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Assessing the trophic ecology of top predators across a recolonisation frontier using DNA metabarcoding of diets

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ABSTRACT: Top predator populations, once intensively hunted, are rebounding in size and geographic distribution. The cessation of sealing along coastal Australia and subsequent recovery of Australian *Arctocephalus pusillus doriferus* and long-nosed *A. forsteri* fur seals represents a unique opportunity to investigate trophic linkages at a frontier of predator recolonisation. We characterised the diets of both species across 2 locations of recolonisation, one site an established breeding colony, and the other, a new but permanent haul-out site. Using DNA metabarcoding, high taxonomic resolution data on diets was used to inform ecological trait-based analyses across time and location. Australian and long-nosed fur seals consumed 76 and 73 prey taxa, respectively, a prey diversity greater than previously reported. We found unexpected overlap of prey functional traits in the diets of both seal species at the haul-out site, where we observed strong trophic linkages with coastal ecosystems due to the prevalence of benthic, demersal and reef-associated prey. The diets of both seal species at the breeding colony were consistent with foraging patterns observed in the centre of their geographic range regarding diet partitioning between predator species and seasonal trends typically observed. The unexpected differences between sites in this region and the convergence of both predators' effective ecological roles at the range-edge haul-out site correlate with known differences in seal population densities and demographics at these and other newly recolonised locations. This study provides a baseline for the diets and trophic interactions for recovering fur seal populations and from which to understand the evolving ecology of predator recolonisation.

KEY WORDS: DNA metabarcoding · Trophic ecology · Predator–prey interactions · Recolonisation · Fur seals · *Arctocephalus forsteri* · *Arctocephalus pusillus doriferus* · Otariid

INTRODUCTION

Pinnipeds are common high trophic-level predators in many ecosystems globally and may play a key role in structuring temperate food webs. Pinnipeds have experienced severe rates of population depletion globally through historical overexploitation and many species are currently recovering (Magera et al. 2013, IUCN 2015, McCauley et al. 2015). Nevertheless, most pinniped species are facing new anthro-

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pogenic threats through climate change and competition for resources with fisheries (Goldsworthy et al. 2003, Forcada & Hoffman 2014). Yet, the role of pinnipeds in the dynamic structure and function of temperate ecosystems remains a key knowledge gap (Connell 2002, Estes et al. 2013).

In southeastern Australia, 2 sympatric seal species, Australian fur seals *Arctocephalus pusillus doriferus* (hereinafter: AUFS), and long-nosed fur seals (formerly New Zealand fur seals) *A. forsteri* (hereinafter: LNFS), are undergoing population and range recovery following historical overexploitation and near-extinction (Shaughnessy et al. 2001, Goldsworthy et al. 2003, Burleigh et al. 2008, Kirkwood et al. 2010). A breeding colony and several new haul-out sites have recently established in New South Wales (NSW; eastern Australia), and these populations represent the first for nearly a century (Warneke 1982, McIntosh et al. 2014, Shaughnessy et al. 2014) in a region peripheral to the core geographic range of the species in Australian waters. Newly recolonised locations represent a frontier for species range recovery and/or expansion, where predator densities are still low, affording an opportunity to document predator diets and ecological interactions at an early stage of recolonisation. Additionally, frontier populations, due to their low densities, may be especially vulnerable within the greater population as they come into conflict with anthropogenic activities.

Knowledge of the diets of these species is based almost entirely on single predator studies from the central parts of their geographic ranges: Bass Strait for AUFS and South Australia and New Zealand for LNFS, and the majority are from breeding colonies (Gales & Pemberton 1994, Fea et al. 1999, Harcourt et al. 2002, Page et al. 2005, 2006, Kirkwood et al. 2008, Deagle et al. 2009). These studies report a broad diet in both species, as well as resource partitioning between species, whereby AUFS diets are reported as benthopelagic and LNFS as mostly pelagic. Both species exhibit seasonal variations in diet that correlate with prey availabilities and fur seal reproductive cycles, namely, a greater prevalence of benthic and demersal prey for both fur seal species in the summer compared to winter, when adult fur seals typically forage further offshore (Harcourt et al. 2002, Page et al. 2005, Arnould et al. 2011). Diet studies using morphological analyses of prey remains typically identify between 20 and 50 prey taxa, mostly bony fishes and cephalopods (Gales & Pemberton 1994, Fea et al. 1999, Page et al. 2005, Kirkwood et al. 2008). In contrast, the only other DNA-based study (Deagle et al. 2009) from one of the fur seal species studied here (AUFS) revealed a total of 62 prey species in only a single season of sampling. There is currently no published information on the diets of these species at their northern geographic range edge, a frontier for population and range recovery in Australia and an area distinct in its oceanography and biogeography compared to that of the rest of their range (Connell & Irving 2008).

A predator's diet represents the direct pathway of interaction with their ecosystems and forms the basis for understanding food web structure (Tollit et al. 2009, Pompanon et al. 2012). Dietary information at high taxonomic resolution (i.e. to genus/species) enables accurate identification of key drivers that underpin food web processes (Pompanon et al. 2012, Eisenberg et al. 2013). A suite of methods exist to study the diets of predators: from traditional morphological analyses of prey remains extracted from a predator's digestive tract to various molecular methods analysing chemical signals from predator tissues, including stable isotope, fatty acid and DNA-based methods (Bowen & Iverson 2013). However, many methods of diet analysis suffer problems and biases that impede fine-scale taxonomic identification of diet components (Tollit et al. 1997, Deagle et al. 2005, Casper et al. 2007, King et al. 2008, Bowen & Iverson 2013). DNA-based metabarcoding approaches have proven to be taxonomically sensitive, detecting prey items where traditional methods have not, as well as enabling higher taxonomic resolution identification of prey, requiring molecular expertise rather than extensive taxon-specific expertise (Deagle et al. 2009, Tollit et al. 2009, Pompanon et al. 2012, Peters et al. 2014, Berry et al. 2015). This method is ideally suited to explore predator diets (Leray et al. 2012, Pompanon et al. 2012), as it enables the identification of the ecological function of prey taxa (i.e. their trophic level and the type of ecosystem from which prey were likely obtained), and thus to characterise the role of predators in ecosystems (Spitz et al. 2014).

We investigated trophic interactions in 2 sympatric fur seal species in the newly recolonised region of eastern Australia, using DNA-based methods to extract high taxonomic resolution data from scats obtained from 2 main sites in this region. Our aims were to: (1) characterise the diets of these sympatric predators at a frontier of recolonisation and range expansion, and (2) identify important trophic interactions and investigate how the ecological function of prey taxa varies between seal species, sampling sites and time in these newly recolonised areas. We expected that the broad dietary patterns and prey resource partitioning observed between these seal species in southern

Australia would be reflected in their diets in our study region in eastern Australia. We therefore hypothesised that diet composition would differ between seal species and across time, but that within seal species, diets would be similar across the eastern Australian sites.

MATERIALS AND METHODS

Study populations, sites and sample collections

The study populations of AUFS and LNFS in NSW are at the northeastern range-edge of these species' geographic distributions — an area experiencing rapid population growth (McIntosh et al. 2014). To date, the majority of the NSW population of both seal species occurs at breeding colonies on Montague Island (MI), whereby breeding colonies are defined as locations harbouring the birth of at least 15 pups within each species (McIntosh et al. 2014). As such, MI harbours a relatively large representation in the population of adult females, as well as large breeding males in eastern Australia, similar to the demographic composition of colonies elsewhere in Australia (R. Harcourt, Macquarie University, pers. comm., N. Hardy pers. obs.). Additionally, growing haul-out sites around Jervis Bay (JB) (Burleigh et al. 2008) and new haul-out sites at the Five Islands Nature Reserve typically harbour juvenile and sub-adults of either sexes, and some adult seals (R. Harcourt, pers. comm.; N. Hardy pers. obs.) (Fig. 1). There are no ongoing surveys of seals in these areas, so accurate estimates of population size or gender/size/age structure are not available.

