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UPDATE ON THE PRESENCE OF JAPANESE SARDINE (*SARDINOPS MELANOSTICTA*) IN THE CALIFORNIA CURRENT LARGE MARINE ECOSYSTEM 2025

Gary Longo[1], Kelsey James[2], Kristen Hinton[3], Jennifer Topping[3], and Matthew Craig[2]

[1]Ocean Associates, Inc., under contract with NOAA Southwest Fisheries Science Center
Fisheries Resources Division
La Jolla, California

[2]NOAA Southwest Fisheries Science Center
Fisheries Resources Division
La Jolla, California

[3]Washington Department of Fish and Wildlife
Olympia, WA

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INTRODUCTION

The Southwest Fisheries Science Center (SWFSC) has conducted surveys of coastal pelagic species in the California Current Large Marine Ecosystem (CCLME) using the acoustic trawl method since 2006 (Renfree, et al., 2024). This survey primarily targets northern anchovy (*Engraulis mordax),* chub mackerel (*Scomber japonicus*) also known as Pacific mackerel, and Pacific Sardine (*Sardinops sagax*). Data from this survey are used to calculate biomass for use in management of these ecologically and (at one time) economically important species. The Pacific Sardine biomass in U.S. waters is currently low and its periodic fluctuations in biomass are closely monitored by the SWFSC. The directed fishery has been closed since 2015 with an exception for the live bait fishery.

During the course of a genetic analysis of population structure of the Pacific Sardine (*S. sagax*) across the northeast Pacific (Longo et al., 2025), the presence of Japanese Sardine (*S. melanosticta*) was documented (Longo et al., 2024). Longo et al. (2024) reported the presence of Japanese Sardine in 2022 and 2023, but did not detect its presence from 2013-2021. The presence of Japanese Sardine in U.S. waters poses several questions that relate to the management and ecosystem dynamics of Pacific Sardine and as such genetic methods continue to be used to track its presence in the CCLME. This report presents the results from the 2025 survey and updates the genetic methods used.

METHODS

Tissue samples from *Sardinops* spp. (n=1045) were collected during the 2025 Joint USA Canada Integrated West Coast Pelagics Survey (IWCPS; Clemons et al., 2026). The NOAA Ship Bell M. Shimada conducted offshore acoustic and trawl sampling from the U.S./Mexico border to U.S./Canada border while two chartered commercial purse seine vessels (F/V Lisa Marie and F/V Long Beach Carnage) conducted these activities from nearshore waters (details in Stierhoff et al., 2026). Tissue samples (caudal muscle) were preserved at sea in 100% ethanol. DNA was extracted by combining a tissue subsample and 150µL 10% (w/v) Chelex 100 resin beads suspended in deionized water and heating to 60˚C for 20 minutes, followed by 103˚C for 25 minutes.

Previous reports (Longo et al., 2024, Longo et al., 2025, Longo et al., 2025b) used a mitochondrial DNA-based assay to identify Japanese and Pacific Sardine. An updated, nuclear DNA gtSeq panel was developed to simultaneously identify sardine species and screen for possible hybridization. At the current time, we have not fully developed the assay for the detection of hybrids, however, it is ready to deploy for species identification of *Sardinops* in the eastern Pacific. Finalized methods will be presented in a forthcoming paper, and we report methods sufficient to replicate the species identification herein.

Genome data from Pacific Sardine (*Sardinops sagax*) and Japanese Sardine (*Sardinops melanosticta*; Longo et al.2024) were queried for highly differentiated loci to develop a GTseq identification panel (Campbell et al. 2015). Large differences in pairwise F_{ST} estimates were used to identify candidate loci. Primers were designed to include the identified SNP and 150bp upstream and downstream flanks. 179 candidate loci were tested and after optimization 84 primer pairs targeting 88 informative SNPs were chosen.

