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UPDATE ON THE PRESENCE OF JAPANESE SARDINE (*Sardinops melanosticta*) IN THE CALIFORNIA CURRENT LARGE MARINE ECOSYSTEM 2024

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Update on the presence of Japanese Sardine (*Sardinops melanosticta*) in the California Current Large Marine Ecosystem 2024

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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 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 its northeast Pacific range, 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 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 2024 survey.

Methods

Sardinops spp. were collected during the 2024 coastal pelagic species survey conducted by the SWFSC which deployed three vessels to collect data and biological samples. The NOAA Ship Reuben Lasker conducted offshore sampling from northern Baja California to Vancouver Island, British Columbia, while two chartered commercial purse seine vessels (F/V Lisa Marie and F/V Long Beach Carnage) collected acoustic and catch data from nearshore waters (details may be found in Renfree, et al., 2024). Aboard the NOAA Ship Reuben Lasker, a new multi-function trawl net was used in 2024 whereas in previous years a Nordic 264 trawl was used (for details see Renfree, et al., 2024). Tissue samples (caudal muscle) were preserved at sea in 100% ethanol. While sardine were collected by all three vessels, only a subset of tissues were available from those collected aboard the F/V Long Beach Carnage (see Discussion). A subsample of each tissue sample was removed and DNA was extracted by combining the 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. Detailed genetic methods may be found in Longo et al. (2024). Briefly, a modified GTseq protocol was developed which included the design of a custom panel to target three regions of the mitochondrial genome with putative, diagnostic (fixed) single nucleotide polymorphisms. Two rounds of PCR were performed to amplify the target regions and add Illumina i5 and i7 indexes. Indexed PCR samples were cleaned and normalized. Pooled, normalized PCR reactions (libraries) were subjected to size-selection using Ampure XP beads. Libraries were prepped for sequencing using an Illumina MiSeq following Illumina protocols. Bioinformatic methods can be found in Longo et al. (2024).

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. Only fish assigned to the Northern Subpopulation of Pacific sardine were aged and these were aged prior to genetic identification as Japanese sardine. No fish from the F/V Long Beach Carnage were aged in 2024.

Results

613 sardine samples passed quality filters from the 2024 survey. Among those, 112 (18.3%) were determined to be Japanese sardine (Table 1). Japanese sardine were detected across the survey area from Washington state to just south of the U.S./Mexico border. The largest concentration of Japanese sardine was located in the waters adjacent to San Francisco Bay (Figure 1). In many instances, Japanese and Pacific sardine were caught in the same trawl. Japanese sardine in 2024 were age 0 and ages 3-5 years (Figure 2).

Discussion

The presence of Japanese sardine in the CCLME in 2024 marks the third consecutive year in which it has been detected. 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 is not evidence of hybridization, rather it is a remnant of the shared evolutionary history between the two species.

The geographic extent of the distribution of Japanese sardine in the CCLME in 2024 extends further south than in 2022 (Figure 3) and 2023 (Figure 4) but its northward limit remains consistent among all three years. The relative proportion of Japanese sardine to Pacific sardine decreased in 2024 (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 three-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 ~170 of the ~1300 sardine collected in the southern portion of the nearshore survey had associated tissue samples available for genetic analysis (see Figure 4). 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 appears to show a cohort effect with the most common age class becoming successively older in the second and third year of collection. Additionally, it is interesting to note that no age zero fish were collected in 2022 but were collected in 2023 and 2024. This may indicate that Japanese sardine are reproducing in the CCLME as age zero fish would be unlikely to have the ability to move long distances and it is highly unlikely that egg or larval transport from distant localities could have resulted in successful recruitment. However, it is important to note that the sampling

of sardine tissue for genetic analysis was not synoptic over the three-year period which could bias the data. 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 where sardine are known to recruit. 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 may be related to changing 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 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. No samples have been analyzed for the presence of hybridization, however a method for detecting hybridization between these two species is currently being developed at the SWFSC which may help to understand the dynamics between these two species in the CCLME.

