The following supplement accompanies the article

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

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Table S1 Primers used and designer references, (5'-3' end specifications + references). All qPCR reactions were carried out in 25µL: 15.4µL of H_2O, 1X Taq Gold buffer (Applied Biosystems [ABI], USA), 2nM $MgCl_2$ (ABI, USA), 0.4mg/mL BSA (Fisher Biotec, Australia), 0.25mM dNTPs (Astral Scientific, Australia), 0.4µM each of forward and reverse primers (Integrated DNA Technologies, Australia), 0.6µL of 1/10,000 SYBR Green dye (Life Technologies, USA) and 0.05U/µL of Taq polymerase Gold (ABI, USA) with 2µL of DNA. Each qPCR was run on Applied Biosystems® step-ONE qPCR thermocycler (ABI, USA): 95˚C for 5 min, then 40-50 cycles of 95˚C for 30s, 50–57˚C for 30s (as per primer annealing temperature; Table S.1) and 72°C for 45s. This was followed by a 1s melt curve and a 10 min final extension of 72˚C.

Target amplicon	Gene	Primer name	Sequence (5'-3')	Product size (bp)	Annealing temperature	Reference
Mammal	mtDNA 16S	Mam16S1	CGGTTGGGGTGACCTCGGA	90	57˚C	Taylor (1996)
Mammal	mtDNA 16S	Mam16S2	GCTGTTATCCCTAGGGTAACT			
Fish	mtDNA 16S	Fish16S_F	GACCCTATGGAGCTTTAGAC	200	54˚C	F: Deagle et al. (2007)
Fish	mtDNA 16S	Fish16S_R	CGCTGTTATCCCTADRGTAACT			R: Murray D. (unpublished)
Cephalopod	mtDNA 16S	S_Cephalopoda_F	GCTRGAATGAATGGTTTGAC	70	50˚C	Peters et al. (2014)
Cephalopod	mtDNA 16S	S_Cephalopoda_R	TCAWTAGGGTCTTCTCGTCC			
Crustacea	mtDNA 16S	Crust16s_F(short)	GGGACGATAAGACCCTATA	150	51˚C	Berry T. (unpublished)
Crustacea	mtDNA 16S	Crust16S_R(short)	ATTACGCTGTTATCCCTAAAG			
Bird	mtDNA 12S	Bird12sa	CTGGGATTAGATACCCCACTAT	230	57˚C	Cooper (1994)
Bird	mtDNA 12S	Bird12sh	CCTTGACCTGTCTTGTTAGC			

References

Cooper A (1994) DNA from museum specimens. Springer, New York, United States

Deagle BE, Gales NJ, Evans K, Jarman SN, Robinson S, Trebilco R, Hindell MA (2007) Studying Seabird Diet through Genetic Analysis of Faeces: A Case Study on Macaroni Penguins (Eudyptes chrysolophus). PloS one 2:10

Peters KJ, Ophelkeller K, Bott NJ, Deagle BE, Jarman SN, Goldsworthy SD (2014) Fine-scale diet of the Australian sea lion (*Neophoca cinerea*) using DNA-based analysis of faeces. Molecular ecology 36:347–367

Taylor PG (1996) Reproducibility of Ancient DNA Sequences from Extinct Pleistocene Fauna. Molecular Biology and Evolution 13:283–285

Table S2 Taxonomic assignment and percentage frequency of occurrence (FO%) for samples of Australian (n = 60) and long-nosed (n = 53) fur seals for prey taxa occurring in <10% of samples (Primer sets: Fish16S and S_Cephalopoda, Crust16S and Bird12S). See below table for abbreviations.

