 1905 and 1915, landing a total of 1,730 animals (Allison, 2016b; Tønnessen and Johnsen, 1982). The New Island catches are detailed in Weir (2017) and have been estimated as: 1,121 sei whales (*Balaenoptera borealis*), 284 fin whales (*B. physalus*), 201 humpback whales (*Megaptera novaeangliae*), 17 blue whales (*B. musculus*), three sperm whales (*Physeter macrocephalus*) and one southern right whale (*Eubalaena australis*).

In 1952, Hamilton wrote that "*during coastal passages large cetacean are commonly to be seen* [around the Falklands]*, at any rate during the first six months of the year*" and "*the sei whale is the commonest, but the fin whale is tolerably frequent, the humpback is occasionally seen and the blue whale has been reported*." Hamilton's (1952) paper originates just prior to the heaviest decades of Southern Hemisphere sei whale captures that occurred from the 1950s to the 1970s (Horwood, 1987).

The International Whaling Commission (IWC) moratorium on whaling went into effect in 1986, by which time most Southern Hemisphere baleen whale populations were heavily depleted. Relatively little new information on whales around the Falklands has emerged in subsequent decades. Between 1998 and 2000, the UK's Joint Nature Conservation Committee and Falklands Conservation (FC) conducted at-sea surveys around the Falklands to collect information on the distribution of seabirds and marine mammals (White et al., 2002). Those surveys recorded 14 cetacean species, including fin whales (57 animals), sei whales (45 animals), and minke whales (68 animals). Additionally, a small number of humpback whales (7 animals) and southern right whales (7 animals) were observed.

Interview surveys with local residents and a compilation of opportunistic data in the Falkland Islands by Frans and Augé (2016) suggested a large increase in baleen whale sightings (especially fin and sei whales) since the 1990s, which they attributed to post-whaling population recoveries. The species composition reported by interviewees for the period from the 1990s to 2015 in nearshore waters included sei whales (49.8%), fin whales (12.1%), minke whales (*B. acutorostrata*/*bonaerensis*: 5.2%), southern right whales (1.2%) and humpback whales (*Megaptera novaeangliae*: 0.2%). However, the species identification was unconfirmed for most records, and confusion is known to occur amongst local communities in the Falklands between especially sei, fin and minke whales.

Between January and May 2017, the first systematic and targeted field study of baleen whales in coastal waters occurred in Berkeley Sound (Weir, 2017). Almost all of the baleen whales recorded in the summer and early autumn comprised sei whales. However, at the end of autumn (May) an influx of southern right whales to the wider Berkeley Sound region was also recorded (Weir, 2017; Weir and Stanworth, 2020). The latter was an unexpected result, since no indication of a regular presence of southern right whales had been provided prior to the project commencing. An extensive systematic survey of the west coast of the Falklands was conducted in Feb–Apr 2018, and generated the first available abundance estimate for sei whales in the Islands (Weir, 2018). These studies led directly to the current project, which aimed to carry out two intensive years of work to assess populations of sei whales and southern right whales at two sites.

1.4 Focal species

1.4.1 Sei whale

The sei whale is a species of large baleen whale reaching average lengths of around 15 m. The species is characterised by a slender body, a prominent erect dorsal fin positioned two-thirds of the way along the back (Figure 1.6), a light chevron marking extending over the back behind the blowholes, and a distinctive forward-angled and upsweeping "brush mark" located on the upper flank, approximately midway between the blowholes and the dorsal fin.

Figure 1.6. *Sei whale surfacing in the Falkland Islands.*

Sei whales occur worldwide from polar to tropical waters. Densities appear to be highest in mid-latitude temperate areas, in water temperatures of 8°C to 18°C (Horwood, 1987). In most geographic areas it is considered to be primarily oceanic in habitat, being found along the continental slope or in deep ocean basins (Horwood, 1987). However, in the Falkland Islands, and elsewhere around the southern tip of South America, it routinely also occupies neritic and nearshore habitats (Acevedo et al., 2017; Häussermann et al., 2017; Weir, 2017). Despite their global distribution, sei whales are categorised by the International Union for Conservation of Nature (IUCN, 2018) as having Endangered (EN) global conservation status, due to heavy exploitation by commercial whaling operations that occurred particularly during the 1960s and 1970s.

In the Falklands, sei whales are known to be present in coastal waters between (at least) November and June, but with a strong seasonal peak during February and March (Weir, 2017, 2018; Weir et al., 2019; this report). The underlying driver for this strong seasonal occurrence appears to be the use of Falklands' waters as a feeding ground, evidenced by observations of surface feeding on squat lobster krill (*Munida gregaria*) and amphipods (*Themisto gaudichaudii*), and of regular defecations by whales (Weir, 2017; Weir et al., 2019). Like most large baleen whale species, the sei whale undertakes seasonal migrations between winter subtropical areas where mating and calving occur, and summer temperate and polar feeding areas (Horwood, 1987). The locations of feeding and breeding areas remain poorly understood. However, in the south-west Atlantic a link has been shown between the Falkland Islands feeding ground and a wintering area located off Brazil (Weir et al., 2020).

DPLUS082 aimed to collect targeted data on sei whales during the 2019 and 2020 seasons, aimed at acquiring additional spatio-temporal information (including at a new site at Falkland Sound), identifying habitat preferences and drivers of occurrence, and assessing variation between years through the acquisition of a multi-year dataset. In many other geographic regions, sei whales are traditionally thought to fluctuate markedly in occurrence between years (Horwood, 1987); one core motivation for this study was to demonstrate whether sei whales persistently use Falklands' coastal waters for feeding across years, such that the region may qualify as a KBA for the species.

1.4.2 Southern right whale

The southern right whale is a stocky baleen whale reaching around 14 to 15 m body length on average. The species has a robust body shape and lacks a dorsal fin. It is characterised by a large head with a strongly arched jawline. Each whale has a unique pattern of roughened patches of skin called 'callosities' on their head, which become infested with crustaceans and appear cream or yellow in colour (Figure 1.7). The majority of southern right whales of both sexes are black in colour, sometimes with irregular white patches on their belly. However, a small number of (mostly male) calves are born white with black mottled spotting, and become pale grey with black mottling (or 'brindle') as adults (Schaeff et al., 1999). Several other pigmentation patterns occur, and are further discussed in **Chapter 3**.

Figure 1.7. *Head of a southern right whale in the Falkland Islands.*

Southern right whales are distributed across temperate and polar waters of the Southern Hemisphere, including well-documented winter mating and calving areas located along the coasts of South America (particularly in Argentina and Brazil), South Africa, southern Australia and New Zealand (Cooke and Zerbini, 2018). The pelagic foraging grounds occupied by southern right whales during the austral summer are far less well known, but are thought to be concentrated at latitudes of 40–50°S. Although the global population remains well below the estimated pre-exploitation size of 55,000 to 75,000 animals, in many regions southern right whales are steadily recovering from centuries of severe exploitation during the early whaling era (<1920s: IWC, 2001). Consequently, their global conservation status has been categorised as Least Concern (LC) since 2008 (Cooke and Zerbini, 2018).

Whaling data, occasional sightings, and recent satellite-tracking work indicate that southern right whales use the pelagic waters around the Falklands during summer and autumn (Townsend, 1935; Zerbini et al., 2016, 2018; Weir and Stanworth, 2020), presumably for both foraging and during seasonal migrations between foraging and breeding areas (Weir, 2021). However, during 2017, a novel occurrence of southern right whales in nearshore waters during the winter was discovered (Weir, 2017; Weir and Stanworth, 2020), which was repeated to some extent in 2018 (Weir and Stanworth, 2020). The 2017 wintering aggregation included observations of socialising and apparent mating, leading to the suggestion that Falklands' waters may comprise a non-calving winter breeding area for right whales (Weir and Stanworth, 2020). This finding led directly into the current Darwin Plus project, which planned to conduct surveys throughout the winters of 2019 and 2020 to specifically target wintering southern right whales and determine whether their occurrence was persistent across years, provide insight into the numbers and composition (i.e. age, sex) of the animals involved, and assess the underlying drivers for their winter presence in coastal areas.

1.5 COVID-19

DPLUS082 was initially planned to comprise two full seasons of boat-based survey work in 2019 and 2020 and a two-year acoustic monitoring programme (Dec 2018 to Dec 2020). However, like many field projects worldwide, the project experienced various challenges associated with the outbreak of the COVID-19 pandemic, including:

- Loss of 6.5 weeks of boat survey work during the peak season for sei whales in 2020 due to local lockdown restrictions in the Falkland Islands. Consequently, the 2020 sei whale field season was incomplete, and the project was extended to carry out a third sei whale season from February to May in 2021 to compensate; and
- Delayed sample shipments and restricted laboratory access for project partners, resulting in significant delays to the analysis of acoustic, genetic and dietary data. In some cases (see **Chapter 4**, **Chapter 5** and **Chapter 7**), this has resulted in only partial datasets or analyses being included in this report.

The authors of each chapter have included as much information as was feasible under the circumstances, but readers are advised to check for scientific manuscripts and updated versions of this report later in 2022 which may include more complete data analyses.

1.6 Report format

The remainder of this report comprises standalone chapters that cover individual components of the DPLUS082 project. These include chapters on the visual surveys (**Chapter 2**), photo-identification (**Chapter 3**), diet (**Chapter 4**), genetics (**Chapter 5**), suction cup tagging (**Chapter 6**), and acoustic monitoring (**Chapter 7**).

1.7 Research permits

All of the work described in this report was conducted with the following permits issued by Falkland Islands Government:

- R11/2017: *Monitoring of baleen whales in the Falkland Islands*. Covering non-invasive work including visual surveys, photo-identification, acoustic monitoring, and faecal sampling.

- R23/2018: *Genetic sampling and suction-cup tagging of baleen whales in the Falklands.* Covering more invasive components, including suction-cup tag deployments and biopsy sampling to collect tissue samples.
- R40/2018: Covering sampling of stranded (dead) marine mammals for scientific studies.

Additionally, licenses were obtained from FIG under the Food and Environment Protection Act 1985 ('FEPA') for the deposit of anchors on the seabed associated with the acoustic monitoring work.

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Chapter 2: Whale distribution during small boat surveys

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2.1 Introduction and aims

The collection of robust data on whales in the Falklands has been limited to date, comprising a targeted study of sei whales in Berkeley Sound during 2017 (Weir, 2017), whale sightings recorded during a systematic aerial survey for dolphins in 2017 (SAERI, unpublished data), and a yacht survey aimed at assessing the distribution and abundance of sei whales off West Falkland in 2018 (Weir et al., 2021). Large baleen whales are highly mobile, and their occurrence can vary markedly between years depending on oceanographic conditions and prey availability (e.g. de Vos et al., 2014; Murase et al., 2002; Schleimer et al., 2019). The datasets available for the Falklands to date all originate from single sites and/or single years. Consequently, understanding of the occurrence of whales in Falklands' waters remains limited, and the acquisition of multi-year datasets a priority. This project aimed to address this point through the collection of targeted data on baleen whales at two study sites and over at least two seasons. Visual survey work provides information on the spatial and temporal distribution of cetacean species, and has advantages over some other methods in: (1) providing confirmation of species identification through the use of experienced personnel and the acquisition of supporting photographic images; (2) providing data on group size and composition (e.g. the presence of calves); and (3) facilitating observations of behaviour (albeit limited to the surface). Additionally, a range of other techniques can be carried out concurrently with sighting surveys during boat survey work, including photo-identification of individual animals (see **Chapter 3**), and the collection of samples that are critical to understanding other aspects of species conservation and management. In the Falklands, visual surveys using small boats have the disadvantage of being highly limited by prevailing weather conditions, resulting in a relatively low sampling frequency.

During this project, standard visual sighting surveys were conducted to achieve two core objectives:

1. To collect information on the spatial and temporal distribution of whales; and
2. To collect data on species group size and composition.

The visual survey was also intended to identify suitable whale individuals and groups that could be approached for photo-identification (**Chapter 3**), faecal sampling (**Chapter 4**) and biopsy sampling (**Chapter 5**).

Some data from the visual surveys carried out in 2019 and 2020 as part of DPLUS082, have already been published as scientific manuscripts, including:

- Weir, C.R. (2021). Southern right whale (*Eubalaena australis*) surveys in the Falkland Islands (Malvinas) during winter 2019 and 2020: preliminary results. Paper SC/68C/CMP/09Rev1 submitted to the International Whaling Commission. https://archive.iwc.int/?r=19176&k=b90b93a1de
- Baines, M. and Weir, C.R. (2020). Predicting suitable coastal habitat for sei whales, southern right whales and dolphins around the Falkland Islands. PLOS ONE 15(12): e0244068. https://doi.org/10.1371/journal.pone.0244068
- Weir, C.R., Stanworth, A., Cartwright, S., Jelbes, P.A.Q., Taylor, M. and Pompert, J. (2019). Distribution and movements of sei whales (*Balaenoptera borealis*) on coastal feeding grounds

in the Falkland Islands (Malvinas). World Marine Mammal Conference, Barcelona, Spain, December 2019.

2.2 Materials and methods

2.2.1 Study area

Small boat surveys were carried out at two focal sites: (1) Falkland Sound (FS), comprising areas within a working radius of New Haven; and (2) north-east Falklands (NEF) comprising the area between Cape Pembroke and Cape Carysfort, including Berkeley Sound (see Section 1.2 and Figure 1.2).

2.2.2 Survey plan

Small boat surveys were carried out for baleen whales across two defined seasons in 2019 and 2020: (1) January to May (austral summer and autumn), targeting the sei whale; and (2) May to August (late autumn and winter), targeting the southern right whale.

In FS, three predetermined routes were selected for boat surveys that comprised loops of the northern, central, or southern, portions of the study area respectively. It was planned to survey FS only between February and May, since the specific focus of that site was the sei whale. Small boat surveys in NEF were planned to occur across both the sei whale and the southern right whale seasons. During the sei whale season (Jan to May) the standard route comprised a loop of Berkeley Sound and south of the Cape Pembroke lighthouse to the Wolf Rocks. The loop extended inside Berkeley Sound as far west as Long Island and Johnson Harbour; the water depths further west of that area were considered too shallow to regularly support sei whales. During the southern right whale season (May to September), the route either comprised a return trip across the mouth of Berkeley Sound to the Volunteer Point/Cow Bay area, or a loop inside Berkeley Sound.

While the desired routes were determined prior to each survey and planned to optimise spatial coverage, the exact route taken on each boat survey was subsequently determined by factors including prevailing weather conditions, whale encounters and, on occasion, the need to also visit specific locations to conduct acoustic deployments or recoveries.

For reasons of both safety and productivity (it was difficult to work effectively with cameras and sampling equipment once spray began to come over the bow), small boat surveys could only occur on days where low wind speeds (≤10 knots) were forecast. Weather conditions were therefore the primary constraint in the number of surveys carried out per month. Additionally, surveys in some months (particularly May and June) were limited by the presence of coastal fog.

2.2.3 Data collection

A 6.5 m rigid-hulled inflatable boat with twin 125-hp engines (survey speed of around 13 to 14 knots) was chartered through the Shallow Marine Surveys Group (SMSG) for the whale surveys. The open nature of the boat facilitated easy communications between observers and skipper, and provided a clear view of the 180º observation area forwards. The author was the Survey Leader on every effort-related survey, which ensured consistency in survey methods. On the majority of surveys at least one additional observer was also present.

The boat position was continuously logged at 1-min intervals using a handheld Garmin GPS, with all other data collected during the survey being linked to the GPS via a correlated timestamp. Effort status was continuously logged as: (1) Active Search effort (while observers were actively scanning the sea surface in search of cetaceans); or (2) Cetacean Encounter effort (while working with cetaceans and not actively searching for new animals). Periods where the observers were not actively engaged in any of these "on effort" activities (e.g. during lunch breaks or engine maintenance) were considered as “off effort” and no data were logged. For every period of effort, the start and end times were recorded, along with environmental data and effort status. A new set of data was recorded whenever conditions changed, for example when changing from Active Search to Cetacean Encounter effort. Standardised environmental data were recorded throughout the survey in order to assess the quality of the effort data with regard to detecting the target cetacean species. Those data comprised Beaufort sea state, swell height (m), visibility (km), precipitation, and sun glare (see Weir, 2017, 2018 for definitions).

During periods of Active Search effort, the observers searched for cetaceans using the naked eye, with each observer scanning a separate 90° quarter (port and starboard) from the beam to the bow. A digital voice recorder was used by the Survey Leader to log data throughout the surveys.

Whenever cetaceans were observed, the following standardised information was recorded: sighting start and end times (recorded directly from the GPS to ensure accurate correlation with positional data), effort status, species identification, group size (minimum, maximum, best estimate), group composition (adults, juveniles, calves, unknown age), and overall behaviour.

For baleen whale sightings, the Survey Leader decided whether to divert the boat to approach the animal(s) to collect additional data. When an approach was made, a second time and distance were logged when the boat was within 200 m of the animal(s) and used to reflect actual animal location. When it was decided not to approach whales (e.g. due to time constraints or adverse weather), the time, vessel heading, relative bearing, and estimated distance to the sighting were recorded, in order to subsequently calculate a more accurate sighting position.

2.2.4 Data analysis

Effort and sightings data were entered into standardised Excel databases as soon as possible after each survey. The analysis positions for sightings were determined as: (1) the boat position at initial sighting if animals were within 300 m; (2) the boat position at encounter start time for whales approached in closing mode; or (3) when animals were at distances exceeding 300 m from the boat in passing mode, the sighting position was recalculated based on angle and estimated distance from the boats GPS position using an Excel worksheet (MacLeod, 2011). The best visual estimate of group size was used throughout analysis.

All mapping was carried out in Quantum Geographic Information System (QGIS: V3.12; https://qgis.org) using the WGS 84 / UTM zone 21S projection.

Cetacean relative abundance was calculated as both the number of sightings, and the number of individuals, recorded per km of survey coverage. This represents a measure of relative abundance and is not a calculation of density or absolute abundance (Evans and Hammond, 2004). Relative abundance was calculated using only effort and associated sightings data collected in conditions considered to be favourable for the visual detection of whales. Favourable conditions were defined for this report as Beaufort sea state ≤4, swell of ≤2.5 m, and visibility of >5 km.

The sighting positions used for relative abundance mapping were the uncorrected boat positions at the time of each initial sighting, since the sightings had to match the associated effort in each grid cell. One consequence of this approach is that the actual positions of the animals may have been located in an adjacent grid cell. The relative abundance maps were produced at 5 km grid cell size. Only grid cells in which >1 km survey coverage was achieved were included in the maps, to minimise falsely-inflated relative abundance values when sightings were recorded in grid cells where very small amounts of survey effort had occurred.

2.3 Results

2.3.1 Survey effort

2.3.1.1 Overview

Small boat surveys were carried out on 94 dates between January 2019 and June 2021 (Table 2.1). A total of 632.7 hr and 9,215.9 km of survey effort was collected, comprising:

- Active Search effort: 333.7 hr / 7,306.9 km;
- Active Search in favourable conditions: 323.2 hr / 7,104.1 km;
- Cetacean Encounter effort: 299.0 hr / 1909.0 km:
    - Sei whale: 147.2 hr / 1,215.9 km;
    - Southern right whale: 78.7 hr / 345.3 km;
    - Humpback whale: 7.8 hr / 44.1 km;
    - Dolphins: 65.3 hr / 303.7 km.

Active Search effort was highest during 2019, reduced during 2020, and was lowest in 2021 (Table 2.1; Figure 2.1). This reflected impacts on the survey work associated with the COVID-19 pandemic during 2020 and 2021. In 2020, a 6.5 week gap in fieldwork occurred during the sei whale season due to the local COVID-19 lockdown in the Falkland Islands, resulting in no coverage at all during April 2020 (Table 2.1). As a result, the project was extended for an additional year in 2021, but only to cover the peak months of the sei whale season (Feb to May). In total, the coverage included two complete sei whale seasons (2019 and 2021), one partial sei whale season (2020), and two southern right whale seasons (2019 and 2020).

The amount of Active Search effort collected in favourable conditions for the visual detection of large whales was 323.2 hr / 7,104.1 km. The majority of that effort occurred in sea conditions with no whitecaps (Beaufort sea state 0–2: 70.3%), low swells of ≤1.0 m (73.7%), and in excellent visibility (88.7%: Figure 2.2).

When considering all three study years combined, most Active Search effort occurred between February and June (Figure 2.3). This reflects the long daylight hours of the austral summer and autumn, which provided more scope to work around weather windows and facilitated more coverage over the core period for sei whale occurrence compared with the southern right whale season.

Monthly search effort varied quite markedly between years (Figure 2.4), reflecting: (1) availability of good weather days for boat work; (2) differences in the relative amounts of time spent in search effort versus encounter effort (depending on the number of whales present in the study area each survey); and (3) logistical factors including the COVID-19 pandemic (e.g. no data in April 2020), boat and vehicle availability, and personnel availability. While every effort was made throughout the project to maximise time at sea, the evident variability in Active Search effort between months and years does have implications for data interpretation (as highlighted throughout the Results and Discussion).

The level of search effort in favourable weather conditions varied between the two focal study sites (Table 2.2): NEF received 83.0% (5,893.7 km) of the total search effort, while FS received only 17.0% (1,210.4 km). Some of this discrepancy was because FS was only scheduled to receive survey coverage during the sei whale season (between January and May), whereas the fieldwork was planned to continue at NEF throughout June, July and August for the right whale season. Nevertheless, even in the February to May period for sei whales, NEF received 77.2% of the total search effort while FS received only 22.8% (Table 2.2; Figure 2.5). This situation was especially pronounced during 2020, when only a single small boat survey was accomplished at FS.

Table 2.1. *Summary of boat-based effort collected on 94 survey dates in 2019–2021.*

Year	Month	No. of survey dates	All survey effort (search and encounter)		Active Search effort		Active Search effort in favourable weather	
			hr	km	hr	km	hr	km
2019	Jan	2	13.6	223.1	8.8	195.6	8.8	195.6
	Feb	6	39.6	625.0	23.5	511.3	23.1	502.5
	Mar	8	65.7	932.3	31.3	675.3	30.8	660.7
	Apr	5	36.1	558.4	18.2	435.3	17.8	423.6
	May	5	30.9	483.4	16.4	385.4	16.4	385.4
	Jun	8	44.8	657.4	26.6	567.9	23.6	509.3
	Jul	5	35.4	431.6	17.3	350.8	14.3	303.9
	Aug	3	23.6	294.3	12.0	251.0	10.1	217.7
2020	Jan	1	5.8	113.1	4.9	108.8	4.9	108.8
	Feb	4	27.8	444.7	16.9	362.7	16.9	362.7
	Mar	6	38.2	549.1	17.4	402.3	17.2	399.5
	Apr	0	0.0	0.0	0.0	0.0	0.0	0.0
	May	4	24.6	367.6	14.3	324.1	14.2	320.7
	Jun	4	24.2	350.4	13.8	302.9	13.8	302.9
	Jul	4	24.8	354.7	14.6	311.3	14.6	311.3
	Aug	4	22.7	357.1	14.1	323.9	13.5	307.2
	Sep	2	14.0	226.6	9.7	212.7	9.7	212.7
2021	Jan	0	0.0	0.0	0.0	0.0	0.0	0.0
	Feb	2	15.5	258.2	8.9	211.5	8.9	211.5
	Mar	5	31.6	466.4	15.9	355.9	15.9	355.9
	Apr	7	50.7	661.9	20.4	413.8	20.4	413.8
	May	7	49.7	666.3	21.7	458.9	21.4	452.9
	Jun	2	13.2	194.3	7.0	145.6	7.0	145.6
2019	Total	42	289.7	4,205.5	154.1	3,372.6	144.9	3,198.7
2020	Total	29	182.2	2,763.3	105.7	2,348.6	104.7	2,325.8
2021	Total	23	160.8	2,247.1	73.9	1,585.6	73.6	1,579.7
All	Total	94	632.7	9,215.9	333.7	7,306.9	323.2	7,104.1

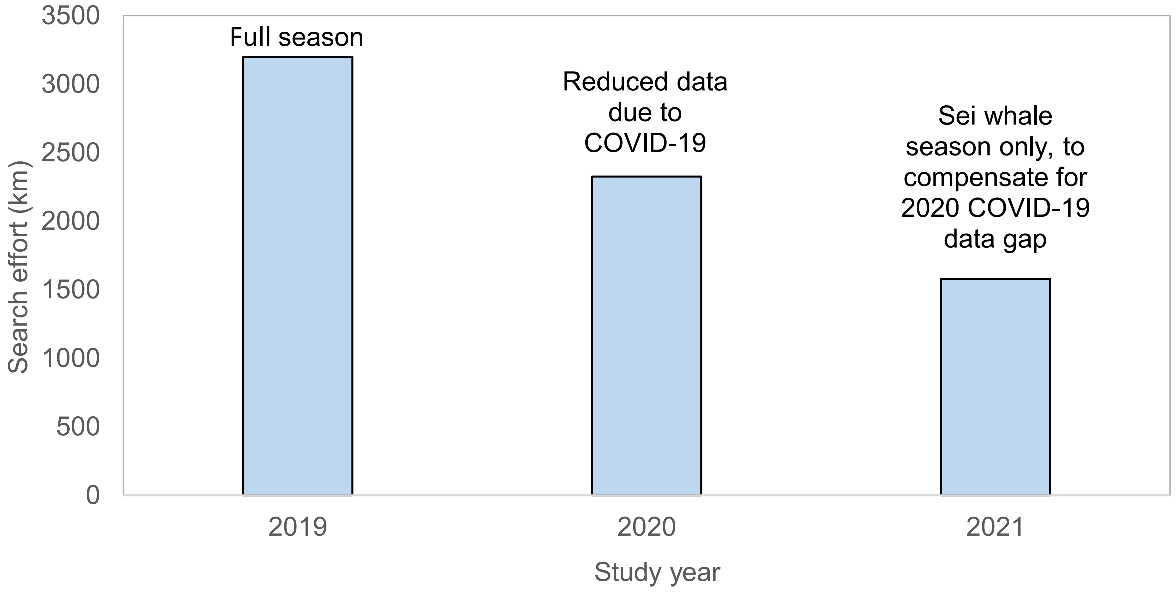


Figure 2.1. *Annual distribution of 7.104.1 km of Active Search effort collected in favourable weather conditions.*

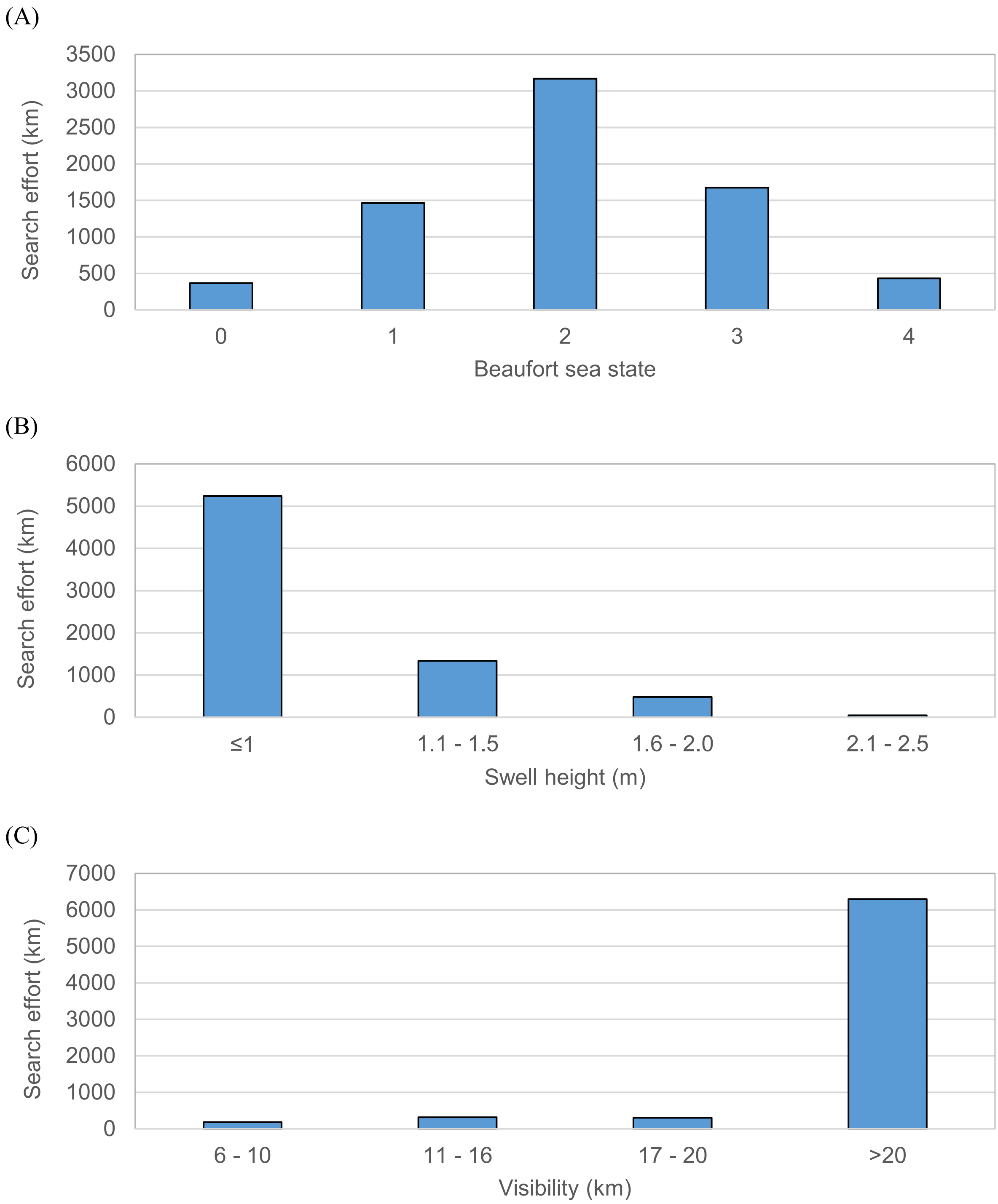


Figure 2.2. *Environmental data during 7.104.1 km of Active Search effort collected in favourable weather conditions (i.e. after adverse data collected in Beaufort sea state >4, swell of >2.5 m, and visibility of <5 km had been removed): (A) Beaufort sea state; (B) swell height; and (C) visibility (km).*

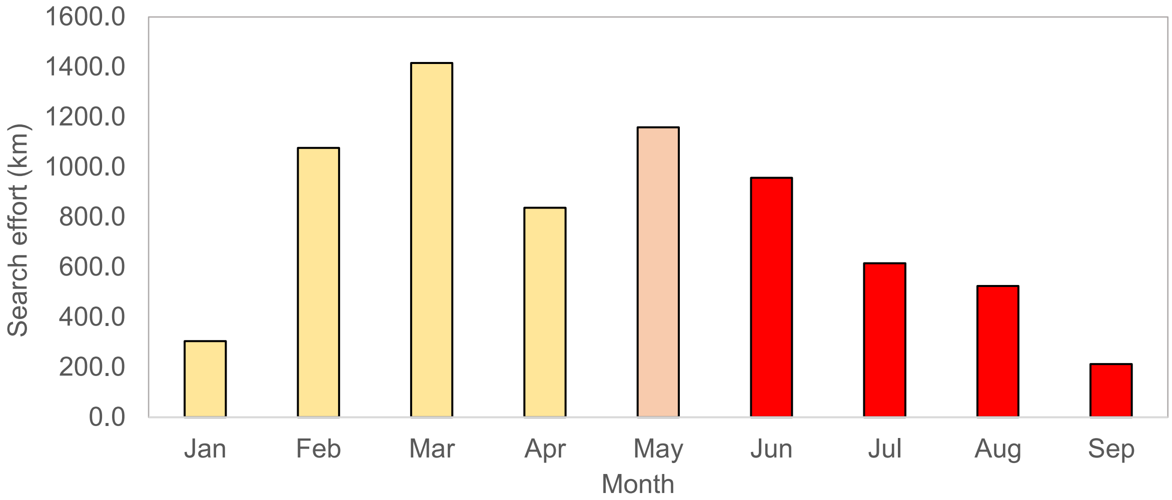


Figure 2.3. *Monthly distribution of 7.104.1 km of Active Search effort collected in favourable weather conditions for all three survey years (2019–2021) combined. Yellow: core sei whale season; Red: core southern right whale season; Orange: both species (relative occurrence varying by year).*

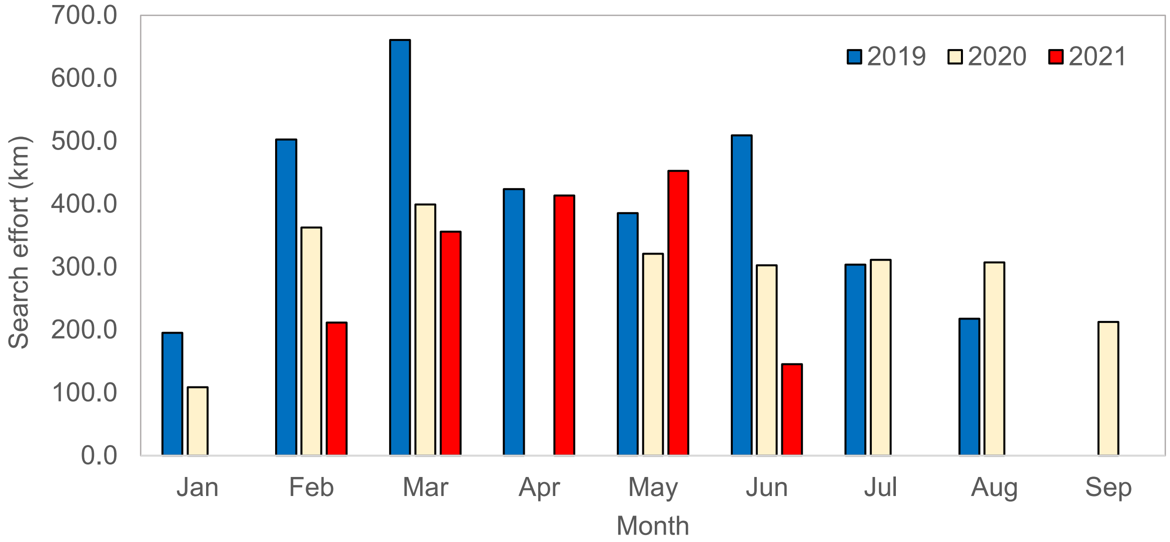


Figure 2.4. *Monthly distribution of 7.104.1 km of Active Search effort collected in favourable weather conditions for each of the three survey years.*

Table 2.2. *Annual and monthly distribution of 7.104.1 km Active Search effort at Falkland Sound (FS) and in the north-east Falklands (NEF) during small boat surveys in favourable weather conditions.*

Year	Month	FS		NEF	
		hr	km	hr	km
2019	Jan	0	0	8.8	195.6
	Feb	3.5	71.4	19.6	431.1
	Mar	8.5	182.0	22.4	478.7
	Apr	3.6	79.3	14.2	344.3
	May	7.6	175.2	8.8	210.2
	Jun	4.2	100.3	19.4	408.9
	Jul	0	0	14.3	303.9
	Aug	0	0	10.1	217.7
2020	Jan	0	0	4.9	108.8
	Feb	0	0	16.9	362.7
	Mar	0	0	17.2	399.5
	Apr	0	0	0	0.0
	May	2.3	59.4	12.0	261.4
	Jun	0	0	13.8	302.9
	Jul	0	0	14.6	311.3
	Aug	0	0	13.5	307.2
	Sep	0	0	9.7	212.7
2021	Jan	0	0	0	0
	Feb	0	0	8.9	211.5
	Mar	10.7	247.1	5.1	108.9
	Apr	3.3	76.0	17.1	337.8
	May	6.4	134.8	15.0	318.1
	Jun	4.1	84.9	2.9	60.6
2019	Total	27.4	608.3	117.5	2590.4
2020	Total	2.3	59.4	102.5	2266.4
2021	Total	24.5	542.8	49.1	1036.9
All	Total	54.2	1,210.4	269.0	5,893.7

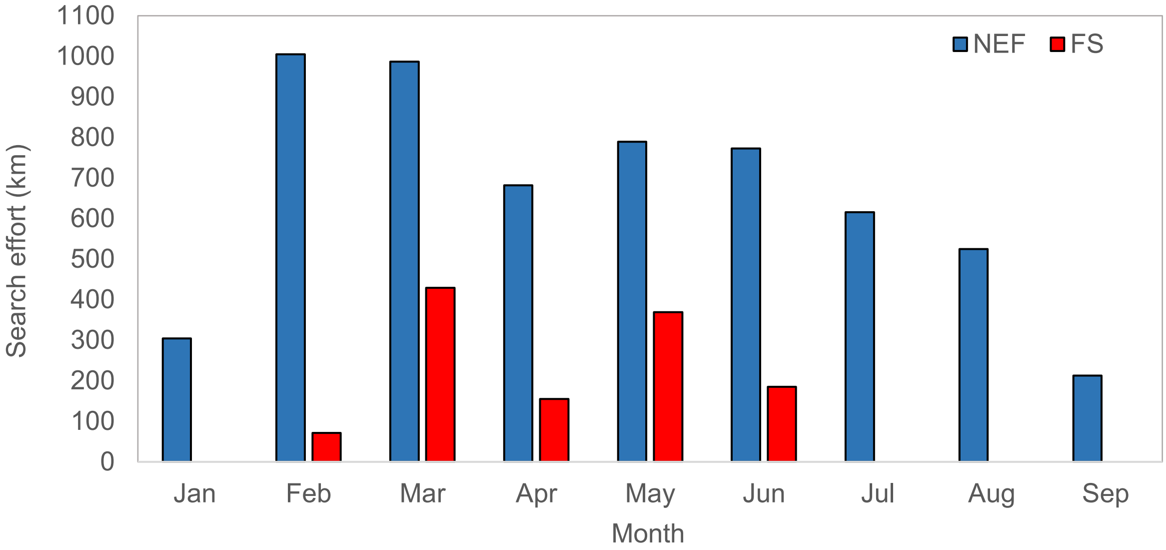


Figure 2.5*. Monthly distribution of 7.104.1 km of Active Search effort collected in favourable weather conditions at the two focal study sites: Falkland Sound (FS) and the north-east Falklands (NEF).*

The discrepancy in the relative amounts of coverage achieved at the two sites was primarily the result of logistical constraints while trying to operate in two locations with a single survey boat and team. These included:

1. NEF was located in immediate proximity to Stanley, and therefore required less forward planning and provided more flexibility for taking advantage of short weather windows;
2. Working at FS required towing the survey vessel for 2–3 hrs each way from Stanley along large expanses of gravel road; those trips involved long days and significant resources, and could only be reasonably undertaken when the weather conditions for working at the site were likely to remain optimal for at least 6 hrs and when the forecasts were sufficiently stable for trips to be planned a day in advance;
3. FS experienced strong tide; during early experiences in 2019 it was apparent that tidal currents in addition to marginal weather conditions made it very difficult to work with whales. Consequently, FS coverage became further limited to days with particularly favourable weather forecasts;
4. On occasions where two simultaneous good weather days were available, it was often not logistically possible to spend one day at FS and the other at NEF (due to logistics around boat and vehicle fuel, tide times for launching and recovering the boat etc.). Consequently, in those situations a choice had to be made regarding which site to focus on. In most cases NEF was the most viable site because it required less planning ahead in case the weather forecast changed.

As a result of these challenges, May 2019 was the only month during the project where reasonably comparable amounts of search effort were collected at both sites (Table 2.2). In all other months the coverage at NEF far exceeded that achieved at FS (Table 2.2), with the exception of March 2021 when FS received double the coverage of NEF after significant effort was made to prioritise FS during the project extension.

Comparisons of whale occurrence at the two focal sites therefore have to take into account the disparities in survey effort. In general, the data presented for NEF will be more representative of relative whale occurrence and seasonality than that at FS, since search effort at the former site was more evenly distributed across months and years.

2.3.1.2 Falkland Sound

Over the three combined survey years, the realised spatial search effort spanned most of the central and northern regions of FS, and was distributed from White Rock Point and Race Point in the north, to Tyssen Island and Swan Island in the south (Figure 2.6).

When assessed by month, the spatial distribution of search effort was most extensive during March and May (Figure 2.7). May was also the only month for which search effort occurred in all three of the survey years.

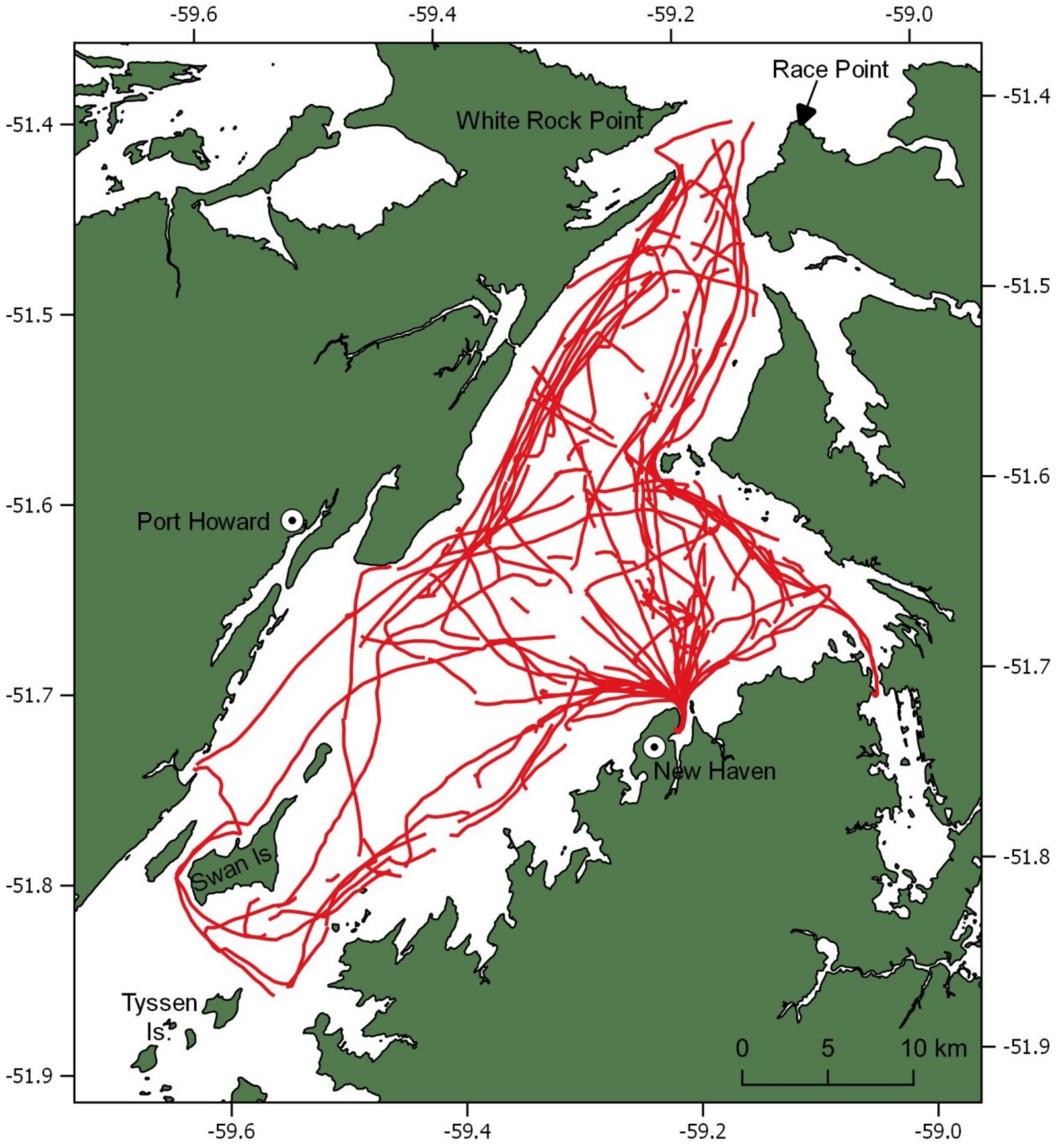
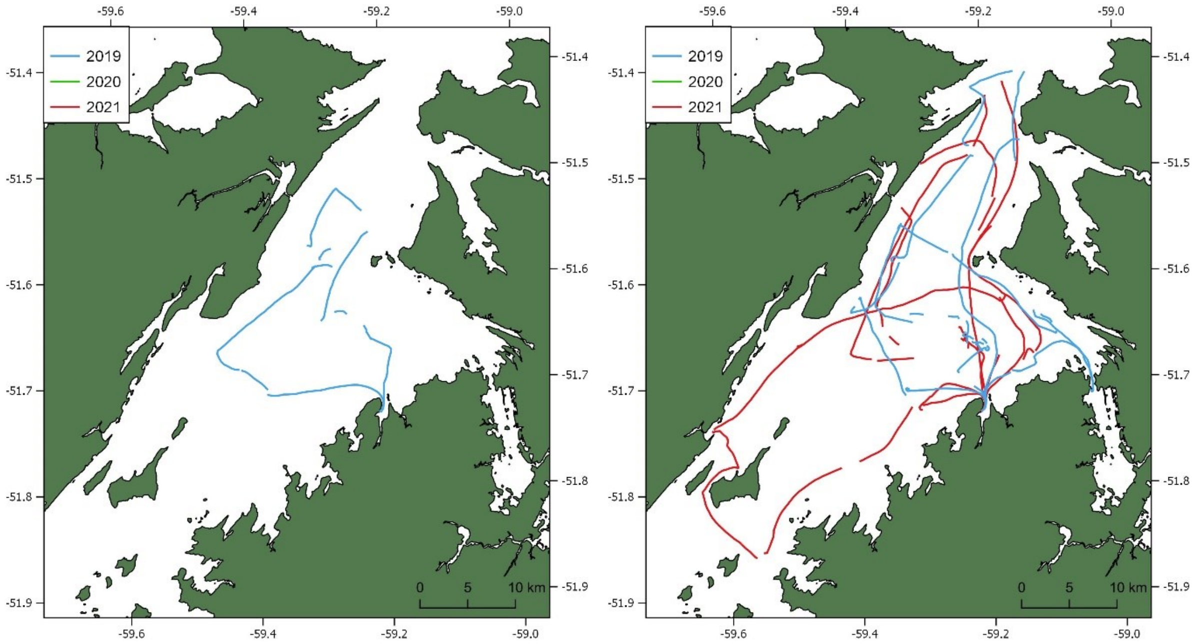


Figure 2.6. *Spatial distribution of 1,210.4 km of Active Search effort collected in favourable weather conditions at Falkland Sound, 2019–2021.*

(A) February (B) March



(C) April (D) May

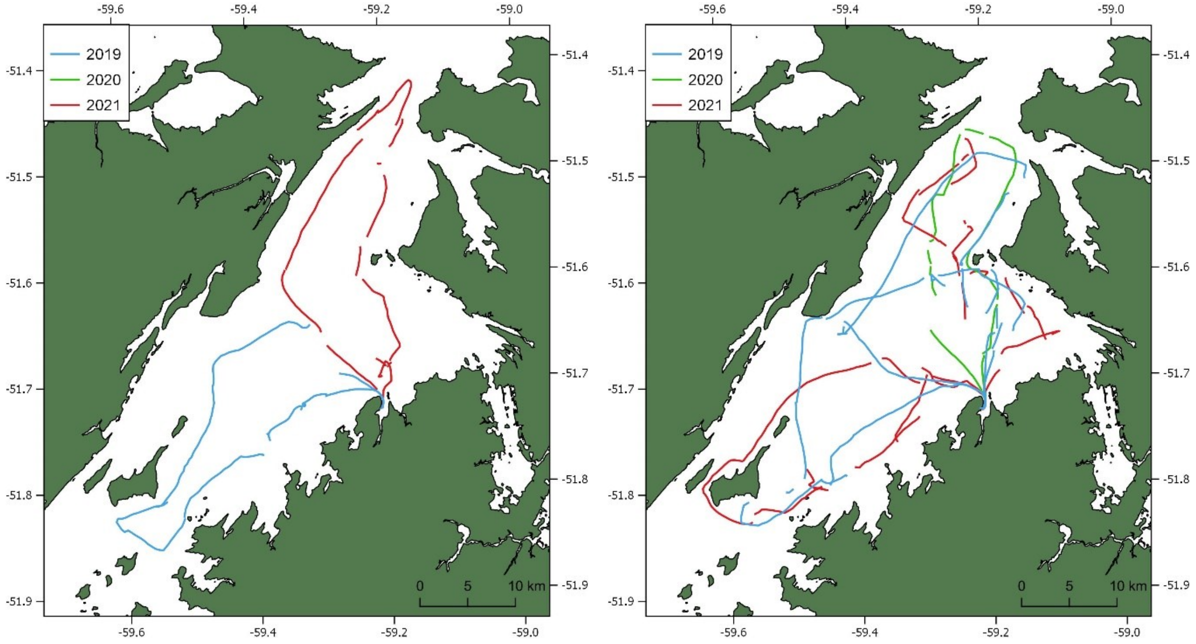


Figure 2.7. *Monthly spatial distribution of 1,210.4 km of cetacean Active Search effort collected in favourable weather conditions in Falkland Sound, February to June 2019–2021.*

(E) June

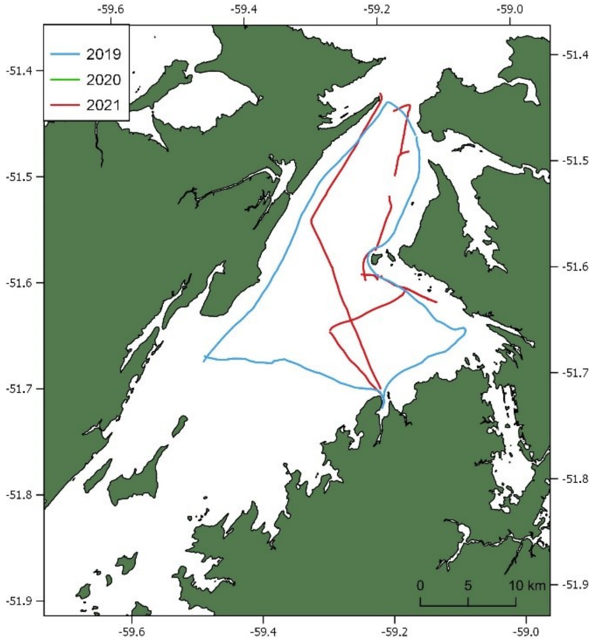


***Figure 2.7**. Contd.*

2.3.1.3 North-east Falklands

The spatial coverage at NEF focussed on the large inlet of Berkeley Sound, and also included exposed coastal waters spanning the area from the Wolf Rocks in the south to Cape Carysfort in the north (Figure 2.8). Since NEF was surveyed for both sei whales and southern right whales, coverage extended across more months than FS. Between January and April, the boat surveys exclusively covered Berkeley Sound and the open coastal waters between Volunteer Point and the Wolf Rocks (Figure 2.9A–D). In May, most boat surveys also covered Berkeley Sound, but there was some coverage in the northern part of the study area between Volunteer Point and Cape Carysfort (Figure 2.9E).

From June through to September, the focus was primarily on southern right whales and the majority of surveys included coverage of Port William, Kidney Island, and the Volunteer Point to Cape Carysfort area (Figure 2.9F–I). Coverage acquired in the inner areas of Berkeley Sound over the latter period was mostly associated with acoustic deployments. There was no spatial coverage after June in 2021, because the boat work for that year did not continue into the right whale season.

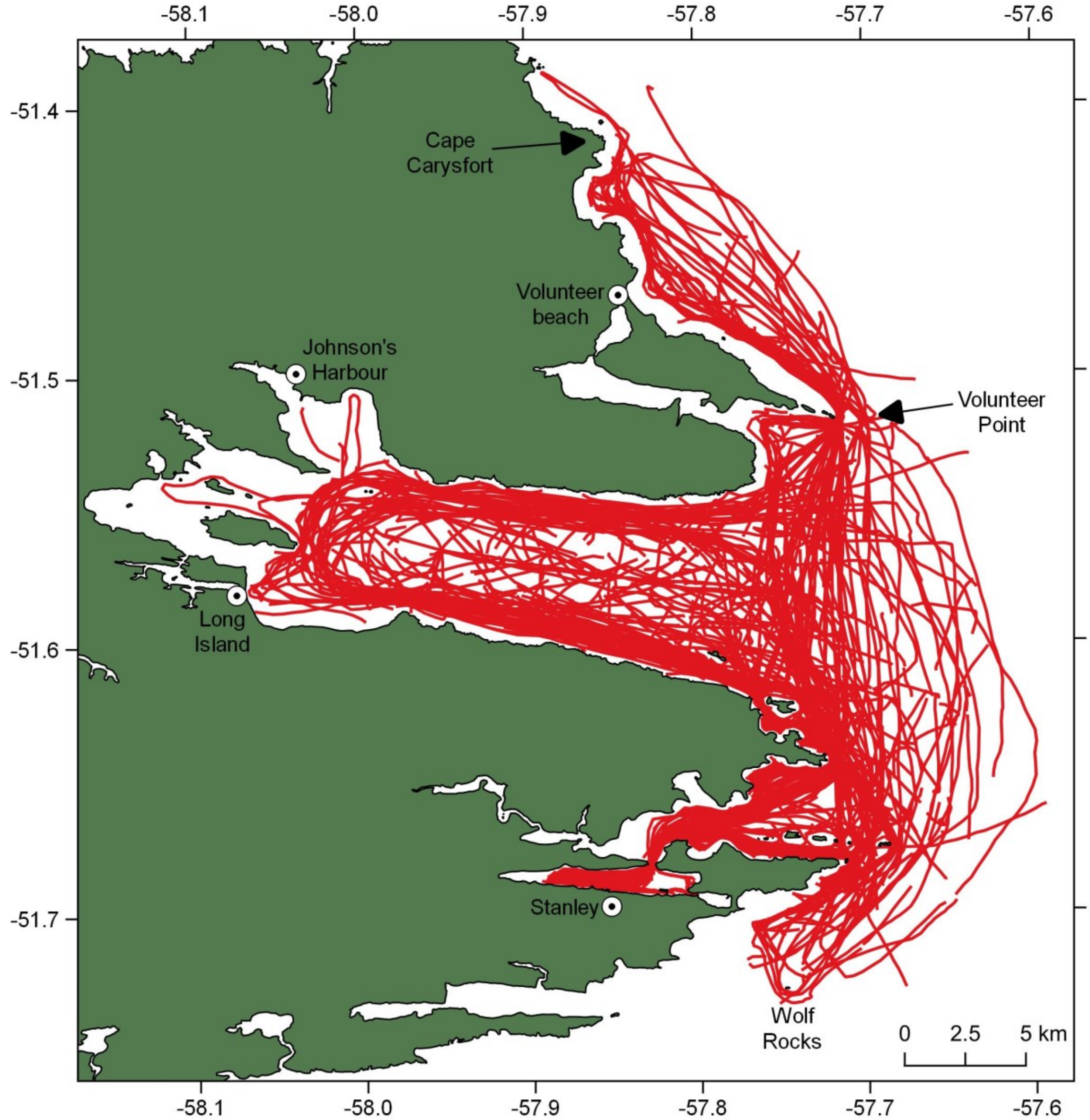
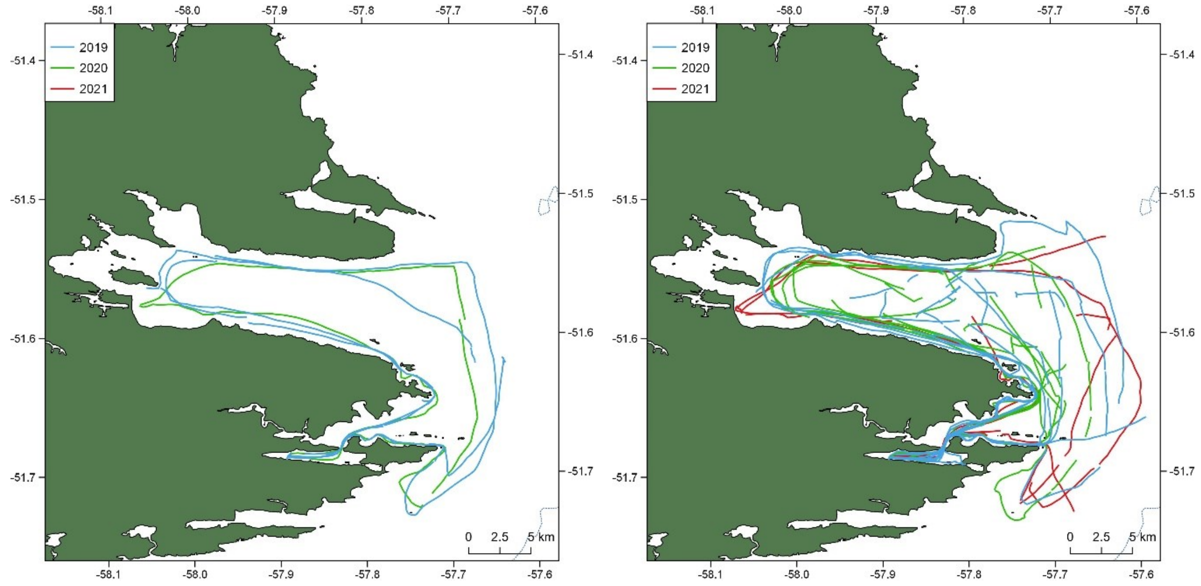


Figure 2.8. *Spatial distribution of 5893.7 km of Active Search effort collected in favourable weather conditions in the north-east of the Falklands, 2019–2021.*

(A) January (B) February



(C) March (D) April

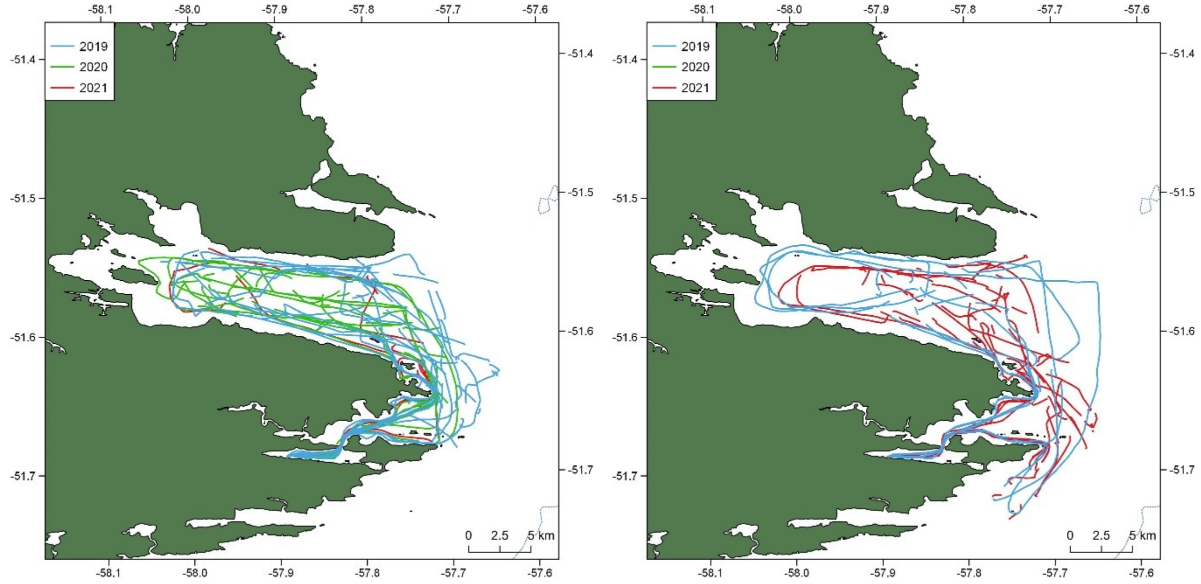
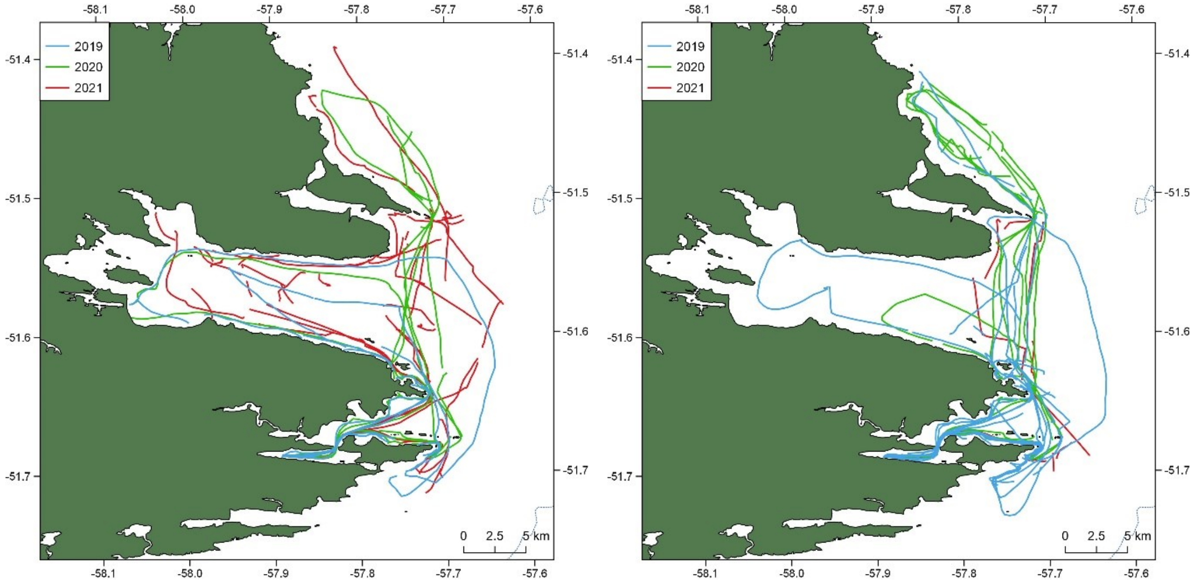


Figure 2.9*. Monthly spatial distribution of 5893.7 km of Active Search effort collected in favourable weather conditions in the north-east of the Falklands, January to September 2019–2021.*

(E) May

(F) June



(G) July

(H) August

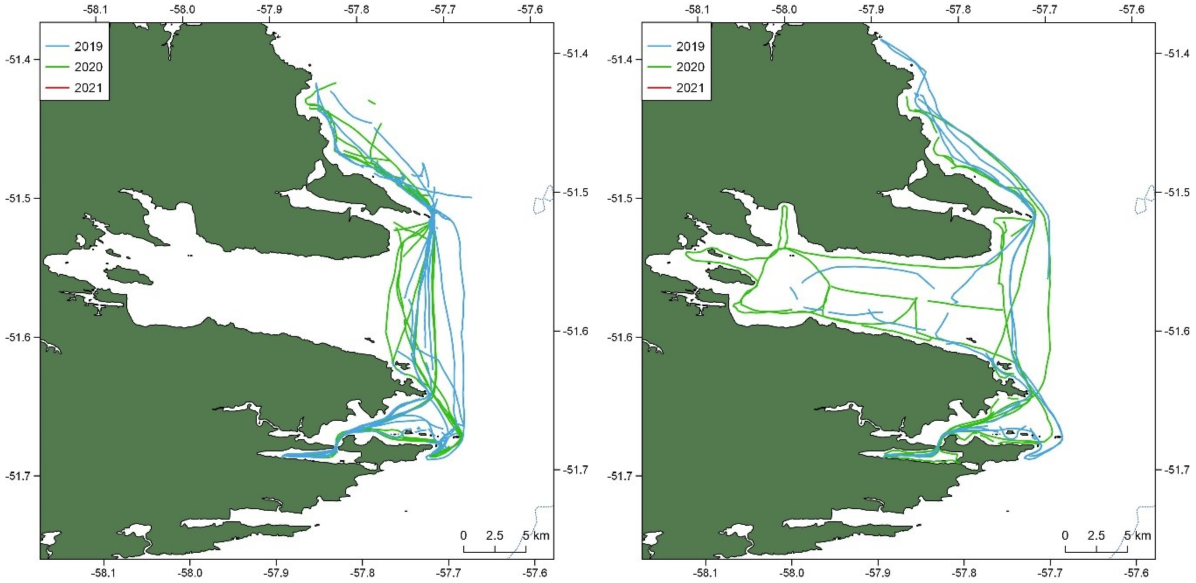


***Figure 2.9**. Contd.*

(I) September

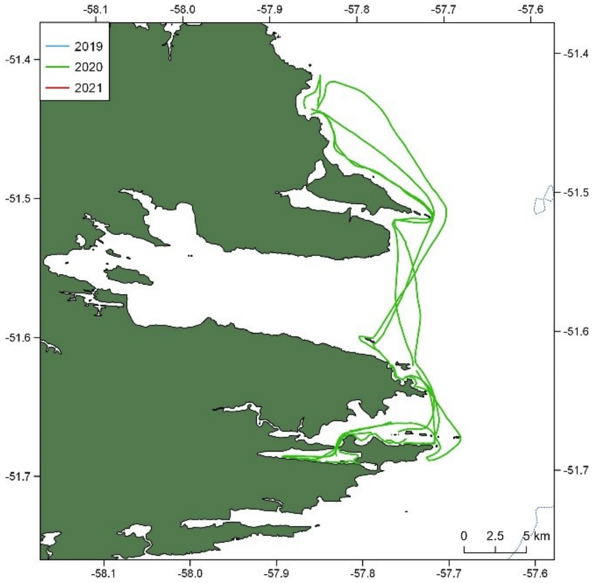


Figure 2.9. *Contd.*

2.3.2 Baleen whale occurrence

2.3.2.1 Sightings summary

A total of 1,619 cetacean sightings was recorded during the small boat survey work carried out from 2019 to 2021, comprising a best estimate of 4,821 animals of at least seven species (Table 2.3). The 1,619 sightings included four species of baleen whale, predominantly of sei whales (n = 566) and southern right whales (n = 220). The Peale's dolphin (*Lagenorhynchus australis*) was the most commonly-sighted and numerous cetacean species recorded overall during the surveys, followed by the sei whale (Table 2.3). A total of 1,183 sightings of 3,454 animals were associated with Active Search effort in favourable weather conditions (Table 2.4), and were therefore available for relative abundance calculations. That included 429 sightings of sei whales and 164 sightings of southern right whales (Table 2.4).

Consistent with the discrepancy in survey effort between the two focal study sites, a far higher number of cetacean sightings was recorded in NEF (1,389 sightings comprising 4,375 animals) compared with FS (230 sightings comprising 446 animals: Table 2.5). Additionally, there were differences in species composition between the two sites. For example, while sei whales, humpback whales and Commerson's dolphins (*Cephalorhynchus commersonii*) were recorded at both sites, southern right whales and Peale's dolphins were sighted only in NEF (Table 2.5). The number of recorded cetacean sightings also differed between years (Table 2.6), reflecting inter-annual variation in both the spatial and temporal survey coverage, and in species occurrence. For example, humpback whales were not recorded at either site during 2019 or 2020, but occurred at both sites in 2021 (Table 2.6). The temporal occurrence and spatial distributions of sei whales and southern right whales are considered below in Sections 2.3.2.2 to 2.3.2.4.

Table 2.3. *Summary of all cetacean sightings (combined Active Search, Cetacean Encounter effort and off-effort) recorded during boat surveys in the Falklands (both sites combined) during 2019–2021. The numbers of animals comprise summed survey totals that may include re-sightings of some individuals.*

Species	2019		2020		2021		2019–2021	
	Sightings	Animals	Sightings	Animals	Sightings	Animals	Sightings	Animals
Sei whale	190	326	136	251	240	518	566	1,095
Southern right whale	98	219	97	214	25	47	220	480
Humpback whale	0	0	0	0	18	21	18	21
Minke whale	0	0	1	1	0	0	1	1
Unidentified baleen whale	42	53	14	19	62	78	118	150
Peale's dolphin	312	1,365	171	640	120	656	603	2,661
Dusky dolphin	4	4	1	1	2	2	7	7
Commerson's dolphin	32	128	34	167	19	110	85	405
Unidentified dolphin	0	0	1	1	0	0	1	1
Total	*678*	*2,095*	*455*	*1,294*	*486*	*1,432*	*1,619*	*4,821*

Table 2.4. *Summary of cetacean sightings by effort status during boat-based surveys in the Falklands (both sites combined) during 2019–2021. The numbers of animals comprise summed survey totals that may include re-sightings of some individuals.*

Species	Active Search (all)		Active Search (favourable weather)		Cetacean Encounter effort		Off effort		Group size		
	Sightings	Animals	Sightings	Animals	Sightings	Animals	Sightings	Animals	Mean	Range	SD
Sei whale	429	818	429	818	87	163	50	114	1.9	1–12	1.40
Southern right whale	181	384	164	343	29	66	10	30	2.2	1–17	2.00
Humpback whale	15	18	15	18	2	2	1	1	1.2	1–2	0.38
Minke whale	1	1	1	1	0	0	0	0	1	–	–
Unidentified baleen whale	97	127	97	127	18	20	3	3	1.3	1–3	0.52
Peale's dolphin	442	1921	417	1,846	145	689	16	51	4.4	1–55	3.90
Dusky dolphin	3	3	3	3	4	4	0	0	1	–	–
Commerson's dolphin	57	298	57	298	14	43	14	64	4.8	1–27	5.46
Unidentified dolphin	0	0	0	0	1	1	0	0	1	–	–
Total	*1,225*	*3570*	*1,183*	*3,454*	*300*	*988*	*94*	*263*	*N/A*	*N/A*	*N/A*

***Table 2.5**. Summary of cetacean sightings by effort status during boat surveys at two focal study sites in the Falklands during 2019–2021. The numbers of animals comprise summed survey totals that may include re-sightings of some individuals.*

Species	Active search (all)		Active search (favourable weather)		Encounter effort		Off effort		Group size		
	Sightings	Animals	Sightings	Animals	Sightings	Animals	Sightings	Animals	Mean	Range	SD
Falkland Sound											
Sei whale	142	227	142	227	20	27	6	17	1.6	1–12	1.22
Humpback whale	2	3	2	3	0	0	1	1	1.3	1–2	0.58
Unid. baleen whale	31	38	31	38	5	5	3	3	1.2	1–2	0.39
Commerson's dolphin	10	68	10	68	4	27	6	30	6.8	1–27	7.77
Total	*185*	*336*	*185*	*336*	*29*	*59*	*16*	*51*	*N/A*	*N/A*	*N/A*
North-east Falklands											
Sei whale	287	591	287	591	67	136	44	97	2.1	1–9	1.45
Southern right whale	181	384	164	343	29	66	10	30	2.2	1–17	2.00
Humpback whale	13	15	13	15	2	2	0	0	1.1	1–2	0.35
Minke whale	1	1	1	1	0	0	0	0	1	–	–
Unid. baleen whale	66	89	66	89	13	15	0	0	1.3	1–3	0.57
Peale's dolphin	442	1,921	417	1,846	145	689	16	51	4.4	1–55	3.90
Dusky dolphin	3	3	3	3	4	4	0	0	1	–	–
Commerson's dolphin	47	230	47	230	10	16	8	34	4.9	1–26	5.57
Unid. dolphin	0	0	0	0	1	1	0	0	1	–	–
Total	*1,040*	*3,234*	*998*	*3,118*	*271*	*929*	*78*	*212*	*N/A*	*N/A*	*N/A*

***Table 2.6**. Summary of cetacean sightings recorded by year during periods of Active Search effort in favourable weather conditions at two focal study sites in the Falklands during 2019–2021. The numbers of animals comprise summed survey totals that may include re-sightings of some individuals.*

Species	2019		2020		2021		2019–2021	
	Sightings	Animals	Sightings	Animals	Sightings	Animals	Sightings	Animals
Falkland Sound								
Sei whale	55	80	17	25	70	122	142	227
Humpback whale	0	0	0	0	2	3	2	3
Unidentified baleen whale	13	15	1	1	17	22	31	38
Commerson's dolphin	6	41	0	0	4	27	10	68
Total	*74*	*136*	*18*	*26*	*93*	*174*	*185*	*336*
North-east Falklands								
Sei whale	89	161	81	158	117	272	287	591
Southern right whale	71	151	74	157	19	35	164	343
Humpback whale	0	0	0	0	13	15	13	15
Minke whale	0	0	1	1	0	0	1	1
Unidentified baleen whale	16	24	13	18	37	47	66	89
Peale's dolphin	189	849	137	529	91	468	417	1,846
Dusky dolphin	1	1	1	1	1	1	3	3
Commerson's dolphin	16	53	25	137	6	40	47	230
Total	*382*	*1,239*	*332*	*1,001*	*284*	*878*	*998*	*3,118*

2.3.2.2 Sei whale

A total of 566 sei whale sightings (1,095 animals) was recorded across the three years of the project (Table 2.3), of which most (429 sightings, 818 animals) were associated with Active Search effort (Table 2.4).

The best estimate of sei whale group size ranged from 1 to 12 animals (Figure 2.10), with an overall mean of 1.9 animals (Table 2.4). Over 50% of the total sightings comprised single animals, and a further 43% of sightings were of small groups of 2 to 4 animals (Figure 2.10). The proportion of sightings comprising single animals was much higher at FS (67.3%) than at NEF (45.4%: Figure 2.10). Similarly, the median group size was significantly different between the two sites (Mann-Whitney test, W=41018.5, p=<0.001), with mean group size being higher at NEF (2.1 animals) than at FS (1.6 animals; Table 2.5). Kruskal-Wallis tests revealed no significant difference in group size by month in FS (*H*=3.9, df=4, *P*=>0.05), but a highly significant difference at NEF (*H*=19.2, df=4, *P*=0.001). Mann-Whitney U-tests indicated that the differences in group size at NEF existed between March and all other months (February, April, May and June). In all cases, March had a significantly lower group size than the other months (Figure 2.11).

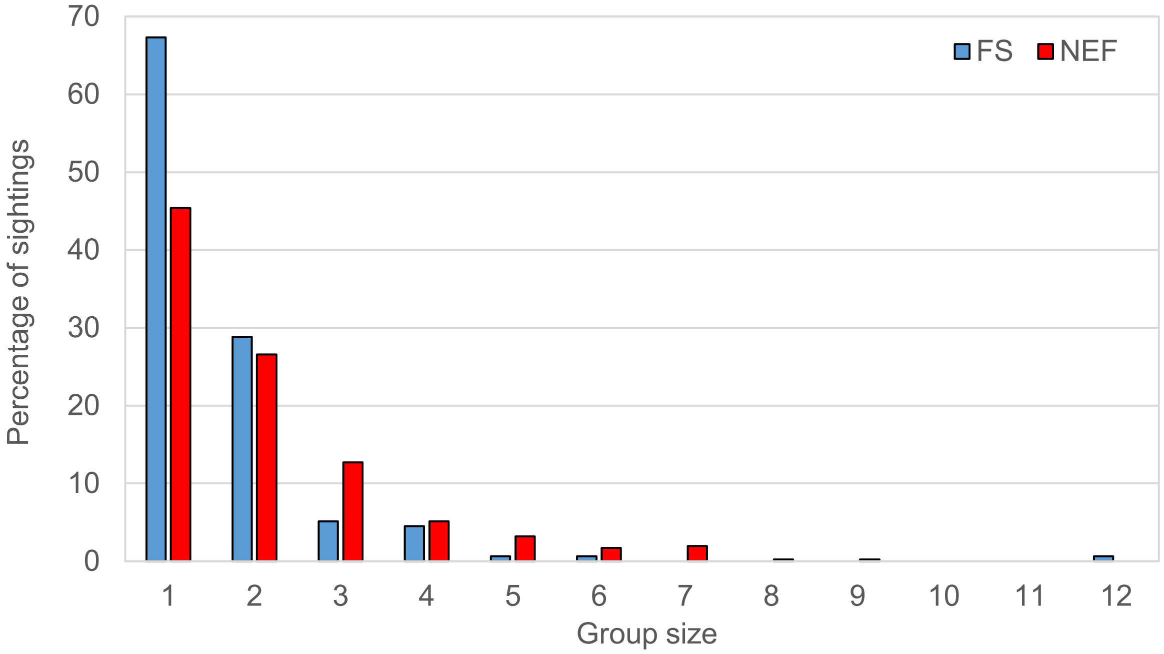


Figure 2.10. *Visual estimates of sei whale group size in Falkland Sound (FS) and the north-east Falklands (NEF).*

Individuals identified as calves (based on small body size and close association with an adult: Figure 2.12) were noted during 18 sightings, occurring in February, March and April. Most sei whale sightings comprised animals that were travelling or presumed to be foraging subsurface (based on arched dives, unpredictable directions of travel, and the presence of clouds of faecal matter). Surface foraging (both lunges and skimming) was also exhibited by sei whales under particular conditions when the sea was very calm and the sky overcast (e.g. Figure 2.12).

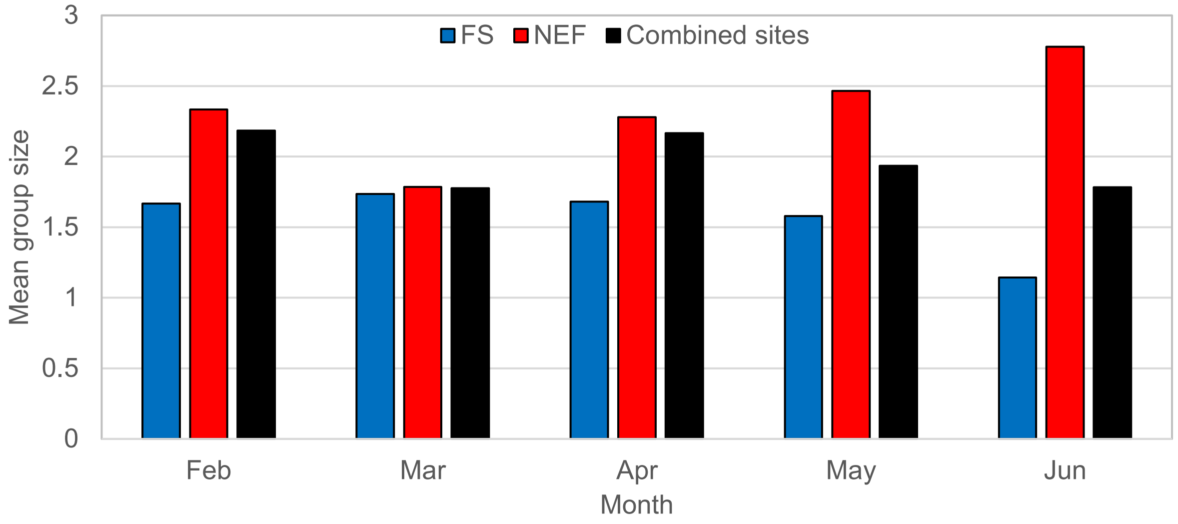


Figure 2.11. *Visual estimates of sei whale group size by month in Falkland Sound (FS) and the north-east Falklands (NEF). January was excluded from the comparison in NEF, since it had only one sighting.*

The occurrence of sei whales in Falklands' waters varied markedly between years, with the relative abundance (both sightings/km and individuals/km) being approximately three times higher during 2021 compared to 2019 and 2020 (Figure 2.13). Sei whale occurrence also showed strong seasonal variation. Considering both sites combined, the relative abundance of sei whales was highest between February and May, with a strong seasonal peak evident during March and April (Figure 2.14). When the two study sites were considered separately, a strong seasonal peak during March and April was still apparent at NEF, but the seasonality at FS was less consistent (Figure 2.15). At FS the relative abundance during March and April was lower than in February and May. Generally, the seasonality in the NEF dataset is supported by higher overall amounts, and a better seasonal spread, of survey effort (see Figure 2.5), and is therefore considered to be more representative of sei whale seasonality in the Falklands than the dataset for FS. Nevertheless, some differences in sei whale seasonal occurrence likely occur between the sites. For example, a longer temporal span of peak occurrence was evident at FS compared to NEF (Figure 2.15).

The survey data also support inter-annual variation in the temporal occurrence of sei whales. In FS, the available survey coverage was too sparse and too variable between years to provide a robust indication of seasonality in sei whale relative abundance (Figure 2.16). However, in NEF it was apparent that the seasonality of sei whales during 2019 and 2020 differed markedly from 2021 (Figure 2.17). While all years exhibited similar values for February, the occurrence of sei whales had a strong seasonal peak during March in 2019 and 2020. No data were available for April 2020 due to the COVID-19 lockdown, but in both years the relative abundance of sei whales was zero from May onwards. In contrast, during 2021 the relative abundance peaked during April and remained high through May and into early June (Figure 2.17).

Figure 2.12. *Images of sei whales taken during DPLUS082, including a group of three animals travelling together (top), a mother with her calf surfacing alongside (centre), and a surface-lunge during feeding (bottom).*

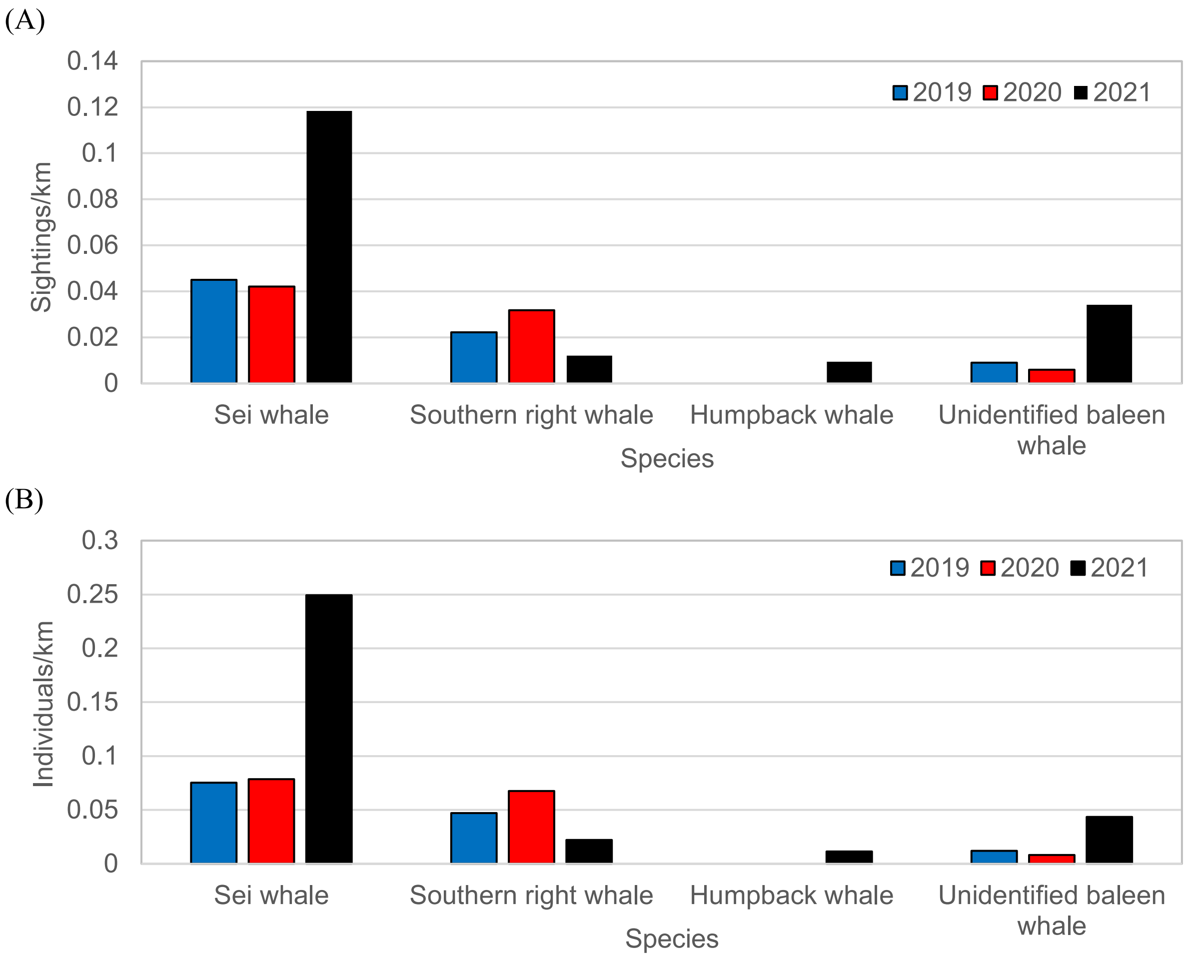


Figure 2.13. *Relative abundance of whale species in Falklands' nearshore waters (both sites combined) during boat surveys from 2019 to 2021: (A) sightings/km; and (B) individuals/km. Relative abundance was calculated using 7,104.1 km of Active Search data collected in favourable weather conditions.*

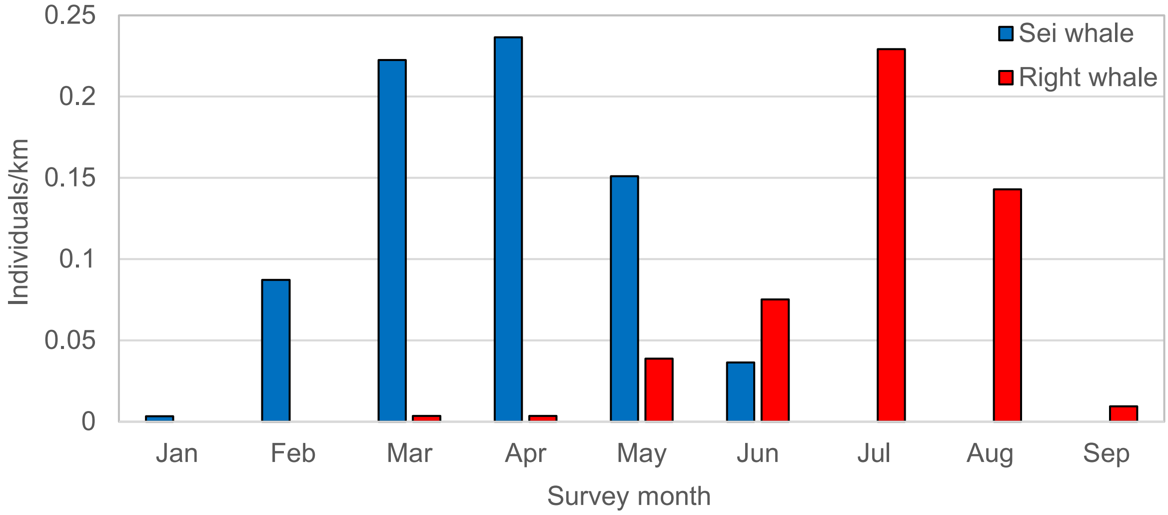


Figure 2.14. *Monthly relative abundance (individuals/km) of sei whales and southern right whales recorded during small boat surveys at two sites (combined) from 2019 to 2021. Relative abundance was calculated using 7,104.1 km of Active Search effort collected in favourable weather conditions.*

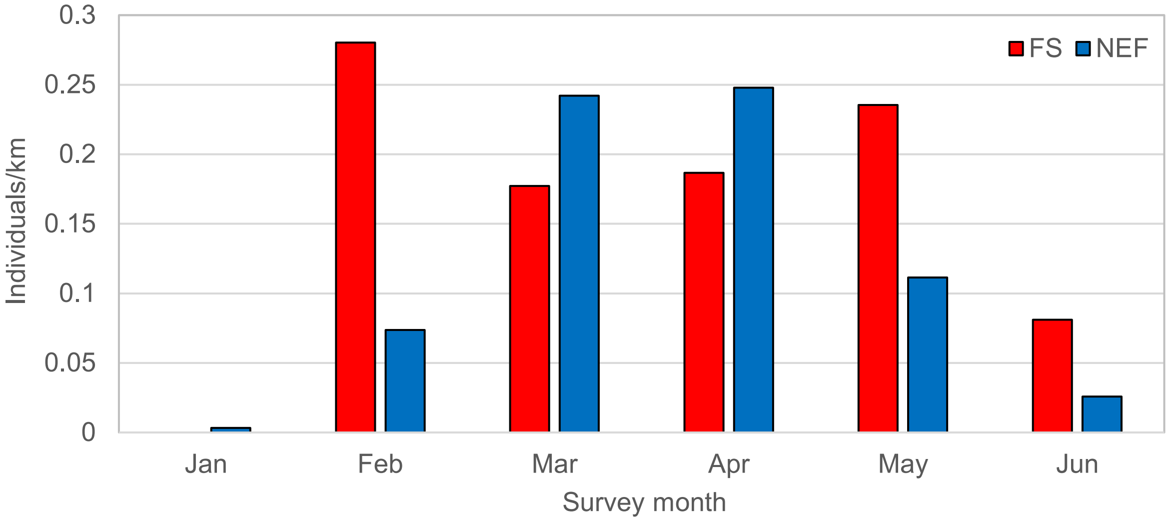


Figure 2.15. *Monthly relative abundance (individuals/km) of sei whales at Falkland Sound (FS) and the North-east Falklands (NEF) from 2019 to 2021. Relative abundance was calculated using 7,104.1 km of Active Search effort collected in favourable weather conditions. No sei whale sightings were recorded during surveys from July to September, and consequently those data are not plotted. No coverage was available for FS in January.*

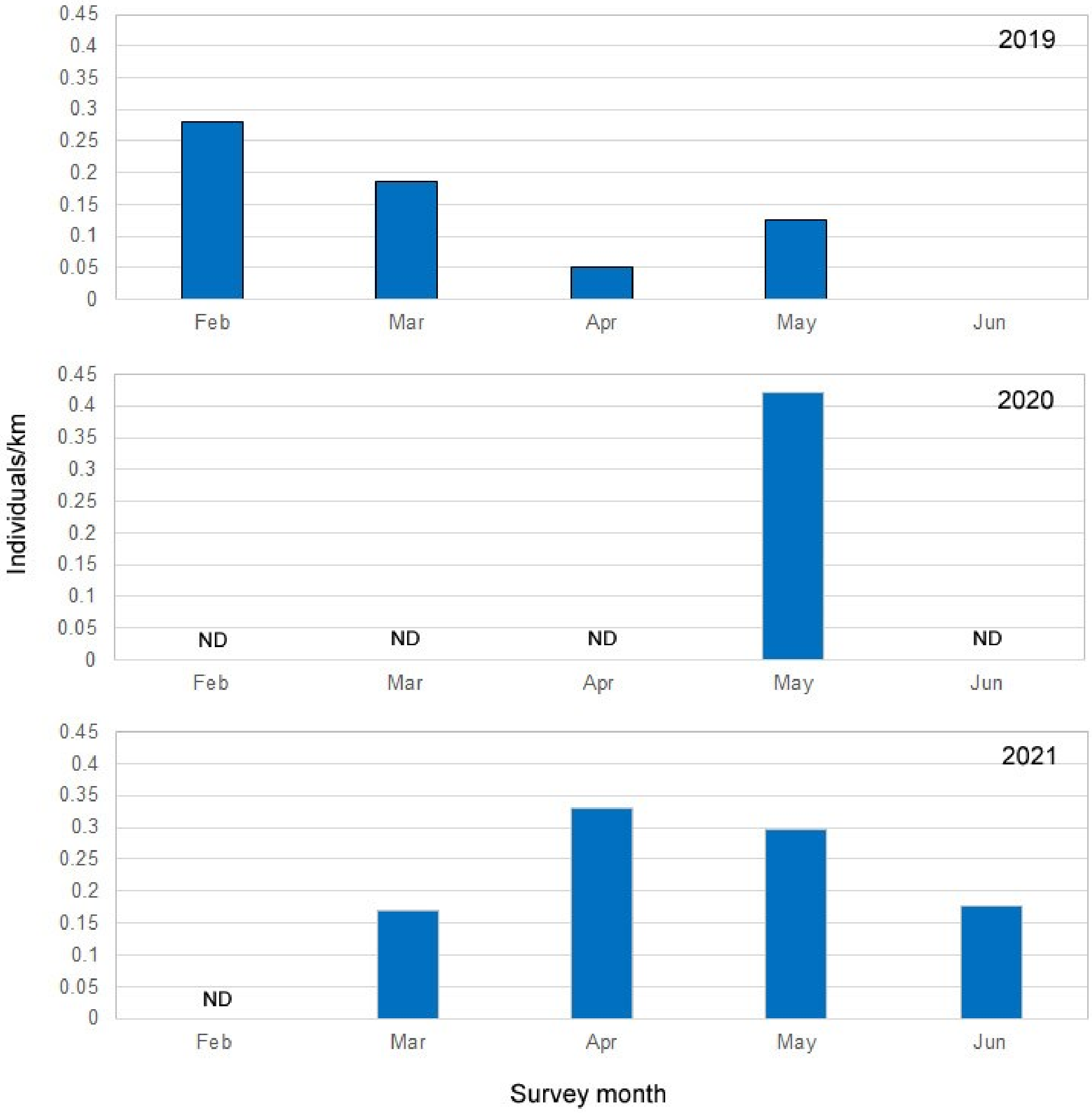


Figure 2.16. *Monthly relative abundance (individuals/km) of sei whales at Falkland Sound during each of the survey years from 2019 to 2021. Months where no survey coverage was carried out are shown as ND – No Data.*

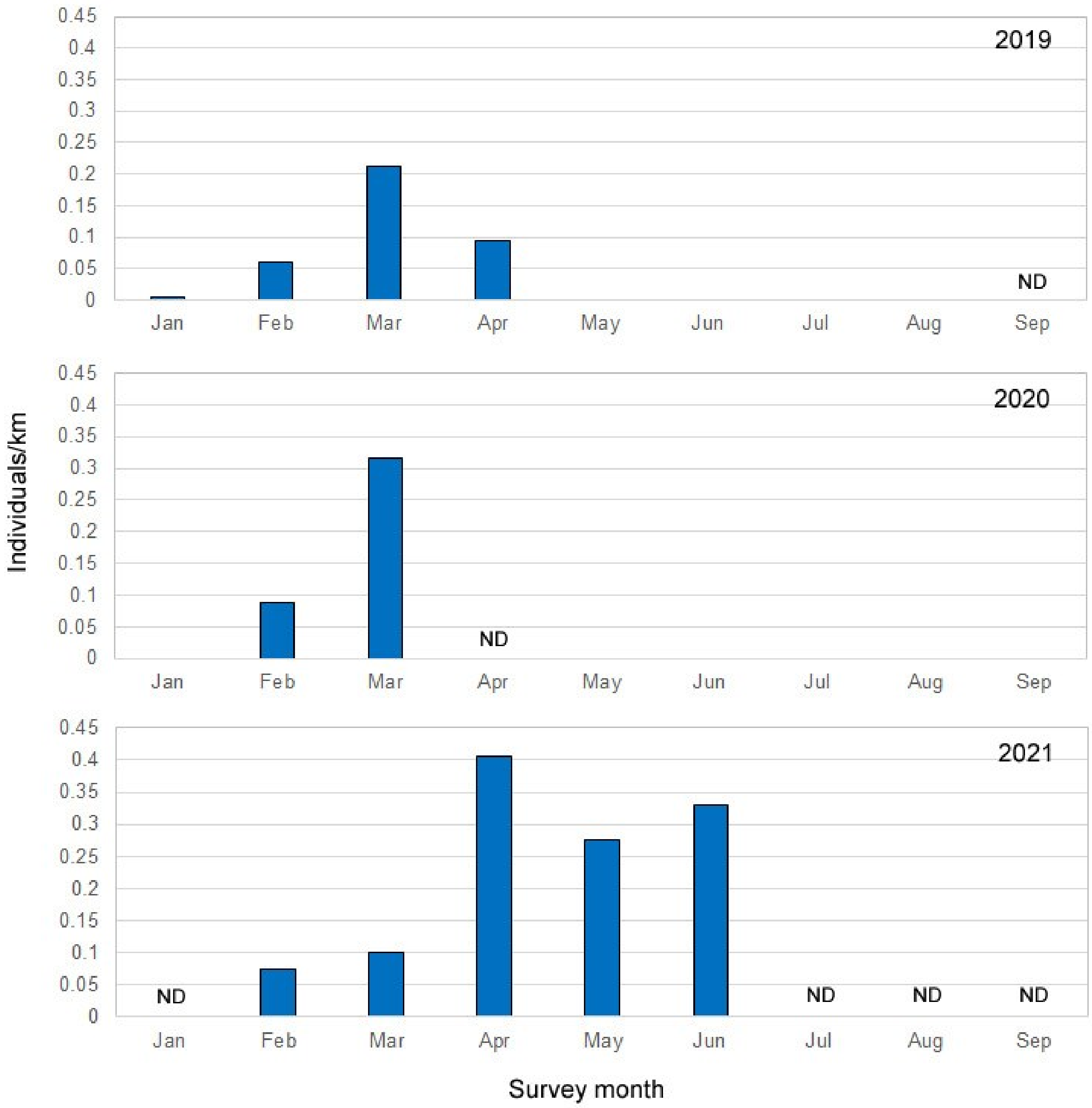


***Figure 2.17**. Monthly relative abundance (individuals/km) of sei whales off the North-east Falklands during each of the survey years from 2019 to 2021. Months where no survey coverage was carried out are shown as ND – No Data.*

In FS, sei whale sightings were widespread, including some that were located at the northernmost and southernmost limits of the surveyed area. These highlight that sei whales are likely to have a wider distribution in FS than was indicated by the spatially-restricted survey coverage (Figure 2.18). Sightings were most numerous in the expanse of Grantham Sound, where they occurred inshore to the north of Rabbit Island at the mouth of Brenton Loch (opportunistic sightings of sei whales were also reported inside the confines of Brenton Loch during the project). However, it should be noted that Grantham Sound also received the highest amount of survey coverage due to its close proximity to New Haven and relatively sheltered location in some wind directions. The north channel of Falkland Sound was well used by sei whales, including the entrance to San Carlos Water. In particular, the area between West Falkland and the North-west Islands produced many sightings. The southern part of the study area received less survey effort than the central or northern areas, but several sightings were recorded between Swan Island and the Tyssen Islands (Figure 2.18). The western side of the southern study area (i.e. the waters around Swan Island, to Port Howard and Bold Point) received relatively little survey coverage, and the lack of sightings in that region may well reflect low effort rather than an absence of sei whales; the species is sometimes reported anecdotally in that area by passengers on the New Haven–Port Howard ferry. The mean water depth of sei whale sightings in FS was 34.2 m (Table 2.7).

