APPENDIX

METHODS

Primer optimization

We obtained samples of possible prey items (fish, squid, and krill) from fish trawls conducted off the coast of California and Oregon. We extracted genomic DNA from prey tissue using a Qiagen DNeasy Blood and Tissue Kit (69540, Qiagen) following the manufacturer's protocol. Genomic DNA was PCR amplified using universal COI (Folmer *et al*. 1994) and 16S primers. We confirmed positive amplification on gel electrophoresis and cleaned any positive amplifications using ExoSAP-IT (Applied Biosystems). All cleaned PCR products were Sanger sequenced at the University of Wisconsin-Madison Biotechnology Center. We visualized the sequence chromatograms, and we aligned the forward and reverse sequences using MUSCLE (Edgar 2004) as implemented in MEGA 7.0.26 (Kumar *et al*. 2016). After alignment, we BLAST (Altschul *et al*. 1990) searched the sequences to determine identity. Additionally, we downloaded reference sequences for the species of interest or closely related species from NCBI database (NCBI Resource Coordinators 2018) and added these sequences to our alignment. We used the Primaclade (Gadberry *et al*. 2005) web-based platform (The Santos Lab http://webhome.auburn.edu/~santosr/primaclade.htm) to create a selection of possible primers. We then chose from the list those primers that flanked the targeted insert size, were ~200bp, and had no repeats at the 3' end. Using the IDT OligoAnalyzer Tool (Owczarzy *et al*. 2008), we analyzed the selected primers for hairpins, self-dimers, and hetero-dimers. To confirm that our designed primers amplify prey DNA, we PCR amplified the prey DNA using the newly created

primers, cleaned the positively amplified PCR products, Sanger sequenced, visualized chromatograms, aligned sequences, and identified taxa following the same protocol as above.

Prey DNA amplification and library building

We amplified a fragment of the 16S gene in prey DNA using four primer sets: 16S (fish, Deagle *et al*. 2007), SQ16S (cephalopods), Cala16S (copepods and amphipods), and Mala16S (malacostracans). Although previous crustacean primers (Crust16S) were created for malacostracens in Berry *et al*. (2017), given this project started in 2016, we opted to keep the primers we originally created and optimized at the start of this project. Each primer was tagged with an Illumina TruSeq Universal adapter to allow for the attachment of unique barcodes. Each PCR master mix consisted of 1X PCR buffer (Agilent Technologies), 0.2 µM of forward and reverse primer, 0.25 mM dNTPs (Qiagen), 1 mM $MgCl_2$ (Agilent Technologies), 5 µg μL^{-1} BSA (Fisher Scientific), 0.02 units pfu Ultra HotStart (Agilent Technologies), 5 µL DNA extract, and water for a total of 25 µL in each reaction. The PCR parameters for each primer set consisted of an initial denaturation of 98°C for 30 seconds, followed by 40 cycles of 98°C for 30 seconds, 50-52° for 20 seconds, 68°C for 30 seconds, followed by a final elongation step of 68°C for 7 minutes.

For ocean water extractions, we first filtered the ocean water through a funnel apparatus to remove inhibitors and then we extracted genomic DNA from the filter using a Qiagen DNeasy Blood and Tissue kit (69540, Qiagen) following the protocol for tissue extraction with the following modifications: filter paper remained in the extraction tube until transference to the spin-column and DTT (dithiothreitol) was added at the original lysis stage. We performed three

extractions for each ocean water sample. All ocean water extractions were PCR amplified for each primer set.

We cleaned amplified PCR product with AMPure XP (Beckman Coulter) beads and quantified DNA concentrations of cleaned amplified product using a Qubit fluorometer. We then combined the amplified products for each sample in equimolar proportions and custom i5 and i7 indices with stem adapters were attached using KAPA HiFi HotStart ReadyMix (KAPA Biosytems). We cleaned the indexed products with AMPure XP beads to remove any unincorporated indices, quantified concentrations, and combined all samples equimolar into a single library. Libraries were then sequenced on Illumina MiSeq 2x250 nano runs with one library per lane by the University of Wisconsin-Madison Biotechnology Center.