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Actinopterygii									
Congridae	*Gnathophis* sp. (conger eel)	PR, Benthic Predator	0.00	0.00	6.67	0.00	6.67	0.00	0.00
Aulopidae	*Latropiscis purpurissatus* (sergeant baker)	PR, Benthic Predator	0.00	7.69	0.00	0.00	6.67	0.00	0.00
Belonidae	Unknown Belonidae (needlefishes)	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	9.52	0.00
Hemiramphidae	*Hyporhamphus regularis* (river garfish)	OM, Coastal Pelagic Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Clupeidae	*Etrumeus teres* (maray)	IN, Coastal Pelagic Invertivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Sardinella gibbosa (gold-belly sardinella)	OM, Coastal Pelagic Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Engraulidae	*Engraulis australis* (australian anchovy)	IN, Coastal Pelagic Invertivore	0.00	0.00	0.00	6.67	0.00	0.00	0.00
Moridae	*Lotella rhacina* (rock cod, beardie)	PI, Reef Piscivore	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Pseudophycis barbata (bearded rock cod)	PR, Reef Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Mugilidae	*Aldrichetta forsteri* (yelloweye mullet)	OM, Demersal Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Liza argentea (goldspot mullet)	OM, Demersal Omnivore	0.00	7.69	0.00	0.00	0.00	9.52	0.00
	Mugil cephalus (sea mullet)	OM, Demersal Omnivore	0.00	7.69	0.00	0.00	0.00	9.52	0.00
	Myxus elongatus (sand mullet) †	OM, Demersal Omnivore	5.88	0.00	0.00	0.00	0.00	4.76	0.00
	Unknown Mugilidae (mullets)	OM, Demersal Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Myctophidae	*Lampadena* sp. (lanternfishes)	IN, Pelagic Invertivore	0.00	0.00	6.67	0.00	0.00	0.00	0.00
	Myctophum sp. (spotted lanternfish)	IN, Pelagic Invertivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00
	Hygophum hanseni (hansen's lanternfish)	PL, Pelagic Planktivore	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Ophidiidae	*Genypterus* sp. (cusk-eels)	PR, Demersal Predator	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Bramidae	*Brama brama* (atlantic pomfret)	PR, Pelagic Predator	0.00	0.00	0.00	0.00	0.00	4.76	0.00

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Carangidae	*Seriola lalandi* (yellowtail kingfish)	PR, Coastal Pelagic Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Centrolophidae	*Seriolella brama* (blue warehou)	PR, Demersal Predator	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Cheilodactylidae	*Nemadactylus douglasii* (grey morwong)	PR, Reef Predator	5.88	7.69	0.00	6.67	6.67	0.00	0.00
	Unknown Cheilodactylidae (morwongs)	PR, Reef Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Nemadactylus macropterus (jackass morwong)	PR, Reef Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Chironemidae	*Chironemus marmoratus* (eastern kelpfish)	PR, Reef Predator	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Dinolestidae	*Dinolestes lewini* (longfin pike)	PR, Reef Predator	5.88	0.00	0.00	0.00	6.67	4.76	0.00
Emmelichthyidae	*Emmelichthys nitidus* (redbait)	PR, Continental Pelagic Predator	0.00	7.69	0.00	6.67	0.00	0.00	0.00
Gempylidae	*Nealotus tripes* (black snake mackerel)	PR, Pelagic Predator	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Girellidae	*Girella* sp. (greenfish)	OM, Demersal Omnivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Girella tricuspidata (luderick)	HE, Demersal Herbivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Kyphosidae	*Kyphosus sydneyanus* (silver drummer)	HE, Reef Herbivore	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Kyphosus vaigiensis (brassy drummer)	OM, Reef Omnivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Unknown Kyphosidae (drummers)	OM, Reef Omnivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00
Labridae	*Achoerodus viridis* (eastern blue groper)	IN, Reef Invertivore	5.88	0.00	0.00	6.67	0.00	0.00	0.00
	Austrolabrus maculatus (blackspotted wrasse)	IN, Reef Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Bodianus sp. (pigfish)	IN, Reef Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Mullidae	*Upeneichthys* sp. (goatfish)	IN, Benthic Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Odacidae	*Odax cyanomelas* (herring cale)	HE, Reef Herbivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00
Pempheridae	*Parapriacanthus* sp. (sweepers)	IN, Reef Invertivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00
Pentacerotidae	Unknown Pentacerotidae (armourheads)	PR, Demersal Predator	0.00	0.00	0.00	0.00	6.67	0.00	0.00
Pinguipedidae	*Parapercis allporti* (barred grubfish)	IN, Benthic Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Polyprionidae	*Polyprion oxygeneios* (hapuku)	PR, Pelagic Predator	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Sphyraenidae	*Sphyraena* sp. (pikes)	PR, Reef Predator	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Tetragonuridae	*Tetragonurus atlanticus* (bigeye squaretail)	IN, Pelagic Invertivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Trichiuridae	*Lepidopus caudatus* (silver scabbardfish)	PR, Continental Pelagic Predator	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Scorpaenidae	*Scorpaena* sp. (scorpionfishes)	PR, Reef Predator	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Scorpaenodes scaber (pygmy scorpionfish)	PR, Reef Predator	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Sebastidae	*Helicolenus* sp. (ocean perch)	PR, Demersal Predator	5.88	0.00	6.67	6.67	0.00	0.00	0.00
Triglidae	*Chelidonichthys* sp. (gurnard)	PR, Benthic Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Lepidotrigla argus (eye gurnard)	IN, Benthic Invertivore	5.88	0.00	6.67	0.00	0.00	0.00	0.00
	Lepidotrigla papilio (spiny gurnard)	IN, Benthic Invertivore	0.00	0.00	6.67	0.00	0.00	0.00	5.88
Diodontidae	*Allomycterus pilatus* (australian burrfish)	IN, Reef Invertivore	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Monacanthidae	*Cantherhines dumerilii* (barred leatherjacket)	OM, Reef Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Meuschenia australis (brownstriped leatherjacket)	HE, Reef Herbivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00
	Meuschenia scaber (velvet leatherjacket)	IN, Reef Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Eubalichthys mosaicus (mosaic leatherjacket)	HE, Reef Herbivore	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Ostraciidae	*Anoplocapros inermis* (eastern smooth boxfish)	IN, Reef Invertivore	5.88	0.00	0.00	0.00	0.00	0.00	0.00