The spatial distribution of sei whales in FS by month is shown in Figures 2.19–2.23. Interpretation of the figures needs to consider the discrepancies in survey effort at FS during different months, with the coverage being most comprehensive during March and May (Table 2.2). The use of relative abundance does account for variation in the total amounts of survey effort, but clearly the variation in the extent of the spatial coverage achieved in each month (see Figure 2.7) also affects the reported whale distributions.

During February, the survey effort was limited to the central portion of the study area; sei whales were widely-distributed within that region (Figure 2.19). March received much higher spatial coverage; sei whales were encountered throughout the central and northern regions of FS, particularly in the area between Brenton Loch and Bold Point (including Grantham Sound: Figure 2.20). The encounter tracks indicate that within individual sightings, whales were moving quite widely and rapidly within this area. A single survey of the southern study area during March 2021 produced one sei whale sighting to the north-east of the Tyssen Islands (Figure 2.20); although no whales were encountered in other southern areas, this may partly reflect the low amount of survey effort and the fact that this area was surveyed in March during only one year.

Although only two surveys were carried out in FS during April, the resulting survey coverage was reasonably widespread (see Figure 2.7). Sei whales were distributed mostly along the eastern half of FS, with no sightings in the western portion except in the far north of the study area (Figure 2.21).

As noted in Section 2.3.1.2, May was the only month where survey effort occurred at FS in all three of the survey years (Figure 2.7). Sei whales were widespread across the study area in May, with over half of the 4 km^2 grid cells producing relative abundance values ranging from 0.08 to 0.87 individuals/km (Figure 2.22). Sightings were most numerous in the central area between New Haven, Bold Point and Grantham Sound. However, sei whales were also encountered frequently in the north channel, especially on the west side between Many Branch and Jersey Point. This month also produced the highest relative abundances in the grid cells located in the south-east part of the study area, between the Tyssen Islands, Swan Island, and seaward of Egg Harbour (Figure 2.22).

In June, single surveys occurred in FS during 2019 and 2021 (Figure 2.7). There were no confirmed sightings of sei whales on the June 2019 survey, although a blow from an unidentified large baleen whale was seen that was likely to have been a sei whale. However, in June 2021 a good number of sei whales were still present in FS; they were distributed primarily along the eastern half of FS with no sightings confirmed along the west side (Figure 2.23).

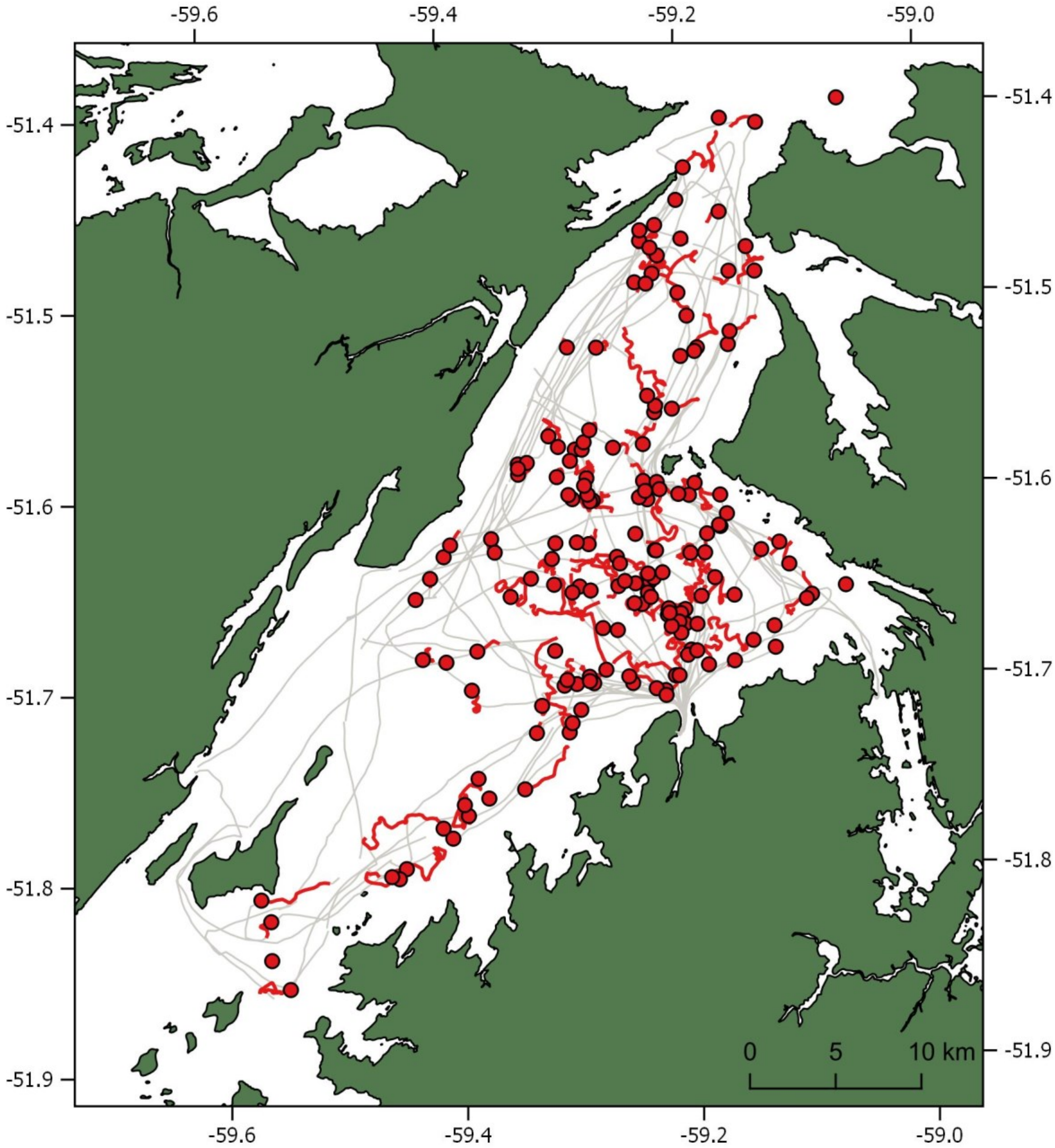


***Figure 2.18**. Spatial distribution of sei whale sightings (all records, n = 168: circles), sei whale encounter effort (red lines: 372.5 km) and Active Search effort in favourable conditions (grey lines: 1,210.4 km) in Falkland Sound, 2019–2021. Sighting locations have been recalculated to reflect animal positions rather than location of the boat.*

***Table 2.7**. Water depths at recalculated analysis sighting positions (see Section 2.2.4) for cetacean species encountered during boat surveys in the Falklands during 2019–2021. Only species with ≥10 sightings are presented. Water depths were acquired from bathymetric data provided by Premier Oil and converted into a 500 m grid cell raster using QGIS; consequently, depths represent central values for 500 m grid cells.*

Species	Combined sites			Falkland Sound			North-east Falklands		
	Mean	Range	n	Mean	Range	n	Mean	Range	n
Sei whale	31.9	0.6-75.7	566	34.2	15.3-57.2	168	31.0	0.6-75.7	398
Southern right whale	22.2	0.8-54.9	220	–	–	0	22.2	0.8-54.9	220
Humpback whale	20.4	0.8-41.7	18	30.3	22.6-37.8	3	18.4	0.8-41.7	15
Unidentified baleen whale	34.1	9.0-67.3	118	33.5	11.7-54.3	39	34.3	9.0-67.3	79
Peale's dolphin	19.1	0.7-67.3	603	–	–	0	19.1	0.7-67.3	603
Commerson's dolphin	13.7	1.1-54.3	85	23.7	1.1-54.3	20	10.6	4.0-28.8	65

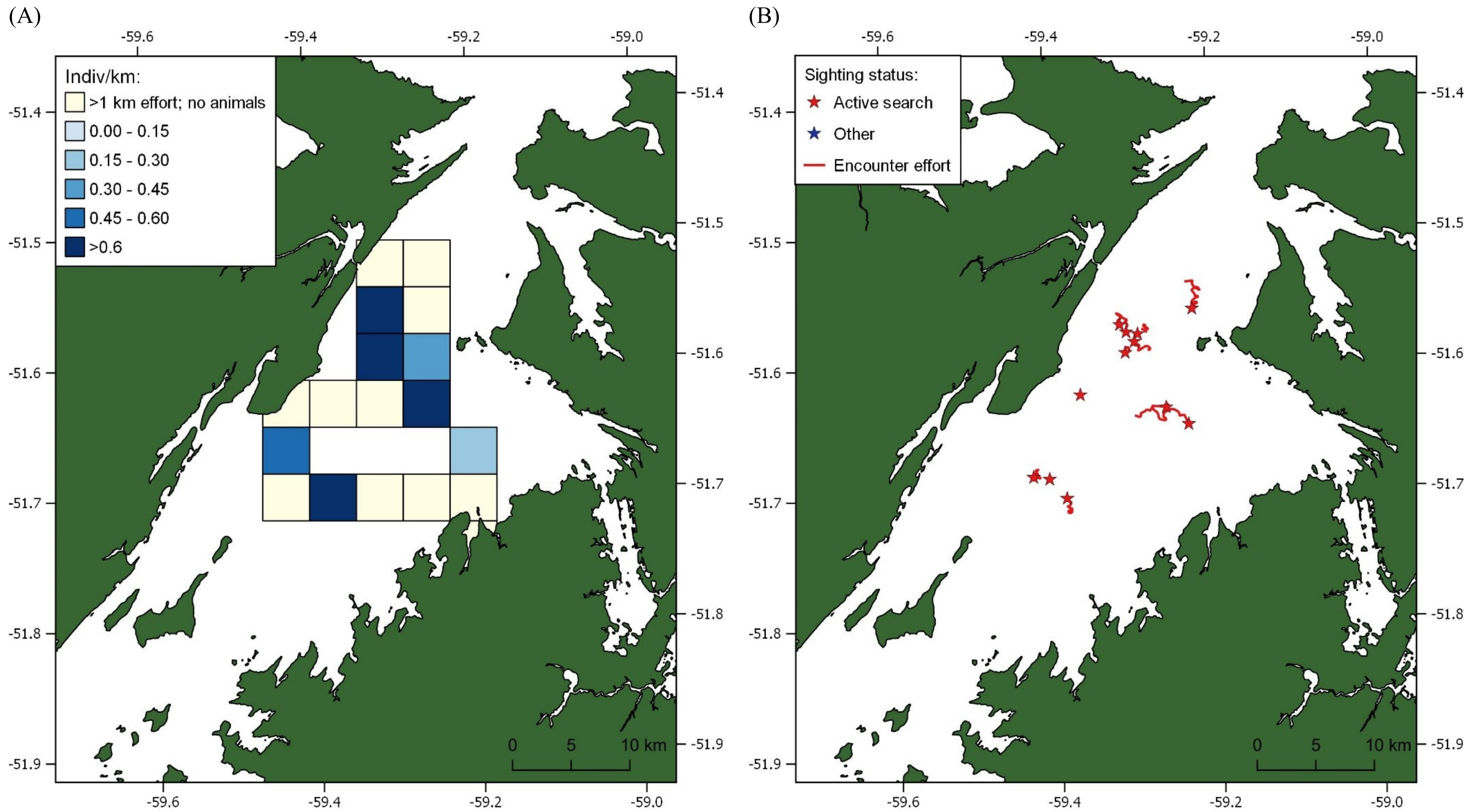


***Figure 2.19**. Distribution of sei whales in Falkland Sound during February (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

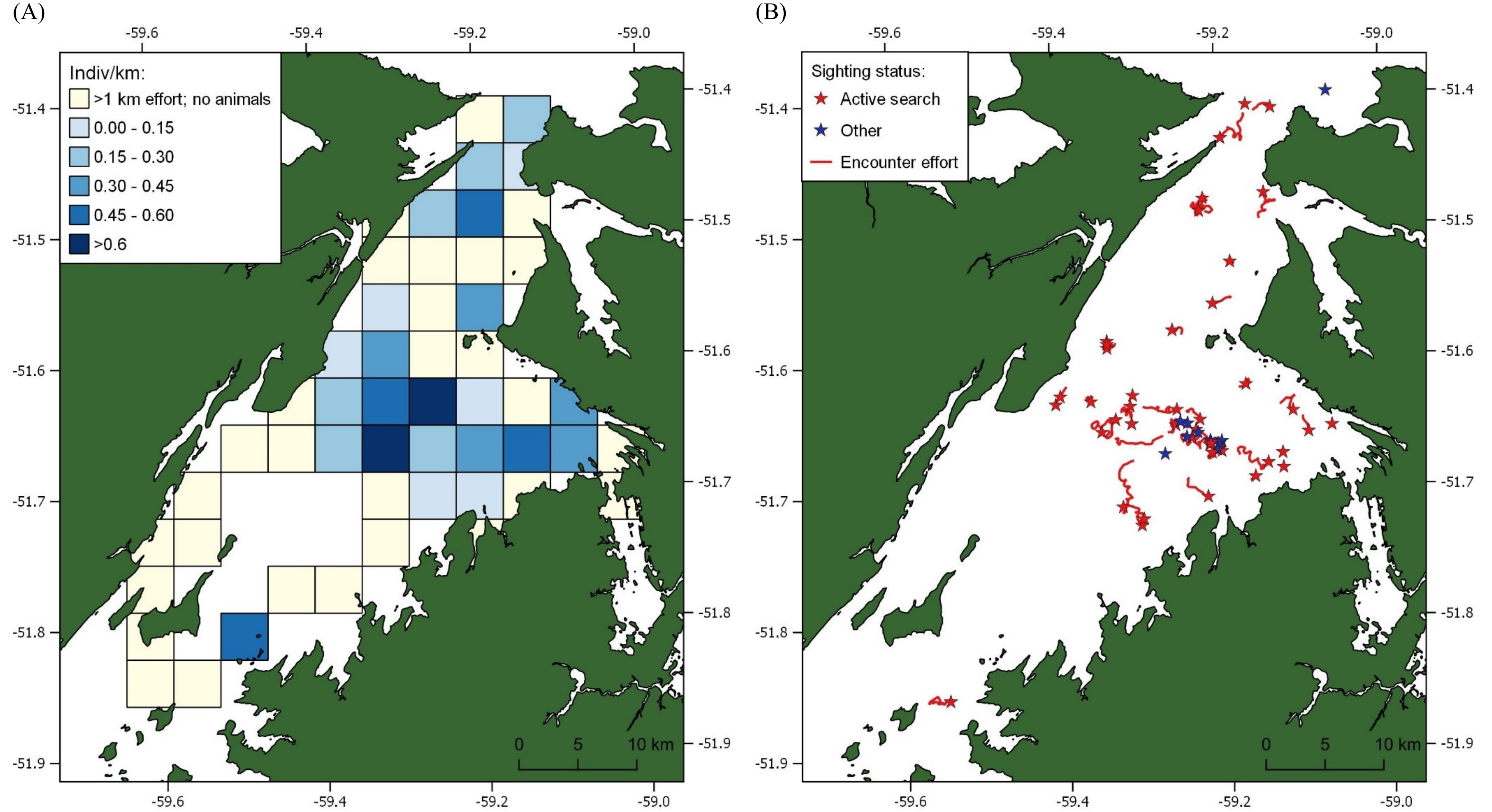


Figure 2.20. *Distribution of sei whales in Falkland Sound during March (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

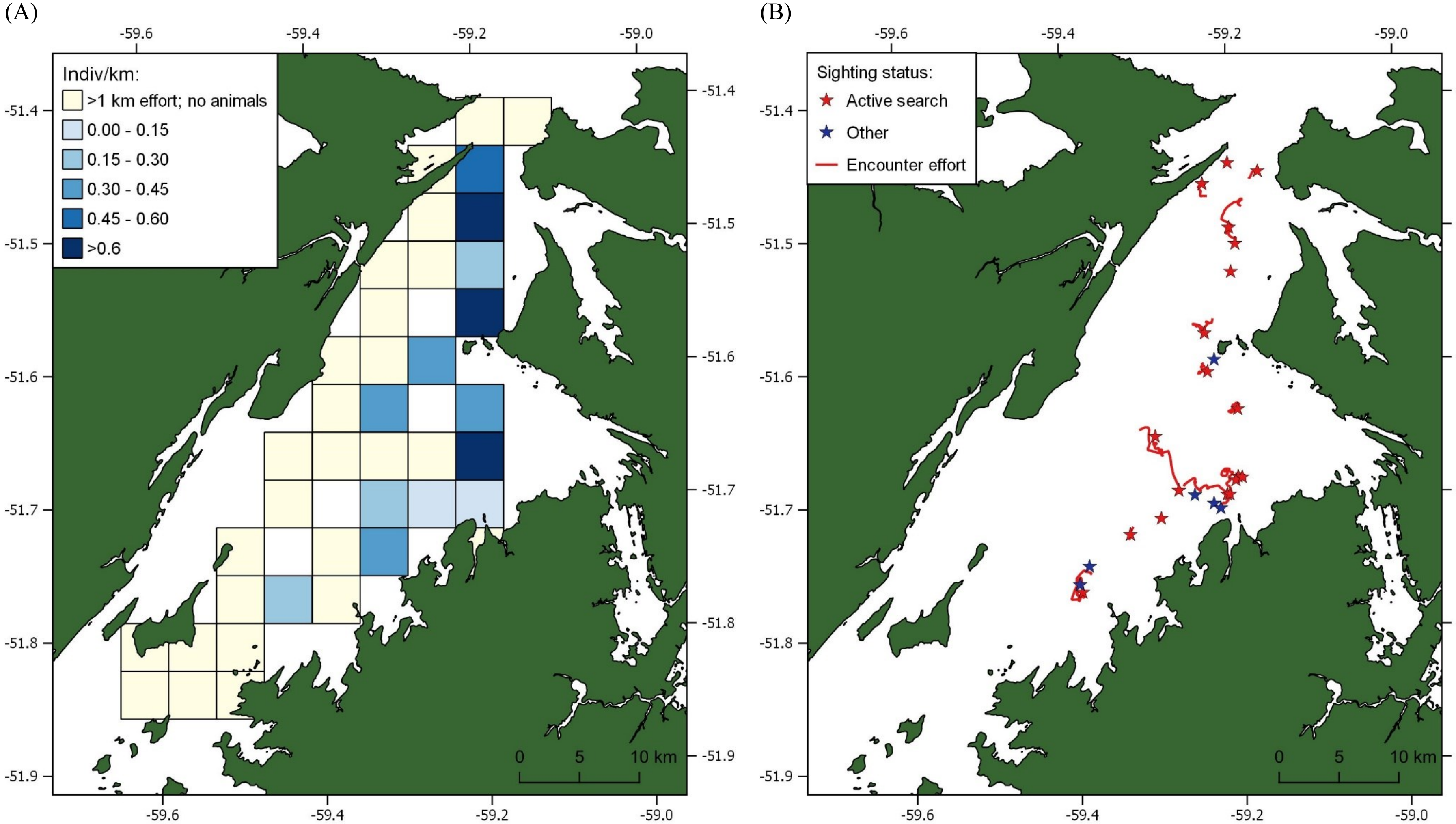


Figure 2.21. *Distribution of sei whales in Falkland Sound during April (2019–2021 combined): (A) 4 km² grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

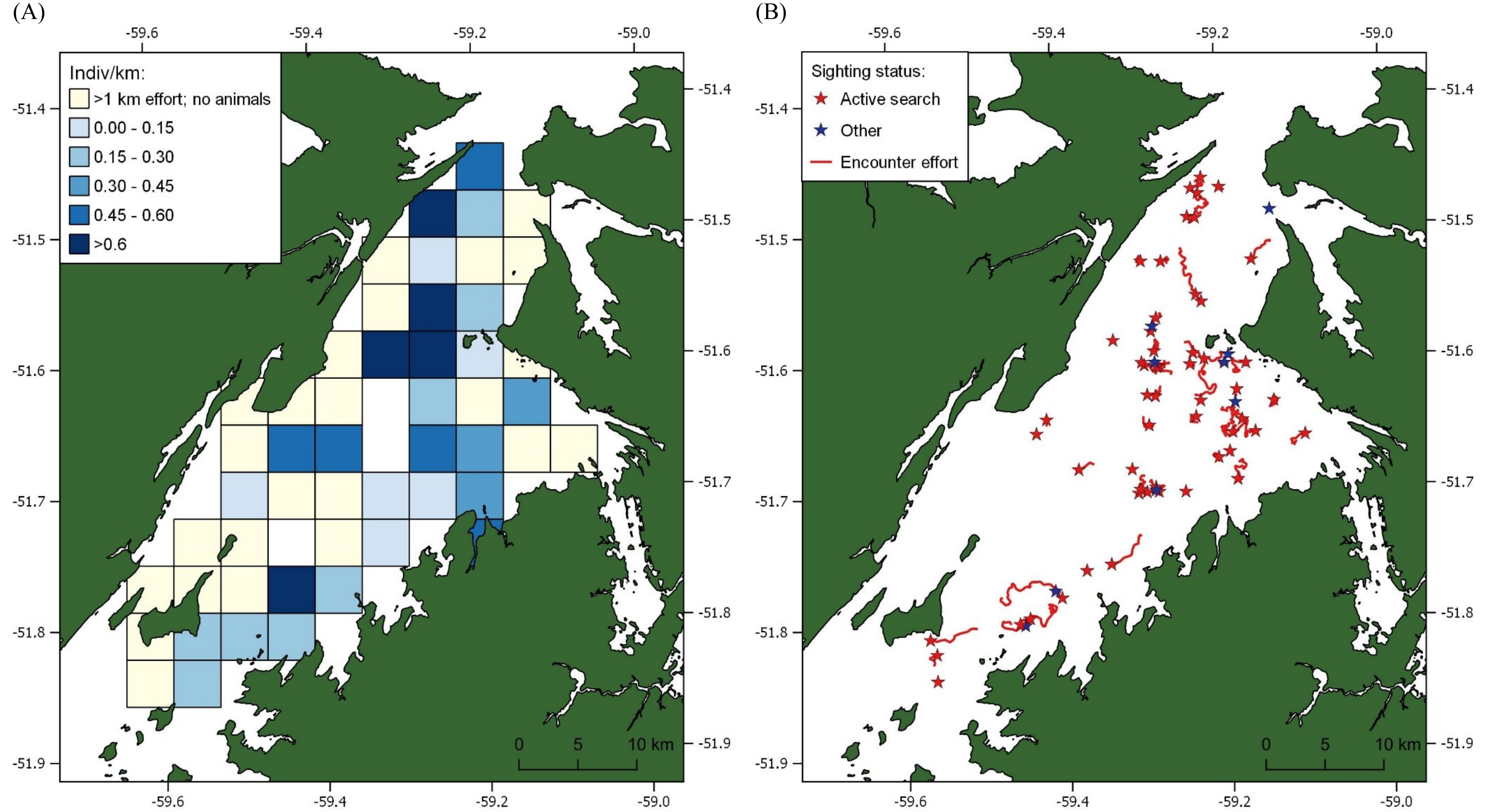


Figure 2.22. *Distribution of sei whales in Falkland Sound during May (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

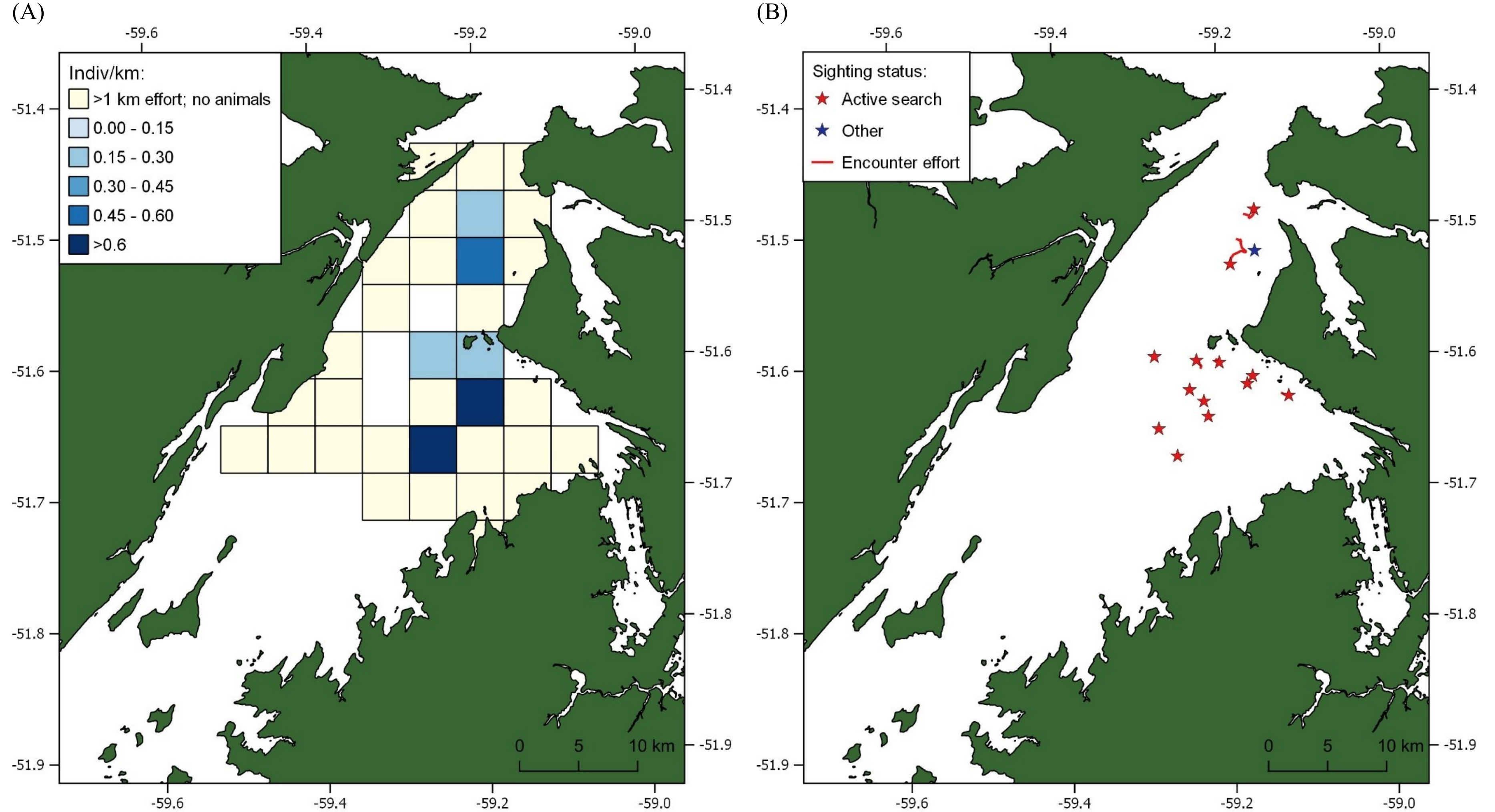


Figure 2.23. *Distribution of sei whales in Falkland Sound during June (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

In NEF, sei whale sightings occurred predominantly in Berkeley Sound, including the more open waters off Mengeary Point and Kidney Island, located close to the entrance to the Sound (Figure 2.23). Sei whales were seen throughout Berkeley Sound, as far as the innermost area where water depths of >10 m occur between Long Island and Duclos Point. However, sightings inside Port William were scarce, despite high survey coverage and the presence of suitable water depths. Sightings were also relatively infrequent in the outermost surveyed areas (Figure 2.23). However, those regions received the least survey coverage and tended to be surveyed on dates where whales were not found in Berkeley Sound and time allowed exploration of additional areas; it is plausible that whales were simply not present in the wider area at all on those dates. Similarly, the lack of sei whale sightings in the area north of Volunteer Point reflects a lack of survey effort in that region during the peak sei whale season (Feb–Apr); this area was predominantly targeted during winter for southern right whales. The mean water depth of sei whale sightings in NEF was 31.0 m (Table 2.7).

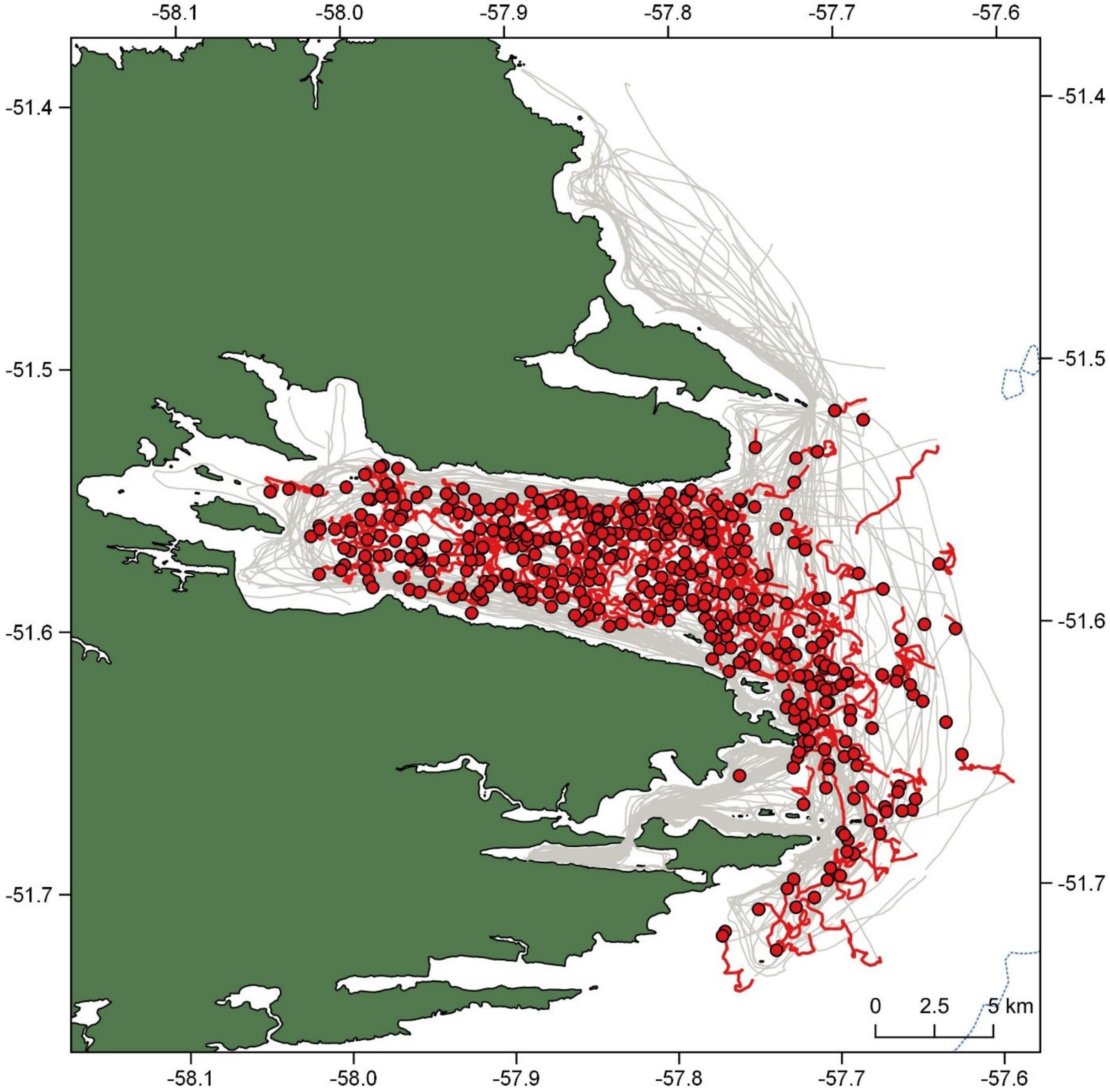


__Figure 2.24__. Spatial distribution of sei whale sightings (all records, n = 398: circles), sei whale encounter effort (red lines: 843.4 km), and Active Search effort in favourable conditions (grey lines: 5893.7 km), in the North-east Falklands 2019–2021. Sighting locations have been recalculated to reflect animal positions rather than location of the boat.

The spatial distribution of sei whales in NEF by month is shown in Figures 2.25–2.30. During January, sei whales were scarce in the study area, and none were encountered within Berkeley Sound during January surveys in 2019 and 2020; the only sighting recorded during January in NEF was located offshore of Kidney Island and occurred at the very end of the month on 31 January 2019 (Figure 2.25).

During February and March, Berkeley Sound received good survey coverage in all three of the survey years (Figure 2.9). In February, sei whale distribution was concentrated in the more open coastal waters located offshore of the mouth of Berkeley Sound, and in the outer half of Berkeley Sound to the west of Cochon Island and Eagle Point (Figure 2.26). Relatively few sightings were recorded in the innermost half of Berkeley Sound (Figure 2.26). In contrast, during March the spatial distribution of sei whales expanded to include the entirety of Berkeley Sound, with some of the highest relative abundance values recorded in the innermost grid cells to the west of Monkey Point and Strike Off Point, including the waters of Uranie Bay, east of Long Island, and south of Johnson Harbour (Figure 2.27). Sei whales also used the waters of outer Port William during March (Figure 2.27).

Due to the COVID-19 pandemic, survey effort for April occurred only in 2019 and 2021; however, Berkeley Sound was extensively covered in both of those years (Figure 2.9). The distribution of sei whales in April showed a seaward contraction compared to March, with little activity in the innermost part of Berkeley Sound (Figure 2.28). Rather, most sightings and the grid cells of highest relative abundance, were located in the central and outermost portions of Berkeley Sound, particularly around the mouth of the Sound (Figure 2.28). Additionally, sei whales were observed in outer Port William and along the exposed coast offshore of, and south of, Cape Pembroke lighthouse (Figure 2.28); the latter area was especially used by sei whales during 2021.

May and June are more difficult to interpret, since the occurrence of sei whales clearly differed markedly between years in those months. No sei whales were recorded in May or June during 2019 or 2020, and consequently all of the sightings and relative abundance shown in Figure 2.29 relate to 2021. Considering that the relative abundance was calculated using combined data from all three years, the resulting values must underestimate occurrence in 2021 and overestimate occurrence in 2019 and 2020. The data indicate that sei whales were still widely distributed across the study area during May 2021, using both the innermost part of Berkeley Sound west of Monkey Point and Strike Off Point, as well as the central area and the waters at the mouth (Figure 2.29). There was also a good number of sightings recorded in more exposed coastal waters during May, including around Volunteer Point, offshore of Berkeley Sound, and south of the Cape Pembroke lighthouse (Figure 2.29). During June, the focus at NEF switched to southern right whales and there was relatively little coverage inside Berkeley Sound (Figure 2.9). However, sei whales were still present in (at least) the outer regions of Berkeley Sound and in exposed coastal waters south of Volunteer Point during June 2021 (Figure 2.30).

Good amounts of survey coverage were achieved in NEF July and August, with some coverage also acquired in September (Figure 2.9). While the proportion of coverage inside Berkeley Sound was relatively low, those surveys did include consistent effort across the mouth of the Sound, and also in other areas well-used by sei whales between February and April such as off Kidney Island, Mengeary Point, and off the Cape Pembroke lighthouse. However, no sei whales were recorded on boat surveys between July and September.

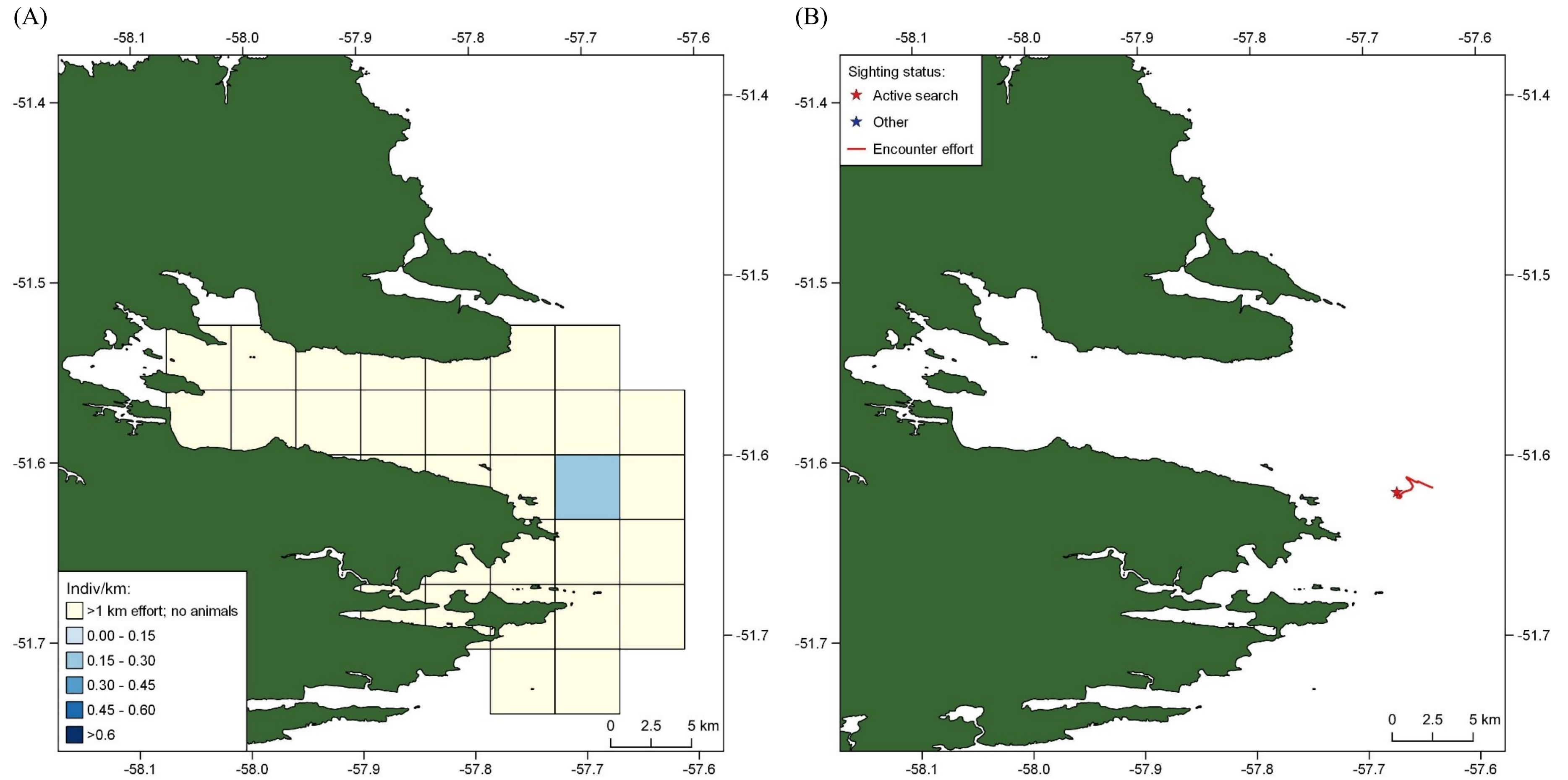


Figure 2.25. *Distribution of sei whales in the North-east Falklands during January (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

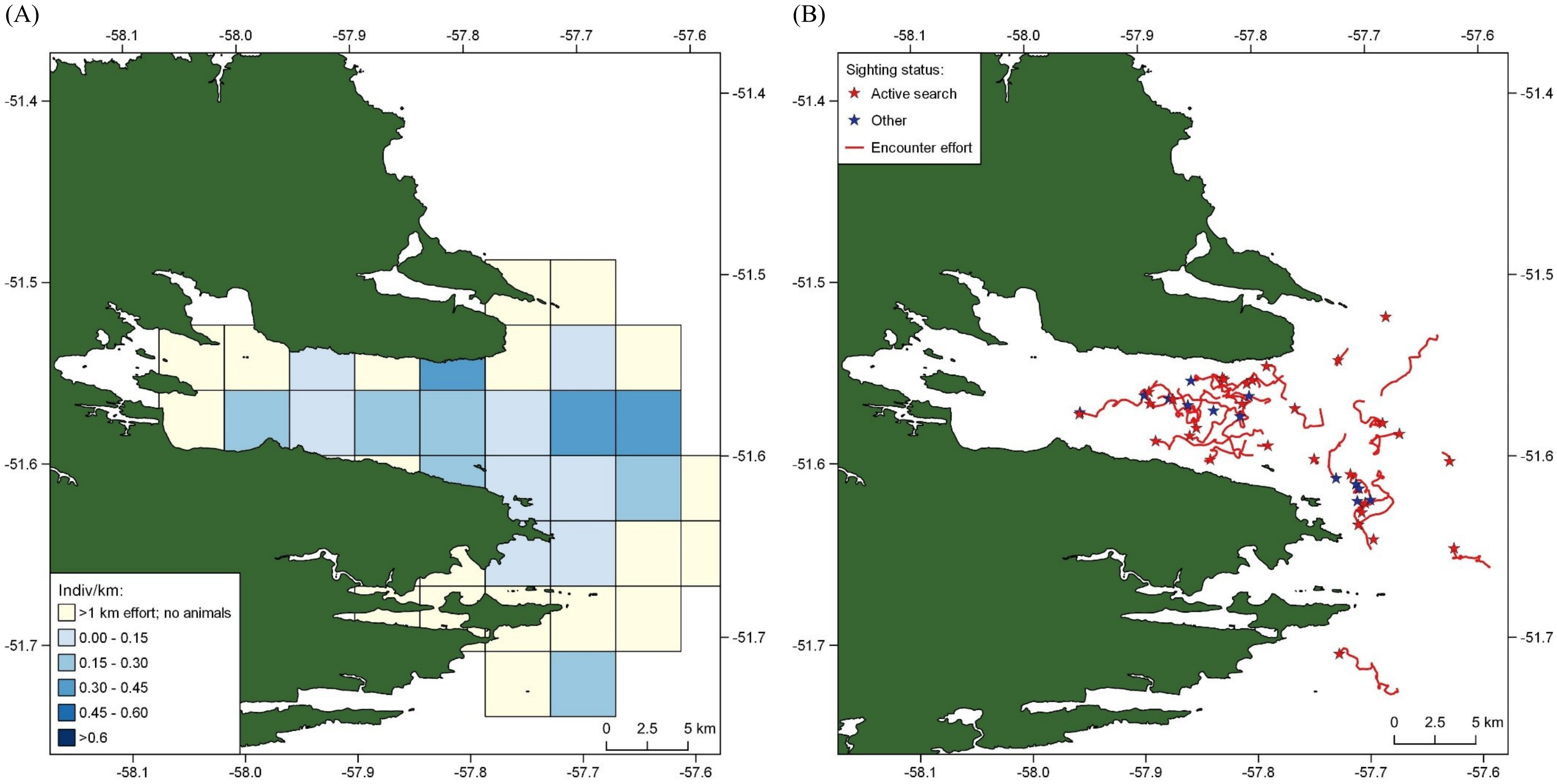


__Figure 2.26__. Distribution of sei whales in the North-east Falklands during February (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.

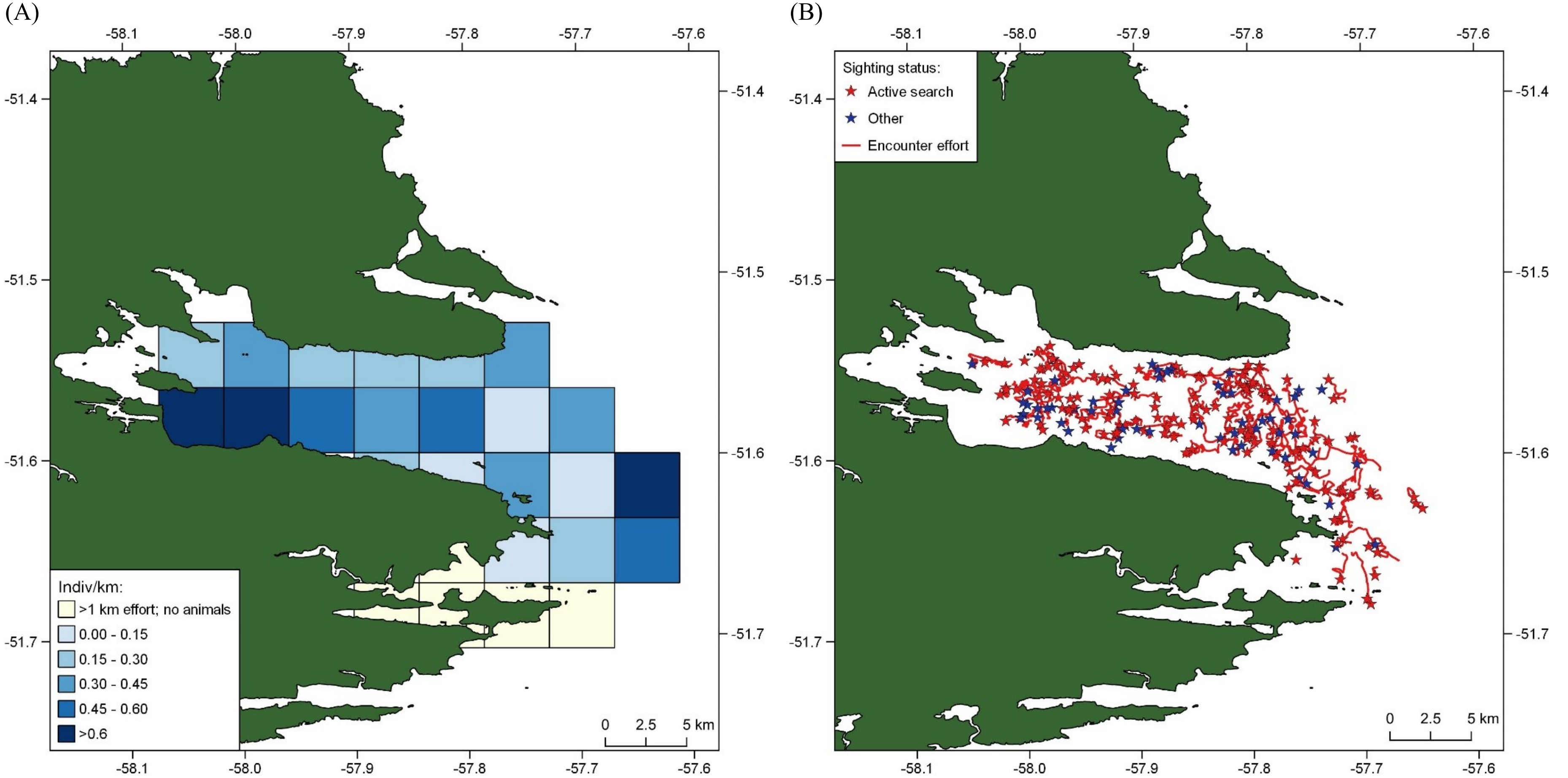


Figure 2.27*. Distribution of sei whales in the North-east Falklands during March (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

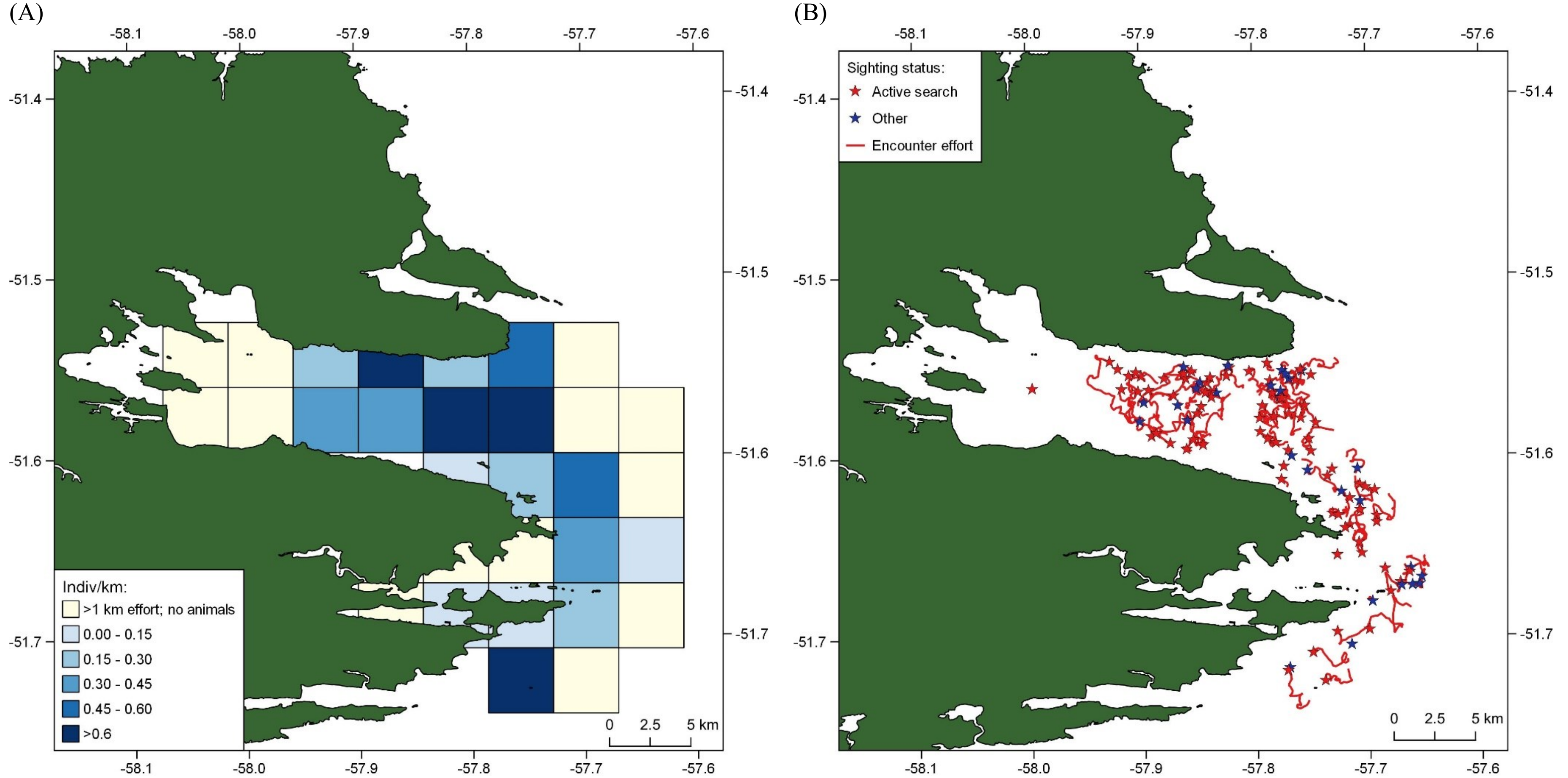


Figure 2.28. *Distribution of sei whales in the North-east Falklands during April (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

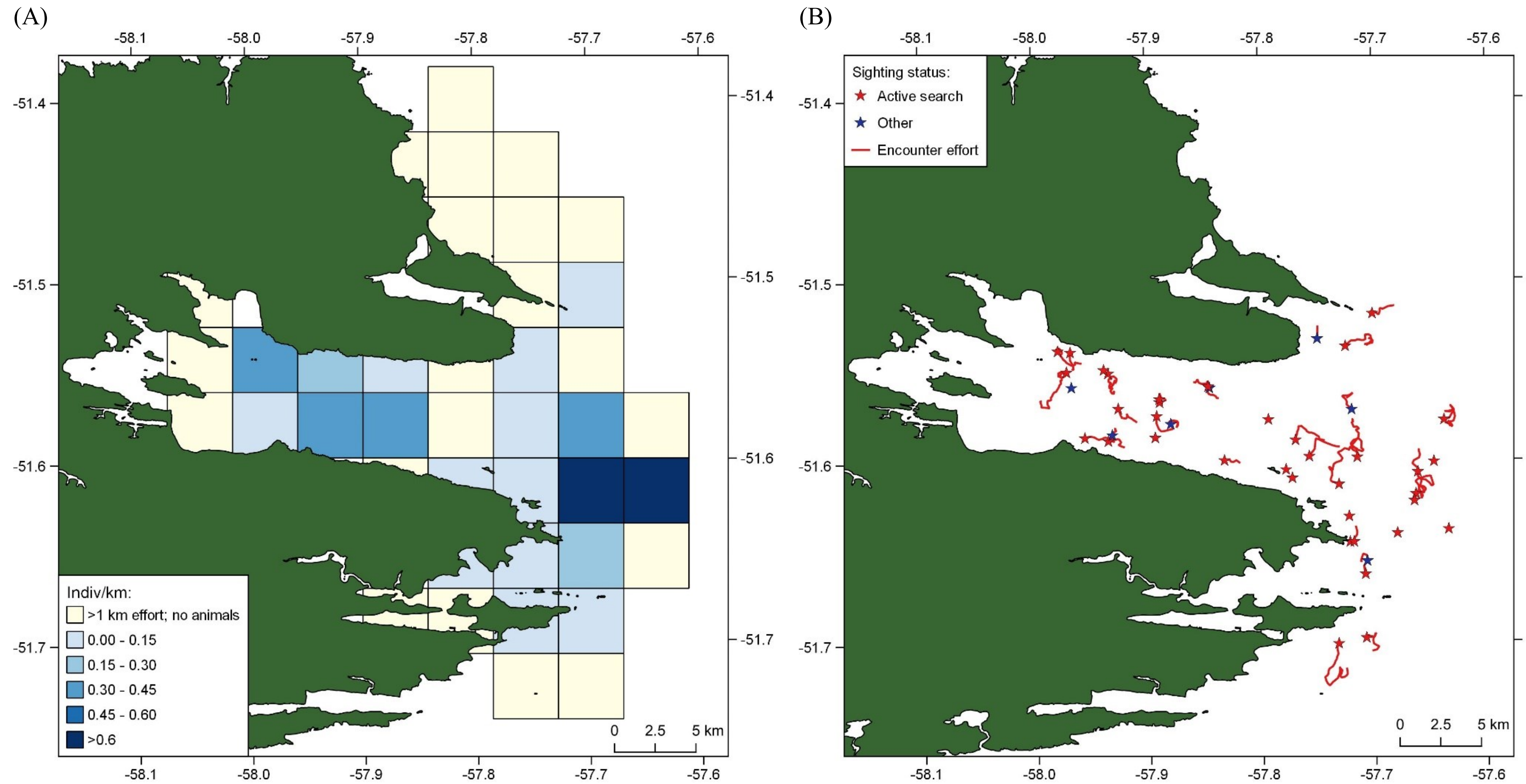


Figure 2.29. *Distribution of sei whales in the North-east Falklands during May (2019–2021 combined): (A) 4 km*^{2} *grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

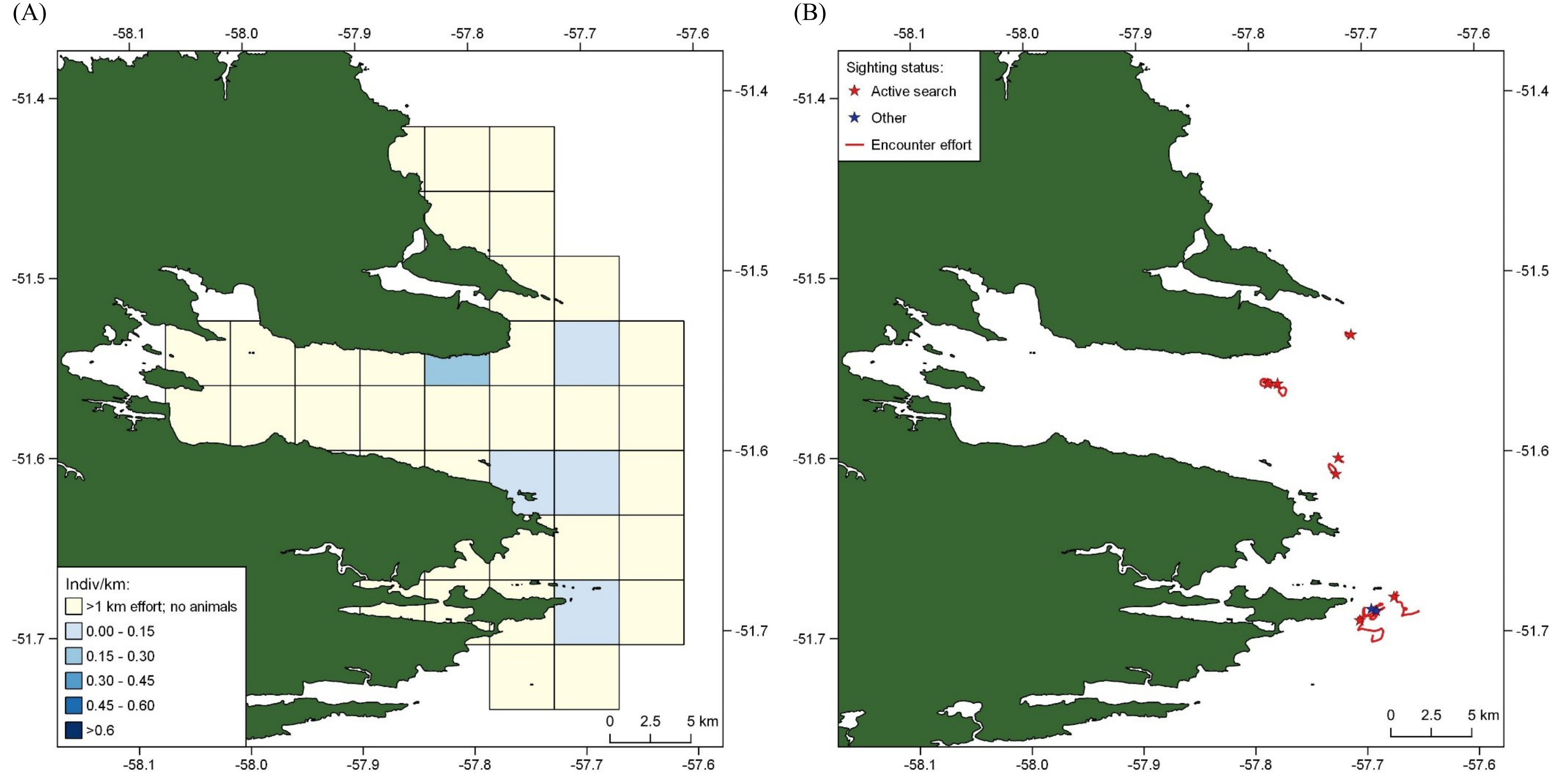


Figure 2.30. *Distribution of sei whales in the North-east Falklands during June (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

2.3.2.3 Southern right whale

A total of 220 southern right whale sightings (480 animals) was recorded across the three years of the project (Table 2.3), of which most (181 sightings, 384 animals) were associated with active search effort (Table 2.4). Right whales were recorded as single animals and in groups containing up to an estimated 17 animals, with an overall mean group size of 2.2 animals (Table 2.4). Over 48% of right whale sightings were of single animals (Figure 2.31). However, some of those individuals were encountered moving purposefully between surface active groups (SAGs[2]); sightings were logged as a group size of one animal if they were initially observed as singletons, even if they subsequently joined groups. An additional 25% of sightings comprised pairs, while small groups of three or four animals made up another 20% of the total sightings (Figure 2.31). Groups of ≥5 animals comprised the final 6.8% of sightings. Kruskal-Wallis tests revealed no significant difference in group size by month (Figure 2.32), either when all months were included (H=2.6, df=6, P=>0.05) or when only May to August (i.e. those months with higher sample size) were included (H=2.1, df=3, P=>0.05).

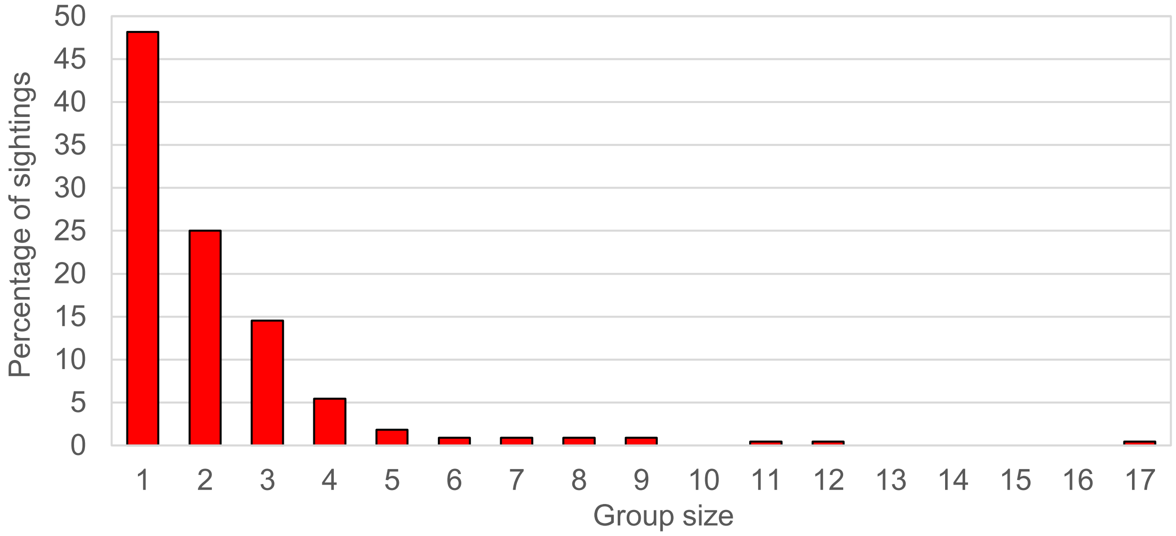


Figure 2.31. *Visual estimates of southern right whale group size in the North-east Falklands.*

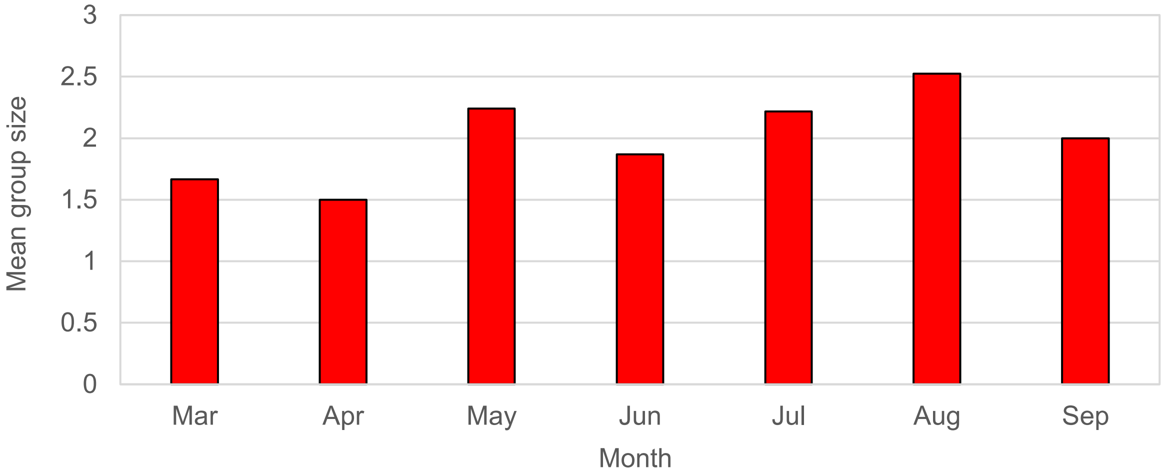


Figure 2.32. *Visual estimates of southern right whale group size by month in the North-east Falklands.*

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[2] A SAG was defined as two or more whales interacting at the surface with frequent physical contact (Parks and Tyack, 2005).

No calves were observed in NEF during any of the study years. While both juveniles and adults were present, distinguishing between large juveniles and small adults was challenging, and therefore the relative proportions of each were largely undetermined. The behaviour of right whales in the study area was often complex and variable, most typically comprising combinations of travel, resting/milling, and surface active. The latter category included behaviours variously comprising SAGs, breaching, flipper slapping, courtship, spy-hopping, rolling in kelp, and interactions with Peale's dolphins. Clear mating attempts were observed and photographed in several SAGs (Figure 2.33).

The occurrence of southern right whales varied between years. Using the larger combined dataset (i.e. FS and NEF), the relative abundance showed a clear peak during 2020 and was lowest in 2021 (Figure 2.13). However, given that southern right whales were not recorded at all at FS (primarily because winter effort targeted only NEF), an assessment of annual variation in their relative abundance was repeated using only the NEF dataset (Figure 2.34). The relative abundance was again lowest in 2021, which is unsurprising because survey effort in that year terminated in mid-June and prior to the onset of the core right whale season (Table 2.1). However, the difference in relative abundance between 2019 and 2020 was much reduced using the NEF-only dataset (Figure 2.34).

Southern right whales exhibited strong seasonality in the study area. Similar trends are apparent using both the combined site dataset (Figure 2.14) and the NEF-only dataset (Figure 2.34); these datasets differ only in values for the March to June period, where the relative abundances using NEF-only data are slightly higher. A marked peak in the relative abundance of southern right whales is evident during July, in the middle of the austral winter (Figure 2.35).

However, the seasonal trend was inconsistent between years (Figure 2.35). During 2019, no right whales were observed on boat surveys prior to June. This may partly reflect lower survey effort at NEF during May 2019 compared with the following years (Table 2.2). However, the relative abundance (i.e. corrected for effort) of right whales was also lower in June 2019 compared with June 2020 or 2021 (Figure 2.36), despite the total amount of June survey effort being highest in 2019 (Table 2.2). Consequently, the main concentration of wintering right whales appeared in nearshore Falklands' waters later in 2019 compared to the other years. Additionally, the relative abundance in July 2019 was almost double that recorded in July 2020, consistent with an influx of animals during that month in 2019. In contrast, southern right whale relative abundance in 2020 was more consistent across months; although a July peak was still apparent, the values across May to August were more similar and indicative of a more prolonged season in 2020 (Figure 2.36).

The distribution of southern right whales at NEF indicated an overall nearshore occurrence and a preference for coastlines situated adjacent to the more open and exposed parts of the study area (Figure 2.37). This included the waters around the Cape Pembroke peninsula, the outer portion of Port William, around Mengeary Point and Kidney Island, and along the stretch of coast between Volunteer Lagoon and Cape Carysfort (Figure 2.37). Although sightings and encounter effort were clustered in those localities, right whales were also sighted several kilometres from the coast including off the mouth of Berkeley Sound, and well inside Berkeley Sound, particularly along the southern coast (Figure 2.37). Right whales were also seen in Johnson Harbour (Figure 2.37), indicating that the species sometimes enter very shallow and semi-enclosed inshore areas. The mean water depth of southern right whale sightings in NEF was 22.2 m (Table 2.7).

Generally, the winter right whale surveys travelled up to the Volunteer Point area when weather allowed rather than looping around Berkeley Sound, and consequently the occurrence of the species inside Berkeley Sound shown in Figure 2.37 is likely to be under-estimated. The occurrence of the species is therefore better indicated using effort-related relative abundance for the months of March to September (Figures 2.38 to 2.44).

Figure* *2.33. *Some southern right whale behaviour observed during DPLUS082: interactions with Peale's dolphin (top left) and kelp (top right), female laying belly-up to avoid mating attempts (centre), and mating attempts within a surface-active group (bottom).*

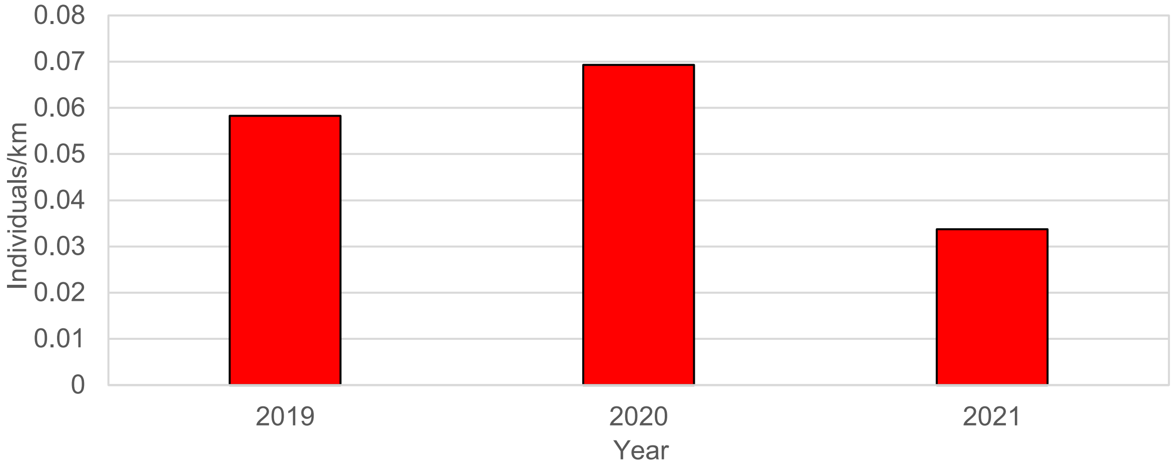

Figure 2.34. *Annual relative abundance (individuals/km) of southern right whales in the North-east Falklands. Relative abundance was calculated using 5,893.7 km of Active Search data collected in favourable weather conditions.*

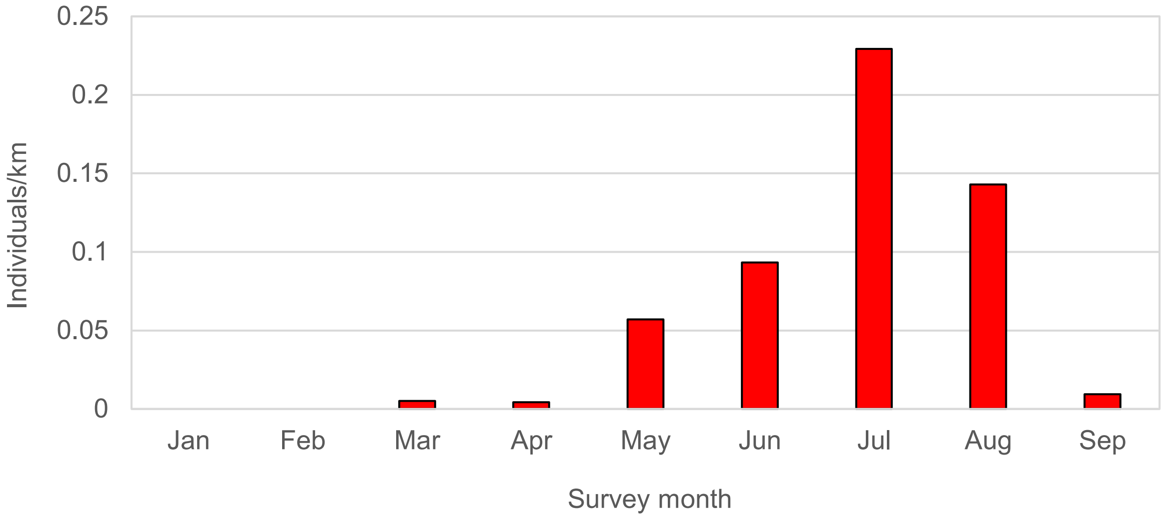

Figure 2.35. *Monthly relative abundance (individuals/km) of southern right whales in the North-east Falklands from 2019 to 2021.*

During March, a small number of right whale sightings occurred in the Mengeary Point and Kidney Island areas (Figure 2.38). Despite significant amounts of boat survey effort within Berkeley Sound during that month, no right whales were recorded within the Sound. A similar situation was apparent during April, with small numbers of right whales encountered off the south side of Cape Pembroke but no sightings anywhere else (Figure 2.39). The March and April sightings were all related to 2021 survey effort, and no early right whales were recorded in NEF during March and April in 2019 or 2020.

During May, the presence of southern right whales at NEF markedly increased, with low relative abundance off Cape Pembroke, Mengeary Point and Kidney Island, and some areas of high relative abundance in the Volunteer Point and Cow Bay areas (Figure 2.40). It should be noted that the latter areas were only surveyed during 2020 and 2021, and with relatively low effort, since the May surveys were usually still focussed on sei whales within Berkeley Sound.

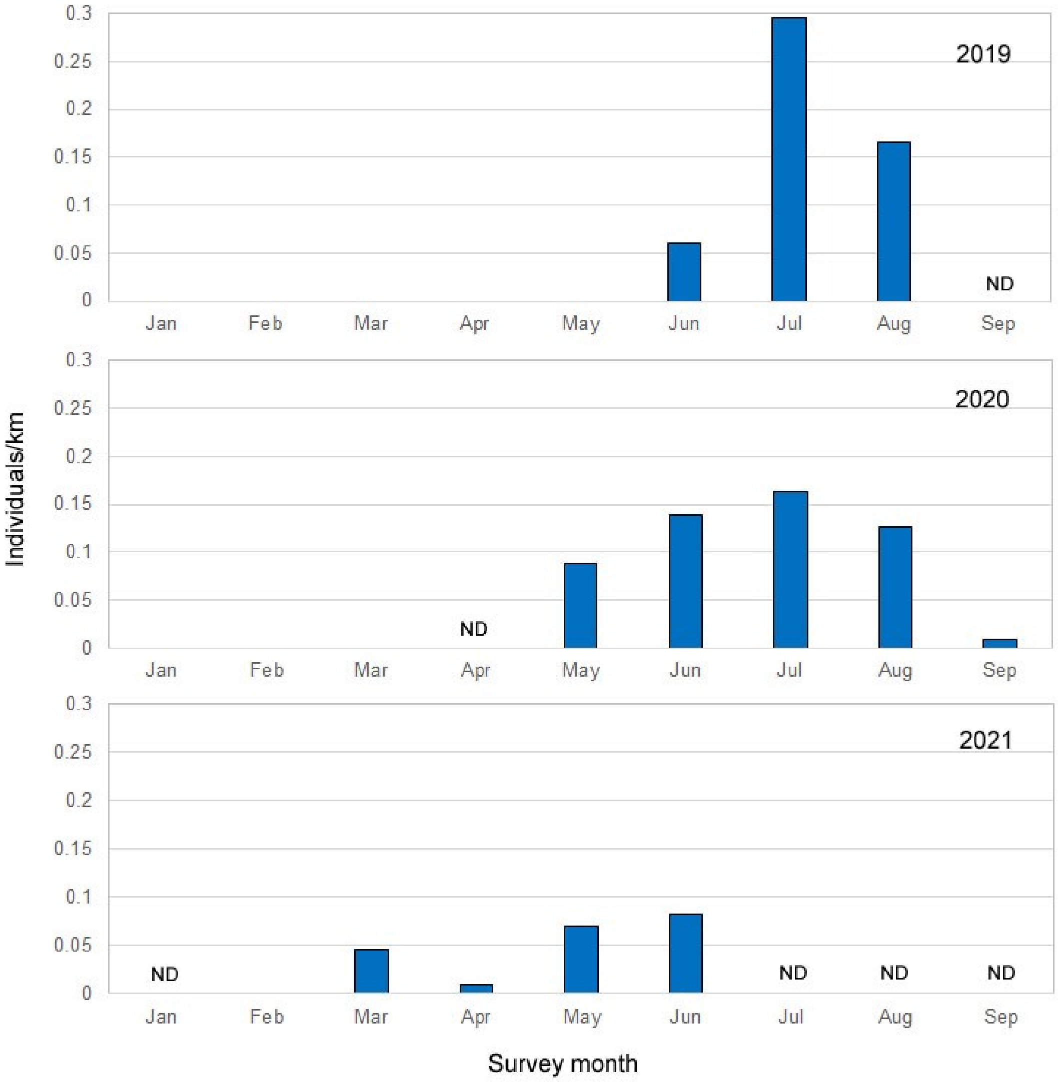

***Figure 2.36**. Monthly relative abundance (individuals/km) of southern right whales in the North-east Falklands during each of the survey years from 2019 to 2021. Months where no survey coverage was carried out are shown as ND – No Data.*

Between June and August, most boat survey effort was focussed outside of Berkeley Sound and with emphasis predominantly on southern right whales. In all of those months, and particularly during July, high relative abundances of right whales were recorded in the northern part of NEF along the coast between Volunteer Point lagoon and Cow Bay (Figures 2.41–2.43). Low to moderate relative abundances were also recorded in the Cape Pembroke–Mengeary Point–Kidney Island areas in all three months, reflecting a reasonably high and sustained use of those areas by right whales. This region includes the shipping lane serving Port William and Stanley. In contrast, lower relative abundances were recorded in more open waters across the mouth of Berkeley Sound, between Kidney Island and Eagle Point. Within Berkeley Sound itself, the lack of survey effort limits conclusions on right whale occurrence (see Figure 2.9). In June, only a single boat survey was carried out within the Sound, and that occurred very early in the season (1 June) during a year (2019) when right whales were late to arrive to NEF. Consequently, the dataset is not considered sufficiently robust to interpret the use of Berkeley Sound by right whales during most of the winter. The possible exception is during August, when a small number of surveys occurred in Berkeley Sound during 2019 and 2020 (Figure 2.9), and yielded numerous right whale sightings especially along the south coast of the Sound (Figure 2.43). Although right whales were clearly very widespread at NEF during August, the area off Volunteer Beach and Cow Bay still produced the highest relative abundances (Figure 2.43). Only two surveys were carried out during September (both in 2020); however, a notable decrease in right whale sightings was apparent by that month (Figure 2.44).