Sampling occurred in January–April and September 2014 (hereinafter: austral 'summer' and 'winter' samples, respectively), representing the warmest and coldest months of the year in terms of water temperature (data from Batemans Bay, NSW, at 20–60 m depth; Fig. 1) (IMOS 2014). Sampling locations included: colonies at MI (36° 14.645′ S, 150° 13.439′ E); and 3 haul-out sites at JB which were: Steamer's Head (for AUFS; 35° 10.725′ S, 150° 43.895′ E), Drum & Drumsticks and Lamond Head (for AUFS and LNFS respectively; 35° 2.799′ S, 150° 50.552′ E) (Table 1, Fig. 1). These sites are adjacent to extensive networks of complex shallow and intermediate depth

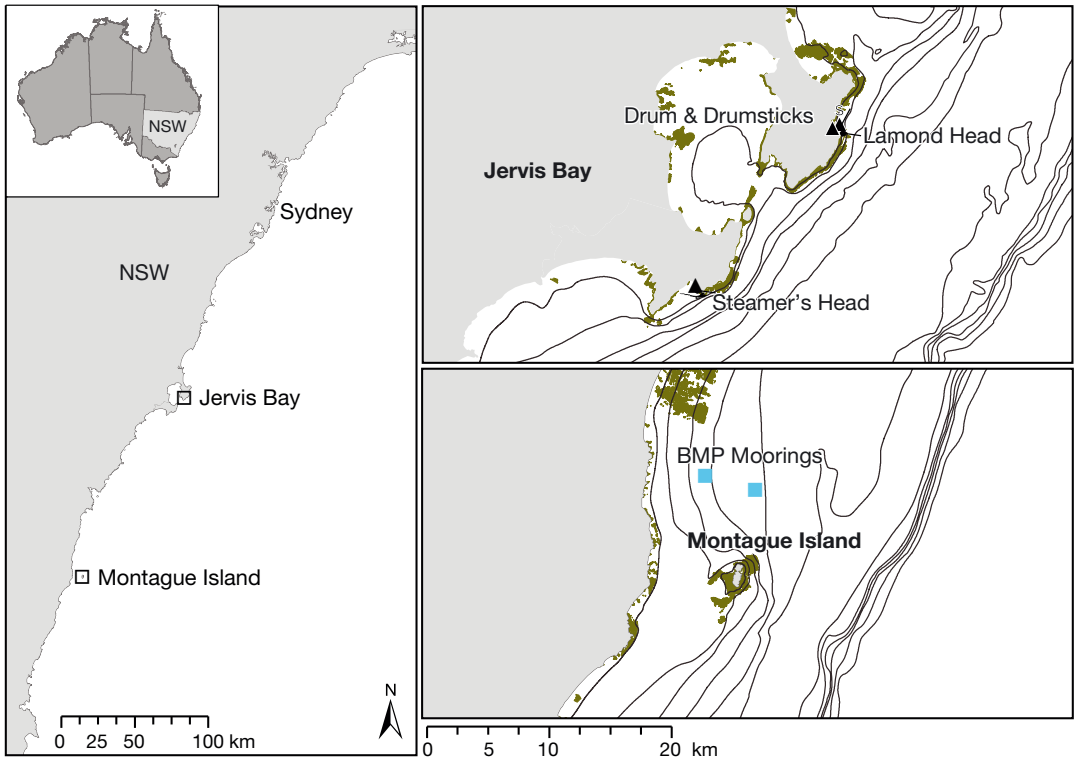

Fig. 1. Fur seal (*Arctocephalus pusillus doriferus* and *A. forsteri*) haul-out sites at Jervis Bay and Montague Island on the New South Wales (NSW) coast in relation to continental shelf (depth contours displayed every 20 m from 20–300 m) and shallow to intermediate reef habitat (up to 60 m, shaded in green) (OEH 2015). Water temperature data are from the Batemans Marine Park (BMP) moorings north of Montague Island (IMOS 2014). Black triangles indicate the locations of seal haul-out sites at Jervis Bay

Table 1. Collection locations, seasons and sample sizes for Australian (AUFS) and long-nosed fur seals (LNFS)

Location	Time period (2014)	Sample size AUFS	LNFS
Montague Island	Jan–Apr	15	21
	Sep	15	17
Jervis Bay	Jan–Apr	17	1[a]
	Sep	13	15

[a]Few LNFS are present in Jervis Bay in the summer; 1 sample was collected opportunistically at a predominantly AUFS haul-out site and confirmed to belong to LNFS from DNA analyses. This sample was not included in the statistical analyses, but prey items identified in this sample are indicated in Table 2 and Table S2 in the Supplement at www.int-res.com/articles/suppl/m573p237_supp.pdf

rocky reefs (MI and JB) (Fig. 1), a narrow continental shelf influenced by the warm East Australian Current and proximal to pelagic waters (MI and JB); MI is particularly close to one of the deepest sections of the continental shelf (at 132 m depth) and JB to a large shallow and sheltered bay with mixed seasonal estuarine influences (Jordan et al. 2010).

We collected a total of 129 faecal samples (n_{AUFS} = 67, n_{LNFS} = 62) across both fur seal species at 2 key locations (MI and JB) and times (Table 1). However, LNFS are typically absent from JB in the summer months at the time of this study, and only a single LNFS sample from this time point and location was opportunistically obtained in a predominantly AUFS haul-out site (Table 1). Fresh faecal samples were collected in zip-lock bags. Each whole scat was homogenised in the field using disposable spatulas, creating a mixed substrate, from which a 2 ml sub-sample was taken. Whole scats and sub-samples were immediately stored at −10°C in a portable freezer (WAECO) for up to 10 d and later transferred to −20°C freezer facilities for longer-term storage (maximum of 6 mo) (Murray et al. 2011).

Molecular analyses

Extractions were carried out on 120–200 mg of scat sub-sample using a QIAmp DNA Stool Mini Kit (QIAGEN) as per manufacturer's instructions. As a result of the laboratory optimisation of extraction procedures, we also included an overnight digestion (at 55°C) prior to extraction and we used half an InhibitEX tablet (an inhibitor absorption reagent, QIAGEN) per sample in accordance with Deagle et al. (2005). DNA was eluted in 50 µl of AE buffer (10 mM) at 3 dilutions in water (neat, 1:10, 1:100) and stored at −20°C. Quantitative PCR (qPCR) was used to optimise the selection of samples and DNA concentration for subsequent fusion tagging. DNA extracts were screened using qPCR to assess DNA quality and quantity, and to detect possible PCR inhibition (Deagle et al. 2009, Murray et al. 2011). Details on PCR reactions are described in Table S1 in the Supplement at www.int-res.com/articles/suppl/m573p237_supp.pdf. Four previously designed group-specific primers were used to bind directly to and amplify short regions of the 16S mtDNA gene, targeting mammals, fishes, cephalopods and crustaceans, and the 12S mtDNA gene for birds (Table S1).

Each DNA extract was then assigned a unique MID (Multiplex IDentifier) tag combination along with the next-generation sequencing (NGS) adaptors, using the same reaction conditions as for qPCR (Table S1). The resulting tagged amplicons were combined in pools of up to 5 samples of similar DNA molarity. Amplicon pools were then purified (Agencourt AMPure XP beads, Beckman Coulter Life Sciences) and combined again in accordance with their DNA concentrations to produce a single DNA library of 60–100 samples for sequencing. Each sequencing library was quantified alongside a set of standard synthetic oligonucleotides of known molarity (Bunce et al. 2012) before sequencing. Sequencing was performed on an Illumina MiSeq platform (300 bp V2 Nano kit) using single-end sequencing.

Bioinformatics

Sorting, filtering, clustering and identification of sequences were executed using specialised software. Samples were demultiplexed, and sequences were assigned to the correct sample using the unique MID tag combinations, after which identifiers, NGS adaptor sequences and primers were trimmed, in the program Geneious R8.1.5 (Kearse et al. 2012), leaving just the target sequences. Any sequences that did not contain exact matches to both the forward and reverse PCR primers, tags and adaptor sequences were discarded, as well as sequences that were significantly shorter than the primer product length. Discarded sequences at this stage typically corresponded to primer dimer or low-quality reads.

For each sample, target sequences were filtered with FastQ using a maximum error of 0.5 and dereplicated into clusters of unique sequences, using 97% similarity for clustering, in USEARCH (Edgar 2010). Sequence

clusters containing <1% of the total number of unique sequences detected in the sample were discarded. This minimises the risk of erroneous sequences and false positives from sequencing and other error, and vastly improves confidence in the subsequent analysis of the remaining sequences. Sequence clusters were then queried against the GenBank database using the algorithm BLASTn (Basic Local Alignment Search Tool).