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Tetraodontidae	*Arothron firmamentum* (starry toad)	IN, Demersal Invertivore	0.00	0.00	6.67	0.00	0.00	4.76	0.00
Osmeriformes	Unknown Osmeriformes (argentines)	UN, Unknown	0.00	0.00	0.00	6.67	0.00	0.00	0.00
Cephalopoda									
Argonautidae	*Argonauta nodosa* (knobby argonaut)	IN, Pelagic Invertivore	0.00	0.00	0.00	6.67	0.00	0.00	0.00
Octopodidae	*Octopus tetricus* (Common Sydney Octopus)	IN, Benthic Invertivore	0.00	7.69	6.67	0.00	0.00	4.76	0.00
	Unknown Octopodidae (octopus)	IN, Benthic Invertivore	0.00	0.00	0.00	6.67	0.00	0.00	0.00
	Octopus sp. #2 (*O. berrima* or *O. pallidus*)	IN, Benthic Invertivore	0.00	7.69	0.00	6.67	0.00	4.76	0.00
Ocythoidae	*Ocythoe tuberculata* (tuberculate pelagic octopus)	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	9.52	0.00
Cranchiidae	*Leachia* sp.	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	0.00	5.88
	Taonius sp. (glass squid)	UN, Pelagic Unknown	0.00	0.00	0.00	6.67	6.67	0.00	0.00
Enoploteuthidae	*Abralia* sp. (midwater squid)	UN, Pelagic Unknown	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Abraliopsis sp.	UN, Pelagic Unknown	0.00	0.00	0.00	6.67	0.00	0.00	0.00
Ommastrephidae	*Eucleuteuthis* sp. (luminous flying squid)	UN, Pelagic Unknown	0.00	0.00	6.67	0.00	0.00	0.00	0.00
	Ornithoteuthis volatilis (flying squid)	UN, Pelagic Unknown	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Pyroteuthidae	*Pterygioteuthis microlampas*	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Sepiidae	Unknown Sepiidae (cuttlefish)	PR, Reef Predator	5.88	7.69	6.67	0.00	6.67	0.00	5.88
Aves									
Spheniscidae	*Eudyptula minor* (little penguin)	PR, Pelagic Predator	0.00	0.00	0.00	0.00	6.67	0.00	0.00

* PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown

† Prey items found in the single LNFS sample from JB in Jan-Apr (*Myxus elongatus*).

Table S3 Taxonomic assignment and percentage frequency of occurrence (FO%) for taxa considered likely secondary predation, for samples of Australian (n = 60) and long-nosed (n = 53) fur seals (Primer set: Crust16S).