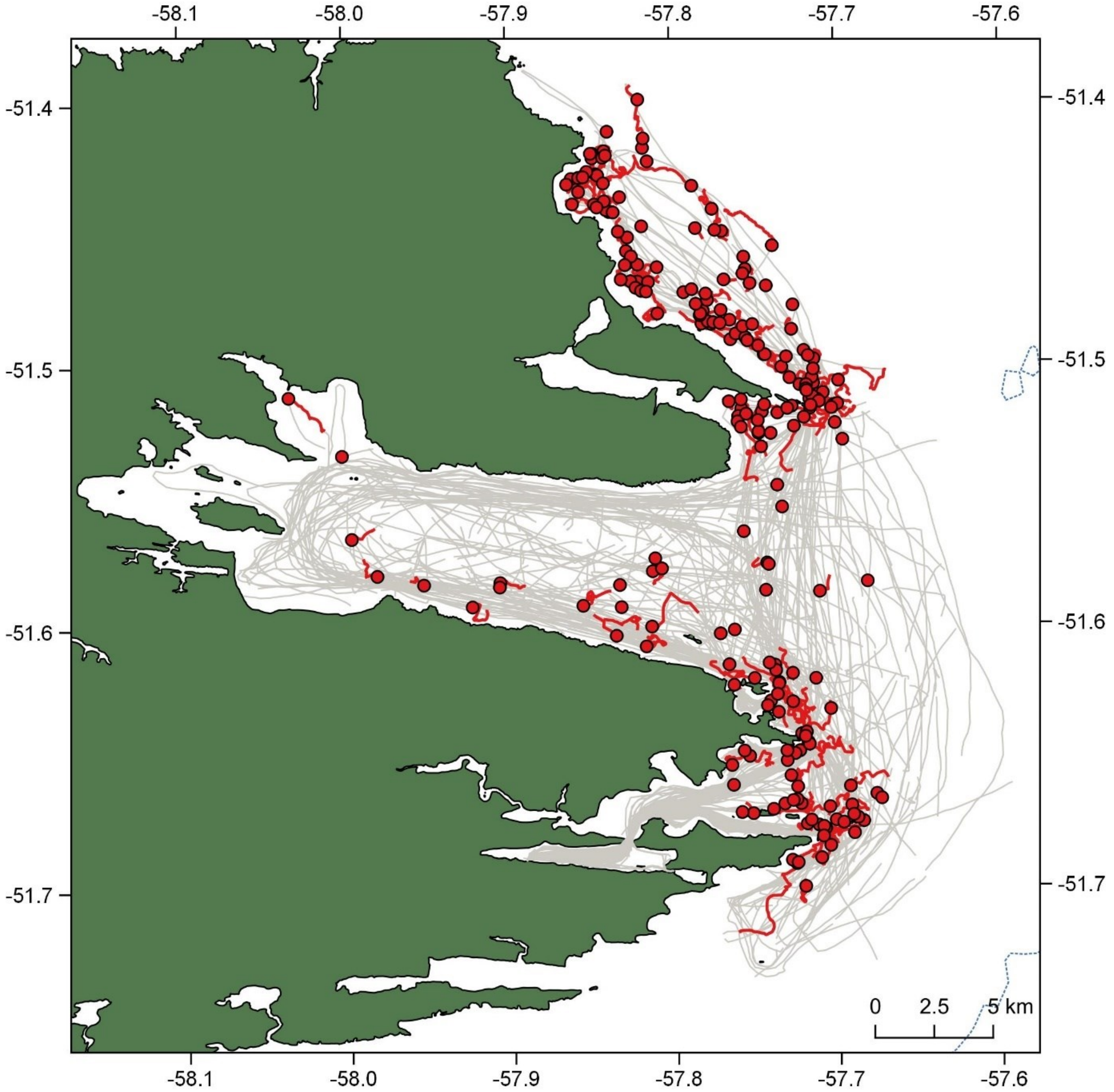

Figure 2.37*. Spatial distribution of southern right whale sightings (circles), southern right whale encounter effort (red lines), and Active Search effort (grey lines) in the north-east Falklands, 2019–2021. Sighting locations have been recalculated to reflect animal positions rather than location of the boat.*

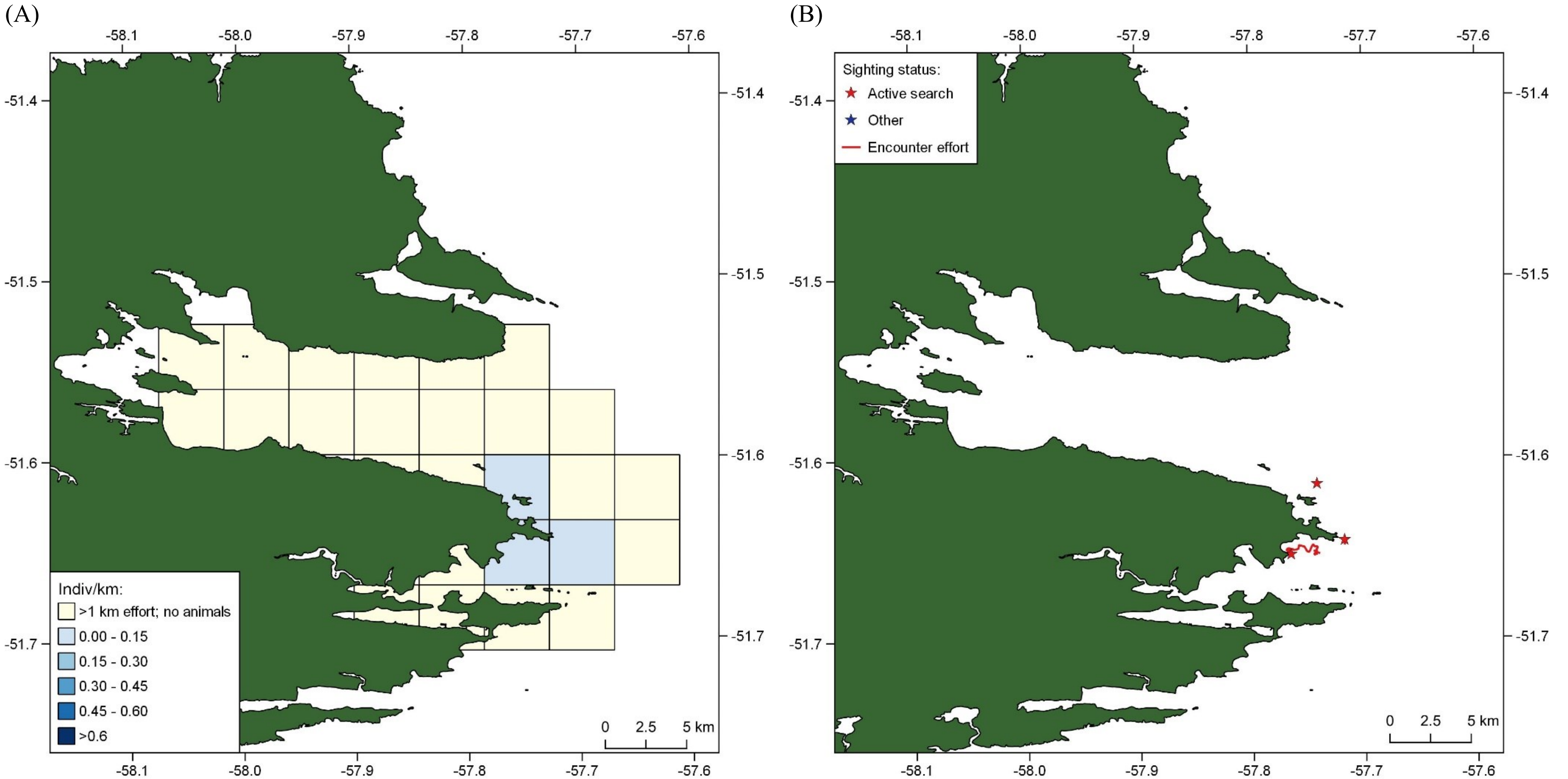

Figure 2.38. *Distribution of southern right whales in the North-east Falklands during March (2019–2021 combined): (A) 4 km*^{2} *grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

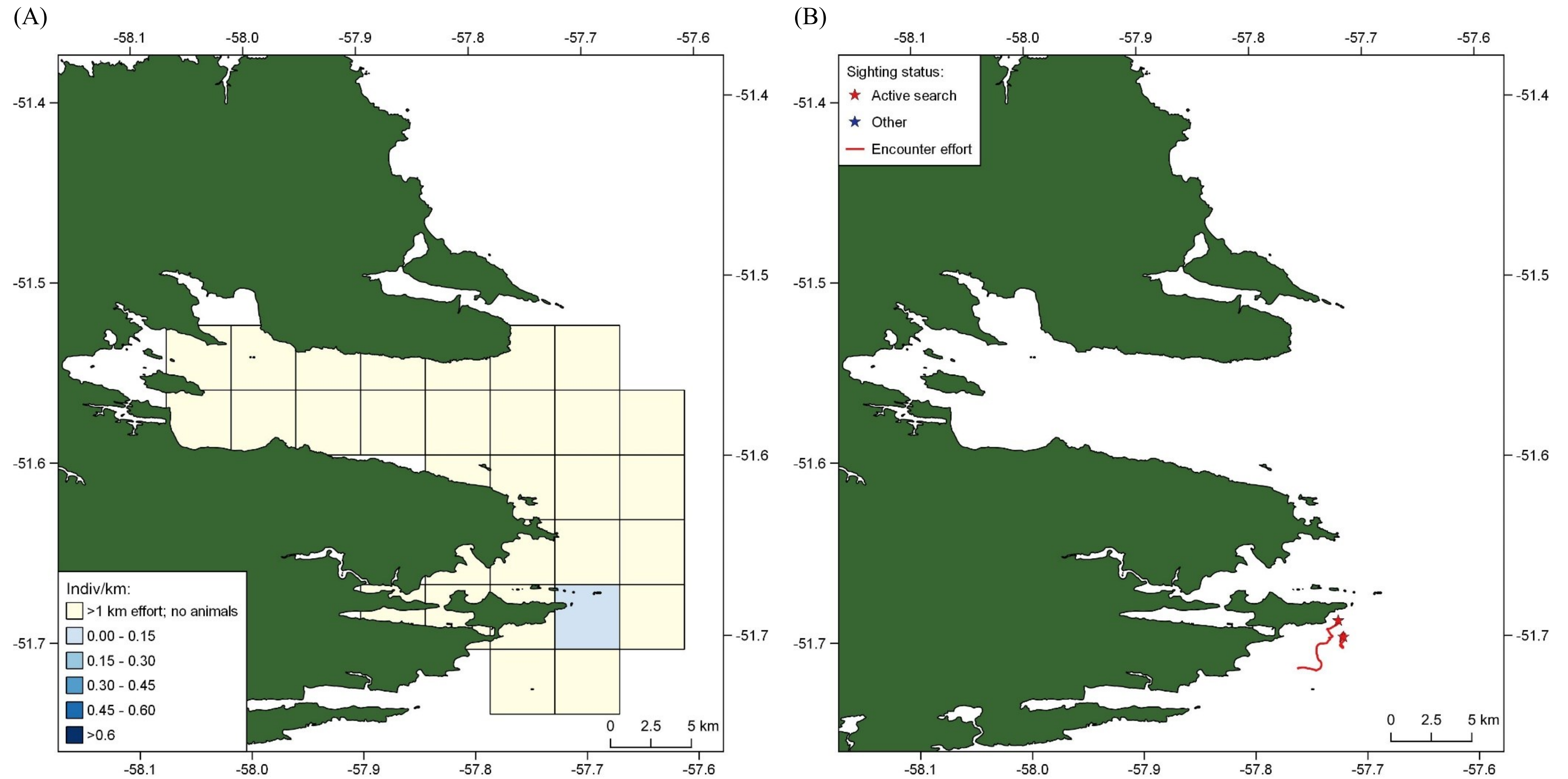

Figure 2.39. *Distribution of southern right whales in the North-east Falklands during April (2019–2021 combined): (A) 4 km*^{2} *grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

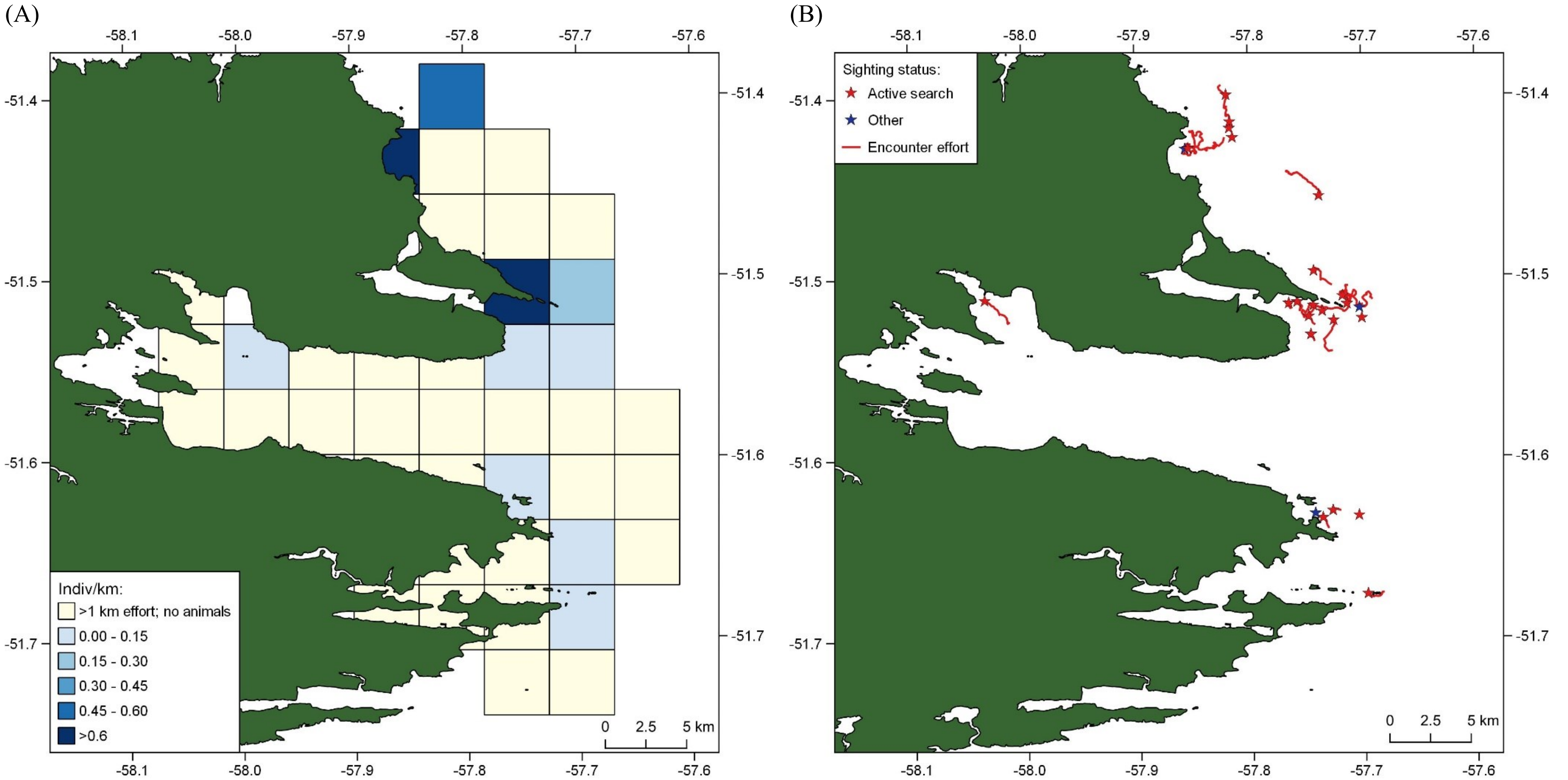

Figure 2.40. *Distribution of southern right whales in the North-east Falklands during May (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

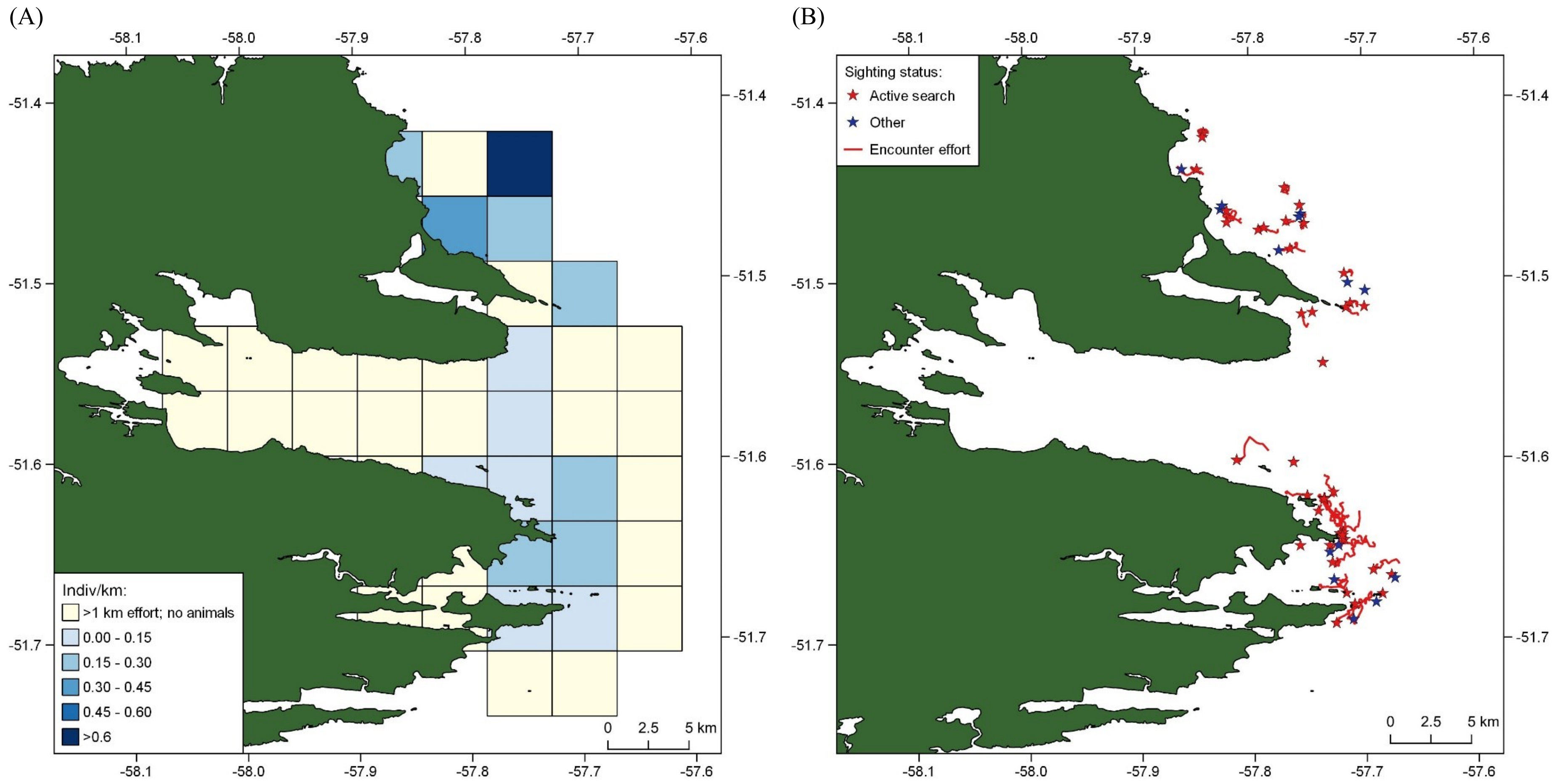

__Figure 2.41__. Distribution of southern right whales in the North-east Falklands during June (2019–2021 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.

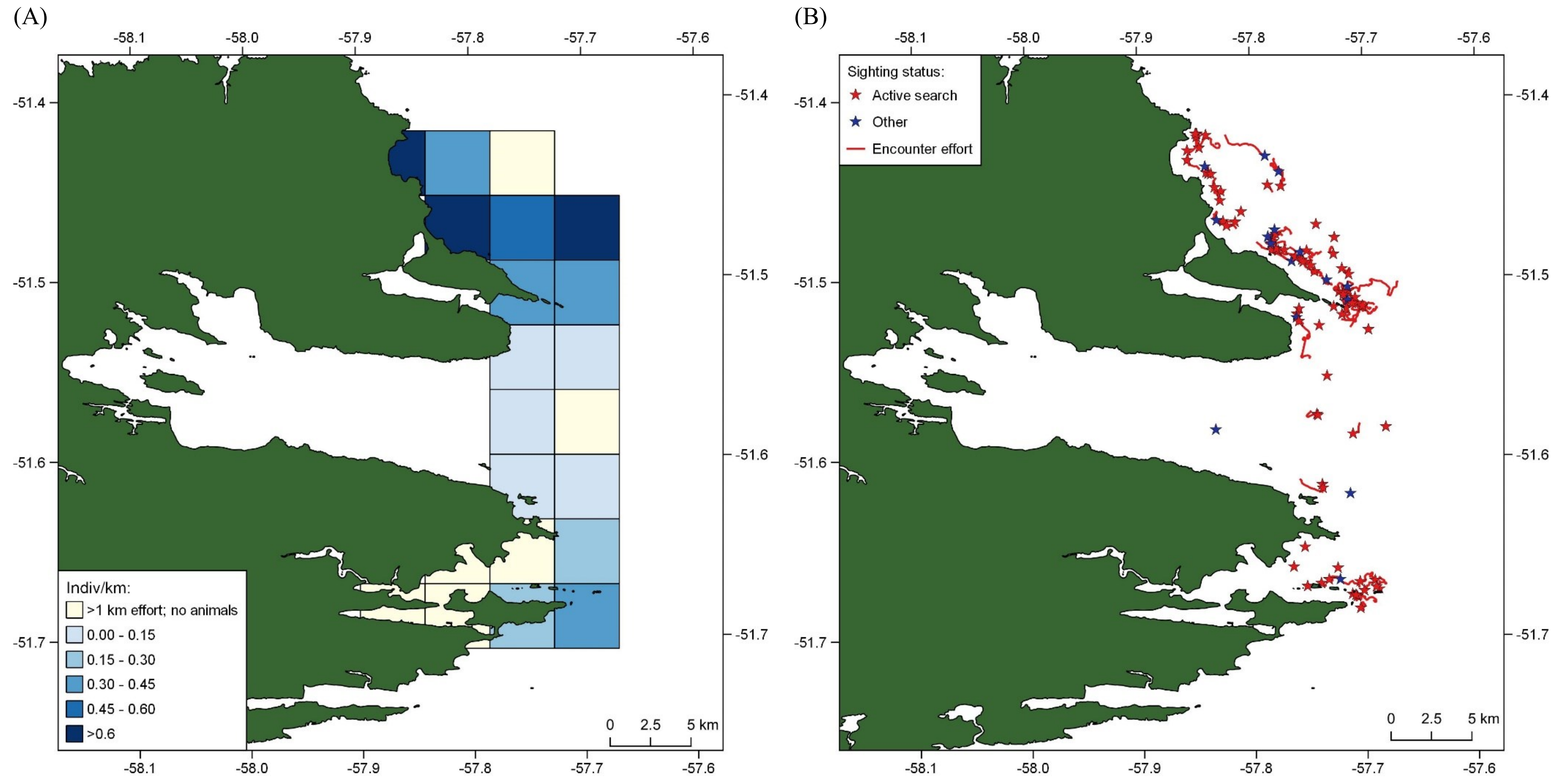

Figure 2.42. *Distribution of southern right whales in the North-east Falklands during July (2019–2020 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

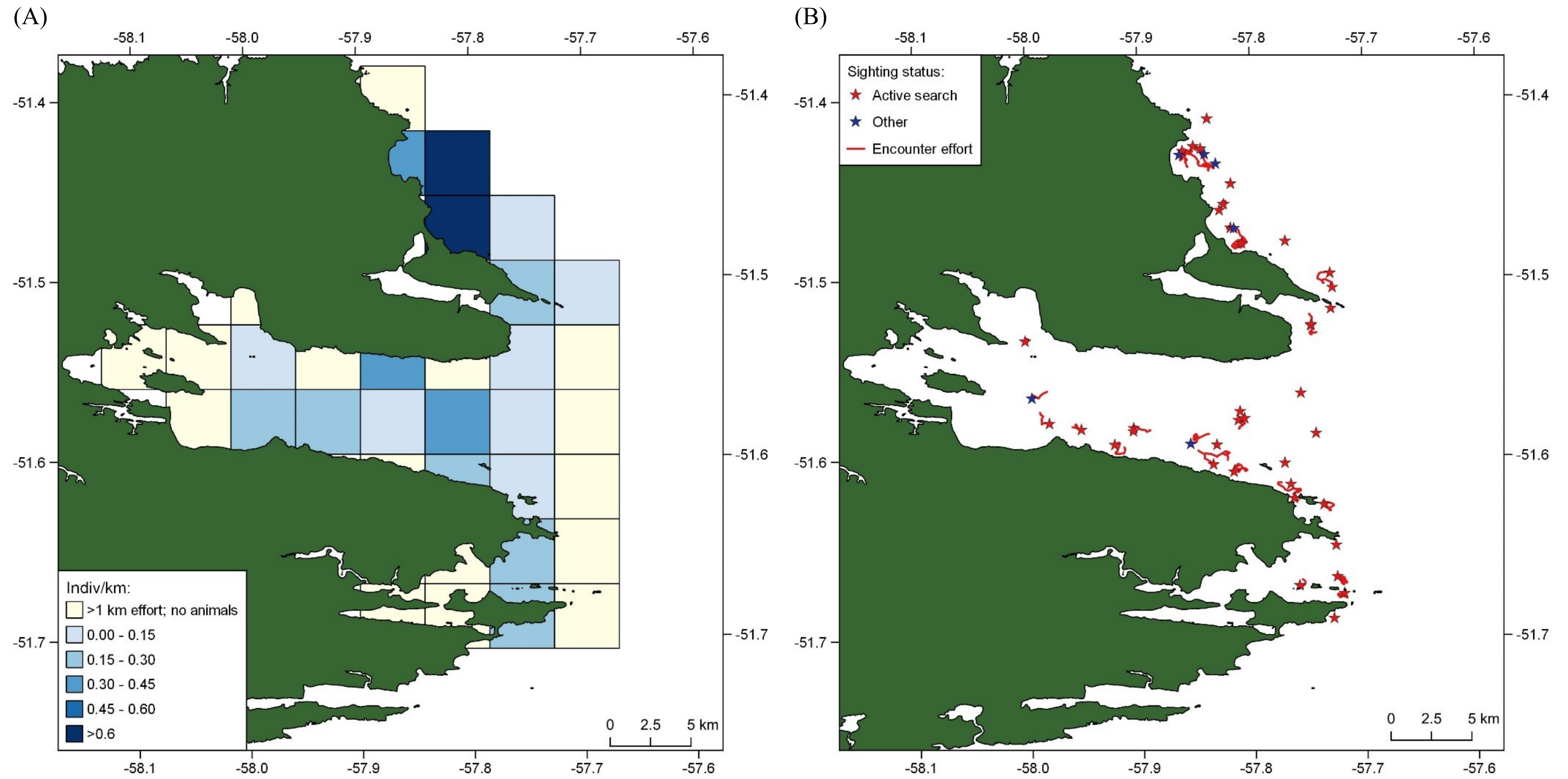

Figure 2.43. *Distribution of southern right whales in the North-east Falklands during August (2019–2020 combined): (A) 4 km^2 grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

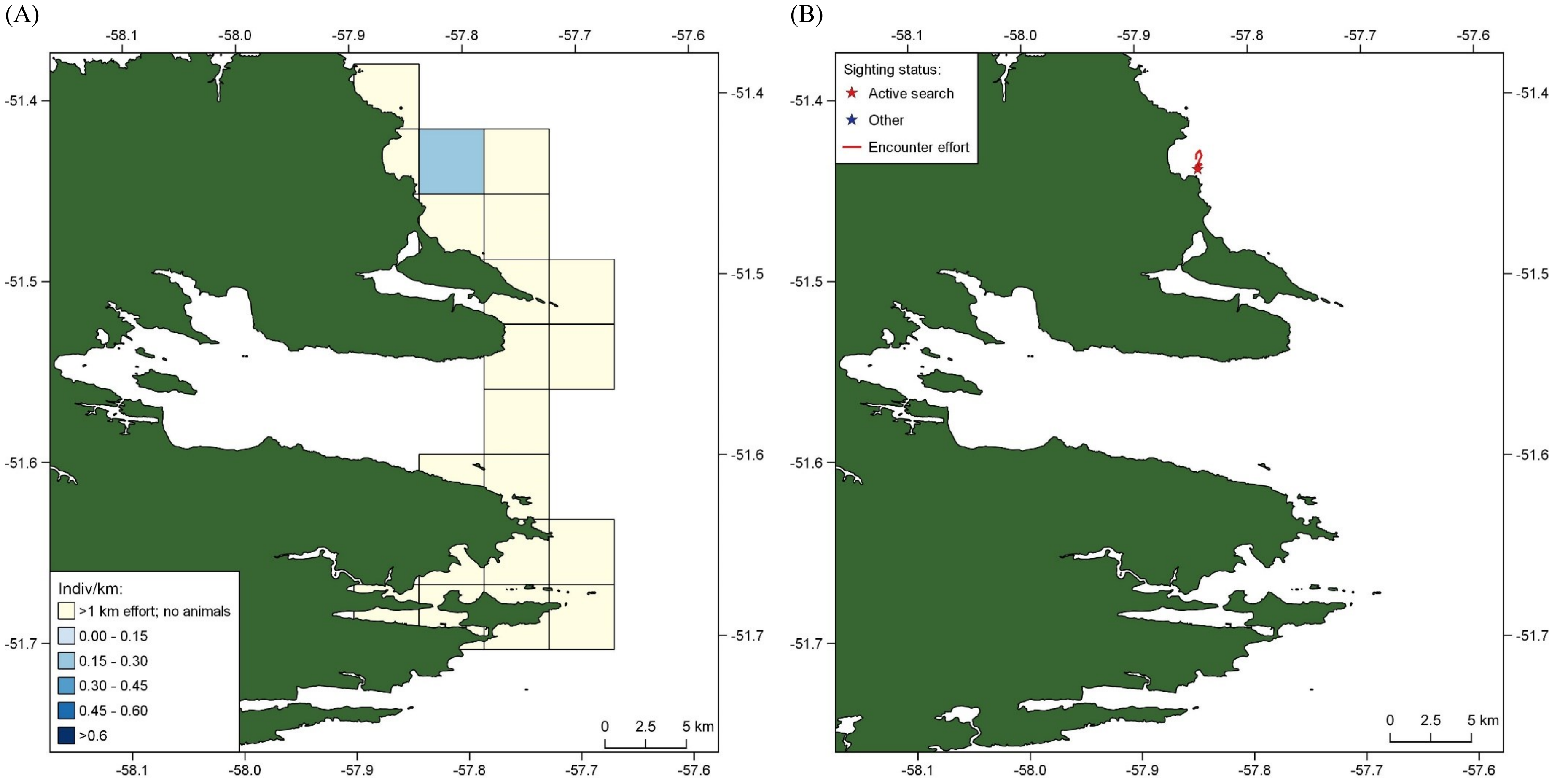

Figure 2.44. *Distribution of southern right whales in the North-east Falklands during September 2020: (A) 4 km*^{2} *grid of relative abundance (calculated with active search data collected in favourable conditions); and (B) plot of sightings (recalculated to reflect animal positions rather than location of the boat) and encounter effort.*

2.3.2.4 Unidentified baleen whales

Sightings logged as 'large unidentified baleen whales' (LBAL) comprised animals that were seen too distantly, too briefly, or in too poor weather conditions, to identify the animal(s) with certainty to species level. These predominantly comprised whale blows observed at distance. In total, 118 LBAL sightings (150 animals) were recorded during the DPLUS082 fieldwork from 2019 to 2021 inclusive (Table 2.3). The sightings comprised groups of 1 to 3 animals, with a mean group size of 1.3 animals (Table 2.4).

The temporal distribution of LBAL sightings is shown in Figure 2.45. The relative abundance shows marked seasonality, with presence recorded between February and June and a strong peak evident during April at both sites (Figure 2.45). When compared with Figure 2.14, this seasonality shows close similarity to that of sei whales. Indeed, the seasonal trend in relative abundance of LBAL at NEF (Figure 2.45B) matches very closely with that of sei whales at the same site (Figure 2.15). At FS, a strong peak in LBAL relative abundance during June (Figure 2.45B) also markedly influenced the reported seasonality of LBAL in the combined dataset for that month (Figure 2.45A). It should be noted that the relative abundance of LBAL was more than double in 2021 compared with 2019 and 2020 (Figure 2.13), and that sei whales remained much later in the Falklands during 2021 compared with the earlier years (see Figures 2.16 and 2.17). Consequently, it might be reasonably concluded that even the June peak may most likely relate to sei whales that were seen too poorly to confirm their identification. However, the incursion of humpback whales into Falklands' waters during 2021 may also have been a factor in the higher number of LBAL recorded in that year. It is not considered likely that many LBAL sightings comprised southern right whales, both because of the strong seasonality evident in the records and also due to the conspicuous features of the latter species even at distance; it was usual to see tail flukes, characteristic v-shaped blows, and/or flippers/heads when right whales were sighted.

The distribution of LBAL sightings in FS (Figure 2.46) exhibited similarities to that of the sei whale (see Figure 2.18), with a concentration located in the waters off New Haven and in the open expanse in the central portion of the study area. These likely represent animals recorded distantly and 'off effort' while the survey team was already working with other sei whales, and animals seen while returning to New Haven at the end of surveys when time constraints prevented closing for identification purposes. The distribution of LBAL sightings at NEF (Figure 2.47) also bore most similarity to that of sei whales (see Figure 2.24), occurring widely throughout Berkeley Sound and in more open Atlantic waters offshore of Cape Pembroke, Mengeary Point and off the mouth of Berkeley Sound. The mean water depth of LBAL sightings (34.1 m) was similar to, but slightly deeper than, that of sei whales (Table 2.7).

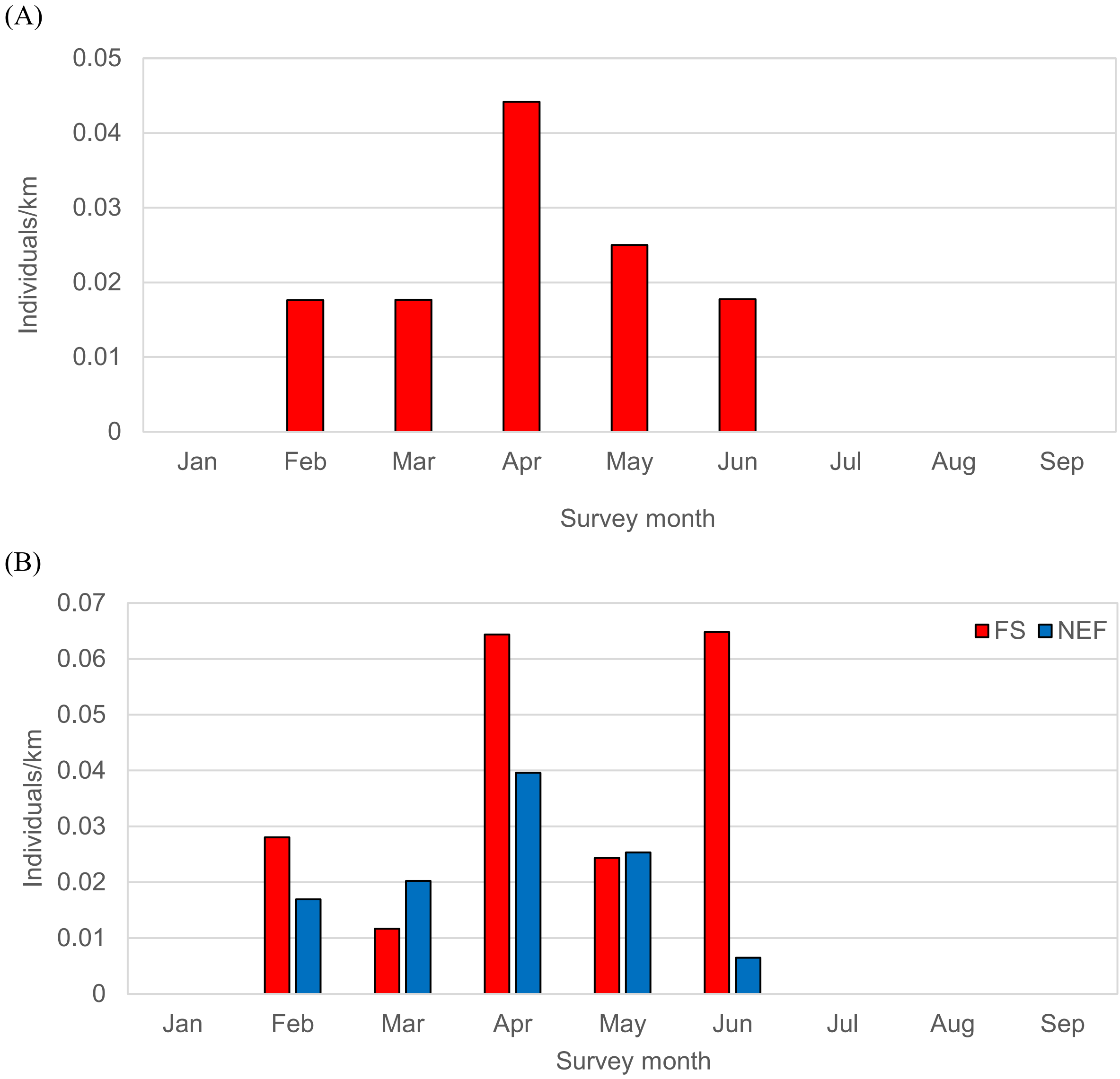

***Figure 2.45**. Relative abundance (individuals/km) of unidentified large baleen whales in Falklands' nearshore waters during boat surveys 2019-2021: (A) at both sites combined; and (B) by study site. Relative abundance was calculated using 7,104.1 km of Active Search data collected in favourable weather conditions.*

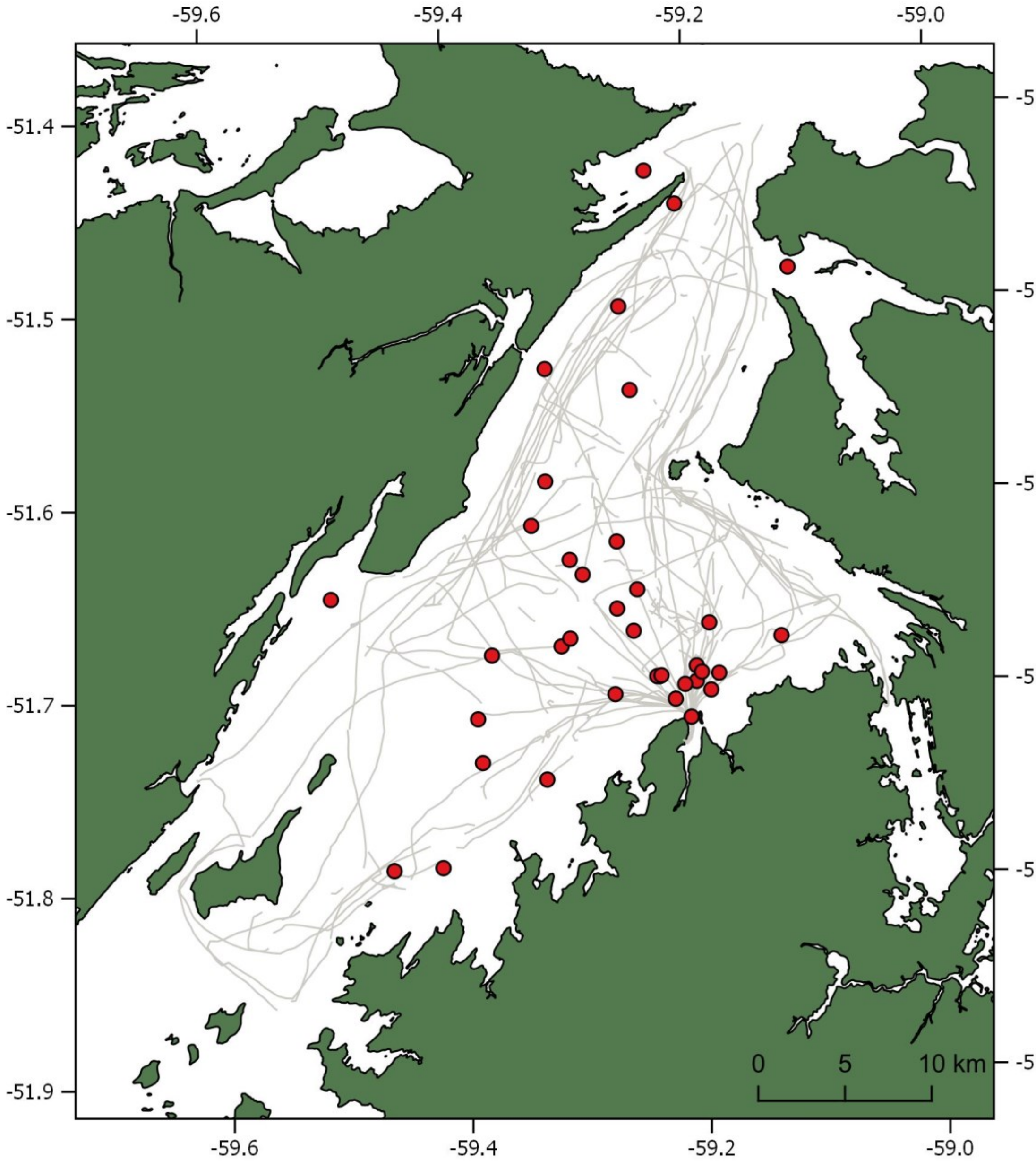

***Figure 2.46**. Spatial distribution of unidentified large baleen whale sightings (circles) and Active Search effort (grey lines) in Falkland Sound, 2019–2021. Sighting locations have been recalculated to reflect animal positions rather than location of the boat.*

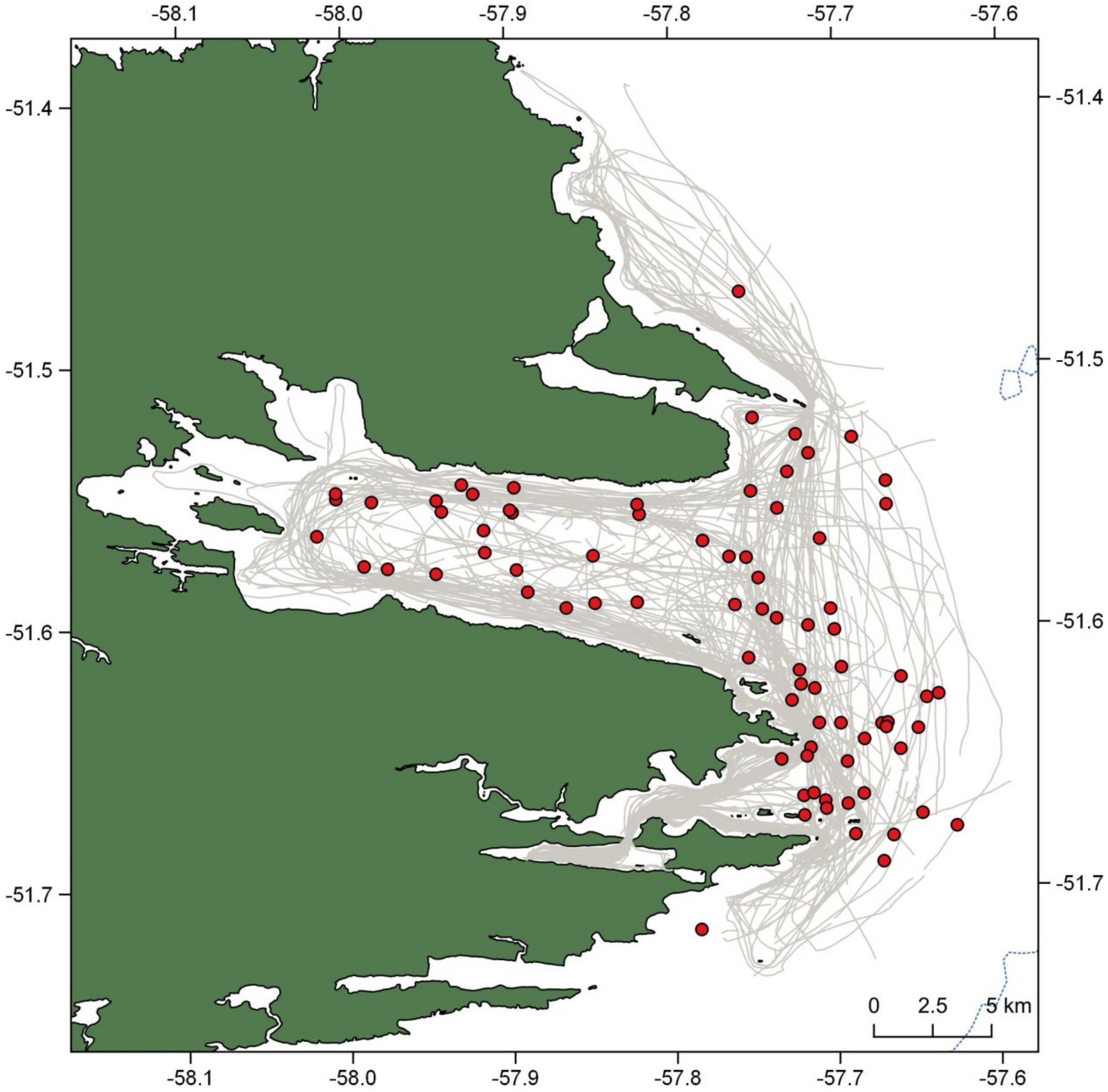

***Figure 2.47**. Spatial distribution of unidentified large baleen whale sightings (circles) and Active Search effort (grey lines) in the north-east Falklands, 2019–2021. Sighting locations have been recalculated to reflect animal positions rather than location of the boat.*

2.4 Discussion

2.4.1 Survey constraints

The project successfully completed small boat surveys on 94 dates over the project duration, which is a significant amount for a geographic region where weather and logistical constraints are challenging. However, interpretation of the visual survey dataset collected during DPLUS082 needs to carefully consider the discrepancies in survey effort achieved at the two sites, and across months. Unfortunately, the Falkland Sound dataset was significantly impacted by logistical constraints, and the realised coverage was highly inconsistent between years, particularly during 2020 when only one small boat survey was carried out at FS. It also varied between months, with May being the only month where coverage was achieved at FS in all three years. Even at NEF, where logistical constraints were far fewer, the amount of achieved survey coverage was impacted by the COVID-19 lockdown and the challenges of working at both sites simultaneously. Nevertheless, the coverage achieved at NEF greatly surpassed the amount of coverage at FS in most months, and the results from NEF are therefore considered to be more robust overall with regard to sei whale occurrence. The use of relative abundance throughout the analyses in this chapter also takes discrepancies in survey effort over space and time into account to some extent; however, the results and interpretation are still limited by lack of coverage in some months or years at FS.

In contrast to the sei whale dataset, the survey coverage acquired for southern right whales was far more comparable across months and between years due to the project operating at only one site during winter and the timing of the right whale season which occurred outside of the COVID-19 lockdown.

2.4.2 Sei whales

As found in Berkeley Sound during 2017 (Weir, 2017) and West Falkland during 2018 (Weir, 2018), the sei whale was the most numerous baleen whale species observed in both of the DPLUS082 coastal study sites during the summer and autumn. One of the unique aspects of DPLUS082 was the inclusion of a new study area in Falkland Sound that was both intermediate in geographic locality between those earlier studies, and encompassed slightly different habitat comprising a semi-enclosed channel of water situated between East Falkland and West Falkland. Although the dataset collected at FS was impacted by low survey coverage, the results indicated that sei whales routinely used that region between (at least) February and May. In addition to the 2017 and 2018 datasets, this result suggests that during those months then sei whales are distributed around most of the Falklands' coasts. This is also supported by the documented movements of individual sei whales between all three regions (NEF, FS and West Falkland: see **Chapter 3**), again indicating a widespread use of the entirety of Falklands' coastal waters. These results have already been used in support of recognising the entire region as the *Falkland Islands Inner Shelf Waters KBA* for sei whales (Weir, 2021), rather than the initial small sites initially highlighted as potential KBAs by Taylor et al. (2016).

The DPLUS082 project provided the first multi-year study of sei whales at specific sites within the Falklands, and therefore the first opportunity to investigate inter-annual variation. With regard to FS, the marked differences in realised survey coverage between years limited conclusions on inter-annual sei whale occurrence. However, at NEF there were three years of regular data collection on sei whales, which facilitates some consideration of the seasonal variation in sei whale occurrence between years.

Fieldwork was not scheduled to commence until late January or early February in all years, and so information on sei whale occurrence during the earliest part of the season isn't available from boat surveys. Additionally, the latter part of the sei whale season from the end of March through to mid-May was not surveyed at all in 2020 due to COVID-19. Nevertheless, seasonal differences in occurrence were apparent between 2019 and 2021. While 2019 had a strong seasonal peak in relative abundance during March and zero relative abundance by May, the peak in relative abundance during 2021 occurred later in the year during the April to June period.

Considering these four years of boat survey data combined, it may be concluded that sei whales use NEF most consistently between February and April. Their presence in good numbers during May in 2017 and 2021, indicates that the season can be more protracted if sufficient food is present. And the 2021 dataset supports an ongoing presence of sei whales even into June at the commencement of the austral winter, if conditions are suitable. Conversely, although no boat survey data were collected in April 2020 due to COVID, the acoustic dataset suggests that sei whale activity at NEF during April 2020 was much lower than that recorded in April 2019 (see Figures 7.14 and 7.16), indicating that the season can also end early in some years. Certainly, in 2019 and 2020 numerous boat surveys were carried in NEF during May and June on which no sei whales were observed, and it is reasonable to conclude that they had departed the site earlier in those years compared to 2017 and 2021. This inter-annual variation is supported by the acoustic dataset (see **Chapter 7**), which revealed notably less sei whale vocal activity during May and June in 2019 and especially in 2020, compared with the February to April period. Although the acoustic monitoring programme ended in December 2020, it is notable that an unusually extended 2021 season was also being indicated by the onset of high vocal activity in the early part of the season during November and December 2020 compared with the same period in 2018 or 2019. This was especially evident on the acoustic device located in the innermost region of Berkeley Sound (see Figures 7.14, 7.16 and 7.17). The 2021 season was therefore greatly extended compared to all of the earlier years, with sei whales present regularly from late November (acoustic dataset), observed regularly during boat surveys in February and March, and then reaching their highest relative abundance in NEF from April to June.

The reasons for these differences in the onset and extent of the sei whale core season between years are unclear. However, since nearshore Falklands' waters are utilised as a feeding ground by sei whales (**Chapter 4**; Weir et al., 2019), it is likely that this variation is primarily driven by inter-annual variation in the distribution and abundance of their prey. This may include both prey availability in different parts of the Falklands, and also in the wider adjacent waters of the south-west Atlantic which can influence how much time sei whales spend in different geographic regions. Many studies have reported correlations between the density and distribution of baleen whales and that of their prey (e.g. Laidre et al., 2010; Feyrer an Duffus, 2015), and related seasonal variation in the distribution and abundance of baleen whales to preferred prey availability (e.g. Piatt et al., 1989; Macleod et al., 2004). Sei whales in the Falklands are known to predate at least squat lobster krill (*Munida gregaria*) and the amphipod *Themisto gaudichaudii* based on visual observations of surface feeding (Weir, 2017; Weir et al., 2019). Faecal sampling has documented a range of additional prey species (Buss et al., In Prep; **Chapter 4**). However, the spatio-temporal occurrence of these prey species in the Falklands, and their relative importance as preferred prey for sei whales, remain poorly known and require investigation before trends in the occurrence of sei whales can be better understood. With regard to local prey availability in different parts of the Falklands, it was notable that at FS the sei whale seasons in both 2019 and 2020 were longer than at NEF; in both years, sei whales were still present in good numbers during May in FS when none were observed at NEF that month. This suggests that prey availability at FS was higher than at NEF during May, supporting a presence of foraging sei whales later into the season. This was particularly the case in 2020, when surveys on consecutive dates at NEF and FS in mid-May encountered no sei whales at NEF but recorded 20 sightings in FS. It is also plausible that sei whales arrive at, and depart from, the Falklands via the Patagonian Shelf. For example, sightings off Golfo San Jorge in Argentina (46°S) during March, May, and August to October, indicate that sei whales may seasonally migrate through, and/or forage in, other areas of the Patagonian Shelf (Iñíguez et al., 2010). If the shallow Patagonian Shelf is used by sei whales as part of their seasonal movements to reach the Falklands from mainland South America, then it is plausible that the east coast of the Falklands (including NEF) may have a shorter overall season than the west coast of the Falklands, with animals arriving later to and leaving sooner from the eastern region. Similarly, FS may then be expected to have a slightly longer overall season than NEF. While speculative, this possibility could be investigated using a series of acoustic deployments around the Falklands over the same season.

The temporal variation (both month and year) of sei whales within and between sites in the Falklands is important to incorporate into the development of management measures for the species. The four years of data at NEF indicate that while there are several months where the species is present in all years, some other months (e.g. May and June) may have little sei whale activity in some years but high activity in other years. It is likely that this variation in temporal occurrence is also the case in other regions of the Falklands, although multi-year datasets at other sites are currently lacking. With regard to understanding when sei whales are using a particular area to inform the management and mitigation of human activities, it should therefore be considered that sei whale activity could potentially be high within the entire period from late November through to mid-June, and the exact months of core occurrence will vary within that period between years and sites. The emphasis is therefore on monitoring throughout those months, during potentially-adverse human activities that require the implementation of real-time mitigation measures. This temporal variation also has implications for the timing of future survey work targeting sei whales, since the peak period of sei whale occurrence varies between years. The *Falkland Islands Inner Shelf Waters KBA* for sei whales (Weir, 2021) requires reassessment every 8 to 12 years, and the planning of surveys to facilitate such an assessment should incorporate this information on temporal variation in order to optimise overlap of monitoring effort with the peak period for sei whales – in practice, given the variability in whale occurrence between years, this likely means a full season of data would be required.

With regard to spatial distribution, the DPLUS082 survey work recorded sei whales throughout the two study sites, and generally whales used all of the habitats that were surveyed across the sei whale season. By their nature, these sites were relatively shallow, coastal areas, with water depths of primarily <60 m. Within that depth range, sei whales at both sites predominantly occurred in mid-depth areas, with mean values (per 500 m grid cell) of 31.0 m at NEF and 34.2 m at FS. These values are comparable to the 2017 mean depth for sei whales in Berkeley Sound of 34.7 m (Weir, 2017), but lower than the West Falkland 2018 mean depth of 41.7 m (Weir, 2018). The latter site was surveyed to deeper water depths (>100 m) during transect work, and the presence of sei whales in those deeper areas explains the higher mean value. One spatial trend that was apparent, was the seasonal shift in sei whale distribution at NEF, with a much higher occurrence in the innermost part of Berkeley Sound during March compared to other months. This result correlates well with the findings of the acoustic dataset (**Chapter 7**), suggesting that there is a genuine seasonal change in the distribution of sei whales within Berkeley Sound that results in higher usage of the innermost portion during March. Conversely, in other months the relative abundance of sei whales was highest in the outer half of the Sound and/or in exposed waters adjacent to the open Atlantic. The distribution shift further inside the Sound during March is noteworthy, and could reflect factors such as prey availability, changes in anthropogenic noisescapes that potentially influence where the whales occur, or other seasonal changes in behaviour (for example, the 'L-calls' associated with sei whale song also increase in early March, marking the onset of singing behaviour in Berkeley Sound: **Chapter 7**).

It should be recognised that the presence of sei whales in the more exposed parts of NEF may be underestimated in the seasonal relative abundance maps. The waters located offshore and especially those located off, and to the south of, Cape Pembroke lighthouse, regularly experienced higher swells due to topography and prevailing swell direction, and consequently significant portions of data were omitted from those grid cells during some months even though sei whales were present in those areas.

Sei whale group sizes recorded during DPLUS082 were comparable to earlier studies. In Berkeley Sound during 2017, sei whales were recorded in groups of 1–7 animals with a mean of 2.0 animals (Weir, 2017). During DPLUS082, very similar values were recorded at NEF of 1–9 animals with a mean of 2.1 animals (this chapter). In West Falkland, groups ranged from 1 to 11 animals, with a mean of 1.8 animals (Weir, 2018). And in FS, groups had the largest range (1–12 animals) but the smallest mean group size (1.6 animals). In all of the studies, single animals comprised the majority of sightings (Berkeley Sound 2017 = 46.3%; NEF 2019–2021 = 45.4%; FS 2019–2021 = 67.3%; West Falkland 2018 = 53.8%). This suggests that sei whales occur in similar group structure throughout the Falklands, which would be expected if whales were primarily using the region for the same purpose, i.e. foraging. The lack of large aggregations of whales also suggests that much of the feeding behaviour exhibited by sei whales in the Falklands comprises individual subsurface foraging, rather than coordinated group foraging, perhaps reflecting available prey densities and depth distribution.

The boat surveys carried out as part of DPLUS082 also recorded the first sightings of mother-calf pairs in the Falklands; none were confirmed during the survey work in 2017 or 2018. Calves were present during 17 sightings in both of the study areas and in all three years, including at least eight different individual calves that were photo-identified in 2019 and 2020 (images for 2021 are yet to be analysed). The sightings of mother-calf pairs in the Falklands between February and April does not necessarily mean that calving is occurring in the waters around the Falklands (although it also doesn't exclude it). Rather, these calves may have been born at lower latitudes. Whaling data from the Southern Hemisphere indicate that the conception of sei whale calves occurs between January and November, with a strong peak between May and July (Horwood, 1987). Given that gestation lasts for around 12 months (Horwood, 1987, 2018), this implies that the peak period for calving may also be May to July but with some calves also born as late as October/November. Most calves are weaned after approximately seven months and after migrating to higher latitude feeding areas with their mothers (Horwood, 2018). Calves observed in the Falklands between February and April are therefore likely to have been born <7 months earlier, from July onwards. Sei whales migrate seasonally to lower latitude wintering grounds to give birth (Horwood, 1987), with one movement documented to date between the Falklands feeding ground and a wintering area off Brazil (Weir et al., 2020). Mother-calf pairs have been observed in Brazilian waters between May and October (Heissler et al., 2016; Wedekin et al., 2018; Weir et al., 2020); a calving period over these months corresponds well with the observations of mother-calf pairs on the feeding ground. These observations suggest that mother-calf pairs observed in the Falklands are likely to comprise calves that were born in the subtropics or tropics during winter, and have subsequently migrated south with their mothers. It is plausible that lactating mothers find sufficient prey around the Falklands to meet their energetic demands, and that calves are weaned in the region and feed there during their first critical months of self-sufficiency. The use of Falklands' coastal waters by mother-calf pairs and (probably) newly-weaned sei whales, further emphasises the importance of the region as an important foraging ground for the species.

2.4.3 Southern right whales

DPLUS082 provided the most comprehensive information available to date for the occurrence of southern right whales in the Falkland Islands, comprising two full winter seasons of surveys to target the species during 2019 and 2020. Little information existed beforehand, and most related to right whales foraging in more pelagic areas during summer (see Weir and Stanworth, 2020). The occurrence of southern right whales in the nearshore waters of NEF during the austral winter was first documented during 2017, when independent and targeted cetacean work in the Islands commenced on sei whales (Weir, 2017) and inshore dolphins (DPLUS042); those projects recorded right whales during boat surveys at NEF in May and June 2017 respectively. Recently, more information has emerged about the presence of southern right whales in the Falklands prior to 2017. One was seen inside Stanley Harbour in around 2005, while regular sightings of the species were observed just outside of the kelp beds along Volunteer Beach by the wardens during the winters of 2010 to 2012 (Micky Reeves, pers. comm). These reports confirm that the winter occurrence of right whales in the Falklands pre-dates 2017, although their full history is still not understood.

In 2017, shore watches and opportunistic reports during July and August indicated that the right whales first observed during May remained in NEF throughout the winter; the species was also reported again to a lesser extent during the winter of 2018 (Weir and Stanworth, 2020). DPLUS082 therefore aimed to carry out systematic boat surveys over the winters of 2019 and 2020 to determine whether the southern right whale wintering occurrence was persistent across years, clarify the spatio-temporal distribution of the whales, and determine why the whales were using Falklands' waters. The project has been successful in achieving these goals.

The targeted surveys carried out during 2019 and 2020, confirmed that 2017 was not an anomalous year for southern right whales. Rather, a significant wintering aggregation of right whales has now been documented within NEF across several years, with a seasonal peak in relative abundance during July evident in both 2019 and 2020. Consequently, it can be concluded that the north-east coast of the Falkland Islands represents a persistent, and newly-reported, wintering ground for the species. It remains unclear whether these wintering aggregations occur throughout the Falklands or whether NEF is of particular importance.

As with the sei whales, the systematic dataset indicated that some inter-annual variation occurred in the timing of right whale winter aggregations in NEF. Intra-annual variation in arrival times has also been noted at calving grounds, such as Península Valdés (Crespo et al., 2019). At NEF, right whales were present throughout June to August in both years, and this may be considered the core period for wintering aggregations (since sightings occurred less consistently in other months). However, as noted in Section 2.3.2.3, there were inter-annual differences in occurrence during the early part of the right whale season. During 2017, southern right whales were present in NEF from mid-May (Weir, 2017), and that was also the case in 2020. In contrast, they were relatively late to arrive in 2019, with regular sightings only commencing from 12 June despite ample survey coverage in May and early June. Their presence in 2021 was unusually early, with small numbers of right whales being encountered on boat surveys during March and April, as well as in May and June. These differences in the onset of the arrival of wintering aggregations at NEF presumably reflect inter-annual variation in feeding conditions during the preceding months. Given the geographic location of NEF, it is plausible that many of the right whales arriving there may have been foraging to the south or east of the Falklands, perhaps including South Georgia, the South Shetland Islands, the Scotia Sea, and the pelagic waters around the Falklands. Variations in environmental conditions across this wide region, and associated changes in prey availability, may be factors determining the annual arrival times. For example, whales may extend their time on foraging areas when prey availability is high and subsequently delay their movements towards wintering grounds, or those movements may simply take longer if areas of high prey resources are located further south in some years.

Weir and Stanworth (2020) noted that the potential uses of NEF by right whales during winter could include: (1) a short-term resting and socialising stop-off for animals migrating from foraging grounds located further east or south, before they continue northwards to established calving areas; (2) a wintering destination used for courtship and mating; (3) a winter gathering area for sub-adult and non-breeding adult right whales, primarily for social interaction; and (4) recolonisation of a historical winter calving ground. The datasets collected during 2019 and 2020 cannot discount any of those possibilities, with the exception that no calves have been confirmed to date. At the Península Valdés calving ground in Argentina, most new calves appear in August, and the final ones as late as October (Rowntree et al., 2013). While the surveys carried out in the Falklands during 2019 and 2020 had ended by early September, it would be expected that at least some new calves would have been apparent during August if the area was being used as a calving area by females. The behaviour observed in the Falklands over both winters is strongly supportive of the study area being used as an area for social interactions among juveniles and adults, and as an area where courtship and mating occur. Consistent with those observations is the results of the two-year acoustic monitoring programme (see **Chapter 7**), where the presence of gunshot song also supports the use of the area for socialising and mating purposes.

The International Whaling Commission (IWC) does have a Conservation Management Plan (CMP) in place for the south-west Atlantic population of southern right whales (Iñíguez Bessega et al., 2012). The CMP aims to protect right whale habitat, minimise anthropogenic impacts, and ensure that the recovery of the species continues to pre-exploitation levels. The Falklands Islands are mentioned in the CMP solely in the context of the “Brazil/False Banks/Falkland Islands” summer feeding ground, an offshore area extending between latitudes 30° and 55°S where a large number of right whales were captured during the whaling era (Townsend, 1935; Richards, 1993). However, two years of winter boat surveys in the Falklands have demonstrated the importance of the region as a wintering destination for southern right whales, and the genetic work confirms that these animals are part of the same south-west Atlantic population that uses calving areas in Argentina and Brazil (see **Chapter 5**). The increasing importance of the Falkland Islands as a foraging, migratory and wintering habitat for southern right whales, merits future recognition by, and inclusion in, the IWC CMP.

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Chapter 3: Photo-identification

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3.1 Introduction and aims

Cetacean photo-identification studies rely on the acquisition of high-quality images of the naturally-occurring markings that can be used to recognise individuals (Würsig and Jefferson, 1990; Hammond, 2017). The markings used to catalogue individual animals vary between species, depending on their morphology and behaviour. In all cases, the selected markings need to persist over the timeframe of the study, such that animals can continue to be recognised. Over longer timeframes, the recognition of individuals can provide valuable information including population size, movements, habitat use, social affiliations, survivorship, and life history parameters (Hammond, 2017).

Some cetacean species have been studied for decades using photo-identification, for example bottlenose dolphins (*Tursiops truncatus*; using nicks and notches in their dorsal fin edges: Urian et al., 2013), killer whales (*Orcinus orca*; using scarring in and shape of the saddle patch, and dorsal fin nicks: Kuningas et al., 2014), and humpback whales (*Megaptera novaeangliae*; using the pigmentation pattern on the underside of the tail flukes: Friday et al., 2000). One of the longest-running cetacean photo-identification studies anywhere worldwide is of southern right whales (*Eubalaena australis*) at Peninsula Valdés in Argentina, where the cataloguing of individual animals began in the 1970s (Payne, 1986). Right whales are born with a unique pattern of roughened, raised patches of skin on their heads, known as 'callosities' (Payne, 1986). The callosities are naturally colonised by light-coloured cyamid crustaceans (or 'whale lice') over time (Rowntree, 1983), which make them appear white or yellow in colouration, and aids their visibility to researchers. The pattern of callosities on the heads of right whales is their most conspicuous and stable feature, and forms the basis for photo-identification work. Traditionally, that work has relied on aerial images obtained of the entire dorsal surface of the head of each whale (e.g. Bogucki et al., 2019), from clifftops, planes, helicopters or unmanned aerial vehicles (UAVs). Photo-identification images taken laterally of right whales during boat surveys have several potential limitations compared to aerial imagery including: (1) reduced set of available features to catalogue and match individuals (as only part of the head is visible above the water); (2) increased difficulty with angles affecting the perceived appearance/placement of callosities; (3) challenges with matching left and right side images to the same individual, when multiple animals are present; and (4) challenges with matching animals to the long-running aerial catalogues maintained in most other geographic regions. In the Falkland Islands, systematic photo-identification of southern right whales had not been carried out prior to the DPLUS082 project.

In contrast to the southern right whale, concerted photo-identification of sei whales (*Balaenoptera borealis*) globally has been sparse. This largely reflects an absence of regular encounters with this species in many of the geographic areas where whale scientists are based, but is also the result of the low amount of natural markings on sei whales compared with many other cetacean species. Consequently, while a few small and fairly opportunistic photo-identification results have been published for sei whales (e.g. Schilling et al., 1992; Acevedo et al., 2017), concerted multi-year cataloguing of this species is generally lacking. The first intensive and targeted photo-identification study of sei whales across a full season anywhere worldwide was carried out at Berkeley Sound in the Falkland Islands during 2017 (Weir, 2017), and individuals were found to be recognisable using nicks and notches along the edges of the dorsal fin, pigmentation and scars on the fin, and the pattern of cookie-cutter shark (*Isistius brasiliensis*) scarring on the flanks. The method produced a minimum population size of 87 sei whales (Weir, 2017), and also provided useful information on the duration that individual animals spent within the Sound, and on their spatial movements. This was followed by a similar concerted effort on the west coast of the Falklands during 2018, which included photographic recaptures of two individuals from the 2017 Berkeley Sound study (Weir, 2018). Consequently, the technique was found to be applicable to sei whales, and formed the basis for the proposed photo-identification work carried out at two sites during DPLUS082.

The DPLUS082 photo-identification study had the following aims:

1. Assessing whether photo-identification could be systematically applied to southern right whales using lateral head images obtained from a small boat;
2. Calculating the minimum number of sei whales present at Falkland Sound (FS) and the North-east Falklands (NEF) over the study period;
3. Investigating whether individual sei whales were recaptured between: (1) FS and NEF over the study period; (2) FS/NEF and the 2017 Berkeley Sound dataset; or (3) FS/NEF and the 2018 West Falkland dataset;
4. Calculating the minimum number of southern right whales present at NEF over the study period;
5. Investigating whether recaptures of individual whales (both species) occurred in different years at either site; and
6. Determining the residency of individual whales (both species) in the study areas each year.

3.2 Materials and methods

3.2.1 Data collection

Images were taken of sei whales and southern right whales during small boat surveys carried out at FS and NEF (see **Chapter 2** for general methods and results from small boat surveys). One day of survey effort, including photo-identification, was also spent in FS on 25 January 2020 from the yacht *Saoirse* (see Weir, 2020), primarily focussed on the southern part of FS and outside of the normal area covered during the DPLUS082 small boat surveys.

The author acted as Survey Leader, and made decisions about whether or not to deviate the survey to approach whales for photo-identification (and other sampling) on a case-by-case basis. These decisions depended on factors including the behaviour of the whales encountered, prevailing weather conditions, and survey time constraints. If the decision was made to approach whales, photo-identification was usually the initial priority activity carried out. The coxswain was asked to carefully manoeuvre the boat to position the photographers parallel with the animal(s) and to travel slowly alongside them.

High-resolution images suitable for photo-identification work were taken with Canon 100–400 mm zoom lenses, and either a Canon 7D Mark II DSLR or a 5D Mark III DSLR camera body. The clocks on all camera bodies were synchronised with the GPS to ensure that images could be cross-referenced with particular sightings. Whenever possible, two members of the team initially took images simultaneously. Where groups were followed, equal effort was made to photograph every individual in the group. Both sides of the animals were photographed whenever possible. Photo-identification effort focussed on obtaining images of the dorsal fin region during sei whale encounters, and on the callosity pattern on the heads of southern right whales (Figure 3.1). When the Survey Leader determined that a reasonable amount of photo-identification effort had been achieved for a given encounter and conditions were appropriate for other work, a switch was made to genetic or faecal sampling; at those times the volunteer assistant continued to take photo-identification images to identify sampled individuals.

The Survey Leader monitored whales for signs of disturbance throughout photo-identification effort, and encounters were aborted when repeated avoidance was observed or as soon as it was felt that sufficient images had been obtained of each individual.

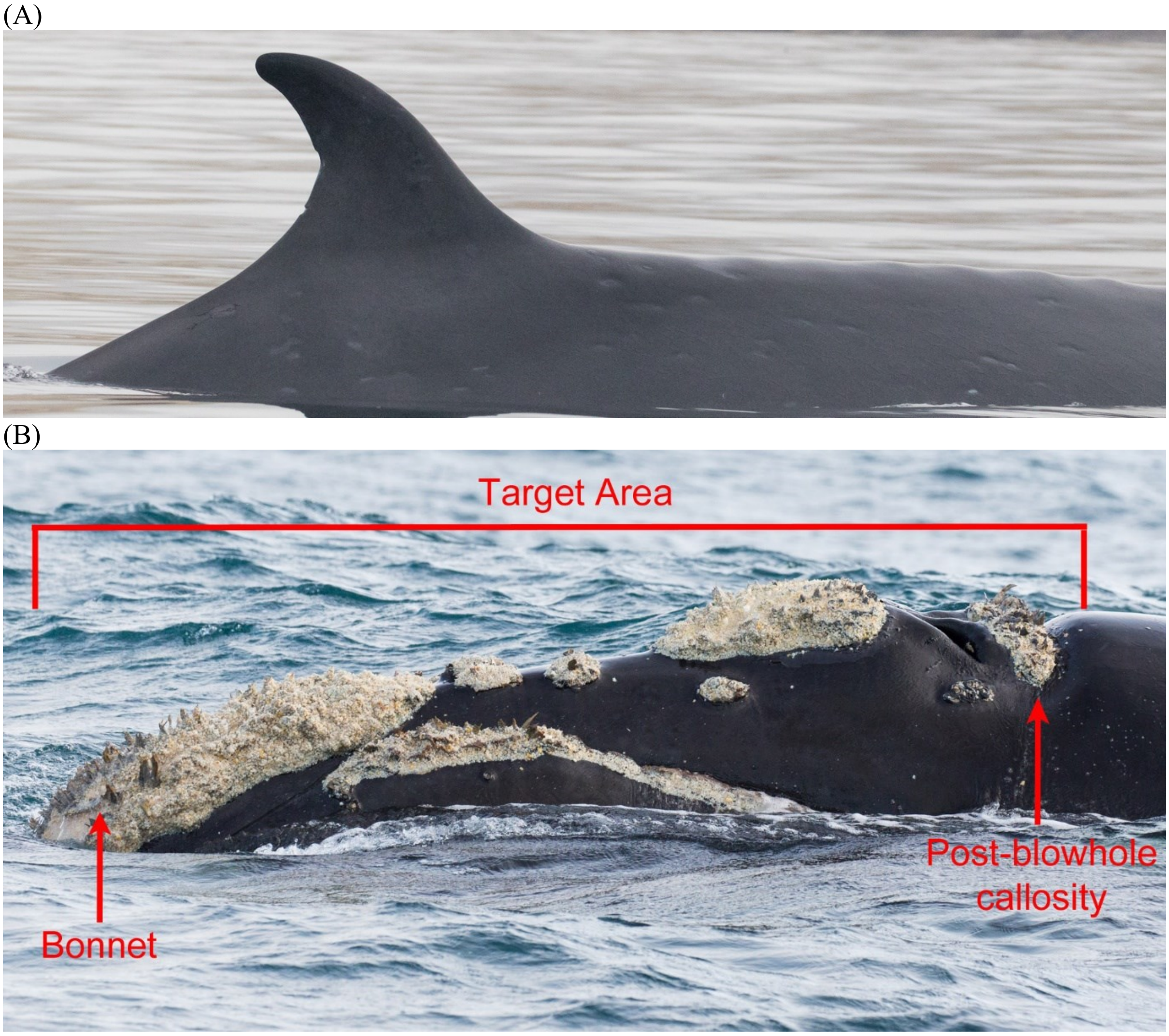


Figure 3.1. *The target areas used for photographic effort on: (A) sei whales, comprising the dorsal fin and scar pattern on the flank; and (B) southern right whales, comprising a lateral view of the head callosities.*

3.2.2 Cataloguing

At the end of each survey day, all images were downloaded, filed by sighting reference number, and renamed with a unique code/number. The Survey Leader subsequently worked through every photo-identification encounter, visually-assessing each image and assigning it to a sub-folder for a particular individual where possible based on unique markings. Left- and right-side images were matched to the same animal where possible, using distinctive features. The highest-quality images of the left and right sides of each distinctive individual were then selected for the cataloguing process.

For sei whales, images from NEF were firstly matched to the existing 2017 catalogue for Berkeley Sound. If the individual had already been catalogued in 2017, then it was considered to be a photographic 'recapture' and the same unique Identification Number was used to catalogue it in the 2019 and 2020 catalogues developed as part of DPLUS082. If it was a new animal then it was entered into the catalogues with a new code. Separate sei whale catalogues were developed for Berkeley Sound in 2019 and 2020, with the best-available images for each year used in each. The amount of photographic effort in Falkland Sound was lower overall, and consequently a single catalogue was established for that site using the combined data from 2019 and 2020.

For southern right whales, images from 2019 and 2020 were entered into separate annual catalogues. Individual animals in each new encounter were firstly matched with any animals already catalogued in earlier encounters/years, and either allocated the same unique Identification Number for a recapture, or assigned a new number if they had not been previously catalogued.

At the end of this process, the DPLUS082 project generated five catalogues:

- Sei whales in Berkeley Sound in 2019 – BS coded ID numbers (continuing from 2017);
- Sei whales in Berkeley Sound in 2020 – BS coded ID numbers (continuing from 2017 and 2019);
- Sei whales in Falkland Sound in 2019/2020 – FS coded ID numbers;
- Southern right whales in North-east Falklands in 2019 – FEA coded ID numbers;
- Southern right whales in North-east Falklands in 2020 – FEA coded ID numbers (continuing from 2019).

Example catalogue pages from the sei whale and southern right catalogues are illustrated in Figures 3.2 and 3.3. Each catalogue was cross-checked for false positives (i.e. matching images to the same animal that actually originate from two separate individuals) and false negatives (i.e. allocating images from the same animal to two different individuals). False positives can be reduced with care and by using only good-quality images. False negatives are more common in cetacean studies, and can result from: (1) matching images of insufficient quality (including different light conditions that might affect the visibility of scarring); (2) attempting to match individuals that are very poorly-marked; and (3) changes in the natural markings between encounters caused by acquisition or healing of scars or nicks.

3.2.3 Quality control

3.2.3.1 Sei whales

The photographic quality (PQ) of each image in the sei whale catalogues was rated 1–4 (excellent, good, fair or poor) according to the focus, camera angle, exposure, and the size of the dorsal fin/flank region relative to the frame (e.g. Gendron and Ugalde de la Cruz, 2012; Tezanos-Pinto et al., 2017). Separate PQ ratings were allocated to the best-available left and right side images. Photographs with a PQ of 4 contained features that may be useful to identify the individual, but were not of sufficient quality for population parameter estimations (Gendron and Ugalde de la Cruz, 2012).

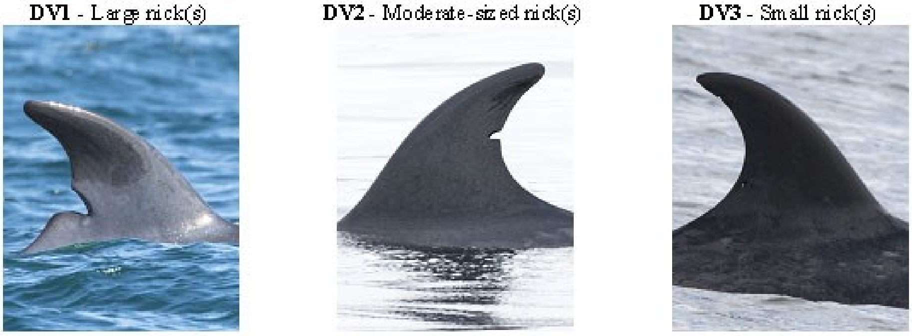
Each catalogued sei whale individual was assigned a Distinctiveness Value (DV) based on: (1) permanent marks along the edges of the fin or posterior to the fin on the tailstock, or (2) scar patterns in the TA (Table 3.1 and Figure 3.4). The lowest applicable value was used; i.e. if an animal had both moderate nicks in the dorsal fin and heavy scarring it was allocated to DV2 rather than DV6A. See Weir (2018) for more details on the allocation of DV's to sei whales.

Table 3.1. *Definitions of Distinctiveness Value (DV) allocated to individual sei whales.*

DV	Criteria	
1	Conspicuous large nick(s), hole through the dorsal fin or fin deformity/injury.	
2	Moderate-sized nick(s), hole through the dorsal fin or fin deformity/injury.	
3	Subtle/shallow nick(s) in fin edge or holes through the dorsal fin.	
4	Uniquely-distinctive fin shape, such as wavy indents in edges of dorsal fin.	
5	One or more dorsal notch on the surface of the tailstock, in the area posterior to the dorsal fin.	
6	No evidence of nicks, notches or other permanent marks. Classified from scarring in the target area:	
	6A	Extensively scarred/lesioned, including conspicuous marks suitable for identification.
	6B	Moderately scarred/lesioned.
	6C	Animal only very lightly scarred/lesioned/pigmented and generally poorly-marked.

Figure 3.2. *Example page from the sei whale photo-identification catalogue*

Figure 3.3. *Example page from the left side southern right whale photo-identification catalogue.*



DV1 - Large nick(s)

DV2 - Moderate-sized nick(s)

DV3 - Small nick(s)

DV4 - Distinctive shape

DV5 - Tailstock notch

DV6A - Extensive scarring in target area

DV6B - Moderate scarring in target area

DV6C - Light scarring in target area

Figure 3.4. *Illustrative examples of Distinctiveness Value (DV) allocated to individual sei whales. Red boxes show the defined target area (TA) for scar-based photo-identification. From Weir (2018).*

3.2.3.2 Southern right whales

The photographic quality (PQ) of each image in the southern right whale catalogues was rated 1–4 (excellent, good, fair or poor) according to the four factors detailed in Table 3.2. Each factor was scored '1' if the image was optimal, and '2' if the image was suboptimal. Scores were then tallied to assign PQ values of: 4 = Excellent; 5 = Good; 6 = Fair; and 7 = poor. Separate PQ ratings were allocated to the left- and right-side catalogues, such that an individual right whale could have different scores for the images taken on either side.

Table 3.2. *Factors scored to assess Photographic Quality (PQ) for lateral southern right whale images in the Falkland Islands.*

Factor	Quality	
	Optimal	Suboptimal
1. Visibility of Target Area (TA)	Entire TA is visible; not significantly obscured by spray	Part of TA is obscured by waves, other whales, spray, or angle
2. Sharpness (post-image cropping to the TA)	TA is well-focussed and sharp	Not sharp/poor resolution
3. Exposure (post digital manipulation)	Image is sufficiently well-exposed to readily view the callosity pattern	Image is too bright or too dark to easily make out the callosity pattern
4. Angle of the whale to the photographer	Perpendicular	Angled image (whale is orientated towards or away from the photographer)

Given that all southern right whales have callosity patterns, it was considered that most photographed (non-calf) individuals would be inherently distinctive. Each left and right-side lateral image in the photo-identification catalogues was assigned a DV according to the definitions provided in Table 3.3. These DVs were allocated based on permanent features available in images of the TA (i.e. on the head: Figure 3.1). Examples of the DVs used for right whales are provided in Figure 3.5.

3.2.4 Data analysis

The minimum population size (MPS) was defined as the total number of distinctive whales that were photographically-captured in the study area over the duration of the study (i.e. with no genetic or absolute abundance implications). It is intended as a crude initial indicator of the number of whales using the sites.

For sei whales, the MPS comprised the sum of: (1) all permanently-marked animals (DV1–5; which should be identifiable from both sides), and (2) all animals identified from scar patterns (DV6) on one side (left or right, depending which was highest). Calculations of MPS were made using only a subset of animals for which images of PQ1–3 were available for at least one side. A separate MPS was calculated for FS and NEF.

For southern right whales, the MPS consisted of all individuals coded as DV2–4, using images of PQ4–6. Separate MPS values were calculated for 2019 and 2020, and for the left- and right-side catalogues in each year.

Table 3.3. *Definitions of Distinctiveness Value (DV) allocated to individual southern right whales using lateral images in the Falkland Islands. Note that some very distinctive features on the body (e.g. white blaze colour pattern, or large and pigmented dorsal scars) were not available in images of the Target Area (see Figure 3.1).*

DV	Description	Definition
0	Calf	Callosity pattern not yet sufficiently stable for recognition
1	Not distinctive	No rostral islands or accessory callosities, plus moderate to heavy lip callosity obscuring jaw crenulations.
2	Slightly distinctive	No rostral islands or accessory callosities, but low to zero lip callosity allowing jaw crenulation pattern to be visible; or One rostral island or accessory callosity or tubercle visible laterally.
3	Distinctive	Two rostral islands or accessory callosities or tubercles visible laterally.
4	Highly distinctive	Any individual with a grey morph colour pattern; or Any individual with a serious head injury; or ≥3 rostral islands and/or accessory callosities and/or tubercles visible laterally; or Any individual with continuous (unbroken) callosity between the bonnet and the coaming.

*Visible laterally means that the feature is either located on that side of the head, or is on the dorsal surface of the head such that it is visible on both sides of the animal when perpendicular to the photographer.

3.3 Results

3.3.1 Overview

A total of 128,087 photo-identification images were taken of the two target whale species over the three years of DPLUS082, comprising 83,580 images of sei whales and 44,507 images of southern right whales (Table 3.4). A similar number of images was taken in 2019 and 2020 (Table 3.4); however, a far larger number of images was taken in 2021 despite the shorter timeframe of that season (see Chapter 2). This was primarily due to the high number of sei whales encountered in 2021. The locations of photo-identification encounters with sei whales and southern right whales are shown in Figures 3.6 and 3.7 respectively.

Table 3.4. *Number of photo-identification images collected for sei whales and southern right whales during small boat surveys, 2019-2021.*

Species	Site	2019	2020	2021	Total
Sei whale	FS	5,537	2,978	14,839	23,354
Sei whale	NEF	13,422	15,274	31,530	60,226
Southern right whale	NEF	17,233	19,211	8,063	44,507
Total	FS/NEF	36,192	37,463	54,432	128,087

3.3.2 Sei whale

A total of 273 sei whales were initially catalogued in FS and/or NEF in 2019 and 2020. Of those, there were seven individuals for which only images of PQ4 (poor quality) were available. Those animals were retained and catalogued solely in case better images of them were obtained in the future, but were not considered to be of sufficient quality to be certain of their uniqueness or to include in further analyses. An additional eight individuals were identified as definite ($n = 7$) or probable ($n = 1$) calves, and were also removed from the analysis.

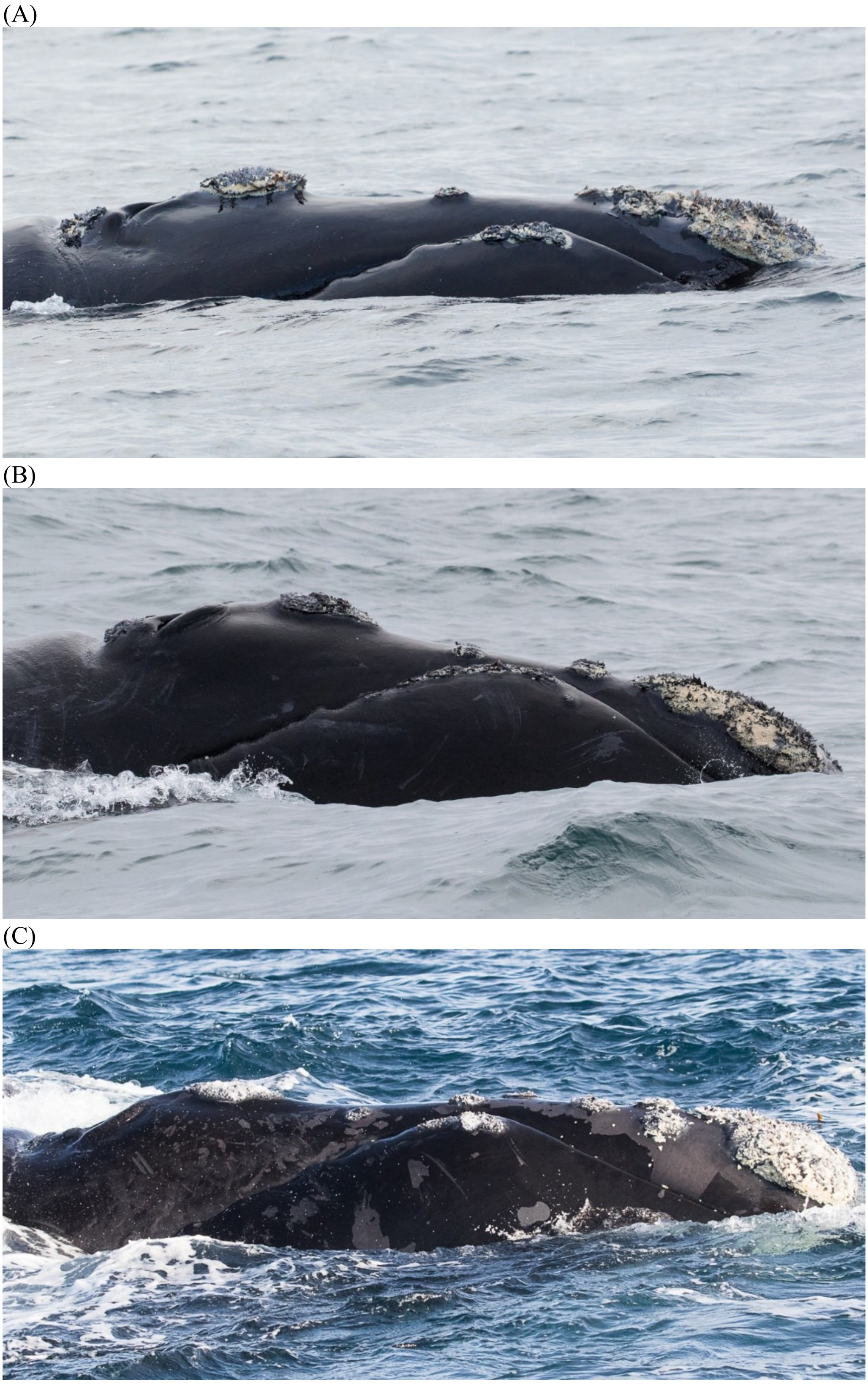


Figure 3.5. *Illustrative examples of Distinctiveness Value (DV) allocated to individual southern right whales using images of the defined target area shown in Figure 3.1: (A) DV2; (B) DV3; and (C) DV4.*

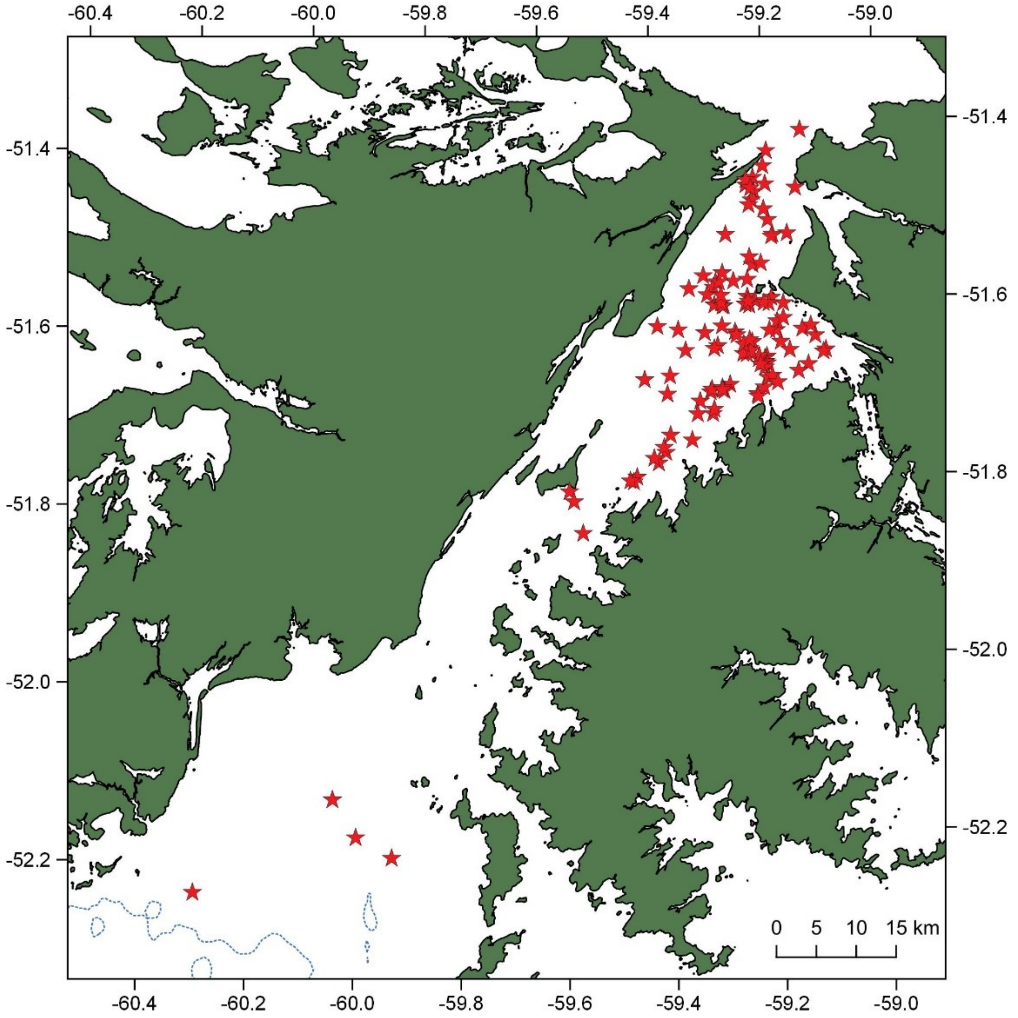


Figure 3.6. *Location of photo-identification encounters with sei whales in Falkland Sound, 2019–2021. Sighting locations have been recalculated to reflect animal positions rather than the location of the boat. The four locations at the southern end of Falkland Sound were encounters recorded from the yacht* Saoirse *in January 2020.*

The remaining 258 animals had images of PQ1 to PQ3 available for at least one side of their body. For 212 individuals, images of both the left and right sides were acquired; however, for 20 of those animals then only PQ4 images were available for one of the sides. Only left side images were available for 24 individuals, and only the right side for 22 individuals. A total of 79.8% of the 258 individuals were of DV1–5, and had permanent markings (Table 3.5). Animals lacking permanent markings that were catalogued from scar patterns (DV6) comprised 20.2% of the total (Table 3.5).

Table 3.5. *Summary of the distinctiveness values (DVs) of 260 non-calf sei whales catalogued from images of fair to good photographic quality (PQ1–3) in 2019 and 2020.*

DV	Falkland Sound	North-east Falklands	Both sites	Total	
				No. of animals	% of animals
DV1	7	9	0	16	6.2
DV2	21	34	0	55	21.3
DV3	35	71	2	108	41.9
DV4	1	6	0	7	2.7
DV5	9	11	0	20	7.8
DV6	17	35	0	52	20.2
Total	90	164	2	258	100.0

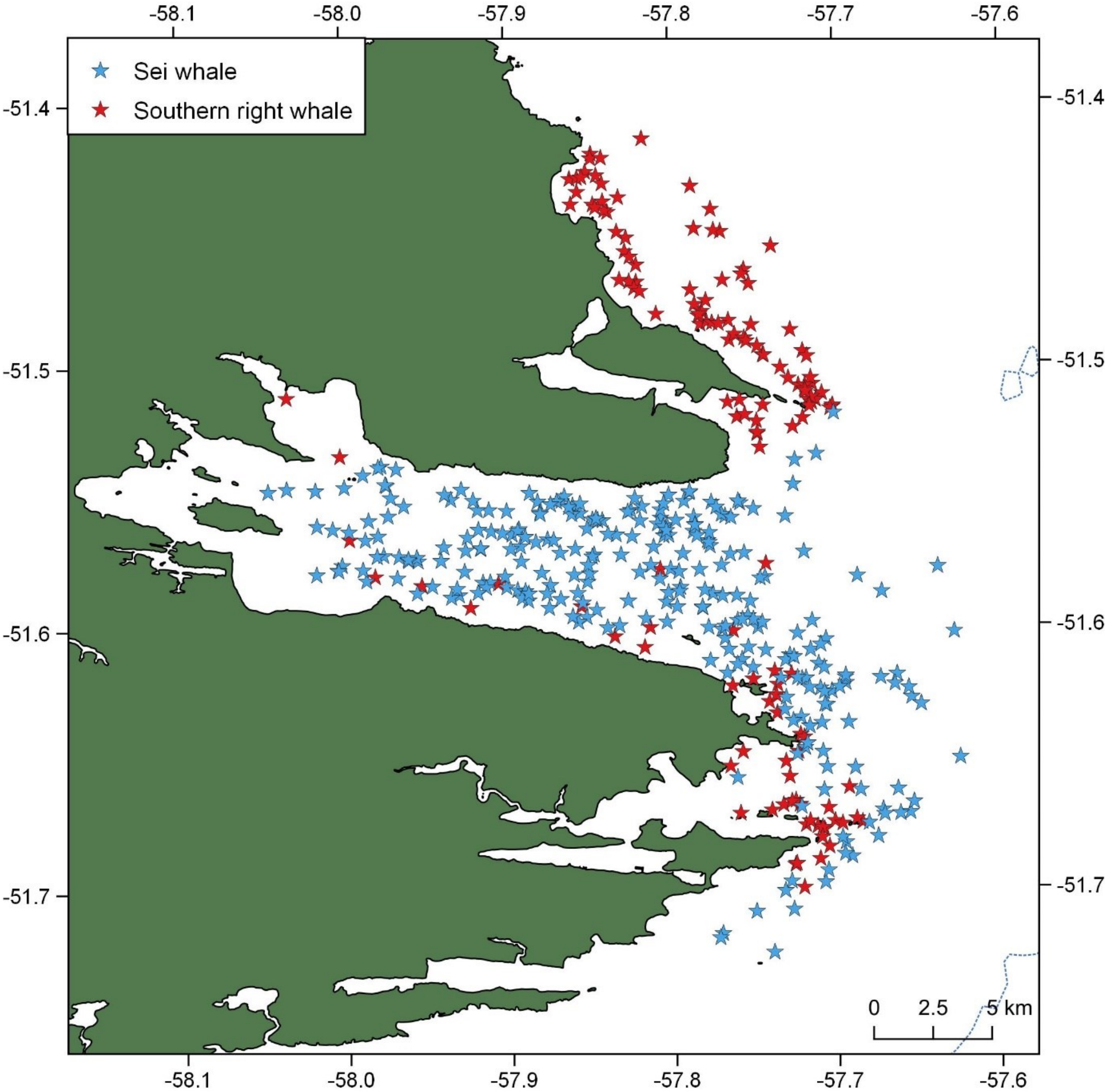


Figure 3.7. *Location of photo-identification encounters with sei whales and southern right whales during small boats surveys in the north-east Falklands, 2019–2021. Sighting locations have been recalculated to reflect animal positions rather than the location of the boat.*

In NEF, photo-identification work was most productive during March in 2019 and 2020 (Figure 3.8), both in terms of the total number of data collection days available in favourable weather, and with regard to the numbers of whales in the study area. Monthly coverage at FS was not sufficiently consistent over months or years to carry out a similar analysis for that site.

3.3.2.1 Minimum population size

The MPS for FS, NEF and both sites combined is provided in Table 3.6. In combination across the two years and both sites, the MPS for sei whales was 254 animals. A higher MPS was recorded in NEF compared with FS, which likely reflects the discrepancy in survey effort at the two sites (see **Chapter 2**).