GTSeq libraries were prepared using a two-step PCR process. PCR1 reactions were prepared using Qiagen Multiplex Plus Master Mix (3.5µL master mix, 1.5µL pooled primer mix [250 nM each primer], and 2µL template DNA). Following an initial denaturation at 95˚C for 5

min thermal cycling parameters were as follows: 5 rounds of 95˚C for 30 sec, 57˚C for 90 sec, 72˚C 2min, 10 rounds of 95˚C for 30 sec, 65˚C for 90 sec, 72˚C 30 sec. PCR2 was performed to add Illumina i5 and i7 index sequences. PCR2 reactions were prepared using Qiagen Taq PCR Mastermix (5µL master mix, 2µL i5 index at 10µM, 1 µL i7 index primer at 10µM, and 3µL 1:10 diluted PCR 1). Following an initial denaturation at 95˚C for 5 min, thermal cycling parameters were as follows: 12 rounds of 98˚C for 10 sec, 65˚C for 90 sec, 72˚C 30 sec, followed by a final extension at 72˚C for 5 min.

Indexed PCR samples were cleaned and normalized using Charm Biosciences Just-a-Plate Purification and Normalization kit following manufacturer's protocol. Pooled, normalized PCR reactions (libraries) were size-selected using Ampure XP beads following manufacturer's protocol. Libraries were prepped for sequencing on an Illumina MiSeq following Illumina protocols.

Demultiplexed FASTQ files were genotyped using the bioinformatic pipeline *GT-score* (McKinney et al. 2020; https://github.com/gjmckinney/GTscore). *ProbeDesign_GTscore.Rmd* was used to generate in silico primer-probe sequences to target the forward locus-specific primers of PCR1 and probes targeting desired SNPs and flanking regions. *AmpliconReadCounter.pl* was used to match primer-probe sets to each sample's amplicon sequences to count the occurrence of each unique sequence and call genotypes. Sequences that did not align were excluded as off-target. Individuals were flagged as potentially contaminated when conscore (percent of heterozygous loci with significantly different allele depths) was > 0.25 and heterozygosity was > 0.25. As a further safeguard against potential contamination, individuals with heterozygosity > H = 0.25 were excluded from analysis.

The R package *Rubias* v0.3.2 (Moran & Anderson 2018) was used to perform individual assignments through comparisons to GTseq genotype data from the reference sample set. For species identification, we ran *infer_mixture()* using default parameters and calls from the reference sample set. Individual calls were made based on the highest posterior mean of group membership (PofZ) for the two possible species.

A subset of individuals collected during the survey (those allocated to the Northern Subpopulation and collected on NOAA Ship Bell M. Shimada and F/V Lisa Marie) were aged. Whole sagittal otoliths were collected with corresponding tissue samples. Otoliths were extracted, cleaned with water, placed in 0.6 mL microcentrifuge tubes, and allowed to dry overnight. For age reading the whole otoliths were submerged in water or ethanol in a small dish with a black background and viewed under reflected lighting using a Leica MZ10 F or M80 stereomicroscope. Ages were generated from counting annuli without knowledge of species, date of capture, sex, length, or weight. Fish were aged prior to their species allocation.

RESULTS

Of the 1045 sardine processed, 901 sardine samples passed quality filters from the 2025 IWCPS. Among those, 71 (7.88%) were determined to be Japanese Sardine (Table 1). In general sardine were scarce north of latitude 40° (Figure 1). Japanese Sardine were detected from Mendocino, CA, to just north of the U.S./Mexico border. The largest concentration of Japanese Sardine was located in the stretch of water adjacent to the Big Sur coastline in Central California (Figure 1). In many instances, Japanese and Pacific Sardine were caught in the same trawl or seine. Six of the 71 Japanese Sardine from 2025 were allocated to the Northern Subpopulation and were aged (one age 0, four age 4, and one age 5).

DISCUSSION

The presence of Japanese Sardine in the CCLME in 2025 marks the fourth consecutive year of detection. It should be noted here that one specimen from 2014 was previously reported in presentations of this work as being identified as a Japanese Sardine. That sample was subsequently determined to be a Pacific Sardine with an introgressed Japanese mitogenome (i.e., with 100% of the Pacific Sardine nuclear genome; Longo et al., 2024). This may be evidence of hybridization from a past incursion of Japanese sardine to the east Pacific, or it could be a remnant of the shared evolutionary history between the two species.