		Australian fur seal				Long-nosed fur seal		
		JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Malacostraca								
Acanthephyridae	*Notostomus* sp. (shrimp)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Callianassidae	*Biffarius* sp. (ghost shrimp)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Diogenidae	Unknown Diogenidae (hermit crabs)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Euphausiidae	*Euphausia recurva* (krill)	0.00	0.00	0.00	0.00	0.00	0.00	5.88
	Nyctiphanes australis (euphausiid)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Unknown Euphausiidae (krill)	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Pandalidae	*Chlorotocus crassicornis* (green shrimp)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Penaeidae	*Melicertus plebejus* (eastern king prawn)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Metapenaeus sp. (school prawn)	0.00	0.00	0.00	13.33	0.00	0.00	0.00
Polybiidae	*Ovalipes* sp. (sand crab)	0.00	0.00	6.67	0.00	6.67	0.00	23.53
	Unknown Polybiidae (swimmer crabs)	0.00	0.00	20.00	0.00	0.00	0.00	0.00
Portunidae	*Portunus sanguinolentus* (blue-spot swimming crab)	0.00	0.00	6.67	0.00	0.00	19.05	0.00
	Thalamita admete (swimming crab)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Thalamita sima (four-lobed swimming crab)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Raninidae	*Ranina ranina* (spanner crab)	11.76	0.00	0.00	0.00	0.00	0.00	0.00
Scyllaridae	*Galearctus rapanus* (slipper lobster)	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Sergestidae	Unknown Sergestidae (sergestid shrimps)	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Squillidae	*Busquilla plantei* (stomatopod crustacea)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Oratosquillina sp. (mantis shrimp)	0.00	0.00	0.00	0.00	0.00	9.52	0.00
	Unknown Squillidae (mantis shrimps)	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Xanthidae	Unknown Xanthidae (rubble crabs)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
	Unknown Decapod	11.76	0.00	13.33	0.00	13.33	4.76	17.65
	Unknown Dendrobranchiata (shrimps)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
	Unknown Euphausiacea (krill)	0.00	0.00	0.00	0.00	0.00	0.00	5.88
	Unknown Stomatopoda (mantis shrimps)	0.00	0.00	0.00	0.00	0.00	4.76	0.00

Table S4 Analysis of deviance table for multivariate generalised linear models (mvGLM) for trophic and spatial group analyses of prey composition between fur seal species, at different locations and time points sampled, tested on four models. Where significant interactions occurred in the full model, reduced models tested the differences between levels of explanatory variables. Significance denoted by: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; † denotes where outliers were removed prior to analysis of the final model

	Explanatory variables	**SPATIAL**			
MODELS	*Factors*	*R.df*	*Df.diff*	*Dev*	*P-value*
(i) AUFS	*Intercept*	*59*			
	Time	58	1	12.37	0.185
	Location	57	1	11.38	0.245
	Time×Location	56	1	25.03	0.011*
AUFS in Summer	*Intercept*	31			
	Location (Summer)	30	1	33.37	0.006**
AUFS in Winter	*Intercept*	27			
	Location (Winter)	26	1	26.49	0.034*
AUFS at MI	*Intercept*	29			
	Time (MI)	28	1	36.44	0.001**
AUFS at JB	*Intercept*	29			
	Time (JB)	28	1	12.09	0.492
(ii) LNFS	*Intercept*	51			
	Group (Location+Time)	49	2	83.46	0.001**
LNFS in Winter	*Intercept*	30			
	Location (Winter)	29	1	31.55	0.009**
LNFS at MI	*Intercept*	35			
	Time (MI)	34	1	34.67	0.006**
(iii) MI	*Intercept*	65			
	Time	64	1	33.98	0.001**
	Seal sp.	63	1	36.68	0.002**
	Seal sp.×Time	62	1	24.47	0.008**
MI in Summer	*Intercept*	35			
	Seal sp. (Summer)	34	1	40.17	0.002**
MI in Winter	*Intercept*	29			
	Seal sp. (Winter)	28	1	20.98	0.031*
(iv) JB	*Intercept*	45			
	Group (Seal sp.+Time)	43	2	15.74	0.457

Table S5 Analysis of variance table for species richness between fur seal species, locations and time points sampled, tested on four models. Significance denoted by: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

	Explanatory variables	**SPECIES RICHNESS**				
MODELS	*Factors*	*Df*	*Sum Sq*	*Mean Sq*	*F value*	*P-value*
(i) AUFS	*Time*	1	14.8	14.8	0.208	0.65
	Location	1	2.8	2.82	1.095	0.3
	Time×Location	1	0.3	0.28	0.021	0.886
	Residuals	56	757	13.517		
(ii) LNFS	*Group (Location+Time)*	2	80.02	40.01	6.887	0.002**
	Residuals	49	284.66	5.81		
(iii) MI	*Time*	1	25	25.03	4.155	0.081
	Seal sp.	1	19	19.01	3.155	0.046
	Seal sp.×Time	1	37.5	37.51	6.227	0.015*
	Residuals	62	373.5	6.02		
(iv) JB	*Group (Seal sp.+Time)*	2	1.8	0.87	0.106	0.9
	Residuals	43	355.7	8.27		