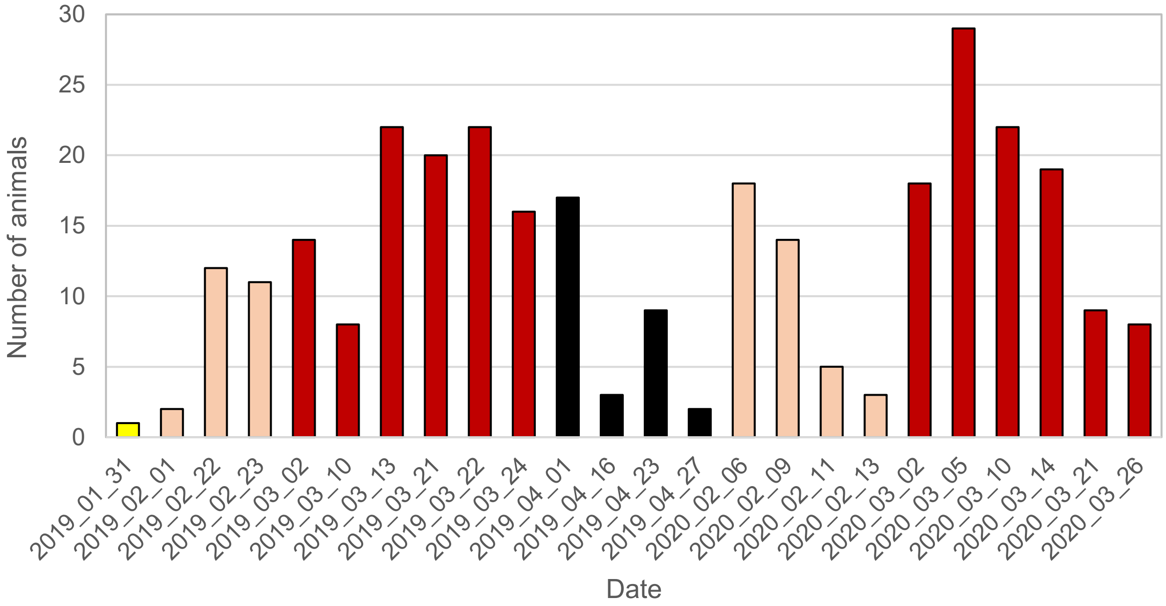


Figure 3.8. *Number of non-calf sei whales (PQ1–3) photographed by survey date in the NEF; some individuals were photographed on more than one date (see Section 3.3.2.4). Data collection in 2020 stopped on 27 March due to the COVID-19 outbreak. Bars are shaded by survey month: Jan = yellow; Feb = orange; Mar = red; April = black.*

Table 3.6. *The minimum population size (MPS) of sei whales calculated for different site and year combinations based on Distinctiveness Value (DV). Some individuals were photographed in more than one site or more than one year, and consequently the combined values may be different to the summed totals for individual sites/years.*

Site	Year	DV1–5	DV6 left side	DV6 right side	MPS
Falkland Sound	2019	35	8	9	44
	2020	42	6	8	50
	2019+2020	76	14	17	93
North-east Falklands	2019	71	24	21	95
	2020	65	10	12	77
	2019+2020	133	32	31	166
Combined sites	2019	104	32	30	136
	2020	106	16	20	126
	2019+2020	206	46	48	254

3.3.2.2 Inter-site recaptures

Prior to DPLUS082, two photographic recaptures of sei whales had been documented between Berkeley Sound in 2017 and the west coast of the Falklands in 2018 (Ref 1 and 2 in Table 3.7: Weir, 2018). This study has added another eight inter-site recaptures, including a third recapture of BS-100/WF-115 which has now been recorded in NEF in 2017, West Falkland in 2018, and in NEF again in 2020. There were two additional recaptures of sei whales between West Falkland and NEF (Figure 3.9), attesting to the wide use of Falklands' waters by this species. There were also two recaptures in Falkland Sound of sei whales that had been originally recorded in West Falkland (Table 3.5; Figure 3.9). Finally, three movements were recorded of sei whales between Falkland Sound and NEF; this included one mother-calf pair photographed at both sites in 2019 (Table 3.5; Figure 3.9).

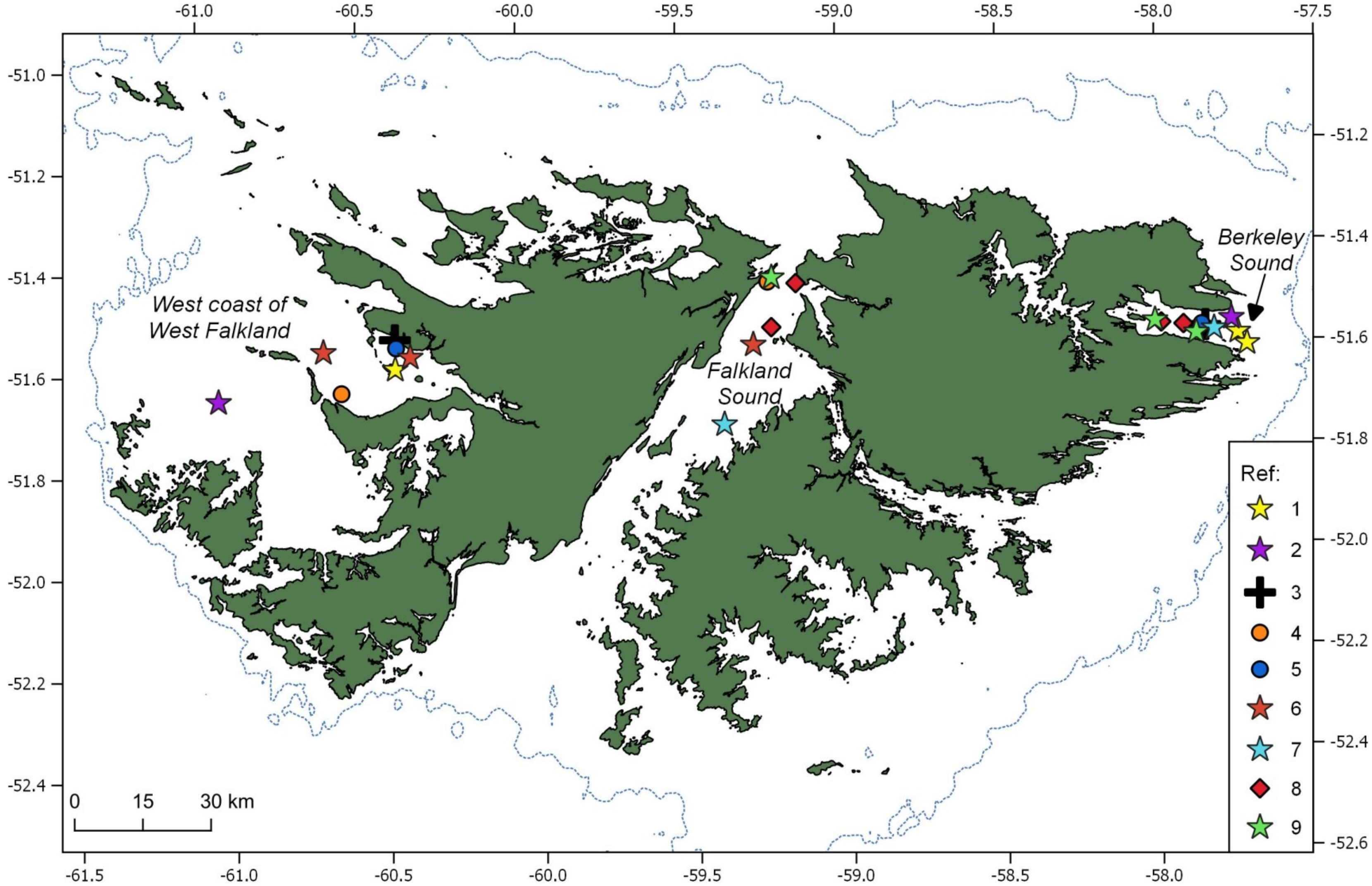


Figure 3.9. *Locations of inter-site photographic recaptures of individual sei whales from 2017 to 2020. Reference numbers match those provided in Table 3.7.*

The multi-site nature of DPLUS082 provided a novel opportunity to investigate movements of individual sei whales between sites *within* the same year. Three such recaptures between NEF and FS were recorded within the same year, including one adult-calf pair (Refs 7 to 9: Table 3.7, Figure 3.9). The pair were seen in FS on 17 February and 1 March 2019, before being recaptured inside Berkeley Sound 12 days later on 13 March 2019. The other two recaptures comprised reverse movements with the initial encounter taking place in Berkeley Sound and the subsequent recapture occurring in FS; the timespans between these captures were longer, at 46 days (Ref 7: Table 3.7) and 74 days (Ref 9: Table 3.7) respectively. Using conservative estimates if the whales had moved in straight lines between headlands around the coast, the distance travelled between sightings at each site was ~140 km for Ref 8 and 9, and ~170 km for Ref 7.

Table 3.7. *Photographic captures and recaptures of individual sei whales between different regions of the Falkland Islands: FS = Falkland Sound; NEF = North-east Falklands; WF = west coast of West Falkland.*

Ref	Individual	Sites documented	Years of photographic capture		
			First capture	Recapture 1	Recapture 2
1	BS-100 / WF-115	NEF – WF – NEF	2017	2018	2020
2	BS-80 / WF-31	NEF – WF	2017	2018	–
3	WF-77 / BS-196	WF – NEF	2018	2019	–
4	WF-12 / FS-96	WF – FS	2018	2020	–
5	WF-130 / BS-214	WF – NEF	2018	2020	–
6	WF-7 / FS-89	WF – FS	2018	2020	–
7	BS-138 / FS-35	NEF – FS	2019	2019	–
8	FS-4 / BS-149 (adult) & FS-5 / BS-150 (calf)	FS – NEF	2019	2019	–
9	BS-227 / FS-97	NEF – FS	2020	2020	–

3.3.2.3 Inter-annual recaptures

Five new inter-annual recaptures documented during DPLUS082 also involved movements between sites; those data are summarised in Refs 1 and 3–6 in Table 3.7. The recapture of BS-100 / WF-115 during 2020 was the third separate year that any individual sei whale has been documented around the Falklands, and supports longer-term fidelity of some animals to the Falklands feeding ground. Of the four other inter-annual recaptures summarised in Table 3.7, all originated from the West Falkland 2018 survey and were then recorded again during DPLUS082; one was an animal documented again in 2019, while three were recaptured during 2020. Additionally, there were 11 inter-annual recaptures that occurred within the same site (Table 3.8), predominantly occurring at NEF which received the most survey coverage during DPLUS082 and for which a photo-identification catalogue had already been established in 2017 and formed the baseline for a number of recaptures (Weir, 2017).

Table 3.8. *Inter-annual photographic recaptures of individual sei whales within the same site.*

Individual	Capture 1	Capture 2	Site
BS-19	2017	2019	North-east Falklands
BS-26	2017	2019	North-east Falklands
BS-36	2017	2019	North-east Falklands
BS-56	2017	2019	North-east Falklands
BS-60	2017	2019	North-east Falklands
BS-112	2019	2020	North-east Falklands
BS-122	2019	2020	North-east Falklands
BS-124	2019	2020	North-east Falklands
BS-169	2019	2020	North-east Falklands
BS-191	2019	2020	North-east Falklands
FS-22	2019	2020	Falkland Sound

3.3.2.4 Intra-annual recaptures

Totals of 98 and 87 non-calf individuals for which PQ1 to PQ3 images were available were photographed in NEF during 2019 and 2020 respectively (those totals include some of the same individuals encountered in both years: Table 3.7). The majority of animals (62% in 2019 and 60% in 2020) were photographed on only one date in NEF over the course of the season (Figure 3.10). Similar proportions were also recorded in 2019 and 2020 for animals with two recaptures (22 and 23%), three recaptures (8 and 10%), and four recaptures (5 and 6%). Two animals in 2019 were recorded on five dates each (BS-131 and BS-184), while one whale in 2020 (BS-218) was photographed on six separate dates.

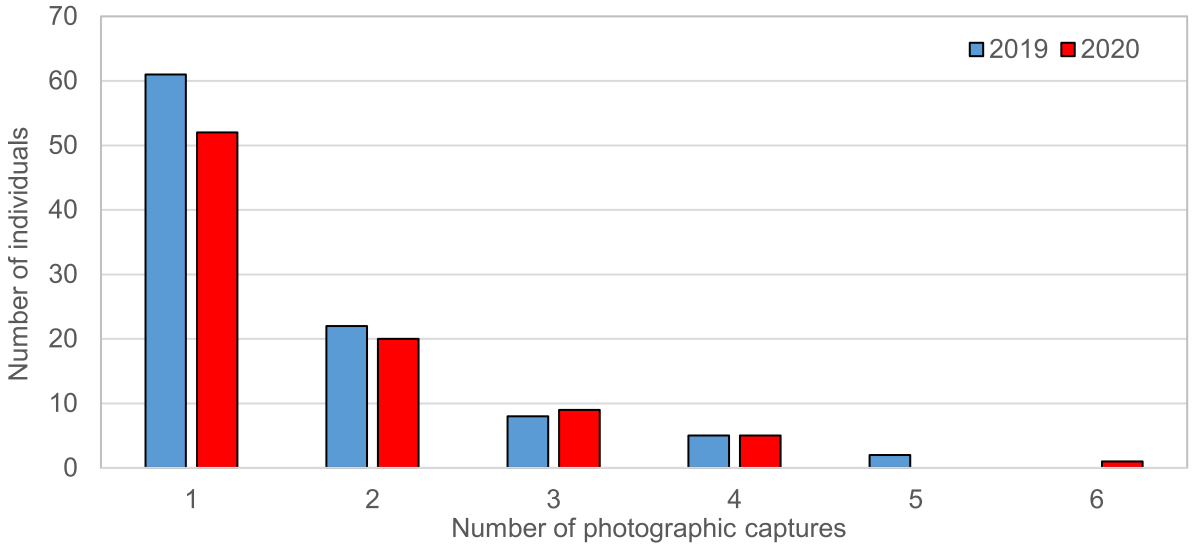


Figure 3.10. *Number of photographic captures for individual sei whales in NEF during 2019 and 2020.*

A total of 37 (non-calf) individuals were sighted in NEF on more than one date in 2019, and 35 in 2020 (Figure 3.11). The maximum number of days between first sighting and final recapture within each season was 52 days in 2019 (BS-132) and 46 days in 2020 (BS-218). However, the 2020 sei whale season ended early on 27 March due to COVID-19 restrictions, which greatly limited the scope for recording additional longer-duration recaptures. In general, the two years showed a similar trend, with approximately 40% of recaptures occurring within a week of the initial capture, and very few whales recaptured over intervals exceeding 40 days (Figure 3.11). A robust comparison of the average duration between recaptures in different years was hindered by: (1) the short season in 2020; and (2) the inconsistent survey effort across weeks and months due to weather and the logistics of operating at two sites. However, and acknowledging those constraints, the average number of days between recaptures of sei whales in NEF was 15.8 days in 2019 and 13.3 days in 2020, which is broadly similar between the years.

In FS, totals of 44 and 50 non-calf individuals for which PQ1 to PQ3 images were available were photographed during 2019 and 2020 respectively (those totals include one individual encountered in both years: Table 3.8). In 2019, photo-identification images were collected on six dates in FS between 17 February and 15 May. Most animals (86.4%) were captured on only one survey date. However, five animals were recaptured once, and one animal was photographed on three separate survey dates:

- Two animals were photographed on the 6 and 15 May (9 day interval);
- Two were photographed on the 17 Feb and the 1 Mar (12 day interval);
- One was photographed on 28 Mar and then recaptured on both the 6 and 15 May (48 days in total): and

- One individual was recorded on 1 March and then again on 25 April (55 day interval).

Due to combined factors including weather, logistics and COVID-19, FS was surveyed on only two dates in 2020 (see **Chapter 2**): the 25 January from the yacht *Saoirse* (35 animals photographed) and 15 May from the RIB (15 animals photographed). There were no recaptures between those two dates, and little can be concluded due to the very low survey effort.

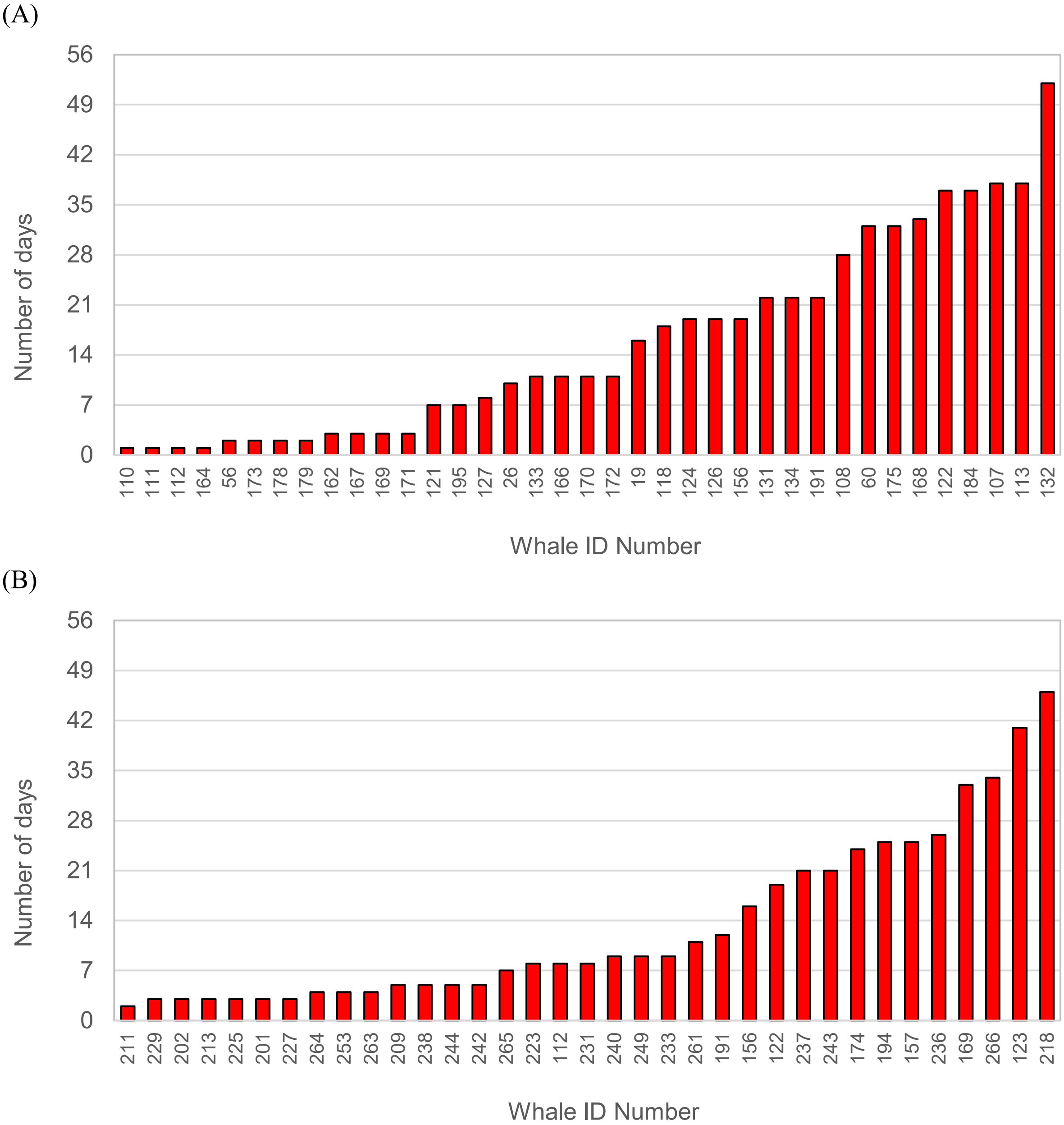


Figure 3.11. *Number of days from the first to the last sighting of 35 sei whales that were photographically-captured on more than one survey date in NEF: (A) 2019; and (B) 2020.*

3.3.2.5 Scar longevity

Eight individuals were photographically recaptured (see Tables 3.7 and 3.8) two years after their initial capture (in 2017 and 2019, or in 2018 and 2020), while one individual (BS-100 / WF-115) was recaptured three years after its initial capture (in 2017, 2018 and 2020). These durations of 2 to 3 years between captures, provided the opportunity to assess the timeframes over which the natural marks used to recognise sei whales remained consistent. Three examples of consistency in marks over time are illustrated for different sei whale individuals in Figures 3.12 to 3.14. The lack of nicks in the fin edges of BS-19 (Figure 3.13) and BS-60 (Figure 3.14) meant that scarring was the primary method of confirming matches for those animals. It is apparent that natural scars remain conspicuous over at least a 2 to 3 year timeframe; however, new scars do have the potential to obscure existing marks (Figure 3.14).

3.3.2.6 Scar appearance

The photo-identification of sei whales carried out during DPLUS082 also highlighted known issues with photographic quality. In particular, it was apparent that variations in angle, light conditions, and the flexing of an animal's body as it surfaced, impacted the visibility and appearance of flank scars. Examples are shown in Figures 3.15 and 3.16.

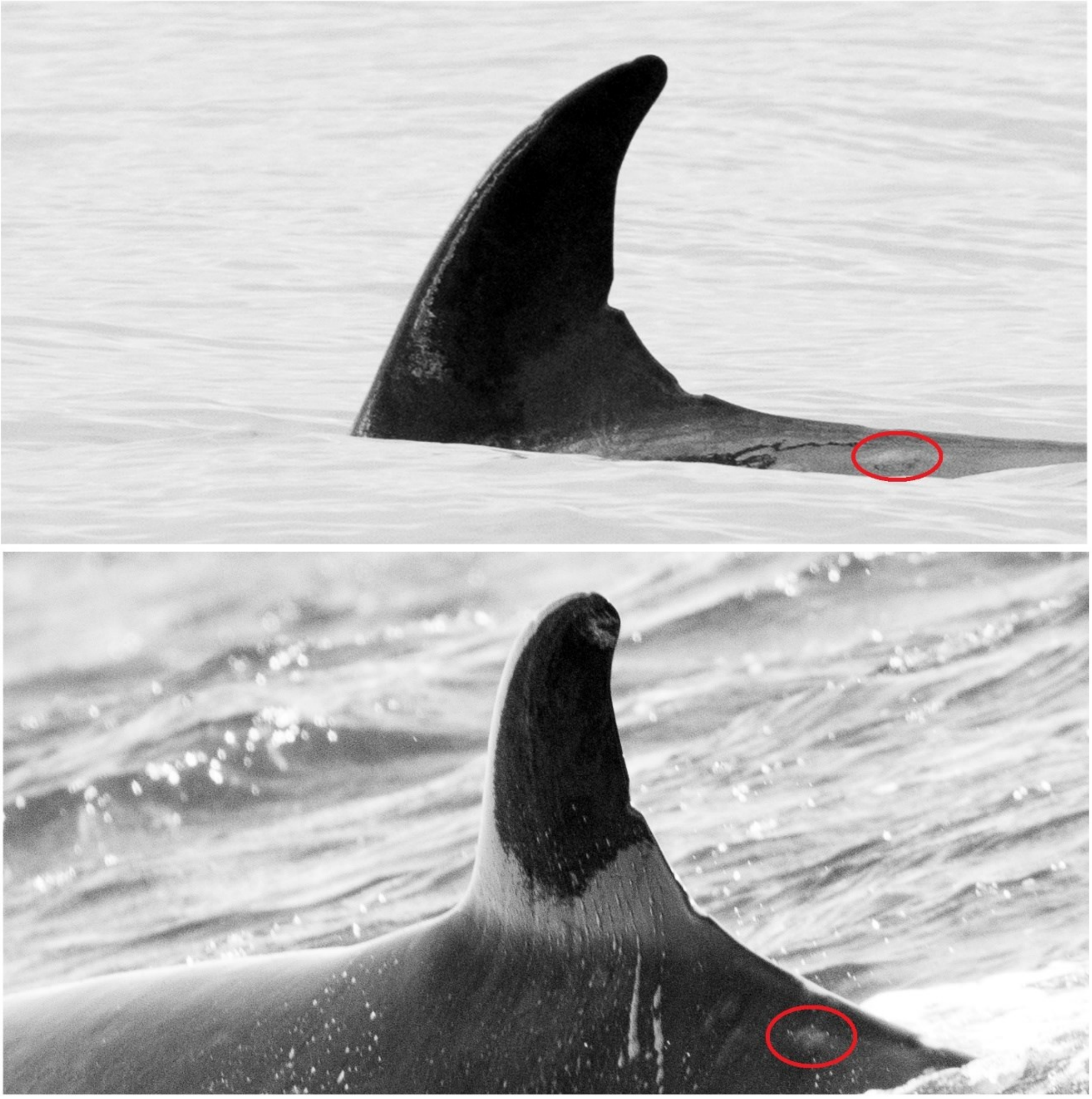


Figure 3.12. *Individual BS-100 / WF-115 was photographed in three years: 2017 (top image), 2018 and 2020 (bottom image). Dorsal fin marks were primarily used to recognise this animal. Although images of each side were inconsistent in quality between years, it was apparent that some scars remained visible three years later (example circled in red).*

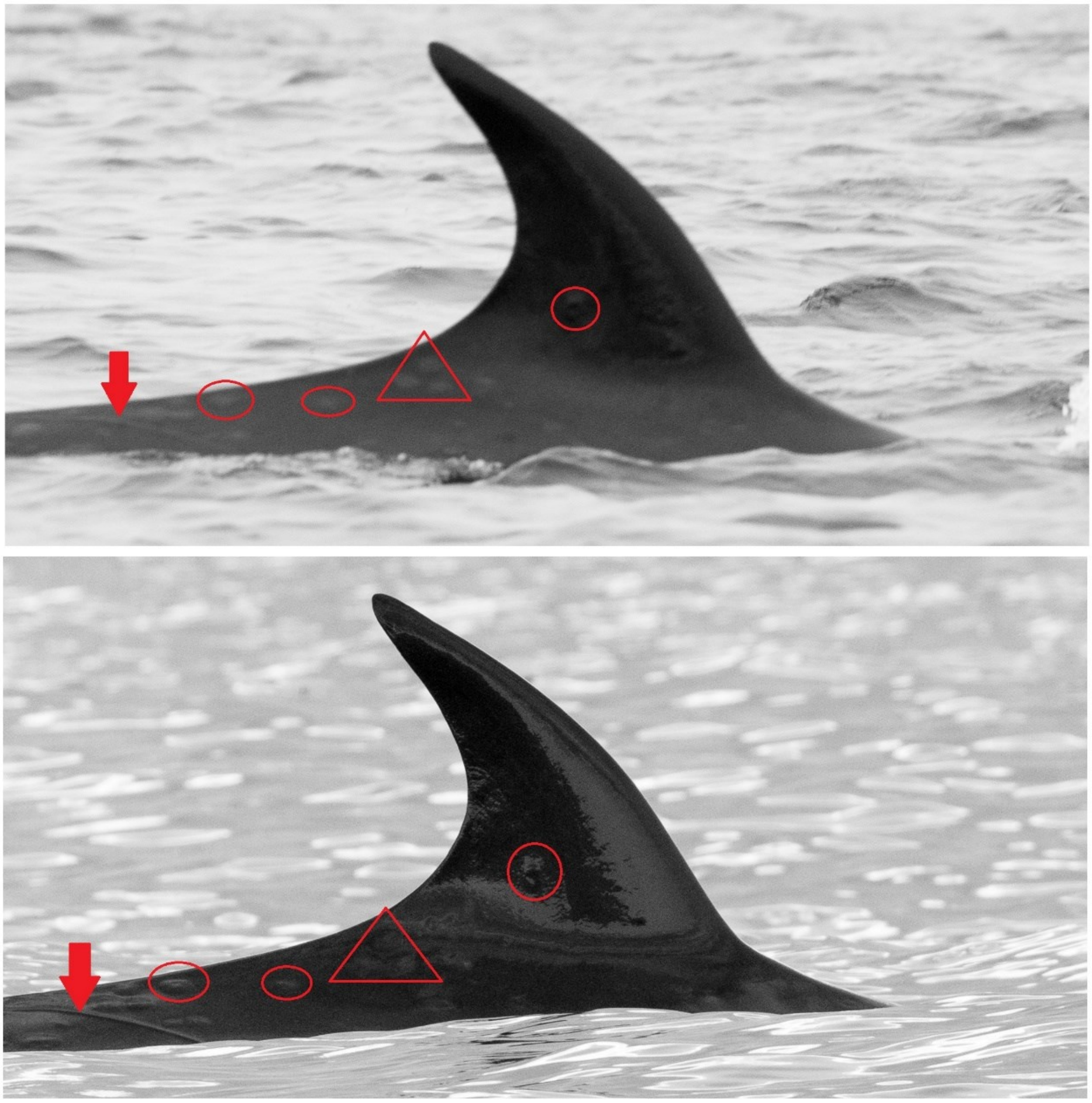


Figure 3.13. *BS-19 was photographed in 2017 (top image) and again in 2019 (bottom image). Even though the quality of the 2017 image is poor, a number of scars are clearly visible in both images, including a raised oval scar on the dorsal fin (circled), patterns of scars on the flank and tailstock (circles and triangle), and a linear scar on the tailstock (arrow).*

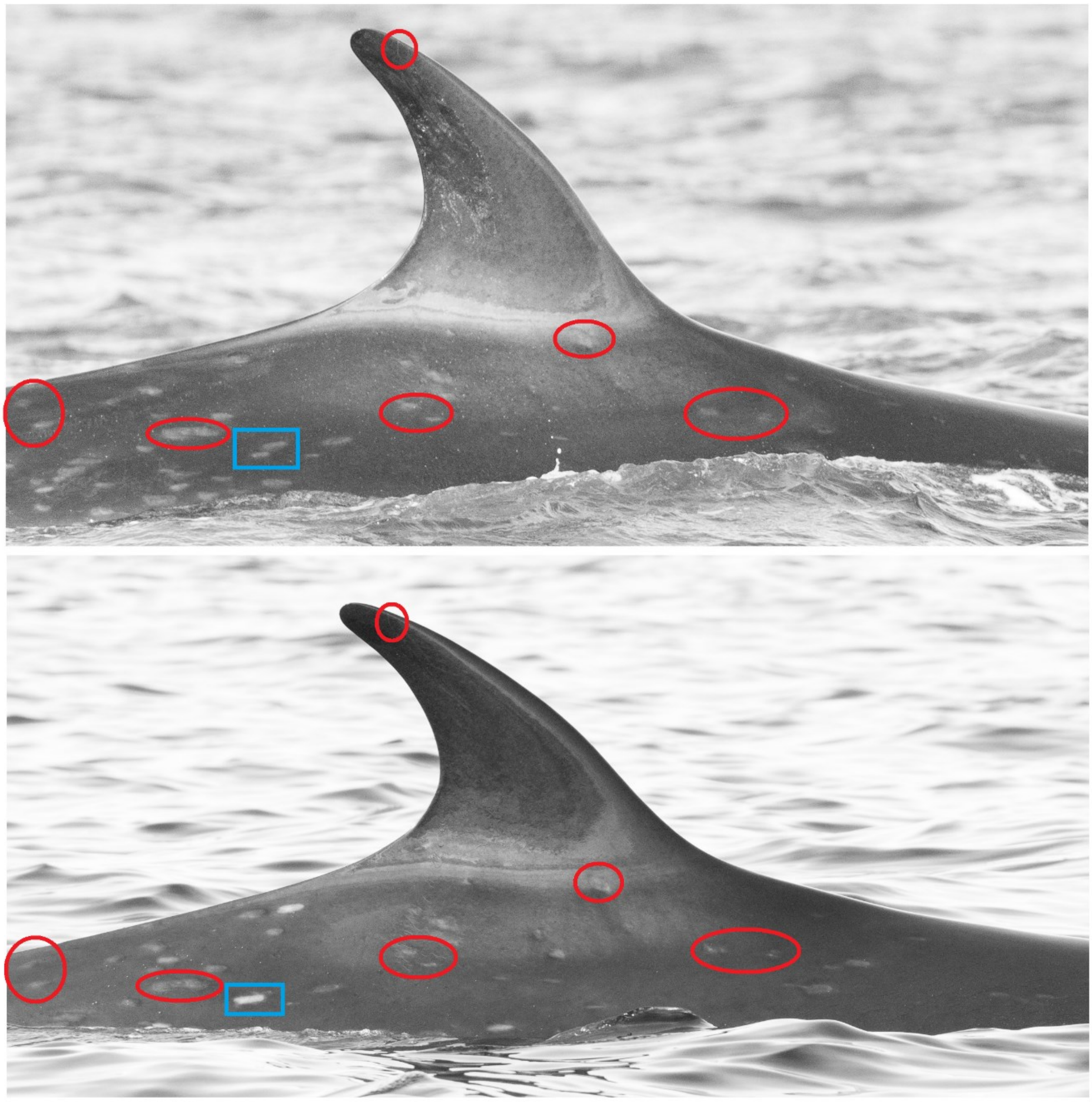


Figure 3.14. *BS-60 was photographed in 2017 (top image) and again in 2019 (bottom image). Many of the flank scars are readily apparent in the images from both years (some examples circled in red). In the 2019 image, the animal also has some more recent scars that have appeared since 2017 and are a whitish colour. An example of where a new mark has overlapped and obscured existing marks, is shown by the blue box.*

Figure 3.15. *Individual BS-19 was photographed in 2017 (left image) and again in 2019 (centre and right images). Of the two 2019 images, the centre one has the highest photographic quality, being sharp, perpendicular to the photographer, and reasonably well-exposed. However, these images serve as an example of where the visibility of certain scars changes with angle and exposure. In this case, the visibility of the three circled raised oval scars in the 2017 image is much reduced in the centre 2019 image, despite the high quality of the latter image. The scar pattern is however very obvious in the righthand 2019 image where the light and angle are different and overall photographic quality is lower. As this example illustrates, matching of sei whales can be optimised by using a range of images, rather than relying on the single best-quality image from each encounter.*

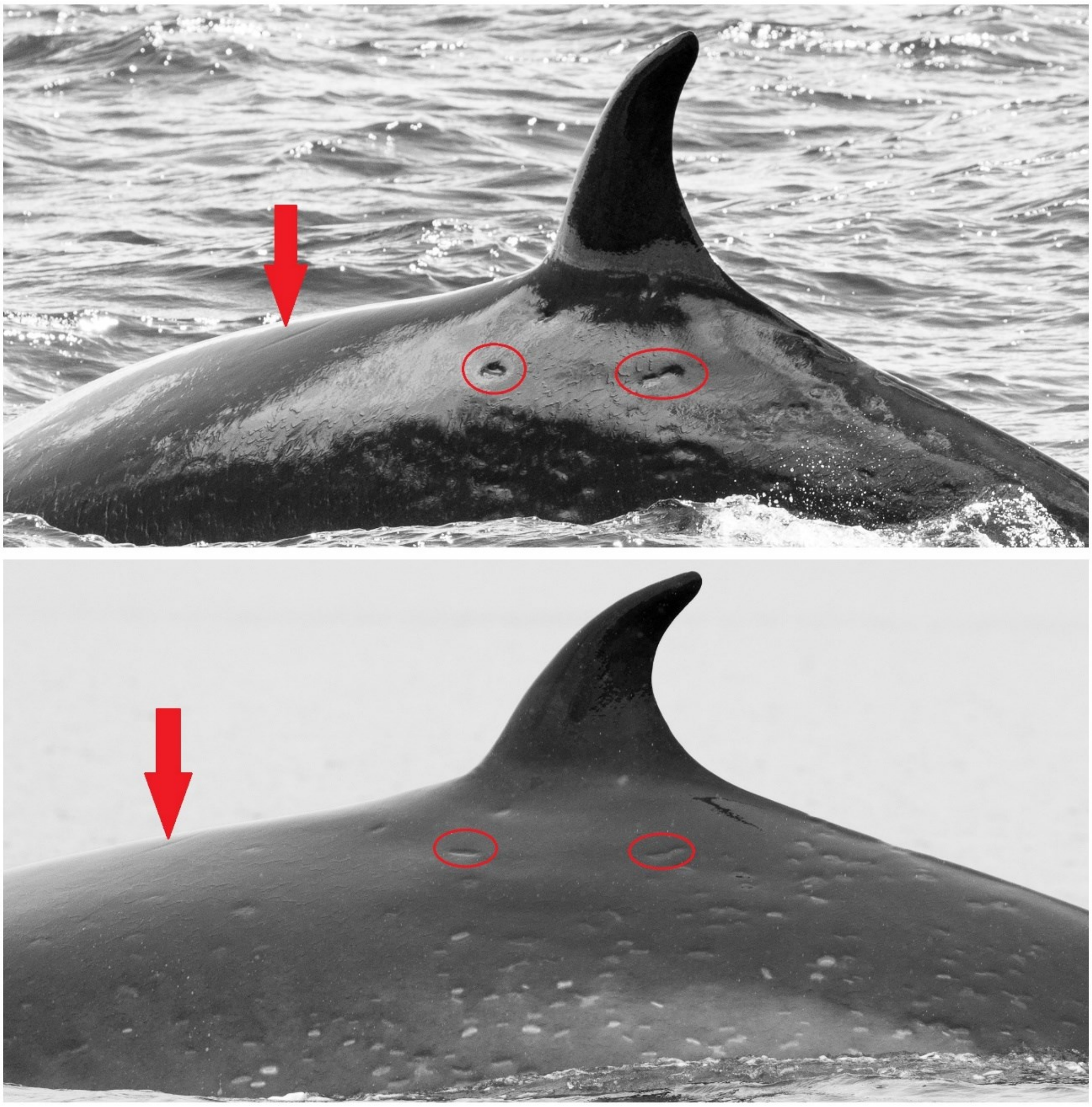


Figure 3.16. *Individual BS-26 was photographed in 2017 (top image) and in 2019 (lower image). Differences in angle, lighting, the amount of body above the water, and the degree of flexing of the animal's back, change the visibility and perceived relative positioning of various scars on the flank and tailstock. In particular, due to the slight increase in angle in the 2017 image, a reduced number of scars are visible overall because of light reflection, while certain scar craters (circled) appear much deeper and more obvious. While healing may have slightly reduced the depth of the scar craters over the two years between the images being taken, much of the difference in their appearance is likely the result of light and angle.*

3.3.3 Southern right whale

All 44,507 southern right whale images taken during DPLUS082 during small boat surveys between 2019 and 2021 (Table 3.4), were assessed and catalogued. The locations of southern right whale photo-identification encounters primarily occurred in the area between Volunteer Point and Cape Carysfort, and in the Cape Pembroke to Kidney Island region (Figure 3.7), reflecting their core areas of occurrence during winter boat surveys (see Chapter 2). Photo-identification effort was most productive (in terms of the total number of individuals identified per trip) during July and August (Figure 3.17).

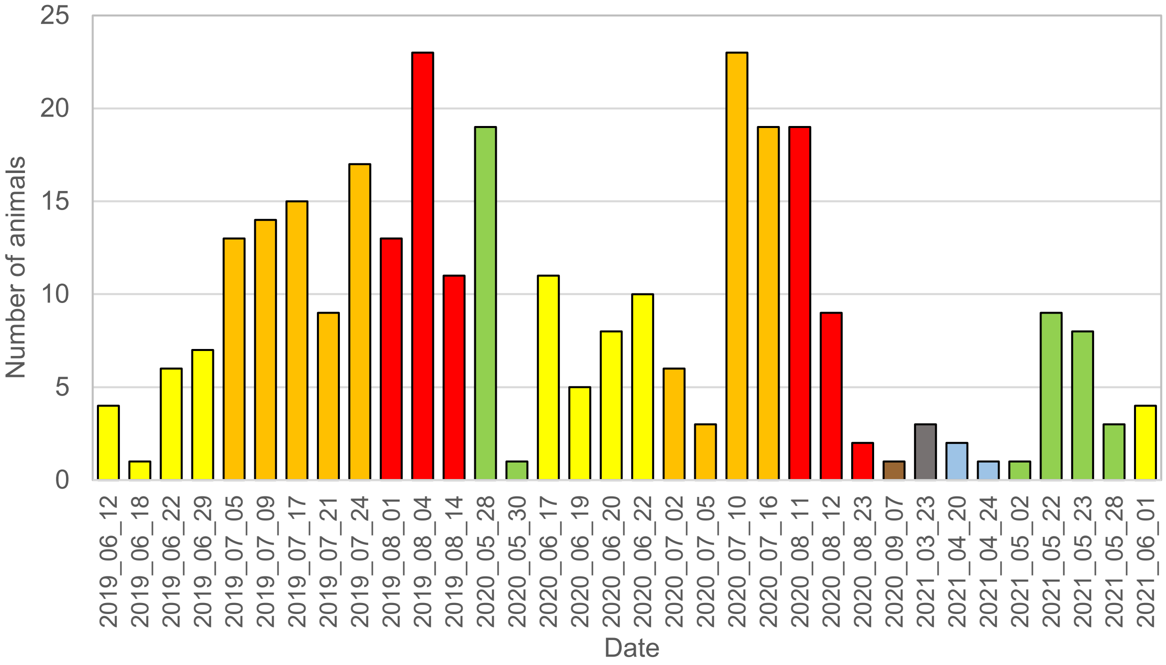


Figure 3.17. *Number of southern right whales photographed (using images of all quality) by survey date in NEF during DPLUS082. Bars are shaded by survey month: Mar = grey; Apr = blue; May = green; Jun = yellow; Jul = orange; Aug = red; Sep = brown.*

A total of 2,806 right whale images from May/June 2017 (Weir, 2017) were reassessed as part of DPLUS082 and are included in some of the analyses presented in this report; that small dataset yielded 17 catalogued animals. A total of 272 individuals were initially catalogued (i.e. prior to quality control, and including animals photographed on only one side) using the 2019–2021 DPLUS082 dataset, comprising 125 in 2019, 128 in 2020, and 29 in 2021 (also including some matches of the same animals between years – see Section 3.3.3.2). Altogether, a total of 289 animals were initially catalogued over the 2017 to 2021 period. It is emphasised that boat surveys carried out in 2021 were aimed at sei whales, and therefore occurred only between January and early June (see Chapter 2); consequently, the smaller number of right whales photographed in 2021 is due to the lack of boat work during their core season (July–August) and does not reflect genuinely lower numbers of animals in that year. In fact, 2021 was the only year when right whales were observed in March and April.

Of the 289 animals initially catalogued between 2017 and 2021, images of both the left and right sides were available for 214 individuals. The remaining animals had images available of only their left (n = 39) or right (n = 36) side. Fifteen animals (5.2%) had ‘grey morph’ colouration. Only four individuals (1.4%) had continuous callosity extending between the bonnet and the coaming; all other animals had broken callosity patterns.

A total of 2.8% of left-side images, and 6.0% of right-side images, were of poor photographic quality (Table 3.9) and were deleted from the analysis. Following this step, 286 individuals remained in the catalogue, comprising 195 animals for which both sides were photographed, 51 animals with left side only images, and 40 animals with right side only images. Of the 286 individuals for which fair to excellent quality images were available, all were marked and distinctive (Table 3.10). None were scored as being a calf (DV0) or 'not distinctive' (DV1). A relatively low number were scored as 'slightly distinctive' (DV2); however, even in that category, animals still had clear and permanent features that should facilitate their photographic recapture. Consequently, all 286 animals were included in the analyses.

Table 3.9. *Photographic Quality (PQ – see Table 3.2) allocated to the left and right sides of 289 individual southern right whales. Scores range from PQ4 (excellent) to PQ7 (poor).*

PQ	Left sides		Right sides	
	Total no. of animals	% of total animals	Total no. of animals	% of total animals
PQ4	101	39.9	73	29.2
PQ5	103	40.7	98	39.2
PQ6	42	16.6	64	25.6
PQ7	7	2.8	15	6.0
Total	253	100.0	250	100.0

Table 3.10. *Distinctiveness Values (DV – see Table 3.3) allocated to the left and right sides of 289 individual southern right whales.*

DV	Left sides		Right sides	
	Total no. of animals	% of total animals	Total no. of animals	% of total animals
DV0	0	0	0	0
DV1	0	0	0	0
DV2	6	2.4	10	4.0
DV3	40	15.8	57	22.8
DV4	207	81.8	183	73.2
Total	253	100.0	250	100.0

3.3.3.1 Minimum population size

The MPS results were similar using the left-side and right-side catalogues (Table 3.11). The combined value across the four years was 246 and 235 animals using the left- and right-side catalogues respectively. Using only data from 2019 and 2020, when photographic effort spanned the core right whale season (July-August), the MPS was 208 and 198 animals for the left- and right-side catalogues respectively.

Table 3.11. *The minimum population size (MPS) of southern right whales photographed at NEF from 2017 to 2021.*

Catalogue	Minimum Population Size					
	2017	2019	2020	2021	2019+2020	2017–2021
Left side	15	102	113	26	208	246
Right side	13	104	101	27	198	235

3.3.3.2 Inter-annual recaptures

Ten inter-annual photographic recaptures were recorded during DPLUS082 (Table 3.12, Figures 3.18 and 3.19). The first comprised an animal photographed in 2017 that was subsequently recaptured two years later during 2019. Two individuals were first photographed in 2019 and 2020 respectively, and then recaptured in 2021. The remaining seven recaptures were of individuals initially recorded in 2019 and then recaptured during 2020 (Table 3.12). Remarkably, four of those recaptures involved animals observed in different encounters in 2019 (FEA-65, FEA-93, FEA-121 and FEA-131) that were recaptured within a single large mating aggregation seen offshore of Volunteer Beach on 28 May 2020. Also remarkable, is that another of the 2019 animals (FEA-35) was also recaptured on 28 May 2020, but at a location off Cape Pembroke, around 25 km south of the other four animals (Figure 3.20).

The close proximity of the encounters of each individual in different years within NEF is also noteworthy (Figure 3.20). For example, three initial encounters with FEA-35 on 29 June 2019, 28 May 2020 and 30 May 2020, were all at locations within ~5 km of one another.

Table 3.12. *Inter-annual photographic recaptures of individual southern right whales at NEF.*

Ref	Individual	Capture 1	Capture 2
1	FEA-10	2017	2019
2	FEA-21	2019	2020
3	FEA-30	2019	2020
4	FEA-35	2019	2020
5	FEA-65	2019	2020
6	FEA-93	2019	2020
7	FEA-121	2019	2020
8	FEA-126	2019	2021
9	FEA-131	2019	2020
10	FEA-144	2020	2021

Figure 3.18. *Inter-annual match of individual FEA-35: (A) 2019; and (B) 2020.*

Figure 3.19. *Inter-annual match of individual FEA-121: (A) 2019; and (B) 2020.*

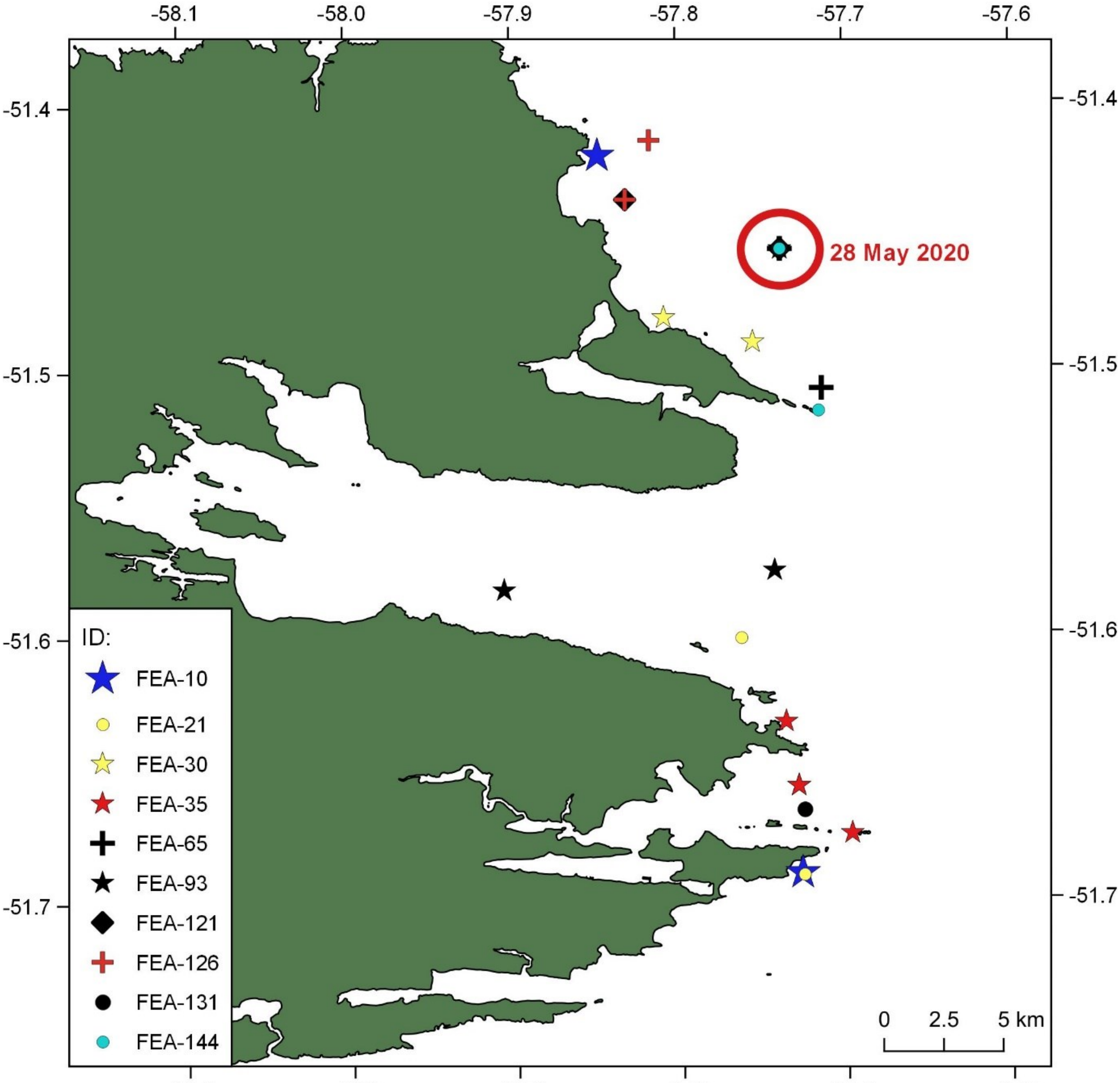


Figure 3.20. *Locations of inter-annual photographic recaptures of southern right whales (see Table 3.12) at NEF. The circled location marks the four recaptures on 28 May 2020 – the black symbols for each of these individuals are overlaid at this location, since they were photographed within the same mating group on that date. Where more than two symbols occur for an individual whale, that reflects photographic captures occurring on more than one date in some years.*

3.3.3.3 Intra-annual recaptures

Intra-annual recaptures were investigated in 2019 and 2020 only, since these were the only years that ran full winter southern right whale seasons including June, July and August. Totals of 122 and 128 right whales for which PQ4–6 images were available were photographed in 2019 and 2020 respectively (those totals include some of the same individuals encountered in both years). The vast majority of animals (>93% in both years) were only photographically captured once per year (Figure 3.21), with only 16 animals being captured on more than one date within a year (eight each in 2019 and 2020). The longest recorded interval between within-season recaptures was 32 days (Figure 3.22). However, most (*n* = 9) of the within-season recaptures occurred at intervals of <12 days. As with the sei whale dataset, it is important to note that the time intervals between within-season recaptures was impacted by survey coverage, since surveys occurred intermittently around favourable weather conditions.

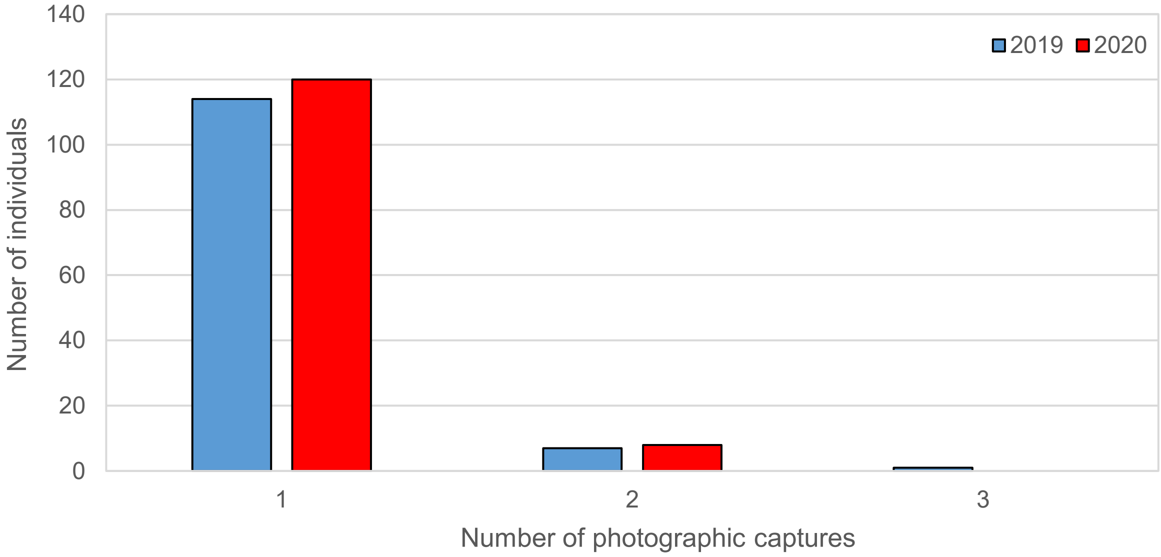


Figure 3.21. *Number of within-year photographic captures for individual southern right whales during 2019 and 2020.*

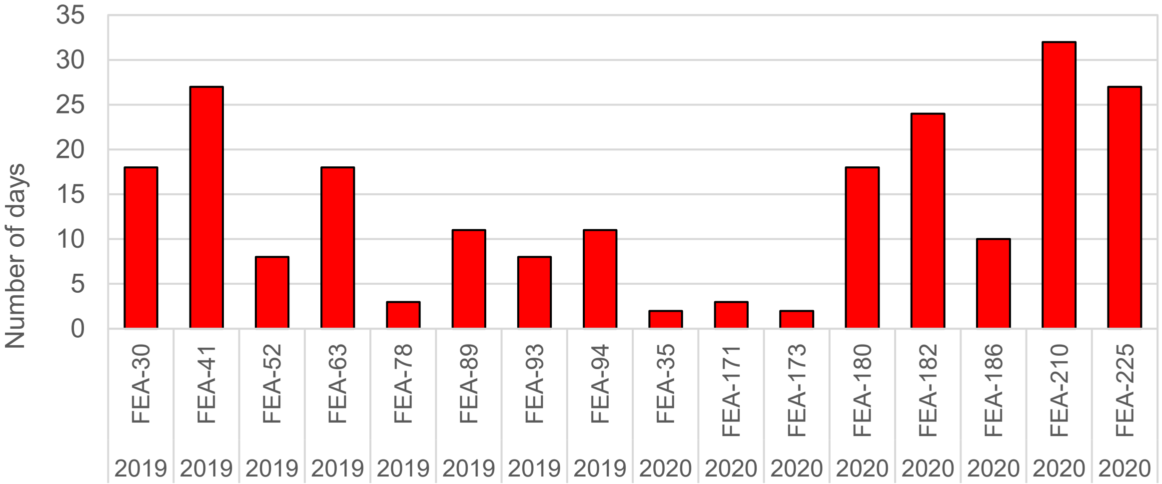


Figure 3.22. *Number of days from the first to the last sighting of 16 southern right whales that were photographically-captured on more than one survey date within a year.*

3.4 Discussion

3.4.1 Sei whales

As reported during earlier studies in the Falklands (Weir, 2017, 2018), sei whales are not a particularly well-marked species for photo-identification work. The DPLUS082 study, combined with studies in 2017 and 2018 (Weir, 2017, 2018), brought the total number of sei whales catalogued in the Falklands to 508 animals. Of those, 485 animals had images of PQ 1–3 available for at least one side. Taking the 431 animals for which either both sides were photographed (*n* = 369), or for which only left side images were available (*n* = 62, as opposed to *n* = 54 for right side only), the proportions of DV1 (large nicks) and DV2 (moderate nicks) in the entire Falklands catalogue (2017–2020) were 6.5% and 23.4% respectively. This means that less than a third of individual sei whales catalogued in the Falklands had large or moderate sized features such as nicks in the fin edges. Rather, most were catalogued using subtle features such as small nicks and scar patterns. The appearance of these subtle features in images is more prone to being affected by angle, exposure (in the case of scars), and low resolution due to heavy cropping when the animal was too far away. Consequently, obtaining high quality images, while *always* important for mark-recapture work on cetacean species, is particularly vital for the individual recognition and recapture of sei whales. The species is challenging to acquire high quality images from, being fast-swimming, sometimes surfacing without revealing the entirety of the dorsal fin, and often surfacing just ahead of, and at a slight angle away from, the boat, which reduced the usability of the images for identification. Additionally, since Falkland Islands' waters comprise a foraging ground, sei whales often behaved unpredictably with regard to where they surfaced, changing directions regularly and with variable dive durations.

Despite these challenges, DLUS082 has greatly expanded the photo-identification catalogues for sei whales in the Falklands, including at a novel site (Falkland Sound), and successfully addressed the core goals of this component of the project. The MPS at FS and NEF over the study period (2019–2020) was calculated as 93 and 166 animals respectively. The NEF estimate was almost double that recorded in Berkeley Sound during 2017 (85 animals: Weir, 2017), but this would be expected given that the survey coverage was also double and comprised two years of data compared with only one year in 2017. However, the MPS recorded in West Falkland (133 animals: Weir, 2018) likely reflects the highest sei whale numbers out of all three of the study sites, considering that it related to photo-identification effort carried out during only a ~5 week period and within a single year.

Prior to DPLUS082, the only inter-site recaptures of sei whales documented in the Falklands were two animals photographed in Berkeley Sound during 2017 that were subsequently recaptured off West Falkland during 2018 (Weir, 2018). During 2019 and 2020, an additional eight inter-site recaptures were recorded, demonstrating movements of individual sei whales between NEF, FS and West Falkland. Interestingly, even though the Falkland Sound catalogue was much smaller than at the other sites, and the overall coverage achieved there was lower, five of the recaptures included records from that site. This suggests that FS may be visited by many of the whales using wider Falklands' waters, even though it is a relatively enclosed channel. This may be due to its geographic location midway between the east and west coasts of the Falklands, or it is plausible that characteristics of the channel (which has strong tidal current but is reasonably sheltered from swell) is particularly productive for sei whales with regard to prey availability or their foraging efficiency.

The distances of 140–170 km evidenced here between photographic captures, represent the longest such *intra*-annual movements documented within Falkland Islands' waters to date, and were only possible due to the multi-site nature of DPLUS082. Previously, movements between different sites around the Falklands were only documented from different years, and did not exclude the possibility that individual sei whales used one coast during one year, and returned to inhabit a different part of the coastline the next year. However, these intra-annual recaptures in different study areas indicate that at least some sei whales do move extensively around Falklands' inshore waters *within* a season. The distance of ~140 km travelled by the mother-calf sei whale pair over 12 days in March 2019, equates to a straight-line average swim speed between the sites of ~12 km/day. Given that this movement was unlikely to have been straight and nonstop, the actual distance covered by these animals may have been significantly higher. Although the photo-identification data provide a useful broad indication of movements, only three sites have been investigated to date and the coverage at each site was interspersed with multiple days of no photographic survey effort. Consequently, it remains unclear how sei whales on the Falklands' foraging ground use the region over finer spatial and temporal scales. For example, whether they spend prolonged periods exploiting prey resources within relatively small spatial areas and then make rapid linear movements to another location, or whether they forage continuously and make their way slowly across different regions of the Falklands. For large and mobile marine species such as sei whales, and in a geographic location as remote as the Falklands, these types of finer-scale data can only realistically be acquired using satellite telemetry.

In combination, the inter-site+inter-annual, inter-site+intra-annual, and intra-site+inter-annual recaptures recorded during DPLUS082 add significant support to earlier suggestions (e.g. Weir, 2018, 2020) that individual sei whales: (1) return to the region in different years and therefore exhibit a degree of long-term fidelity to the Falklands feeding ground; and (2) visit multiple sites within the Falklands and therefore range widely around the Islands both within and between years. Indeed, together with habitat modelling that indicated the presence of potentially suitable habitat for sei whales across large swathes of coastal waters (Baines and Weir, 2020), a subset of these findings formed part of the evidence-base for including the entirety of inshore waters around the Islands as the *Falkland Islands Inner Shelf Waters KBA* for sei whales (Weir, 2021). Since foraging appears to be the primary driver for their occurrence in Falklands' waters, it may be expected that the distribution and movements of sei whales reflects spatio-temporal variation in the availability of their prey, potentially including switching between preferred prey species over the course of the season. However, it is also clear that there is inter-individual variation in the within-season residency of sei whales at particular sites, such that most animals are photographed only once in the season, while a small number are encountered multiple times over periods of several weeks and thus have a higher degree of residence in the site (this chapter; Weir, 2017, 2018). It remains unclear what drives such differences.

As highlighted during earlier studies of sei whales (Schilling et al., 1992; Weir, 2017), multi-year datasets are required in order to better understand the persistence of scar patterns and their reliability for mark-recapture techniques, since the presence of long-lasting identifiable natural marks is a prerequisite for the method (Evans and Hammond, 2004). Neither the healing rate of existing scars, nor the rate of acquisition of new scars is currently known, although it might be expected that many sei whales acquire some cookie-cutter shark scars annually during their winter occurrence in the (sub)tropics. DPLUS082 contributed another two years of photo-identification data for the Falklands (with the 2021 dataset still to be analysed), and provided a larger number of recaptures to assess the longevity of scar patterns. The additional years of data included matches of some animals with a 2-yr separation and one with a 3-yr separation between the initial and final photographic encounters. These data indicated that existing scar patterns remained visible over 2–3 yr timeframes, although new scars can be overlaid over them and, in some cases, mask the appearance of older scar patterns. Generally, if the flank and tailstock areas were visible in image sequences, then multiple areas could be checked for matching scar patterns which reduced the impact of new scars distorting the scar pattern on particular areas of the body. Given the continual gradation of dorsal fin shape, and the similarity in nick patterns (placement and size) amongst many sei whales (especially given that many whales had just single fin nicks), scarring was used as a secondary feature to confirm *all* matches made in the Falklands even when permanent markings were available.

While the DV and PQ ratings applied to the sei whale dataset were sufficient for the basic analyses carried out in this chapter, the future application of mark-recapture analysis techniques to the dataset will require both a more rigorous PQ scoring method and a more robust assessment of what constitutes a 'marked' versus a 'non-marked' animal (Hammond, 2017). The refinement of these processes will be carried out as part of DPLUS126.

It is remarkable that over 400 individual sei whales have been catalogued in the Falklands to date (not including the 2021 data which are still to be analysed), considering the small sizes of the study sites and the relatively low numbers of survey dates achieved each month due to weather and logistical constraints. Moreover, the relatively small number of recaptures across sites and years, and the continued high rate of discovery of previously-uncatalogued animals, suggests that the total number of sei whales using the Falklands feeding ground could potentially run into the thousands, and that the cataloguing of new animals is limited by the amounts of survey effort achieved. To our knowledge, this has become the largest photo-identification catalogue available for sei whales anywhere globally, and will provide invaluable information on the species for many years to come.

3.4.2 Southern right whales

The two winter seasons of surveys carried out for southern right whales during DPLUS082 have greatly increased knowledge of the status of this species in the Falklands (this chapter; **Chapter 2**, **Chapter 5** and **Chapter 7**). The photo-identification component of DPLUS082 aimed firstly to assess whether lateral images taken of right whales from a boat (as opposed to the standard approach of photographing the species from above) would be appropriate to catalogue and recapture individual animals. The project was successful in applying this approach to right whales in the Falklands, developing indexes of photographic quality and distinctiveness value that could be applied to lateral head images. Although time-consuming, it proved less problematic than originally envisaged to match images of the left and right sides of individuals *within* an encounter, based on factors including the use of multiple images taken at different angles, the presence of dorsal scars/marks, and the differing shapes and amounts of growth on the bonnet and coaming callosities (which are visible from both sides of the animal). However, matching of left- and right-side images taken during different encounters was considered to be too difficult and prone to error, and was not attempted.

It remains unclear whether the catalogues of lateral head images established in the Falklands can be readily compared and matched with the aerial catalogues from other regions of the South Atlantic, but for the purposes of long-term monitoring within the Falklands then the method is applicable. This was evidenced by the photographic recaptures of individual whales both within years and between years. Therefore, it is recommended that acquisition of lateral head images should continue during future boat work in the Falklands. It would be desirable to also establish an aerial catalogue of right whales in the Falklands that would better facilitate between-region matching; however, the logistical constraints (i.e. weather, lack of suitable platforms) currently make this challenging and costly to implement.

A total of 208 individual right whales were catalogued during DPLUS082 (considering the full winter seasons covered in 2019 and 2020), which builds significantly on the 15 animals catalogued in May and early June 2017 (Weir, 2017). The number of catalogued animals does not accurately reflect the true number of right whales using the nearshore waters around the Falklands each winter, since not all animals encountered during boat surveys were photographed. Additionally, the number of small boat surveys and photo-identification work completed during DPLUS082 was greatly restricted by weather; of the 78 possible days for boat work between 15 June and 31 August (comprising the core of the right whale season in the Falklands), only 11 survey days were achieved in 2019 and 12 days in 2020. This equates to only 14.1 and 15.4% coverage of the core season during 2019 and 2020 respectively. Furthermore, the NEF study site constitutes only a small part of the Falklands coastline. Consequently, it can be reasonably supposed that the actual number of right whales using the nearshore waters around the Falklands over the winter is substantially higher than what has been indicated here via photo-identification. The International Whaling Commission (IWC) modelled global right whale population size for 2009, producing estimates of 13,611 animals across the Southern Hemisphere and 4,029 animals in the south-west Atlantic (IWC, 2013). In that context, the >200 animals catalogued to date in the Falklands is significant, and suggests that the Islands are of at least regional importance for the species. The work carried out during DPLUS082 will contribute in the future to a more robust population size analysis of southern right whales using mark-recapture modelling, which may provide a better indication of numbers.

One of the key aims of the right whale photo-identification work was to assess intra- and inter-annual recaptures at the NEF. The project recorded 10 inter-annual recaptures, indicating that at least some right whales return annually to Falklands' nearshore waters during the winter. The data don't necessarily imply that whales make large deviations in their movements specifically to come to the NEF; rather, it might be the case that the foraging areas and/or migration routes used by some right whales during autumn are sufficiently consistent between years that the animals travel past this part of the Falklands annually anyway. Whatever the driver, the inter-annual recapture data suggest some longer-term fidelity of a portion of the south-west Atlantic right whale population to the Falklands, whether as a temporary migratory stop-off or for more sustained use as a wintering destination for mating and socialising.

With regard to the latter point, the low number of intra-annual recaptures recorded during DPLUS082 was a significant and somewhat unexpected result. Less than 7% of right whales were photographed more than once in either 2019 or 2020. Compared with the sei whale dataset, the total proportion of individuals captured more than once per season, the total number of intra-annual survey dates that individual animals were captured on, and the number of days between first and last capture dates within the season, were all much lower for southern right whales. These parameters add more evidence for the two species using the area in different ways, with right whales having a more transitory presence in the NEF than sei whales. These results are consistent with the occurrence of a steady succession of different right whale individuals moving along the coast of the NEF throughout the winter, with most animals staying in the site for relatively short periods and then moving on. This finding has several conservation and management implications. Firstly, it strongly implies that the total number of right whales using the NEF during 2019 and 2020 was significantly higher than the number catalogued. As described above, only around 15% of available survey days during the core right whale season received survey coverage, which combined with the low number of recaptures (and high rate of discovery of new animals), indicates that only a small proportion of the total population was sampled. Secondly, this result suggests that a significant proportion of the south-west Atlantic right whale population may have been using feeding areas located eastwards of the Falklands, or to the south or south-east of East Falkland, immediately prior to their movements towards wintering areas, such that those movements brought them close to the north-east region of the Falklands and past the NEF.

The genetic work presented in **Chapter 5**, confirms that the right whales observed in the Falklands wintering area are from the south-west Atlantic population that uses calving grounds in Argentina and Brazil. However, not all right whales visit the calving grounds each year; mature females may only return every 3–5 years for calving (e.g. Watson et al., 2021), while mature males and juveniles of both sexes may not always migrate to the calving areas, particularly if their reproductive (mating) and socialising requirements can be achieved in areas located at higher latitudes and closer to rich feeding areas. Consequently, it remains unclear where the individual whales photographed at the NEF in a particular year travel on to. It is possible that they continue to move on to other sites located along the coast around the Falklands, but the NEF is currently the only site to date to have received systematic survey effort during winter. However, they may also move offshore into pelagic areas or migrate on to adjacent geographic regions. Weir and Stanworth (2020) considered that the seasonality in the Falklands (peaking July, into August) did not exclude the possibility that right whales observed in the nearshore waters in the Falklands subsequently continued on to established calving grounds in Argentina or Brazil. Peak numbers at the Península Valdés calving/mating ground usually occur at the end of August through to mid-September (Crespo et al., 2019), and at Santa Catarina in Brazil then numbers peak from August to October (Seyboth et al., 2015). A comparison of photo-identification images taken of right whales in those calving areas during the 2019 and 2020 seasons with the Falklands catalogue from the same years, might reveal whether animals have continued on to the calving areas after departing the Islands. Satellite-tracking of right whales in the Falklands would also help to ascertain movements within and beyond the Falklands, and better clarify how individual whales are using both the Falklands' coasts and the wider south-west Atlantic. Satellite-tracking will be carried out as part of another DPLUS project (DPLUS126) in 2022 and 2023. The Falklands catalogue will also be compared with a photo-identification catalogue for South Georgia as part of DPLUS126, to establish linkage between those areas.

3.5 Acknowledgements

Most photo-identification work occurred during the small boat surveys described in **Chapter 2**. In addition to all of the acknowledgements mentioned for that chapter, I am grateful to Maria Taylor for assistance with photo-ID work carried out in 2017 and 2018 which facilitated some of the inter-annual matching. Many thanks also to Keri Lee Pashuk and Greg Landreth of Patagonia Projects who generously made the sailing vessel *Saoirse* available for some sei whale photo-identification work in January 2020.

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Chapter 4: Diet of sei and southern right whales

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4.1 Introduction and aims

The Falkland Islands are located on an extension of the Patagonian Shelf in the south-west Atlantic (see Figure 1.1), in a region influenced by the Falklands Current, a northwards-travelling branch of the Antarctic circumpolar current, which creates strong local upwelling patterns and areas of high productivity across the Patagonian Shelf. Sei whales and southern right whales are seasonal visitors to Falkland Islands' waters, with evidence suggesting that both species feed in the region during summer and autumn; sei whales utilise nearshore waters (**Chapter 2**; Weir, 2018; Weir et al., 2019), while southern right whales are thought to feed in more pelagic habitats (Weir and Stanworth, 2020). During the winter months, southern right whales also aggregate close to shore for breeding and socialising (**Chapter 2**; Weir and Stanworth, 2020).

Baleen whales are thought to have a significant top-down role in the ecosystems in which they forage, impacting prey distribution and behaviour, and also enhancing local nutrient flux through their defecation and diving behaviour (Ratnarajah et al., 2014; Roman et al., 2014). Understanding sei and southern right whale foraging ecology in the Falkland Islands is therefore important to help establish how they impact the local ecosystem, and the importance of this ecosystem for their own recovery. In the Southern Hemisphere, sei whale diet has primarily been documented from historical whaling data at South Georgia and in the Antarctic, evidencing a wide diet that includes copepods, amphipods and euphausiids, and also small shoaling fish and squid species (Horwood, 1987; Kawamura 1994). Prey preferences are known to vary latitudinally and regionally (Horwood, 1987; Flinn et al., 2002). The dietary preferences of sei whales using the waters of the south-west Atlantic are only slowly becoming documented (Buss, 2022). The diet of southern right whales has been characterised in more detail in the South Atlantic, with historical whaling data and stable isotope ecology suggesting that this species predominantly feeds on copepods in Patagonian Shelf waters, and on Antarctic krill in sub-Antarctic and Antarctic waters (Tormosov et al., 1998; Valenzuela et al., 2009; Valenzuela et al., 2018). Their differing seasonal habitat use patterns suggest that sei and southern right whale might target different prey species in Falkland Islands' waters, with sei whales feeding in inshore waters and southern right whales foraging further offshore (Baines and Weir, 2020; Weir and Stanworth, 2020).

Studying the diet of highly mobile oceanic species is extremely challenging, with much data traditionally based on information from whaling-era stomach contents (e.g., Kawamura 1980; Tormosov et al., 1998) or field observations (Matthews 1932). Stable isotope analysis, using the ratio of nitrogen and carbon isotopes found in whale skin, can be used to infer the relative trophic level of species foraging, and identify the approximate geographic location of foraging (Newsome et al., 2010). Where skin samples can be collected, this approach can provide insights about whale foraging behaviour during the few months prior to sample collection, with the timeframe of the insight depending on the turnover rate of whale skin. This turnover rate is poorly known, but estimates from blue whales suggest that skin samples represent feeding in the 3–7 months prior to sample collection (Busquets-Vass et al., 2017).

A second important technique for studying the diet of marine predators is to recover DNA from faeces, an approach which has been widely used for marine species that come ashore (e.g. penguins and sea lions: Deagle et al., 2007; Berry et al., 2017). This approach has only been used in a small number of whale studies to date (e.g., de Vos et al., 2018; Carroll et al., 2019), due to the challenges and limited opportunities for collecting faeces at sea on feeding grounds. To identify prey within faecal samples, the dietary components are genetically identified using a 'metabarcoding' approach, and those genetic identifications are then compared with publicly available reference sequences to determine the taxonomic units of DNA sequences recovered from faecal samples. Sei whales in the Falkland Islands represent one of the few aggregations of baleen whales where regular faecal sample collection has been possible, enabling such an approach to be taken.

In this chapter, a DNA metabarcoding approach is applied to investigate the taxonomic groups of prey species being consumed by sei whales while foraging in the Falkland Islands, and stable isotope analysis is carried out to assess the trophic foraging ecology of the sei and southern right whales that visit Falklands' coastal waters.

4.2 Materials and methods

4.2.1 Sample collection

4.2.1.1 Faecal samples

Faecal samples were collected during the DPLUS082 small boat surveys between 2019 and 2021 (see **Chapter 2** for boat survey methodology and coverage). Sample collection relied on the visual detection of faecal material at the surface, and therefore usually occurred while actively working in proximity to whales during photo-identification and biopsy sampling work. Whenever faeces were observed and conditions allowed, other activities ceased and the collection of a sample was prioritised. The boat was manoeuvred quickly towards the faeces, and a long-handled 150 µm mesh dipnet was used to collect as much faecal material as possible using sideways swoops through the water column (Figure 4.1). The better digested faecal material tended to sink relatively quickly (leaving only undigested crustacean hard parts at the surface, such as carapaces), and effort was therefore made to deploy the net as deep in the water column as possible during the initial period of collection.

Following collection, each faecal sample was stored in a sterile sample pot or a clean freezer bag. They were then placed on ice in a cooler. To limit cross-contamination between samples while out in the field, any residue left in the net was rinsed out using seawater. The net was then sprayed with 10% bleach solution to sterilise it prior to the next sampling attempt. Between surveys, the net was thoroughly washed with fresh water and soaked in 10% bleach solution for 10 min before being rinsed again in fresh water and air-dried.

In addition to the sampling of live animals, faecal material was extracted from the intestines of a dead sei whale that stranded at Saladero (East Falkland) during 2019.

Faecal samples were sub-divided for future analysis:

- One part was stored in 96% ethanol (EtOH) and frozen at -20ºC for DNA-based prey analysis; and
- Remaining material was stored frozen without storage medium (in multiple vials depending on quantity).

Additionally, any parasites observed while processing the samples were extracted and placed into 96% EtOH.

4.2.1.2 Skin samples

Skin samples were acquired from: (1) live whales, via biopsy sampling carried out during small boat surveys; and (2) dead whales reported by local landowners. The methods used to collect and process these samples are outlined fully in **Chapter 5** of this report which covers the genetic work. The skin samples used for isotope analysis were placed in foil and frozen at -20ºC.

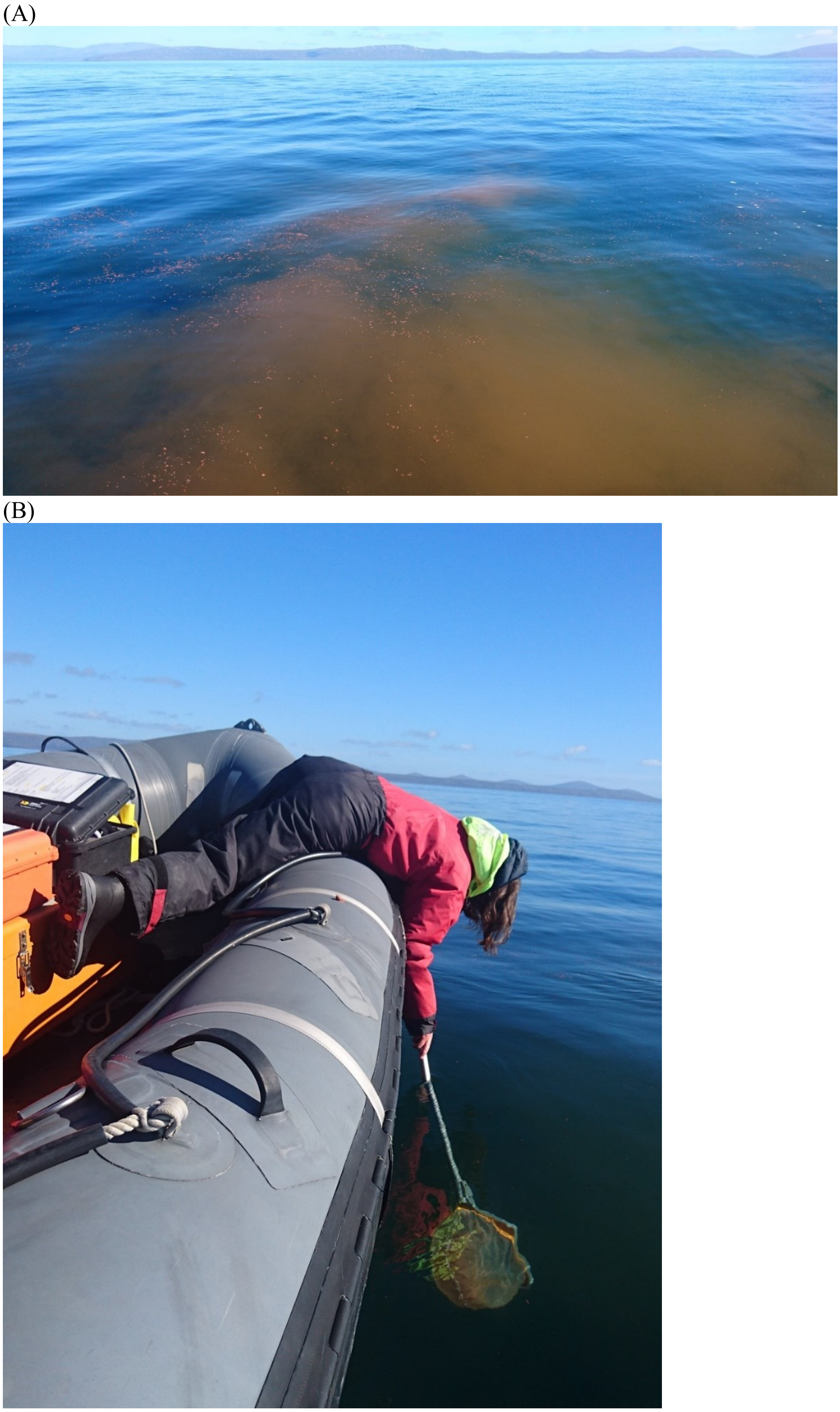

Figure 4.1. *Sampling the faeces of a sei whale during DPLUS082: (A) the cloud of faecal material; and (B) collecting the material with the sampling net.*

4.2.1.3 Shipments

All faecal and skin samples were stored in the Falkland Islands during the field seasons, and were subsequently transported north to the UK once per year on the BAS vessel. Since the vessel usually departed in April or May, any samples collected from April onwards could not be shipped until the following year; as a result, none of the 2021 samples could be included in these analyses. Relevant Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) export/import permits were acquired for skin samples, and Falkland Islands Government provided export licences for the faecal samples.

4.2.2 Prey DNA analysis

DNA from faecal samples was extracted using QIAamp Fast DNA Stool Minikit (Qiagen, Hilden, Germany) following the manufacturer's instructions with an extended overnight digestion step at 55ºC. Inhibitors were removed using the Zymogen One StepTM PCR Inhibitor Removal kit. DNA extracts were quantified by visualisation on a 1.5% agarose gel and by use of a Nanodrop DNA quantifier. Samples with high yields were diluted to equimolar concentrations of 5ng/µL using sterile water. Low-yield samples (<5ng/µL) were kept at initial concentration.

To identify which prey species were present in each faecal sample, three genetic loci (mitochondrial 12S and 16S ribosomal DNA rDNA, and nuclear 18S rDNA) were amplified by polymerase chain reaction (PCR). One fragment of each of the two mitochondrial genes was amplified, using primers that were specifically designed to target fish species (Deagle et al., 2007; Miya et al., 2015; Berry et al., 2017). The 18SrDNA gene was amplified using universal (i.e. taxonomically nonspecific) primers (Machida and Knowlton, 2012). Three different fragments of this gene were amplified in order to minimise the impact of amplification bias (i.e. differential amplification of different prey species) on the results, and maximise insights from the taxonomic reference database Genbank. All amplification primers contained adapters compatible with the Nextera 500 Mid Output Kit (Illumina Inc.).

In order to minimise amplification of whale DNA during metabarcoding, 'blocking' primers were used (e.g. Vestheim and Jarman, 2008). These are designed to bind to whale DNA fragments and therefore stop them from being amplified during PCR. Blocking primers were used in the 12S fish amplification, and two of the three 18S amplifications (Table 4.1).

PCR amplifications were performed in reaction volumes containing 1 x 'Q' solution, 2.5x buffer, 1.2 mM $MgCl_2$, 0.2mM dNTP mix, 0.4µm of each amplification primer, 0.1 units/µl Takara Ex Taq Hot Start DNA polymerase, and 1µl faecal DNA extract. For reactions containing a whale block, 3.2 mM of blocking primer was also added. The solution was made up to 20 µl with double-distilled, sterile water. Each amplification was performed independently per primer pair on a PCR thermal cycler with temperature increments fixed at 0.3°C per second. Thermal cycling conditions were 94°C for 4 min, followed by 30 cycles of 94°C for 45 s, an optimised annealing temperature (see Table 4.1) for 45 s, 72°C for 45 s, and a final extension step of 72°C for 10 min. PCR reactions were repeated in triplicate until triplicate reactions amplified concurrently for each faecal sample. Template amplification success was monitored by eye using agarose gel electrophoresis.

For each faecal sample and DNA fragment, triplicate reactions were pooled and then equimolar concentrations of the five loci were pooled for each faecal sample, resulting in 34 unique libraries. Dual-indexing of sample libraries and preparation for amplicon-sequencing was outsourced to the University of Bristol Genomics Facility, where libraries were sequenced on the Illumina NextSeq 500 system (mid-output 150 bp paired-end reads).

Table 4.1. *Primer pairs and primer blocks used for 12S, 16S and 18S metabarcoding.*

Region	Primer name	Primer Sequence	Anneal temp (°C)	Citation
18S-v7	18S-3-F	[Adaptor sequence] GYGGTGCATGGCCGTTSKTRGTT	50	Machida and Knowlton (2012)
	18S-4RC-R	[Adaptor sequence] CKRAGGGCATYACWGACCTGTTAT		Machida and Knowlton (2012)
Block	18S-4R-CetBlock	CCUCUAAGAAGUUGGGGGAC[SpC3]		Buss (2022)
18S-v8	18S-4-F	[Adaptor sequence] GTCWGTRATGCCCTYMG	57	Machida and Knowlton (2012)
	18S-5RC-R	[Adaptor sequence] GTGTGYACAAAGGBCAGGGAC		Machida and Knowlton (2012)
Block	18S-4F-CetBlock	GACUGGCUCAGCGUGUG[SpC3]		Buss (2022)
18S-v9	18S-5-F	[Adaptor sequence] GTCCCTGVCCTTTGTRCACAC	50	Machida and Knowlton (2012, #2979)
	18S-1510R-R	[Adaptor sequence] CCTTCYGCAGGTTCACCTAC		Amaral-Zettler et al., (2009)
Fish16S	16SF/D	[Illumina Adaptor sequence] GACCCTATGGAGCTTTAGAC	50	Berry et al. (2017)
	16S2R-degenerate	[Illumina Adaptor sequence] CGCTGTTATCCCTADRGTAACT		Deagle et al. (2007)
Fish12S	MiFish_F	[Illumina Adaptor sequence] TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTCGGTAAAACTCGTGCCAGC	54	Miya et al. (2015)
	MiFish_R	[Illumina Adaptor sequence] GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCATAGTGGGGTATCTAATCCCAGTTTG		Miya et al. (2015)
	MiFishU_CetBlock	AGTGTTAAGGAACTACATAAAATTAAAGTCA[SpC3]		Buss (2022)

The University of Bristol Genomics Facility provided downloadable FASTQ files that were demultiplexed per sample (*n* = 24) using the Illumina BaseSpace Server (Illumina Inc.). Primers were used to identify samples by locus (allowing errors in up to 15% of each primer sequence, corresponding to ~3 mismatches) and were removed using *cutadapt* (Martin, 2011). All remaining functions were carried out using the *dada2* package (Callahan et al., 2016) in R version 4.2.1. To remove low-quality tails on the 150bp paired end reads, we truncated forward reads to 110bp and reverse reads to 105bp using the *filterAndTrim* function in dada2. We then used the parametric error model *learnErrors* in *dada2* to learn errors in the dataset, and used that to inform the dada sample inference algorithm (function *dada*) with default settings. Paired end reads were merged using *mergePairs*, allowing up to three mismatches in the overlap area, and setting a minimum overlap of 12bp. Chimeras were removed using *removeBimeraDenovo*. Finally, taxonomic identification of the resultant reads was conducted using the *assignTaxonomy* function with bespoke databases compiled by Danielle Buss (Buss, 2022), available at https://github.com/DannyLBuss/SeiWhaleDiet_MANUSCRIPT. Relative read abundance was calculated for each sample by dividing the number of reads per amplicon sequence variant (ASV) by the total reads in that sample. Only reads of >1% frequency were retained for downstream statistical analysis, following McInnes et al. (2017). Prior to statistical analysis, we also excluded all >1% reads that identified cetaceans, bacteria, dinoflagellates, protists, worms (annelids and platyhelminthes), and echinoderms ("non target species"). All remaining reads were subject to a BLAST search in the Genbank database (https://blast.ncbi.nlm.nih.gov, 13th August 2022) to confirm species identity. Where 100% correspondence with database items was found across all three 18S fragments, samples were identified to genus level; where correspondence was 85–95%, samples were identified to higher taxonomic levels, depending on the taxonomic breadth of different species blasting to that sequence at that level of identity.

Reads identified to family level were aligned using Clustal Omega within Geneious Prime, and manually reviewed to identify any genetic variants (reads that differed from each other by one base-pair or more). Where the same family-level BLAST identification was made for a single genetic variant across the same set of samples for multiple 18S fragments v7, v8 and v9, the 18S fragments were concatenated for that sample set, and BLAST was then used to investigate species identity by alignment against a concatenation of the three fragments (>450bp length).

Once taxonomic identifications had been made, each amplicon sequence variant was reviewed to determine the likelihood that they were targeted (rather than incidental) sei whale prey. Historical literature on whale diet was combined with information about zooplankton distribution, size and swarming habit in order to suggest whether the item was likely, probable, unlikely, or not whale prey. Mean RRA values were estimated across samples to review the dietary patterns by month and locus. To provide an alternative means of visualizing dietary content, we also examined weighted percent of occurrence (wPOO), weighting each food occurrence according to the number of items identified in the sample (see Deagle et al., 2019).

Variation in samples by month, season and year was tested across the three 18S fragments, including just items identified as likely food (DIET), and all items, including those considered unlikely food (ALL). Since there was a single sample available from December 2018 (DW03), this was excluded due to the small sample size; the sample from a stranded animal collected in April 2019 (DW18) was also removed due to the possibility of anomalous feeding by that animal prior to death. Differences in RRA values at the Class, Order, Family and Genus level relative to month, season (summer = February and March, autumn = April and May) and year (2019; 2020) were tested using Bray-Curtis dissimilarity indices and PERMANOVA (2,500 permutations) for all dietary items, and items hypothesized to be putative prey following taxonomic review. Temporal factors were tested separately and in combination. Analyses were implemented in R package *vegan* (Oksanen et al., 2022). To analyse if groups of samples have significant differences in intra-group community variation, the PERMDISP2 procedure (Anderson et al., 2006) was implemented in the function *betadisper* (*vegan* package). Where there was a globally significant result from *betadisper*, pairwise group differences in heterogeneity of multivariate dispersion were analysed using Tukey's Honest Significant difference (HSD) test.

4.2.3 Stable isotope analysis

4.2.3.1 Laboratory methods

For isotope analysis, the whale skin samples were freeze-dried for 48 hours prior to lipid extraction. Lipids were extracted using three alternating extractions with chloroform:methanol (2:1 v/v, $n = 2$) and methanol:chloroform (2:1 v/v, $n = 1$) until all lipid was removed. The samples were subsequently rinsed with deionised water. To remove all inorganic material the skin was then submerged in 0.5N hydrochloric acid (HCl), refrigerated for 48 hours and again rinsed with deionised water to remove all excess acid. Finally, to extract collagen from the skin, samples were heated in a HCl solution (HCl in deionised water, pH=3) for 48 hours. The extracted collagen was freeze-dried for 48 hours and aliquots (0.6–0.8 mg) were weighed into tin capsules prior to isotope analysis.

The $\delta^{13}C$ and $\delta^{15}N$ isotopic analysis of the samples was carried out at the Scottish Universities Research Centre (SUERC) in Glasgow, UK. Carbon and nitrogen isotopes were measured on a Thermo-Fisher-Scientific (Bremen, Germany) Delta XP Plus Isotope-Ratio Mass Spectrometer linked to an Elementar (Hanau, Germany) Pyrocube Elemental Analyser. The internal reference materials were GEL (gelatin solution), ALAGEL (alanine-gelatine solution spiked with ^{13}C-alanine), and GLYGEL (glycine-gelatine solution spiked with ^{15}N-alanine), each dried for two hours at 70°C. Four United States Geological Survey (USGS) 40 glutamic acid standards 106,107 were used as independent checks of accuracy. Delta values were corrected for instrument drift (changes in isotopic composition of gases through the mass spectrometer) and linearity (variability in sample masses).

4.2.3.2 Analytical challenges

Whale diet and the locations where a whale has been feeding can be inferred using stable isotope analysis of proteinaceous tissues, since the isotopic composition of a consumer's protein is closely related to the isotopic composition of the proteins in their diet (Hobson, 1999). Whereas the nitrogen in an animal's tissue is mainly sourced from the proteins in its diet, the carbon is supplied by dietary proteins, lipids and carbohydrates, which potentially differ in their carbon isotope composition (Newsome et al., 2010). Lipids tend to be depleted in the heavier isotope ^{13}C compared to proteins (Post et al., 2007), so that the presence of lipids in the analysed tissue can reduce bulk tissue $\delta^{13}C$ values and may lead to erroneous conclusions about diet or feeding grounds. Therefore, lipid rich tissues such as whale skin should be lipid-extracted prior to carbon isotope analysis. All skin tissue samples of sei whales (n = 9) and southern right whales (n = 69) were analysed in October 2021 according to the protocol described in Section 4.2.3.1.

Following isotopic measurement, the atomic ratio of carbon to nitrogen (C:N) is generally used to evaluate the presence of lipids in the sample. Ratios below 3.5–3.7 are considered to be fully lipid extracted (Post et al., 2007). However, as of yet there is no consensus regarding acceptable marine mammal C:N ratios, with some studies considering values between 4 and 5 acceptable (Smith et al., 2020).