Data analysis

We used QIIME2 (Bolyen *et al*. 2019) to filter and process raw sequence data. First, we used DADA2 (Callahan *et al*. 2016) plug-in to trim and filter our demultiplexed sequences and identify unique reads in each sample. We built a custom sequence database with possible prey sequences downloaded from GenBank (Clark *et al*. 2016) and the sequenced prey from our primer optimization. We also included Marbled Murrelet in our database for the identification of host sequences. To filter out spurious sequences before taxonomic assignment, we excluded sequences that did not query against our database based on BLAST search criteria. We only used those sequences with hits to the database in the rest of the downstream analysis. We trained a naïve Bayes classifier on our custom database in QIIME2 and then assigned OTUs to our

sequenced reads. We re-ran this procedure with multiple filtering, trimming, and taxonomic assignment cut-offs to maximize the number of identified taxa and minimize spurious or incorrectly classified mOTUs. To further ensure correct taxonomic assignment, we also performed closed and opened clustering. For any taxonomic assignment with less than 95% confidence in taxonomic assignment, we BLAST (Altschul *et al.* 1990) searched against the NCBI database to confirm taxonomic assignment and percent sequence coverage. We then further filtered our final assignments by removing any assignment to an individual sample with less than 15 reads for fish and less than 30 reads for invertebrates.

REFERENCES

ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.W. & LIPMAN, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403-410. doi:10.1016/s0022-2836(05)80360-2

ANTHONY, J.A., ROBY, D.D. & TURCO, K.R. 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. *Journal of Experimental Marine Biology and Ecology* 248: 53–78. doi:10.1016/S0022-0981(00)00159-3

BERRY, T. E., OSTERRIEDER, S. K., MURRAY, D. C., ET AL. 2017. DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (*Neophoca cinerea*). *Ecology and Evolution* 7: 5435-5453. doi:10.1002/ece3.3123

CALLAHAN, B.J., MCMURDIE, P.J., ROSEN, M.J., HAN, A.W., JOHNSON, A.J.A. & HOLMES, S.P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* 13: 581-583. doi:10.1038/nmeth.3869

CLARK, K., KARSCH-MIZRACHI, I., LIPMAN, D.J., ET AL. 2016. GenBank. *Nucleic Acids Research* 44: D67-D72. doi:10.1093/nar/gkv1276

DEAGLE, B.E., GALES, N.J., EVANS, K., ET AL. 2007. Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (*Eudyptes chrysolophus*). *PLoS One* 2: e831. doi:10.1371/journal.pone.0000831

EDGAR, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792-1797. doi:10.1093/nar/gkh340

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–9.

GADBERRY, M.D., MALCOMBER, S.T., DOUST, A.N. & KELLOGG, E.A. 2005. Primaclade—a flexible tool to find conserved PCR primers across multiple species. *Bioinformatics* 21: 1263-1264. doi:10.1093/bioinformatics/bti134

KUMAR, S., STECHER, G. & TAMURA, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874. doi:10.1093/molbev/msw054

LAWSON, J.W., MAGALHÃES, A.M. & MILLER, E.H. 1998. Important prey species of marine vertebrate predators in the northwest Atlantic: Proximate composition and energy density. *Marine Ecology Progress Series* 164: 13–20. doi:10.3354/meps164013

LOGERWELL, E.A. & SCHAUFLER, L.E. 2005. New data on proximate composition and energy density of Steller Sea Lion (*Eumetopias jubatus*) prey fills seasonal and geographic gaps in existing information. *Aquatic Mammals* 31: 62–82. doi:10.1578/AM.31.1.2005.62

MORISSETTE, O., PARADIS, Y., POULIOT, R. & LECOMTE, F. 2018. Spatio-temporal changes in littoral fish community structure along the St. Lawrence River (Québec, Canada) following Round Goby (*Neogobius melanostomus*) invasion. *Aquatic Invasions* 13: 501–512. doi:10.3391/ai.2018.13.4.08