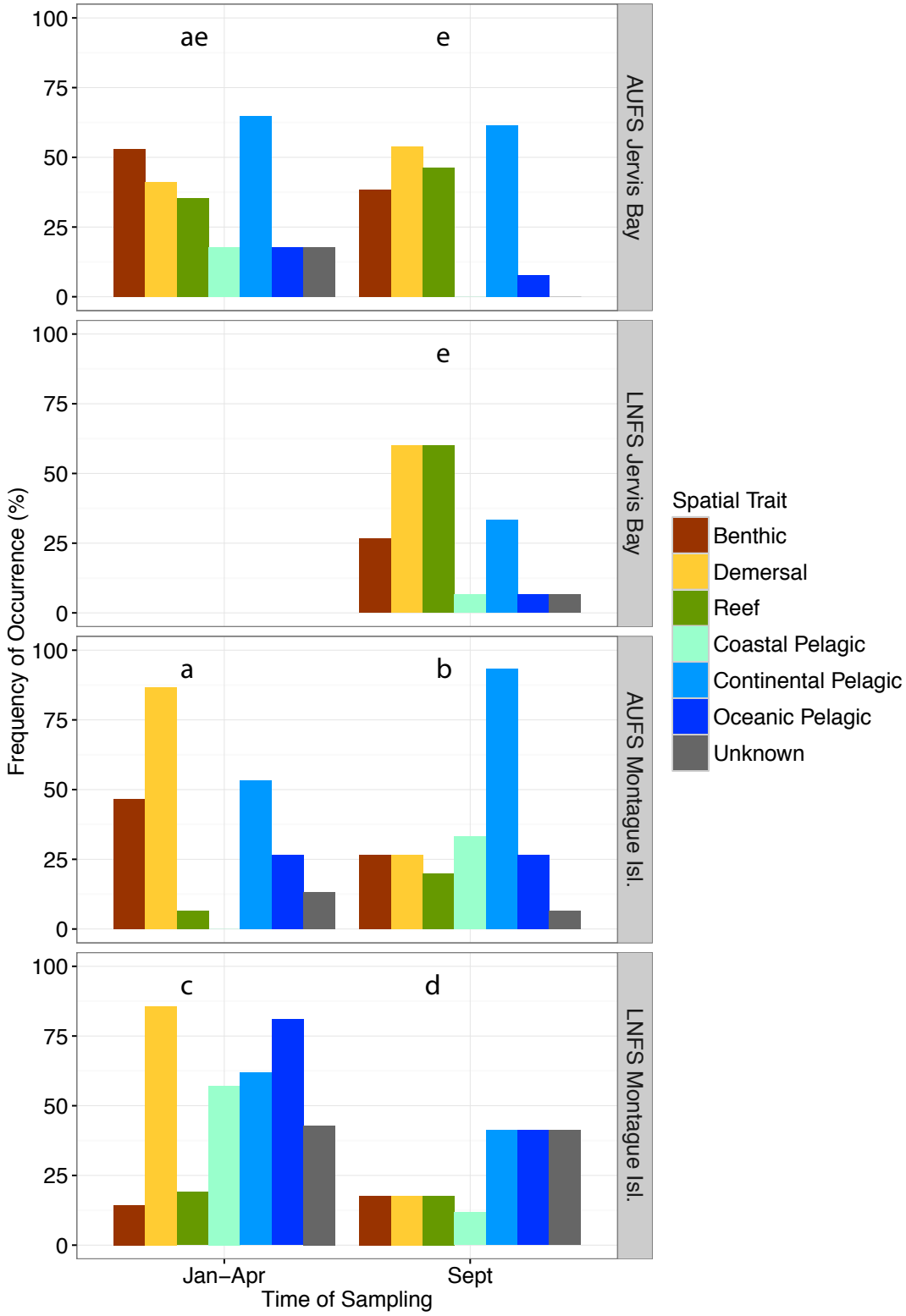

Figure S1 Percentage frequency of occurrence (FO%) of spatial groups across (AUFS and LNFS), location (MI and JB) and sampling time (Jan-Apr/summer* and Sept/winter 2014). Different letters denote significant differences in the prey assemblage between pairwise combinations of explanatory variables: seal species, location, and time. No FO% data are available for LNFS at JB in the summer months.