4.3 Results

4.3.1 Samples

4.3.1.1 Faecal samples

Over the course of DPLUS082, a total of 51 faecal samples were collected from sei whales (Table 4.2), comprising 50 from at-sea sampling during boat surveys and one from a stranded animal. Of the 50 samples collected at sea, 11 originated from Falkland Sound and 40 from Berkeley Sound (Figure 4.2). The samples were collected from five different survey months, but the majority (73%) were collected during March and April in the austral autumn (Figure 4.3). Only the 34 samples collected from 2018 to 2020 were shipped to the UK in time for inclusion in the diet analyses.

One additional faecal sample was collected in the vicinity of southern right whales during 2021, but it was unclear whether that material originated from the right whales or from another source; that sample will be identified genetically once it has been received by BAS.

Table 4.2. *Summary of 54 cetacean faecal samples collected in the Falkland Islands over the course of DPLUS082.*

Species	At-sea faecal sampling				Collected from stranded animals			Total
	2018	2019	2020	2021	2019	2020	2021	
Sei whale	1[1]	20	12	17	1	0	0	51
Peale's dolphin	0	2	0	0	0	0	0	2
Unidentified[2]	0	0	0	1	0	0	0	1
Total	1	22	12	18	1	0	0	54

[1] Sample collected by visiting researchers in December 2018 and donated to the project.

[2] Sample collected during May 2021 in the vicinity of southern right whales, but ID needs to be confirmed.

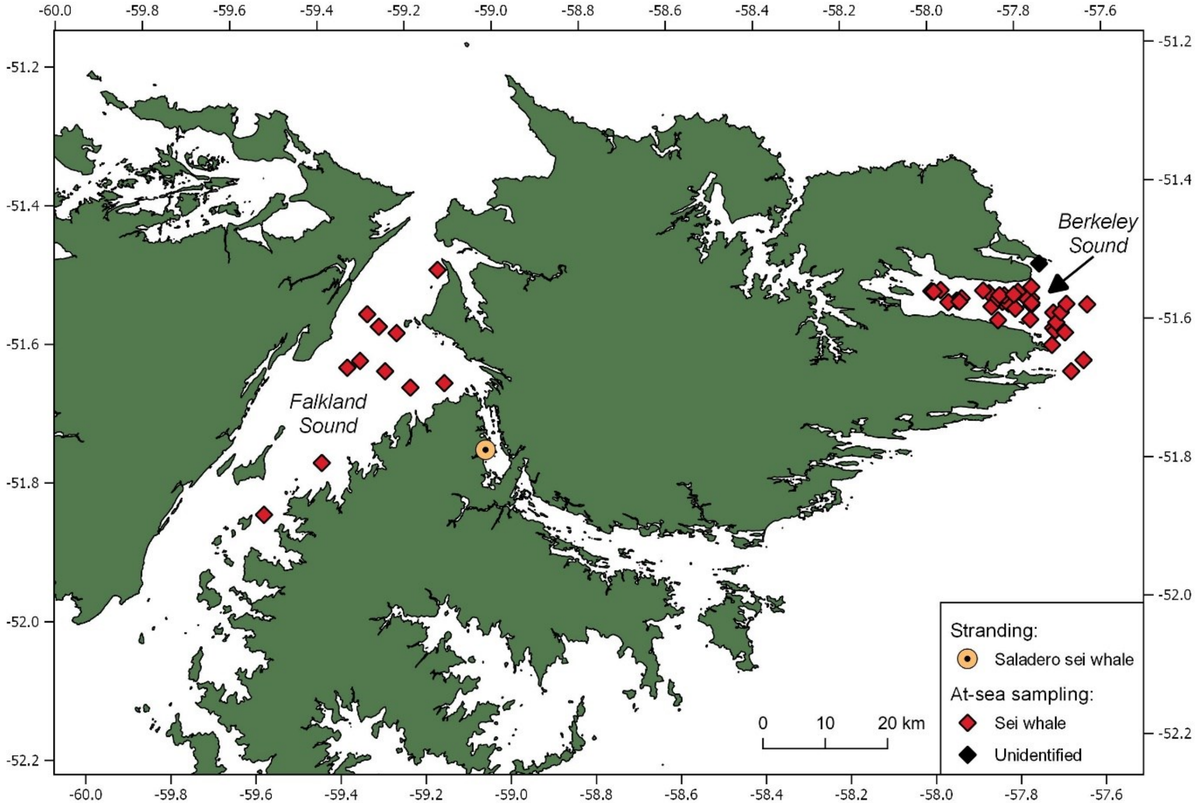

Figure 4.2. *Locations of the faecal samples collected during DPLUS082.*

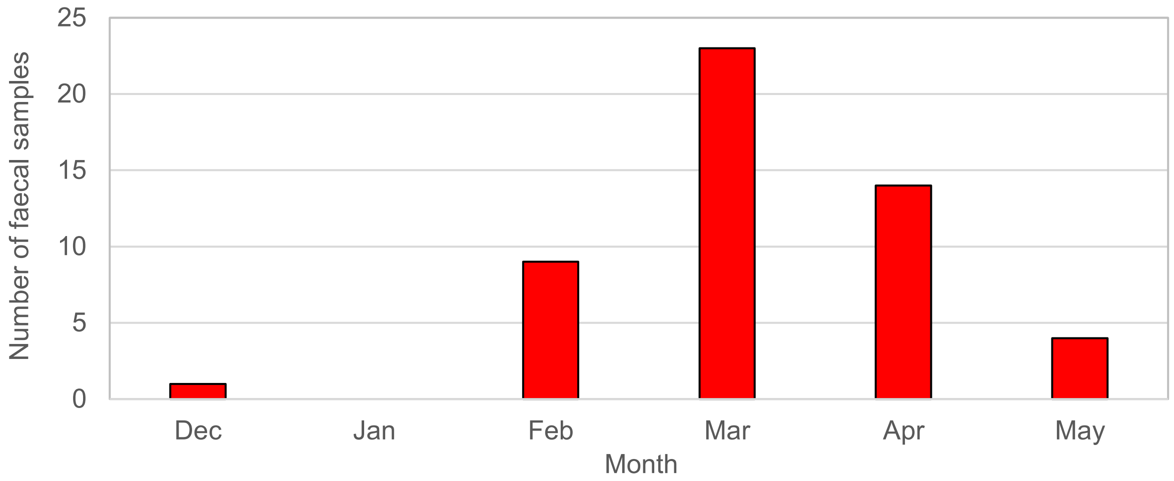

Figure 4.3. *Seasonal distribution of the faecal samples collected during DPLUS082.*

4.3.1.2 Skin samples

A total of nine sei whale skin samples (all samples from 2019 and 2020) and 96 southern right whale samples (all biopsy samples from 2019 and 2020, plus the 2019 stranding) were exported to the UK in time for inclusion in analysis (see Table 5.2 for details of total tissue sampling). Of those, all nine sei whale samples included subsamples of skin that had been frozen for stable isotope analysis (Table 4.3). However, some of the southern right whale samples collected in the field comprised only very small amounts of skin, which were prioritised for the genetic work (**Chapter 5**). A total of 69 right whale tissue samples included sufficient skin to facilitate subsampling for isotope analysis, comprising 36 in 2019 and 33 in 2020 (Table 4.3). The 2019 and 2020 totals included known duplicate samples from four different southern right whale individuals; three had one duplicate and one had two duplicates (confirmed by photo-identification and genetic analysis). Duplicate samples were retained in the analysis, in order to infer potential isotopic variation within individuals.

Table 4.3. *Summary of skin samples from sei whales and southern right whales in 2019 and 2020 that were available for $\delta^{15}N$ and $\delta^{13}C$ analysis.*

Species	Year	Number of samples		
		Total	Unique	Duplicate
Sei whale	2019	5	5	0
	2020	4	4	0
	Total	*9*	*9*	*0*
Southern right whale	2019	35	33	2
	2020	33	30	3
	Total	*68*	*63*	*5*

4.3.2 Prey DNA analysis

4.3.2.1 Data description

Amplicon sequencing resulted in a total of 65.8 million paired end reads across all samples and loci (Table 4.4). All faecal samples amplified for 6 loci and all datasets had <10,000 reads, so were retained for further analysis. After quality control filtering, merging and removal of chimeras, 50.3 million reads remained, representing 12,139 amplicon sequence variants (ASV, genetically unique reads). When retaining reads of >1% relative read abundance and excluding non-target species, the 18Sv7, 8 and 9 fragments yielded 150, 151 and 139 ASV respectively, summed across all samples. Alignment and review of these reads identified that many ASV were genetically identical between samples, with only 1–2 variant reads identified within each family-level identification (see Tables 4.6 and 4.7).

Table 4.4. *Summary of total metabarcoding reads over all samples at each filtering step. #ASV refers to the number of Amplicon sequence variants identified (summed over all samples).*

Locus	Initial reads	Filtered reads	% passing QC	# merged reads	# reads chimeras removed	% chimeras	Read lengths (bp)	# ASV	Median # final reads / sample
18Sv7	14,285,182	13,111,052	91.8	11,726,206	11,237,132	4.2	131-157	3,022	262,514
18Sv8	13,633,449	13,458,630	98.7	13,294,656	12,640,014	4.9	166-183	3,240	311,537
18Sv9	10,681,007	10,325,898	96.7	9,745,177	9,616,976	1.3	132-166	3,347	253,739
12S	17,472,563	16,792,612	96.1	14,729,270	14,683,444	0.3	–	1,849	390,347
16S	9,773,623	9,480,740	97.0	2,144,782	2,144,488	0.0	–	681	41,874

4.3.2.2 Taxonomic identification

Taxonomic identification of these ASV yielded identification of species across 11 orders, summarised in Table 4.5. The most predominant order was the calanoid copepods, which were present in >90% of samples and were also the predominant order recovered in terms of relative read abundance (RRA, Figure 4.4, Figure 4.5). Within Calanoida, sequence alignment and BLAST searches with 18Sv7, v8 and v9 identified five families represented by 6, 7 and 7 ASV respectively. Two ASV were identified within the family Calanidae, two within Clausocalanidae, one in Subeucalanidae, one in Centropagidae. One ASV was genetically distant from all available calanoids (~90% identical at 450bp), and placed most closely to the Acartiidae. Decapod crustaceans were the next most frequent order identified; these were present in ~80% of samples, and were represented by a single ASV at all 18S loci, identified as a member of the family Munididae. This ASV was genetically identical to published squat lobster krill *Munida gregaria*/*M. subrugosa* sequences (559bp alignment).

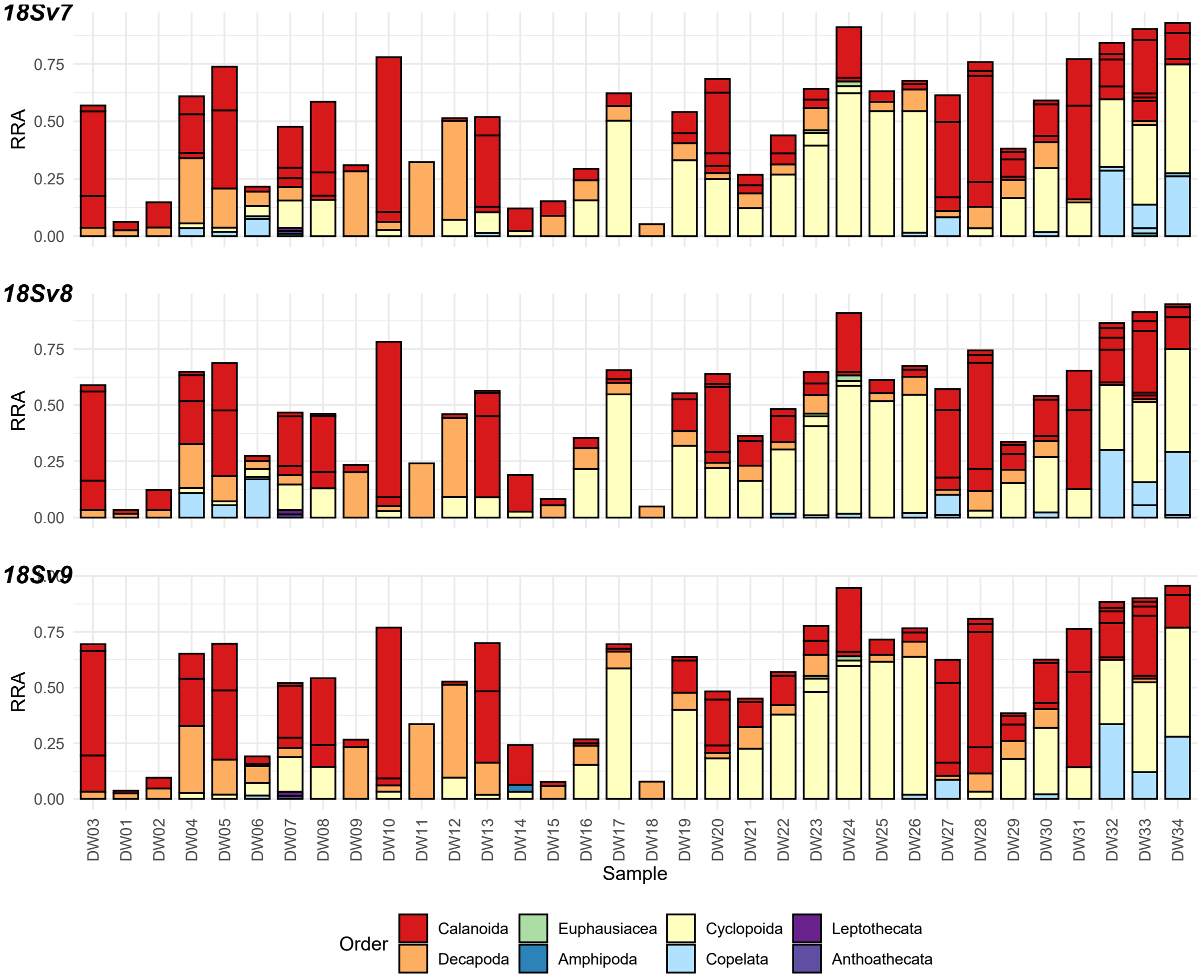

Figure 4.4. *Relative read abundance of different taxonomic orders of animals recovered in sei whale faecal samples.*

Table 4.5. *Taxonomic orders of species found in faecal samples, and the number of samples in which they occurred at >1% total read abundance. Values in parentheses refer to the percentage of samples including each order.*

Class	Subclass	Order	18Sv7	18Sv8	18Sv9
Maxillopoda	Copepoda	Calanoida	32 (94%)	32 (94%)	32 (94%)
		Cyclopoida	26 (76%)	26 (76%)	26 (76%)
	Cirripedia	Balanomorph	2 (6%)	0	0
Malacostraca	Eumalacostraca	Decapoda	28 (82%)	27 (79%)	28 (82%)
		Euphausiacea	2 (6%)	2 (6%)	2 (6%)
		Amphipoda	0	0	1 (3%)
Appendicularia		Copelata	10 (29%)	12 (35%)	7 (21%)
Hydrozoa	Hydroidolina	Anthoathecata	1 (3%)	1 (3%)	1 (3%)
	Hydroidolina	Leptothecata	1 (3%)	1 (3%)	1 (3%)

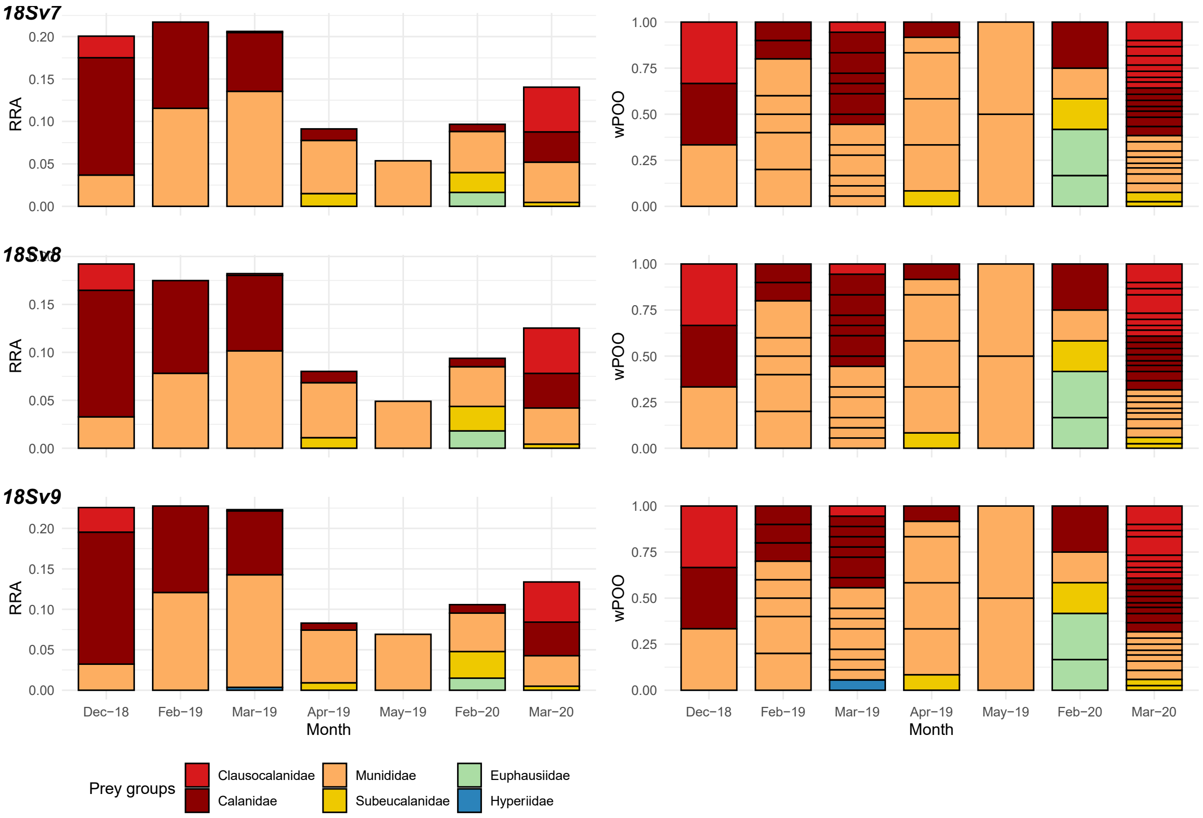

Figure 4.5. *Sei whale putative dietary items (family-level identifications) shown by month, visualised as mean relative read abundance (RRA) and mean weighted proportion of organisms (wPOO) for each 18S fragment (v7, v8 and v9).*

The next most common order was the cyclopoid copepods (76% of samples, Table 4.5), represented by two ASV (Table 4.7), both identified within the genus *Oithona*, a small, widespread and highly abundant copepod species. The predominant ASV (O1, 26 samples) is identical to *O. similis* over 444bp. The second ASV (O2, two samples DW23 and DW24) is most closely related to *O. atlantica* (one bp difference over 328bp). This copepod genus is small in size (<1mm) and so is not likely be a target species for whales but may instead be an incidental capture associated with areas of oceanic productivity. Similarly, the next most common order identified was tunicates within the order Copelata (35% of samples); due to their size and energy content, these are also likely to be incidental prey rather than target species (Table 4.6). Two groups of tunicates were identified. One fell within family Oikopleuridae (two ASV: T1 in 8 samples, T2 in 2 samples DW32 and DW34), with the predominant ASV (T1) genetically identical to *Oikopleura fusiformis* over 470bp. The minor variant T2 was not genetically identical to any available species, but fell genetically closest to *O. fusiformis*. The other group fell within family Fritillaridae (9 samples, one ASV), and was genetically identical to *Fritillaria borealis* over 348bp.

Euphuasiid krill were identified in two samples (one ASV), and were genetically identical to *Euphausia. vallentini* across all three 18S loci, but also identical to a partial 18S sequence of *E. lucens*; both of these species are found in the Patagonian Sea so are potential targets. Balanomorph barnacles were also identified in two samples (DW07 and DW33); these are not whale target food but may have been attached to other dietary items. Two hydrozoan species (orders Anthoathecata and Leptothecata) were found in a single sample (DW07), as was one amphipod species (DW14), which was genetically identical to members of the *Themisto* genus over 141bp.

None of the amplified gene fragments at >1% read occurrence comprised fish.

Table 4.6. *Review of orders recovered in multiple sei whale faeces to establish likelihood that they are target prey for sei whales. Here we exclude species found in only one sample (e.g. Hydrozoans), but retain Amphipoda, as those were recovered by Buss (2022) in previous sei whale faecal collections.*

Class	Subclass	Order	Identified families	Previously identified as sei whale food?	Previously identified as baleen whale food?	Summary of evidence	Verdict (likely, probable, unlikely, no)
Maxillopoda	Copepoda	Calanoida	Clausocalanidae, Calanidae, Subeucalanidae, Acartiidae (uncertain), Centropagidae	Y	Y	Calanoida have been previously identified as sei whale food (Nemoto, 1962; Nemoto and Kawamura, 1977; Horwood, 1987). Particular whale prey candidates are those species that form large (multi-metre), dense swarms and are large in size (>1 mm length).	See Table 4.7
		Cyclopoida	Oithonidae	N	N	Oithonidae are small relative to usual whale zooplankton prey (<1 mm length). Consumption may be a consequence of high water column abundance in productive areas, rather than targeted feeding.	Unlikely
	Cirripedia	Balanomorph	Balanidae	N	N	Balanomorphs (barnacles) are filter feeders that attach to substrates and are not target species for whale diet. One ASV was identified (18Sv7 135bp, two samples). A number of Balanidae are genetically identical to this sample.	No
Malacostraca	Eumalacostraca	Decapoda	Munididae	Y	Y	*Munida* species have previously been identified as whale food in Patagonia, particularly *M. gregaria,* both through visual observations and stomach contents studies (Matthews, 1932; Nemoto, 1970). Sei whales have been observed actively surface-feeding on *M. gregaria* in the Falklands and hard parts of the species (i.e. carapaces, eye balls, pincers) have been confirmed in their faeces (Weir et al. 2019).	Likely
		Euphausiacea	Euphausiidae	Y	Y	Krill is primary baleen whale food in polar waters. In temperate waters there is evidence of feeding on e.g. *E. vallentina* and *E. lucens* in South African waters (Best, 1967).	Likely

Class	Subclass	Order	Identified families	Previously identified as sei whale food?	Previously identified as baleen whale food?	Summary of evidence	Verdict (likely, probable, unlikely, no)
		Amphipoda	Hyperiidae	Y	Y	*Themisto gaudichaudii* has previously been reported as whale food (e.g., Kawamura, 1980). Sei whales have been observed actively surface-feeding on *T. gaudichaudii* in the Falklands (Weir et al. 2019). However, there is only one sample containing this genus in the dataset.	Probable
Appendicularia		Copelata	Fritillariidae, Oikopleuridae	N	N	Incidental feeding by minke whale reported in Mediterranean (Fraija-Fernández et al., 2018). Never recorded as a significant dietary component. Two *Oikopleura* ASV were identified (T1, 8 samples, T2, 2 samples). Most frequent variant is identical to *O. fusiformis* over 470bp. *Fritillaria* samples contain one ASV (P1, found in 9 samples) which is identical to *Fritillaria borealis spp* over 331bp.	Unlikely

Table 4.7. *Genetic and distributional review of calanoid copepods recovered in sei whale faeces to determine most likely genus and assess likelihood that they are target prey for sei whales.*

Family	Candidate sp south Patagonia Sea (Cepeda et al., 2018)	Candidate sp on Genbank (spanning 18S target region)	# genetic variants (ASV), name and # samples	Genetic identity (over # sequenced base-pairs)	Notes	Verdict (likely, probable, unlikely, no)	References
Calanidae	*Calanoides patagoniensis*, *Calanus australis*, *Calanus simillimus*, *Neocalanus tonsus*	None	2 (C1, 17; C2, 1)	C1: not identical to any Genbank sp over 480bp. *Calanus* sp. closest with 99.1% identity.	C1: 99.8% (one basepair difference) identity to environmental sample collected in Gulf of California. Only candidate sp with a distribution including both locations is *C. australis*. Mean female size is 2.6–3.59 mm, males 2.9–3.47 mm (large copepod). However, *Neocalanus tonsus* should not be excluded as possible source, as it also has Pacific occurrence and was proposed as a primary sei whale food source in Horwood (1987).	Both ASV are likely food items. All candidates are relatively large copepods and there are multiple reports of this genus as whale food (summary in Horwood, 1987).	Horwood, 1987; Cepeda et al., 2018; Horton et al., 2022.
				C2: 100% identical to *C. helgolandicus*, *C. pacificus*, *C. finmarchicus*, *C. glacialis* and *C. chilensis* over 480bp.	C2: none of the identical *Calanus* sp. are local to Patagonia, but local species are not represented on Genbank, so these cannot be excluded as possible candidates.		
Clausocalanidae	*Clausocalanus brevipes*, *Clausocalanus furcatus*, *Clausocalanus laticeps*, *Ctenocalanus vanus*, *Drepanopus forcipatus*	*Clausocalanus furcatus*, *Ctenocalanus vanus*	2 (D1, 23; D2, 10)	D1: *Microcalanus pygmaeus* has 100% identity over 447bp.	D1: *Microcalanus.* The mean female size is 0.755 mm (*n* = 4; SD = 0.2479), and in male 0.813 mm (from: https://copepodes.obs-banyuls.fr/en/fichefam.php?fam=13#g140). This species is very broadly distributed and very small. Potentially incidental food rather than target.	D1 *Microcalanus* sp. unlikely food.	Nemoto, 1962; Best, 1967; Bucklin and Frost, 2009; Cepeda et al., 2018; Horton et al., 2022.
				D2: *Clausocalanus furcatus* 100% identical over 457 bp, 5. bp different	D2: *Clausocalanus furcatus*, mean female size is 0.92–1.31 mm, males 0.7–0.92 mm. If the species is actually *C. brevipes*, this is larger (1.24–1.62 mm females, 1.12–1.20 mm males). Previous reports of *Clausocalanus* as sei whale		

Family	Candidate sp south Patagonia Sea (Cepeda et al., 2018)	Candidate sp on Genbank (spanning 18S target region)	# genetic variants (ASV), name and # samples	Genetic identity (over # sequenced base-pairs)	Notes	Verdict (likely, probable, unlikely, no)	References
				from *C. vanus*. Genetically, *C. furcatus* falls basal within *Clausocalanus* genus (Bucklin and Frost 2009) without close sister-group, strengthening likelihood that the 18S match is with *C. furcatus*.	food off South Africa and Kerguelen Islands (Nemoto, 1962; Best, 1967). For example, Best (1967) reports sei stomach contents including *Clausocalanus arcuicornis* off South Africa (similar size to *brevipes*).		
Subeucalanidae *	*Subeucalanus longiceps*, *Rhincalanus nasutus*	*Rhincalanus nasutus*	1 (G1, 4)	G1: not identical to any Genbank species over 485bp. *Rhincalanus nasutus* is 90% identical.	G1: not genetically close to *R. nasutus*; if this species is *S. longiceps*, female size is 4.2–4.9 mm, males 3.2 mm, sufficient size to be target whale food. This species has not previously been recorded as whale food although other eucalanids have been identified (Horwood, 1987). It is abundant at 100–250 m depth, north of the sub-Antarctic frontal zone (Ward et al., 2014), so may be a proxy for deeper water feeding.	G1: *Subeucalanus* sp. probable food.	Horwood, 1987; Goetze, 2003; Cepeda et al., 2018; Horton et al., 2022.
Acartiidae	*Acartia tonsa*	*A. tonsa*	1 (A1, 11)	A1: genetically distant from all available Genbank species and 75% identical to *A. tonsa*.	A1: given the substantial 18S genetic distance from *A. tonsa*, this species is *incertae sedis*, potentially representing a genetically unidentified calanoid family. However, note that *Acartia* is a highly diverse cryptic species complex (including *A. tonsa*) so this species could still fall within the Acartiidae family (Figueroa et al., 2020). Animals within this group can swarm, but swarm sizes are often much smaller than e.g. for *Calanus* species	A1: unlikely whale food but needs better level of identification; members of Acartiidae are not regularly recorded as whale food.	Ambler, 2002; Cepeda et al., 2018; Figueroa et al., 2020; Horton et al., 2022.

Family	Candidate sp south Patagonia Sea (Cepeda et al., 2018)	Candidate sp on Genbank (spanning 18S target region)	# genetic variants (ASV), name and # samples	Genetic identity (over # sequenced base-pairs)	Notes	Verdict (likely, probable, unlikely, no)	References
					(Ambler 2002) so tend to be prey for smaller animals.		
Centropagidae	*Centropages brachiatus*	None	1 (1)	Genetically identical to *C. typicus* at 480bp, but this species is not found in the South Atlantic. Comparison with *C. brachiatus* needed.	While there is evidence *C. brachiatus* can swarm at high abundance off South America (Gonzalez and Marín, 1998), there is scant records of this as whale food (low occurrence in sei whale stomachs off South Africa reported in Kawamura, 1980) and the low occurrence in the dataset suggests this species may be incidental rather than target prey. Size of *C. brachiatus* 1.73–2.3 mm females, 1.58–1.9 mm males.	Unlikely whale food.	Kawamura, 1980; Gonzalez and Marín, 1998; Cepeda et al., 2018; Horton et al., 2022.

* This family designation has recently changed. Sometimes designated as Eucalanidae.

4.3.2.3 Community analysis

Curtis-Bray dissimilarity analysis of relative read abundances by sample for all dietary items showed no significant difference between months at any taxonomic level (Class, Order, Family, Genus), both with April and May separate and April and May combined due to small sample sizes. Dissimilarity between years and (year + season) was significant at all taxonomic levels, with the interaction of year and season the most strongly supported model (Table 4.8). Significant differences by year were also found at all taxonomic levels with Tukey's HSD (Table 4.8). This model also showed significant differences between the three seasonal groups (Summer 2019, Autumn 2019 and Summer 2020), with the strongest differences between Summer and Autumn 2019, followed by Summer 2019 and Summer 2020.

Jaccard dissimilarity analysis of wPOO data showed similar but weaker patterns, with differences between years only significant at the Family level, but significant differences between seasons and years seen at all taxonomic levels (Table 4.8) again support the seasonal group model as the best explanation for community-level differences. As with the RRA data, the Tukey's HSD results for the wPOO data also consistently found a significant difference between Summer and Autumn 2019 at all taxonomic levels except Class, but was less consistent in finding differences between the other seasonal groupings, with the Family level showing significant differences between all groups (Table 4.10), and Order and Family levels both finding significant differences between Autumn 2019 and Summer 2020 (in contrast to the RRA results which never found a significant difference between these groups). Patterns were very similar between 18S fragments; only 18Sv7 is therefore shown here as representative. Multidimensional plots of patterns by seasonal group are shown in Figures 4.6 and 4.7, and those by year in Figure 4.8.

When analysing only taxonomic groups thought to be putative sei whale prey, the seasonal groups model was also the most strongly supported compared to month and year models, with significant differences found between seasonal groups overall (Table 4.8). However, in contrast to the analysis of all dietary items, year differences were only significant at the Class level both with RRA and wPOO (Table 4.8). Seasonal groupings were still strongly supported by RRA, but wPOO showed weaker support for these, with support for this model only significant at the Order level. Looking at pairwise differentiation between seasonal groups, Tukey's HSD again found differences between Summer and Autumn 2019 to be significant at all taxonomic levels with RRA (Table 4.9). Differentiation between the other seasonal groups varied across the taxonomic levels with no consistent pattern seen. However, wPOO only found significant differences between Summer and Autumn 2019 at the Order level, and did not show a consistent pattern across taxonomic levels in terms of pairwise difference between other seasonal groups.

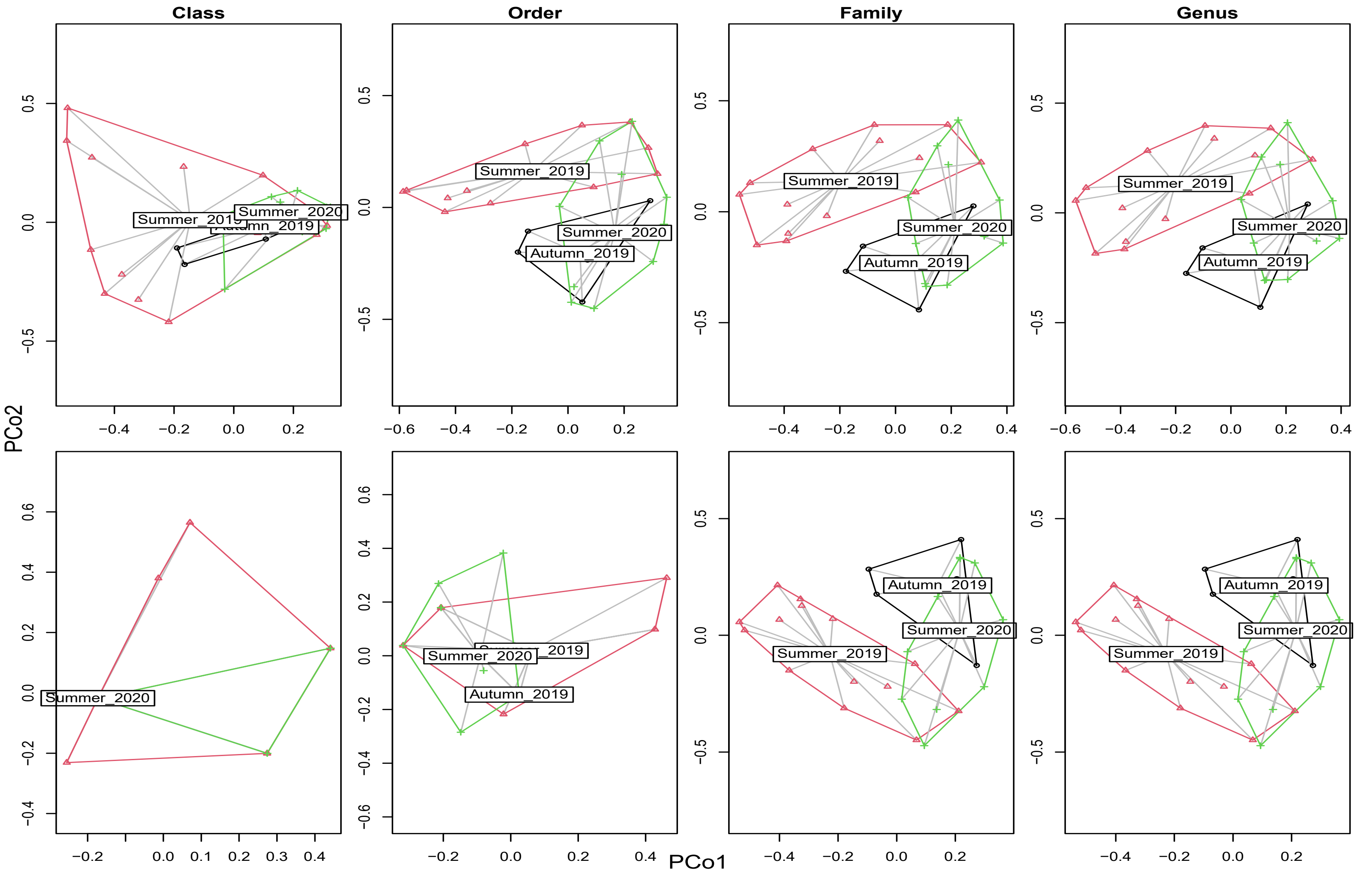

Figure 4.6. *Effect of seasonal group on beta diversity of all items identified in sei whale faecal samples (ALL, fragment 18Sv7) at the Class, Order, Family and Genus level using the betadisper function. Top row, Relative read abundance, Bottom row, weighted proportion of occurrence. PCoA1 (x-axis) and PCoA2 (y-axis) are the first and second sort axes in the betadisper analysis, respectively.*

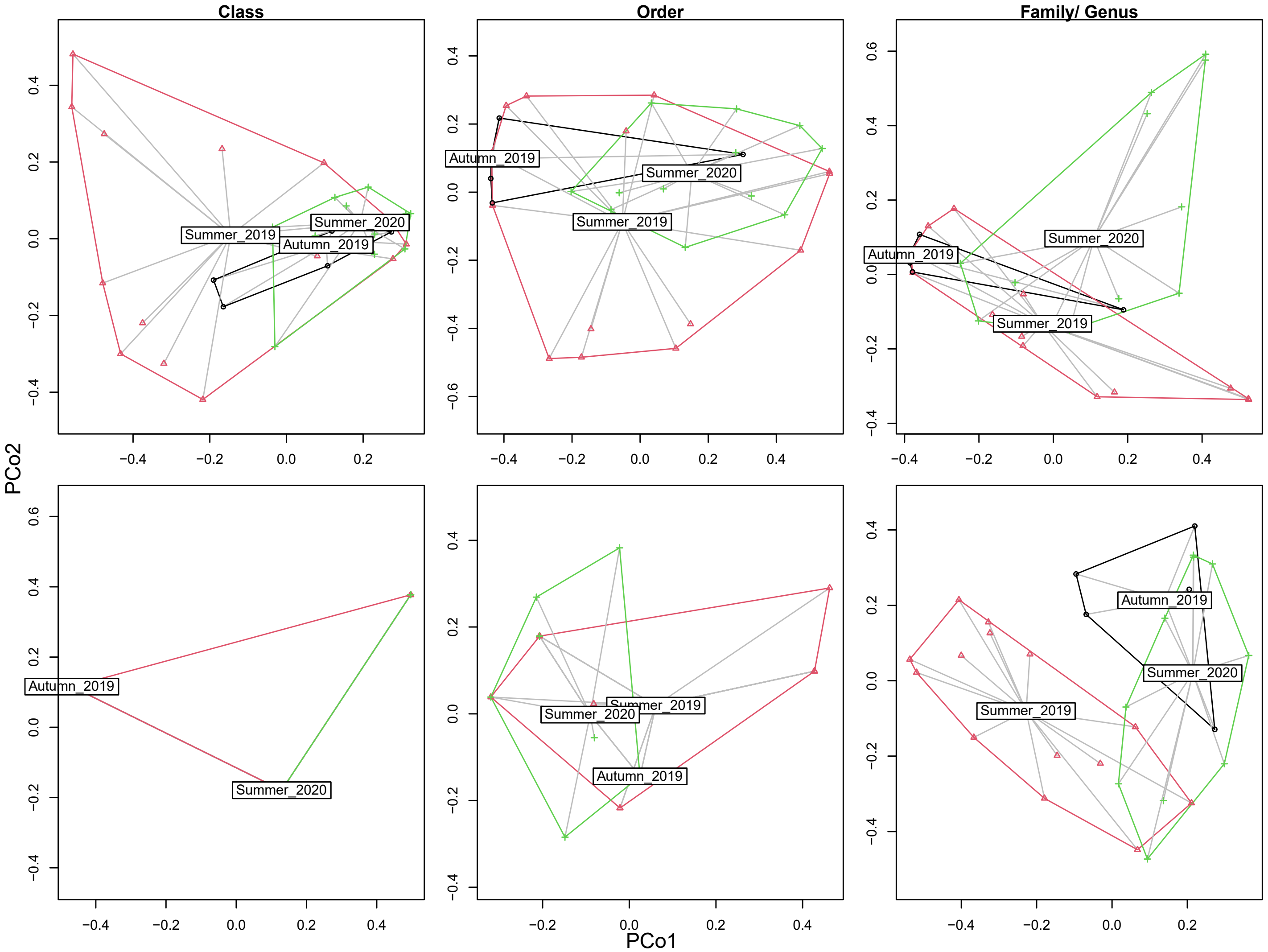

Figure 4.7. *Effect of seasonal group (Summer 2019, Autumn 2019 and Summer 2020) on beta diversity of putative food items in sei whale diet (DIET, fragment 18Sv7) at the Class, Order and Family/Genus level using the "betadisper" function. Top row, Relative read abundance, Bottom row, weighted proportion of occurrence. PCoA1 (x-axis) and PCoA2 (y-axis) are the first and second sort axes in the betadisper analysis, respectively.*

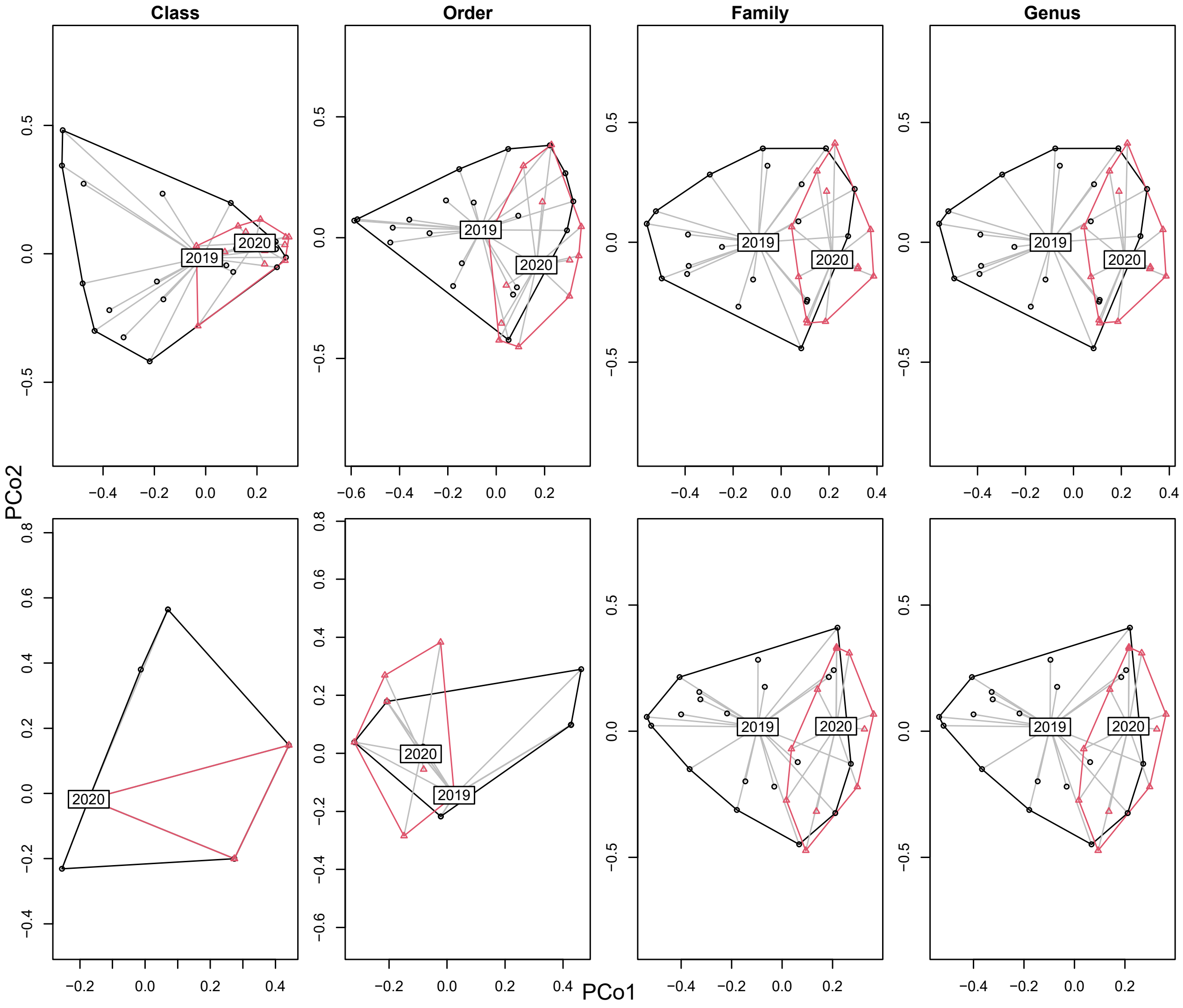

Figure 4.8. *Effect of year (2019 and 2020) on beta diversity of all items identified in sei whale faecal samples (ALL, fragment 18Sv7) at the Class, Order, Family and Genus level using the "betadisper" function. Top row, Relative read abundance, Bottom row, weighted proportion of occurrence. PCoA1 (x-axis) and PCoA2 (y-axis) are the first and second sort axes in the betadisper analysis, respectively.*

Table 4.8. *Significance tests to detect differences in whale faecal samples in 18S fragment v7 across the sampling period. ANOVA: D.F. reflects the degrees of freedom (between and within-group), F-value and p-value of the ANOVA of the multivariate homogeneity of group dispersions, to test for significant differences in sample diversity between year (2019 and 2020) and seasonal group (Summer 2019, Autumn 2019 and Summer 2020). PERMANOVA reflects the significance of multi-factorial permutational multivariate analysis of variance, using Bray-Curtis distance for RRA and Jaccard distance for wPOO. Tukey's HSD describes pairwise group differences in heterogeneity of multivariate dispersion via a beta value and p-value. Tukey's HSD results for seasonal groups can be found in Table 4.9.*

Taxonomic level	Year						Seasonal group			
	ANOVA D.F.	F-value	*p*-value	PERMANOVA	Tukey's HSD *beta*	*p*-value	ANOVA D.F.	F-value	*p*-value	PERMANOVA
Relative read abundance				Bray						Bray
All items										
Class	1,30	4.04	**0.004**	**0.01**	-0.17	**0.01**	2,29	12.6	**0.0001**	**0.001**
Order	1,30	5.34	**0.03**	**0.02**	-0.11	**0.03**	2,29	8.7	**0.0011**	**0.002**
Family	1,30	6.65	**0.02**	**0.02**	-0.12	**0.02**	2,29	12.5	**0.0001**	**0.001**
Genus	1,30	6.15	**0.02**	**0.03**	-0.12	**0.02**	2,29	12.7	**0.0001**	**0.001**
Items hypothesised to be target prey										
Class	1,30	6.82	**0.01**	**0.02**	-0.17	**0.01**	2,29	12.6	**0.0001**	**0.001**
Order	1,30	2.16	0.15	0.15	-0.10	0.15	2,29	5.95	**0.0068**	**0.008**
Family/Genus[1]	1,30	0.03	0.86	0.88	0.01	0.86	2,29	5.68	**0.0083**	**0.007**
Weighted Proportion of Occurrences				Jaccard						Jaccard
All items										
Class	1,30	0.17	0.69	0.67	0.044	0.69	2,29	3.19	0.056	**0.048**
Order	1,30	0.04	0.85	0.86	0.016	0.85	2,29	19.6	**<0.0001**	**0.001**
Family	1,30	7.51	**0.01**	**0.02**	-0.098	**0.01**	2,29	12.9	**0.0001**	**0.001**
Genus	1,30	0.7	0.41	0.39	-0.047	0.41	2,29	6.57	**0.0044**	**0.011**
Items hypothesised to be target prey										
Class	1,30	4.26	**0.048**	**0.048**	-0.27	0.05	2,29	3.16	0.058	0.064
Order	1,30	0.04	0.85	0.86	0.016	0.85	2,29	19.6	**<0.0001**	**0.001**
Family/Genus[1]	1,30	0.06	0.81	0.83	0.033	0.81	2,29	2.7	0.084	0.086

[1] Community composition is the same at family and genus level for hypothesised target prey because only one genus per family was identified as potential prey.

***Table 4.9**. Pairwise community distances between seasons for 18Sv7 at each taxonomic level using relative read abundances (RRA), calculated using the Bray-Curtis dissimilarity coefficient and post-hoc comparisons (Tukey HSD test). Below diagonal shows beta dispersion distances. Above diagonal shows p-value. Significant values at $p < 0.05$ in bold.*

Taxonomic level	Seasonal group	Summer 2019	Autumn 2019	Summer 2020
All items				
Class	Summer 2019	–	**0**	**0**
	Autumn 2019	0.26 (0.98, 0.42)	–	0.88
	Summer 2020	-0.22 (-0.35, -0.10)	0.03 (-0.13, 0.20)	–
Order	Summer 2019	–	**0**	**0.04**
	Autumn 2019	0.24 (0.09,0.39)	–	0.16
	Summer 2020	-0.12 (-0.24, -0.00)	0.12 (-0.04, 0.27)	–
Family	Summer 2019	–	**0**	**0.01**
	Autumn 2019	0.29 (0.14,0.44)	–	0.07
	Summer 2020	-0.15 (-0.27, -0.03)	0.14 (-0.01, 0.30)	–
Genus	Summer 2019	–	**0**	**0.02**
	Autumn 2019	0.29 (0.15, 0.44)	–	0.05
	Summer 2020	-0.14 (-0.26, -0.02)	0.15 (0.00, 0.30)	–
Items hypothesised to be target prey				
Class	Summer 2019	–	**0**	**0**
	Autumn 2019	0.26 (0.10,0.42)	–	0.88
	Summer 2020	-0.22 (-0.35,-0.10)	0.033 (-0.13,0.20)	–
Order	Summer 2019	–	**0.01**	0.12
	Autumn 2019	0.27 (0.07,0.48)	–	0.23
	Summer 2020	-0.13 (-0.30,0.03)	0.14 (-0.07,0.35)	–
Family/Genus[1]	Summer 2019	–	**0.01**	0.95
	Autumn 2019	0.28 (0.07,0.49)	–	**0.02**
	Summer 2020	-0.02 (-0.19,0.15)	0.26 (0.04-0.47)	–

[1] Community composition is the same at family and genus level for hypothesised target prey because only one genus per family was identified as potential prey

4.3.3 Stable isotopes

4.3.3.1 Sei whales

Sei whale skin samples showed very little variation in carbon isotope values with an overall mean of -17.4 ± 0.2 ‰ (range -17.7 to -17.1 ‰). Nitrogen values showed a larger variation ranging from 10.6 to 13.6 ‰ with an overall mean of 11.8 ±1.0 ‰. Sample sizes were too small to test for differences based on sex class or year. However, when comparing averages in Table 4.10, there seems to be very little difference between sex classes or years of sampling for either δ13C or δ15N values. While the narrow range in carbon values indicates that all of these whales utilise feeding grounds of similar latitude or water mass, the wider range of nitrogen values suggests a possible specialisation on different prey species with differing nitrogen isotope signatures.

4.3.3.2 Southern right whales

The C:N ratios after initial lipid extraction were not consistent between sei and southern right whales. Southern right whale C:N values were higher (mean 3.85 ± 0.20, range 3.48–4.48) than those for sei whales (mean 3.55 ± 0.06, range 3.44–3.64). Since 26% (18 out of 68) of southern right whale skin samples showed C:N ratios >4.0, repeat measurements were carried out during January 2022 to investigate whether lipid extraction was complete for those tissues. Replicates of seven samples with C:N ratios over 4.0 were lipid-extracted and also measured in bulk (non-lipid extracted) to test for the expected difference in δ13C between extracted and non-extracted samples. Extractions were doubled (*n* = 6) with solvents alternating between Chloroform:Methanol 2:1 (*n* = 3) and Methanol:Chloroform 2:1 (*n* = 3).

Table 4.10. *δ13C and δ15N values of nine skin samples of sei whale (*Balaenoptera borealis*) collected in the Falkland Islands in 2019 and 2020. Isotope values are given as mean ± SD.*

Category	N per group	δ13C (‰)	δ15N (‰)	C:N (mol)
Year				
2019	5	-17.2 ± 0.1	11.9 ± 0.9	3.6
2020	4	-17.5 ± 0.2	11.8 ± 1.3	3.5
Sex				
Female	3	-17.3 ± 0.1	12.4 ± 0.9	3.6
Male	6	-17.4 ± 0.2	11.6 ± 1.0	3.5

Southern right whale results are presented in Table 4.11. While lipid extraction should lead to a decrease in C:N ratio and associated enrichment in δ13C, this relationship was not observed for all samples. Rather, the C:N ratios of two-thirds of the samples were higher after the first set of lipid extractions than for the untreated bulk samples. A second, longer, extraction (*n* = 6) lowered the C:N ratios slightly below the bulk values, but for one-third of the samples the trend was reversed. However, apart from one specimen (EA-DW-03), skin samples with lower C:N ratios showed more enriched δ13C values.

Table 4.11. *Stable isotope ratios of $\delta^{13}C$ and corresponding C:N ratios of southern right whale (*Eubalaena australis*) skin samples. LE = lipid extracted, NLE = non-lipid extracted.*

Sample ID	Bulk NLE		LE (*n* = 3)		LE (*n* = 6)	
	δ13C	C:N ratio	δ^{13}C	C:N ratio	δ^{13}C	C:N ratio
EA-DW-03	-17.88	4.11	-17.64	4.48	-17.38	3.86
EA-DW-28	-17.88	3.88	-17.95	4.08	-18.69	4.50
EA-DW-35	-18.60	4.07	-18.32	4.07	-19.10	4.37
EA-DW-67	-18.80	3.93	-19.30	4.06	-18.30	3.85
EA-DW-72	-18.26	3.95	-18.54	4.11	-17.68	3.77
EA-DW-91	-18.81	3.79	-19.08	4.04	-18.37	3.63
EA-DW-92	-20.58	4.32	-20.49	4.18	-19.84	4.11

When compared to other studies of balaenopterid whales (including southern right whales) the C:N ratios generated in our study fell within the acceptable range (Valenzuela et al., 2009; Ryan et al., 2012; Smith et al., 2020). However, the increase in C:N ratios with increasing lipid extractions in some southern right whale samples remains confounding.

The tissue samples were freeze-dried but not ground prior to lipid extraction. One reason for the values could therefore be that the solvents used to extract the lipids could not penetrate the tissue, and the resulting C:N ratios between treatments simply reflect the natural within tissue variation of untreated samples. However, both the sei whale samples (and one minke whale sample) were analysed using the exact same method as used for the right whale samples, and their C:N ratios all measured between 3.4 and 3.5 after only one set of extractions (*n* = 3 extractions).

Another reason for the highly variable C:N ratios in the southern right whale samples could relate to the anatomy and biochemical composition of the integument. Southern right whale skin seems to be structurally different to most other baleen whales, in that the epidermis has a fat-free zone in the reticular dermal layer and more elastic fibres and keratin in the dermal and hypodermal layers, more reminiscent of odontocetes (Reeb et al., 2007). The isotopic values measured in this study are based on the extraction and measurement of collagen, the main protein reflecting dietary intake. While collagen has a theoretical C:N ratio of 3.1 (Doherty et al., 2021), the presence of other non-collagenous proteins such as elastin (C:N 5.8) and keratin (C:N 3.4) could skew the results (Doherty et al., 2022).

The southern right whale skin samples showed a wide range of δ15N nitrogen values with an overall mean of 12.3 ± 1.4 ‰ (range 8.0 -14.3‰). The combined data set of δ15N was non-normally distributed (*n* = 63, Ryan-Joiner, $P < 0.01$) and thus non-parametric tests (Kruskal-Wallis, K-W) were used to test for differences in isotopic values due to sex class and year of sampling. Although showing slightly lower values in 2020 than in 2019 for both sexes (Figure 4.9), the isotopic signatures of the collected skin samples were not significantly different between sampling years (K-W, H = 2.09, p = 0.148). Nor were any significant differences found between male and female southern right whales (K-W, H = 0.0, p = 0.987) when all of the data were pooled.

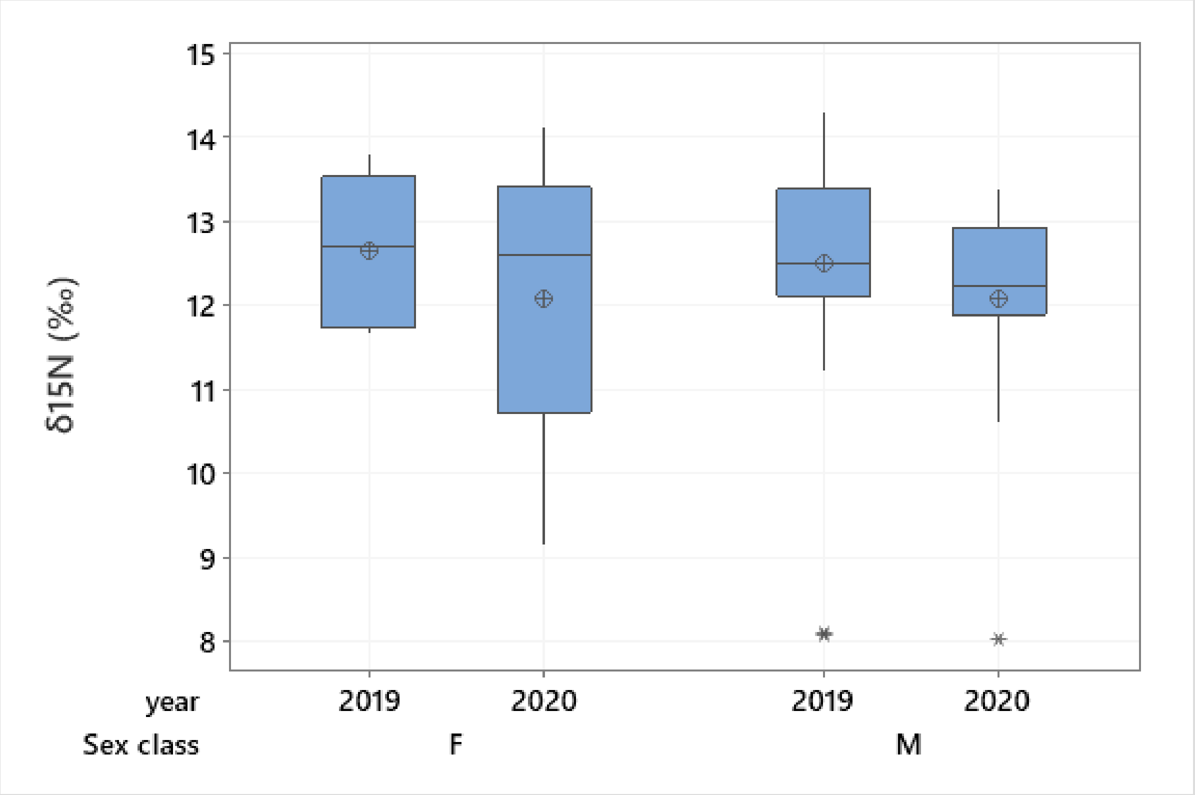

Figure 4.9. *Boxplot of δ15N values from 63 skin samples collected from southern right whales in the Falkland Islands, grouped by year and sex class. 2019: Females (F) = 5, Males (M) = 28; 2020: F = 11, M = 19. Boxplots represent the median, mean and 25th and 75th percentiles.*

However, the nitrogen data seemed to be distributed as two groups, one with a lower mean δ15N value of 8.5±0.6‰ (*n* = 5; range: 8.0–9.3‰) and a higher group with a mean δ15N value of 12.6±0.9‰ (*n* = 58; range: 10.6–14.3‰). This was similar to the δ15N distribution found by Valenzuela et al. (2018) for southern right whales at Peninsula Valdés in Argentinian waters.

4.4 Discussion

4.4.1 Sei whales

Metabarcoding of sei whale faeces recovered one genus of decapods (Munididae, likely representing *Munida gregaria*) and multiple genera of calanoid copepods within three families. Of these, Calanidae (composed of two *Calanus* species) was most predominant in the diet, followed by Subeucalanidae (genus uncertain) and then Clausocalanidae (two genera, and the likely prey item may be *Clausocalanus furcatus*). Krill were identified in two samples, while the amphipod genus *Themisto* was identified in one sample. Both dietary items were also recovered at low frequency in 2017 and 2018 (Buss, 2022). A number of additional species were recovered that were considered unlikely to be target prey species due to their small size, low nutritional content and/or small swarm size- these included cyclopoid copepods (*Oithona*), an unidentified crustacean perhaps affiliated with Acartiidae and a small calanoid genus within the Clausocalanidae (*Microcalanus*). These species may represent non-target consumption by sei whales, or in the case of highly abundant species *Oithona* and *Microcalanus*, they may be present at detectable levels in water samples, i.e. as environmental DNA present in the water column when faeces were collected. At least two tunicate species (Fritillariidae and Oikopleuridae) were also identified in multiple faecal samples; salps are traditionally considered not to have the energy content to be candidate prey for whales, and are rarely recovered in any numbers during stomach sampling by whalers. However, tunicates were also recovered in a metabarcoding study of New Zealand Bryde's whales *Balaenoptera edeni* (Carroll et al., 2019), and their value as a prey item may be underestimated (Henschke et al., 2016). These taxonomic identifications can be used to guide more detailed investigation into the local distribution of potential key prey species' such as *Calanus*, *Munida* and the subeucalanid species. Genetic identification of local zooplankton is very patchy and remains a key data gap for improving resolution of some putative prey species (for example including the subeucalanid species and the acartiid crustacean). Genetic barcoding of many marine zooplankton species has been done using the cytochrome oxidase I (CO1) marker (e.g., Bucklin, 2011; Blanco-Bercial, 2014), but this locus has proven unreliable in many metabarcoding studies (e.g., Deagle, 2014), and taxonomic coverage of the more conserved 18S locus is much poorer. This study points to candidate species that would benefit from more detailed genetic study, to confirm their important in sei whale diet. In turn, the results presented here can be used as a guide to conduct more taxonomically-specific CO1 amplification of faecal samples where particular groups (e.g. krill, calanoids) have already been identified.

Comparisons of samples between years using relative read abundance and weighted proportion of occurrence showed the most significant difference in sample composition between summer and autumn 2019 and between years, the latter predominantly driven by a significant difference in sample composition between seasons. This pattern was consistent whether all dietary items were considered (potentially including incidental prey and eDNA samples) or only hypothesised prey items. This pattern was driven by a diet very dominant in Munididae in autumn 2019 (Figure 4.5), in contrast to summer 2019 when the diet was more mixed between Munididae and copepods. Interestingly, an opposing pattern of inter-seasonal differentiation was seen in previous years (2017 and 2018: Buss, 2022), with Munididae more predominant in the summer than autumn. This suggests temporally varying occurrence of sei whale prey in local waters may be driving this pattern, rather than seasonal prey preferences by sei whales.

Sei whales migrate seasonally between subtropical wintering grounds and higher latitude feeding areas, and the Falkland Islands represent one of the feeding destinations within the south-west Atlantic with peak abundances recorded between February and April (Baines and Weir 2020; Weir et al., 2021; **Chapter 2**). If we assume a potential integration time of 3–7 months of the prey isotopic signature into the skin of whales (Busquets-Vass et al., 2017), the isotope values presented in this report are indicative of the diet on either their wintering grounds or feeding along their migration route, prior to the period represented by the metabarcoding results.

Metabolic isotope fractionations modify the isotopic composition of a predator relative to its diet which leads to an enrichment in predators' tissues relative to their prey (Peterson and Fry, 1987). Borrell et al. (2012), in a study on various tissues in fin whales (*Balaenoptera physalus*), suggested a diet-tissue fractionation factor of 1.3 for carbon and 2.8 for nitrogen in skin tissue. If we adopt these values to our study then the average δ13C values of -17‰ compare well with potential copepod and euphausiid prey isotope data (range of -18 to-19‰ for euphausiid species and -19 to -23‰ for copepod species) from Patagonian shelf waters (Valenzuela et al., 2018 and references therein). Compared to other baleen whales, sei whales have a relatively broad diet, foraging on copepods, euphausiids, decapods and occasionally fish (e.g., Kawamura, 1974; Kawamura, 1980). The range for δ15N‰ found in our study would therefore suggest that sei whale individuals might have preferentially fed on prey in other areas (perhaps, for example, the northern Patagonian sea or subtropical frontal zones) prior to their arrival in the Falkland Islands. The metabarcoding data suggest that sei whales feed on copepods much more predominantly than krill in the summer and autumn, but as stable isotope data represent the feeding period 3–5 months prior to the summer (outside of the core season for sei whales in the Falkland Islands), higher levels of krill feeding during winter and spring cannot be ruled out.

4.4.2 Southern right whales

Southern right whales are known to feed on euphausiid and copepod species in the South Atlantic and Southern Ocean. In waters south of the Polar Front, nitrogen isotope values often overlap between euphausiid and copepod species (Stowasser et al., 2012) and similar overlaps exist in Patagonian shelf waters (Valenzuela et al., 2018, and references therein). This makes it difficult to isotopically distinguish between these two prey groups as the preferred prey of the southern right whales sampled in the Falkland Islands. If we assume a trophic discrimination factor of 2.8 between prey and the skin of southern right whales (adopted from the study by Borrell et al. (2012) for fin whale skin) then our sample group putatively spans at least two trophic levels. Considering the potential integration time of prey isotopic signature into the skin of whales (3–7 months: Busquets-Vass et al., 2017), this suggests that sub-groups used different feeding grounds or prey fields over an extended period of time prior to their migration to the Falkland Islands.

Continuing challenges with the carbon isotope analysis prevented data completion in time for this report, and additional work is still needed to resolve these issues. Without the addition of carbon isotope data, which shows a distinct latitudinal decline with increasing latitude (e.g., Magozzi et al., 2017), it is more difficult to interpret δ15N distributions and allocate them to specific areas in the South Atlantic and Southern Ocean. However, Valenzuela et al. (2018), in their study on southern right whales at Peninsula Valdés, have identified two potentially isotopically distinct food webs in the South Atlantic and Southern Ocean, with low δ15N values measured below the Polar Front and relatively high δ15N values measured on the Patagonian Shelf. This distinction would suggest that the group of whales with low nitrogen values represent a part of the population that feeds primarily in polar waters, while whales with intermediate and high nitrogen values are more likely to have fed along the Patagonian shelf or other shelf waters. This suggests that right whales visit the Falkland Islands following visits to both low and high latitude feeding areas, rather than the Islands comprising a preferred destination for one feeding group. Once it becomes available, the addition of carbon isotope data to the analysis might discriminate the migration and feeding grounds of right whales in the Falkland Islands more clearly in the future.

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Chapter 5: Genetics

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5.1 Introduction and aims

Commercial exploitation of whales was carried out around the Falkland Islands from the start of the 20th century, and intensive whaling across the South Atlantic led to rapid declines in most local species. Following the international moratorium on commercial whaling, populations of many species are slowly recovering in the South Atlantic (Harcourt et al., 2019), and in recent decades sightings of whales have become more common in Falklands' waters, with sei whales (*Balaenoptera borealis*) and southern right whales (*Eubalaena australis*) the most regularly encountered species in nearshore areas (Weir and Stanworth 2020; Weir et al., 2021).

This chapter presents the results of a genetic investigation into the population biology of sei and southern right whales in the Falkland Islands, using mitochondrial DNA (mtDNA) to investigate maternally inherited patterns of diversity and population connectivity, and biparentally inherited microsatellites to investigate their genetic diversity following whaling.

5.2 Materials and methods

5.2.1 Sample collection and transportation

Tissue sampling of baleen whales was carried out between 2019 and 2021 during DPLUS082 via: (1) biopsy sampling of live animals during survey work from a rigid-hulled inflatable boat between 2019 and 2021 (see **Chapter 2** for additional information); and (2) sampling of dead animals that stranded around the Falklands coast.

Biopsy sampling was conducted by a single trained person (CW) using a Barnett BCR Recurve crossbow (150 lb draw weight) fitted with bolts and stainless steel biopsy tips from CETA-DART. All tips were sterilised prior to use. During sampling attempts, the boat was manoeuvred to within ~15 m of a whale, and a shot was aimed at the flank of the whale as it rolled at the surface. Only healthy non-calf animals (i.e., well-rounded, and without obvious disease or injuries) were selected for biopsying. Whenever possible, a photo-identification image was taken for each sample, in order to minimise repeat attempts on the same animals. Following recovery, the samples were retained inside the biopsy tips and stored on ice in a freezer bag. Samples were processed on return to Stanley at the end of each survey day, and subdivided into three parts: (1) a skin sample placed in 95% ethanol (EtOH) and stored in a freezer (-20°C) for genetic work; (2) a skin sample placed in foil and frozen for isotope analysis; and (3) blubber placed in tinfoil and frozen for a range of future analyses including hormone work.

Strandings of baleen whales in the Falklands were either attended by CW (when located on East Falkland and some islands) or were sampled by local landowners under the guidance of CW when located on West Falkland or some remoter islands. In all cases, samples of skin (and bone and baleen) were collected that could be used for genetic work (processed and stored as described above). Species identification of stranded animals was usually established via photographs; however, when an animal was too decomposed to ascertain the species, then the identification was confirmed genetically.

In addition to tissue samples, whale genetic material was also present within the faecal samples that were collected primarily to investigate diet (see **Chapter 4** for details of collection methods). The samples used for genetic work were stored in EtOH at -20°C prior to extraction.

Tissue and faecal samples were transported to BAS in the UK for analysis once per year, using the BAS vessel to ensure that they remained frozen throughout the journey. The transport north usually occurred in April or May, meaning that samples collected from April onwards had to wait until the following year to be sent to the UK. Consequently, some batches of samples collected during DPLUS082 (including all of the 2021 samples) were not available for analysis in this report.

5.2.2 DNA extraction, amplification and genotype profile construction

Total genomic DNA was extracted from skin tissue samples using a Qiagen DNEasy Blood and Tissue kit according to the manufacturer's instructions. Total genomic DNA was extracted from faecal samples as described in **Chapter 4**. Genomic DNA was then visualised on a 2% agarose gel to assess DNA quality, and DNA was quantified using a Nanodrop. DNA concentrations were then standardised to 10 ng/ul by dilution with double distilled water. The sex of sampled whales was identified by amplification of the male-specific SRY gene, multiplexed with an amplification of the ZFY/ZFX region as a positive control (Bérubé and Palsbøll, 1996). See Table 5.1 for polymerase chain reaction (PCR) cycle details.

The mitochondrial DNA (mtDNA) control region of all samples (~950 bp) was amplified by PCR using standard protocols (Oremus et al., 2007). Primers dlp1.5 (also known as tPro-whale: Baker et al., 1998) and dpl5 (CCA TCG WGA TGT CTT ATT TAA GRG GAA, Baker et al., 1993) were used for sei whales (biopsy and faecal samples), while dlp1.5 and tphe (ANN CAT TTT CAG TGY WTT GCT TT: Carroll et al., 2011a) were used for southern right whales. PCR amplicons were purified by LGC Genomics, and forward and reverse strands were sequenced using Sanger sequencing. MtDNA sequences were aligned and edited in Geneious Prime 2021.1.1 (www.geneious.com). Phred scores were used to evaluate the quality of individual bases, and poor quality base calls were manually reviewed and edited where necessary.

For sei whales, fifteen microsatellite loci were amplified for 10 samples. For southern right whales, seventeen microsatellite loci were amplified from the 95 samples that were collected in 2019 and 2020. Individual 10 µl PCR reactions were carried out under the amplification conditions and reaction mixtures described in Table 5.1. Amplification of each locus included a set of samples for which alleles had previously been sized; three for sei whales and five for right whales (Carroll et al., 2020). These were used as internal controls to ensure consistent allele sizing, and a negative control was included in all amplifications to detect contamination. Sei whales were genotyped at Eurofins Genomics, with allele size calls conducted by DB. Southern right whales were genotyped and allele size calls conducted at Oregon State University.

5.2.3 Mitochondrial DNA analysis

For the mtDNA data, the number of haplotypes, haplotype diversity, and nucleotide diversity was calculated using Arlequin v3.5 (Excoffier and Lischer, 2010).

5.2.3.1 Sei whale

Sei whale haplotypes were aligned with haplotypes from the North Atlantic (Huijser et al., 2018), and with a previously sequenced dataset from the Falkland Islands ($n = 37$ at 456bp: Buss, in prep), using Clustal Omega 1.2.3 in Geneious Prime. A median-joining haplotype network was constructed from this alignment using PopART (Leigh and Bryant, 2015). Haplotype and nucleotide diversity and differentiation (F_{ST} and ϕ_{ST}) measures were calculated in Arlequin (Excoffier and Lischer, 2010), and population differentiation from Chile and the North Atlantic was estimated using F_{ST} and ϕ_{ST} with 50,000 permutations; uncorrected pairwise distances were used to calculate the latter.

Table 5.1. *PCR conditions for mtDNA and genotype amplifications of sei whale and southern right whale (SRW) tissue.*

Locus[1]	Species	Primer reference	Primer labels	TA (°C)	mM Mg	*n* cycles	Approx size (bp)
mtDNA							
Dlp1.5 – tPhe	SRW	Baker et al. (1998); Carroll et al. (2011a)		55	2.5	35	950
Dlp1.5 – Dlp5	Sei	Baker et al. (1993)		55	2.5	35	500
Sex							
ZFYX0582F, ZFY0752R, ZFX0784R	SRW, sei	Bérubé and Palsbøll (1996)		52	2.5	37	200-300
Microsatellites							
GATA28	SRW, sei	Palsbøll et al. (1997)	NED	50/57	2.5/3.0	35/33	162-182
GATA98	SRW, sei	Palsbøll et al. (1997)	VIC/FAM	50/57	2.5	35/33	104-124
EV1	SRW, sei	Valsecchi and Amos (1996)	NED	60/57	2.5/2.7	35/33	122-150
EV14	SRW, sei	Valsecchi and Amos (1996)	VIC/HEX	60/62	2.5	35/33	122-143
EV37	SRW, sei	Valsecchi and Amos (1996)	NED	54/56	2.5/3.0	35/34	187-207
EV94	SRW	Valsecchi and Amos (1996)	FAM	55	2.0	31	196-202
GT023	SRW, sei	Bérubé et al. (2000)	VIC	63/60	2.0/3.5	35/33	110-122
GT122	SRW	Bérubé et al. (2005)	FAM	55	2.5	30	133-150
GT310	SRW, sei	Bérubé et al. (2000)	NED/ ATT0550	59/57	2.0/3.5	31/33	94-102
RW18	SRW	Waldick et al. (1999)	FAM	63	2.5	35	185-241
RW31	SRW	Waldick et al. (1999)	FAM	54	2.0	35	117-131
RW48	SRW	Waldick et al. (1999)	NED	50	2.5	35	106-128
RW410	SRW	Waldick et al. (1999)	VIC	50	2.5	35	195-213
TR3G1	SRW	Frasier et al. (2006)	FAM	*T	2.5	35	206-238
TR3G2	SRW	Frasier et al. (2006)	VIC	50	2.5	35	168-188
TR3F4	SRW	Frasier et al. (2006)	FAM	59	2.0	35	301-349
CA232	SRW	Bérubé et al. (2005)	FAM	55	2.5	33	145-157
CA128	Sei	Bérubé et al. (2005)	ATTO565	55	3.5	32	53-77
GATA53	Sei	Palsbøll et al. (1997)	ATTO550	60	2.5	33	180-220
GATA417	Sei	Palsbøll et al. (1997)	HEX	57	2.5	32	180-300
GT011	Sei	Bérubé et al. (1998)	FAM	55	2.5	33	90-150
GT211	Sei	Bérubé et al. (2000)	FAM	57	2.5	32	185-220
GT541	Sei	Bérubé et al. (2005)	ATTO550	56	3.5	34	95-113
GT575	Sei	Bérubé et al. (2000)	FAM	55	2.5	33	140-211
AC087	Sei	Bérubé et al. (2005)	YAKYE	55	3.5	33	151-200

[1] These reactions had cycling conditions of (i) an initial denaturing step at 94°C for 3 min; (ii) *n* cycles at 94°C for 30 sec, annealing at TA for 30 sec and extension at 72°C for 30 sec; and (iii) a final extension step at 72°C for 10 min. *T indicates this primer pair had a touchdown PCR protocol: for the cycling, each annealing temperature is used for five cycles before stepping down to the next annealing temperature; the final annealing temperature is used for 10 cycles, resulting in a total of 30 cycles. Annealing temperatures are 68°C, 64°C, 61°C, 58°C and 57°C.

5.2.3.2 Southern right whale

Southern right whale (SRW) haplotypes were truncated to 381 bp length, and matched with a global dataset of SRW haplotypes, to identify frequencies of new and existing SRW mtDNA haplotypes in the Falkland Islands. To visualise the position of Falkland Islands mtDNA haplotypes within the broader South Atlantic migratory network, the Falkland Islands dataset was aligned with haplotype data from Brazil, Argentina, South Georgia and South Africa (Valenzuela et al., 2009; Carroll et al., 2019; Carroll et al., 2020) in Geneious Prime using Clustal Omega 1.2.3 (Sievers et al., 2011). A median-joining haplotype network was constructed from this alignment using PopART (Leigh and Bryant, 2015). Haplotype and nucleotide diversity and differentiation (F_{ST} and ϕ_{ST}) measures were calculated in

Arlequin (Excoffier and Lischer, 2010). Significance was assessed using permutation tests (50,000 permutations). For ϕ_{ST}, Kimura 2-parameter genetic distances were used, and the exact test of differentiation was conducted (1,000,000 Markov chain steps; 1,000,000 dememorisation steps), with significance set at $p = 0.05$.

5.2.4 Genotype analysis

Replicate samples were identified using CERVUS v3.0.7 to calculate the probability of identity (Waits et al., 2001). CERVUS was also used to identify null alleles and deviations from Hardy-Weinberg equilibrium (Kalinowski et al., 2007). Levels of linkage disequilibrium were examined using GENEPOP v4.7.5 (Rousset, 2008), with p-values adjusted for multiple comparisons. Levels of genetic diversity were calculated using GenoDive v3.05 (Meirmans, 2020). For southern right whales, CERVUS was also used to check for matches at 8 or more microsatellite loci between individuals identified in the Falkland Islands and those previously profiled in other South Atlantic grounds (Carroll et al., 2020): South Africa ($n = 123$), Argentina ($n = 46$), Brazil ($n = 50$) and South Georgia ($n = 11$).

5.3 Results

5.3.1 Samples

A total of 27 tissue samples were collected from sei whales during DPLUS082, comprising 22 biopsy samples, four samples from stranded whales, and a single piece of skin that was recovered from a suction cup tag (Table 5.2). Of those, 10 samples collected in 2019 and 2020 were shipped to the UK in time for inclusion in these analyses.

A total of 128 tissue samples were acquired from southern right whales, the vast majority of which were from biopsy sampling carried out over the three years (Table 5.2). Additionally, samples were collected from two right whales that stranded in the Falklands in 2019 and 2020 respectively (Table 5.2). Of those, 49 biopsy samples from 2019, 45 biopsy samples from 2020, and tissue from the 2019 stranding, were transported to the UK in time for inclusion in this report. Although 50 biopsies were taken in 2019, two of the samples were quickly ascertained to originate from the same individual whale and only one of that pair was included in the analysis.

Additionally, 51 faecal samples were collected from sei whales during DPLUS082 including 50 from live animals and one from a dead stranded whale (Table 5.2, Chapter 4). Of those, the 34 samples collected between 2018 and 2020 were shipped to the UK in time for inclusion in these analyses.

Table 5.2. *Summary of 166 baleen whale tissue samples collected in the Falkland Islands over the course of DPLUS082. Numbers refer to the total samples collected rather than the number of individual whales sampled (the totals include some known duplicates).*

Species	Biopsy sampling			Sampling of stranded animals			Other[1]	Total
	2019	2020	2021	2019	2020	2021	2019	
Sei whale	3	3	16	2	1	1	1	27
S. right whale	50	45	31	1	1	0	0	128
Humpback whale	0	0	9	0	0	1	0	10
Minke whale	0	0	0	0	1	0	0	1
Total	53	48	56	3	3	2	1	166

[1] Comprising one skin sample obtained from a suction-cup tag.

5.3.2 Sex ID and mitochondrial DNA analysis

5.3.2.1 Sei whale

Of the 44 sei whale samples amplified (10 tissue samples and 34 faecal samples), good quality DNA sequence of 482 bp consensus length was obtained from 40 samples, containing 21 polymorphic sites.

One tissue sample (DW4) and one faecal sample (FS-DW18) were acquired from the same stranded whale, and consequently the duplicate was removed to leave a total of 39 samples for the sei whale genetic analyses.

Sex was obtained from biopsy samples only (*n* = 10), with three females and seven males identified. All biopsy-sampled individuals were unique.

Forty-two samples analysed from the Falklands in 2017 and 2018 had already yielded 22 haplotypes from 37 sei whales. On comparison with this dataset, 13 new haplotypes were identified within the 2018–2020 dataset at 482 bp alignment length; the remaining sequences matched with previously identified haplotypes, yielding a total Falkland Islands dataset of *n* = 77 samples, containing 34 unique haplotypes. This dataset shared one haplotype with the North Atlantic (Huijser et al., 2018), and 23 haplotypes with sei whales from Chilean waters (Pérez-Álvarez et al., 2021).

The Falkland Islands dataset showed high haplotype and nucleotide diversity, very similar to that reported in Chile, and significantly higher than comparable data from the North Atlantic (Table 5.3). The median joining haplotype network (Figure 5.1) illustrates the high genetic diversity of the Falkland Islands and level of genetic overlap with sei whales from the Chilean population. Population differentiation measures are summarised in Table 5.4, and show that the Falkland Islands has significant haplotype frequency (F_{ST}) differentiation from Chile and the North Atlantic. However, there was no evidence of long-term ϕ_{ST} differentiation between the Falklands and Chile suggesting that while these two populations are differentiated, there is likely to be a degree of interchange between the two.

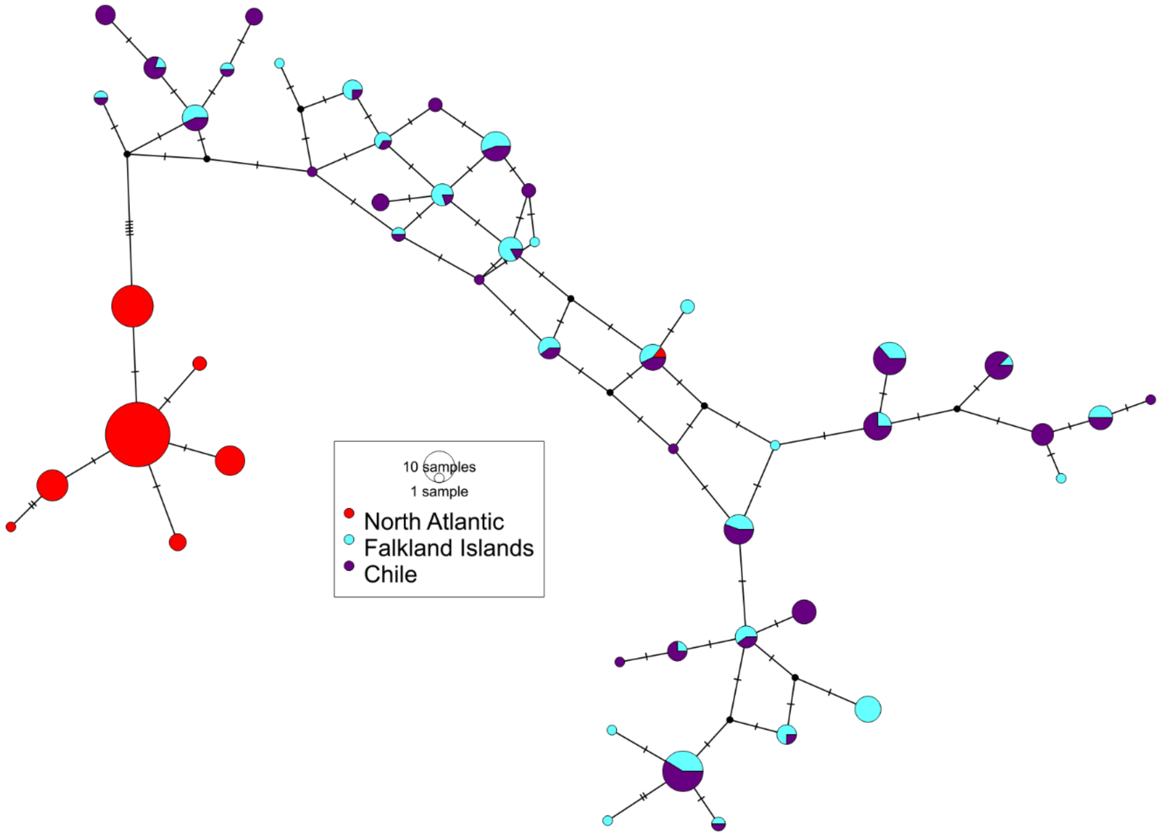

Figure 5.1. *Median joining mtDNA haplotype network, showing the distribution pattern of contemporary sei whale haplotypes from the Falkland Islands (blue) compared with haplotype data from Chile and the North Atlantic.*

Table 5.3. *Summary of mtDNA diversity statistics for sei whales in the Falkland Islands, compared with those in Chile and the North Atlantic.*

Statistic	Falkland Islands	Chile	North Atlantic
No. sequences	77	98	87
No. variable sites	21	20	15
No. haplotypes	34	35	8
Haplotype diversity	0.968 ± 0.006	0.967 ± 0.005	0.695 ± 0.040
Nucleotide diversity	0.016 ± 0.009	0.017 ± 0.009	0.003 ± 0.002
Tajima's D	1.066 (p = 0.89)	1.672 (p = 0.95)	-1.722 (p = 0.02)
Fu's F_S	-13.803 (p = 0.002)	-13.235 (p = 0.000)	-1.578 (p = 0.24)
Source	Buss in prep; this study	Pérez-Álvarez et al. (2021)	Huijser et al. (2018)

Table 5.4. *Population differentiation between sei whales in the Falkland Islands, Chile and the North Atlantic.* F_{ST} *is shown above the diagonal and* ϕ_{ST} *below the diagonal, with comparisons showing significant differentiation at* $p<0.05$ *given in bold.*

Country	Chile	Falklands	North Atlantic
Chile	–	**0.009**	**0.167**
Falklands	0.008	–	**0.170**
North Atlantic	**0.689**	**0.710**	–

5.3.2.2 Southern right whale

Of the 95 southern right whale samples amplified, 94 produced good quality DNA sequences of 959 bp consensus length, containing 48 polymorphic sites. All samples collected in 2019 and 2020 had microsatellite profiles available, from which duplicates (i.e. repeated sampling of the same individuals) could be identified (see Section 5.3.3.2). Following the removal of duplicates from these datasets, there were 82 'unique' mtDNA sequences available; these were genetically identified to comprise 65 males and 17 females.

On alignment with the global dataset of SRW haplotypes, two novel haplotypes were identified within the Falkland Islands dataset (samples DW06 and DW29); the remaining sequences matched with previously identified haplotypes at 381 bp consensus length.

The genetic diversity statistics for SRWs are summarised in Table 5.5. Higher values (e.g. of H_{obs}, h and π) represent high levels of genetic diversity in the region in question. The Falkland Islands dataset showed high haplotype and nucleotide diversity, similar to that seen on other SRW South Atlantic wintering grounds. The median joining haplotype network (Figure 5.2) also illustrates the high genetic diversity of the Falkland Islands, with the Falklands-only network (Figure 5.2 inset) showing a spread of haplotypes very similar to the haplotype pattern of the whole South Atlantic (Figure 5.2 main).

Population differentiation patterns (Table 5.6) showed the strongest differentiation between the Falkland Islands and South Africa (significant for F_{ST} and ϕ_{ST}) although a number of mtDNA haplotypes are common to both locations (Figure 5.2). There was no evidence of long-term ϕ_{ST} differentiation between the Falklands and Argentina or Brazil, but significant differences in haplotype frequencies were evident from these areas F_{ST}, which may suggest that the Falkland Islands contain a mixture of whales from more than one calving ground. No differentiation was seen between the Falkland Islands and the South Georgia feeding area; however, the latter also has a small sample size (*n* = 11) so strong inference cannot be drawn from this finding.

Table 5.5. *Summary of various microsatellite and mtDNA diversity statistics for southern right whale winter nursery/socialising and summer feeding grounds. Sample size (2N), and observed (H_{obs}) and expected (H_{exp}) heterozygosity, are reported for the microsatellite loci used in the analysis. Sample size (N), number of haplotypes (n_{hap}), haplotype diversity (h), and nucleotide diversity (π,) are reported for the 381 bp fragment of the mtDNA control region analysed.*

Region	Microsatellites			Mitochondrial DNA				Source
	2N	H_{obs}±SD	H_{exp} ±SD	N	n_{hap}	*h* ± SD	π (%) ± SD	
South Africa	246	0.74 ± 0.03	0.77 ± 0.03	416	39	0.94 ± 0.01	2.4 ± 1.2	Carroll et al. (2019)
Argentina	92	0.71 ± 0.04	0.76 ± 0.03	208	28	0.94 ± 0.01	2.3 ± 1.2	Carroll et al. (2019)
Brazil	100	0.74 ± 0.04	0.76 ± 0.03	50	21	0.94 ± 0.02	2.6 ± 1.4	Carroll et al. (2020)
South Georgia	22	0.75 ± 0.06	0.77 ± 0.06	11	10	0.98 ± 0.05	2.2 ± 1.3	Carroll et al. (2020)
Falklands	82	0.76 ± 0.044	0.78 ± 0.03	82	29	0.96 ± 0.01	2.4 ± 1.2	This study

Table 5.6. *Population differentiation between the Falkland Islands and other SRW grounds in the South Atlantic. F_{ST} is shown above the diagonal and ϕ_{ST} below the diagonal, with comparisons showing significant differentiation at p <0.05 given in bold.*

Country	South Africa	Argentina	Brazil	South Georgia	Falkland Islands
South Africa		**0.048**	**0.034**	**0.025**	**0.038**
Argentina	**0.081**		**0.024**	0.002	0.004
Brazil	**0.022**	**0.029**		0.004	**0.0214**
South Georgia	**0.085**	0.009	0.017		0.000
Falkland Islands	**0.044**	0.001	0.009	0.004	

5.3.3 Nuclear genotype analysis

5.3.3.1 Sei whale

Fifteen microsatellite loci were successfully amplified and profiled for sei whales. Combined with previous Falkland Islands data, this constituted a dataset of *n* = 26 animals. All individuals in this dataset were identified as unique with no duplicates. Allelic genotype calls differed for two loci out of 36 comparisons, a per-locus error rate of 5.6%. The dataset was identified as showing significant deviation from Hardy Weinberg equilibrium (HWE, $p = 0.016$), with GATA28 and EV37 rejecting it at $p<0.05$. GATA98 also showed an instance of null alleles at a frequency >0.05. After removal of GATA98, GATA28 and EV37, the dataset (now 12 loci) no longer showed significant deviation from HWE ($p = 0.96$). After Bonferroni correction, none of the remaining loci showed significant linkage disequilibrium.