The resulting 'blasted' sequences were then assigned to taxa, a part of the analyses that is necessarily done manually and follows a set of criteria outlined below (see also Deagle et al. 2009) and performed in the program MEGAN (MEtaGenome ANalyser) (Huson et al. 2007). Reads were reported based on the LCA-assignment algorithm parameters of a minimum bit score of 65.0, reports were limited to the top 10% of matches, and a minimum support of 1 (Huson et al. 2007), whereby the program MEGAN returns a shortlist of likely taxonomic assignments based on genetic similarity to the sequence. From that list, an assignment was considered reliable only when the match was made across the whole of the queried sequence. Potential prey identifications were individually investigated by consulting reference resources to assess the likelihood of prey assignments. The factors considered prior to identification include: (1) ensuring that the identified prey's geographic distribution broadly matched that of the likely southeast Australian foraging areas for fur seals, and (2) checking the diversity of closely related species and the presence/absence of voucher sequences for these in GenBank to ensure that any other likely prey species were not overlooked for want of genetic reference information. A broad range of reference databases were consulted and include: FishBase (Froese & Pauly 2016), Atlas of Living Australia (ALA 2016), reference books for coastal and pelagic fishes of southeastern Australia (Hutchins & Swainston 1986, Kuiter 2002), the Australian Museum (2016) reference base and Redmap (2016), the latter to check for out-of-range species.

In addition to this first assessment of the likelihood of the identified taxon being encountered by the predator, a further qualitative assessment was made on a case-by-case basis to classify the likely pathway of interaction (i.e. primary or secondary consumption) in order to remain conservative in our analyses of ecological interactions. This was largely based on, and limited by, knowledge of the biology of prey, and consisted of a sequential checklist of the following criteria: (1) whether the prey taxon were recorded in the literature either at a family, genus or species level, and if so, previously corroborated records were generally considered sufficient evidence that the prey was likely consumed by the predator. If not, further criteria were examined: (2) the frequency of detection of the taxon and whether it consistently occurred with a known mesopredator (i.e. likely secondary predation), or whether it appeared as the sole prey item in a sample (i.e. likely primary predation); (3) the known maximum size and average size of the species identified (FishBase, the Australian Museum, Hutchins & Swainston 1986, Kuiter 2002). Whilst DNA does not provide information on the actual size of the taxon ingested by the predator, all taxa presented as likely primary prey (see Table 2 and Table S2 in the Supplement) belonged to species that matched size-based criteria for consideration as potential prey, based on morphological studies that have estimated prey consumed by fur seals can range from 4000 g to 20 g for example (Page et al. 2005). Where there was insufficient evidence to support consideration for direct consumption of prey, these were considered likely to be the result of secondary consumption and were excluded from statistical analyses to reduce the risk of false positives influencing the analyses. Primary and secondary prey taxa are presented in separate tables (see Table 2 and Tables S2 & S3 in the Supplement).

Data processing and statistics

Response variables

This study aimed to evaluate trends in both fine-scale diet using species-level data, and secondly, to evaluate key trophic interactions for 2 predator species by analysing data based on prey ecological traits. Prey taxa were assigned to collective trait-based schemes that including traits relating to trophic niche, the known spatial association of prey and a combination of these 2 traits which we refer to as the prey's 'functional trait' (see Table 3). The spatial attributes do not assume exactly where the predator encountered that prey, but rather where that prey species most commonly occurs to the best available knowledge, and are thus necessarily broad (see Table 3). Analyses of seal diet composition were then performed at species-level or trait-based groupings of the data, taken as the presence of identified taxa. Additionally, differences in prey species richness were investigated and defined as the number of species in a scat sample.

Statistical analyses

All statistical models included 3 categorical explanatory variables with 2 levels each: seal species (AUFS, LNFS), location (MI, JB) and time sampled (summer, winter). For the purposes of statistical analyses, each specific combination of the levels of the explanatory variables (species, location and time) can be considered an independent 'group' of seals that were sampled, and for which replicate faecal samples were collected. As we obtained only 1 sample from LNFS from the JB location in the austral summer, it was not possible to test a fully orthogonal model of location, time and species. Instead, differences in diet composition between groups of seals were tested by running 4 reduced models that included explanatory variables in combinations where they were replicated: (i) for AUFS, prey assemblage ~ location × time; (ii) for LNFS, prey assemblage ~ group (combination of location and time, i.e. MI-summer vs. MI-winter); (iii) at MI, prey assemblage ~ seal species × time; (iv) at JB, prey assemblage ~ group (combination of seal species and time, i.e. AUFS-winter vs. LNFS-winter) (see Table 4).

Differences in diet composition were tested using multivariate generalised linear models (mvGLMs) and were fitted using a binomial distribution for multivariate presence/absence data on species-level and trait-based diet assemblages (spatial and functional trait-based grouping of the species-level response variable). The mvGLMs were performed in the mvabund package in R version 3.2.4 (R Development Core Team 2011, Wang et al. 2012). Broad trends, overdispersion and outliers in multivariate space were checked graphically by non-metric multidimensional scaling (nMDS) plots (Field et al. 1982) using the vegan package in R (Oksanen et al. 2015), whilst normality in multivariate data were checked using quantile-quantile (Q–Q) plots (Wang et al. 2012, Bates et al. 2015).

Model fit was assessed by analysis of deviance, tested using log-likelihood ratios and p-values calculated from 999 resampling iterations via probability integral transform (PIT) resampling (Wang et al. 2012). For significant interactions between explanatory variables in the full model, the differences between levels of these variables were tested (see Table 4). To then identify which response variables (i.e. species or functional traits) contributed most to the difference between levels, we performed post hoc univariate tests with adjusted p-values fitted to each response variable (i.e. species or functional trait) (Wang et al. 2012). Response variables were ranked based on the test statistic and we calculated how many response variables were required to capture at least 50% of the deviance explained compared to the full model comprising all response variables. The deviance was calculated by taking the ratio of the percentage deviance explained by a subset of the response variables and the deviance explained by the full model containing all response variables (Guisan & Zimmermann 2000). Response variables (i.e. taxa) with the highest univariate test statistic, significant p-values, and capturing in aggregate at least 50% of the deviance explained by the full model therefore had the greatest effect size and were considered to have the strongest evidence for an effect of explanatory variables and thus likely to be contributing to differences between levels of the explanatory variables.

Additionally, differences in prey species richness were tested using ANOVA in the base package 'stats' in R (R Development Core Team 2011). Trends in the data and model assumptions, including homogeneity of variances and normality of errors, were checked graphically using boxplots, co-plots and Q–Q plots. Model validity was assessed by plotting residuals against fitted values.

The percentage frequency of occurrence (FO%) of prey items was used to graphically represent the data using ggplot2 in R (Wickham 2009). Percentage frequency of occurrence of a given food item is defined as the number of samples in which that food item occurred, expressed as a proportion of the total number of samples that contained food (Amundsen et al. 1996, Davis et al. 2015). Thus, the total FO% of multiple diet items can exceed 100% due to the occurrence of multiple food items in samples.

RESULTS

Overview of sequencing and broad trends

A total of 112 faecal samples passed our quality filtering (no human DNA, sufficient quantity and quality of prey DNA) and thus were included in further analyses (n_{AUFS} = 60, n_{LNFS} = 52; Table 1). One additional sample from LNFS from the JB summer time point also passed quality filtering, but could not be included in statistical analyses as it was the only sample found from that location and time (Table 1). The taxa identified in this sample are indicated in Table 2 and Table S2 in the Supplement. The sequencing runs produced in excess of 1.8 million DNA sequen-

Table 2. Taxonomic assignment and percentage frequency of occurrence for samples of Australian (AUFS; n = 60) and long-nosed (LNFS; n = 53) fur seals for prey items occurring in ≥10% of samples. **Bold**: species occurring in at least 20% of samples for ≥1 given location or time sampled. Infrequent taxa occurring in <10% of samples are in Table S2 in the Supplement. JB: Jervis Bay, MI: Montague Island, PR: predator, PI: piscivore, HE: herbivore, PL: planktivore, IN: invertivore, OM: omnivore, UN: unknown