The geographic extent of the distribution of Japanese Sardine in the CCLME in 2025 was more restricted than previous years (Figures 2, 3, and 4). In particular its northward limit was much further south than previous years. The relative proportion of Japanese Sardine to Pacific Sardine in 2025 continued to decrease from 2022 and 2023 (Table 1), however it is important to note that sampling effort and the spatiotemporal extent of sampling were not equivalent for each sampling year. In addition, sampling of sardine for genetic material was not synoptic over the four-year period due to variation in sampling efforts which were not originally designed to track the presence of Japanese Sardine. In 2022, genetic samples were only taken for a limited number of sardine from five geographic regions within the survey area. In 2023, tissue samples for genetic analysis were taken from each sardine for which additional biological data was also taken (e.g., otoliths, gonads), but no samples were available from the southern portion of the nearshore survey. In 2024, only a small number of sardine collected in the southern portion of the nearshore survey had associated tissue samples available for genetic analysis (see Figure 2). Therefore, any differences in the relative proportion of each species may be an artifact of sampling rather than an indication of changes in abundance or biomass of either.

The distribution of Japanese Sardine ages from 2022 (the initial year of detection) to 2024 appeared to show a cohort effect with the most common age class becoming successively older in the second and third year of collection (Longo et al., 2025b). However, with only six aged Japanese Sardine in 2025, there are not enough data to assess whether this trend holds. Additionally, it is interesting to note that no age zero fish were collected in 2022 but were collected in subsequent years (2023-2025). This indicates that Japanese Sardine are spawning in the eastern Pacific, a conclusion supported by the discovery of Japanese Sardine larvae off the coast of Oregon, USA (Andrew et al., 2026).

Again, it is important to note that the sampling of sardine tissue for genetic analysis was not synoptic over the four-year period. Additionally, only fish assigned to the northern subpopulation of Pacific Sardine were aged thus excluding some Japanese Sardine that were collected but not aged, particularly in nearshore southern California. These and other variables make it difficult to interpret these age data with certainty.

The precise mechanism for dispersal of Japanese Sardine into the CCLME is not known, however Longo et al. (2024) hypothesized that it was likely related to anomalous ocean conditions in the far north Pacific. The impact of Japanese Sardine on the CCLME is also unknown. The presence of both species in the same trawl or seine indicates that they likely occur in mixed schools. However, it is unclear if Japanese and Pacific Sardine are able to hybridize or the degree to which the two species may be competing for resources. The improved method described here will allow for the detection of hybrids should they exist in the population.

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TABLES

Table 1. Number (N) of sardine samples analyzed for the presence of Japanese Sardine for samples collected in the SWFSC Coastal Pelagic Species survey 2013-2025. Samples prior to 2025 were identified to species using the mitochondrial DNA panel (Longo et al., 2024) while samples from 2025 were identified to species using the updated nuclear DNA panel.

Year	N Analyzed	N Pacific Sardine	N Japanese Sardine	% Japanese Sardine
2025	901	830	71	7.88
2024	613	501	112	18.27
2023	825	491	334	40.48
2022	172	100	72	41.86
2021	81	81	0	0.00
2019	198	198	0	0.00
2018	131	131	0	0.00
2017	269	269	0	0.00
2016	622	622	0	0.00
2015	175	175	0	0.00
2014	211	211	0	0.00
2013	892	892	0	0.00