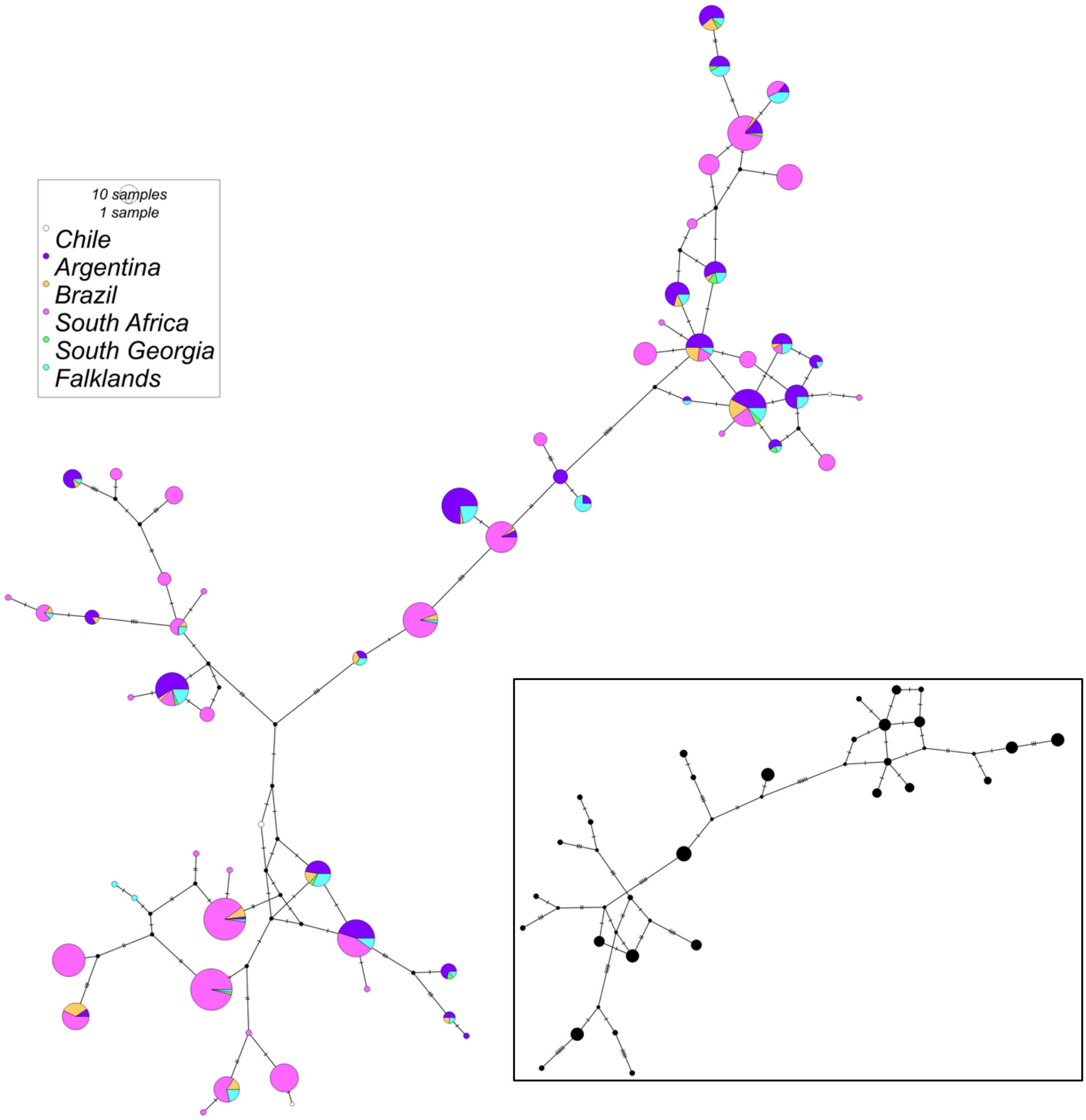

Figure 5.2. *Median joining mtDNA haplotype network, showing the distribution pattern of southern right whale haplotypes from the Falkland Islands (blue) compared with haplotype data from other South Atlantic aggregations. Inset shows the network for Falkland Island haplotypes only.*

Nuclear diversity measurements estimated the number of alleles at 7.917 ± 1.44, with effective number of alleles estimated at 4.69 ± 0.98. Observed heterozygosity was 0.66 ± 0.08 while expected heterozygosity was 0.64 ± 0.08. This estimate cannot be quantitatively compared with the Northern Hemisphere diversity measures estimated by Huijser et al. (2018), since the sets of microsatellite loci amplified for each region are not identical. However, the overall estimated heterozygosity was slightly higher than that estimated from the North Atlantic and substantially higher than estimates from the North Pacific, but not outside the upper confidence bounds of those estimates.

5.3.3.2 Southern right whale

Sixteen microsatellites were successfully profiled for southern right whales; however, CA232 was only successfully profiled for the 2020 samples, so it was excluded from the subsequent population analyses. Four individuals were identified by CERVUS as being sampled more than once. Duplicated samples were: (1) DW10 and DW12; (2) DW17, 18, 19 and 20; (3) DW24 and DW25; (4) DW41, 42 and 43; (5) DW53, 55 and 58; (6) DW67 and 78; (7) DW70 and 83; and (8) DW88 and 90. Additionally, sample DW08 could not be identified by microsatellite genotyping and was removed from subsequent analysis.

This meant that there were 82 unique animals identified out of the 95 samples collected in the field. No microsatellites were identified as being out of Hardy Weinberg equilibrium. Significant linkage disequilibrium was identified between two pairs of alleles: EV1 and TR3F4, and RW18 and TR3G2. These alleles were retained for analysis of diversity, to maintain comparability with previous studies which used the same loci. Two microsatellites were identified as having levels of null alleles above 0.05 (GT23 and TR3G1) and so were removed from the subsequent genetic diversity analysis (conducted on 13 loci).

Levels of nuclear heterozygosity and diversity in the Falklands were most similar to those of SRWs using Brazilian and Argentine calving grounds (Table 5.5), consistent with the hypothesis that the Falkland Islands is seasonally used by whales from both areas. Comparison of n = 82 microsatellite profiles from the Falkland Islands with those obtained from other South Atlantic grounds revealed no matches at 8 loci or more, i.e. no recaptures of individuals previously profiled in other areas.

5.4 Discussion

Southern right whales and sei whales have contrasting exploitation histories in the South Atlantic, with the SRW hunted over 350 years (since the earliest 17th century, ~10–12 generations: de Morais et al., 2017), while the sei whale was hunted intensively using modern methods over ~60 years in the 20th century (2–3 generations). Long periods of exploitation, leading to prolonged, small population sizes, are anticipated to leave a signature on the genome, reducing neutral genetic diversity (Frankham, 2005). However, despite the substantially longer exploitation history of the southern right whale compared to the sei whale, we estimated that they have higher contemporary genetic diversity in the Falklands, both in the nuclear and mitochondrial genome. SRW diversity levels in the Falkland Islands are consistent with high levels of genetic diversity previously reported in other wintering and feeding grounds in the South Atlantic (Carroll et al., 2019, 2020). One possible reason for the diversity difference between the two species could be that sei whales historically (since the Last Glacial Maximum >10,000 years ago) had lower population sizes in the South Atlantic than southern right whales, leading to lower long-term genetic diversity. A second, additional explanation could be that high genetic diversity of SRWs was retained by the long-term fragmentation of their populations.

SRWs are known to have strong migratory fidelity to calving grounds (Carroll et al., 2014), and there is also evidence that they have fidelity to feeding grounds (Valenzuela et al., 2009). During the long period that SRW were exploited, it is possible that there was significant population fragmentation and subsequently genetic drift within small population units (demes) across their South Atlantic range (for example, perhaps fragmented remnant populations survived across their calving ground range in Brazil and Argentina), retaining distinct maternal lineages at local scales. Now that SRW are recovering back into their historical range (Crespo et al., 2019), these demes are overlapping again and descendants of multiple demes are being seen in communal wintering areas such as the Falkland Islands.

Our comparison of sei whale genetic differentiation between the Falklands and a Pacific population in Chile (Pérez-Álvarez et al., 2021) shows that both have similar levels of maternally inherited diversity and low inter-population differentiation. The humpback whale (*Megaptera novaeangliae*) is the only other baleen whale species for which comprehensive mtDNA genetic comparisons have been made between the eastern Pacific and western Atlantic (Cypriano-Souza et al., 2017), and in that case the two populations were more strongly differentiated (e.g. higher F_{ST} values) than seen for the sei whale, suggesting that the Atlantic-Pacific boundary might be less of a barrier for sei whales. However, this is a hypothetical inference, as differences in population size and demographic history between the two species will also influence measured F_{ST}. Comparison of microsatellite profiles between the two regions will help to better establish recent migration rates between the two areas.

For southern right whales, there is strong evidence of maternally inherited substructure even within the South Atlantic Ocean (Carroll et al., 2020), with SRWs from South Africa genetically differentiated from whales wintering in the south-west Atlantic (Brazil and Argentina), and even some evidence of subtle differentiation between those localities. Population differentiation measures showed that the Falkland Islands has the closest affinity to the two south-west Atlantic wintering grounds off Brazil and Argentina, and to the South Georgia feeding area, and is significantly differentiated from South Africa. However, F_{ST} metrics identified marginally significant differentiation between the Falkland Islands and both Brazil and Argentina, suggesting that SRWs in the Falkland Islands might represent a mixture of animals from the two locations.

Sex identification of both species shows that more samples (biopsy and strandings) were collected from males than females, with females representing 30% of the dataset for sei whales (n = 10) and 20.7% of the dataset for SRW (n = 82). There were n = 18 sei whales previously biopsied or stranded in the Falkland Islands in 2017–2018; sex identification of those yielded 55.6% females, suggesting that the sex skew seen in the current study may be the artefact of a small sample size rather than having a biological basis. For southern right whales, the low ratio of females to males may either reflect a preponderance of males at the site, or differential behaviour by the two sexes in relation to the survey boat, such that males are more accessible for biopsy. Genetic surveys of another SRW wintering ground in the sub-Antarctic Auckland islands provided a sex ratio which was not significantly different from parity (Carroll et al., 2011b), but the Auckland Islands is known to be primarily a calving ground (Patenaude and Baker, 2001) while the Falkland Islands is thought to be more of a breeding and socialising ground (Weir and Stanworth, 2020; Weir, 2021; **Chapter 2**). Right whale breeding behaviour has been best studied in the North Atlantic. During breeding, surface active groups composed predominantly of males are seen, with only one or two focal females present (Kraus and Hatch, 2001). Humpback whales also show a sex ratio skew on their winter breeding grounds, with males predominating (Brown et al., 1995). These patterns may reflect there being more males present, males having a longer residency time (so being more available for sampling), or different behaviour patterns by the two sexes, meaning that males are more likely to be sampled. Overall, they add further support to the observations by Weir and Stanworth (2020) that the Falklands may represent a breeding rather than calving area for SRWs.

Sei whales have relatively high genetic diversity in the Falkland Islands and show low genetic differentiation from the South Pacific population in Chile, which means that there may be regular movements of individuals between the two areas. The Falklands' whales may therefore be part of a broader metapopulation that feeds in both Pacific and Atlantic waters. Further collaborative studies between the two regions, for example comparison of photo-identifications and of microsatellite profiles, would be helpful to further investigate these connections. Satellite tracking of sei whale movements in the Falkland Islands would also help to illuminate the extent of their feeding range.

Our genetic results confirm that SRWs in the Falkland Islands are part of the south-west Atlantic breeding population, with no genetic differentiation found between Falklands' whales and calving grounds in Brazil or Argentina, or with the South Georgia feeding ground known to be associated with those breeding areas. Falklands' whales were also significantly genetically differentiated from the South African wintering ground, consistent with previous work showing the same pattern for other south-west Atlantic grounds (Carroll et al., 2020).

5.5 Acknowledgements

All of the samples used in these analyses were acquired as part of DPLUS082. Most were collected during biopsy sampling during small boat surveys – please see **Chapter 2** for Acknowledgements for boat work. We are grateful to landowners within the Falkland Islands for reporting stranded animals and assisting with their sampling. Grateful thanks to Falkland Islands Customs and Immigration, and to Mike Dunn at BAS, for help with CITES export/import permits, and to various BAS personnel for assistance with transporting samples. Thanks to Debbie Steel of Oregon State University for providing genotype calls for southern right whales.

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Chapter 6: Suction cup tagging

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6.1 Introduction and aims

Recent advances in bio-logging technology have revolutionised our understanding of the ecology, physiology, and behaviour of baleen whales (Johnson and Tyack, 2003; Goldbogen et al., 2017). Suction cup attached bio-logging tags are non-invasive (i.e. do not penetrate the animal's skin), and contain diverse sensor suites that provide a wealth of information about what whales are doing when they are subsurface and cannot be directly observed. Bio-logging data can be used for many purposes including to reconstruct geospatial movements (Ware et al., 2006), estimate energetic expenditure (Goldbogen et al., 2011), and understand group dynamics and behaviour (Woodward and Winn, 2006; Simon et al., 2009; Bejder et al., 2019).

Due to their elusive nature, poorly understood distribution patterns, and preference for habitats that are difficult to work in, there have been few bio-logging studies on sei whales. This study aimed to deploy suction cup attached, multi-sensor bio-loggers on sei whales to collect information on three aspects of their ecology and behaviour:

1. *Sei whale movements around the Falklands.* At large scales, a combination of GPS sensors and dead-reckoning can be used to determine the geographic locations of the whales throughout the course of the tag deployment (Wensveen et al., 2015). At smaller scales, depth sensors and accelerometers can be used to reconstruct three-dimensional underwater trajectories, to better understand how sei whales forage and swim through their underwater habitat (Ware et al., 2006);
2. *Foraging behaviour.* Sei whales are unique among baleen whale species in their ability to switch between intermittent lunge-feeding (similar to humpback whales, *Megaptera novaeangliae*) and continuous skim-feeding behaviours (similar to right whales, *Eubalaena* spp.). These two foraging techniques likely require different swimming strategies, physiological structures, and filtration mechanisms (Ingebrigtsen, 1929; Brodie, 2001; Horwood, 2009). However, little is known about the mechanics of how sei whales approach and engulf their prey during either lunge-feeding or skim-feeding events; and
3. *Sei whale calling patterns.* Accelerometers within bio-loggers can detect calls from the tagged whale, and integrated hydrophones can detect calls from both the tagged individual and other nearby whales (Goldbogen et al., 2014). This approach provides information on how often, and in what context, sei whales emit vocalisations, which is useful in interpreting some aspects of the static Passive Acoustic Monitoring (PAM) work being carried out in the Falklands (see **Chapter 7**).

This chapter provides a summary overview of work that has already been published as a detailed open access scientific manuscript, primarily addressing sei whale foraging behaviour:

- Segre, P.S., Weir, C.R., Stanworth, A., Cartwright, S., Friedlaender, A.S. and Goldbogen, J.A. (2021). Biomechanically distinct filter-feeding behaviors distinguish sei whales as a functional intermediate and ecologically flexible species. *Journal of Experimental Biology*, 224(9): jeb238873. https://journals.biologists.com/jeb/article/224/9/jeb238873/263907/Biomechanically-distinct-filter-feeding-behaviors

Additionally, the sei whale suction cup data have been included in a wider publication on the manoeuvring performance in baleen whales that was accepted for publication on 17 January 2022:

> Segre, P.S., Gough, W.T., Roualdes, E.A., Cade, D.E., Czapanskiy, M.F., Fahlbusch, J., Kahane-Rapport, S.R., Oestreich, W.K., Bejder, L., Bierlich, K.C., Burrows, J.A., Calambokidis, J., Chenoweth, E.M., di Clemente, J., Durban, J.W., Fearnbach, H., Fish, F.E., Friedlaender, A.S., Hegelund, P., Johnston, D.W., Nowacek, D.P., Oudejans, M.G., Penry, G.S., Potvin, J., Simon, M., Stanworth, A., Straley, J.M., Szabo, A., Videsen, S.K.A., Visser, F., Weir, C.R., Wiley, D.N. and Goldbogen, J.A. Scaling of maneuvering performance in baleen whales: larger whales outperform expectations. In Press, *Journal of Experimental Biology*.

6.2 Materials and methods

6.2.1 Tag deployment and recovery

A tagging study was carried out over a three-week period in March 2019, during which attempts were made to deploy suction-cup bio-loggers on sei whales in the Berkeley Sound region when weather conditions were favourable and whale behaviour permitted. During the tagging efforts, whales were approached at slow speeds using a 6.5 m long rigid-hulled inflatable boat and, when an animal surfaced within several metres of the boat, an experienced tagging operative (PS) attempted to deploy a tag on its dorsal surface. The tag was located at the end of a 6 m long flexible carbon fibre pole, which the tagging operative manoeuvred into position above the surfacing animal and then dropped onto its back (Figure 6.1).

Figure 6.1. *Suction-cup attached bio-logging tags were deployed on surface-feeding sei whales using a long carbon-fibre pole.*

The tags (Customized Animal Tracking Solutions) were equipped with a suit of sensors, including three-axis accelerometers, three-axis magnetometers, three-axis gyroscopes, pressure and temperature sensors, GPS, video cameras, and hydrophones. The whales swim speed was estimated by calibrating the background accelerometer vibrations with the orientation-corrected depth rate, calculated from the pressure sensors during steeper dives (Cade et al., 2018). Following successful deployments, aerial footage taken with a quadcopter (DJI Phantom 4A), was used to estimate the body dimensions of the tagged whales.

After the suction-cups detached, the tags floated to the sea surface. The geographic coordinates of the floating tags were accessed via a satellite link, and the fine-scale location was determined using VHF telemetry; the tags were then recovered by boat in order to retrieve the recorded data.

6.2.2 Data analysis

Data from the tag accelerometers and magnetometers were used to calculate the pitch, roll, and heading of the whales (Johnson and Tyack, 2003). The body orientation was then combined with the speed estimates and the GPS locations to generate a dead-reckoned track of the whales' positions (Ware et al., 2006).

Feeding events, including surface lunges, subsurface lunges, and skim feeding were identified by searching for characteristic kinematic signatures in depth, speed, and orientation data (Goldbogen et al., 2006; Segre et al., 2021). Surface lunges were mainly identified by 90° rolls that occurred at the sea surface. Subsurface lunges were characterised by rapid accelerations and rapid decelerations that occurred during the approach and engulfment phases. Skim feeding events were indicated by extended bouts at the surface with an upward pitch, suggesting that the mouth was being held above water. Further details on the identification of feeding events can be found in Segre et al. (2021). The timing of the vocalisations, feeding behaviours, and events captured on video were integrated with the trajectories and depth records of the whales.

Spectrograms of the audio from the hydrophones were visually inspected for whale vocalisations using Raven Pro (v1.6). Spectrograms were also created from the accelerometer data in Matlab (Goldbogen et al., 2014) and then visually inspected using Raven Pro.

6.3 Results

The tagging operative was present in the Falklands between 9 and 30 March 2019, during which there were six days of favourable weather suitable for small boat work. Over that period, tags were deployed on two adult sei whales (Figure 6.2). Both individuals were tagged on the 22 March, in separate sei whale groups that were foraging near the entrance to Berkeley Sound. At the time of tagging, the whales were surface feeding on amphipods (*Themisto gaudichaudii*, visible in the tag video and observed from the tagging boat) using a combination of slow lunges and skims (Figure 6.3).

6.3.1 Deployment 1

The first whale was estimated at 16.6 m long, and was feeding in a group with two other individuals when the tag was deployed. The deployment occurred at 15:18 (all times are in local time, UTC-3hr) while the whale was skim feeding to the north-east of Cochon Island at the mouth of Berkeley Sound (Figure 6.4a), after which the whale spent 22 min feeding at the surface using a combination of surface-lunges and skim feeding.

The whale then spent 76 min travelling north-eastwards to a new location further offshore (Figure 6.4a), where it subsequently spent 46 min foraging at the surface. It then travelled south for 70 min, with some intermittent lunge feeding and exploratory dives. At 18:57 the whale spent another 7 min surface-feeding. Shortly after the sunset at 19:00, the animal changed pattern and began subsurface feeding using kinematically variable, upward lunges (Figure 6.4c). It is possible that at this time the whale had switched to feeding on lobster krill (*Munida gregaria*, visible in the tag video).

At 20:29, following 85 min of subsurface feeding, the whale began travelling north along the coast until the tag detached at 03:18 the following morning. Over the course of the 12-hr deployment, the whale performed 67 surface-lunges, 30 skim feeding events, and 120 subsurface lunges.

The average and maximum swim speed and dive depths for the whale are provided in Table 6.1.

Figure 6.2. *Suction-cup bio-logging tag deployed on a sei whale in Berkeley Sound.*

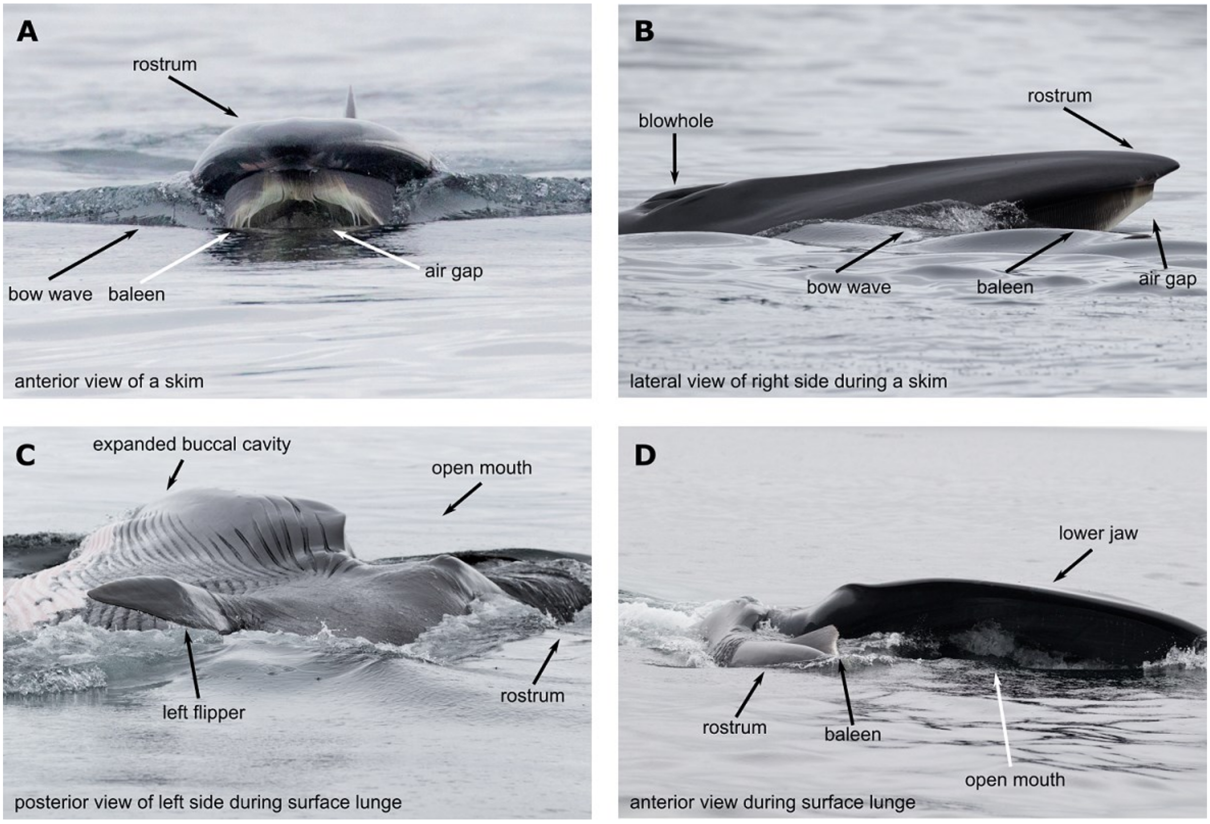

Figure 6.3. *Sei whales skim feeding (A, B) and lunge feeding (C, D) in the Falkland Islands (from Segre et al., 2021).*

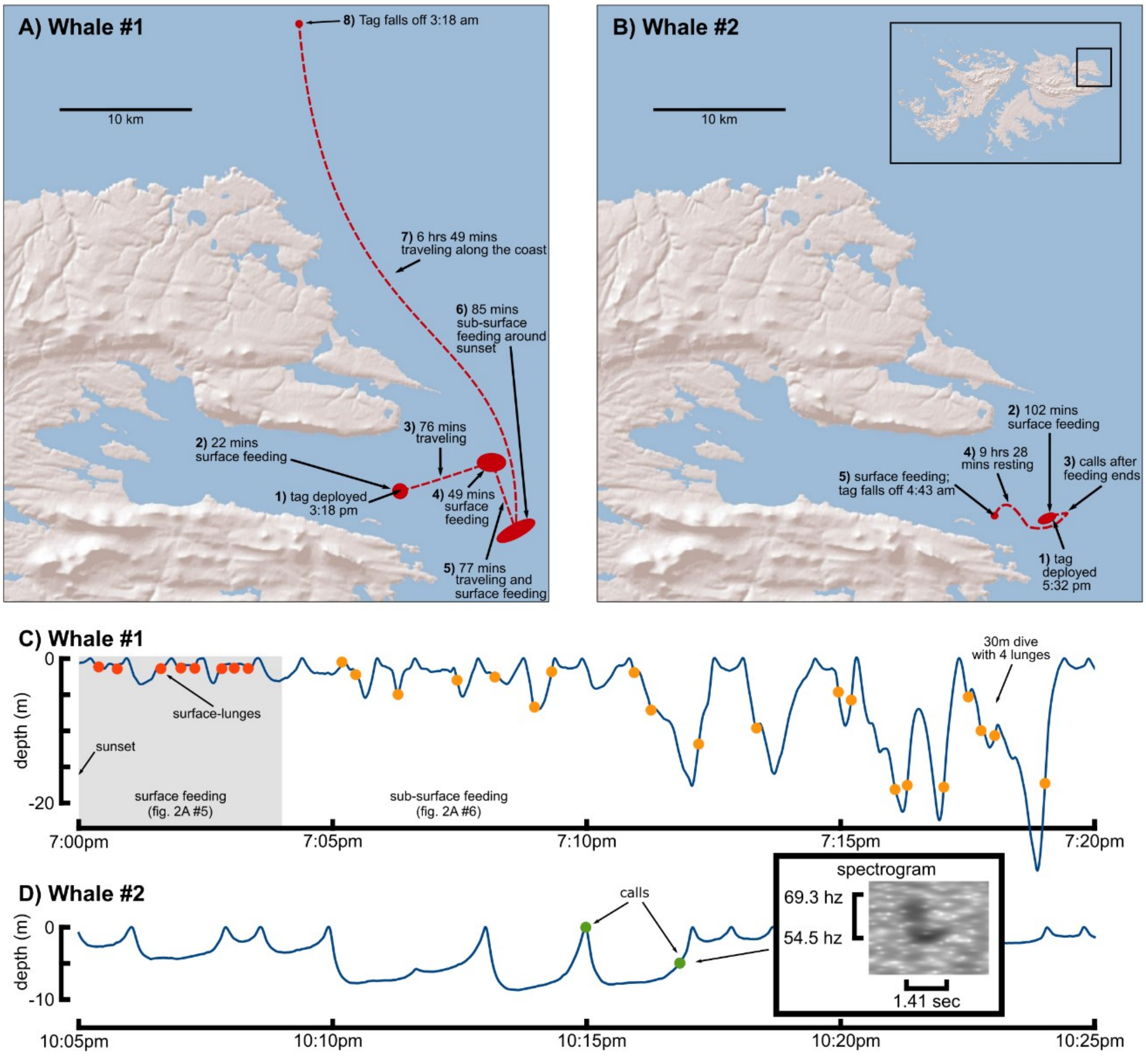

Figure 6.4. *Spatial tracks and foraging bout data for two sei whales tagged in the Falklands (A, B), dive data for Whale 1 (C), and dive and call data for Whale 2 (D). Whale 1 abruptly transitioned from surface lunges (red dots in the grey shaded area) to subsurface lunges (orange dots in unshaded area) shortly after sunset (C). After it stopped feeding, Whale 2 began calling and produced a total of 83 calls over the course of the night. The first two calls are shown as green dots (D).*

Table 6.1. *Swim speed and dive depths of two tagged whales during different behavioural states. Average and maximum values are provided. Whale 2 did not exhibit any subsurface foraging.*

Whale	Surface foraging		Subsurface foraging		Travel	
	Swim speed (m/s)	Depth (m)	Swim speed (m/s)	Depth (m)	Swim speed (m/s)	Depth (m)
Whale 1	2.0 / 5.1	1 / 7	2.3 / 5.1	7 / 29	2.2 / 7.3	3 / 40
Whale 2	1.0 / 3.3	1 / 13	–	–	0.7 / 4.4	4 / 44

6.3.2 Deployment 2

The second whale was estimated at 15.7 m long. The deployment occurred at 17:32, while the animal was skim feeding in a group with four other whales to the north-east of Mengeary Point at the mouth of Berkeley Sound (Figure 6.4b). Following tagging, the whale continued to skim feed and surface-lunge for 102 min. At 19:14 it stopped foraging, and embarked on a slow westwards movement further into Berkeley Sound over a 9 hr period, during which it was presumed to be resting. Early in the morning (04:42) it began to surface-lunge; however, the tag fell off the animal shortly afterwards at 04:43.

Over the course of the 11 hr deployment, the whale performed 115 surface-lunges and 40 skim feeding events. The average and maximum swim speed and dive depths for the whale are provided in Table 6.1.

At 22:15, after the animal had ceased feeding and moved into rest and slow travel behaviour, it emitted two calls with frequency ranges between 52 and 70 Hz (Figure 6.4d). The calls were 1.34 and 1.41 sec in duration and occurred at a 110 sec interval. The calls were recorded in both the accelerometer data and on the hydrophones, suggesting that they were produced by the tagged individual rather than a nearby whale. After the two calls, the whale began a slightly more active phase of travelling. Over the course of the night, the whale emitted a total of 83 calls as it was moving slowly and presumably resting.

6.4 Discussion

Although recent advances in bio-logging technology have vastly improved our knowledge of the underwater behaviours of baleen whales, sei whales have remained a relatively poorly understood species. This study successfully deployed high-resolution, multi-sensor bio-loggers on two sei whales, and the resulting data greatly increase understanding of sei whale behaviour and movements, particularly with regard to their foraging ecology.

6.4.1 Sei whale movements

Both of the tagged sei whales alternated between periods of intense foraging and periods of travelling. The first whale had three long foraging bouts each separated by several kilometres of linear travel. The second whale had two foraging bouts separated by a long overnight period of slow travel and rest. Both whales achieved faster maximum speeds while travelling, although their average swim speeds were similar when foraging and travelling. The average dive depths were shallowest when the animals were surface-feeding, slightly deeper during travel, and greatest during subsurface foraging.

The results indicate that sei whales may remain in relatively small spatial areas for prolonged periods when prey availability is suitable to support repeated foraging bouts. Further, the linear movements exhibited by the two animals were very different, with one whale spending over 9 hr resting and moving slowly over a short distance, while the other whale travelled northwards. The latter whale covered a total linear distance of over 45 km in just under 7 hr of sustained travel, suggesting that it made a distinct decision to move away from the earlier foraging area.

The two sei whales were tagged approximately two hours apart on the same day, with the same prevailing conditions and prey availability. However, the very different movement patterns exhibited by the animals over the hours following tagging indicates high variability in the behaviour and habitat use of sei whales. Additional tag deployments would be needed in order to better understand the factors driving sei whale occurrence in the Falkland Islands. Furthermore, the long-distance movements suggest that longer duration tag deployments would be needed in order to understand how sei whales move around the Falklands. Given the remoteness of the Islands and lack of vessel support in most areas outside of Stanley, achieving longer-term data on sei whale movements would likely require the use of more invasive tag types that transmit for longer and where data could be downloaded via satellite rather than being dependent on tag recovery.

6.4.2 Whale calls

The 83 calls recorded from the second whale after foraging represents the first time that calls have been attributed to an individual sei whale with concurrent behavioural data available. The acoustic data from the tags warrant a full analysis and detailed description, to determine the types of call being emitted and provide interpretation of their context. This analysis will be facilitated by the description of sei whale vocalisation types from the two-year static PAM deployment in Berkeley Sound (see **Chapter 7**), and will potentially provide a unique perspective of the call rates and types emitted by individual whales.

6.4.3 Foraging behaviour

Both of the tagged sei whales had very high foraging rates, compared to the data available for other baleen whale species. Not including periods of travel or rest, the two animals fed at rates of 56 and 91 feeding events per hour respectively. In comparison with similar species, the slightly larger fin whale (*Balaenoptera physalus*) foraged at depth at ~30 lunges per hour (Friedlaender et al., 2020). Minke whales are considered to have much higher foraging rate than most other baleen whale species, but the rates recorded for one of the tagged sei whales was comparable to the 102 lunges/hour recorded for Antarctic minke whales (*Balaenoptera bonaerensis*; Friedlaender et al., 2014). These high foraging rates were likely due to the ease of prey capture and the low volume of buccal cavity inflation associated with surface-lunges and skims, which allowed for a rapid filtration time.

A previous study of tagged sei whales suggested that they did not routinely undertake deep foraging dives to the deep scattering layer at night, but could not preclude the possibility that they foraged at night near the surface (Ishii et al., 2017). Both individuals that were tagged in the Falklands were surface feeding when first encountered, and continued to forage after dark: the first whale foraged for 89 min after sunset (which was at ~19:00), while the second whale foraged for 14 min after sunset and then began to forage again 138 min before sunrise (which was at ~07:00). The first whale switched foraging modes shortly after sunset, transitioning from slow and highly stereotyped surface lunges to faster, highly variable, and more energetically expensive subsurface lunges. By swimming under the prey patches and lunging upwards, the whale may have been taking advantage of the full moon to silhouette its prey against the surface of the water. However, the thick cloud cover on that evening may have limited the brightness of the moon. Overall, this data provides valuable information on how sei whales forage in the coastal waters of the Falkland Islands; however, more deployments are needed to see if these diurnal patterns hold at different times of year and in different conditions.

It should be noted that the types of prolonged surface feeding exhibited by sei whales during the two tagging events on 22 March 2019, are not commonly observed in the Falklands. Active surface feeding has been recorded on only a handful of dates over five seasons of survey work on sei whales in the Falkland Islands since 2017 (C. Weir, pers. obs.), and such observations have been limited to the late March/early April period, often in overcast conditions, and usually when the sea state is especially calm. During the vast majority of surveys (which by their nature are limited to daylight hours), sei whales in the coastal waters around the Falkland Islands have been observed to be foraging subsurface and on longer dives of up to 13 min duration (Weir et al., 2018; **Chapter 2**). Consequently, the results of the tag deployments on 22 March may reflect less usual conditions, and it remains unclear how representative those data are of typical sei whale foraging behaviour around the Islands.

Sei whales are unique among baleen whales in their ability to switch between intermittent lunge-feeding and continuous skim feeding behaviours (Ingebrigtsen, 1929; Brodie and Vikingsson, 2009; Horwood, 2009). However, little is known about the mechanics of sei whale predation and how it differs from the strategies used by related species. By deploying multi-sensor bio-loggers, we found that the sei whales used three biomechanically distinct behaviours to catch their prey (Segre et al., 2021):

1. *Surface-lunges*: these were slow (average peak speed of 2.2 m s^{-1}) and featured consistent 90° rightward rolls. The mouth opened as the whales began to roll and they reached maximum gape halfway through the roll. Unlike other baleen whale species, the sei whales powered their way through the lunges with their mouths open. Generally, whales avoid this because of the high

drag involved in swimming with an open mouth (Cade et al., 2016), but the slow speeds of the sei whale surface-lunges likely mitigated this effect. Surface-lunges also featured relatively low levels of buccal cavity inflation, smaller volumes of engulfed water, and lower filtration times (average of 12.5 s), than were predicted from anatomical studies (Kahane-Rapport and Goldbogen, 2018; Kahane-Rapport et al., 2020).

2. *Skim feeding*: skimming involved the whales extending their rostrums above the water and swimming at extremely slow speeds (average of 1.4 m s^{-1}) with their mouths open, for several seconds (average of 12.6 s). The buccal cavities did not appear to inflate much and the filtration times were short (average of 2.9 s), suggesting that filtration occurred as the whales were swimming. Normally, lunge-feeding rorqual whales filter water after engulfing their prey and closing their mouths. The low filtration times for both surface-lunges and skims allowed the whales to quickly begin their next feeding manoeuvre, and the high foraging rates likely made up for the low volumes engulfed.
3. *Subsurface lunges*: these lunges occurred at faster speeds (average peak speed of 2.9 m s^{-1}) and with more variable body orientations, in a style similar to the lunges used by other species of rorqual. The first tagged whale used these types of lunges when foraging below the surface, after sunset. Subsurface lunges are likely more energetically costly than surface-feeding behaviours, but they are probably important for catching prey that is not trapped near to the surface. This indicates that sei whale have the ability to lunge at faster speeds, but do not need to do so when they are targeting less mobile prey (Segre et al., 2021).

In combination, the results suggest that sei whale surface-lunges and skim feeding events are biomechanically different and involve different filtration mechanisms. When used together they allow the whales to forage at high rates with low energetic costs. Sei whales can also perform lunges that are more similar to those of other whales. Compared to other rorqual species, sei whales are versatile predators that can use different techniques to approach, engulf, and filter prey (Segre et al., 2021).

6.4.4 Conclusions

Despite only achieving two successful tag deployments, the study provided valuable knowledge of the foraging behaviour and movements of sei whales in the coastal waters around the Falkland Islands. The data have several implications for sei whale conservation and management in the Islands, for example:

- Data on movements can inform potential overlap with, and exposure of, sei whales to human activities;
- The information on foraging ecology provides new insights on the prey species being exploited, and prey capture methods being used, by sei whales in the Falklands, that will improve understanding of how whale occurrence is affected by prey distribution and availability;
- The data on swim and dive behaviour suggest that surface-feeding sei whales might be particularly vulnerable to vessel collisions given the slow swim speeds and shallow dive depths that prevail during that behaviour;
- Tag sensors may reveal species-specific behaviours that can put sei whales at elevated risk for entanglements or ship-strikes. Increasing our understanding of behavioural patterns can help to craft mitigation strategies tailored to the conservation of sei whales; and
- Baleen whales have finely tuned energetic budgets. Their foraging behaviours are energetically expensive but highly efficient, when prey patches are dense. For this reason, whales will often travel long distances in a fasting state while searching for their ephemeral prey. Understanding how sei whales use foraging grounds in the Falkland Islands to balance their daily and yearly energetic budgets is critical to creating effective conservation strategies.

Additional tag deployments would be desirable to increase sample size and reach more meaningful conclusions with regard to sei whale conservation management in the Falklands.

6.5 Acknowledgements

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Chapter 7: Passive Acoustic Monitoring

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7.1 Introduction and aims

The Falkland Islands have recently been well established as important habitat for both Southern Hemisphere sei whales (*Balaenoptera borealis*) and southern right whales (*Eubalaena australis*: Weir, 2017; Baines and Weir, 2020; Weir and Stanworth, 2020; Weir et al., 2020, 2021). Sei whales have been documented in the Falklands seasonally during the austral summer and autumn from November to June, and the region is considered to comprise coastal feeding habitat (Weir, 2017; Baines and Weir, 2020). Historical records of southern right whales indicated that they occur throughout the year in the offshore waters around the Falklands, with peaks during the austral summer months; however, more recent work during 2017, 2019 and 2020 supported seasonal peaks in nearshore waters during the austral winter (Weir and Stanworth, 2020; Weir, 2021). Therefore, it was suggested that the Falkland Islands may fulfil multiple habitat needs for southern right whales, including offshore feeding, winter mating, and migratory passage/stop over (Weir and Stanworth, 2020; Weir, 2021). Although boat survey work has provided good information on baleen whale occurrence in the Falklands (see **Chapter 2**), it has been limited to: (1) daylight hours; (2) days of favourable weather; and (3) the core periods of expected whale occurrence for cost-effectiveness. Consequently, the occurrence of whale species at other times of year, at night, and in the many days where the weather is too adverse for boat work, remains unclear.

Long-term Passive Acoustic Monitoring (PAM) is a widely used technique to describe patterns of spatio-temporal variation in the occurrence of vocalising cetaceans. When conducted over multiple years, it has the potential to provide information on seasonal and migratory patterns of occurrence. It can also inform about geographic variation in the occurrence of target species when used at appropriate spatial scales (i.e. across sites). PAM using stationary archival recorders (i.e. recording over long periods while moored at a static site) has distinct advantages over other types of acoustic recording approaches, such as recording from boats with ‘dipping hydrophones’ or towed hydrophone arrays. Archival recorder PAM allows relatively low-cost monitoring of a population for continuous uninterrupted periods (at the scale of months or years), without many of the constraints imposed by weather, field survey logistics, and budget, that exist for boat-based monitoring approaches. In comparison to towed hydrophone arrays, the collected data do not have interference, or ‘masking’, associated with engine noise from the boat towing the hydrophone. However, notable limitations of PAM include the ability to detect and attribute recorded vocalisations to a particular species (in the absence of simultaneous visual observations that occur with boat-based recording methods), and challenges associated with processing the large amounts of data that are collected when devices record continuously in real-time over weeks or months. Additionally, PAM only detects animals when they are actively vocalising; if animals are present at a site but not vocalising, then the method can yield ‘false negatives,’ and consequently, PAM does not provide robust absence data.

With previous knowledge of species-specific vocal behaviour, and application of automated analysis techniques, long-term PAM has proven effective for documenting spatio-temporal trends for both sei whales and right whale species (Davis et al., 2017, 2020; Romagosa et al., 2020). Low frequency (LF: <500 Hz) calls have been attributed to sei whales in several parts of the world (Rankin and Barlow, 2007; Baumgartner et al., 2008; Calderan et al., 2014; Espanol-Jimenez et al., 2019; Tremblay et al., 2019). Tonal downsweeps in the ~100–40 Hz range were first identified as a characteristic vocal behaviour attributed to sei whales in the central North Pacific (off Hawaii: Rankin and Barlow, 2007) and in the western North Atlantic (off Cape Cod: Baumgartner et al., 2008), and subsequently described in other geographic regions, including off Chile (Espanol-Jimenez et al., 2019) and the Azores

(Romagosa et al., 2015, 2020). Repetitive downsweeps (i.e. those that occur in patterns of doublets, triplets or more, with intervals of approximately 3.5 s) have been reported by several of those studies and have been used as a means to distinguish sei whale downsweeps from the similar LF calls produced by other Balaenopterids (e.g. Davis et al., 2020). Additional tonal LF calls have also been reported for sei whales including upsweeps (35–75 Hz range) and "upsweep-downsweep" calls (50–90 Hz range) in the Southern Ocean (Calderan et al., 2014), and lower frequency downsweeps (50–20 Hz range) in the North Pacific and North Atlantic (Rankin and Barlow, 2007; Tremblay et al., 2019). Some authors have also described mid-frequency (MF) vocalisations in the 1.5–3.5 kHz range for North Atlantic sei whales (Thompson et al., 1979; Knowlton et al., 1991), and the 200–1000 Hz range for Southern Hemisphere sei whales (McDonald et al., 2005). These MF sounds have been described as a "*burst of…metallic pulses*" by Thompson et al. (1979), as broadband "*frequency modulated sweeps*" by Knowlton et al. (1991), and as "*broadband…growls or whooshes*" by McDonald et al. (2005). Knowlton et al. (1991) described the sounds as being arranged in phrases, using terminology typically referring to songs, and inferring hierarchal structure. McDonald et al. (2005) suggested that Knowlton et al. (1991) and Thompson et al. (1979) may have recorded reproductive song, distinguishing it from the lower frequency vocalisations that they had recorded for the species. Tremblay et al. (2019) also reported song-like sequences of LF vocalisations from North Atlantic sei whales, comprising a series of LF downsweeps including 82–34 Hz downsweep doublets combined with newly described 50–30 Hz triplet and singlet downsweeps. The individual groupings of downsweeps reported by Tremblay et al. (2019) were clearly stereotyped and suggestive of baleen whale song, but their occurrence and function require further evaluation.

The previously-described vocalisations of right whales include a tonal upsweep call in the 50–200 Hz frequency range, which is produced by both sexes and has been well documented in the North Atlantic, North Pacific and Southern Hemisphere (Clark, 1982; McDonald and Moore 2002; Parks and Tyack, 2005; Webster et al., 2016; Dombroski et al., 2017). The upsweep is relatively stereotyped, particularly compared to other right whale calls, making it useful for automated detection and classification (Davis et al., 2017). Other tonal calls (moans and groans), tending to be more variable and graded, have also been described for several populations of right whales (Payne and Payne, 1971; Clark, 1982; Webster et al., 2016). In addition to tonal calls, characteristic broadband impulsive signals referred to as gunshot calls have been described for North Atlantic, North Pacific and Southern Hemisphere right whales (Clark, 1982; Parks and Tyack, 2005; Parks et al., 2005; Webster et al., 2016; Crance et al., 2017). Parks et al. (2005, 2012) described gunshot calls for North Atlantic right whales, reporting them as part of male stereotyped behaviour, and occurring in stereotyped bouts. These initial studies hypothesised that the calls function as male reproductive displays, but did not describe specific repeated patterns for the display. More recently, Crance et al. (2019) reported gunshot calls for North Pacific right whales, which occurred in sequences that they considered to be a form of baleen whale song. They described four stereotyped song patterns that were repeated in sequences, and were recorded during a period of eight years at multiple locations in the Bering Sea. Furthermore, the behaviour was attributed to males and hypothesised to be a male breeding display. This was the first formal description of male song for a species of right whale.

This chapter reports on the PAM component of DPLUS082. The project originally planned to acquire two years of acoustic monitoring data at two sites: (1) Berkeley Sound on the east coast of East Falkland; and (2) Falkland Sound which extends between East Falkland and West Falkland (see Figure 1.2). However, for combined reasons including equipment failure and constraints around the COVID-19 pandemic, a full year of acoustic data was not acquired for Falkland Sound within the timeframe of the project (acoustic monitoring is currently still underway at that site). Consequently, this chapter reports on the two-year deployment at Berkeley Sound. The analysis primarily focuses on sei whales as the main target species of the acoustic work, with a more limited secondary focus on southern right whales.

The specific aims of the PAM analysis carried out for DPLUS082 were:

1. Assess the success and limitations of the PAM gear deployment methods utilised;

2. Describe the characteristic vocalisations produced by each species, as allowed by confident species attribution;
3. Assess spatial variation in whale detections between the three deployment sites;
4. Describe temporal variation in whale detections over the project duration (ideally at multiple levels of daily presence, hourly presence and call abundance); and
5. Draw conclusions on the applicability of the acoustic method for long-term monitoring of whales in the Falklands.

A scientific manuscript resulting from this work and describing the calls and song of sei whales has been accepted for publication in Royal Society Open Science:

- Cerchio, S. and Weir, C.R. Mid-frequency song and low-frequency calls of sei whales in the Falkland Islands. *Royal Society Open Science*, In Press.

7.2 Materials and methods

7.2.1 PAM gear deployment

7.2.1.1 Recording gear specifications

The PAM gear used for the Berkeley Sound deployments comprised three SoundTrap ST500-STD recorders (Ocean Instruments, New Zealand). The ST500 specifications indicated a flat response from 20–60 kHz (+/- 3 dB) with a -9 dB roll-off at 10 Hz, and a 34 dB re 1V µPa-1 noise floor. Calibration information provided by Ocean Instruments for each specific recorder indicated that hydrophone sensitivity plus system gain ranged from -175.4 to -174.4 dB re 1V µPa-1 among the three units used in Berkeley Sound, and thus the units had comparable sensitivity. Prior to each deployment, the ST500s were programmed to record continuously at 16 bits and a 24 kHz sample rate (SR), to provide a 12 kHz analysis band.

7.2.1.2 Moorings

Each mooring consisted of a weighted anchor on the seabed, from which a rope of approximately 1 m length connected to an Ascent acoustic release (INNOVASEA, Canada). An additional length of rope of approximately 2–3 m length attached the acoustic release to an 11" buoy, which had sufficient buoyancy to maintain the entire mooring in a vertical position from the seabed (and brought the ST500 and acoustic release to the surface, once the acoustic release was triggered). The ST500 was tethered to the latter rope, such that it was positioned ~2 m above the seabed.

7.2.1.3 Berkeley Sound deployment locations

The ST500s were deployed down the centre of Berkeley Sound (BS) at approximately 7 km spacing (Figure 7.1) at three sites as follows:

- Berkeley Sound Outer (BS-Outer): outermost site near to the mouth of Berkeley Sound and approximately 5 km from the north and south coasts. Water depth: 45–47 m; Latitude: -51.58; Longitude: -57.75;
- Berkeley Sound Central (BS-Central): central site within Berkeley Sound, located approximately 3 km from the north and south coasts. Water depth: 29 m; Latitude: -51.57; Longitude -57.85; and
- Berkeley Sound Inner (BS-Inner): innermost site located approximately 3 km from the north and south coasts. Water depth: 27–31 m; Latitude: -51.57; Longitude: -57.95.

7.2.2 Acoustic data analysis

7.2.2.1 Manual spectrographic review and quantitative measurements

Most previously described sei whale and southern right whale vocalisations occur primarily in the low frequency range (<500 Hz), so prior to spectrogram analysis then all files were converted to a lower sample rate to facilitate the browsing and analysis of the data with smaller sized files. The software Avisoft-SASLab Pro was used to down-sample files, using the Sampling Frequency Conversion function in batch processing mode. Since two studies have reported that sei whales may also emit vocalisations in the 1–3 kHz bandwidth (Thompson et al., 1979; Knowlton et al., 1991), a SR of 11,025 Hz was chosen, providing an analysis bandwidth of 5,512 Hz, so that mid-frequency vocalisations (500–5,000 Hz) could also be detected if present.

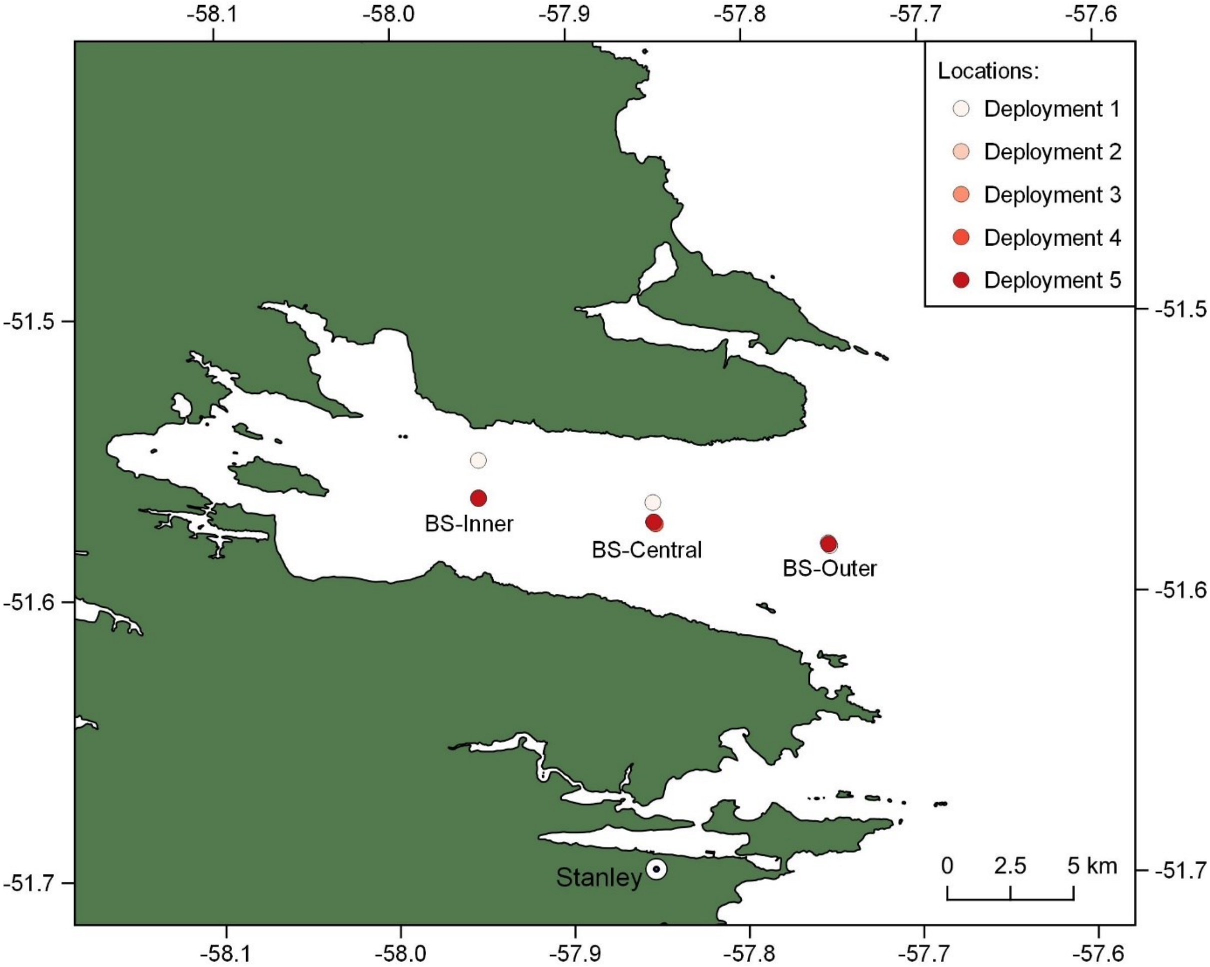

Figure 7.1. *Locations of the three acoustic deployments in Berkeley Sound: BS-Outer, BS-Central and BS-Inner. The positions of the ST500s during Deployment 1 were incorrect at BS-Outer and BS-Central, due to their being misplaced by accident. All subsequent deployments were located as planned, hence the overlap in locations for Deployments 2–5.*

An initial assessment of vocalisations was conducted on the data from Deployment 1 at BS-Central. The deployment was first analysed with the Low-Frequency Detection and Classification System (LFDCS) developed by Baumgartner and Mussoline (2011), using a library of sei whale calls from the North Atlantic (as described in detail in Section 7.2.2.2). This facilitated the detection of previously described sei whale call types (e.g. LF downsweep calls in doublets, triplets or longer series). The vocalisations identified by the LFDCS were reviewed on sound spectrograms generated in Raven Pro 1.5 (Bioacoustics Research Program, 2014). The continuous stream of 11 kHz SR acoustic files recorded during the deployment were loaded into Raven for initial spectrographic browsing using a window size and discrete Fourier transform (DFT) size of 8,192 samples, and 50% overlap respectively. Spectrograms were viewed at 250 Hz bandwidth for LF vocalisations, and 5,512 Hz bandwidth for MF vocalisations. Using the LFDCS detections of downsweeps as an indication of periods of time when sei whales were present, spectrograms of the 4.5 month period from 5 Dec 2018 to 23 April 2019 were browsed in both the LF and MF range to identify and log vocalisations. It was assumed that any LF vocalisations found in association with doublet downsweeps were produced by sei whales. Additionally, visual observations from boat surveys carried out in Berkeley Sound over the Deployment 1 period confirmed that sei whales were by far the predominant baleen whale species in the area (see **Chapter 2**). In addition to downsweeps, a variety of sei whale call types were identified and described.

The logged vocalisations were ordered by received level (using Raven's Peak Power measurement), and a subset of the highest signal-to-noise ratio (SNR) were selected for quantitative measurement. Signal measurements were made by boxing calls on Raven spectrograms using 24 kHz SR files, a window size of 8,192 samples, a DFT size of 16,384 samples, 90% overlap, and a Hann window, with a resultant temporal resolution (time grid spacing) of 34 ms and frequency resolution (frequency grid spacing) of 1.46 Hz. The measurements included duration (call end time - call begin time, s), high frequency (maximum of selection box, Hz), low frequency (minimum of selection box, Hz), slope (frequency range / duration, Hz/s), and peak frequency (frequency of peak power, Hz). The SNR (in dB) was measured using Raven's Inband Power function and the procedure recommended by the Center for Conservation Bioacoustics[3], comparing identical time/frequency-band selections of signal and background noise.

7.2.2.2 Automated detection and classification

In order to assess the spatio-temporal presence of sei whale and southern right whale vocalisations, an automated detection and classification algorithm was applied. The NOAA Northeast Fisheries Science Center (NEFSC) Passive Acoustic Group, and Woods Hole Oceanographic Institute (WHOI), have extensive experience working with the LFDCS developed by Baumgartner and Mussoline (2011). LFDCS is a contour-tracing algorithm that runs on the IDL software platform on an Apple Mac OS, and has been successfully used for detecting North Atlantic sei whales and North Atlantic right whales (*Eubalaena glacialis*) in both archival and real-time detection scenarios (Davis et al., 2017, 2020; Baumgartner et al., 2021). LFDCS first applies a protocol to condition the spectrogram by reducing tonal background noise (e.g. ship noise) and removing transient broadband signals. A contour-tracing algorithm is then applied to detect putative tonal signals, and estimate the frequency variation of the putative call over time. Attributes of the pitch track (e.g. start frequency, end frequency, duration, slope of frequency variation) are extracted, and candidate calls are classified to call type based on comparison with a user-defined call library using quadratic discriminant function analysis (Baumgartner and Mussoline, 2011). The similarity between any classified call in the dataset to a call type category in the library of exemplars is estimated using the Mahalanobis-distance (M-dist) metric, with lower values indicating greater similarity.

With any automated detection/classification approach, the goal is to maximise the actual detection of true calls or true positives (minimising missed detections), while also minimising the number of false positives (detections of noise or non-target signals that the algorithm incorrectly classifies as the targeted signal, in this case whale calls). Typically, as the criteria for acceptance of a classification become more stringent, more false positives are excluded. However, there is a simultaneous reduction in the proportion of actual calls that are detected. In the case of LFDCS, the output results of putative call classifications can be filtered by adjusting a threshold M-dist, in order to balance the ratio of missed detections to false detections to an acceptable level.

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[3] https://ravensoundsoftware.com/knowledge-base/signal-to-noise-ratio-snr

Initially, the LFDCS was run on data from the first two BS deployments, using the established NEFSC call library for sei whales, right whales, and humpback whales, each developed from whale calls in the North Atlantic (Davis et al., 2017, 2020). The resulting classifications were output to log files and converted into selection tables for import into Raven Pro 1.5. This initial LFDCS analysis was also used to identify portions of recordings for the manual review of vocalisations described in Section 7.2.2.1. The initial results indicated that the LFDCS classifier for North Atlantic sei whale downsweeps was partially effective for identifying downsweeps in the Falklands; however, adjusting the M-dist threshold to allow only a moderate amount of false detections, resulted in a high rate of missed calls. Relaxing the threshold criteria improved the detection rate, but produced high numbers of false detections. Therefore, a new call library was created specifically for Falklands' sei whales, drawing exemplar calls from the data collected in BS during the period of peak sei whale occurrence (confirmed by boat surveys: **Chapter 2**) and then manually reviewed. Two different call type categories (downsweeps and L-calls, described in the Results section) were found to be both numerous and stereotyped, and therefore were selected to create the call library. Three different subcategories of downsweep calls were incorporated to further improve the specificity of the detector. The performance of the Falklands-specific call library was quantified (as described below in this section), and was substantially more effective than the LFDCS for detecting sei whale calls (see Section 7.3.4.1).

The NEFSC call library designed for North Atlantic right whale 'up-calls', an upsweep vocalisation that is common among right whale species (Davis et al., 2017), was also applied to the Falklands dataset. As with the sei whales, the North Atlantic call library was partially effective for the detection of southern right whales in Berkeley Sound; however, the M-dist thresholds necessary for an acceptable proportion of false positives resulted in a relatively low detection rate of true calls (i.e. a majority of calls being missed by the detector). The timeframe for this analysis did not allow for the development of a new call library specific to Falklands' southern right whales, so the North Atlantic right whale call library was used, and performance quantified in order to evaluate and qualify the results.

The LFDCS was run on acoustic data from all deployments separately. In order to evaluate performance and choose an appropriate M-dist for the review of data, a subset of test days was chosen to exhaustively browse the continuous spectrogram in Raven (SR 11 kHz, FFT 4096 pts, 90% overlap, Hann window, viewing 3 spectrogram lines of 60 s duration each). During each test day, all detections were manually verified as true (an actual call) or false (noise or non-target signal), and all missed detections of the target vocalisation were logged. For sei whales, this was done for five days spread evenly throughout Deployment 1 at all three Berkeley Sound sites, resulting in 15 total days. An initial M-Dist of 4.8 was chosen as a threshold for the sei whale detector evaluation, in order to determine a lower M-dist that could be applied for determining daily presence, and estimating hourly presence and daily call counts. For southern right whales, six days were evaluated from Deployment 1 and four days from Deployment 2 (since right whales calls were relatively scarce in Deployment 1), at BS-Outer. An initial M-dist of 4.0 was chosen as a threshold for the right whale detector evaluation, because any greater threshold was found to generate a prohibitively large number of false detections. Based on these performance metrics, separate M-dist thresholds were chosen for each species for the evaluation of daily presence, along with the estimation of hourly presence and daily call abundance.

7.2.2.3 Daily presence, hourly presence and daily call abundance

Daily presence. To determine whether vocalisations of each species were present on a given day, a set of criteria were used during a manual verification process for every day of data (summarised in Table 7.1). Detections were reviewed for each day until positive confirmation of occurrence of the species vocalisation was found. Detected vocalisations were classified as either 'confident calls' or 'possible calls' (defined in Table 7.1), and positive confirmation was defined as the occurrence of at least three confident calls for the species during a day. Once three confident calls had been confirmed for a species within a given day, the remainder of detections for that day were skipped and the review process progressed to the next day. If three confident calls were not found, all detections in that day were reviewed in order to eliminate all false detections. This manual verification process ensured a 0% false detection rate for daily presence. For days that did not meet the criteria for 'positive confirmation'

(Table 7.1), but for which one or two confident calls, or at least three possible calls were found, a classification of 'possible presence' was allocated.

Daily presence is a confident indicator of temporal distribution of a species, but does not take into account the relative abundance of animals across the period of occurrence. Therefore, confirmation of daily presence on any given day does not distinguish between a single animal that vocalises several times during a single hour, or many individuals vocalising throughout a day or over multiple hours. A measure of relative vocal activity can therefore provide a better indication of the relative abundance of individuals through the period of occurrence of a species. Two metrics for relative vocal activity that provide greater resolution than daily presence and were calculated during the present study are: (1) hourly presence; and (2) daily call abundance (i.e. total number of calls per day).

Table 7.1. *Criteria used for the verification of calls to assess daily presence.*

Category	Species criteria	
	Sei whale	Southern right whale
Individual Call Classification		
Confident call	(a) A downsweep occurring in a doublet, triplet or quadruplet; or (b) A downsweep in a bout of other known sei whale call types; or (c) An L-call, considered to be diagnostic of sei whales	(a) An upsweep call detected by the LFDCS North Atlantic right whale classifier; or (b) An upsweep call not detected by LFDCS, but occurring within 3 min of a detected upsweep; or (c) A gunshot vocalisation occurring with 3 min of an upsweep detected by LFDCS
Possible call	Singular downsweeps not conforming to (a) or (b) above	Faint, poor SNR, upsweep detected by LFDCS using the right whale classifier
Daily Presence Classification		
Positive presence	Three confident calls	Three confident calls
Possible presence	(a) One to two confident calls; or (b) Three or more possible calls	(a) One to two confident calls; or (b) Three or more possible calls

Hourly presence. This parameter is an estimate of how many hours in each day contained calls. Unlike daily presence, it was not feasible to carry out an exhaustive verification of all hours of every day to ensure a 0% false detection rate for hourly presence. Therefore, hourly presence was based on unverified detections using the LFDCS (acknowledging the constraints outlined in Section 7.2.2.2) that are filtered by two confidence criteria (different M-dist thresholds) representing a conservative and relaxed acceptance of detection respectively. The confidence criteria were defined in terms of % true calls detected and % false detections expected, based upon the LFDCS performance evaluation described in Section 7.2.2.2, such that the conservative criterion minimises false detections at the expense of missing a proportion of true calls, whereas the relaxed criterion maximises true detections while keeping false detections at a moderate rate.

Daily call abundance. Since it was not feasible to manually verify all LFDCS detections in a day to eliminate all false detections, this parameter was an estimate of the total calls that were detected on each day using the LFDCS and acknowledging the constraints outlined in Section 7.2.2.2. Daily call abundance was presented at only the conservative M-dist criterion, to minimise inclusion of false detections, but therefore missing an unknown proportion of true calls.

7.3 Results

7.3.1. Berkeley Sound PAM effort

Five deployments of the three ST500s were carried out over the two-year project in Berkeley Sound, resulting in almost continuous data collection effort between 5 December 2018 and 10 December 2020 (Table 7.2). Recoveries were necessitated every 4–6 months to change batteries in the devices and download data. Some short gaps in data collection occurred over that period, due to the time required to download data and clean the units between deployments, and constraints around weather which sometimes meant that redeployments could not be carried out immediately (Table 7.2, Figure 7.2). Technical issues during the recovery of Deployment 1 resulted in an extended delay in the ST500 redeployments at BS-Outer and BS-Central resulting in a data gap of 34 days between 23 April and 27 May 2019 (Figure 7.2). The duration of individual deployments in Berkeley Sound ranged from 109 to 173 days, producing a combined overall total of 2,042.6 data days (49,015 hours of acoustic data) from the three ST500s.

***Table** 7.2. Details of the ST500 deployments in Berkeley Sound, at the Outer (BS1), Centre (BS2) and Inner (BS3) sites. Deployments are labelled as: Falkland Conservation (FC) - year of project initiation (2018) – Deployment number (D1 to D5) – site. Times are in local time (UTC-3 hr).*

Deployment	Site	Date / time of deployment	Retrieve or stop date / time	Data duration	
				Days	Hours
FC-18-D1-BS1	Outer	05/12/2018 11:29	23/04/2019 11:20	139.0	3,335
FC-18-D1-BS2	Central	05/12/2018 11:52	23/04/2019 12:18	139.0	3,336
FC-18-D1-BS3	Inner	05/12/2018 12:20	27/05/2019 10:30	172.9	4,150
FC-18-D2-BS1	Outer	27/05/2019 10:03	30/09/2019 09:58	126.0	3,023
FC-18-D2-BS2	Central	27/05/2019 09:24	30/09/2019 09:34	126.0	3,024
FC-18-D2-BS3	Inner	01/06/2019 12:06	30/09/2019 10:23	120.9	2,902
FC-18-D3-BS1	Outer	16/10/2019 10:02	21/03/2020 12:21	157.1	3,770
FC-18-D3-BS2	Central	16/10/2019 10:35	21/03/2020 12:57	157.1	3,770
FC-18-D3-BS3	Inner	16/10/2019 11:28	21/03/2020 13:31	157.1	3,770
FC-18-D4-BS1	Outer	26/03/2020 15:38	12/08/2020 10:44	138.8	3,331
FC-18-D4-BS2	Central	26/03/2020 16:12	12/08/2020 11:20	138.8	3,331
FC-18-D4-BS3	Inner	26/03/2020 17:03	12/08/2020 11:53	138.8	3,330
FC-18-D5-BS1	Outer	19/08/2020 10:39	10/12/2020 10:27	113.0	2,711
FC-18-D5-BS2	Central	23/08/2020 10:35	10/12/2020 11:01	109.0	2,616
FC-18-D5-BS3	Inner	23/08/2020 11:17	10/12/2020 11:33	109.0	2,616
Total				2042.6	49,015

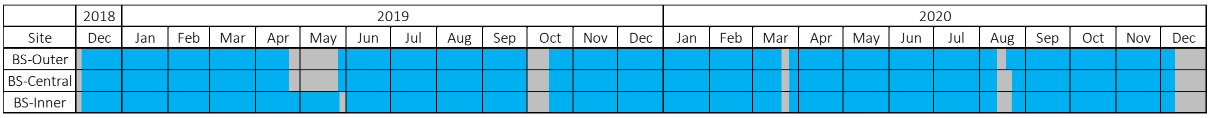

***Figure 7.2.** Temporal schematic of ST500 deployments at the three sites in Berkeley Sound (BS) between December 2018 and December 2020. Grey indicates periods of no data.*

7.3.2 Sei whale vocal behaviour description

7.3.2.1 Low frequency calls

The initial review of spectrograms from BS-Central during Deployment 1 revealed the presence of several categories of LF vocalisations that were attributed to sei whales. Species attribution was determined by: (1) the presence of known sei whale calls (downsweep doublets) among bouts of varied vocalisations; (2) the confirmed presence of numerous sei whales (and no other baleen whale species) during boat surveys in BS over this period (see **Chapter 2**); (3) the use of data relating to the peak period of sei whale occurrence in the Falklands for classification of call types (Feb/Mar: **Chapter 2**); and (4) the use of data from BS-Central, which should limit detected calls to whales within the confines of Berkeley Sound, and thus present the best comparison with the species identifications confirmed during boat surveys.

Of a total of 2,394 calls logged during the review of data, 415 individual vocalisations were isolated for measurement based upon having good SNR with a high received sound level (peak power >100 dB re 1µPa). Calls were selected across different hours and days, to optimise the likelihood of a broad sampling of different individuals and behavioural contexts. Following that process, the resulting calls originated from 148 different hours on 46 different days between 3 January and 21 April 2019. All calls used in calculating the descriptive statistics had a SNR >16 dB, with a mean SNR of 33.6 dB ±6.0 dB (range of 16.3–55.4 dB). Constraining the measured calls to recordings with a high SNR increased the likelihood of accurate time-frequency parameter measurement from the spectrogram.

The 415 calls included at least two types of downsweep (DS1 and DS2), which were somewhat graded in their time/frequency characteristics (Figure 7.3). A total of 157 downsweeps of high SNR were chosen for quantitative measurement, spread across 57 different hours on 28 days between 14 January and 6 April 2019. The bimodal distribution of call high frequency indicated a natural break at 130 Hz, and consequently this frequency was adopted as a criterion to distinguish the two downsweep types (Figure 7.4). DS1 had on average a slightly longer duration than DS2, but with broadly overlapping ranges (0.94–1.67 and 0.86–1.39 s respectively; Table 7.3). While both ended at low frequencies of ~30 Hz, their high frequencies differed from average values of 105 Hz in DS1 to 158 Hz in DS2 (Table 7.3). This resulted in a much steeper slope for DS2 (82–188 Hz/s) compared with DS1 (36–95 Hz/s). DS1 calls commonly exhibited a short initial upsweep or 'hook' (subcategory termed DS1H; Figure 7.3b), as similarly described by Ou et al. (2015) for North Atlantic sei whales. Of the 113 DS1 calls described in Table 7.3, 49 were DS1 (without a hook) and 64 were DS1H; the two sub-categories had nearly indistinguishable time-frequency characteristics. None of the measured DS2 calls exhibited a hook structure.

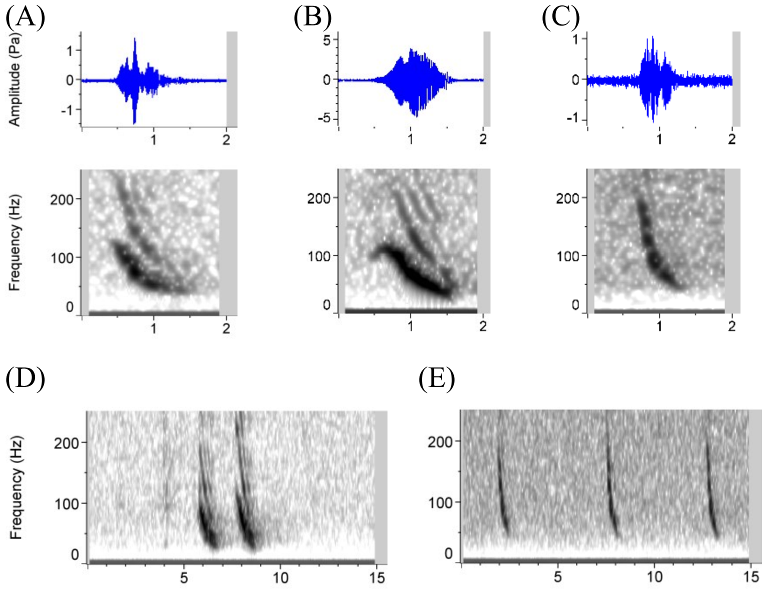

Figure 7.3. *Example downsweep calls of sei whales recorded at BS-Outer; (A) Type-1 downsweep (DS1), characterised by high frequency <130 Hz and sweep from ca. 100–30 Hz; (B) Type-1 downsweep with short initial upsweep, or "hook" (DS1H); (C) Type-2 downsweep (DS2), characterised by high frequency >130 Hz and sweep from ca. 160–30 Hz; (D) commonly occurring doublet of DS1; and (E) a series of three DS2.*

As reported for other geographic regions, DS1 at times occurred in doublets with a separation of approximately 2–4 s (Figure 7.3D), but also singly or in triplets and quadruplets (e.g. Baumgartner et al., 2008). DS2 were often seen in evenly spaced series with a separation greater than exhibited between calls in a DS1 doublet (Figure 7.3E), but at times also occurred in more typical doublets. The two downsweep categories were seen interspersed in the same vocalisation bout on several occasions (Figure 7.5A), and were also mixed with other LF call types (Figure 7.5B).

Table 7.3. *Descriptive statistics for six low frequency call types attributed to sei whales and identified in a manual review of data from BS-Central between 3 January and 21 April 2019 (measured from 415 high SNR calls). Sample size for each call type is indicated by the total number of measured calls, as well as the number of different hours and days from which they were extracted, as an indication of likely broad sampling of individuals. Reported values represent mean ±standard deviation (range). Signal to Noise Ratio (SNR) was measured for each call by comparing identical time/frequency-band selections of signal and background noise. All frequency values are in Hz.*

Parameter	Call type					
	DS Type-1	DS Type-2	L-Call	LDS-Call	Arch-Call	Upsweep
No. of calls	113	44	166	46	93	48
No. of hours, days	48, 28	23, 15	58, 26	14, 8	40, 19	22, 16
Duration (s)	1.20 ±0.15 (0.94-1.67)	1.11 ±0.13 (0.86-1.39)	1.9 ±0.3 (1.33-2.7)	1.4 ±0.1 (1.23-1.63)	1.4 ±0.3 (0.91-2.19)	2.0 ±0.3 (1.4-2.8)
Peak Frequency	57.0 ±9.8 (38-100)	61.6 ±13.5 (44-106)	60.0 ±5.6 (53-73)	52.7 ±1.9 (47-56)	66.1 ±10.8 (41-97)	54.1 ±6.8 (32-67)
High Frequency	104.6 ±10.7 (75-127)	158.4 ±23.9 (131-229)	79.4 ±3.7 (70-90)	76.6 ±2.9 (70-82)	82.8 ±11.1 (66-119)	70.6 ±2.6 (66-78)
Low Frequency	29.9 ±5.0 (20-46)	29.5 ±4.8 (22-44)	22.1 ±3.6 (16-50)	27.5 ±4.9 (20-39)	33.0 ±8.25 (19-65)	25.4 ±3.2 (20-33)
Slope (Hz/s)	63.0 ±11.6 (36-95)	117.0 ±24.7 (82-188)	n/a	n/a	n/a	22.9 ±3.1 (16-31)
SNR (dB)	31.9 ±5.7 (16.7-45.2)	29.1 ±5.7 (16.3-39.2)	35.2 ±6.1 (19.2-53.7)	33.0 ±5.6 (19.1-40.4)	35.2 ±4.9 (21.9-55.4)	28.7 ±5.8 (17.6-40.1)

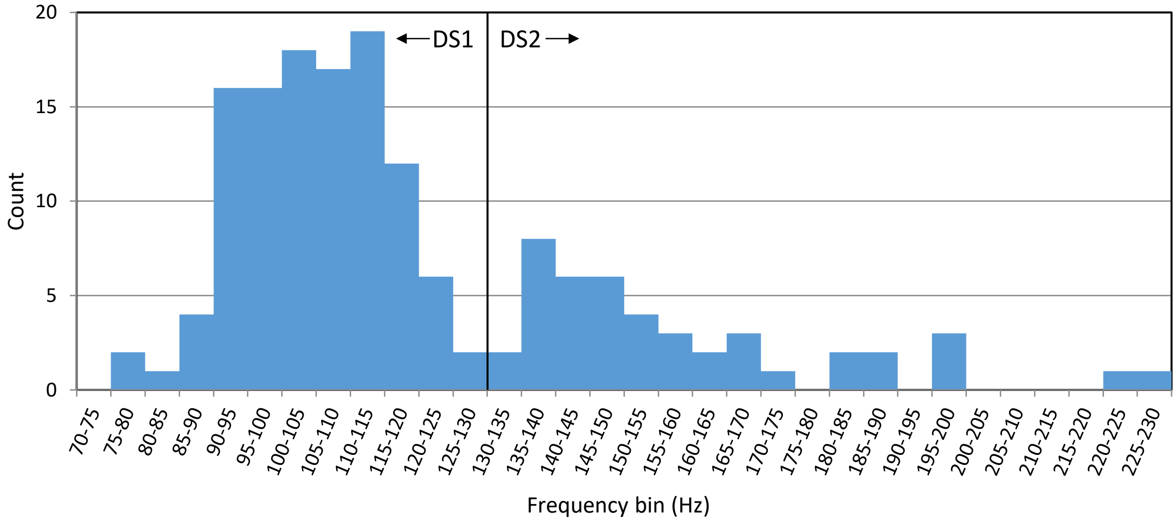

Figure 7.4*. Distribution of high frequency (Hz) measurements for 157 sei whale downsweeps, indicating a bimodal distribution and a break between Type-1 and Type-2 downsweeps (DS1, DS2, respectively) at 130 Hz.*

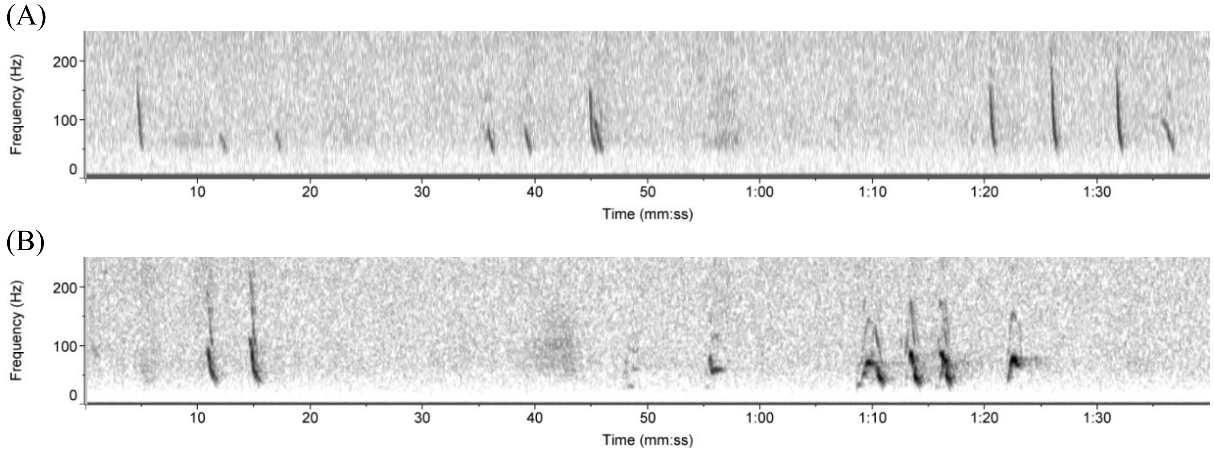

Figure 7.5. *Example sequences of mixed non-song sei whale calls; (A) sequence showing a mix of DS1 and DS2; (B) sequence showing a mix of DS1, L-call, and arch calls.*

In addition to downsweeps, other LF calls were commonly observed throughout Deployment 1, and were found in all of the months of that deployment (Dec to Apr) despite periods with few or no calls:

L-call: Named due to its most typical appearance in the spectrogram of a short rapid downsweep from ~80 Hz to 50 Hz followed by a non-modulated tone at ~60 Hz with a total duration of ~1.5 s (Figure 7.6A). High SNR examples revealed a lower amplitude initial U-shaped component between 20 and 50 Hz and ~0.5 s in duration (Figure 7.6A), resulting in a total duration of 1.9 ±0.3 s and a mean frequency range of 22.1 ±3.6 to 79.4 ±3.7 Hz (Table 7.3). In some cases, the non-modulated tone displayed a frequency oscillation over approximately 10 Hz (**L-OSC** in Figure 7.6A). L-calls were very common throughout the dataset, highly stereotyped, and often occurred in series of 3 to 8 calls (Figure 7.6E).

LDS-call: Considered to be a variation on the L-call, with a similar short rapid downsweep from ~80 to 55 Hz followed by a gradually curving downsweep to a low frequency of 27.5 ±4.9 Hz, and total duration of 1.4 ±0.1 s (Table 7.3, Figure 7.6A). LDS-calls appeared to be relatively rare in the sample, in comparison to the more common L-call.

Arch-call: Arch-calls were among the most variable of call types, ranging from 0.91–2.19 s duration in the 19–119 Hz frequency band (Table 7.3). They graded from calls that were complete arches, to those that were partial arches (Figure 7.6B). The latter primarily comprised an arched downsweep or an arched upsweep with an inflection and short hook at the end or start (Figure 7.6B,F).

Upsweep: Upsweeps were observed throughout the sample, but in lower apparent occurrence than downsweeps or L-calls. They had a duration of 2.0 ±0.3 s and occurred over frequencies sweeping from 25.4 ±3.2 to 70.6 ±2.6 Hz (Figure 7.6C, Table 7.3).

LF-Variable-call: A complex low frequency call, approximately 2 s in duration, in the 20–80 Hz band (Figure 7.6D). The call has mild amplitude modulation at the start and end, and a multi-harmonic tonal portion in the centre with a fundamental frequency at approximately 30 Hz. Due to the complex nature of the call, the acoustic features were not measured. The call was typically repeated in a series, or in a stereotyped pattern of a single LF-Variable call followed by two to six L-calls (Figure 7.6D).

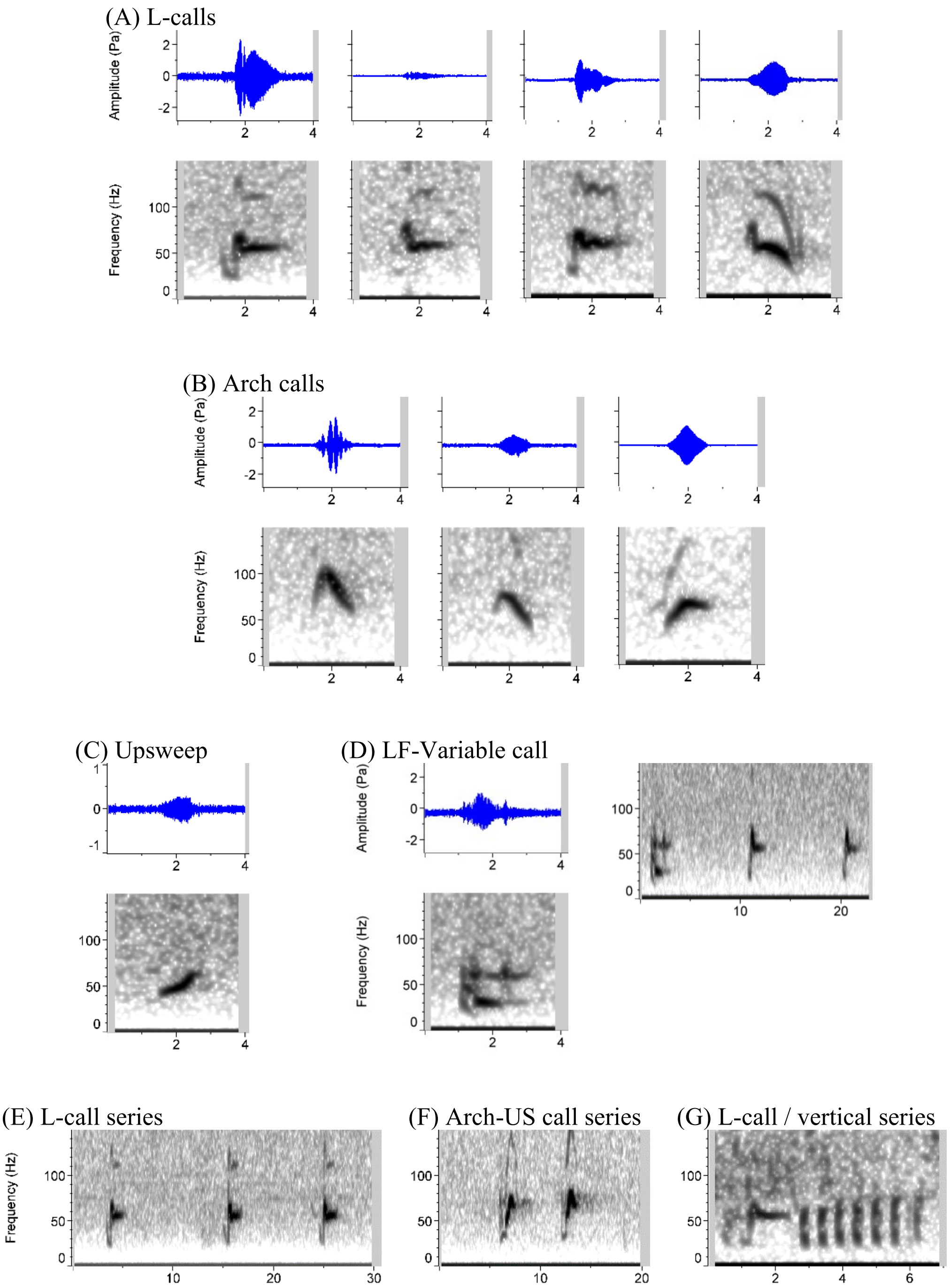

Figure 7.6. *Examples of non-downsweep low-frequency vocalisations of sei whales recorded in Berkeley Sound; (A) examples of L-calls showing a high SNR example that illustrates the introductory U-shaped low-frequency component (left), a lower SNR example illustrating the more commonly observed L-shaped character of the call (centre left), a variant with a frequency oscillation (L-OSC – centre right), and a variant with a terminal downsweep (L-DS – right); (B) three examples of arch calls, illustrating the gradations between complete arch (left), downsweep partial arch (centre), and upsweep partial arch (right); (C) an upsweep call; (D) a LF Variable-call (LF-Var - left), and a common series combining a LF-Var and two L-calls (right); (E) a series of three L-calls; (F) a series of two Upsweep Partial Arch-calls; and (G) a L-call followed by a series of grunts with a vertical spectrographic appearance.*

7.3.2.2 Mid frequency song

During the manual mid-frequency scan of spectrograms from Deployment 1, complex and hierarchically organised sequences of vocalisations were discovered that were consistent with baleen whale song (Figure 7.7). The individual sounds were a mix of broadband frequency sweeps in the 1.0-5.5 kHz bandwidth that had a 'whooshing' aural quality (Figure 7.7C, 3:04 to 3:10, and Figure 7.7D, 3:32 to 3:36) and sequences of noisy squeaks and creaks (Figure 7.7D, 3:36 to 3:46). The vocalisations that have been assessed to date were all arranged into a stereotyped phrase (Figure 7.7B), that can be subdivided into two distinct subphrases. The first subphrase (Figure 7.7C) combines broadband sounds (with peak energy in the 1–2 kHz frequency range), and low frequency L-calls as part of the stereotyped pattern. The second subphrase (Figure 7.7D) combines a broadband 'swooshing' sound (with peak energy in the 2.0–3.5 kHz range) and noisy squeaks and chirps, and was repeated multiple times in sequence (with two to five repetitions observed, but four appearing most common). There were slight variations observed between successive repetitions of the second subphrase, including the presence of L-calls and other variable and graded LF signals below 100 Hz (Figure 7.7B). The entire phrase was repeated in a series, with short but somewhat variable timing between phrases (Figure 7.7A), at times for multiple hours without pause. The application of song terminology introduced by Payne and McVay (1971) to describe humpback whale song is deliberate, due to the similarities in structure. However, unlike humpback whales, which sing multiple different phrase types in a song sequence, only a single phrase type of sei whale song was identified in the Falklands' recordings. Therefore, a sei whale song comprises the repetition of a single phrase, as defined above. Further analysis may indicate more variety and different phrase types, but currently all of the examples that were logged in BS-Central Deployment 1 conformed to the patterns described.

An assessment of hourly presence of song at BS-Central was conducted, browsing long-term spectrograms in Raven (sample rate 11 kHz, FFT 32,768 pts, 0% overlap, Hann window, viewing 3 spectrogram lines of 60 min duration each) for the entirety of Deployment 1 (Figure 7.8A). Sei whale song was not recorded during December 2018 or January 2019, and the first occurrence at BS-Central was detected on 22 February 2019[4]. The commencement of singing during February is in contrast to the detection of non-song vocalisations (downsweeps and L-calls not incorporated into song sequences), which were detected throughout December and January (Figure 7.8B,C; also detailed in Section 7.4.3). Song was recorded extensively during March and April 2019, and was still heard when the ST500 was retrieved on 23 April 2019. Concurrent with the commencement and increase in singing, there was a dramatic increase in the detections of L-calls over this period (Figure 7.8C), due to the incorporation of the L-call into song patterns. Songs were also anecdotally noted to occur at the end of May at the start of Deployment 2, although that deployment has not yet been systematically reviewed.

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[4] However, anecdotally, singing in Berkeley Sound commenced at least 1 week earlier, as it was recorded on the BS-Outer recorder as early as 14 February 2019; a complete review of the BS-Outer recorder has not yet been undertaken.