NCBI RESOURCE COORDINATORS. 2018. Database resources of the national center for biotechnology information. *Nucleic Acids Research*, 46, no. D1: D8-D13. doi:10.1093/nar/gkx1095

OWCZARZY, R., TATAUROV, A.V., WU, Y., ET AL. 2008. IDT SciTools: a suite for analysis and design of nucleic acid oligomers. *Nucleic Acids Research* 36(suppl_2):W163-W169. doi:10.1093/nar/gkn198

TIRELLI, V., BORME, D., TULLI, F., CIGAR, M., FONDA UMANI, S. & BRANDT, S.B. 2006. Energy density of anchovy *Engraulis encrasicolus* L. in the Adriatic Sea. Journal of Fish Biology 68: 982–989. doi:10.1111/j.0022-1112.2006.00987.x

TABLE S1

Primers used in this study to amplify fish, cephalopods, malacostracans, copepods, and amphipods. The PCR parameters consisted of an initial denaturation of 98°C for 30 seconds, followed by 40 cycles of 98°C for 30 seconds, 50-52°C for 20 seconds, 68°C for 30 seconds, followed by a final elongation step of 68°C for 7 minutes.

Taxa/Taxon	Primer name	Sequence	Anneal Temp.
Fish	16S1-F[a]	GACGAKAAGACCCTA	50°C
	16S1-R[a]	CGCTGTTATCCCTADRGTAACT	
Cephalopod	SQ16S-F	CGCCTGTTTATCAAAAACAT	52°C
	SQ16S-R	AAATAGAGACAGSTTAACCTTCG	
Malacostracans (shrimp, krill, prawns, crabs)	Mala16S-F	GACGATAAGACCCTWTAAARCT	52°C
	Mala16S-R	TCAACATCGAGGTCGYAA	
Copepod and amphipod	Cala16S-F	AGACRARAAGACCCTATGAAGC	50°C
	Cala16S-R	AYGCTGTTATCCCTAGAGGAGC	

[a.] Deagle *et al.* 2007

TABLE S2

Average energy density of prey types consumed by Marbled Murrelets in Oregon in 2017 and 2018. Average energy density was estimated from average wet weight energy densities from published sources.

Common Name	Scientific Name	Average Energy Density (kJ g^{-1})	Data Source
smelt spp.	Family: Osmeridae	4.84	a,b,h
invertebrate spp. (crustaceans and cephalopods)		3.15	a,c,d,e,j
flatfish spp.	Family: Pleuronectidae	3.39	b,f,j
hexagrammid spp.	Family: Hexagrammidae	3.72	a,c
northern anchovy	*Engraulis mordax*	6.00	a,j,l
Pacific herring	*Clupea pallasii*	7.77	a,g
Pacific sand lance	*Ammodytes hexapterus*	5.17	a,1l
juvenile rockfish spp.	*Sebastes* spp.	4.51	a,c,g,j
Pacific sardine	*Sardinops sagax*	5.62	a,i,j
sculpin spp.	Family: Cottidae	3.99	b,f
lingcod	*Ophiodon elongatus*	4.00	a,b,j
longnose skate	Family: Rajidae	4.80	h
crescent gunnel	Family: Folidae	4.69	b
bay goby	Family: Oxudercidae	3.20	k

a: Schrimpf *et al*. 2012
b: Anthony *et al*. 2000
c: Van Pelt *et al*. 1997
d: Hunt Jr *et al*. 2005
e: Haynes & Wigley 1969
f: Glaser 2010
g: Lawson *et al*. 1998
h: Logerwell & Schaufler 2005
i: Tirelli *et al*. 2006
j: Sinclair *et al*. 2015
k: Morissette *et al*. 2018
l: Janssen *et al*. 2011