Class/family	Genus and species (common name)	Trophic and functional trait	AUFS				LNFS		
			JB		MI		JB	MI	
			Jan–Apr	Sep	Jan–Apr	Sep	Sep	Jan–Apr	Sep
ACTINOPTERYGII									
Congridae	*Gnathophis* sp. (conger eel)	PR, benthic predator	5.88	0.00	13.33	6.67	6.67	0.00	0.00
Belonidae	***Ablennes hians* (flat needlefish)**	**PI, pelagic piscivore**	0.00	0.00	0.00	0.00	0.00	**42.86**	0.00
Hemiramphidae	***Hyporhamphus melanochir* (southern sea garfish)**	**HE, coastal pelagic herbivore**	0.00	0.00	0.00	0.00	0.00	**47.62**	5.88
Scomberesocidae	***Scomberesox saurus* (king gar)**	**PI, pelagic piscivore**	0.00	0.00	6.67	0.00	6.67	19.05	**23.53**
Berycidae	***Beryx decadactylus* (imperador)**	**PR, demersal predator**	**29.41**	15.38	0.00	6.67	0.00	0.00	0.00
Trachichthyidae	Unknown Trachichthyidae (roughies)	UN, demersal unknown	0.00	0.00	0.00	0.00	13.33	0.00	0.00
Coryphaenidae	*Coryphaena hippurus* (mahi mahi)	PR, pelagic predator	0.00	0.00	13.33	0.00	0.00	4.76	0.00
Clupeidae	***Sardinops sagax* (Australian sardine)**	**IN, coastal pelagic invertivore**	11.76	0.00	0.00	**26.67**	0.00	4.76	5.88
Macrouridae	Unknown Macrouridae (whiptails)	PR, demersal predator	0.00	0.00	0.00	13.33	0.00	0.00	0.00
Ophidiidae	***Genypterus blacodes* (ling, pink cusk-eel)**	**PR, demersal predator**	0.00	0.00	0.00	**20.00**	0.00	0.00	0.00
Aplodactylidae	***Aplodactylus* sp. (marblefishes)**	**HE, reef herbivore**	0.00	0.00	0.00	0.00	**26.67**	0.00	0.00
Carangidae	***Trachurus* sp. (jack mackerel)**	**PR, continental pelagic predator**	**23.53**	**30.77**	13.33	**66.67**	0.00	4.76	11.76
	Pseudocaranx georgianus (silver trevally)	PR, reef predator	11.76	0.00	0.00	0.00	13.33	0.00	0.00
Gempylidae	*Rexea* sp. (gemfish)	PR, continental pelagic predator	0.00	15.38	0.00	0.00	0.00	0.00	0.00
	Thyrsites atun (barracouta)	PR, demersal predator	0.00	7.69	0.00	0.00	13.33	4.76	0.00
Kyphosidae	***Atypichthys strigatus* (mado)**	**PL, reef planktivore**	11.76	7.69	0.00	6.67	**26.67**	0.00	0.00
Latridae	*Latridopsis forsteri* (bastard trumpeter)	IN, reef invertivore	0.00	0.00	0.00	6.67	13.33	0.00	0.00
Nomeidae	*Cubiceps* sp. (drift fish)	PR, demersal predator	0.00	0.00	0.00	0.00	0.00	4.76	11.76
Pomacentridae	*Chromis* sp. (puller)	OM, demersal omnivore	0.00	0.00	0.00	0.00	**20.00**	0.00	0.00
Pomatomidae	***Pomatus saltatrix* (bluefish/tailor)**	**PI, pelagic piscivore**	0.00	0.00	0.00	0.00	0.00	**23.81**	0.00
Scombridae	*Scomber australasicus* (spotted chub mackerel)	PR, pelagic predator	17.65	7.69	13.33	**20.00**	0.00	14.29	5.88
Scorpididae	***Scorpis* sp. (sweep)**	**PL, reef planktivore**	5.88	0.00	0.00	6.67	**20.00**	0.00	5.88
Serranidae	*Caesioperca* sp. (butterfly/barber perch)	PL, reef planktivore	0.00	15.38	0.00	13.33	0.00	0.00	0.00
Sillaginidae[a]	*Sillago flindersi* (eastern school whiting)	IN, benthic invertivore	0.00	7.69	0.00	0.00	13.33	0.00	11.76
Sparidae	*Acanthopagrus* sp. (bream sp.)	PR, reef predator	11.76	0.00	0.00	0.00	0.00	0.00	0.00
Platycephalidae	***Neoplatycephalus richardsoni* (tiger flathead)**	**PR, benthic predator**	17.65	7.69	**20.00**	6.67	6.67	4.76	5.88
Monacanthidae	***Nelusetta ayraudi* (ocean jacket)**	**PR, continental pelagic predator**	**47.06**	**38.46**	**40.00**	**60.00**	**20.00**	**28.57**	11.76
	Unknown Monacanthidae (leatherjackets)	**OM, demersal omnivore**	5.88	0.00	**60.00**	0.00	0.00	9.52	0.00
Tetraodontidae	*Lagocephalus* sp. (rabbitfishes)	IN, pelagic invertivore	0.00	0.00	0.00	0.00	0.00	14.29	0.00
CEPHALOPODA									
Loliginidae	***Sepioteuthis australis* (southern calamari squid)**	**PR, demersal predator**	5.88	15.38	6.67	0.00	**20.00**	**71.43**	5.88
Octopodidae	***Octopus maorum* (Maori octopus)**	**IN, benthic invertivore**	5.88	7.69	6.67	**20.00**	0.00	4.76	0.00
	***Octopus* sp.**	**IN, benthic invertivore**	0.00	7.69	**26.67**	6.67	6.67	9.52	0.00
Enoploteuthidae	*Enoploteuthis galaxias* (galaxy squid)	UN, pelagic unknown	0.00	0.00	0.00	0.00	0.00	19.05	0.00
Ommastrephidae	***Nototodarus gouldi* (red arrow squid)**	**PR, pelagic predator**	17.65	15.38	**26.67**	13.33	**20.00**	**57.14**	**35.29**
	Nototodarus sp. (arrow squid)	PR, pelagic predator	0.00	0.00	0.00	0.00	0.00	14.29	0.00
	***Ommastrephes bartramii* (red flying squid)**	**PR, pelagic predator**	0.00	0.00	0.00	0.00	0.00	4.76	**23.53**
	Todarodes filipovae (fillipova's squid)	UN, pelagic unknown	0.00	0.00	0.00	0.00	0.00	0.00	11.76
Sepiidae	*Sepia apama* (giant cuttlefish)	PR, reef predator	0.00	0.00	0.00	0.00	13.33	0.00	0.00
Oegopsida	Unknown Oegopsida (squid)	UN, unknown	17.65	0.00	6.67	0.00	6.67	4.76	17.65
Decapodiformes	**Unknown Decapodiformes**	**UN, unknown**	5.88	0.00	0.00	0.00	0.00	**42.86**	**35.29**
MALACOSTRACA									
Scyllaridae	***Crenarctus crenatus* (slipper lobster)**	**IN, benthic invertivore**	**23.53**	0.00	0.00	0.00	0.00	0.00	0.00

[a]Prey item found in the single LNFS sample from JB in Jan–Apr

ces of target taxa, of which 1.6 million remained in the dataset after quality filtering, with an average of over 14 200 target sequences per sample using up to 4 primer sets. Sequence data files are available online (see '*Data accessibility*').

A total of 436 taxonomic assignments of fish, cephalopod, crustacean and bird taxa (AUFS: n = 215, LNFS: n = 221) met criteria for consideration in analyses as likely primary prey of AUFS and LNFS (Tables 2 & S2). These represented a total of 115 individual prey taxa, 34 of which were common to both species, and a total of 76 and 73 prey taxa identified in AUFS and LNFS samples, respectively (Tables 2 & S2). A further 48 taxonomic assignments were made of crustaceans (AUFS: n = 21, LNFS: n = 27), belonging to 25 genetically distinct taxa; however, these taxa appeared in <20% of samples, almost all were present in samples alongside possible mesopredators without any prior information on predation by fur seals on these taxa, and they are considered likely to be secondary predation. Prior to removal from further analyses, these taxa represented 10% of all taxonomic assignments made. These assignments are presented in Table S3 in the Supplement.

Fish were the most prevalent taxonomic group across time and location for AUFS, and for LNFS samples from JB, whilst both fish and cephalopods were equally prevalent across time for LNFS at MI (Fig. 2). For AUFS samples, a total of 59 fish taxa occurred in 92–100% of samples, 16 cephalopod taxa occurred in 38–46%, one crustacean species occurred in 23.5% of samples in JB in the summer sampling, and no birds were detected (Fig. 2). A further 13% of AUFS samples contained 14 different crustacean taxa considered likely secondary predation (Table S3). For LNFS samples, 54 fish taxa occurred in 64–100% of samples. We found 18 cephalopod taxa in LNFS samples, with cephalopods occurring in up to 33% of samples at JB in winter compared to 70–86% of samples at MI (Fig. 2). Additionally, 1 bird species, the little penguin *Eudyptula minor*, was identified in 1 LNFS sample from JB in the winter period. We found 14 different crustacean taxa likely to come from secondary predation in ca. 17% of LNFS samples.