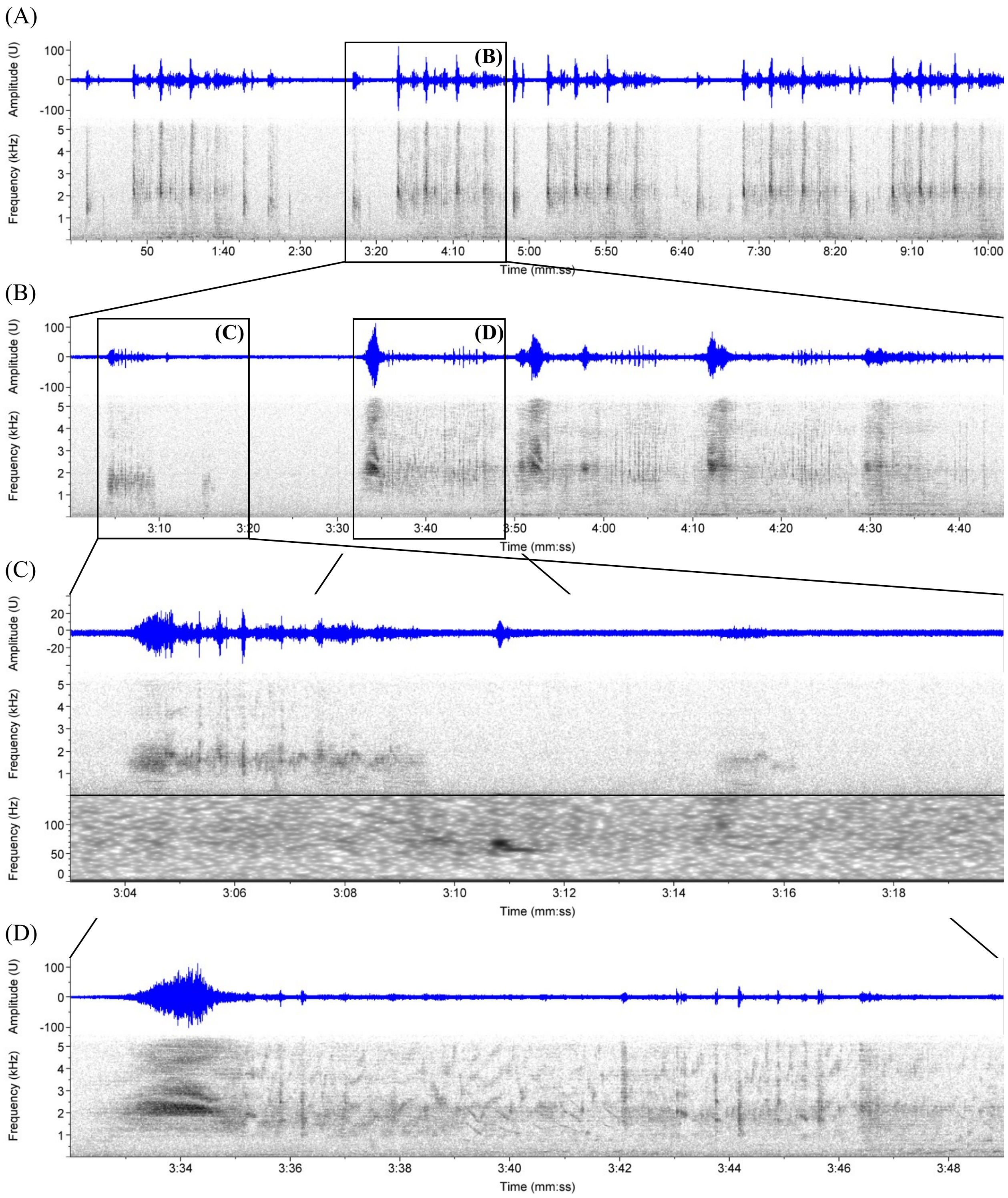


Figure 7.7. *Spectrograms (lower panels) and waveforms (top panels) of sei whale mid-frequency song, illustrating: (A) 10 min sequence of five phrases (2048pt FFT, 90% overlap); (B) 1 min 45 s sequence of a single phrase (2048pt FFT, 90% overlap); (C) 17 s sequence of subphrase 1, with split spectrogram view showing full bandwidth of 0–5.5 kHz (middle panel: 512pt FFT, 90% overlap), and low frequency bandwidth of 0–150 Hz (lower panel: 4096pt FFT, 90% overlap); and (D) 17 s sequence of subphrase 2 (512pt FFT, 90% overlap). All files at a sample rate of 11 kHz.*

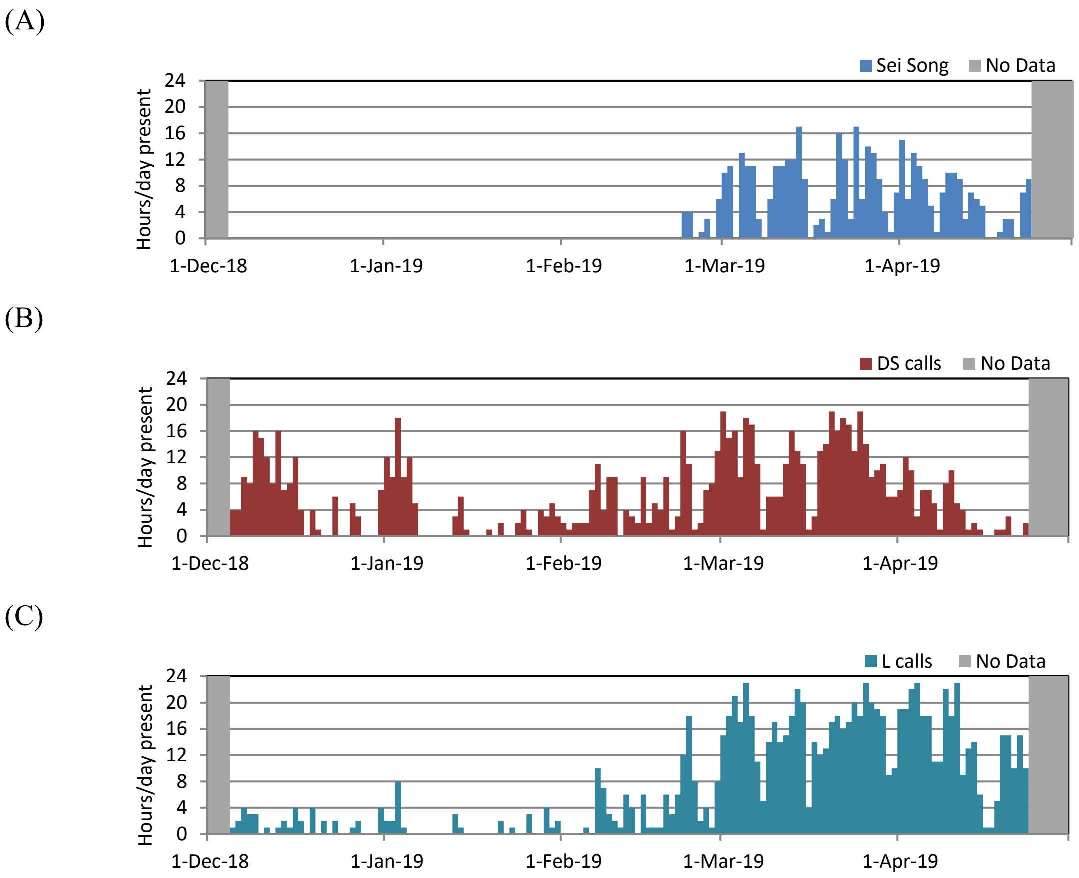


Figure 7.8*. Hourly presence of sei whale calls at BS-Central from 5 December 2018 to 23 April 2019: (A) mid-frequency song; (B) downsweeps (DS); and (C) L-calls.*

7.3.3 Southern right whale vocal behaviour description

7.3.3.1 Upsweep and gunshot calls

Right whale upsweeps were commonly recorded throughout the period when the species was sighted during boat surveys in the study area (see **Chapter 2**; Figure 7.9A). Upsweeps were a diagnostic call for right whales; there were no similar calls in the sei whale repertoire, and therefore upsweeps were a convenient call for the discrimination of right whale presence (see Section 7.3.4). Numerous other tonal calls were present when upsweeps were detected, resembling the typically more variable and graded moans and groans documented for right whales in other populations. Also common throughout the sample were broadband impulsive gunshot calls. Due to the emphasis of this analysis on sei whales, and limited allocation of analysis time, a quantitative description of southern right whale vocalisations from the Falkland Islands is not presented here; however, the similarity of the southern right whale upsweep call to the North Atlantic right whale upsweep call is implicit in the usefulness of the North Atlantic LFDCS call library for the detection of southern right whales (see Section 7.3.4).

7.3.3.2 Gunshot song

During the verification process for southern right whale upsweep call detection in Berkeley Sound (see Section 7.3.4.1), gunshot calls (Figure 7.9B) were commonly found and often occurred in stereotyped sequences consistent with song, as reported in the North Pacific. At least five different distinct stereotyped patterns were observed (see Figure 7.10, for examples of four song types). In each occurrence, the stereotyped song pattern was repeated in long sequences (see Figure 7.11 for a 30 min example sequence of one song type) that at times lasted for multiple hours. There was variable timing between songs within sequences and some variation in structure between repetitions of the song, but the stereotyped pattern of the song type was always unambiguous. Patterns of sounds within a song varied between song types, with some patterns composed solely of gunshots with varying repetition rates (Figure 7.10A), and others composed of combinations of gunshots with other signal types, such as: noisy broadband signals (Figure 7.10B); complex sounds combining gunshots with broadband signals (Figure 7.10C, Figure 7.11); or tonal signals (Figure 7.10D). Song was heard at least during the months of April, May and June, but an exhaustive analysis of occurrence was not completed in the timeframe of this report.

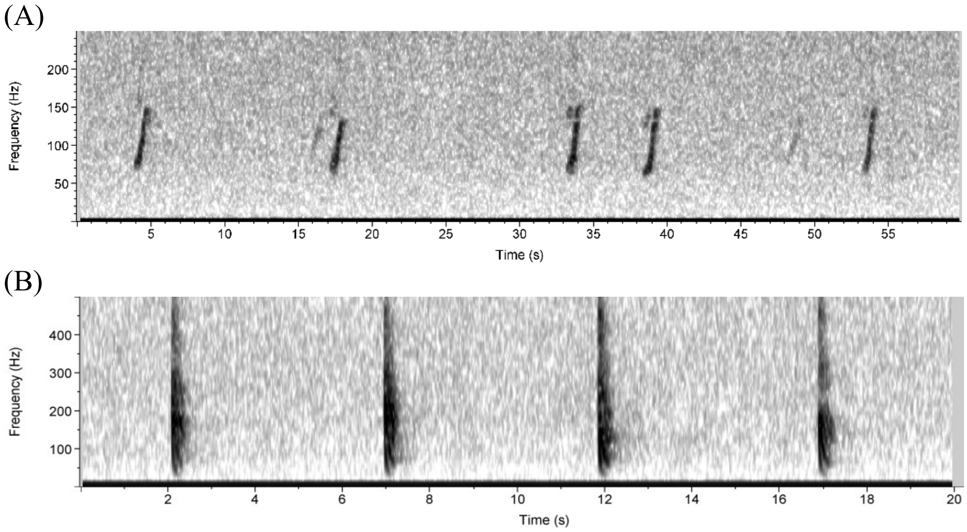


Figure 7.9. *Example southern right whale calls that were commonly recorded in Berkeley Sound: (A) a 60 s series of upsweep calls; and (B) a 20 s series of gunshot calls.*

7.3.4 Spatio-temporal distribution of sei whales and southern right whales

7.3.4.1 LFDCS performance evaluation

The LFDCS results from four sei whale call type categories (DS1, DS1H, DS2 and L-calls) were evaluated together from the 15 days of data that were exhaustively reviewed manually (see Section 7.2.2.2), resulting in 2,962 detections at the test M-dist threshold of 4.8, in a dataset that had 2,954 actual calls (Figure 7.12). In the complete dataset, 76% of the 2,954 actual calls were correctly classified and 24% were missed, whereas 24% of the 2,962 detections were false detections and 76% were correct (note: the similarity of these proportions is coincidental and not linked). As expected, when decreasing M-dist, the missed call rate increased, while the false detection rate decreased, such that at a M-dist threshold of 4.0, 64% of actual calls were detected with an 11% false detection rate, and at a M-dist threshold of 3.0, 40% of actual calls were detected with a 4% false detection rate.

For the assessment of sei whale daily presence, data were manually reviewed at the relaxed criterion of M-dist = 4.0; the data were manually verified for daily presence so that all false positives (days without calls) were removed. The detection rate of 64% was considered sufficiently high to accurately determine the presence of vocalising sei whales on a daily basis, based on the results in the test dataset. Assessing detection at the whole day level on the test days, at an M-dist = 4.0 the detector accurately confirmed sei whale presence in 12 of 15 test days, with the number of detected calls ranging from 1 (of 1 actual call during the day) to 354 (of 551 actual calls during the day). On the other three days there were no actual calls present and number of false detections ranged from 12 to 54. Therefore, in this test set, the 64% detection rate was adequate to ensure that no days with sei whale vocalisations were missed (0% missed detection rate), although one day would be considered "possible" presence because only a single confident call was detected and verified.

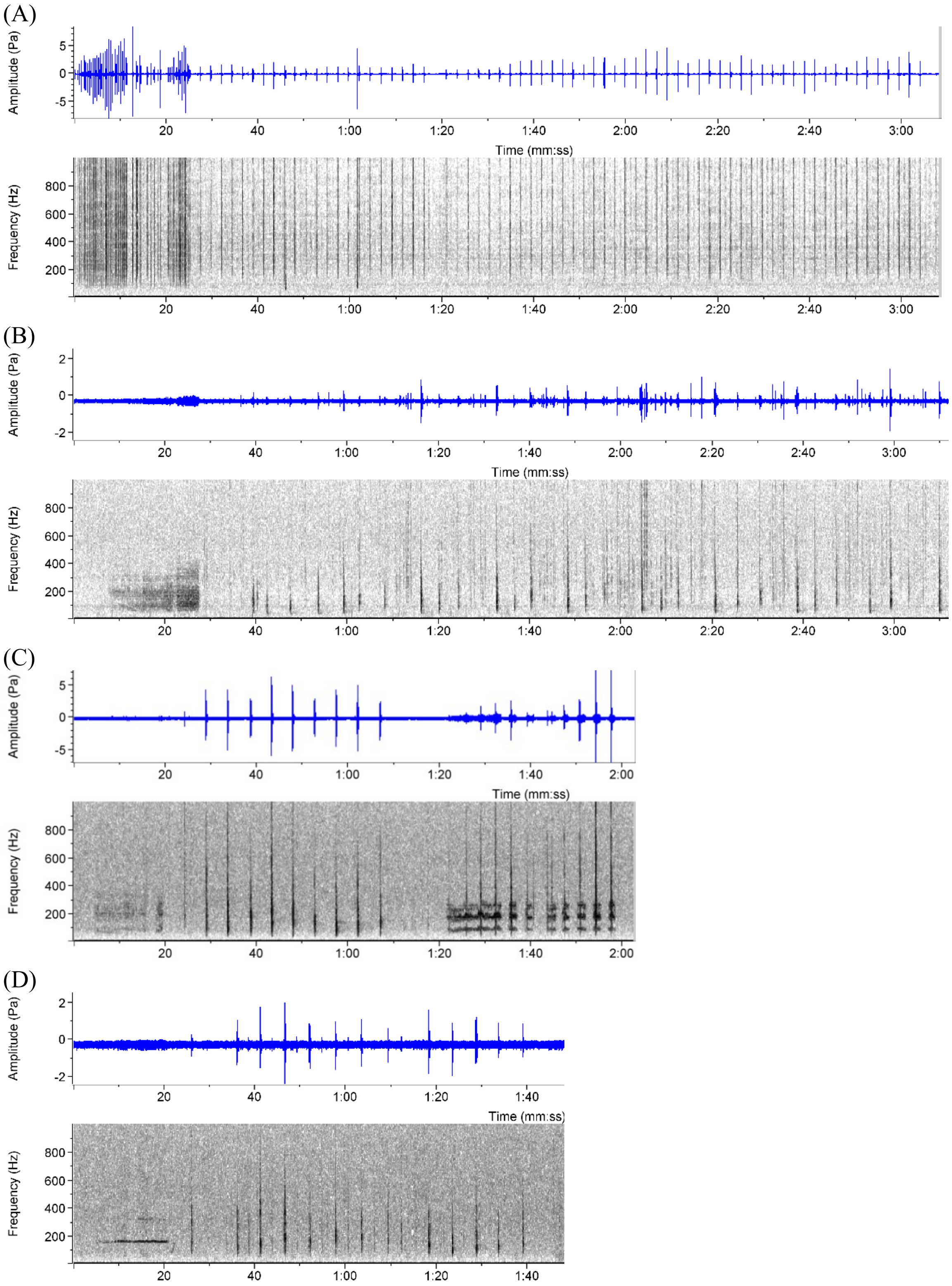


Figure 7.10. *Four examples of distinct stereotyped southern right whale gunshot songs, including spectrograms (lower) and waveforms (upper). Song types included: (A) songs composed solely of gunshots with varying repetition rates; (B) songs with an introductory noisy broadband signal (0:05 to 0:25) followed by a gunshot pattern; (C) songs composed of gunshots and complex sounds combining impulsive and noisy broadband signals (1:20 to 2:00); and (D) songs with an introductory tonal signal (0:05 to 0:22) followed by a gunshot pattern. For each of these song types, the illustrated pattern was repeated in a sequence as illustrated in Figure 7.11. Time and frequency scales are standardised for all songs.*

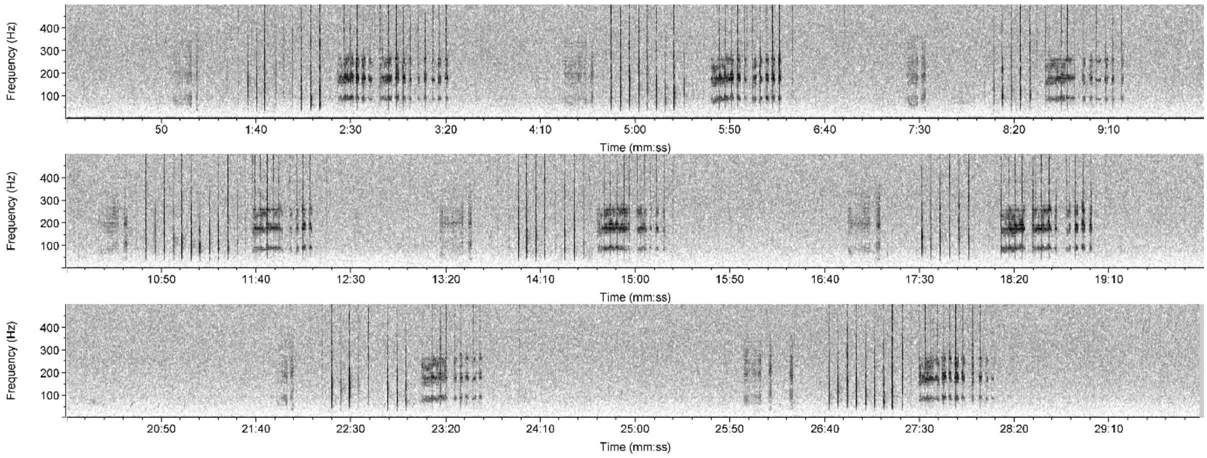


***Figure 7.11**. A 30 min section of a southern right whale gunshot song, illustrating a sequence of the song type from Figure 7.10C. Variable timing between songs and some variation in song structure is evident, but the stereotyped pattern of the song type remains unambiguous.*

	Mahalanobis distance		
	4.8	4.0	3.0
Total Detections	2962	2133	1227
Total Actual Calls	2954	2954	2954
Total True Positives	2248	1893	1184
Total False Positives	714	240	43
Total of Actual Missed	706	1061	1770
Percent True Positives	76%	89%	96%
Percent False Positives	24%	11%	4%
Percent of Actual Detected	76%	64%	40%
Percent of Actual Missed	24%	36%	60%

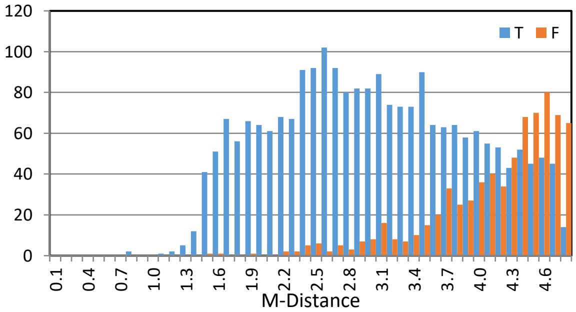


***Figure 7.12**. Performance of the LFDSC for the detection of sei whale vocalisations in the Falkland Islands dataset, evaluated with a test dataset of 15 days of exhaustively browsed data. The table shows the totals and percentages of Detections, Actual Calls, True Positives, False Positives, and Missed Calls (False Negatives) at three M-dist threshold values. The histogram shows the distribution of true positives (T) and false positives (F) across the range of M-dist values from 0 to 4.8.*

For the estimation of the hourly presence of sei whales, both a relaxed criterion of M-dist = 4.0 and a conservative criterion of M-dist = 3.0 are reported, with the assumption that the relaxed criterion retains the majority of calls (and therefore hours), whereas the conservative criterion has a lower false detection rate but may have missed presence in some hours. Assessing detection at the hourly level on the 11 test days for which sei whale vocalisations were confirmed (264 one-hour periods), at a M-dist threshold of 4.0 the true detection rate was 95% of hours, and false detection rate 13% of hours; at a M-dist threshold of 3.0 the true detection rate was 87% of hours and false detection rate 5% of hours.

For daily call abundance, only the conservative criterion is reported, to present the most confident data on actual call counts on a given day, recognising that it is a systematic underestimate. It was noted that comparison of relaxed and conservative criteria applied to daily call abundance did not obviously affect temporal patterns of call distribution, but rather only magnitude of absolute abundance.

The performance of the North Atlantic right whale upsweep call detector was evaluated on the first two deployments, choosing six days from Deployment 1 and four days from Deployment 2 at BS-Outer. In both cases, efficacy was lower than for the sei whale call detector, with a greater overlap of true and false detection distributions across M-dist values. There was also substantially different performance between the two deployments, because during Deployment 1, the upsweep detector was falsely triggered by sei whale song, leading to a higher false detection rate. At an M-dist threshold of 3.0, during Deployment 1 35% of actual calls were detected but with a high 60% false detection rate; this improved during Deployment 2 (when there were few sei whales but many southern right whales) to 35% of actual calls detected and only 9% false detections.

For the assessment of right whale daily presence, data were reviewed at a M-dist threshold of 3.0. Again, all data were manually verified for daily presence so that all false positives were removed, and the detection rate of 35% was considered adequate to provide a conservative assessment of right whale presence on a daily basis. Assessing detection at the whole day level on the test days, at a M-dist threshold of 3.0, the detector accurately confirmed southern right whale presence in 9 of 10 test days, with number of detected calls ranging from 9 (of 10 actual calls during the day) to 755 (of 1,839 actual calls during the day). In the remaining 1 day, there were no actual calls present and the number of false detections was 150. Therefore, in this small test set, the 35% detection rate was adequate to ensure that no days with right whale vocalisations were missed (0% missed detection rate). Based on this evaluation, the detection rate of 35% was likely high enough to accurately determine southern right whale presence on a daily basis during periods when many vocalisations were present (e.g. throughout Deployment 2); however, it is possible that some days may have been missed if few vocalising whales were present (e.g. during Deployment 1). In a more extensive evaluation of 10 years of North Atlantic right whale data on the USA eastern seaboard, Davis et al. (2017) estimated that they missed 31% of days of presence with a M-dist threshold of 3.0; their median number of actual calls on missed days was 7 (range 1–66). The same detector appeared to perform better on our small test set, but this is counter intuitive so we expect a larger subset of test days would reveal a non-zero missed day rate.

For estimation of southern right whale hourly presence, both a relaxed criterion of a M-dist threshold of 3.0 and conservative criterion of a M-dist threshold of 2.0 are reported, under the caveat that the conservative criteria, while having a reduced false detection rate (35% in Deployment 1, 5% in Deployment 2), also had a low detection rate (7% of actual calls). Therefore, M-dist of 2.0 is preferable for Deployment 1, whereas M-dist of 3.0 is preferable for Deployment 2. Similarly, for the estimation of daily call abundance, only the conservative criterion is reported, to reduce the probability of false detections.

7.3.4.2 Daily presence assessment

The LFDCS was run on the total 2,043 days of data from the three Berkeley Sound sites across the two years of monitoring. For sei whales, the LFDCS results were verified for daily presence for all days (5 December 2018 to 10 December 2020) at all sites, encompassing two complete summer/autumn seasons of sei whale occurrence. The results indicate that vocalising sei whales were present for the most days (53%) at BS-Outer, and their presence decreased into Berkeley Sound to be lowest at BS-Inner (Table 7.4).

Table 7.4. *Daily presence assessment, for total complete days (TCD) for which a full 24 hours of data were available (excluding deployment and retrieval days). Results are given for both days with positive confirmation of presence ('Present') and possible presence ('Possible'), referring to definitions in Table 7.2.*

Site	Sei whale			Southern right whale		
	TCD	Present	Possible	TCD	Present	Possible
BS-Outer	669	352 (53%)	101 (15%)	419	192 (46%)	11 (3%)
BS-Central	665	269 (40%)	38 (6%)	419	134 (32%)	12 (3%)
BS-Inner	694	126 (18%)	13 (2%)	448	103 (23%)	10 (2%)

For southern right whales, due to limited analysis time, the LFDCS results were verified for daily presence for only the first three deployments (5 December 2018 to 21 March 21 2020; 1,295 days of data) at all sites, encompassing one complete winter season of southern right whale occurrence. The results indicated that, similar to sei whales, the presence of vocalising southern right whales was highest at BS-Outer (46% of days), and decreased moving further into Berkeley Sound (Table 7.4).

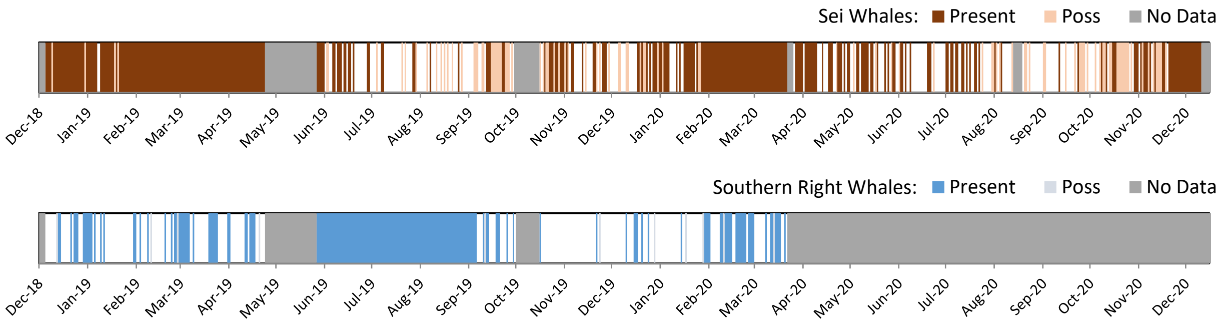
Daily presence results have been presented in two formats: (1) displaying both species together across the entire sampling period to compare the seasonal occurrence of the two species (Figure 7.13); and (2) displaying each species separately in increments of one year to compare variation across years and sites for each species separately (Figures 7.14 and 7.15). The comparison of daily presence revealed marked asynchronous seasonal occurrence of the two species (Figure 7.13), with vocalising sei whales present primarily during the austral summer/autumn months of December to May, and vocalising southern right whales present primarily during the austral winter months of June to August. Hereafter in this chapter these are considered the 'core' seasons for these species, although some inter-annual variation in these core seasons is recognised. Aside from consistent daily presence during these peak months, each species was also intermittently detected outside of the core periods. There was also marked variation between the number of detections across the three sites within Berkeley Sound, with detections being most numerous at BS-Outer and least numerous at BS-Inner (Table 7.4, Figure 7.13). This trend was much more prominent for sei whales, with only one third of the amount of daily detections recorded at BS-Inner compared with BS-Outer (18% vs. 53% of days), as compared to right whales with about one half the number of daily detections at BS-Inner compared with BS-Outer (24% vs. 46% of days) (Table 7.4).

When aligning the plots of daily presence by month, variation in the presence of vocalising sei whales between seasons and between sites is evident (Figure 7.14). At the start of monitoring in early December 2018 (during the 2018/2019 season), vocalising sei whales were consistently present on a daily basis at BS-Outer and BS-Central; this continued until at least late April 2019 when the recorders were recovered. Sei whales were still consistently present for several days in late May 2019 after the delayed redeployment. In contrast, during the 2019/2020 season, vocalising sei whales were not consistently present until mid-January/early February 2020, and their presence became intermittent by early April, inferring a markedly shorter period of occurrence with a season truncated at both ends. The 2020/2021 season appeared to be starting out more similarly to the 2018/2019 season, with consistent presence of vocalising sei whales from mid- to late-November 2020.

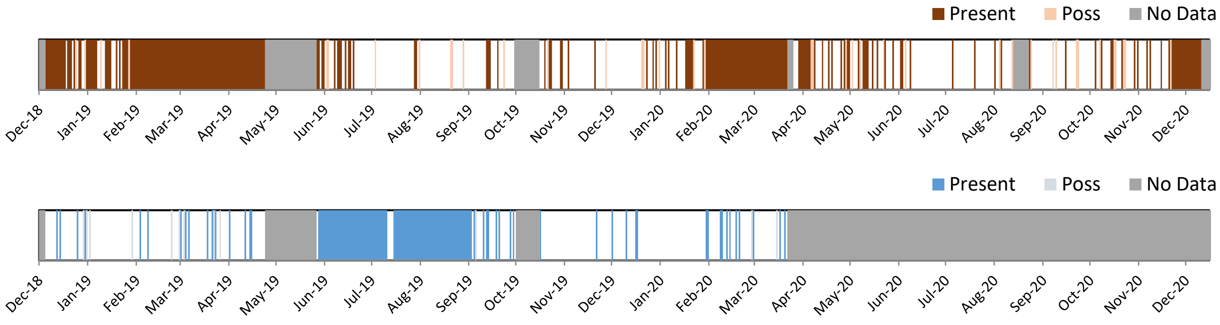
When considering seasonal variation in vocalising sei whales between the sites, the most dramatic difference was evidenced at BS-Inner (Figure 7.14C), which had a markedly shorter period of consistent presence in both seasons than at either BS-Outer or BS-Central (Figure 7.14A,B). Furthermore, there were few sei whale acoustic detections at BS-Inner outside of the core season between the months of May 2019 to February 2020 and April to December 2020. In contrast, acoustic detections outside of the core sei whale season were most common at BS-Outer between June and December 2019, and again between May and November 2020.

It is not possible to assess inter-annual differences in the seasonal occurrence of vocalising southern right whales, since the 2020 data have not yet been fully analysed. Consequently, only the 2019 season is presented in its entirety (Figure 7.15). The core season with consistent daily presence of vocalising right whales appeared to comprise at least late May through to early September during 2019; however, the delayed deployment of D2 at BS-Outer and BS-Central (see Table 7.1) resulted in an absence of data for May 2021 and prohibits a clear assessment of when right whale presence became daily at those sites. Presence of vocalising right whales at BS-Inner was intermittent throughout May; however, the generally lower overall detection rates at BS-Inner limit confidence in projecting this pattern to BS-Outer and BS-Central. With regard to the presence of vocalising animals outside of the core right whale season, there was a greater tendency for intermittent presence prior to the core season from mid-December 2018 to April 2019, and from mid-December 2019 to March 2020, than immediately after the core season between September and December. The tendency for lower occurrence at BS-Inner was also evident, but less pronounced than for sei whales, and the difference was primarily seen during the December to April pre-core season period in both years.

(A) BS-Outer



(B) BS-Central



(C) BS-Inner

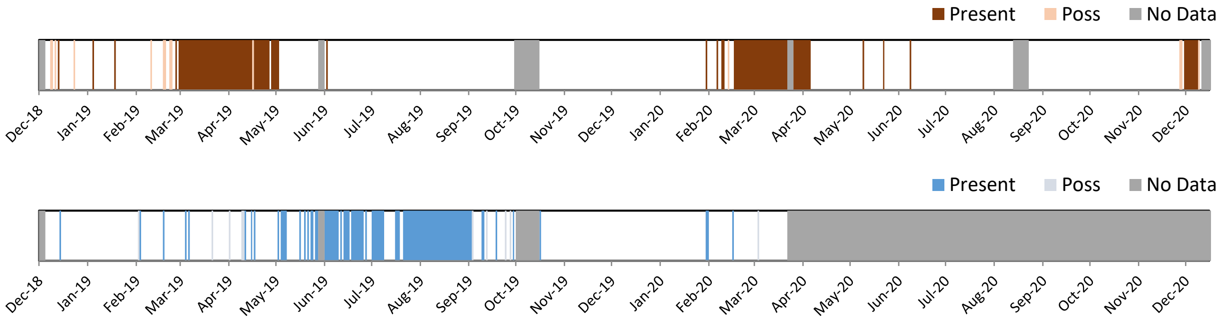


***Figure 7.13**. Daily presence of sei whale vocalisations (red-orange bars) and southern right whale vocalisations (blue bars) at three sites (BS-Outer, BS-Central and BS-Inner) in Berkeley Sound from 5 December 2018 to 10 December 2020. Detections are fully verified (i.e. false positives have been removed) and confidence in presence for each day is indicated as positive confirmation by the acoustic detection of three confident calls ('Present'), or as possible occurrence by the acoustic detection of one or two confident calls, or ≥3 possible calls ('Poss'). White represents areas where data exist and were analysed, but where no vocalising whales were detected. Grey represents periods of no data, either between deployments, or in the case of right whales, also where data exist but haven't yet been analysed.*

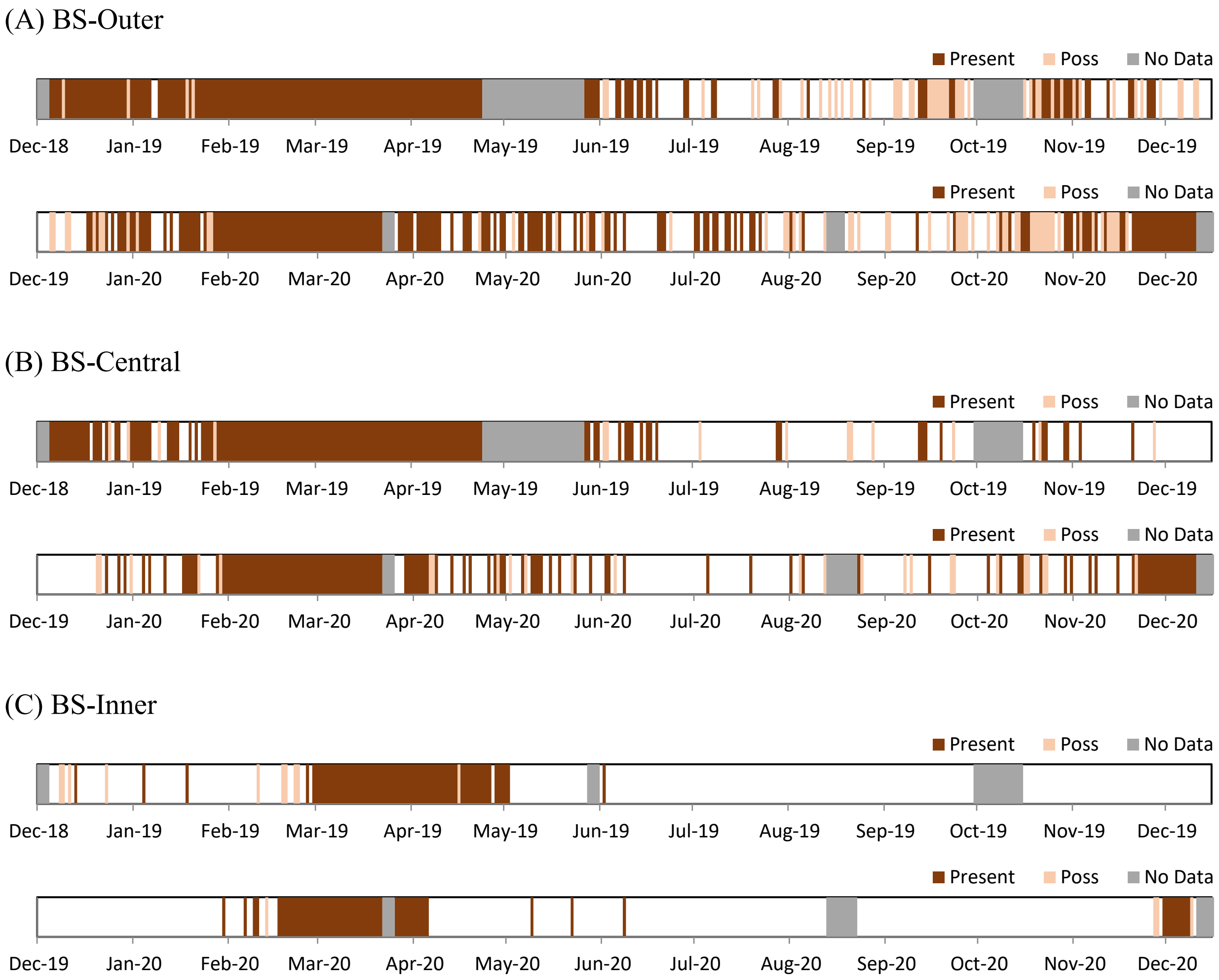
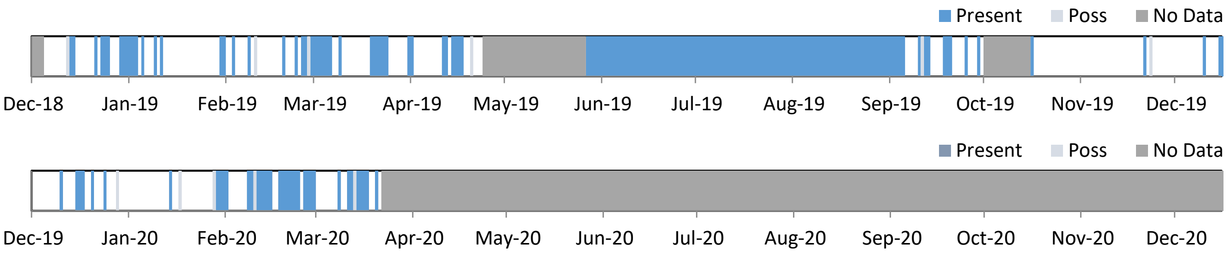
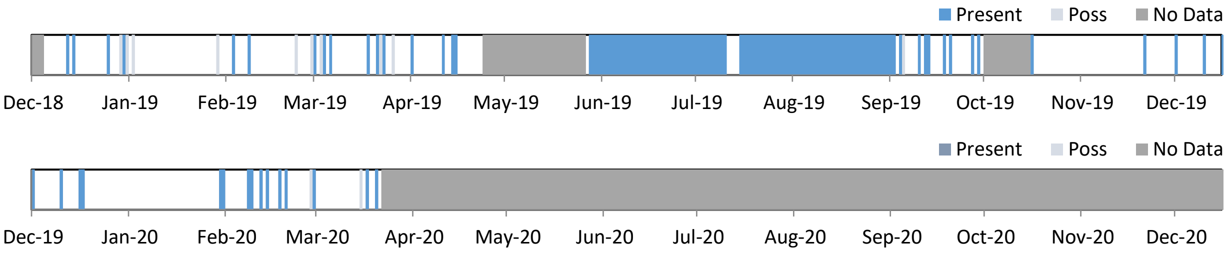


***Figure 7.14**. Daily presence of sei whale vocalisations in Berkeley Sound from 5 December 2018 to 10 December 2020. Detections are fully verified (i.e. false positives have been removed) and confidence in presence for each day is indicated as positive confirmation by the acoustic detection of 3 confident calls ('Present'), or as possible occurrence by the acoustic detection of <3 confident calls, or ≥3 possible calls ('Poss'). White represents areas where data exist but no vocalising whales were detected. Grey represents periods of no data between deployments.*

(A) BS-Outer



(B) BS-Central



(C) BS-Inner

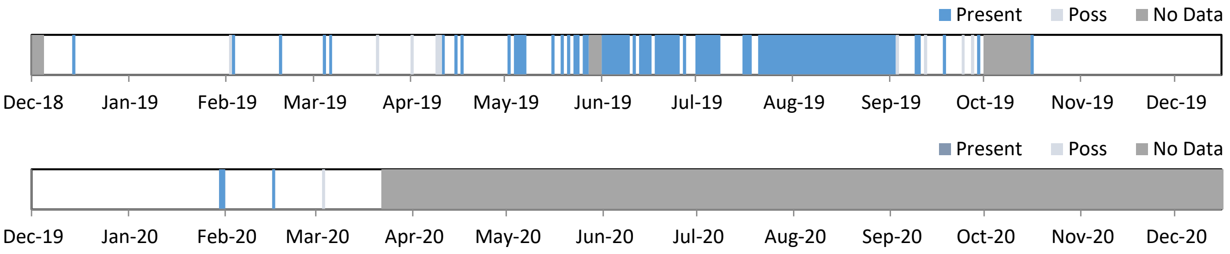
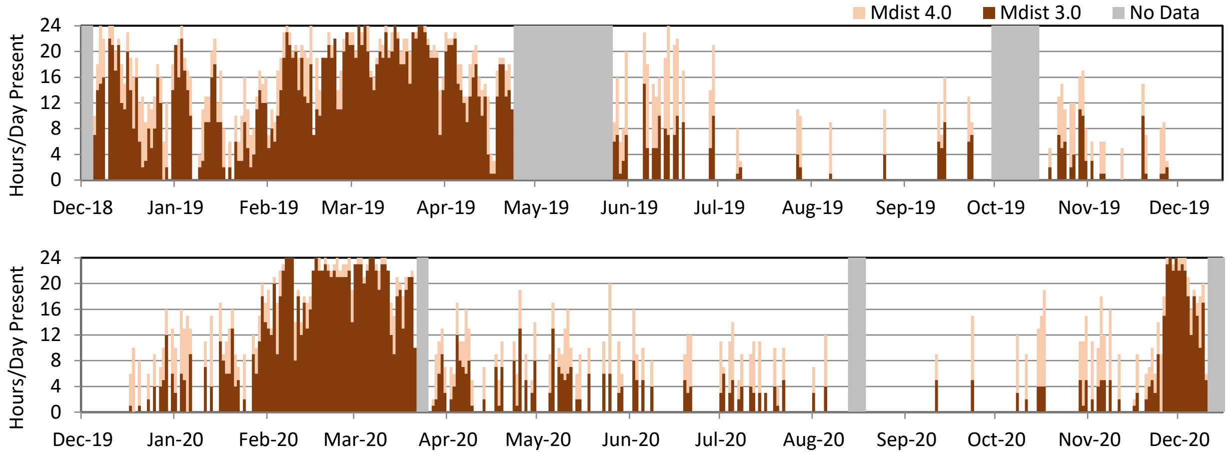


***Figure 7.15**. Daily presence of southern right whale vocalisations in Berkeley Sound from 5 December 2018 to 10 December 2020. Detections are fully verified (i.e. false positives have been removed) and confidence in presence for each day is indicated as positive confirmation by the acoustic detection of three confident calls ('Present'), or as possible occurrence by the acoustic detection of <3 confident calls, or ≥3 possible calls ('Poss'). White represents areas where data exist but no vocalising whales were detected. Grey represents periods of no data between deployments, and data that have not yet been analysed (26 March 2020 onwards).*

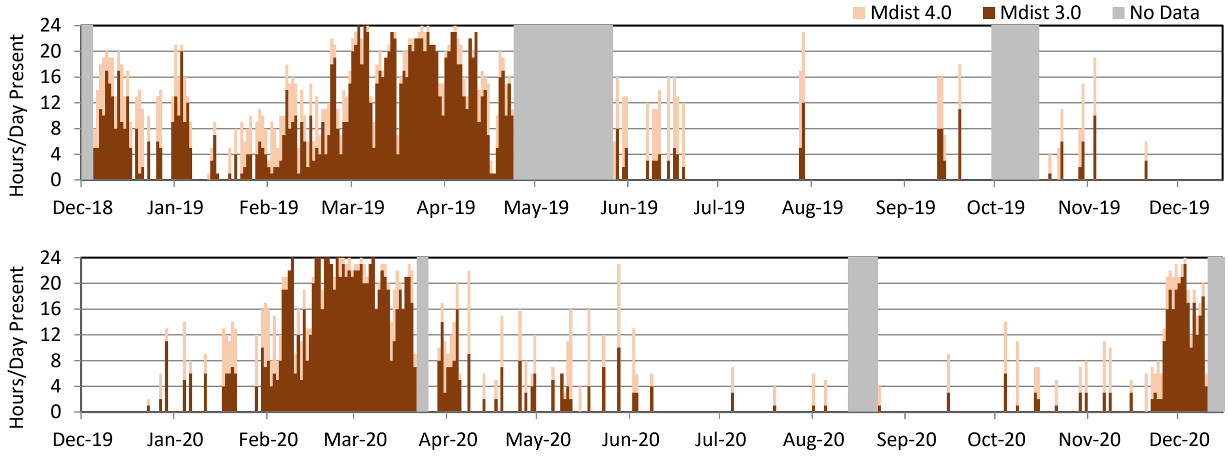
7.3.4.3 Estimates of hourly presence and daily call abundance

The estimates of hourly presence and call abundance provide a more detailed indication of the vocal activity of whales at the sites compared with the daily presence metric considered in Section 7.3.4.2. Sei whales were more acoustically active (i.e. vocalised for a greater proportion of hours per day) during the core seasons as compared to the periods of intermittent presence outside of the core season (Figure 7.16). At BS-Outer and BS-Central, there was a clear peak in activity from early February to mid-April (Figure 7.16A,B) in both 2019 and 2020 (noting that this peak likely continued into May during 2019 but no data were available). Prior to February, there was high but variable vocal activity at BS-Outer and BS-Central during December and January 2019, that was much reduced during 2020 (Figure 7.16A,B). From June through November in both years, sei whale vocal activity was sporadic in terms of both days with activity and the hours per day of activity. In contrast with BS-Outer and BS-Central, sei whale vocal activity at BS-Inner showed a strong seasonal peak centred on March in both years, but peaked earlier in 2020 and continued longer into May during 2019 (Figure 7.16C). That site had virtually no activity during other months. In late November and December 2020, all three of the Berkeley Sound sites experienced high sei whale vocal activity, indicating an early arrival for the 2020/2021 whale season, similar to December 2018.

(A) BS-Outer



(B) BS-Central



(C) BS-Inner

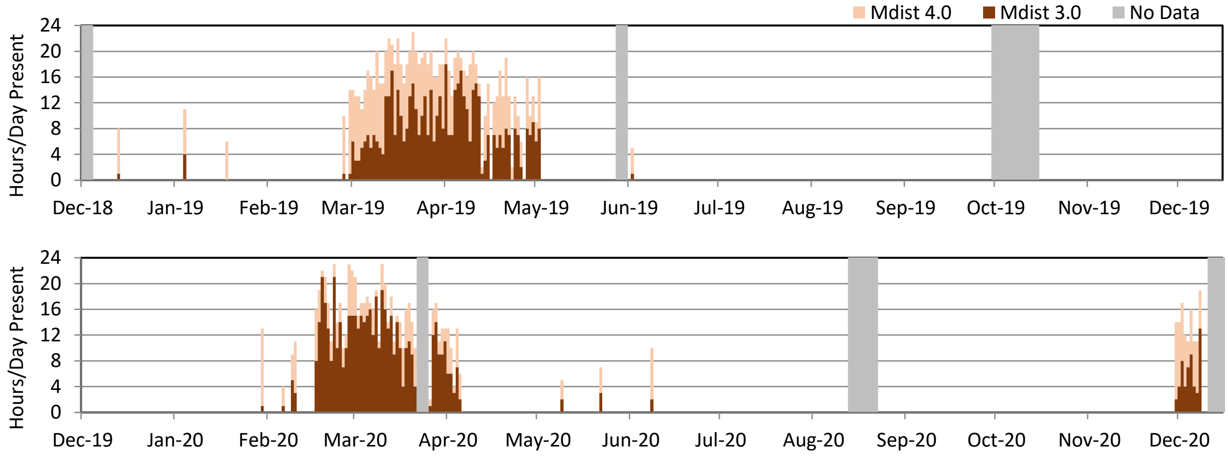
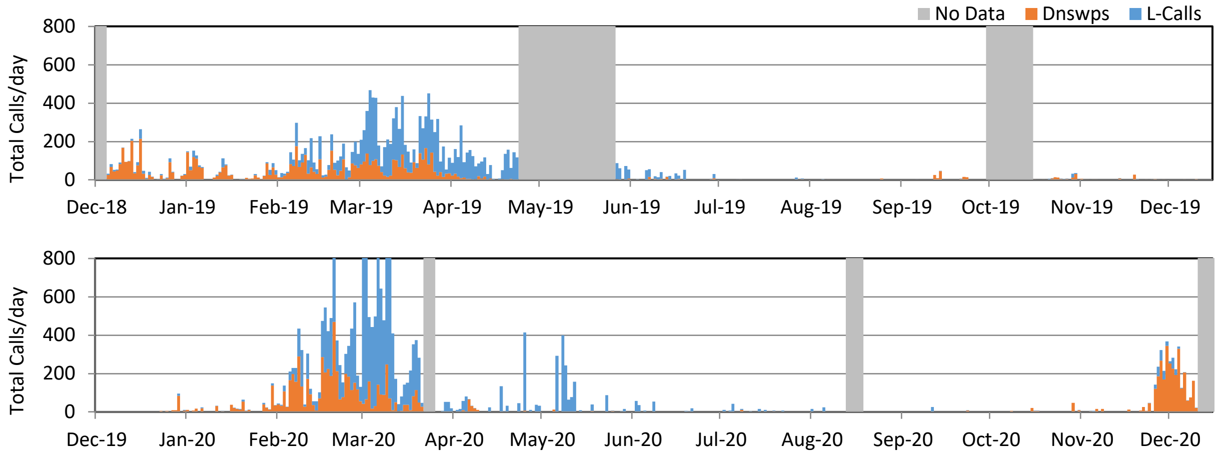


Figure 7.16*. Hourly presence of sei whale vocalisations at three sites in Berkeley Sound from 5 December 2018 to 10 December 2020. The data are presented at two levels of confidence using a conservative criterion with a Mahalanobis distance ("Mdist") of 3.0 that minimises false detections, and a relaxed criterion of 4.0 that maximises true detections but has an increased false detection rate. Data are filtered to include only days confirmed to have confident unambiguous detections in the verified daily occurrence analysis.*

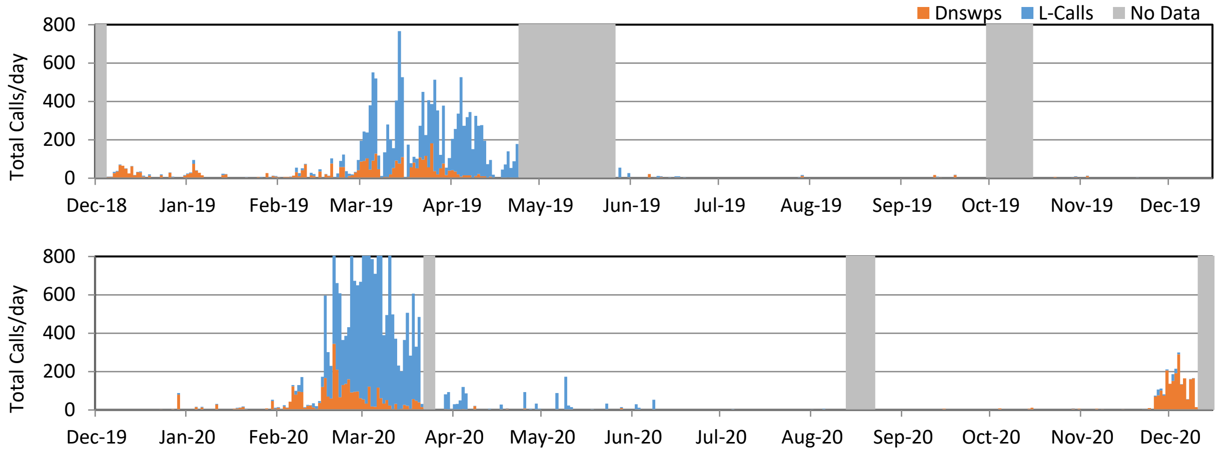
The pattern of daily call abundance generally tracks that of hourly presence for sei whales, further emphasising the difference between the core season and the periods of intermittent daily presence (Figure 7.17). When considering call abundance by call type, a very interesting pattern emerges when comparing downsweeps and L-calls. Downsweeps were produced fairly consistently throughout the periods of high vocal activity (comparing Figures 7.16 and 7.17). However, at BS-Outer and BS-Central, the detection of L-calls greatly increases in early March 2019 and mid-February 2020 respectively (Figure 7.17). L-calls were detected both within sei whale songs, and as calls independent to song (although often in series). As discussed in Section 7.3.2.2, during the 2018/2019 season, song was not recorded until late February and did not become prominent until March (Figure 7.8), despite high rates of hourly acoustic presence during December, January and February (Figure 7.16). Therefore, the surge in L-calls shown in Figure 7.17 is likely correlated to the seasonal onset of singing during March. Moreover, the occurrence of L-calls may potentially be an indicator of increasing male breeding behaviour, under the reasonable assumption that sei whale song represents a male breeding display.

When considering the hourly presence of southern right whale upsweep calls, it is important to recognise the limitations and caveats discussed above, and specifically that when sei whales were present (i.e. Deployments 1 and 3) the conservative criterion estimates presented at an M-dist threshold of 2.0 may be more accurate, whereas during periods when right whales were the predominant whale in the study area (i.e. Deployment 2), the relaxed criterion of 3.0 may be more accurate (Figure 7.18). With that in mind, there is a clear increase in right whale vocal activity during the period from late May to early September 2019 at all three sites within Berkeley Sound (Figure 7.18). As noted in the daily presence assessment, there is some intermittent activity in the months leading up to the core season prior to May, but a rather abrupt drop off following the core season in September and little activity for the remainder of the year. These trends became even more apparent when considering the daily call abundance of upsweeps under the conservative criterion, clearly denoting a marked period of right whale occurrence and vocal activity between June and August (Figure 7.19). There was a noteworthy drop in activity between 6 and 21 July 2019, especially noticeable at BS-Outer and BS-Central (Figures 7.18 and 7.19). This correlated with a period of nearly constant large ship boat noise in Berkeley Sound from 4 to 20 July 2019 (see Section 7.4.4).

(A) BS-Outer



(B) BS-Central



(C) BS-Inner

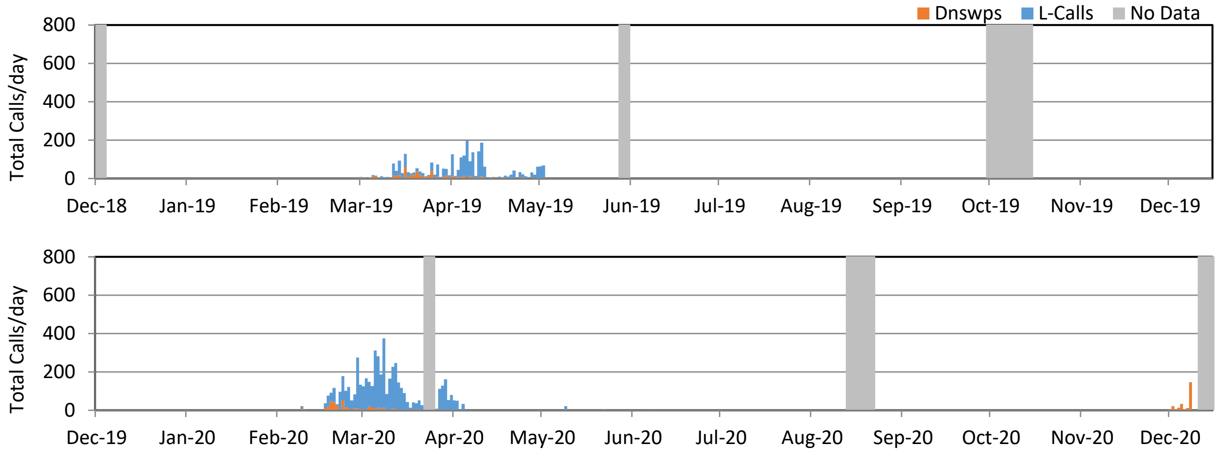
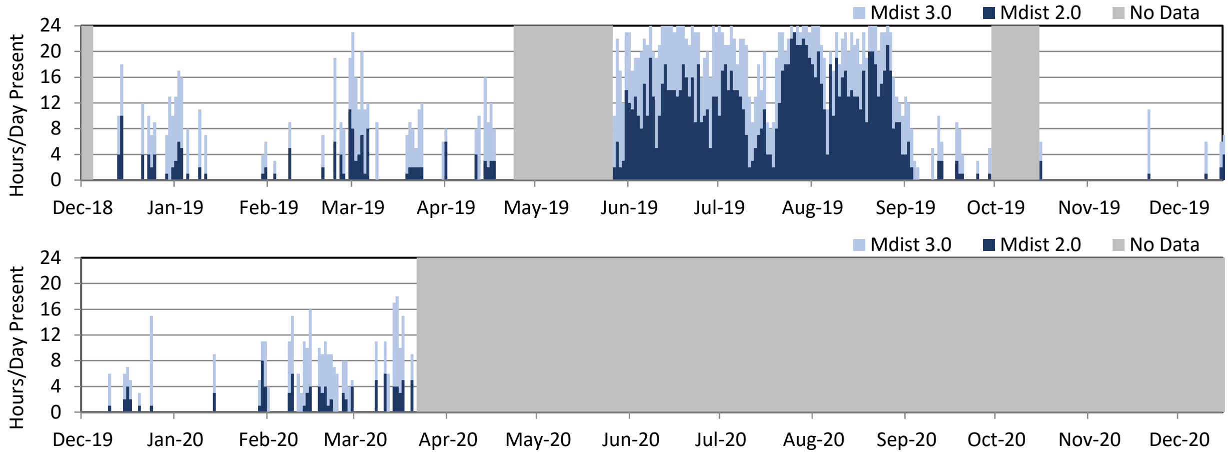
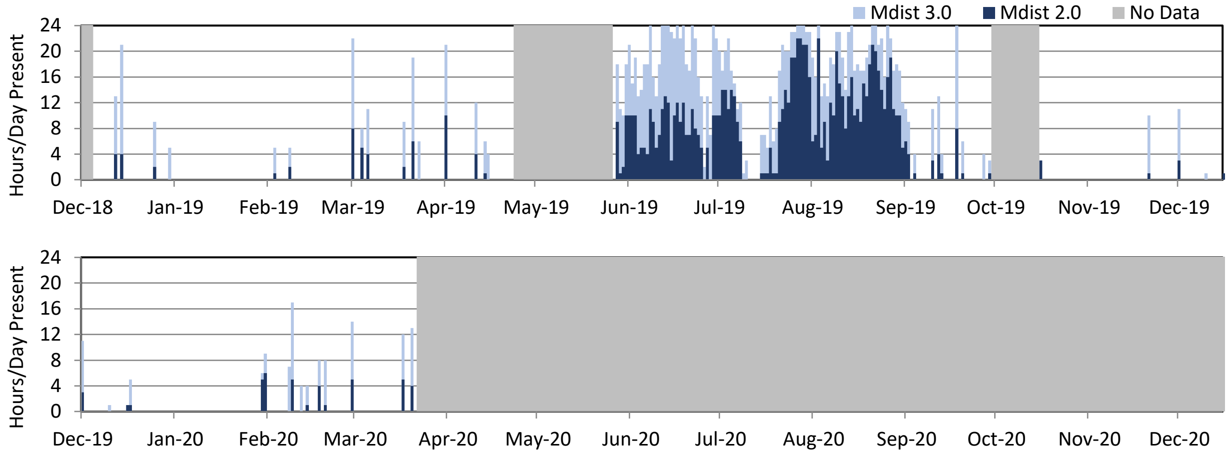


***Figure 7.17**. Sei whale call abundance, as defined by total calls detected per day, subdivided by downsweeps ("Dnswps") and L-calls, at three sites in Berkeley Sound, from 5 December 2018 to 10 December 2020. The data are presented using a conservative criterion with Mahalanobis distance ("Mdist") of 3.0 that minimises false detections. Data are filtered to include only days confirmed to have confident unambiguous detections in the verified daily occurrence analysis. Daily counts are stacked so that each bar represents the proportion of downsweeps and L-calls in the total calls per day. All graphs are standardised to the same y-axis scale for comparison, although maximum values exceeding 800 in February and March 2020 were truncated in order to more clearly present values below 200 in other months.*

(A) BS-Outer



(B) BS-Central



(C) BS-Inner

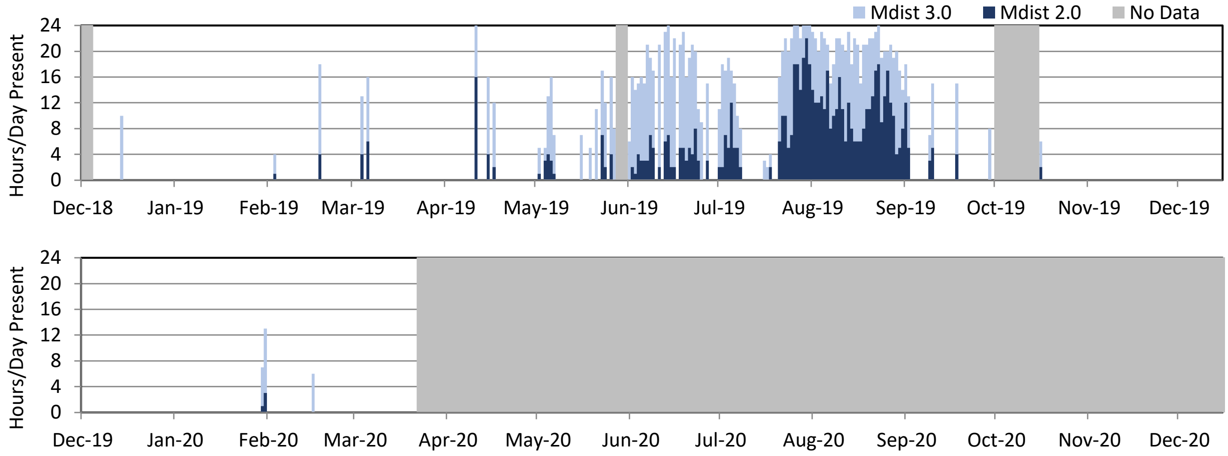
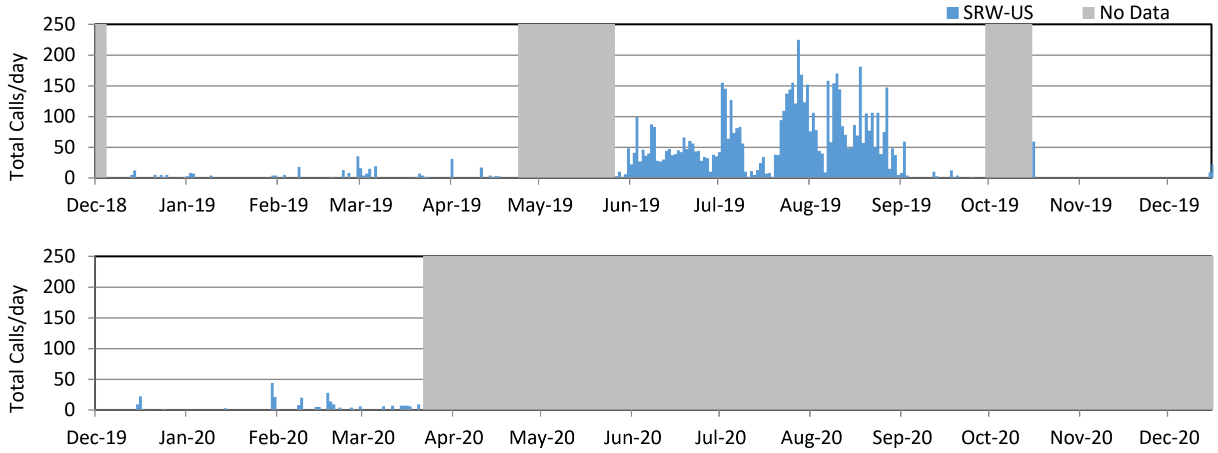
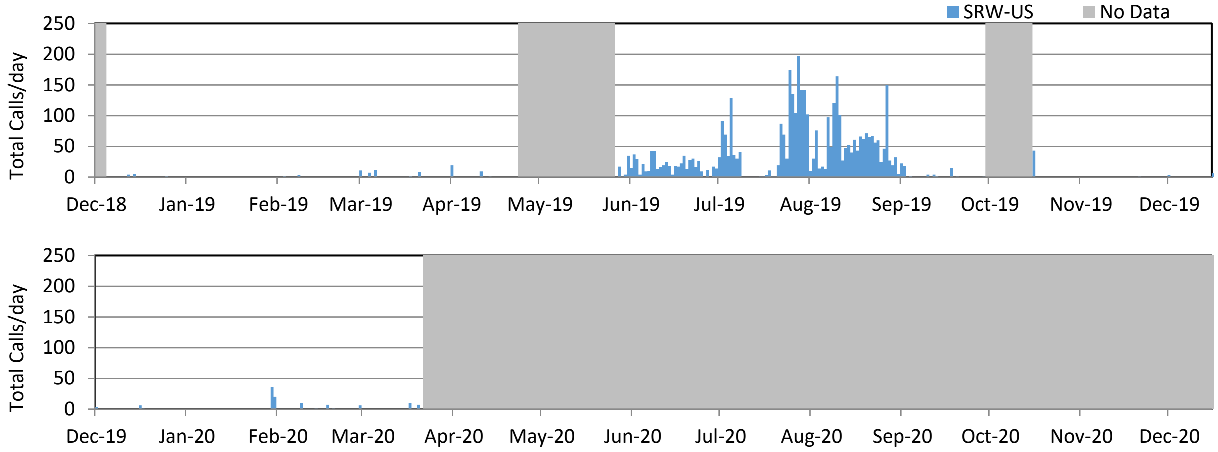


Figure 7.18. *Hourly presence of southern right whale upsweeps at three sites in Berkeley Sound, from 5 December 2018 to 21 March 2020. The data are presented at two levels of confidence, using a conservative criterion with Mahalanobis distance ("Mdist") of 2.0 that minimises false detections, and a relaxed criterion of 3.0 that maximises true detections but has an increased false detection rate. Data are filtered to include only days confirmed to have confident unambiguous detections in the verified daily occurrence analysis.*

(A) BS-Outer



(B) BS-Central



(C) BS-Inner

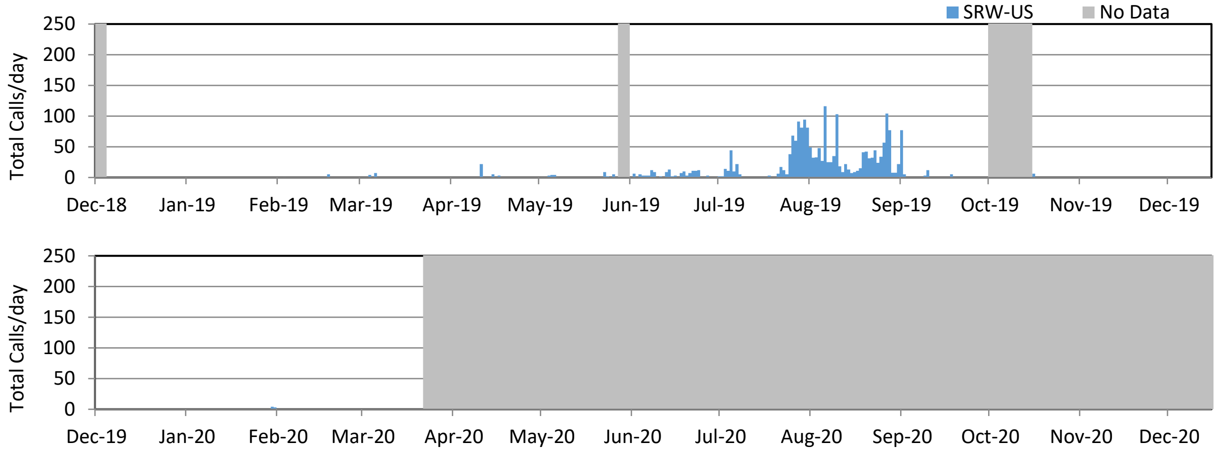


Figure 7.19. *Call abundance, as defined by total calls detected per day of southern right whale upsweeps (SRW-US) at three sites in Berkeley Sound, from 5 December 2018 to 21 March 2020. The data are presented using a conservative criterion with Mahalanobis distance ("Mdist") of 2.0 that minimises false detections. Data are filtered to include only days confirmed to have confident unambiguous detections in the verified daily occurrence analysis.*

7.4 Discussion

7.4.1 Effectiveness of PAM for monitoring sei and southern right whales in the Falkland Islands

This project has demonstrated the value of PAM as a conservation tool for informing effective management decisions for baleen whales in the Falkland Islands. Each individual project aim is discussed below.

7.4.1.1 Assess the success and limitations of the PAM deployments

Despite a few relatively small setbacks during the field component of this work, the two years of data collection should be considered an outstanding success. The month gap in data collection between Deployments 1 and 2 (April/May 2019) at BS-Outer and BS-Central was unfortunate, but resulted from serious damage to the transmitting hydrophone during the first ST500 recovery. This type of error is a typical part of the learning curve for PAM fieldwork, and importantly, was not repeated. Ultimately, this data gap did not have a major impact on the interpretation of the results. The equipment failure of the ST500 deployed in Falkland Sound is also an expected occurrence when working with sophisticated equipment in a harsh marine environment. From prior experience in several geographic regions, the observed success rate for the Falklands team of 94% (only 1 failed deployment out of 17) is outstanding (Cerchio, pers. obs.). The collection of over 50,000 hours of acoustic data represents a major advancement in available information, and the discoveries and observations described herein are testament to the high value of the technique and the success of the approach.

The primary limitations encountered during the work were weather and platform availability. Due to prevailing conditions in the Falklands, it is very rare to have consecutive days of good weather that might facilitate fast turnarounds of recovery and redeployment, and the weather forecasts proved to be unreliable and changeable more than 24 hr in advance which greatly limited forward planning. Additionally, the ST500s were usually recovered during a standard whale visual survey, meaning that the team did not return to Stanley until late at night and the cleaning of fouling and data download did not commence until the following day. A full download of decompressed acoustic files from a single ST500 took 10–11 hours in total, and it was not always possible to download all three devices simultaneously due to available resources. Consequently, a realistic minimum turnaround during the Falklands' deployments was three days, but on almost every deployment the team were then on standby awaiting suitable weather for the redeployment. Platform availability is an additional and ongoing constraint for static acoustic deployments in the Falklands, since away from the east coast of East Falkland there are few vessels available for charter. This added to the challenges of the Falkland Sound deployment, since a small boat had to be specifically towed to that site for recoveries and deployments. As a consequence of these combined challenges, it was frequently the case that turnarounds between acoustic recoveries and their redeployments took longer than would ideally have been desired, sometimes 1–2 weeks rather than a few days. Little could be done in practice to address this loss of data, as it is simply the result of working in this remote and challenging environment. Indeed, these constraints were a key driver for investigating the potential use of PAM in the first instance, because the frequency of visual boat surveys was even more limited by weather.

It should also be considered that an analytical limitation to interpreting the spatial (Section 7.4.1.3) and temporal (Section 7.4.1.4) occurrence of whales using acoustic detections from a PAM study, is the lack of data on the vocal behaviour of individual whales. Individual calling rates likely vary with behaviour, age, sex, group size, and time of year, and it can be challenging to determine how accurately acoustic detections reflect the relative use of environments by whales. While it is typically clear when there are multiple vocalising animals detected in an area (i.e. good presence data), PAM cannot detect animals that are present but not vocalising (i.e. poor absence data). Therefore, the periods with no detections (shown as white space in many of the Figures in this report) do not necessarily indicate an absence of whales. A better understanding of the contexts in which baleen whales vocalise, for instance through representative samples of individually instrumented animals (see **Chapter 6**), could generate estimates of calling rates that would facilitate a more accurate interpretation of acoustic datasets. These considerations do not undermine the usefulness of PAM datasets in providing positive presence data. Particularly in regions like the Falkland Islands, in which visual surveys are severely limited by weather and challenging logistics, and in any region for the interpretation of diel and night-time behaviour, PAM remains an effective means of collecting extensive datasets on the spatio-temporal presence of whales that are not otherwise feasible. Therefore, acoustic and visual techniques should be considered complementary, each with their strengths and limitations, and together create a more informed understanding than either can do individually.

7.4.1.2 Describe detected vocalisations for each species, as allowed by confident species attribution

The success of the PAM study in Berkeley Sound was reliant on being able to confidently assign vocalisations to a particular species, and was optimised by: (1) previous and concurrent boat surveys in Berkeley Sound that provided information on the relative occurrence of baleen whale species at the site (see **Chapter 2**); and (2) previous documentation of common vocalisations for sei whales and right whales from other geographic areas (see Section 7.1). These factors were critical in facilitating the confident species attribution of new, previously undocumented, vocalisations. A fortunate aspect of the site and the timeframe used for this study, was that at any one time there was predominantly one species of baleen whale present (see **Chapter 2**), and therefore previously-undocumented vocalisations that occurred in bouts among known species-specific vocalisations could be positively attributed to that species. This is not the case in geographic regions where several species of baleen whales co-occur temporally in relatively comparable numbers.

Consequently, we have described the vocal repertoire of sei whales in the Falklands for the first time, and in greater detail than any other study globally, adding several previously undocumented sei whale call types. The Type-1 downsweeps (DS1) that we described correlate broadly with a downsweep that has been widely documented in both the Southern and Northern Hemispheres (Rankin and Barlow, 2007; Baumgartner et al., 2008; Calderan et al., 2014; Espanol-Jimenez et al., 2019; Tremblay et al., 2019). With a frequency range of 127–20 Hz, DS1 are most similar to the 41 sei whale downsweeps reported by Espanol-Jimenez et al. (2019) in the eastern South Pacific (off Chile) which had a reported frequency range of 129–30 Hz. The downsweeps from these two Southern Hemisphere locations were slightly higher than those reported for Northern Hemisphere sei whales, which generally sweep from approximately 100–30 Hz (Rankin and Barlow, 2007; Baumgartner et al., 2008; Tremblay et al., 2019). In contrast to DS1, the Type-2 downsweep (DS2) that we reported is novel, and had a much higher maximum frequency and steeper slope then has been previously reported for sei whale downsweeps. The maximum frequency of DS2 calls in the Falklands ranged from 131 to 229 Hz, whereas other studies do not report any downsweeps with a maximum frequency in excess of 130 Hz (Rankin and Barlow, 2007; Calderan et al., 2014; Espanol-Jimenez et al., 2019); therefore, the acoustic features DS2 do not overlap with previously reported downsweeps, and can be considered it a new call-type for sei whales. In addition, the L-calls, and their associated variant LDS-calls, do not appear to have been described in the literature, and thus are completely novel. Arch calls in the Falklands were similar to the upsweep-downsweep call described previously by Calderan et al (2014) from the Southern Ocean, but with a broader frequency range of 19–119 Hz compared to their 49–79 Hz. This discrepancy may be due to the small sample size of Calderan et al. ($n = 4$) compared to the 93 high SNR examples that were measured in the Falklands. The frequency range of upsweeps in the Falklands (20–78 Hz) overlapped broadly in frequency with those in the Southern Ocean (34–76 Hz: Calderan et al., 2014), but were much longer in duration (1.4–2.8 s compared to 0.4–1.7 s, respectively) and thus presented a shallower slope than those recorded in the Southern Ocean.

In addition to the novel LF calls that were described for sei whales in the Falklands during DPLUS082, the new discovery of song for both sei whales and southern right whales is of global significance and is discussed in Section 7.4.2).

7.4.1.3 Describe spatial variation in whale acoustic detections

The spatial distribution of the three ST500s in Berkeley Sound was relatively small, with only 7 km separation between each pair, and a total linear spread of 14 km. Although the realised detection ranges around the ST500s were not determined during the study, the presence of some series of vocalisations simultaneously on adjacent ST500s indicated that the detection ranges overlapped. Therefore, each ST500 had a detection radius of at least 3.5 km. Despite this overlap, a very clear and strong heterogeneity was observed in the number of detections recorded by the three ST500s, particularly with regard to sei whale vocalisations. This suggests heterogeneity in the spatial distribution of sei whales within Berkeley Sound, and an overall reduced preference for the area around BS-Inner, with the exception of during March/April, at the height of the core sei whale season, when distribution appeared to be more even throughout the Sound. Additionally, BS-Outer was located at the mouth of Berkeley Sound and was therefore situated adjacent to the open Atlantic rather than within the semi-enclosed confines of the Sound. Consequently, it may have been monitoring a somewhat larger spatial area than the ST500s at the other locations, and detecting animals further offshore in more open habitat. It's unlikely that it detected whales more than 10 km away, given the limitations of sound propagation in shallow shelf waters. Without a detailed analysis of sound propagation within the region, and an accurate estimation of detection range, it isn't possible to understand how sound propagation may have influenced the relative number of detections at each site.

While highlighting this important caveat, the magnitude in the spatial heterogeneity and the contrast between species strongly suggests that the variation in whale acoustic detections at the different sites within Berkeley Sound reflected actual spatio-temporal variation in whale distribution rather than being solely an artefact of propagation effects. It is noteworthy that the differences between BS-Inner and BS-Outer, and in the vocal activity profiles across the sites, varied substantially between sei whales and right whales. Sei whale detections were made on three times more days at BS-Outer compared with BS-Inner, and the profile of hourly detections was much more similar between BS-Outer and BS-Central, with BS-Inner much reduced. This emphasised the potential preference for the outer half of the Sound, and reduced use of the innermost Sound by sei whales, particularly outside of the core season. In comparison, for southern right whales there were detections on twice as many days at BS-Outer compared with BS-Inner; however, the profile of hourly detections was more similar between BS-Inner and BS-Central, with BS-Outer having the greatest activity. This suggests that the variation between the sites was less pronounced for southern right whales than for sei whales.

There are several possible explanations for this apparent heterogeneity. Since it is clear that sei whales are feeding during the summer and autumn in Berkeley Sound (Weir et al., 2019; see **Chapter 4**), the most obvious causative variable to explore with regard to their spatio-temporal distribution is availability of prey resources. In contrast, the winter occurrence of right whales in nearshore waters around the Falklands is considered to be primarily related to social and mating behaviour (Weir and Stanworth, 2020; Weir, 2021), and the distribution of prey may therefore be less important for that species at that time of year. Another factor to consider is disturbance from anthropogenic activities. If there is more potential for acoustic disturbance deeper inside the Sound, such as from vessel noise or other anthropogenic noise sources, then this may have several potential impacts on the whales that could explain some of the results of this study, including: (1) an impact on the distribution of whales (if they are displaced from noisy areas); (2) altered vocal activity (if they stop calling or otherwise change behaviour in response to noise); and (3) masking of whale calls by high levels of noise, such that the calls are no longer evident at sufficient SNR within the recordings to be detected. We briefly discuss implications of vessel noise in Section 7.4.4.

7.4.1.4 Describe temporal variation in whale acoustic detections

The PAM study successfully provided an evaluation of the temporal occurrence of baleen whales in the Falklands. A significant finding was the marked asynchrony in the core periods of presence of the two target whale species in Berkeley Sound. Sei whale detections were present on a daily basis from mid/late January to early April in both years (with occurrence during the earlier and later parts of the season varying between years), while southern right whale detections occurred continuously on a daily basis between late May and early September in 2019. These findings are consistent with the visual survey data acquired from boat surveys (see **Chapter 2**), and support contrasting seasonality in the respective occurrences of the two species within Berkeley Sound. This variation in the temporal occurrence of the species reflects their use of the region for different purposes, with sei whales using nearshore Falklands' waters as a summer and autumn feeding ground (Weir, 2017; Weir et al., 2019) while southern right whales use the same nearshore area as a winter mating ground (Weir and Stanworth, 2020; Weir, 2021). While the discovery of singing by sei whales later in the season also supports the potential use of Falklands' waters for breeding, it remains likely that feeding is the primary driver for their occurrence in this specific area. For example, singing is known to commence on feeding grounds prior to migration in humpback whales (e.g. Vu et al., 2012; Murray et al., 2014; Kowarski et al., 2019), and this is likely similar for Falklands' sei whales (see Section 7.4.2.1).

Although the core periods of presence of each species differed, the acoustic dataset revealed an intermittent presence of both species in the study area for large portions of the year. For sei whales, monitoring over two complete years also provided an indication of inter-annual variation in their seasonality, which was also evident from the boat surveys (see **Chapter 2**). The presence of sei whales in the early part of the season from late November through to January differed markedly between the study years, with the period of peak presence commencing one to two months earlier in the 2018/2019 and 2020/2021 seasons, than in the 2019/2020 season. Moreover, presence during 2020 was generally truncated with an abrupt reduction in detections occurring at least one month earlier than in 2019. This resulted in consistent presence during 2020 for only about two months, compared to at least five months during the 2018/2019 season. Despite the substantially later arrival and shorter duration of the 2019/2020 season, the peak in presence was approximately two to three weeks earlier than during the 2018/2019 season, most obviously apparent at BS-Inner. This inter-annual variation in the onset and duration of the core season is likely driven by oceanographic conditions and the availability of prey, both within the study area and in other adjacent regions.

Small numbers of intermittent sei whale acoustic detections occurred after the core season, throughout the May to November period in both years, being lowest in August and September. This likely reflects the seasonal migrations of sei whales, with the species thought to move north to lower latitude wintering grounds for mating and calving. In the south-west Atlantic, this includes migrations from the Falklands to a wintering area off Brazil, where calving is known to occur between May and October (Weir et al., 2020). With regard to interpreting sei whale temporal occurrence during this period, it is important to consider a limitation in the PAM technique with regard to uncertainty in the species attribution of remotely recorded vocalisations. This is particularly true with downsweeps, which are a call-type that is common among several species of Balaenopterid whales, with subtle variation between species (Ou et al., 2015). For this reason, we used the approach of qualifying our confidence in daily presence as either confident confirmation or possible presence. During the months of lowest sei whale detections between August and November, there were many days that were classified as 'possible presence' in both 2019 and 2020, the majority occurring at BS-Outer. In those cases, detections comprised a single downsweep that matched the acoustic features of sei whale downsweeps (and thus triggered the detector with low M-dist values), often in repetitive series with long inter-note intervals. It is possible that these calls did not actually originate from sei whales, but rather represented the vocal behaviour of another Balaenopterid whale, such as the Antarctic minke whale (*Balaenoptera bonaerensis*: see Risch et al. 2014 for similar vocalisations) or dwarf minke whale (an unnamed subspecies of common minke whale, *B. acutorostrata* ssp.). Irrespective of uncertainty in these cases, scattered throughout the same periods were a smaller number of days with confident confirmation of sei whale presence, through the detection of typical downsweep doublets, and/or L-calls. Therefore, the data indicate that sei whales maintain a low-level presence in the region throughout much of the year. The predominance of both the possible and confident detections at BS-Outer and BS-Central implies that these individuals may have been transiting through more open waters at, or near, the entrance to Berkeley Sound, rather than occurring well inside the Sound.

Only one full year of southern right whale acoustic data has been analysed to date, and consequently it was not possible to assess inter-annual variation in the detections of that species. However, the dataset did reveal intermittent bouts of right whale occurrence within the study area between December and April in both years, preceding the core winter period for that species. In their review of available historical and contemporary datasets, Weir and Stanworth (2020) concluded that right whales likely occur in open neritic waters around the Falklands year-round, with a peak during the summer, whereas their occurrence in nearshore waters was more limited to the late autumn and winter. They suggested that open neritic waters may be used as summer foraging habitat and for migration, while the nearshore wintering aggregations were engaged in socialising and mating behaviour. In this context, it is plausible that the intermittent acoustic detections of southern right whales during summer and early autumn may have related to animals feeding in the open waters adjacent to Berkeley Sound. Weir (2017) recorded several sightings of foraging southern right whales 10 to 20 km offshore of Berkeley Sound during aerial surveys in March 2017, which also supports this possibility. The far higher detection rate at BS-Outer compared with BS-Central and BS-Inner during the December to April period, further suggests that right whales detected during those months may have been predominantly located near to, or offshore of, the entrance to Berkeley Sound rather than within the Sound itself. Due to the propagation characteristics of right whale upsweeps, it is expected that detections were likely located within, but not beyond, 10 km of the devices. Right whales were not observed within Berkeley Sound during concurrent boat surveys in January to April 2019 or 2020 (see **Chapter 2**); however, boat survey effort in those periods only occasionally occurred more than 4.5 km seaward of BS-Outer, and the total number of boat survey days was only a fraction of the total acoustic monitoring effort. If right whales are feeding in neritic waters just outside of Berkeley Sound during summer and autumn, then it remains unclear why they are not also regularly observed within the Sound over this period when sei whales are actively feeding there. Weir and Stanworth (2020) suggested that sei whales and right whales may avoid competition for resources through establishing different temporal distributions inside the Sound. Alternatively, potential differences in preferred prey types may be more important than competition avoidance in driving these different distributions. The stable isotope work being carried out in the Falklands (see **Chapter 4**) should help to clarify the regional prey preferences of both species.

7.4.1.5 Applicability of the acoustic method for long-term monitoring of whales in the Falklands

It is important to understand that the selection of methods for a whale research project depends entirely on the questions being asked and the goals of the data collection. So, for example, while both boat surveys and PAM can produce data on the spatio-temporal occurrence of whales, the type of data produced is different and therefore its applicability to conservation and management questions will vary. In the case of DPLUS082, the two approaches were considered complementary and were implemented to address different goals. The boat work (**Chapter 2**) covered a wide spatial area and provided multifaceted data on species identification, group size and composition (e.g. presence of calves), photo-identification (for assessing abundance and movements: **Chapter 3**), faecal sampling for diet (**Chapter 4**), genetic tissue sampling (**Chapter 5**), and suction cup tagging (**Chapter 6**). The PAM study focussed on a smaller area (but with three devices was able to cover the entirety of Berkeley Sound), and provided a comprehensive temporal dataset on whale presence over hours, days and seasons that could not have been achieved via boat surveys due to cost and weather limitations. Both approaches come with caveats and need to be considered alongside a careful evaluation of logistics, cost, and applicability to answering research goals. Both methods revealed important aspects of sei whale behaviour that have direct management relevance: feeding, documented during the boat surveys, and singing for reproductive purposes, revealed by PAM.

A major driver for the PAM study at the project outset, was to better understand the temporal presence of baleen whale species in Berkeley Sound, particularly with regard to what was happening at night (when visual surveys from boat are not possible) and during the spring season when no boat survey work was carried out due to perceived low whale occurrence. The PAM study was very successful in fulfilling this objective, providing 24 hr data on the presence of vocalising whales over a two year consecutive period. These data not only expanded knowledge of the seasonality of whales in the Falklands, but facilitated an assessment of inter-annual variation in species arrival and departure times, and provided a means of assessing occurrence over finer timeframes including daily and hourly. Future analyses could also potentially assess this temporal dataset against factors such as tide and sea surface temperature to better understand the drivers of whale occurrence, and investigate fine-scale correlations between whale presence and anthropogenic factors such as vessel noise (see Section 7.4.4).

Expanding the PAM effort to include more sites throughout the Falkland Islands would provide useful information on the relative occurrence of species across the Islands, and may help to answer some of the key remaining questions including: (1) whether the seasonal presence of sei whales is different on the west coast of the Falklands compared with the east coast; and (2) how widely around the Falklands the winter aggregation of southern right whales occurs. Continued monitoring in Berkeley Sound would better clarify how whale presence varies between years; the two-year dataset assessed in this chapter demonstrated variation in sei whale occurrence between years but was too short to clarify what constitutes a 'typical' year. Additionally, longer-term monitoring would provide useful information on changes in whale species composition; the unexpected arrival of humpback whales into Falklands' nearshore waters during 2021 (see **Chapter 2**) occurred following completion of the Berkeley Sound acoustic monitoring program. Such changes in species community composition have management relevance with regard to factors such as inter-specific competition and oceanographic changes in the environment.

It should be recognised that the large volumes of data produced by long-term PAM monitoring do create some challenges for analysis, which can be complex (requiring expertise), time intensive, and therefore expensive. A long-term monitoring programme using PAM in the Falklands therefore needs to consider the commitment required with regard to budget and skillset in analysing and interpreting the datasets, and also whether access is available to detector algorithms that may be developed and maintained by particular institutes.

7.4.2 Wider implications of the discovery of singing behaviour

7.4.2.1 Sei whales

Possibly the most consequential finding of this work on a global scale is the discovery of mid-frequency song in sei whales. Song has been documented as a common male reproductive behaviour among most of the Balaenopteridae; however, unambiguous stereotyped song has not been conclusively reported previously for sei whales. The series of different downsweep types reported by Tremblay et al. (2019) for North Atlantic sei whales are organised into somewhat variable patterns that appear to be a form of Balaenopterid LF song; however, the stereotypy of the sequences and consistency of song structure requires further evaluation and description. In the two-year assessment of sei whale vocalisations in the Falklands, sequences of LF downsweeps resembling those reported by Tremblay et al. (2019) were never observed. Rather, the Falklands' songs occurred in a different frequency band (up to 4,000 Hz) and were composed of different and highly varied vocalisation types (complex combinations of broadband frequency sweeps, mid-frequency chirps and creeks, and low frequency vocalisations). Moreover, they presented a much more stereotyped and hierarchical structure consistent with complex Balaenopterid song, and similar to the phrase structure of humpback whales (Payne et al., 1983; Cholewiak et al., 2013).

This novel finding has several important implications:

1. The existence of song in sei whales is a substantial contribution to global understanding of sei whale behaviour, and will facilitate researchers in other geographic regions to appropriately interpret existing and future datasets to identify the presence of sei whale song and thus identify breeding sites;
2. Singing in Balaenopterids is considered a breeding display produced only by males (Glockner, 1983; Croll et al., 2002; Oleson et al., 2007; Cholewiak et al., 2013), and consequently this may also be true for sei whales. This provides useful information for understanding the sex-age

composition of whales in the Falklands, suggesting that mature reproductive males are at least one of the cohorts present. Although (presumed) females with calves have been encountered during the Falklands' boat surveys (**Chapter 2**), little other information has been available on the sex-age composition of sei whales in the region. Singing also has implications for the detectability of vocalising sei whales which may be higher for males engaged in prolonged bouts of singing, depending on the vocalisation type that is used for detection. Care should be taken in interpretation of the temporal distributions of specific vocalisation types; the increase of L-calls detected concurrent with the commencement of singing behaviour is indicative of the L-call being a component in the song, and may therefore potentially be male-specific. Conversely, downsweeps were recorded throughout the entire period of sei whale detections, indicating that they might be produced by both sexes (as shown for blue whales: Oleson et al., 2007).

3. The occurrence of song indicates that Falklands' waters are used for reproductive display that is integral to sei whale life history, potentially including courtship and mating. The occurrence of these sensitive behaviours has implications for their management (e.g. Section 7.4.4).

Songs were absent early in the recording period from December to February, but became common in early March, and continued through the remaining period of sei whale presence, suggesting distinct seasonality that is congruent with a reproductive song display. Further analysis should seek to confirm this seasonality, to assess whether song consistently commences in the Falkland Islands during the austral autumn (i.e. inter-annually and at other sites), and continues into the winter elsewhere in the migratory range. Within a comparative framework, this pattern of occurrence is similar to what has been described for the seasonality of humpback whale vocal behaviour, where singing is rarely recorded during the summer months in high latitude feeding areas, but commences during the autumn before males migrate to low latitude breeding areas (Vu et al., 2012; Murray et al., 2014; Kowarski et al., 2019). The commencement of singing by sei whales in the Falkland Islands appears to follow a similar pattern, and it is proposed that singing may continue through the winter during the migration of whales to their lower latitude wintering destinations (e.g. offshore Arraial do Cabo, Brazil: Weir et al. 2020).

7.4.2.2 Southern right whales

Similar to the discovery of sei whale song, the documentation of gunshot song from southern right whales in Berkeley Sound is a globally important discovery. Prior to Crance et al. (2019), right whale species were not thought to use male song as part of their mating system or behavioural repertoire. This was an outlier in an evolutionary framework, since singing is widespread among Balaenopteridae, and within the family Balaenidae (to which the right whales belong) the bowhead whale (*Balaena mysticetus*) is known to have one of the most complex singing behaviours among all mysticetes (Stafford et al., 2018). Following the discovery of gunshot song in North Pacific right whales (Crance et al., 2019), the documentation of similar songs produced by southern right whales in the Falkland Islands indicates that stereotyped songs composed of complex sequences of gunshots and other vocalisation types is a behaviour that is likely common to all right whale species and had simply remained undescribed for decades. It has still not been formally reported for North Atlantic right whales, but was suggested by Parks et al. (2012) in their mention of stereotyped sequences of gunshots (but without presentation of examples or using song terminology).

The prevalence of right whale gunshot song in Berkeley Sound during the winter months has important implications for habitat use by the species. Considered together, the consistent occurrence of upsweeps through the winter to early September, the presence of male gunshot song, and observations of surface active groups with breeding activity (Weir and Stanworth, 2020; Weir, 2021; see **Chapter 2**), indicate that Berkeley Sound and (at least) the wider north-east Falklands represent an active mating area. This was initially suggested by Weir and Stanworth (2020) as one non-exclusive explanation for the observed seasonality of visual observations of right whales in nearshore waters around the Falklands. Moreover, since there is little evidence of active calving in the Falkland Islands (Weir and Stanworth, 2020; Weir, 2021), these observations suggest that some spatial segregation may occur in reproductive behaviour, such that certain wintering areas may be used primarily for mating and courtship rather than for calving.