FIGURES

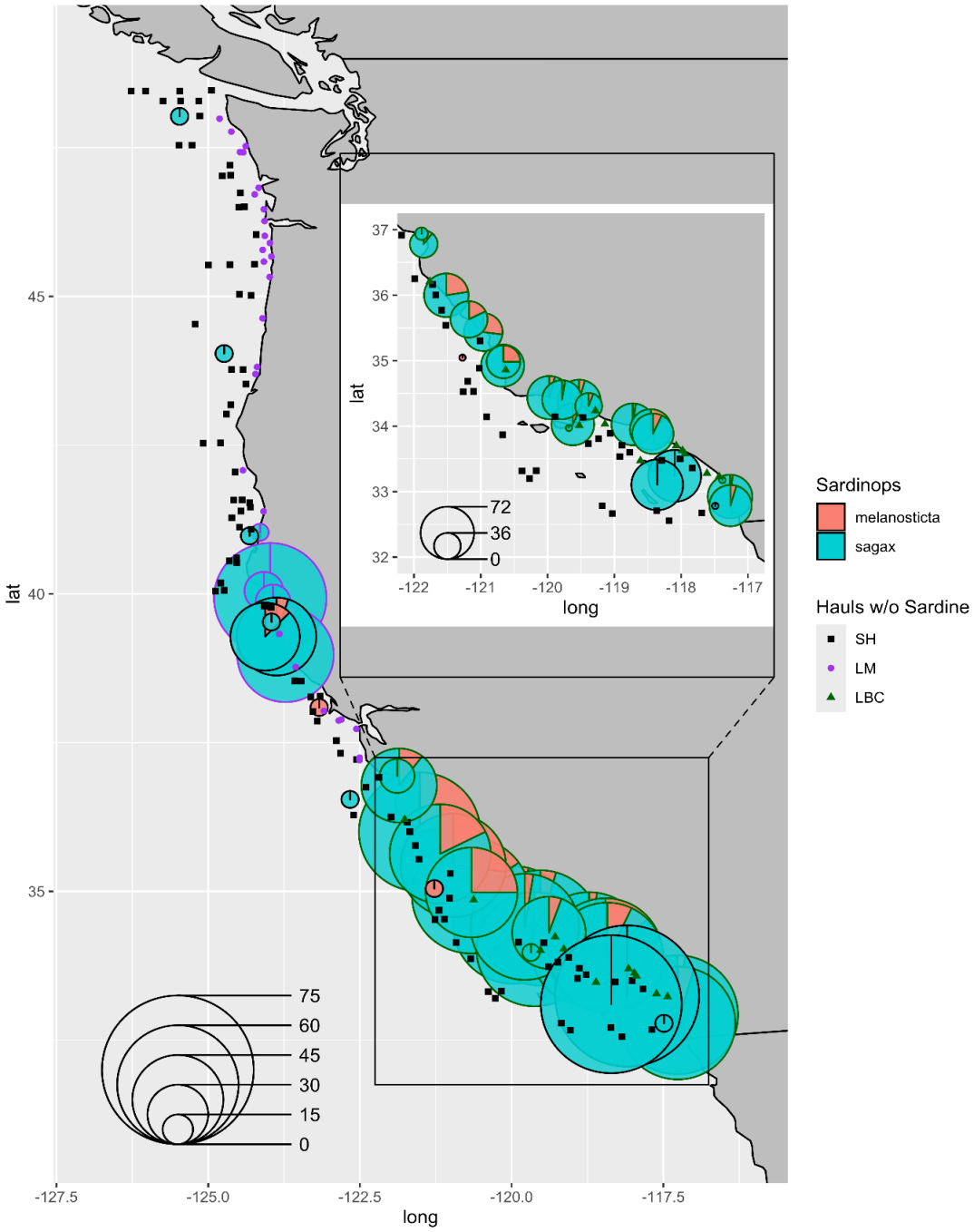

Figure 1. Trawl locations of Pacific and Japanese Sardine taken during the 2025 IWCPS survey aboard NOAA Ship *Bell M. Shimada* (SH) = black, F/V Lisa Marie (LM) = purple, and F/V Long Beach Carnage (LBC = green). Points indicate trawl or purse seine locations where sardine were not caught (color and shape correspond to vessel). Pies represent the relative proportion of species per trawl (pie outlines are color coded by vessel). Size of pie is relative to the number of individuals analyzed from each location (rescaled in the inset for Southern California).