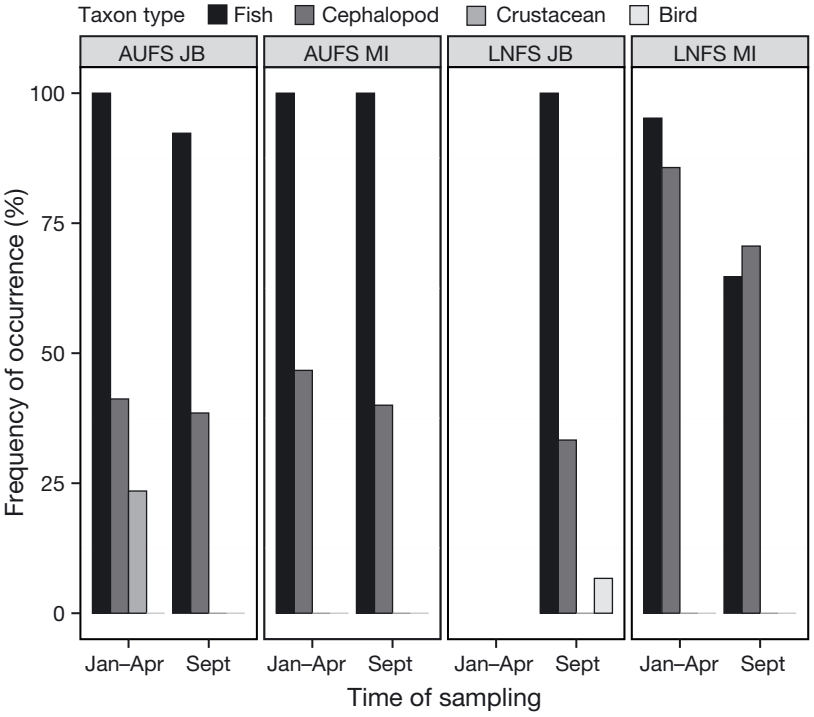

Fig. 2. Prevalence of 4 broad prey taxa for each seal species, location and sampling time (see Table 2). Percentage frequency of occurrence is expressed as % of samples containing each main taxon across predator species, location and time of sampling. No data available for LNFS at JB in the summer months

Trophic, spatial and functional attributes of prey items

Mesopredators were the most common prey by trophic trait, found in 75–100% of samples of either seal species at any location or time (Fig. 3). Analysis of diet composition in both fur seals by spatial traits showed that in samples from MI, benthic and demersal prey were more common in the summer compared to winter, whilst coastal and continental pelagic prey were dominant in the winter samples (Fig. S1 and Table S4 in the Supplement). AUFS typically had greater FO% of benthic prey compared to LNFS at any time and location, whilst the most prevalent spatial traits in the diet of LNFS at MI were pelagics (Fig. S1, Table S4). Samples from JB were not significantly different based on prey spatial traits for all combinations of seal species and sampling time, and were characterised by primarily benthic, demersal and reef-associated prey taxa (Fig. S1, Table S2). This pattern was also observed for functional trait analyses (Table 3, Fig. 4). Reef species and especially reef mesopredators were significantly more prevalent in JB samples, occurring in 23–35% of samples, while

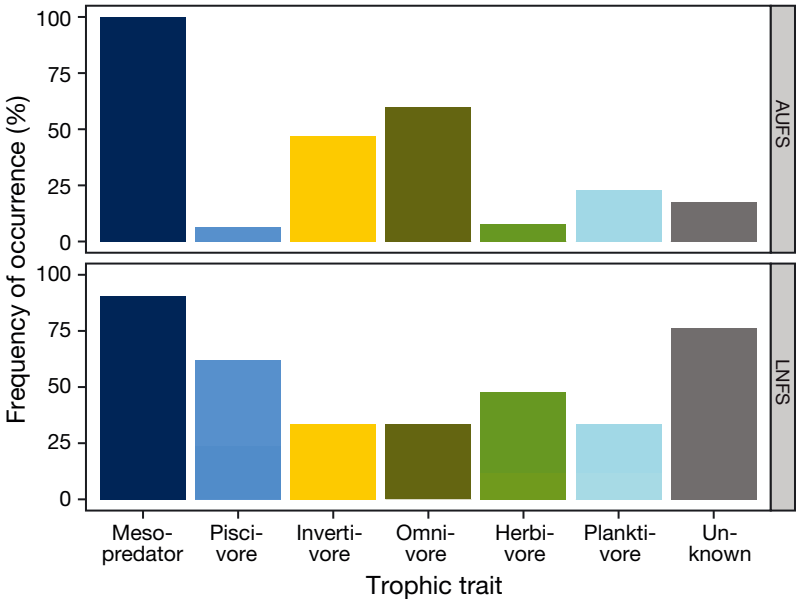

Fig. 3. Summary of percentage frequency of occurrence of trophic traits in the diets of sympatric eastern Australian fur seals; Australian (AUFS) and long-nosed (LNFS) fur seals

these prey traits were rare in MI samples for either species (FO% < 10% at MI) (Fig. 4). As the functional trait includes both the trophic and spatial attributes of the prey taxa, encapsulating both trophic and spatial trait analyses, we present the results of the functional trait analyses in more detail (Fig. 4).

For analyses of the prey assemblage by functional traits, AUFS prey composition varied by sampling time and not location (Table 4, Fig. 4), driven by a significant contribution of demersal omnivores in the summer samples compared to winter samples from MI, which had a higher FO% of coastal pelagic invertivores and continental pelagic mesopredators (Fig. 4). Prey functional traits in AUFS diets at JB were not significantly different between sampling times. However, functional trait analyses for LNFS revealed that prey composition was significantly different for all combinations of location and sampling time (Table 4, Fig. 4). LNFS samples from MI contained significantly greater FO% of demersal mesopredators and omnivores, and coastal pelagic herbivores in summer samples compared to winter (Fig. 4). LNFS samples from winter contained a greater FO% of pelagic mesopredators or prey of unknown trophic guild at MI, compared to greater FO% of demersal omnivores, reef planktivores and reef herbivores at JB (Fig. 4).

Key prey species trends

Species richness varied from 1 to 13 taxa identified as potential primary prey in samples. For AUFS samples, species richness was broadly stable, with an average of 3.6 (±0.4 SE) species per sample (Table S5

Table 3. Functional traits of seal prey species used for trait-based analyses. Placement of species into each category was based on detailed species knowledge and corroboration from reference material (Collette & Nauen 1983, Hutchins & Swainston 1986, Kuiter 2002, Froese & Pauly 2016)

Functional trait	Category	Description
Trophic	Trophic niche	Mesopredator, piscivore, omnivore, herbivore, cleaner and unknown
Spatial	Position of prey in the water column and in relation to the coast	*Benthic:* soft-sediment bottom dweller *Demersal:* associated with the soft-sediment benthos but positioned in the water column *Reef*: any benthic or demersal prey taxon found mostly/exclusively on rocky reefs *Coastal pelagic*: species mostly/exclusively associated with bays, estuaries and shallow coastal habitats *Continental pelagic*: mid- and open-water species known to associate commonly with the continental shelf and slope *Pelagic* (*or 'true' pelagic*): species not known to encounter any coastal or benthic structures and associate exclusively with open-water and oceanographic features *Unknown*: of completely unknown spatial origin
Functional	Trophic interaction	Combination of trophic and spatial traits