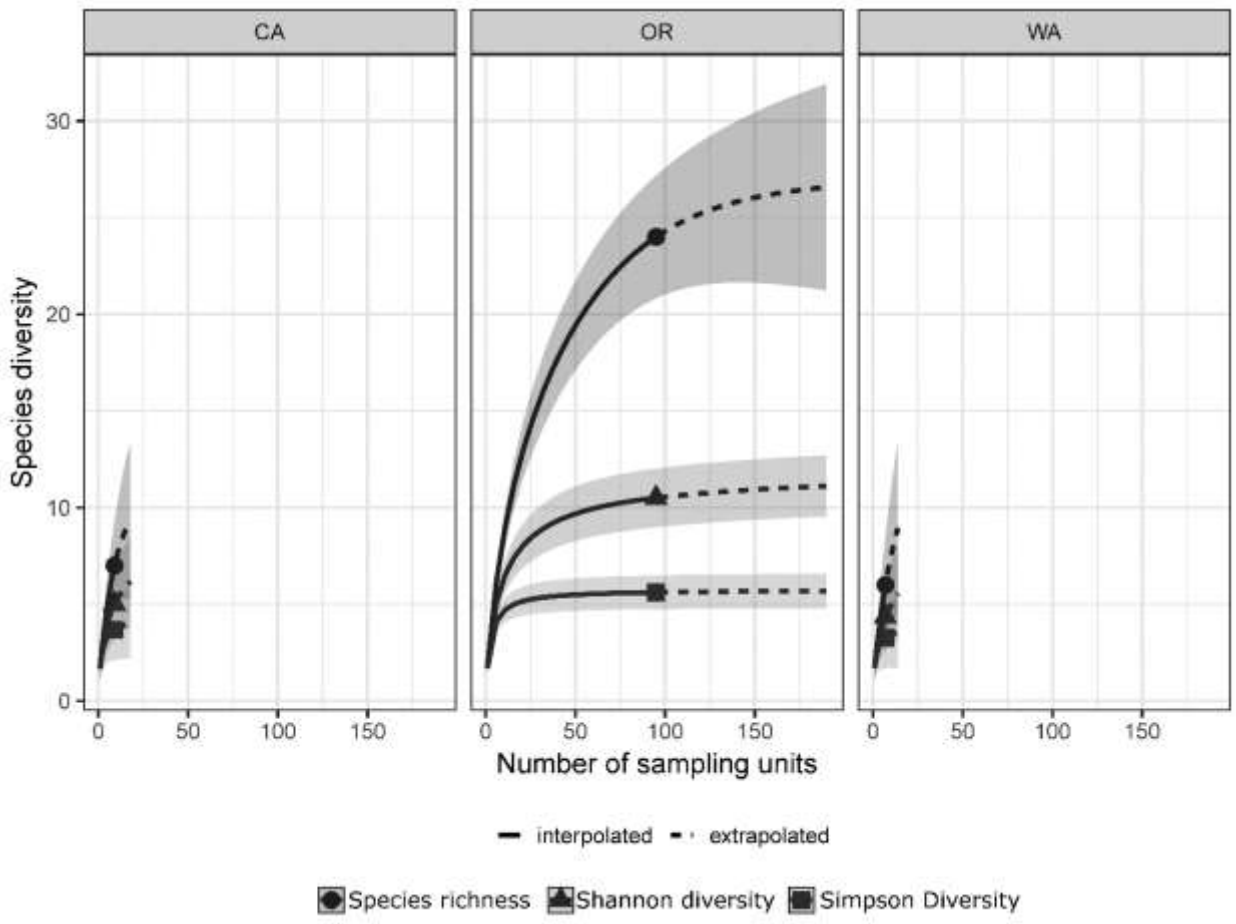

Fig. S1. Sample accumulation curves for each sampling location (California, Oregon, and Washington) with all years combined showing if the number of sampling units is sufficient to determine species diversity using three metrics: species richness, Shannon Diversity and Simpson Diversity.