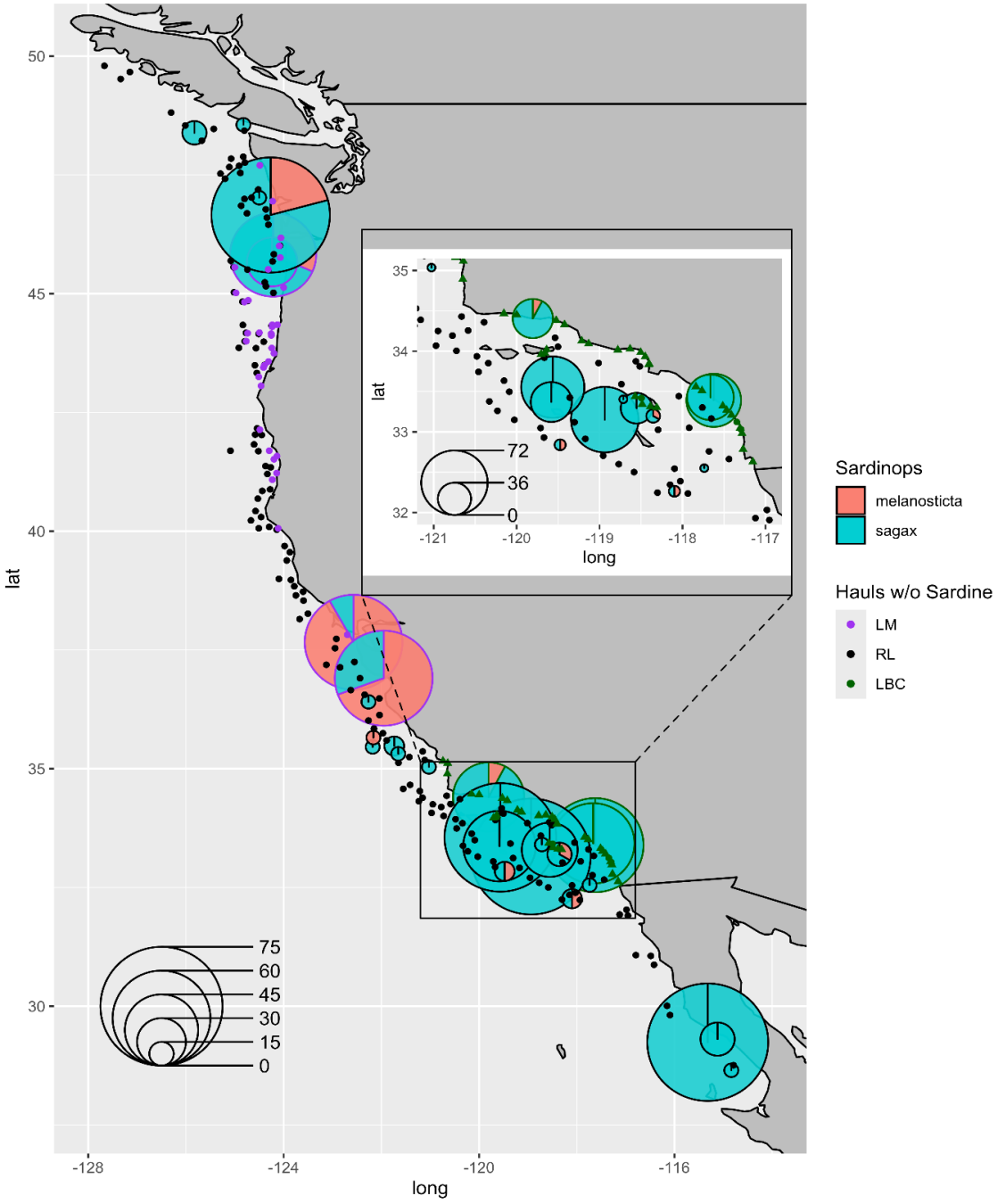

Figure 2. Trawl locations of Pacific and Japanese Sardine taken during the 2024 CCES survey aboard NOAA *Ship Rueben Lasker* (RL) = black, F/V Lisa Marie (LM) = purple, and F/V Long Beach Carnage (LBC = green). Dots indicate additional trawl locations where sardine were not collected, and triangles represent hauls with sardine collected that were not sampled for genetics. Pies represent the relative proportion of species per trawl. Size of pie is relative to number of individuals analyzed from each location (rescaled in the inset of the Southern California Bight).