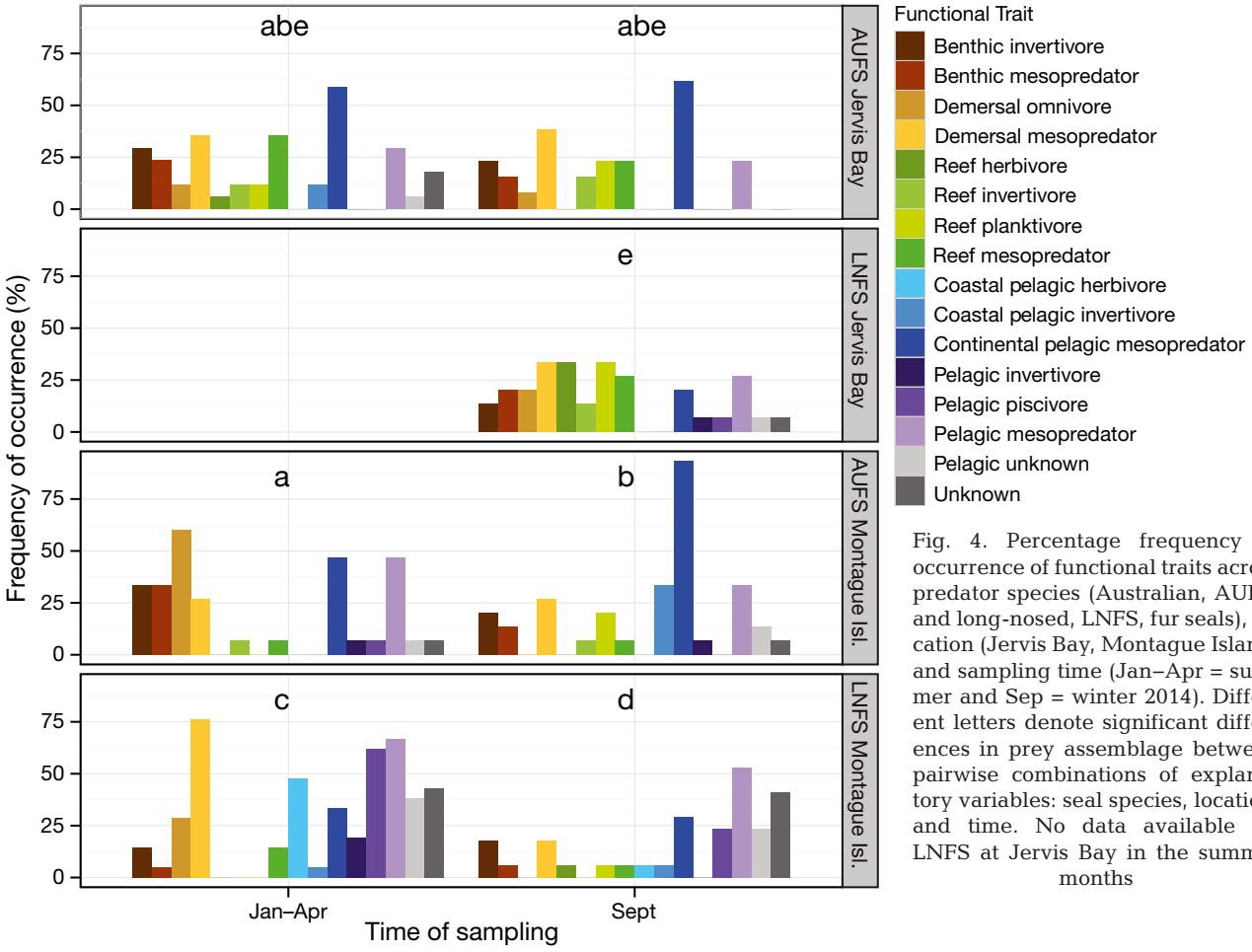

Fig. 4. Percentage frequency of occurrence of functional traits across predator species (Australian, AUFS, and long-nosed, LNFS, fur seals), location (Jervis Bay, Montague Island) and sampling time (Jan–Apr = summer and Sep = winter 2014). Different letters denote significant differences in prey assemblage between pairwise combinations of explanatory variables: seal species, location, and time. No data available for LNFS at Jervis Bay in the summer months

in the Supplement). For LNFS, richness per sample for LNFS was significantly greater in summer, with average richness of (mean ± SE) 5.7 ± 0.5 species compared to 3.2 ± 0.4 species in the winter samples (Table S5), but there was no difference between winter samples from either location.

For AUFS and LNFS, 12 and 13 prey taxa, respectively, were encountered in over 20% of samples for a given location or sampling time (in bold in Table 2), and were considered common prey taxa. Virtually all combinations of the levels of explanatory variables (predator species, location and sampling time) were significantly different when the prey assemblage was analysed at the species level, with the exception of diet composition for AUFS from MI and JB sampled in winter, which were not significantly different even at the species level (Tables 2 & 4). For AUFS from winter, seal diets consisted mainly of forage fish and continental pelagics, Australian sardine *Sardinops sagax* and jack mackerel *Trachurus* sp. (Table 2). In summer, MI samples for AUFS had greater FO% of a taxon assigned to the family Monacanthidae (unknown Monacanthidae) and *Octopus* sp. in summer (Table 2). Species composition in AUFS diets from JB contained greater FO% of less common and reef-associated taxa such as slipper lobster *Crenarctus crenatus*, silver trevally *Pseudocaranx georgianus*, a bream species (*Acanthopagrus* sp.), in the summer compared to winter samples; whilst JB samples from the winter had greater FO% of Australian sardine *S. sagax* (Tables 2 & S2).

For LNFS, species composition within samples was significantly different for all combinations of location and time (Tables 2 & 4). Differences between locations were due to greater FO% of the cephalopod red flying squid and king gar fish at MI, and at JB greater FO% of the reef-associated mado *Atypichthys strigatus*, marblefish *Aplodactylus* sp., puller *Chromis* sp. and bastard trumpeter *Latridopsis forsteri* (Tables 2 & 4). For MI samples, species composition in the diets of LNFS varied in time due to greater FO% of several cephalopod taxa, with a peak in their prevalence in summer (Table 2).

Table 4. Analysis of deviance for multivariate generalised linear models (mvGLM) of species-level analyses and functional trait analyses of prey composition between fur seal species (AUFS: Australian and LNFS: long-nosed fur seals), locations (JB: Jervis Bay, MI: Montague Island) and time points sampled, tested on 4 main models (in **bold**). R.df: residual degrees of freedom; Df.diff: difference in degrees of freedom; Dev: sum-of-deviance values. Where significant interactions occurred in the full model, reduced models tested the differences between levels of explanatory variables. $^{*}p < 0.05$, $^{**}p < 0.01$

Models	Explanatory variables	Species				Functional			
		R.df	Df.diff	Dev	p	R.df	Df.diff	Dev	p
(i) AUFS	Intercept	59				59			
	Time	58	1	106.53	0.051	58	1	34.43	0.044*
	Location	57	1	133.91	0.002**	57	1	32.34	0.065
	Time × Location	56	1	37.85	0.010**	56	1	22.32	0.084
AUFS in summer	Intercept	31				–	–	–	–
	Location (Summer)	30	1	84.09	0.021*	–	–	–	–
AUFS in winter	Intercept	27				–	–	–	–
	Location (Winter)	26	1	68.68	0.104	–	–	–	–
AUFS at MI	Intercept	29				–	–	–	–
	Time (MI)	28	1	92.51	0.003**	–	–	–	–
AUFS at JB	Intercept	29				–	–	–	–
	Time (JB)	28	1	81.59	0.021*	–	–	–	–
(ii) LNFS	Intercept	51				51			
	Group (Location + Time)	49	2	294.55	0.001**	49	2	125.93	0.001**
LNFS in winter	Intercept	29				30			
	Location (Winter)	28	1	94.66	0.003**	29	1	44.48	0.014*
LNFS at MI	Intercept	35				35			
	Time (MI)	34	1	120.20	0.001**	34	1	47.52	0.008*
(iii) MI	Intercept	64				64			
	Time	63	1	188.00	0.001**	63	1	70.08	0.001**
	Seal sp.	62	1	228.93	0.001**	62	1	92.90	0.001**
	Seal sp. × Time	61	1	22.83	0.043*	61	1	15.02	0.296
MI in summer	Intercept	35				–	–	–	–
	Seal sp. (Summer)	34	1	142.75	0.001**	–	–	–	–
MI in winter	Intercept	27				–	–	–	–
	Seal sp. (Winter)	26	1	107.56	0.001**	–	–	–	–
(iv) JB	Intercept	45				45			
	Group (Seal sp. + Time)	43	2	204.78	0.003**	43	2	56	0.104

Several previously rarely recorded or unrecorded taxa, such as mado, puller and silver sweep *Scorpis lineolata* (Table 2) were relatively common in samples from this study. They were all found together in at least 1 sample with no other taxa present, and as planktivores, it is improbable that they were consuming each other, and so they are likely to be primary prey items in eastern Australian fur seals. Several prey were only identified to genus or family level and each represented sequences from a single prey species, all of which were from taxa previously recorded as AUFS or LNFS prey (e.g. Monacanthidae, Macrouridae, Myctophidae, Sillaginidae, Sepiidae) (Tables 2 & S2). Several taxa identified to suborder, order or super-order include, respectively, unknown Osmeriformes, unknown Oegopsida and unknown Decapodiformes (Tables 2 & S2), due to sequences having <90% similarity to any existing sequences in GenBank. These taxa were included in analyses as they belong to taxonomic groups known to be consumed by fur seals, with the caveat that without better coverage of these groups in reference databases, it is not possible to determine whether these prey occur in samples due to primary or secondary predation.