The major south-west Atlantic breeding ground for southern right whales is located at Peninsula Valdés in Argentina. Right whales have been studied in this region since the 1970s, and it has traditionally supported mother-calf pairs, non-breeding whales (mature females between calf years and subadults), and mating groups comprising a female with multiple males (Payne, 1986). However, gunshot song has not been documented at Península Valdés despite extensive recording effort and detailed description of the acoustic repertoire of southern right whales utilising that region in the 1970s (Clark 1982), with researchers confident that it did not occur within the range they monitored (C.W. Clark, pers. comm.). While some fine-scale separation of these different cohorts has always been evident within the Peninsula Valdés breeding ground (Payne, 1986), there has been a much higher amount of segregation in recent years such that mating groups are being displaced into adjacent habitats while the mother-calf pairs dominate the coastal preferred habitat (Crespo et al., 2019). This change is thought to reflect density-dependence and the result of increasing size of the whale population in recent decades, which is causing breeding groups to move into different areas including deeper-water habitat and along new coastlines (Crespo et al., 2019). It is therefore plausible that the occurrence of mating groups of southern right whales in the Falklands is also a response to growing population size in the south-west Atlantic and the suitability of Falklands' nearshore habitat for some reproductive behaviours.

7.4.3 Notes on other species

Several other cetacean vocalisations were opportunistically identified within the acoustic dataset in Berkeley Sound, some of which could be attributed to species based upon known species-specific vocalisations from the literature. The characteristic pulse trains of Antarctic minke whale song (see Risch et al., 2014; Cerchio et al., 2018; Shabangu et al., 2020) were recorded at all three Berkeley Sound sites and over both winters. At least three different song types were found, each previously described in other geographic regions (e.g. off north-west Madagascar: Cerchio et al., 2018). Although a systematic scan for minke whales was not conducted on the Falklands dataset (and consequently the true prevalence of occurrence is unknown), songs were noted on at least seven days between 25 July and 22 October 2019, and on 11 days between 14 September and 28 October 2020, suggesting an occurrence during the austral winter and spring. The presence of sounds attributed to the Antarctic minke whales in the Falklands dataset is surprising, since the species more typically winters in oceanic habitat in other regions. A full analysis of the dataset to specifically target Antarctic minke whales, and also the dwarf minke whale subspecies of the common minke whale, would better elucidate the occurrence of both species around the Falklands.

Fin whales (*Balaenoptera physalus*) have been anecdotally reported in coastal areas around the Falkland Islands, in some areas frequently enough to suggest that their occurrence might support a Key Biodiversity Area (e.g. Frans and Augé, 2016; Taylor et al. 2016). However, there is some debate over whether they actually occur on a regular basis in nearshore waters[5] or if reported sightings are in fact misidentified sei whales (see Weir, 2017). Fin whales produce a characteristic song comprising patterns of short duration downsweeps ranging from approximately 30 to 15 Hz (with peak energy at 20 Hz). These songs tend to be readily identifiable and very commonly heard during acoustic monitoring in areas inhabited by fin whales. They are produced during most months of the year, but predominantly during winter. During the verification process for the sei and right whale detections, there was never definitive indication of fin whale 20 Hz song, despite careful examination of any pulse-like signals at 20 Hz. Given familiarity with Southern Hemisphere fin whale song (e.g. from the Mozambique Channel: Cerchio et al., 2018), we are confident that it did not occur in the portions of the recordings that were reviewed. This could potentially be further verified by the use of LFDCS to automatically detect fin whale song, which would require a completely separate analysis with different parameter settings due to the very low frequency nature of the signals.

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[5] It is clear that fin whales do routinely occur in deeper, offshore waters around the Falklands (e.g. White et al., 2002).

The question remains of whether the series of singlet downsweeps in the 100–50 Hz band that triggered the sei whale detector primarily between August and October could be non-song calls of either minke or fin whales. Those detections resulted in the many 'possible' sei whale presence days during those months, particularly on the BS-Outer recorder for which the detection radius extended into open Atlantic waters. A detailed manual scan through all the 'possible' as well as all the 'confident' sei whale downsweeps from June to September 2019 on BS-Outer indicated that there was no fin whale song associated with any of those downsweeps. This does not absolutely rule out that the 'possible' sei detections during the austral winter could be non-song fin whale downsweeps. However, it is considered highly unlikely because fin whale song is ubiquitous during winter in areas where the species occurs, so that if fin whales were making the non-song calls, we would likely have seen at least distant song in the LF spectrum in addition to the non-song calls. This acoustic review therefore reinforces visual observations indicating that all large Balaenopterid sightings were sei whales, and further supports that anecdotal fin sightings are likely to be misidentified sei whales. With regard to the true species identity of the aforementioned 100–50 Hz singlet downsweep series, it remains possible that they could originate from Antarctic minke whales, since the detection period of these downsweeps overlaps with the period during which their song was documented.

Although toothed whale species were not a focus of DPLUS082 (and the ST500 settings were not optimised for their detection), several opportunistic observations were made of odontocete clicks and whistles during the mid-frequency browse of the recordings for sei whale song. These included at least two likely occurrences of killer whales (*Orcinus orca*) on BS-Central on consecutive dates on the 4 and 5 January 2019.

7.4.4 Noise: a possible stressor and conservation implications

Berkeley Sound, together with wider Falklands' waters, clearly represents important habitat for both sei whales and southern right whales, as demonstrated by multiple lines of evidence (this Technical Report). Whales are acoustically-sensitive animals, which rely on sound for many aspects of their life history including navigation, social communication, and reproductive advertisement. The confirmation of song, associated with breeding activities, further highlights the importance of Falklands' waters for both species. Both feeding (sei whales in summer/autumn) and reproductive activity (both species in autumn/winter) are critical life history behaviours that are vulnerable to disturbance.

Anthropogenic ocean noise is increasingly considered to represent a stressor for marine mammal populations globally (Southall et al., 2008; Clark et al., 2009). The introduction of noise from large vessels has the potential to impact whale populations both directly, through disturbance that can cause displacement from important areas or cessation of important behaviours (e.g. feeding or breeding), or indirectly, by causing changes in prey and by masking important vocalisations such as breeding displays (Nowacek et al., 2007; Cholewiak et al., 2018). Although an assessment of noise was not a specific aim of this study, an overwhelming feature of the Berkeley Sound soundscape was the intermittent presence of vessel noise. Perhaps the most striking aspect of this soundscape was the dramatic difference between the quietness of the Sound when no vessel noise was present (possibly because of its semi-enclosed and sheltered nature) compared to extensive and severe noise that was introduced when a large vessel entered the Sound. Noise introduced into Berkeley Sound, particularly from large vessels such as cruise ships, tankers, or reefers, has the potential to disturb feeding or courting animals, and likely completely masks vocalisations (both social/contact calls and reproductive displays) given the semi-enclosed topography and shallow bathymetry of the Sound.

An example of an episode of extended noise introduction, and a consequent potential impact on whales, was identified during the examination of data from BS-Central and is shown in Figures 7.20 to 7.22. During mid-July 2019, detections of right whale upsweep calls exhibited a four-day gap in daily presence (Figure 7.15B) and an obvious two-week reduction in hourly presence (Figure 7.18B). This coincided with one of the more dramatic noise events that was opportunistically noted while reviewing the data. At ~16:00 on 4 July 2019, a large vessel entered Berkeley Sound and apparently laid anchor or moored for an extended period. There was near constant vessel noise introduced for 16 days until noise levels decreased substantially at ~18:00 on 20 July 2019, and remained reduced for the remainder of the month with the exception of short transient noise events (Figure 7.20). In addition to this near constant elevated background noise, during the period from 14 to 20 July there were also numerous instances of passing vessel noise (see Figure 7.21 for detail of 19 July), as evidenced by the characteristic noise pattern made by vessels at their Closest Point of Approach (CPA). The following ship traffic was logged in Berkeley Sound by FIG FishOps over this period:

1. Tanker *Marlin* visited Berkeley Sound at 15:15 on 4 July 2019, and left to move to Port William on 16 July 2019;
2. Trawler *Sejong* visited at 16:28 on 15 July 2019 and left at 16:37 on 17 July 2019;
3. Reefer *Avadu* visited at 13:17 on 17 July 2019 and left at 18:34 on 20 July 2019; and
4. Tanker *Sea Lion* visited Berkeley Sound at 16:48 on 17 July 2019, and left at 17:50 on 20 July 2019.

Therefore, the observed noise on the ST500s was contributed by several different vessels with overlapping presence in the Sound, and each of the above arrivals and departures can be seen in Figure 7.20. The numerous CPAs between 14 and 20 July (depicted for 19 July in Figure 7.21) are likely due to movements of launches servicing these vessels, or manoeuvres of the ships themselves while inside the Sound. The average hourly received noise level at BS-Central (measured in the 50–200 Hz communication band of southern right whale upsweeps, and reported in dB RMS re: 1µPa) varied by more than 50 dB during this period, with lows of ≤80 dB both before (on 1 and 4 July), and after (on 20, 21, 24 and 25 July) the noise event, and a peak of 130 dB during the event (at 09:00 on 19 July: Figure 7.22A). The daily median of hourly noise levels was elevated by ~15–25 dB during the entire 16 day period when the vessels were present (Figure 7.22A). Since every 6 dB increase represents a doubling of sound pressure levels, these observations represent a dramatic variation in ambient noise level.

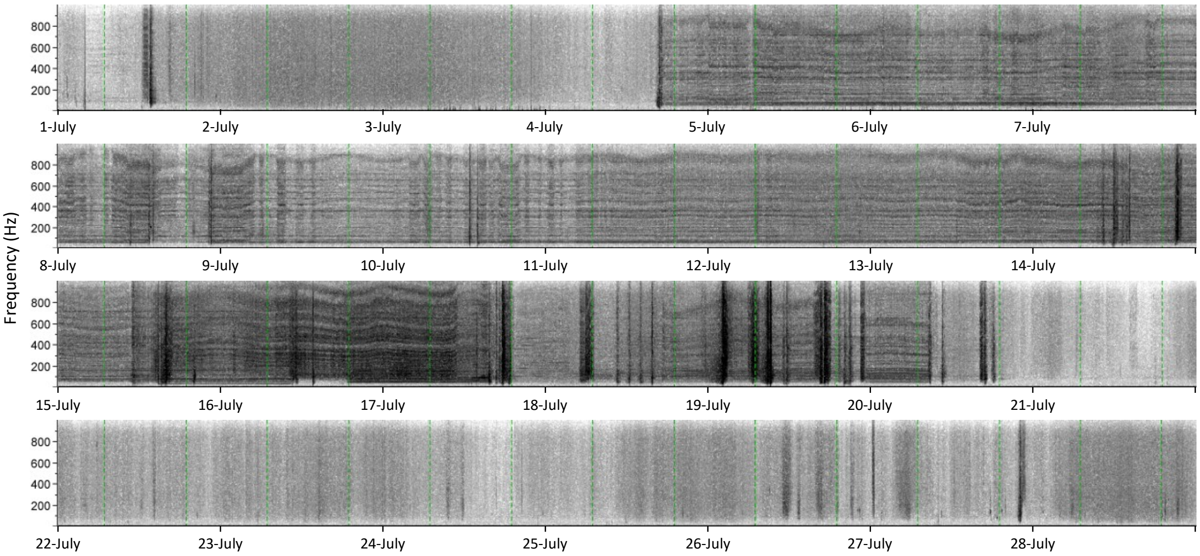

***Figure 7.20**. Continuous spectrogram of four weeks of recording at BS-Central from 1 to 28 July 2019. This example illustrates the near constant presence of ship noise from 16:00 on 4 July to 18:00 on 20 July (appearing as continuous wavy horizontal lines), and numerous more acute noise instances associated with the Closest Point of Approach (CPA) of passing vessels (appearing as dark vertical lines at this time scale). These days are contrasted with the relatively quieter periods prior to 4 July and after 20 July, for which there are only short transient CPA events evident in the spectrogram.*

During this time there was an obvious change in the acoustic detections of southern right whales in Berkeley Sound, most clearly apparent at BS-Central and BS-Inner, and illustrated in detail in Figure 7.22. At BS-Central, the hourly detections of right whale upsweep calls began to decline after 6 July, reducing from confident detections on >12 hr/day to possible detections on only a few hr/day by 9 July, and then to no detections at all from 11 to 14 July (Figure 7.22A). Thereafter, there were few confident hourly detections until 20 July, after which the number of hours with confident detections steadily rose following the departure of the vessel from <12 hrs/day on 22 July to >20 hrs/day by 27 July. This trend was even more apparent on the BS-Inner site with no vocalisations detected from 9 to 15 July, or from 19 to 20 July (Figure 7.22B).

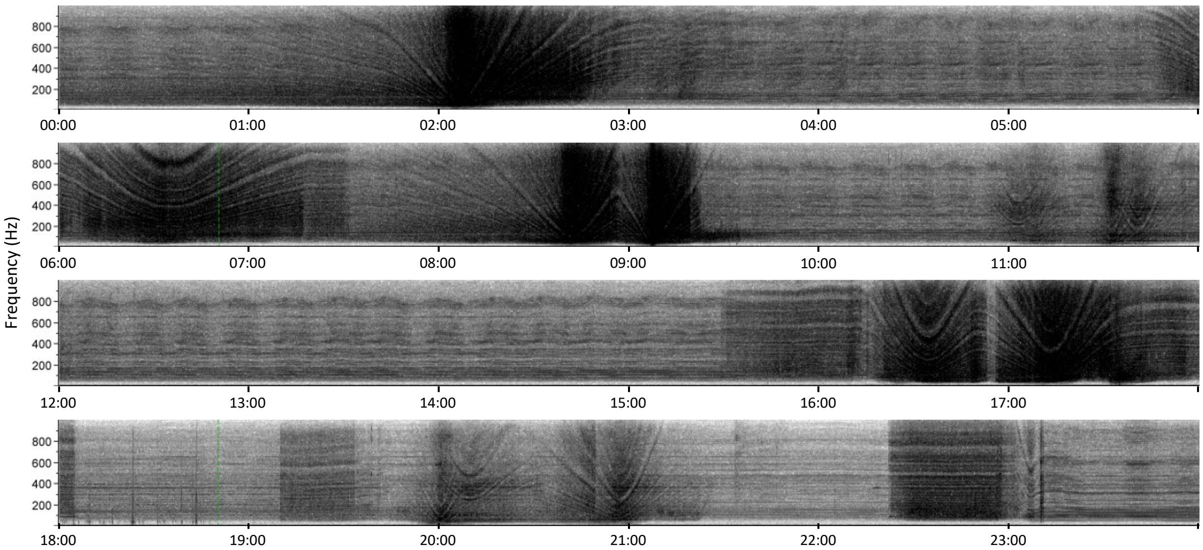

Figure 7.21. *Detailed spectrogram of 24 hours on 19 July 2019, illustrating the constant background noise of a large vessel, and several CPAs as boats of variable loudness passed by the recorder (shown as areas of intense black), with the characteristic U-shaped frequency pattern caused by Doppler shift effects.*

It is not possible to unequivocally attribute this reduction in right whale detections to acoustic disturbance due to the presence of the vessel noise. However, the correlation of the events is noteworthy. Nor is it possible to know whether the reduction in detections was due to a true reduction in the number of calls emitted (either because animals left the area or stopped vocalising) or whether it was due to masking of calls by vessel noise such that the detector became less effective. Given the observed progressive reduction in detections over 5 days from 4 to 9 July after noise levels had risen, and the detection of some calls between 15 and 20 July when noise levels were at their highest (Figure 7.22), it seems likely that the trend represents a real change in the behaviour of the animals and not simply masking of calls. This is also suggested by the gradual increase of vocal activity over several days after the noise event ended on 20 July (Figure 7.22). In addition to a behavioural change, there was also likely at least some masking of low SNR vocalisations that would otherwise have been detected during quieter periods, combining to create the observed pattern. In either case, it represents a potential impact on the right whales within the Sound, either by displacement, reduction in vocal activity, or impeding communication among conspecifics. Importantly, these observations warrant a longer-term focussed and quantitative assessment of the soundscape of Berkeley Sound in relation to the occurrence of sensitive whale species.

Irrespective of measurable impacts, it is clear that whales inhabiting the relatively confined space of Berkeley Sound are regularly being exposed to high noise levels from vessels within the Sound. Frankel and Gabriele (2017) showed substantial exposure of humpback whales to vessel noise from cruise ships in Glacier Bay, Alaska, after evaluating a number of different management scenarios in an effort to reduce cumulative sound exposure levels. Parks et al. (2007) demonstrated changes in vocal behaviour of North Atlantic and southern right whales in response to elevated levels of low frequency noise. And Rolland et al. (2012) suggested that exposure to vessel noise elicited a physiological stress response in North Atlantic right whales in the Bay of Fundy, Canada; they demonstrated that only a 6 dB decrease in underwater noise related to reduced ship traffic resulted in a measurable reduction in stress-related faecal hormone metabolites. These same potential impacts exist in Berkeley Sound for sei and right whales, and need to be considered in context of effective management of these populations, particularly given the recognition of Falklands Inner Shelf Waters as a globally-important Key Biodiversity Area for endangered sei whales.

(A)

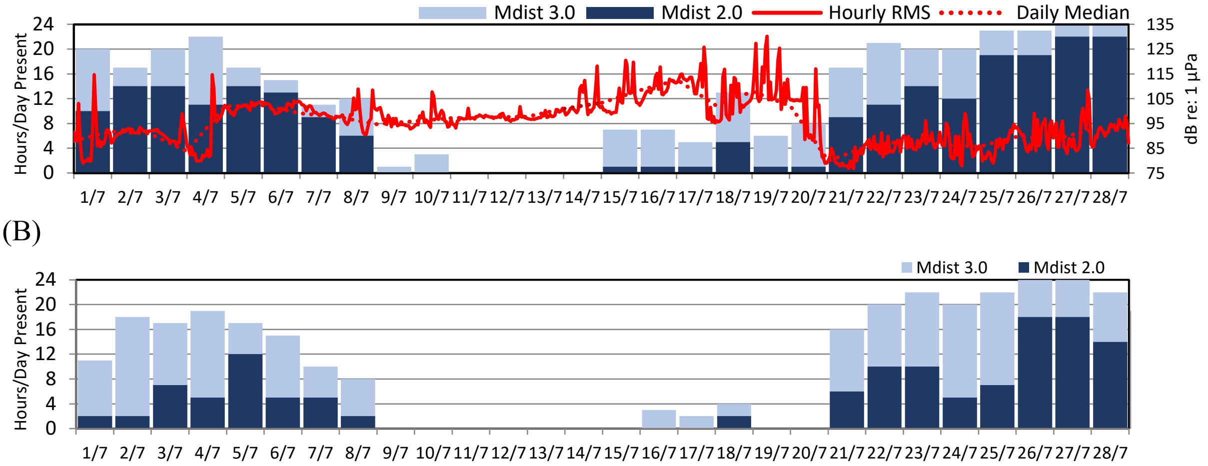

Figure 7.22*. Detail of hourly presence of southern right whale upsweep calls (blue bars, hr/day) and received noise levels in the communication band of southern right whales (red line, dB RMS re: 1µPa) from 1 to 28 July 2019 (corresponding with the noise event in Figure 7.20) at: (A) BS-Central; and (B) BS-Inner. See Figure 7.18B for explanation of hourly presence data and complete depiction of 2019 recordings. Noise levels are represented as the hourly RMS (root mean square, or average received level) for each hour, and the daily median of the hourly RMS for each day, in the 50-200 Hz frequency band of right whale upsweeps.*

7.4.5 Conclusions and recommendations

To conclude, the documentation of the spatio-temporal occurrence of vocal activity adds an important component to understanding the occurrence of sensitive baleen whale species in the Falkland Islands, and is highly useful for recommending informed conservation measures. PAM is also informative for improving understanding of potential acoustic disturbance on these populations. The dataset collected during DPLUS082 is rich and extensive, and the analyses presented here represent only the initial possible outputs. Recommendations for ongoing and/or additional analyses include:

1. Completion of the spatio-temporal analysis for southern right whales by incorporating the fourth and fifth Berkeley Sound deployments (i.e. the second year of data);
2. Improvement of the LFDCS automated detector and classifier for southern right whales by designing a new call library using data from the Falkland Islands, and the inclusion of a broadband detector for gunshot calls in addition to contour trace detector for upsweep calls;
3. Analysis of the dataset from the Falkland Sound site (data collection still underway), using the same tools for comparison to the Berkeley Sound dataset;
4. Completion of a more detailed analysis to describe sei whale and southern right whale song; and
5. An assessment of the potential for acoustic masking and disturbance in Berkeley Sound and elsewhere in the Falkland Islands, with consequent recommendations for managing anthropogenic noise to better conserve whale populations. This would best be achieved through additional targeted analyses on the existing dataset, to quantitatively measure noise levels throughout the recording period, correlate noise measurements with records of vessel traffic (i.e. with harbour/vessel logs and/or AIS data), and further assess the vocal behaviour of whales in relation to vessel traffic and measured noise. The July 2019 example illustrated above was one of multiple cases where a reduction in vocal activity appeared to be correlated with the

introduction of vessel noise. A detailed quantitative analysis using a multivariate approach may help to tease apart the effect of anthropogenic noise levels from other environmental covariates (e.g. Cerchio et al., 2014). Careful consideration of vocalisations recorded during periods of quiet compared to periods of noise, and controlling for both SNR and received level, will allow a distinction to be made between the effects of masking and a true reduction in vocal activity. In this way, inferences could be made on the potential impacts of noise on animals using the Sound, which may inform future management plans and facilitate the introduction of measures to reduce noise levels (e.g. through adjustments in vessel speeds, number of vessels, and vessel activity schedules) during periods of peak whale sensitivity.

7.5 Acknowledgements

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Analysis: We are extremely grateful for the support provided by the NOAA Northeast Fisheries Science Center, including Genevieve Davis, Danielle Cholewiak, Nicole Pegg, and Sofie Van Parijs, for their guidance in the use of LFDCS, to NOAA Hollings Scholar Julie Xu for assistance with acoustic results verification, and to Mark Baumgartner of the Woods Hole Oceanographic Institution for making LFDCS available for our use.

7.6 References

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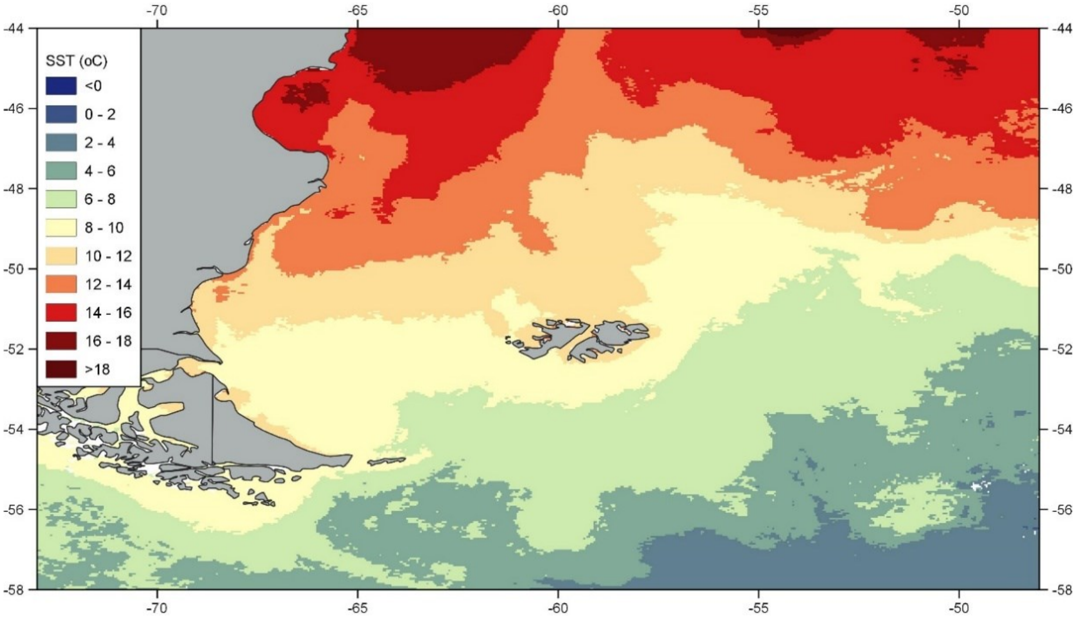
Annex 1: Sea surface temperatures around the Falklands in 2019–2021

Mapped sea surface temperature (SST) is derived from MODIS (MODerate Resolution Imaging Spectroradiometer), and presented as daytime monthly composites of SST at 4.63 km spatial resolution.

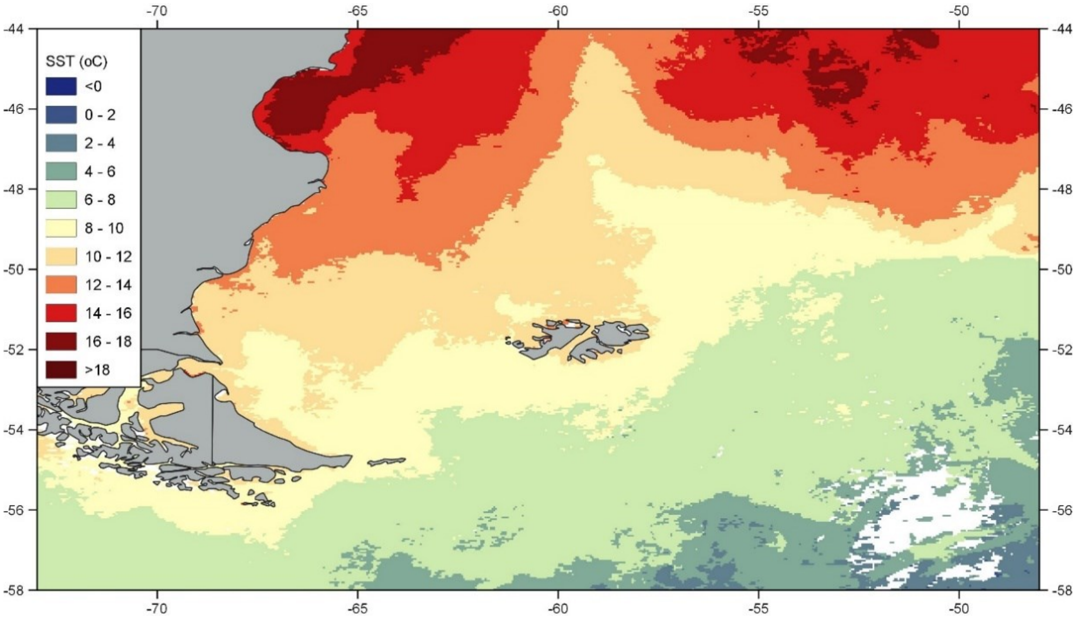
In the maps below, each months average SST is presented for each of the years of the DPLUS082 project (Jan 2019 to June 2021).

JANUARY

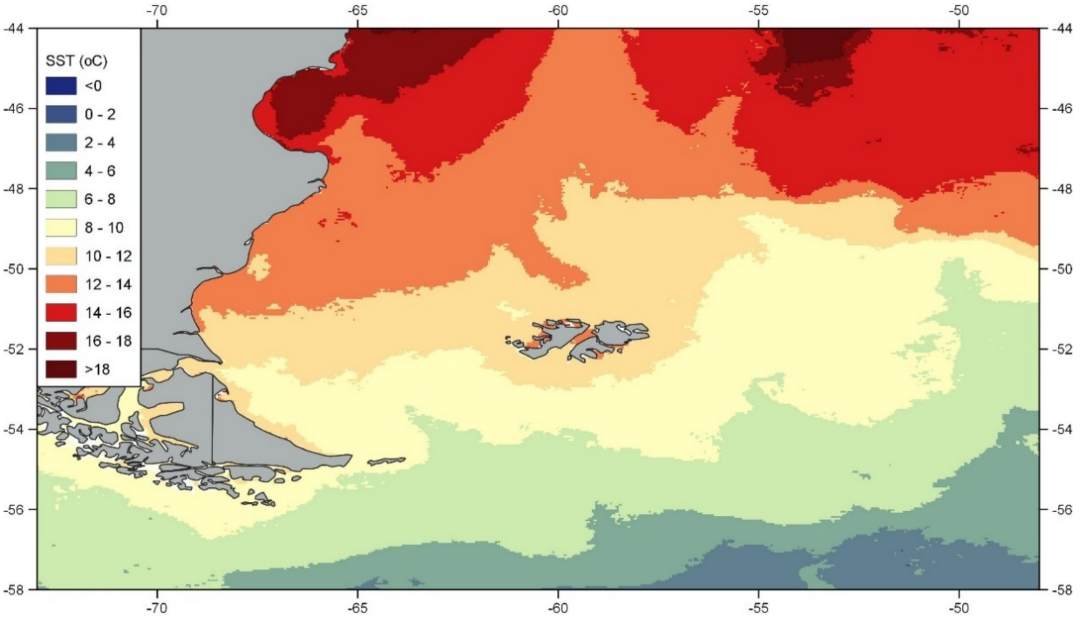
2019



2020



2021



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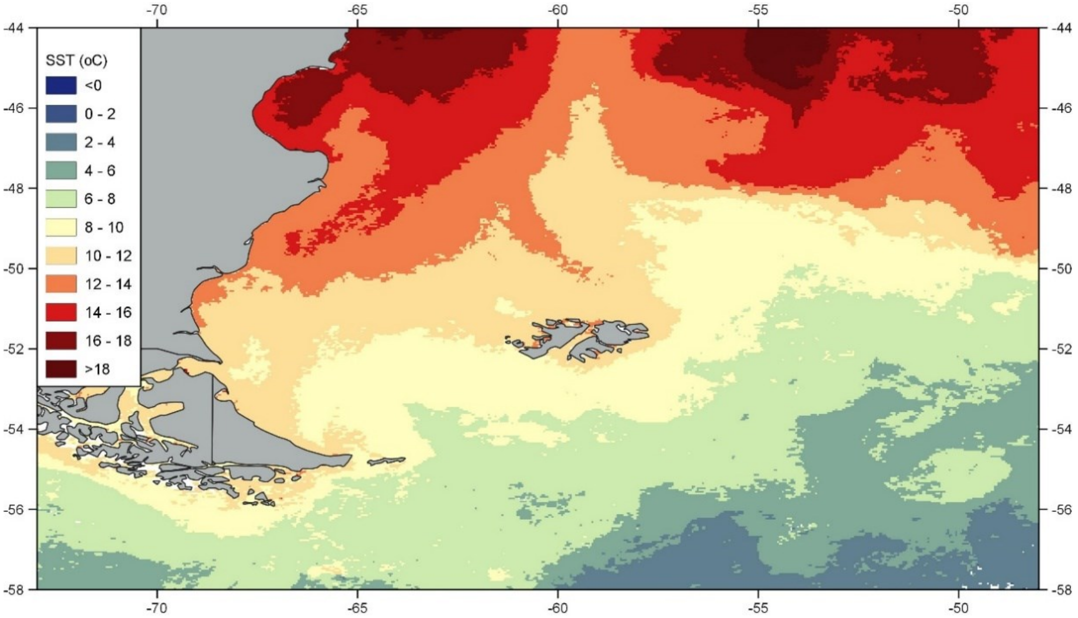

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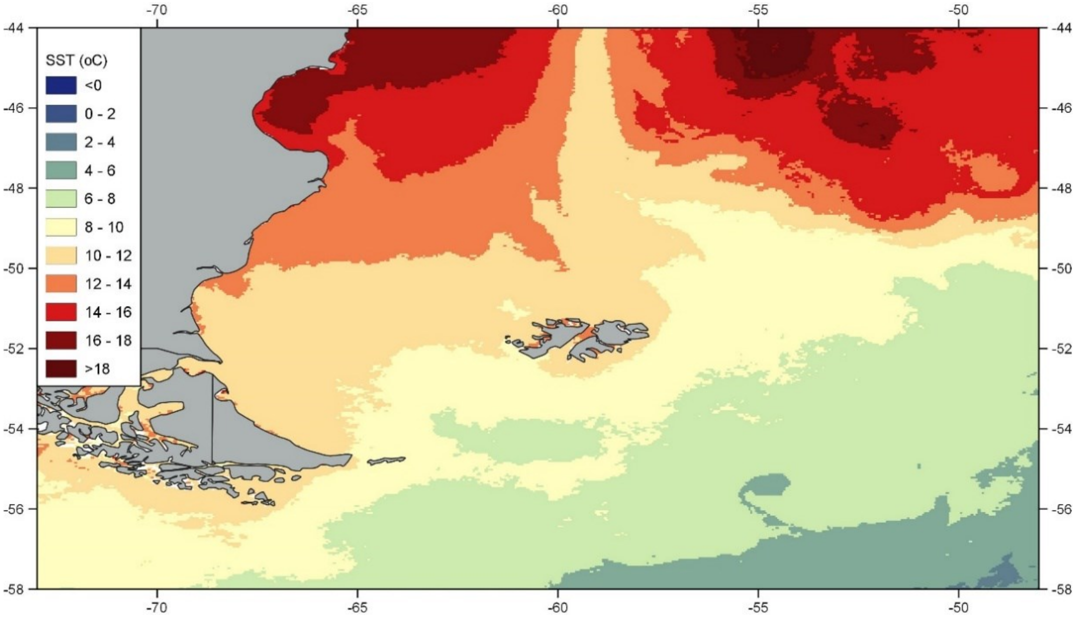
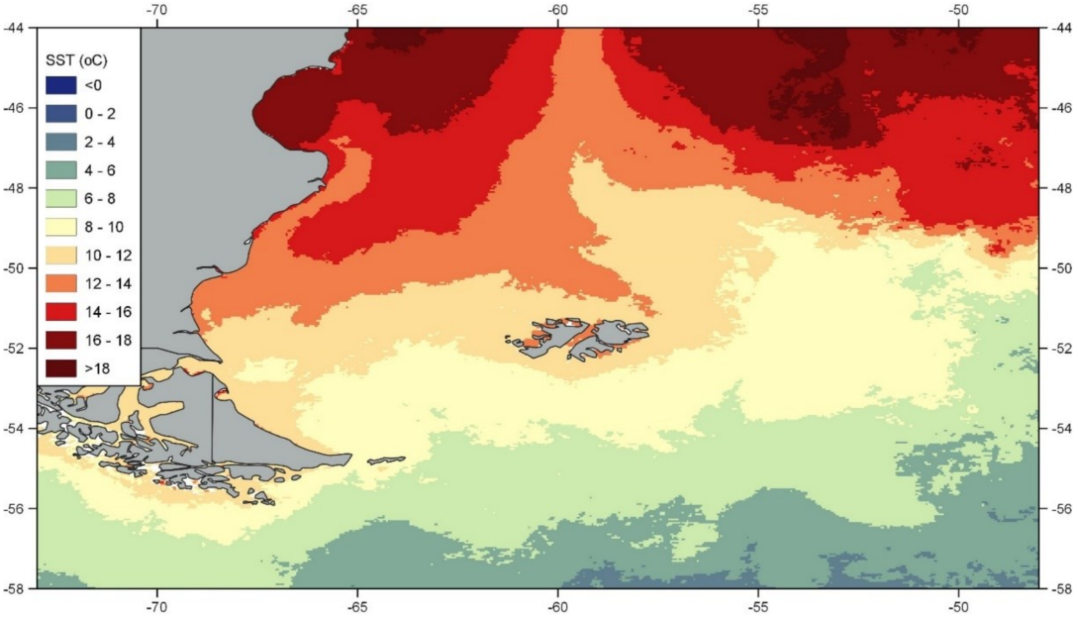


2021



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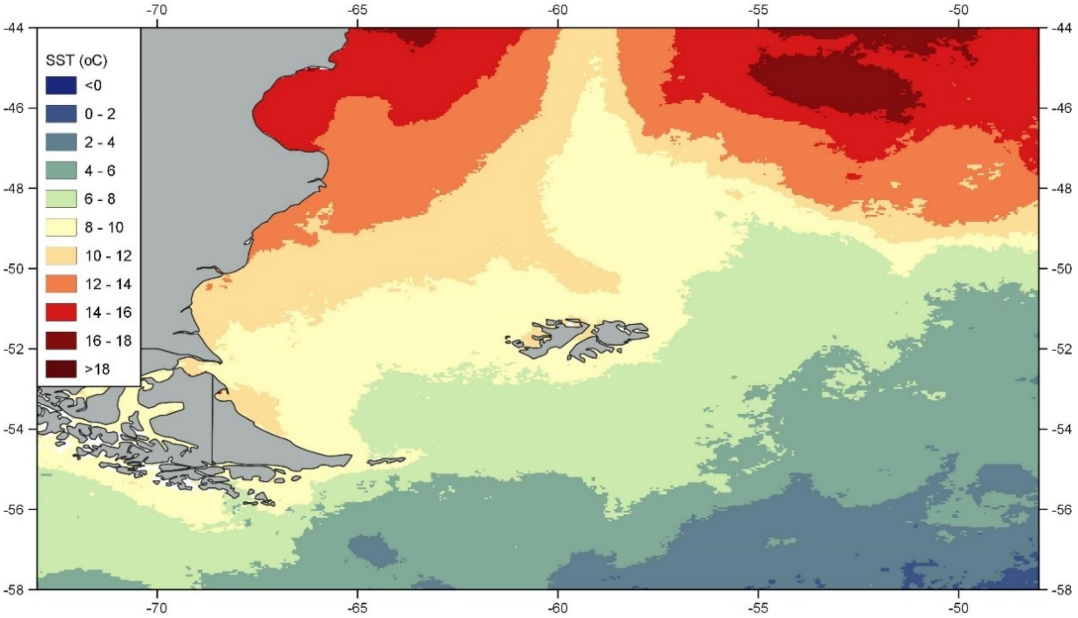
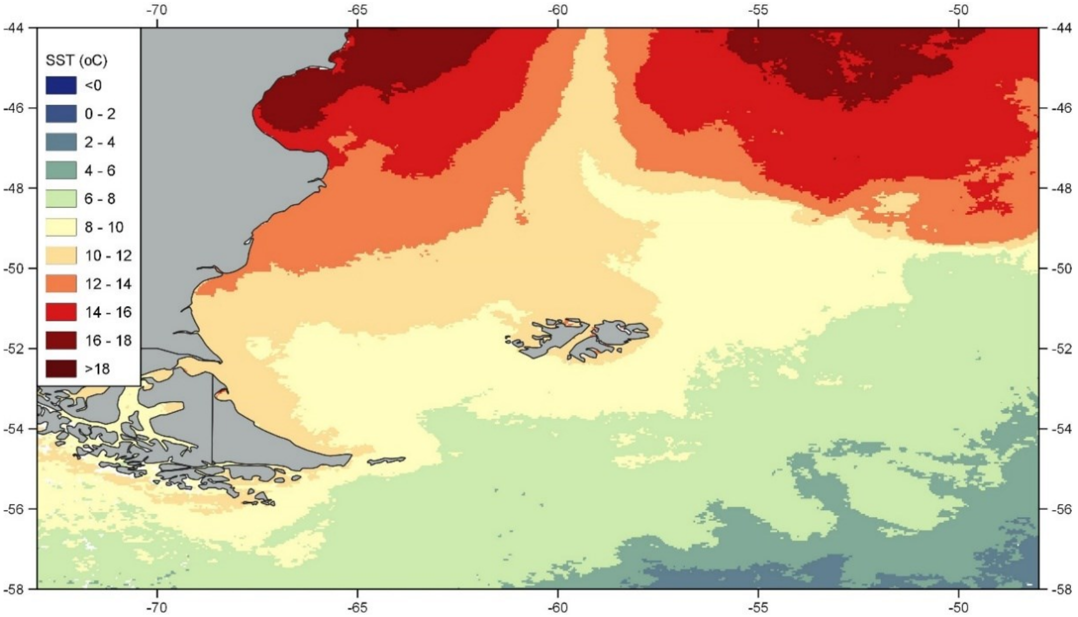
2019



2020



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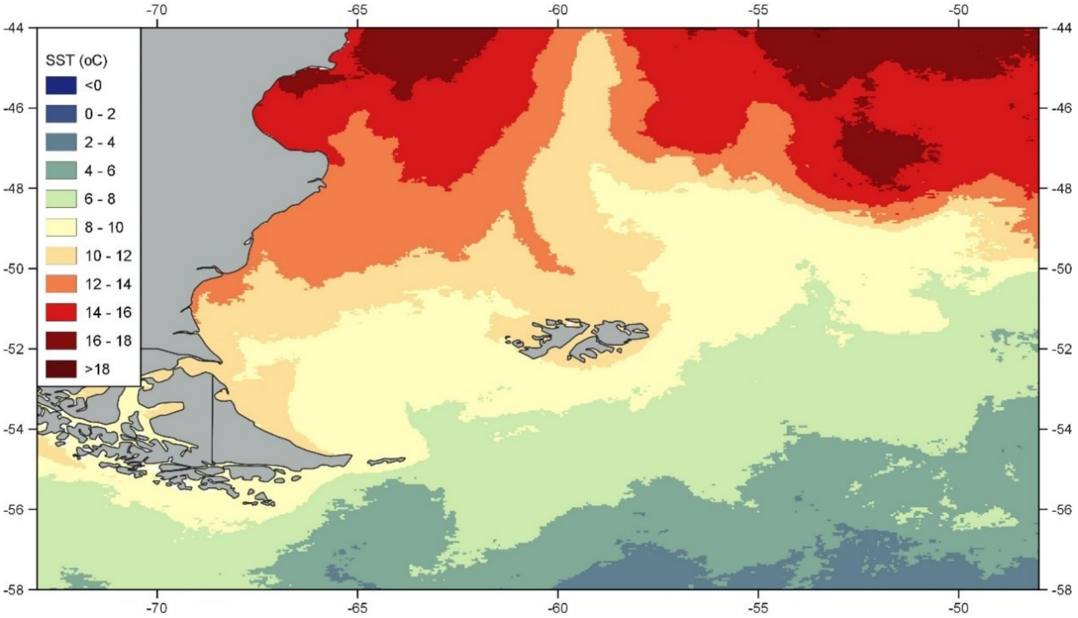

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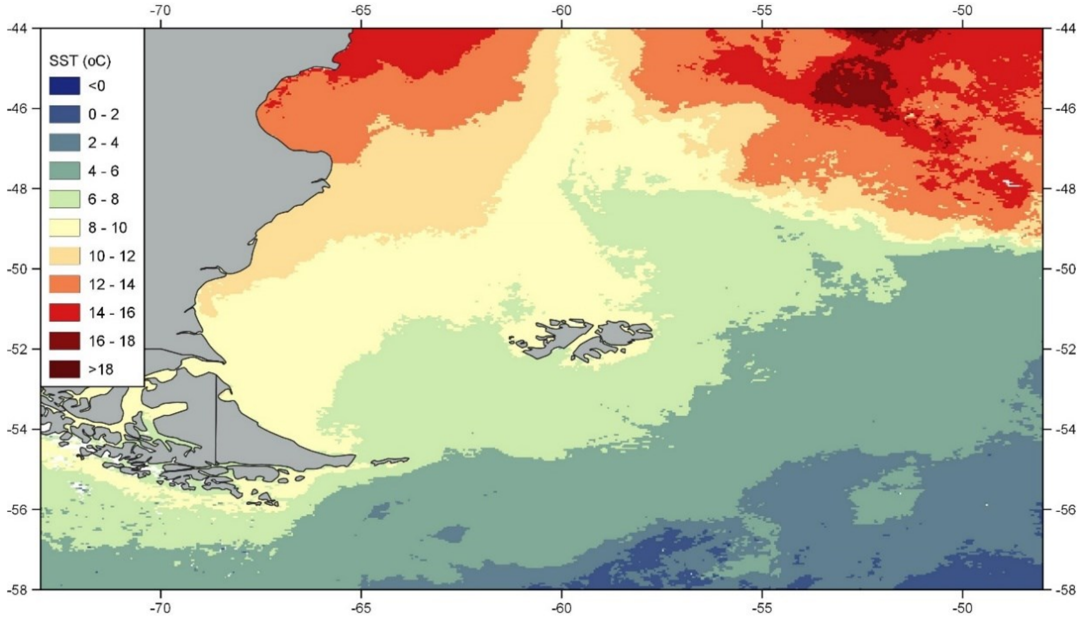

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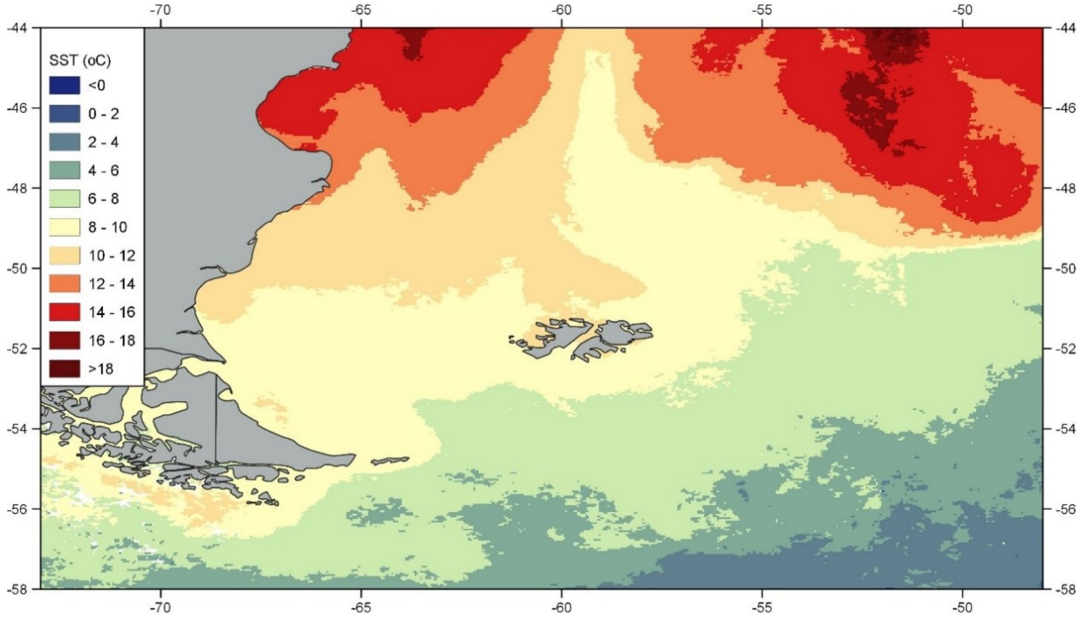

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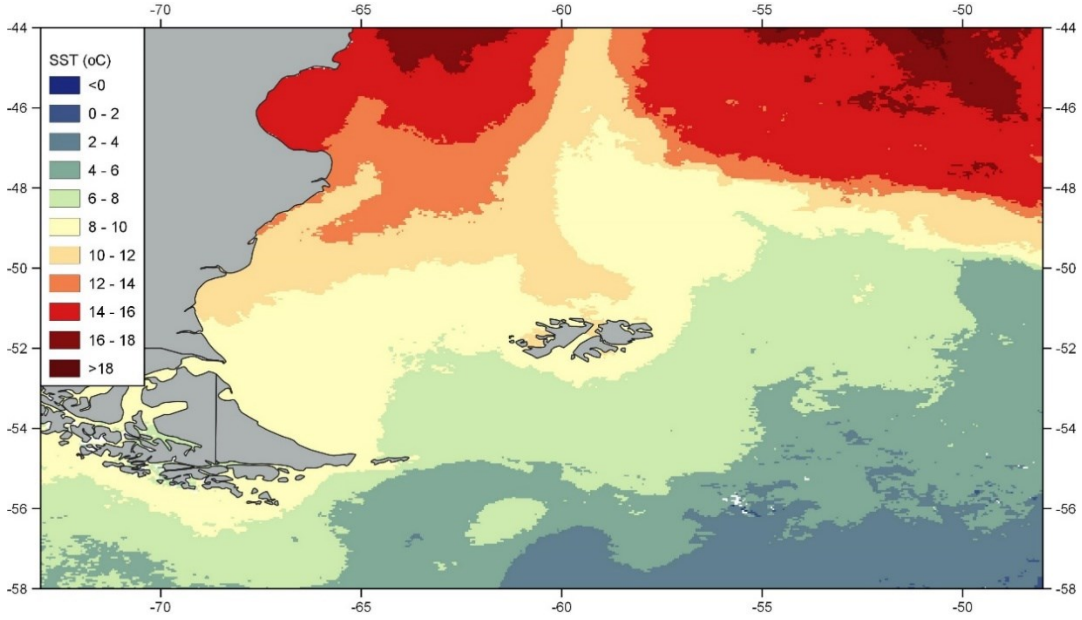

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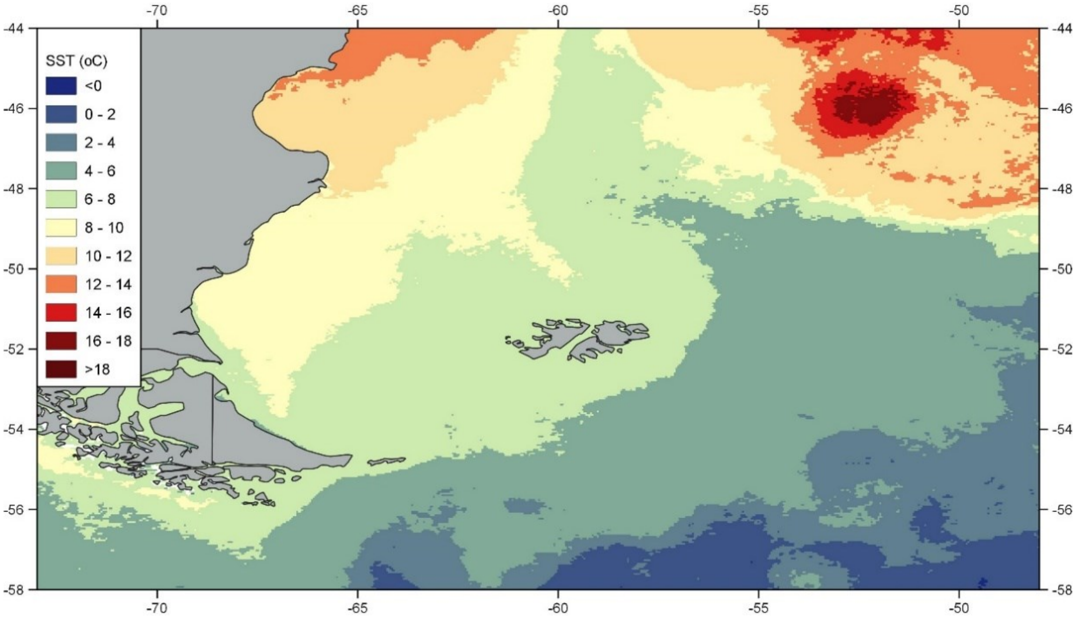

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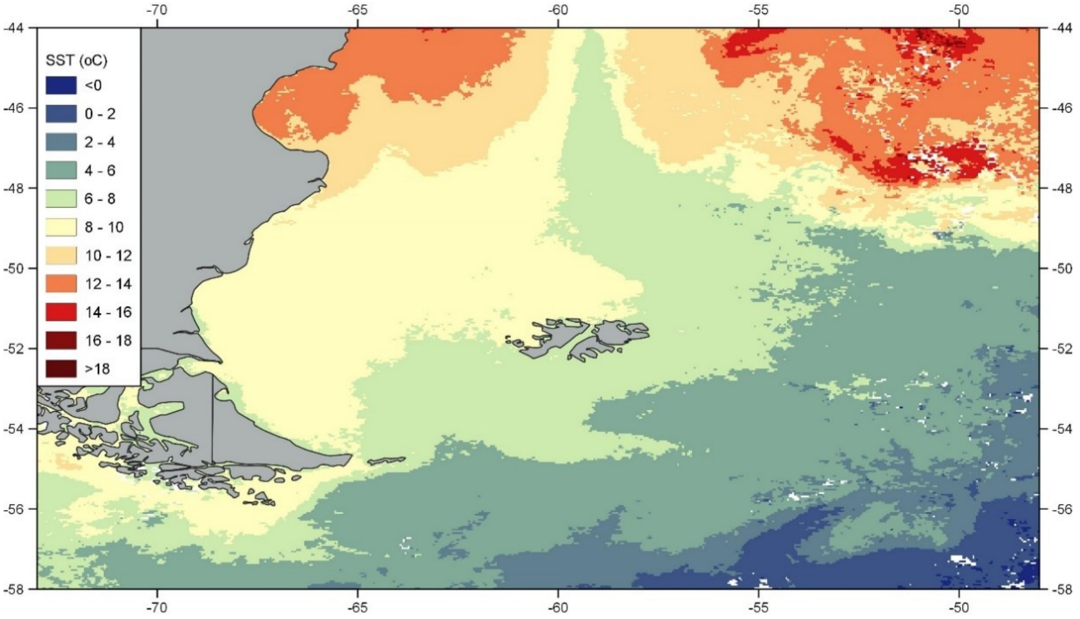

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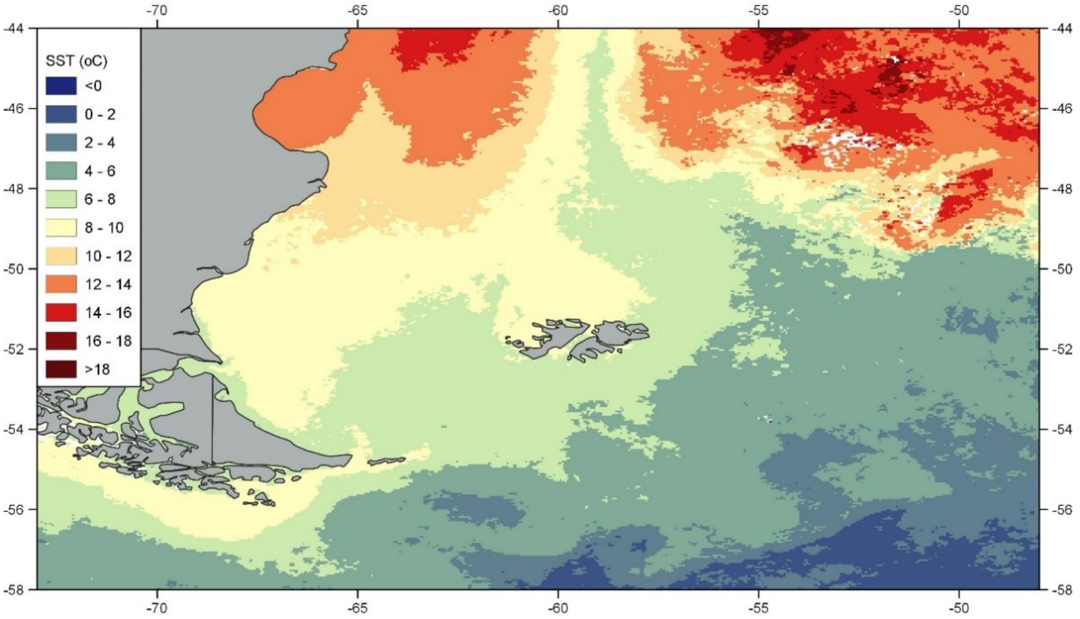

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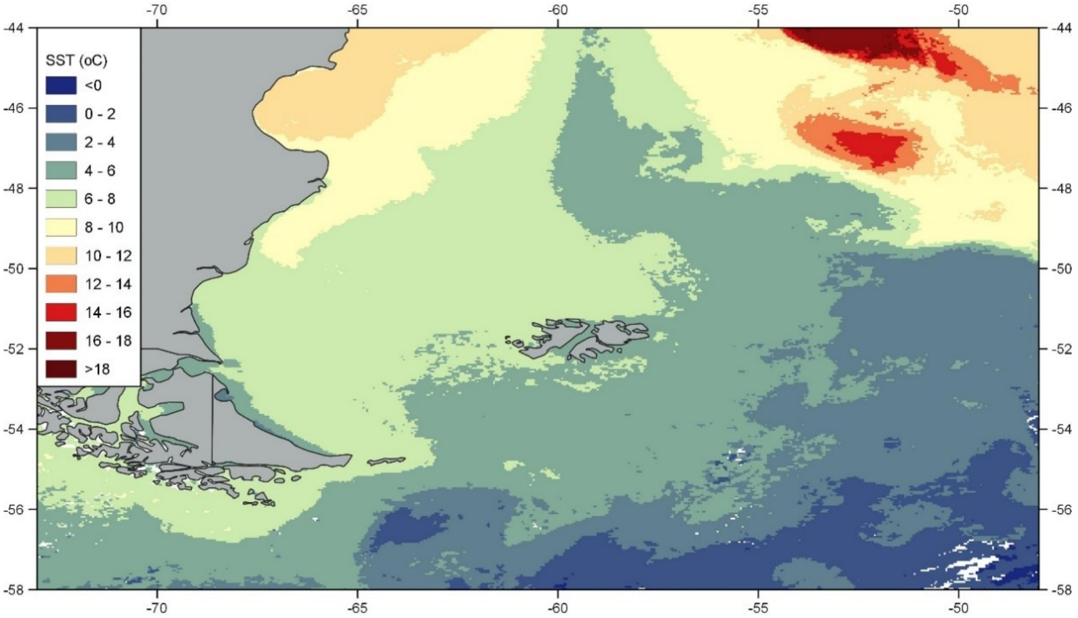

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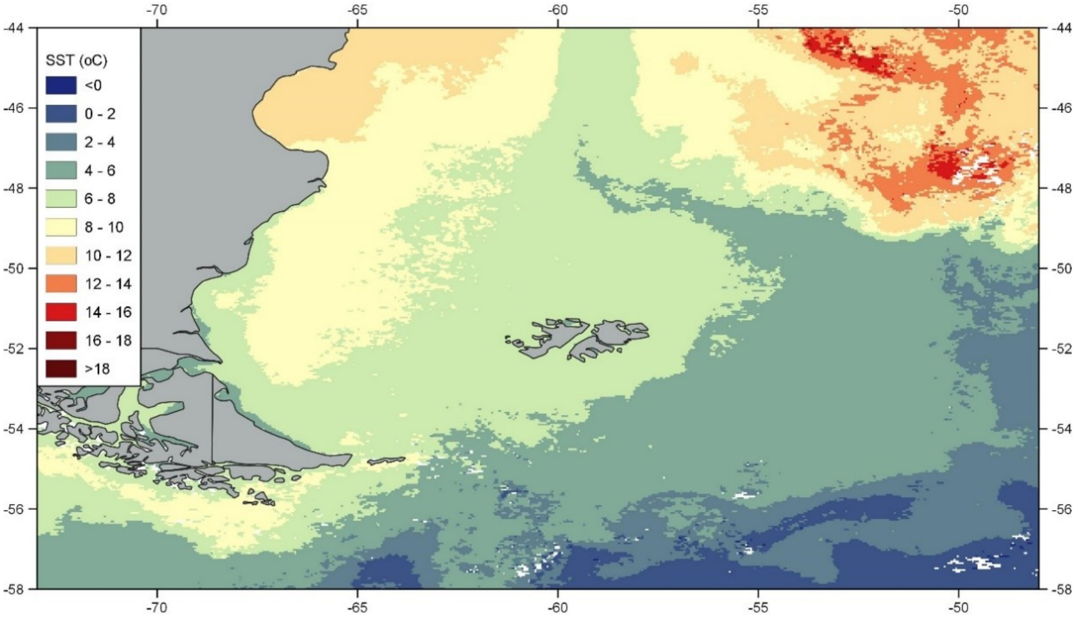

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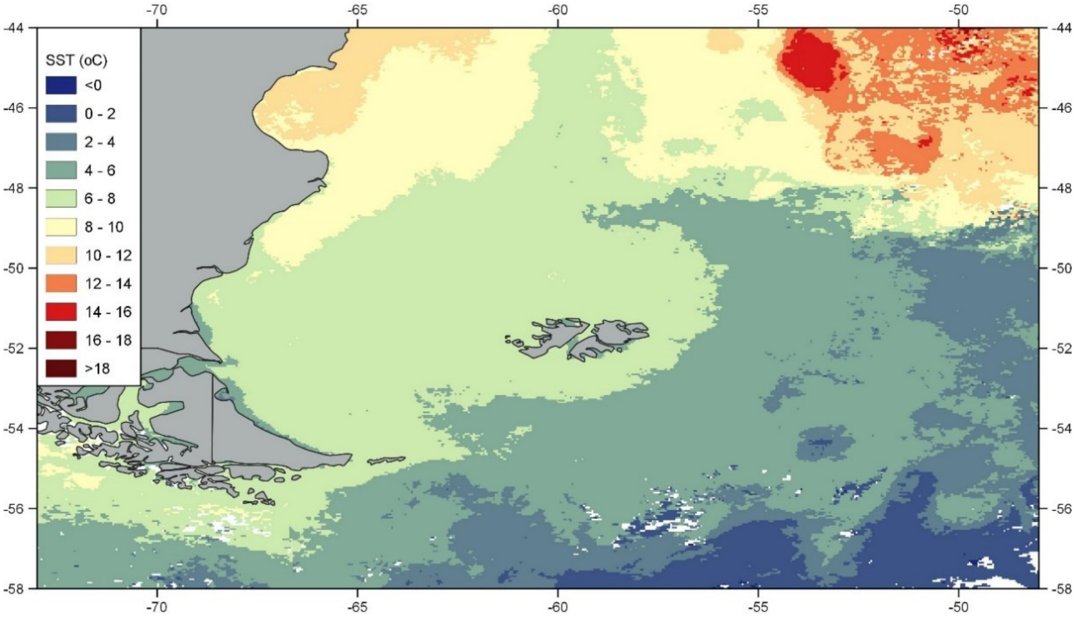

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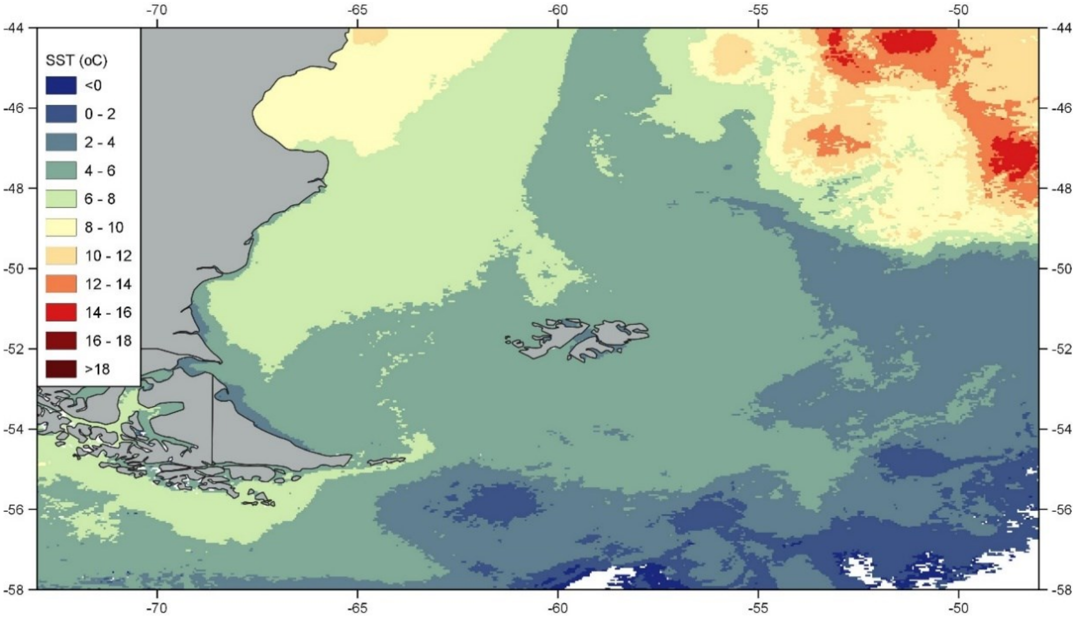

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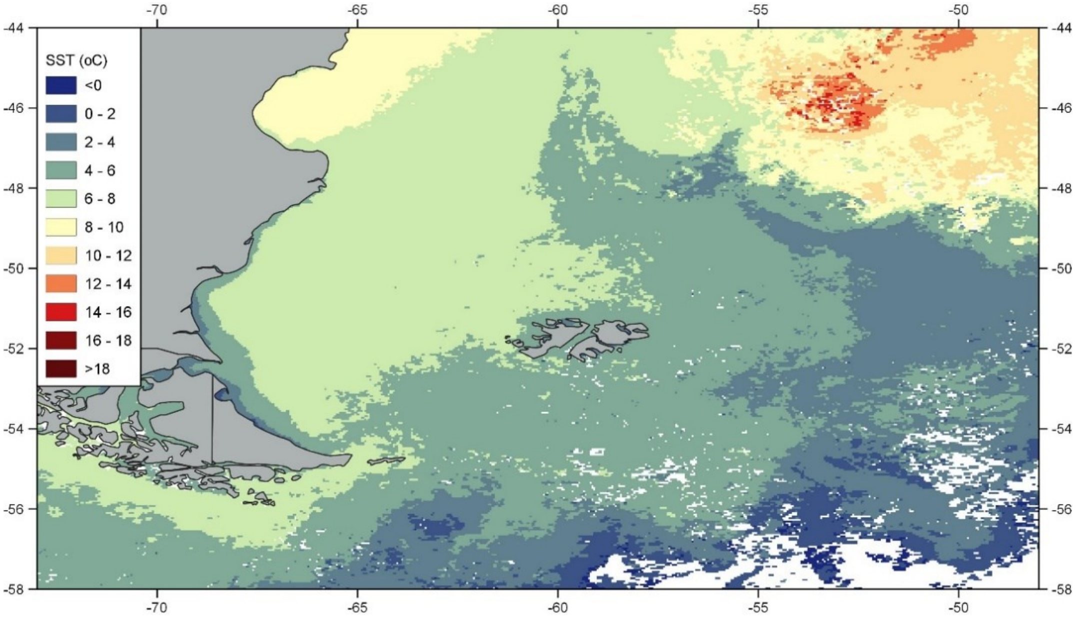

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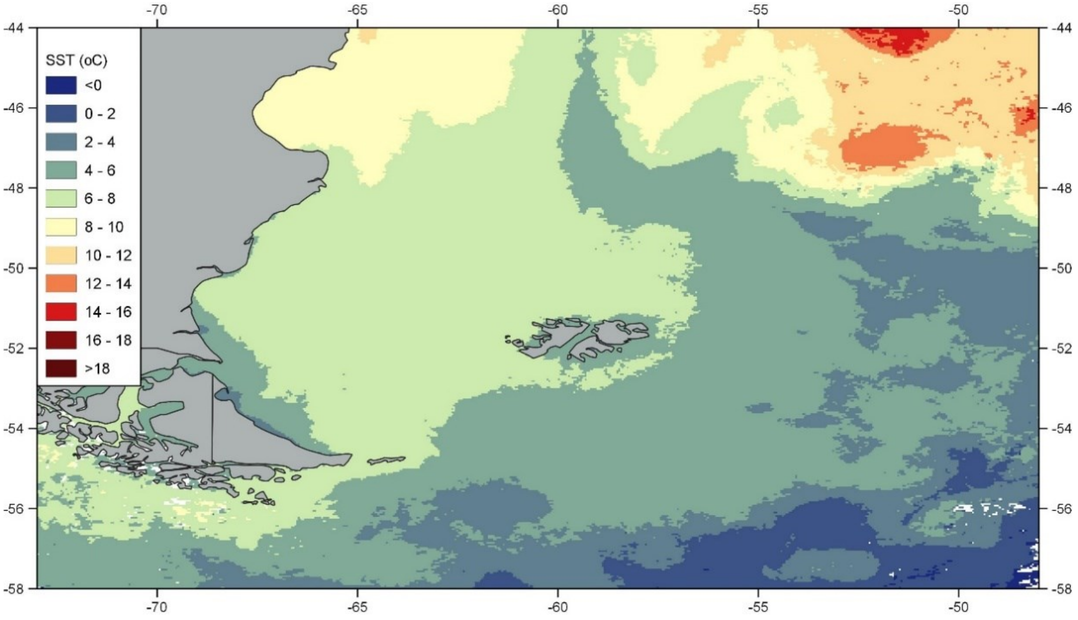

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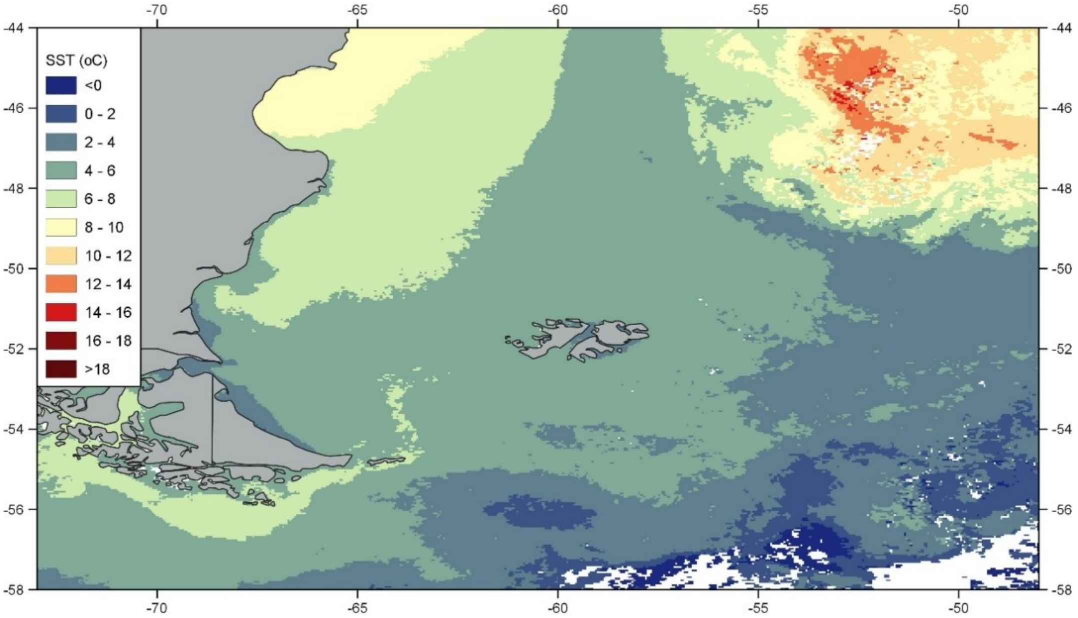

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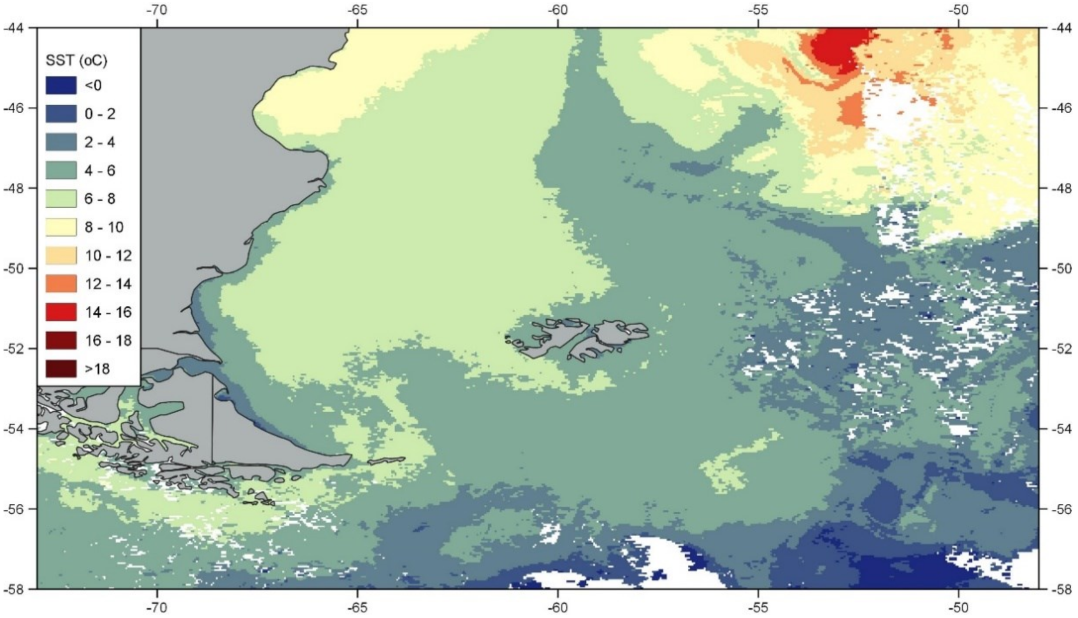

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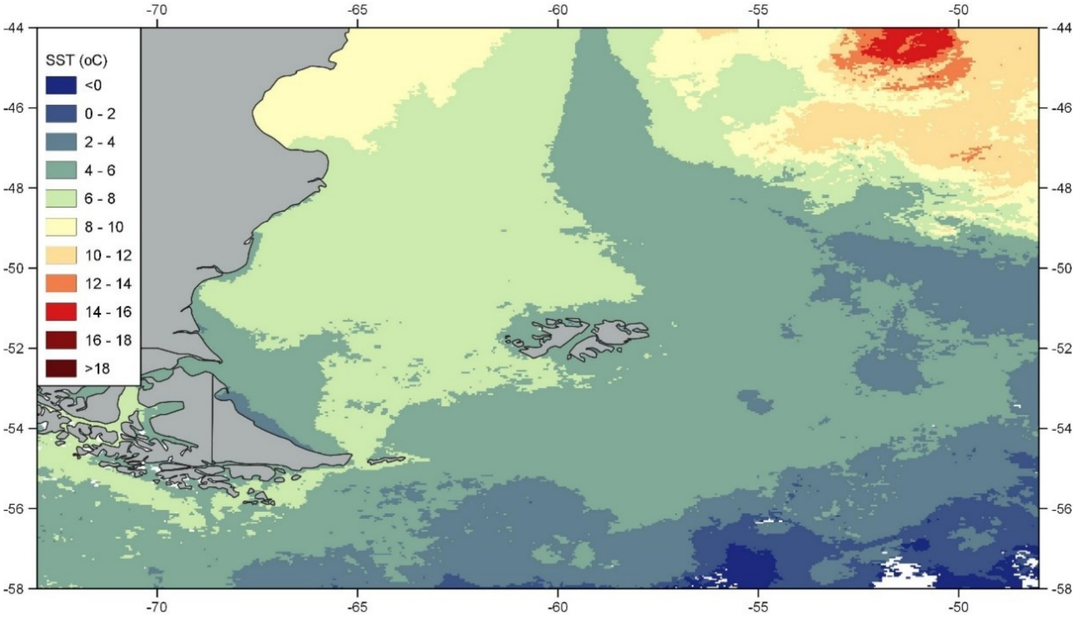

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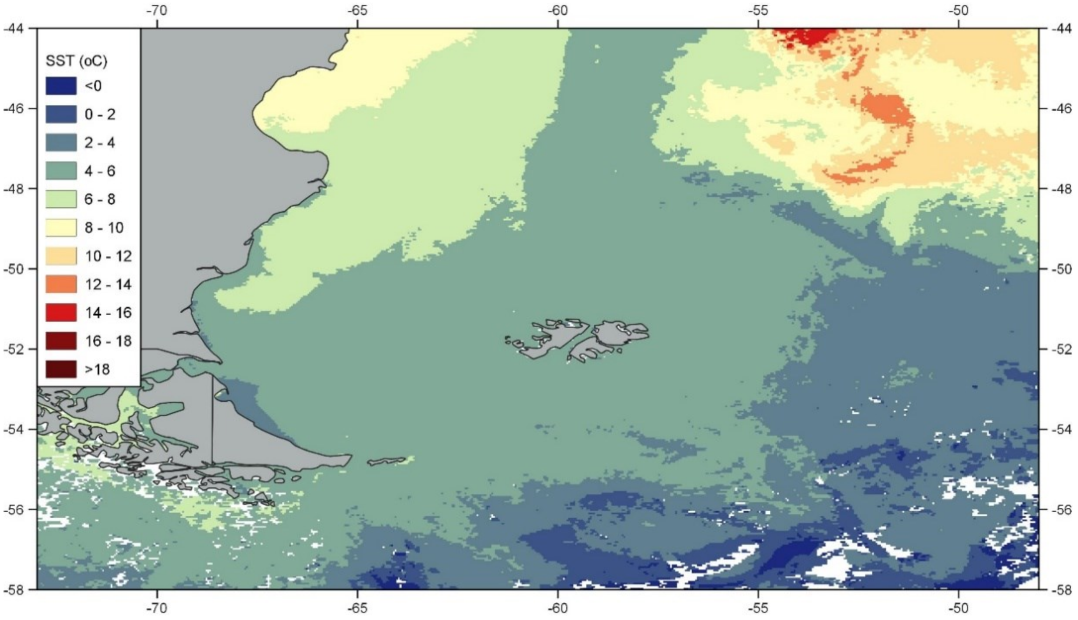

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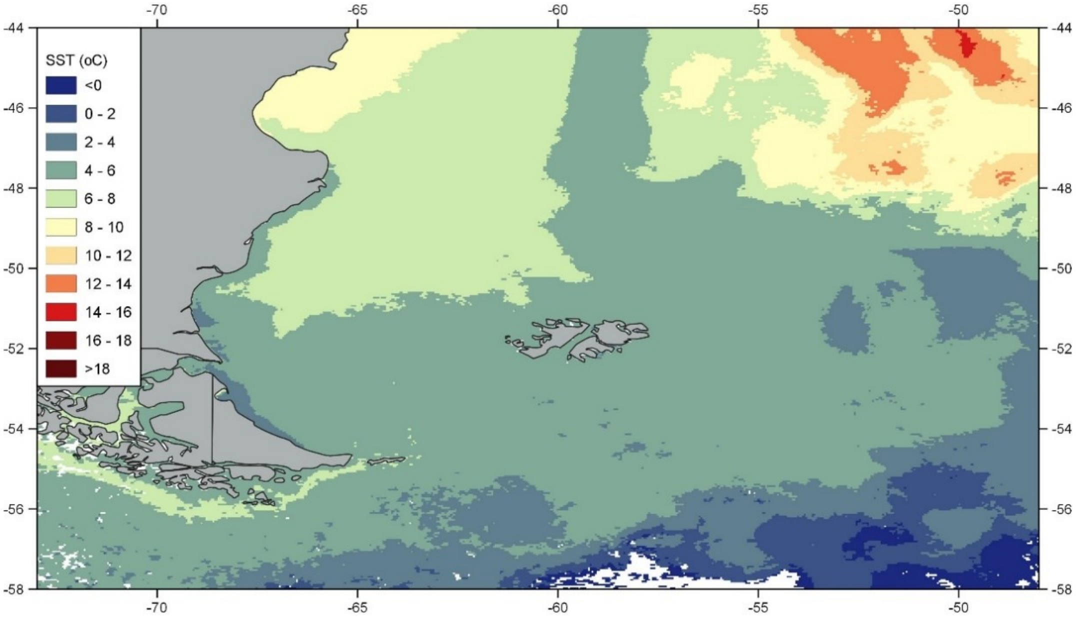

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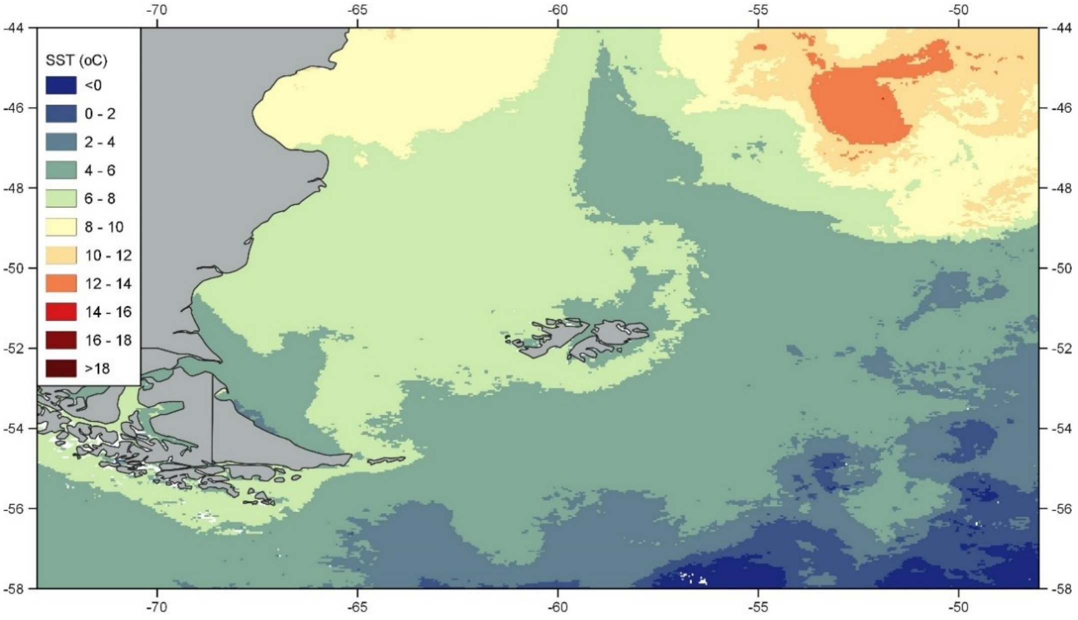

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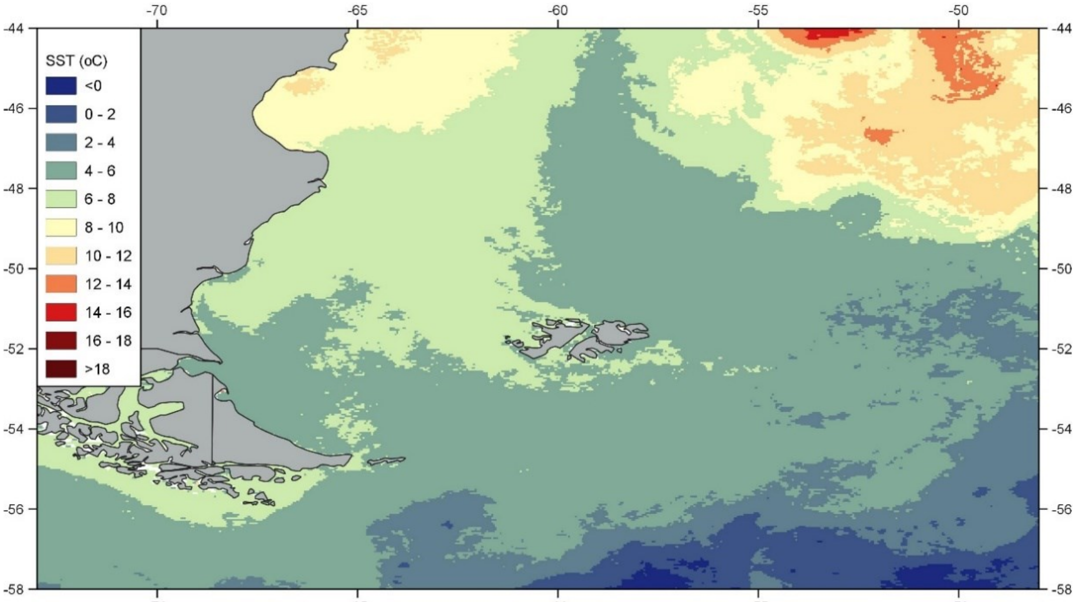
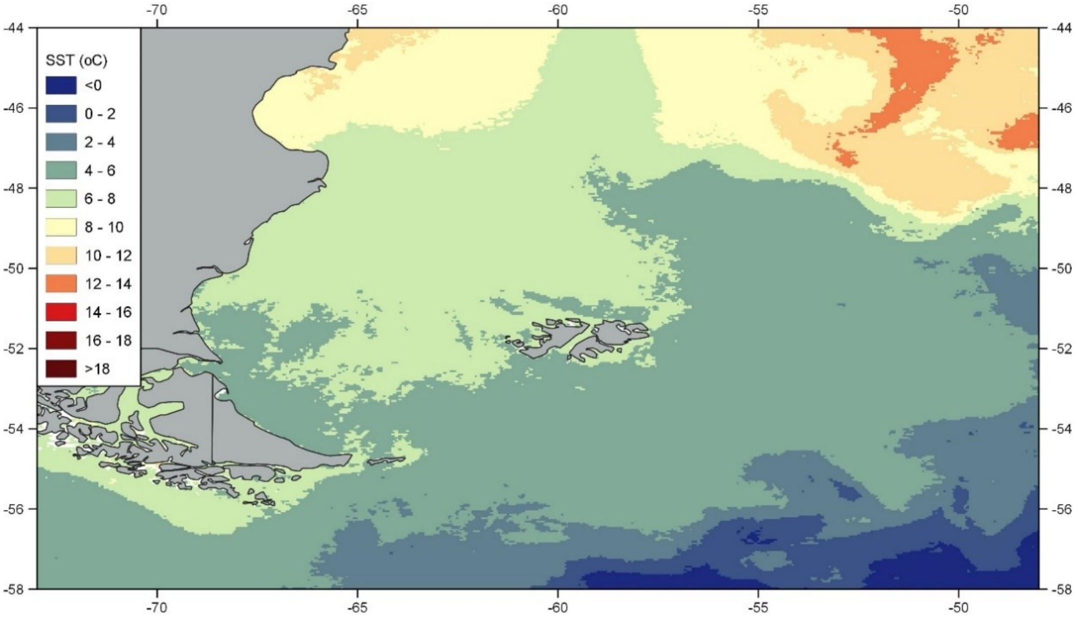
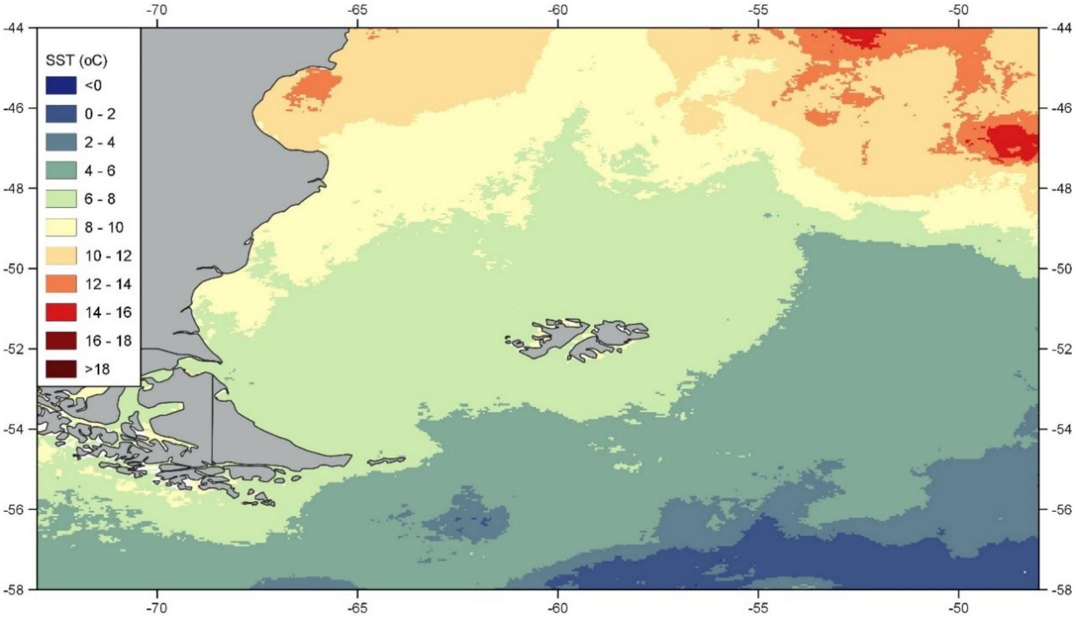


2020

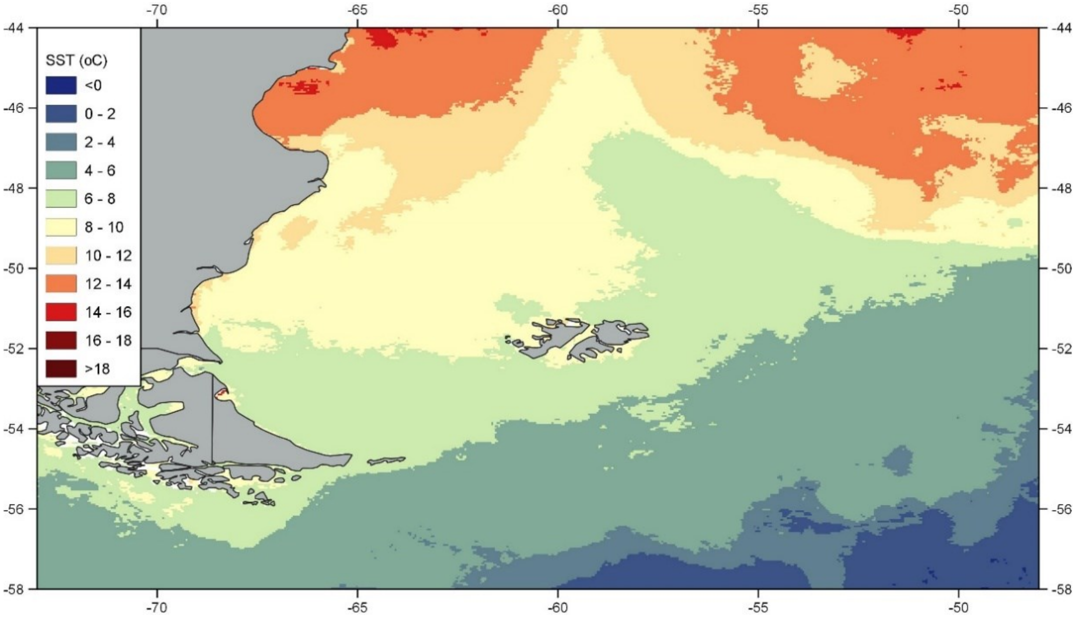


NOVEMBER

2019

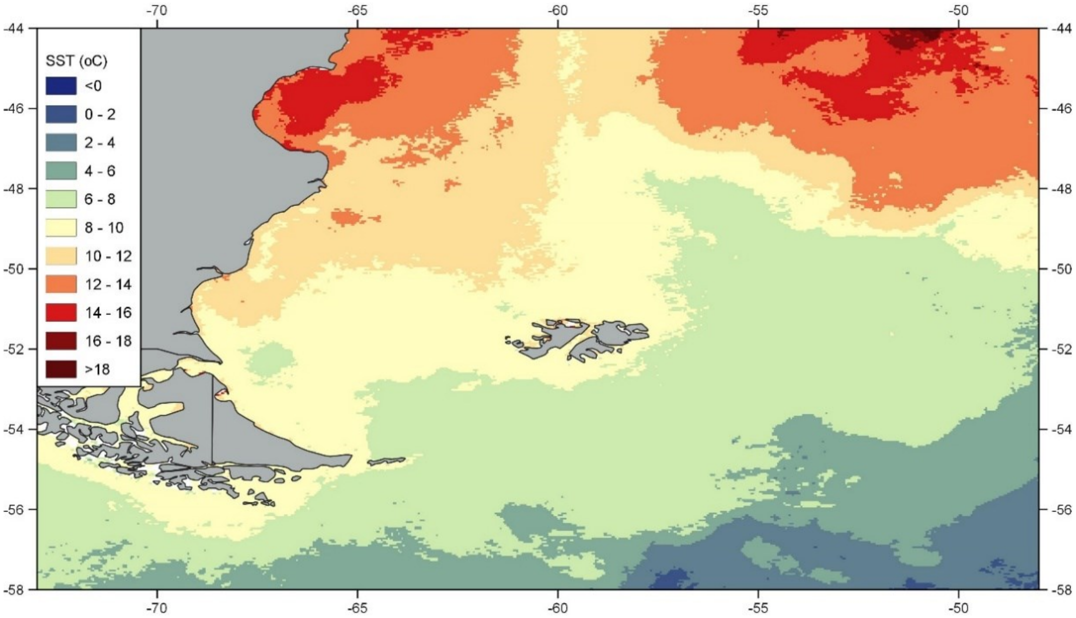


2020



DECEMBER

2019



2020