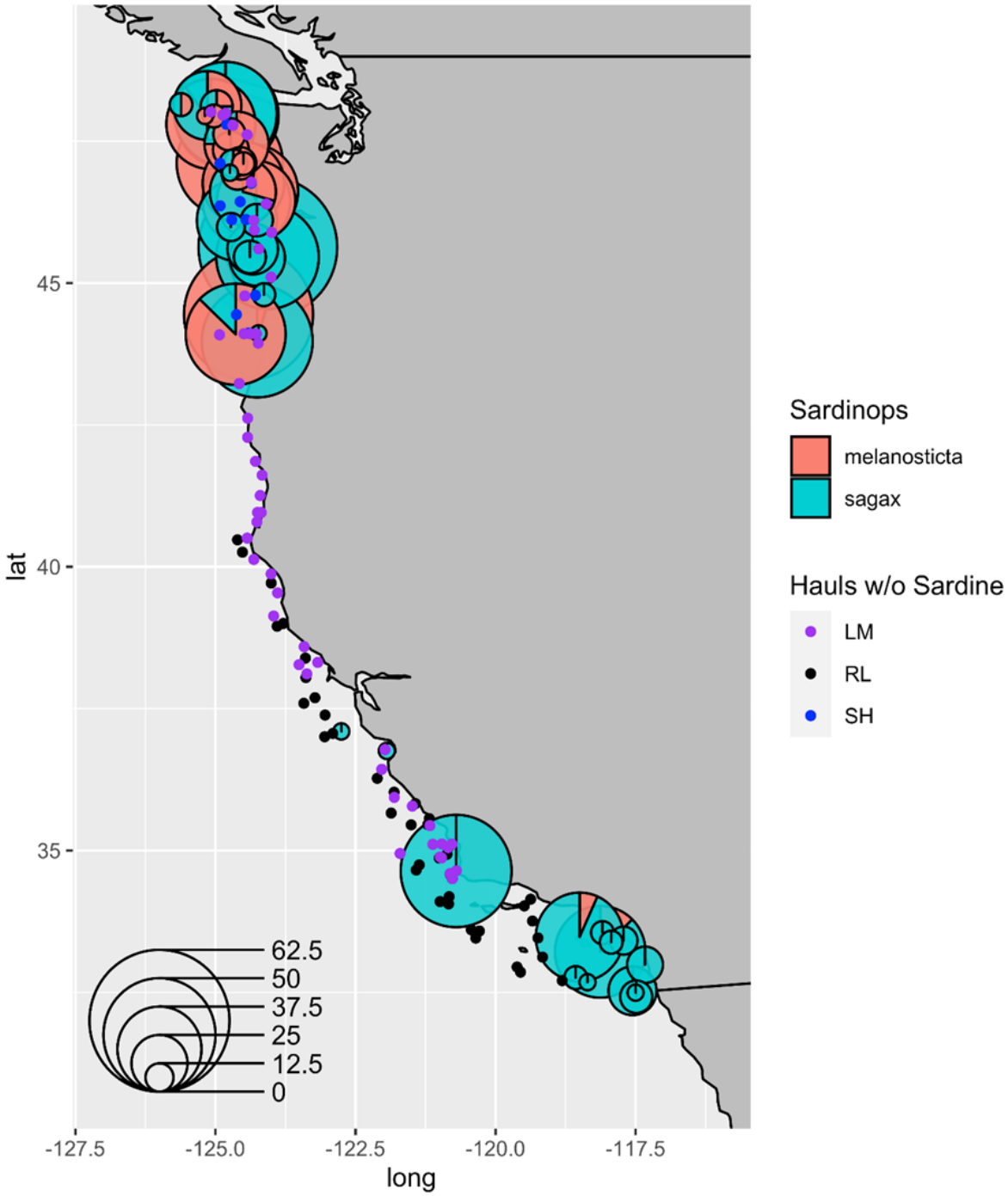

Figure 3. Trawl locations of Pacific and Japanese Sardine taken during the 2023 CCES survey aboard the F/V Lisa Marie (LM) = purple, NOAA Ship *Rueben Lasker* (RL) = black, and NOAA Ship *Bell M. Shimada* (SH) = blue. Dots indicate additional trawl locations that did not catch sardine. Pies represent the relative proportion of species per trawl. Size of pie is relative to the number of individuals analyzed from each location.