DISCUSSION

The recent recolonisation of the coast of southeastern Australia (NSW) by AUFS and LNFS affords a unique opportunity to investigate trophic interactions in 2 sympatric, recolonising predators. Using taxonomically sensitive DNA metabarcoding meth-

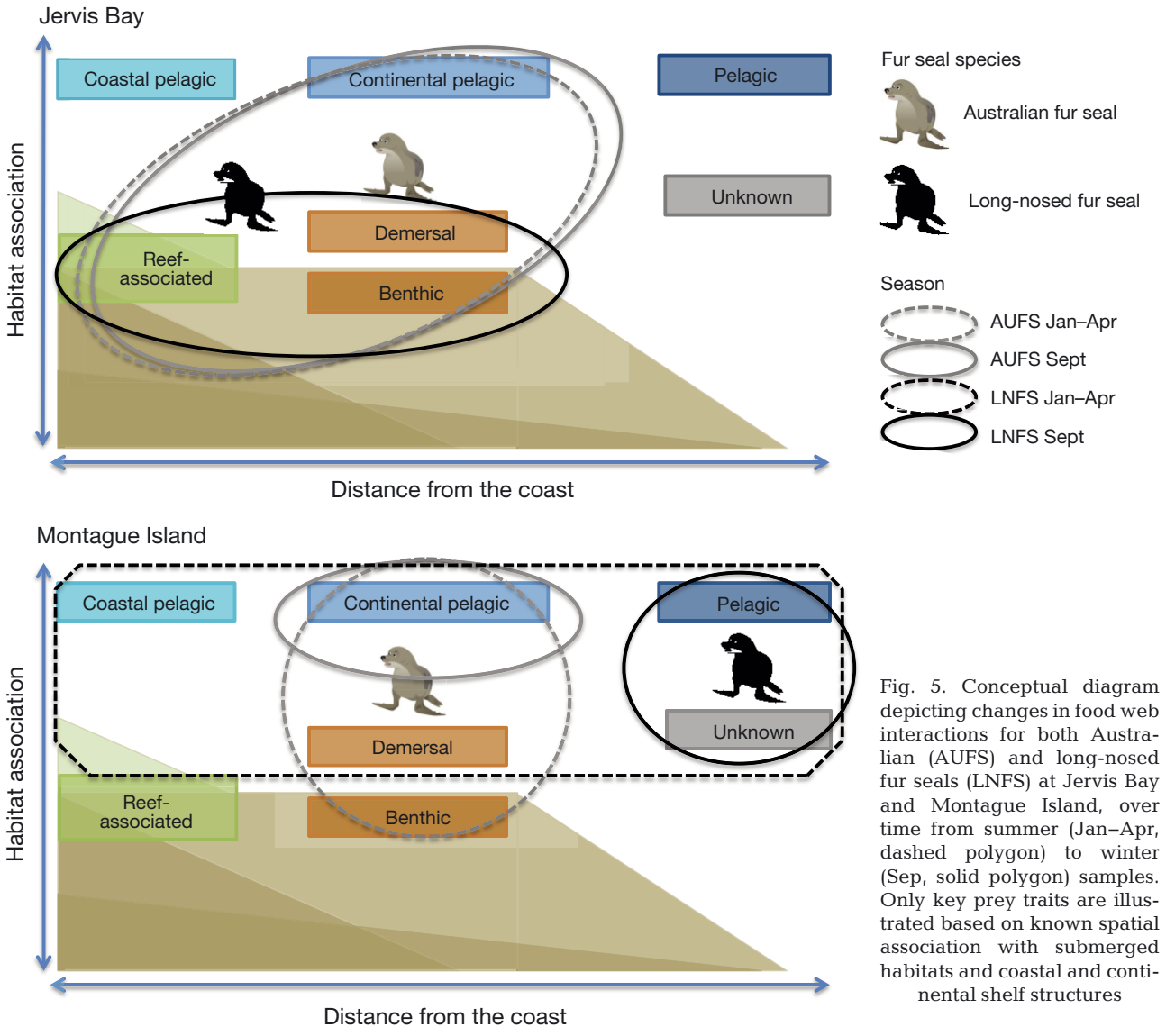

Fig. 5. Conceptual diagram depicting changes in food web interactions for both Australian (AUFS) and long-nosed fur seals (LNFS) at Jervis Bay and Montague Island, over time from summer (Jan–Apr, dashed polygon) to winter (Sep, solid polygon) samples. Only key prey traits are illustrated based on known spatial association with submerged habitats and coastal and continental shelf structures

ods to analyse diets, we identified a greater than expected diversity of prey items within the diets of both predator species and we provide baseline dietary information for 2 recolonising predators in the eastern Australian region. These methods enabled the identification of the ecological function of prey taxa and novel areas of differentiation and overlap in the diets of recolonising predators, affording greater characterisation of trophic interactions occurring within these temperate food webs. Although diet composition at the species level was different between predator species and locations, there was considerable overlap in prey functional traits in the diet of both seal species at the range-edge haul-out site, with the most prevalent traits being benthic, demersal and reef-associated prey at this location (Fig. 5). This result was unlike that of the diet composition of both seal species from the breeding colony, which exhibited a greater prevalence of prey from continental pelagic and true pelagic functional traits overall, and also a spike in benthic and demersal prey in the summer samples. At the breeding colony, diet composition also varied between predators (Fig. 5), as expected from studies in these species from the centre of their geographic range. Therefore the hypothesis that diets would differ between seal species and seasons was supported at the breeding colony but not at the haul-out site. These data support the notion that there may be stronger trophic linkages for both seal species with coastal ecosystems at the haul-out site as compared to the more established breeding colony in eastern Australia.

Ecological interactions of eastern Australian fur seals

Differences in diet composition at the species- and functional trait levels observed between predator species at the breeding colony (MI) were consistent with broad trends observed in the centre of their range (Gales & Pemberton 1994, Harcourt et al. 2002, Page et al. 2005, Kirkwood et al. 2008, Deagle et al. 2009). The main prey for AUFS were from benthic and demersal food webs, as well as from pelagic food webs over continental shelf waters from both the breeding colony (MI) and the haul-out site (JB); whilst for LNFS, the main prey for samples from the breeding colony (MI) were broadly pelagic (Fig. 5). Temporal differences evident in the diet of both seal species at the breeding colony (MI) were also consistent with trends observed at other breeding colonies (Harcourt et al. 2002, Page et al. 2005, Arnould et al. 2011), with greater prevalence of benthic and demersal prey in the summer samples and pelagic prey in the winter samples (Fig. 5). Summer is also the time of year when females are nursing young pups and are known to forage closer to breeding colonies, possibly contributing to these population-level trends in diets at MI (Harcourt et al. 2002, Page et al. 2005, 2006, Kirkwood & Arnould 2011).

At the range-edge haul-out site (JB), however, the diets of both seal species were unexpectedly similar to each other and exhibited patterns atypical of other sites. The diet composition of LNFS samples from JB was more similar to AUFS samples from this location than to their own kind from the breeding colony (MI) (Fig. 5), only several hundred kilometres away. Despite some differences in prey at the species level, our findings indicate that both fur seal species functionally overlap at this location, with prevalent trophic interactions with coastal ecosystems due to the dominance of benthic, demersal and especially reef-associated prey in their diets. Interestingly, the most common prey trait for AUFS at JB were still continental pelagic mesopredators, whilst pelagic prey were rare in LNFS from JB, with the exception of *Nelusetta ayraudi*, contrary to what would be expected from studies from elsewhere for LNFS, mostly based at breeding colonies (Harcourt et al. 2002, Page et al. 2005, 2006, Kirkwood & Arnould 2011), and contrary to what we observed in their diets at MI.

Reef fishes are a particular focus of coastal zone management due to their susceptibility to localised depletion from fishing and the need to mitigate anthropogenic effects through strategies including networks of marine protected areas (MPAs). Although direct trophic effects of pinnipeds on reef communities are not well known, reef-associated prey are occasionally found in the diets of AUFS elsewhere in Australia (Page et al. 2005, Deagle et al. 2009). Fur seals are also suspected to affect reef fish assemblages at MI, where their densities in eastern Australia are highest (Kelaher et al. 2015). Additionally, concerns about the trophic impacts of large predators on coastal reefs by local human communities and marine resource users are usually related to the densities of predators (i.e. the more seals, the greater the concern) (M. Voyer, University of Technology Sydney, pers. comm.). The results of this study instead highlight the possibility that range edge and haul-out sites may experience greater trophic interactions between seals and coastal ecosystems.

Differences in predator diets may be influenced by site-specific differences in prey assemblages (Gales et al. 1993, Cherel & Hobson 2007, Deagle et al. 2009). JB is a large coastal embayment, whereas MI is 2° latitude further south, a unique offshore island, and may be more heavily influenced by oceanographic features that drive the distributions of highly mobile prey (Suthers et al. 2011, Kelaher et al. 2015). This influence may partially explain the greater prevalence of pelagic prey items in both fur seal species at MI. However, both sites are positioned on a narrow continental shelf of ca. 20 km, proximal to a strong western boundary current that strongly affects prey distributions and availabilities, and both sites are associated with extensive networks of shallow and intermediate rocky reefs (Jordan et al. 2010).