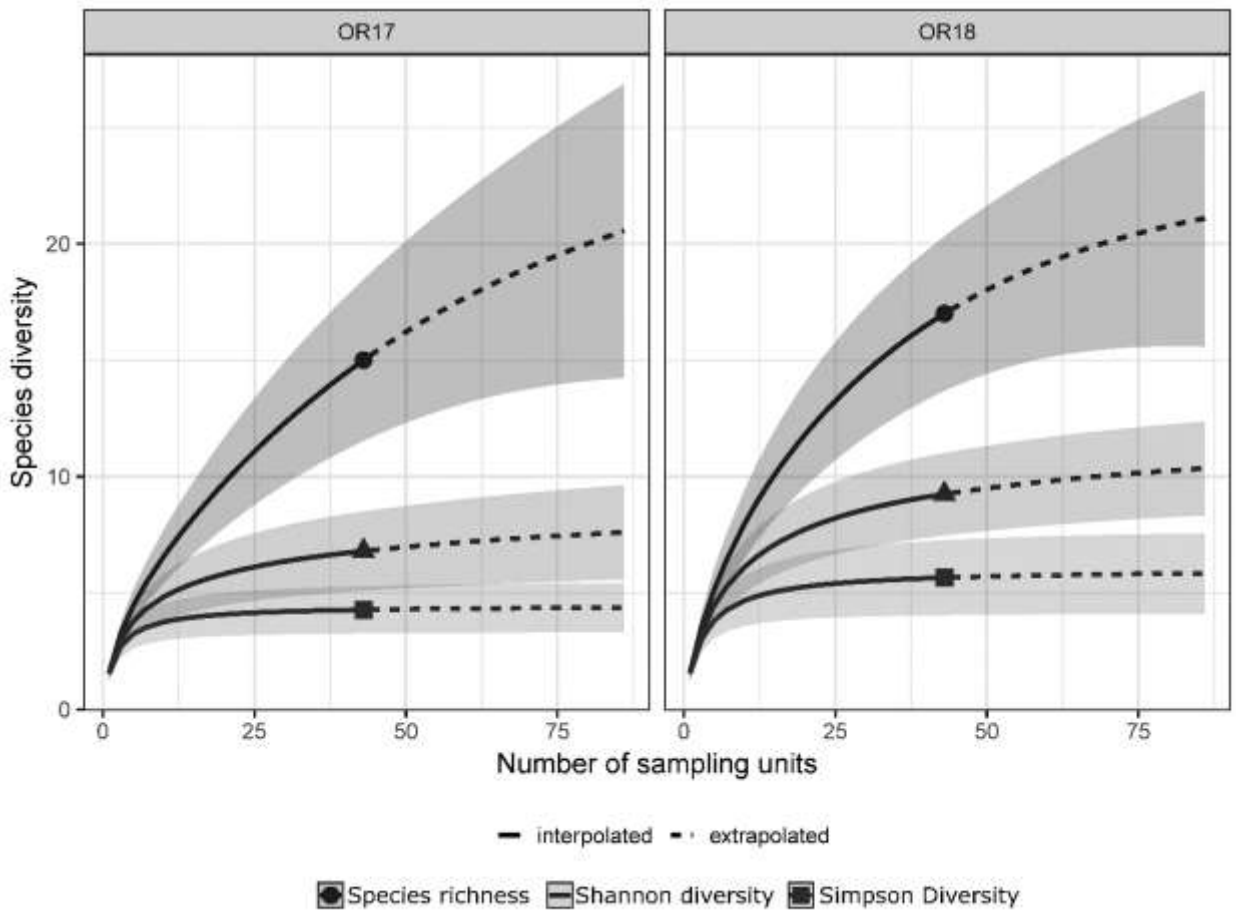

Fig. S2. Sample accumulation curves for each sampling location for Oregon 2017 and Oregon 2018 showing if the number of sampling units is sufficient to determine species diversity using three metrics: species richness, Shannon Diversity and Simpson Diversity.