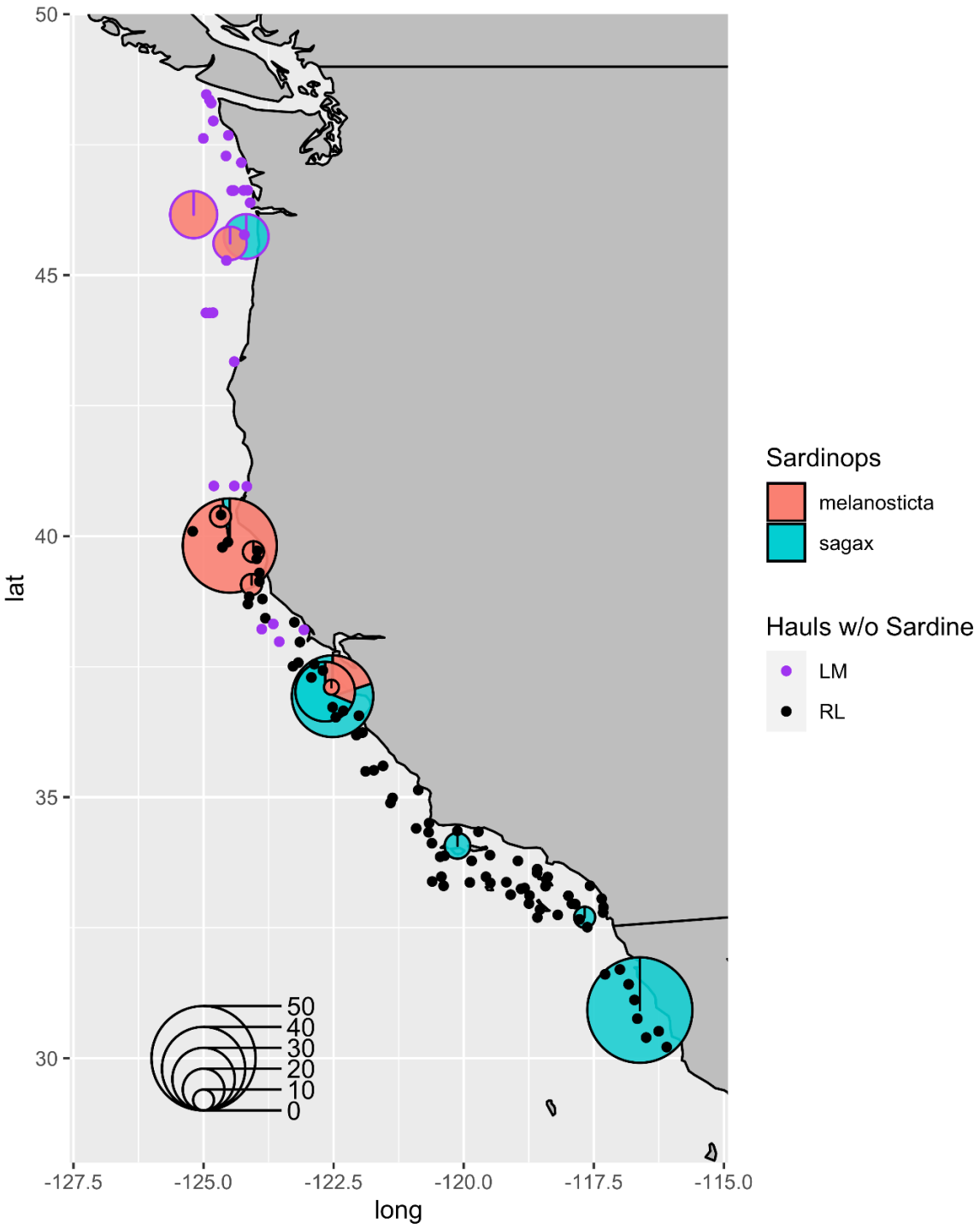

Figure 4. Trawl locations of Pacific and Japanese Sardine taken during the 2022 CCES survey aboard NOAA Ship *Rueben Lasker* (RL) = black and M/V Lisa Marie (LM) = purple. Dots indicate additional trawl locations where tissue samples for sardine were not collected but that may have taken sardine. Pies represent the relative proportion of species per trawl. Size of pie is relative to the number of individuals analyzed from each location.