It is more likely that the broad dietary patterns observed here are driven by differences in fur seal population demographics and densities between recolonised sites. The age cohorts and sex of fur seals differ between breeding colonies and haul-out sites, the latter consisting mainly of juvenile and sub-adult seals (Burleigh et al. 2008; R. Harcourt pers. comm.), differences that are known to influence foraging strategies of seals (Fowler et al. 2006, Page et al. 2006, Lowther et al. 2013). Juveniles have been found to make shorter, shallower and near-shore dives compared to adults in another otariid species, the Australian sea lion (Fowler et al. 2006, Page et al. 2006, Lowther et al. 2013). Additionally, density-mediated effects could be occurring at the breeding colony, observed in other recovering pinniped populations, such as northern fur seals (Kuhn et al. 2014), such that increasing population density, intra- and interspecific competition between predator species, could lead to localised resource depletion at the breeding colony compared to a less established haul-out site over time. This raises the question of whether certain

demographic and frontier cohorts of seals, particularly younger cohorts, may be more likely to forage in and impact shallower, near-shore reef communities before competition drives foraging effort further offshore, and importantly, how long this effect might be observable. The ongoing NSW fur seal population recovery provides a unique opportunity to test these hypotheses for further research using a gradient of recolonising fur seal densities and demographics, as several more haul-out sites have become established since the commencement of this study.

The majority of prey taxa identified in this study were generalist 2nd or 3rd trophic-level mesopredators: invertivores, piscivores or generalist mesopredators; and included wide-ranging, generally schooling prey items that occur in a range of ecosystems. This observation confirms that both seal species are functionally 4th trophic-level generalist predators in the recently colonised east coast ecosystems and throughout their range (Goldsworthy et al. 2013). The direct trophic impact of these seals will therefore be felt primarily towards the middle of the food web, while indirect effects are expected for lower trophic levels through mesopredator release feedback mechanisms (Prugh et al. 2009, Estes et al. 2016). However, detailed information on prey diets, trophic linkages and dynamics are lacking and currently limit further interpretation of local food webs. Whole ecosystem trophodynamic modeling, such as performed for South Australia (Goldsworthy et al. 2013), is required to further evaluate at all trophic levels, the complex interactions between recolonising predators and eastern Australian ecosystems.

We also observed a previously known trophic linkage between LNFS and little penguins. Predation of little penguins is known to occur in both fur seal species, but is more common in LNFS (Gales & Pemberton 1994, Page et al. 2005) and has been observed at MI, where little penguins nest (M. A. Coleman pers. obs.). A relatively high frequency of occurrence of their remains has been recorded in the scats and regurgitates of male LNFS in South Australia (~20% of samples) (Page et al. 2005) and in Victoria (up to 60% of samples; R. McIntosh, Philip Island Nature Parks, pers. comm.). In contrast, we only found little penguin remains in a single scat in 1 season and at 1 location, the haul-out site at JB (<7% of samples), and none have yet been detected at MI, a breeding colony. Given concerns about the potential impacts of recovering fur seal populations on little penguin populations elsewhere throughout their range, further monitoring of the degree of trophic interaction is warranted.

Recommendations for further work on DNA-based methods and predator diet analysis

High-taxonomic resolution was fundamental in identifying key trophic interactions of these recolonising predator species by enabling the identification of broader patterns in these predators' diets based on prey ecological traits. The number of prey species identified in the diets of either predator in this study, using DNA-based methods targeting 4 taxonomic prey groups, so far represents the highest number recorded in any study for either AUFS or LNFS, despite other studies typically employing greater sampling effort. Between 20 and 42 individual species are typically identified for either AUFS or LNFS in studies from Australia and New Zealand, with sampling efforts ranging from several hundred to over 1250 scats over multiple seasons, for 2–9 years of sampling effort (Gales & Pemberton 1994, Fea et al. 1999, Page et al. 2005, Kirkwood et al. 2008). A DNA-based study of AUFS diet from Bass Strait identified 54 bony fish, 4 cartilagenous fish, 4 cephalopods and 1 bird species in only 1 season (n = 90 scats) (Deagle et al. 2009), similar to the numbers found in the present study.

Differences in prey diversity can be influenced by study location, as a function of latitude and oceanographic parameters, and further studies directly comparing these methods across locations are warranted. However, geographic differences (Stuart-Smith et al. 2013) are not sufficient to explain the differences observed in prey diversity between DNA-based and morphological studies in these locations. DNA-based studies are known to be currently the most taxonomically sensitive method for diet analysis for marine predators (Casper et al. 2007, Deagle et al. 2009, Tollit et al. 2009, Berry et al. 2015), and aspects of predator diets may be overlooked by restricting diet investigations to morphological methods alone (Deagle et al. 2009, Berry et al. 2015). This method enabled this study to rapidly capture the breadth of these predator diets in novel locations.

At present, the use of multiple primers for DNA metabarcoding, to produce detailed taxonomic information on diet, incurs the loss of information on prey relative abundance (Deagle et al. 2009, Berry et al. 2015), information that is crucial to assessing the relative importance of prey observed. Biological and technical biases are known to affect sequence abundances and therefore sequence proportions recovered within samples and between primer sets (Deagle et al. 2006, 2013, Thomas et al. 2014). Three recent studies have addressed these biases and offer

solutions in the form of quantifying technical biases (Deagle et al. 2013), and in developing DNA correction factors either based on prey tissue composition or specific species (Thomas et al. 2014, 2016). These developments will likely enable further breakthroughs to confidently using relative abundance or biomass information from DNA-based studies. However, further research and development of corrective factors across different predator species and ecosystems are needed before they can be more broadly applied. Additionally, whilst proportions of prey sequences observed for the same primer were found to be stable across sequencing runs from within the same laboratory group (Deagle & Tollit 2007), we caution that raw sequence abundances are contingent on accurate replication of library build conditions, will necessarily vary across laboratories, and are not comparable between primers. Due to the use of multiple DNA primer sets in this study to target multiple taxonomic groups, we conservatively analyse only presence data at high taxonomic resolution.

A second issue is that of secondary predation or the recovery of DNA not only from fur seal prey taxa but also from the taxa that the prey themselves consumed and that could, in theory, survive digestion twice (Sheppard & Harwood 2005, King et al. 2008). In practice, this has not, to our knowledge, been tested in any vertebrate predator for any method of diet analysis, and secondary predation is a little understood issue common to all methods of diet analysis sampling predator tissues and faeces. Evaluating the extent to which trace amounts or greater quantities of material originating from secondary consumption is present in predator tissues would require complex multi-trophic level captive feeding trials for each method of diet analysis. In the absence of such a study, present studies employ a weight of evidence approach outlined in the methods and rely on sound biological interpretation and prior knowledge of the system to increase confidence in prey identifications. This process is currently done manually, and for most prey items detected, it is relatively straightforward. Some of the lower taxonomic resolution taxa found here could in fact be secondary predation (e.g. unknown Decapodiformes); however, they could also represent species for which reference material is lacking in global genetic databases for entire taxonomic groups. Indeed, poorly resolved taxonomy and a paucity of reference material are limiting factors in genetic analyses, particularly for certain taxonomic groups such as cephalopods and crustaceans (Berry et al. 2015). Our methods were careful to balance the risks of false negatives concerning legitimate prey items and also conservative towards false positives arising from contamination and potential secondary predation. Finally, through stringent quality control and manual curation of the prey database, we are certain that those prey were ultimately consumed, and their relative importance will become clearer through longer-term research.

CONCLUSIONS

The results of this study provide a much-needed prey database for recolonising eastern Australian fur seal populations that can inform future work on their diet, trophic interactions and ecosystem trophodynamics. The differences observed in trophic linkages for predators at the haul-out site compared to the breeding site also highlight the need to further investigate different demographic and frontier cohorts of seals in recovering populations, which could result in different considerations for coastal management targeted to different cohorts of seals. We recommend continued research on seal diets in eastern Australia, a location at the frontier for the population and range recovery of 2 large-bodied predators, to provide valuable insights on the trophodynamics of similar predator recolonisations in temperate coastal ecosystems. Importantly, parallel sampling and analysis of the diets of mesopredators and lower trophic levels using complimentary multi-disciplinary and DNA-based methods will enable better resolution of trophic interactions across whole ecosystems. Reconstruction of ecosystem-scale trophodynamics will be essential to managing the recovery of protected species and the marine resources they depend on.

Data accessibility. Metabarcoding data (raw FASTQ files and filtered FASTA files) are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.2tk0q.

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