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Table 1. Number (N) of sardine samples analyzed for the presence of Japanese sardine for samples collected in the SWFSC Coastal Pelagic Species survey 2013-2024.

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

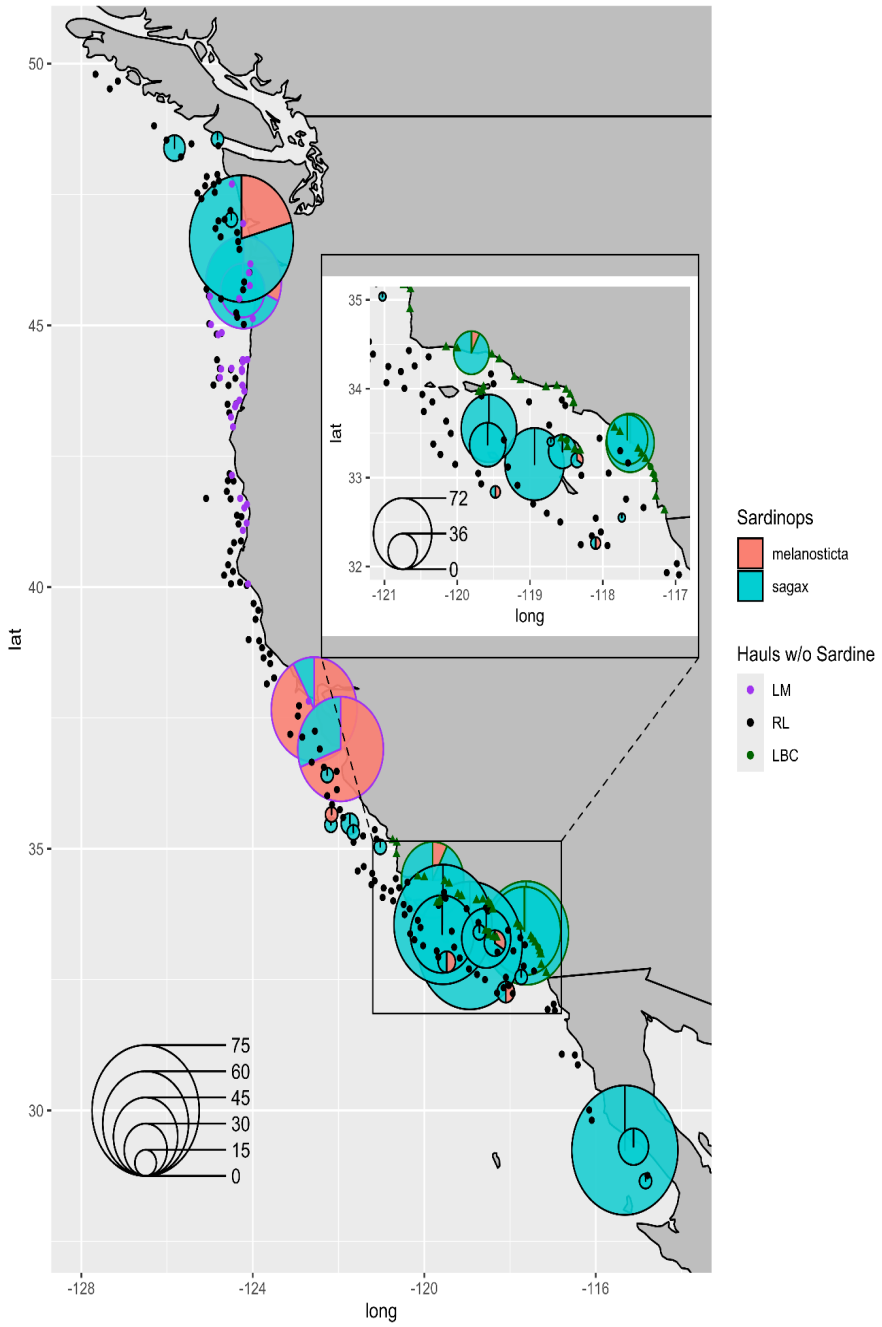
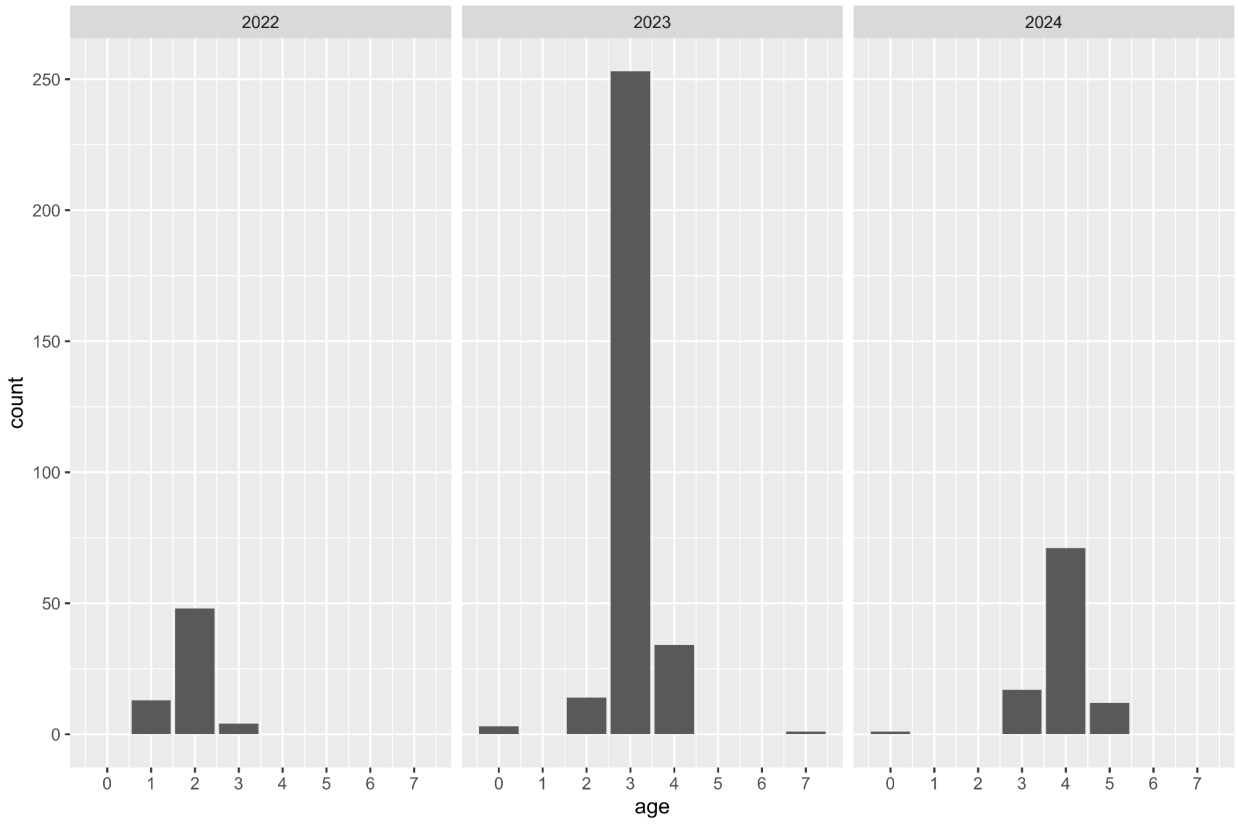


Figure 1. Trawl locations of Pacific and Japanese sardine taken during the 2024 CCES survey aboard the F/V Rueben Lasker (RL) = black, M/V Lisa Marie (LM) = purple, and F/V Long Beach Carnage (LBC = green). Dots indicate additional trawl locations where sardine were not collected, and triangles represent 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 (rescaled in the inset of the Southern California Bight).



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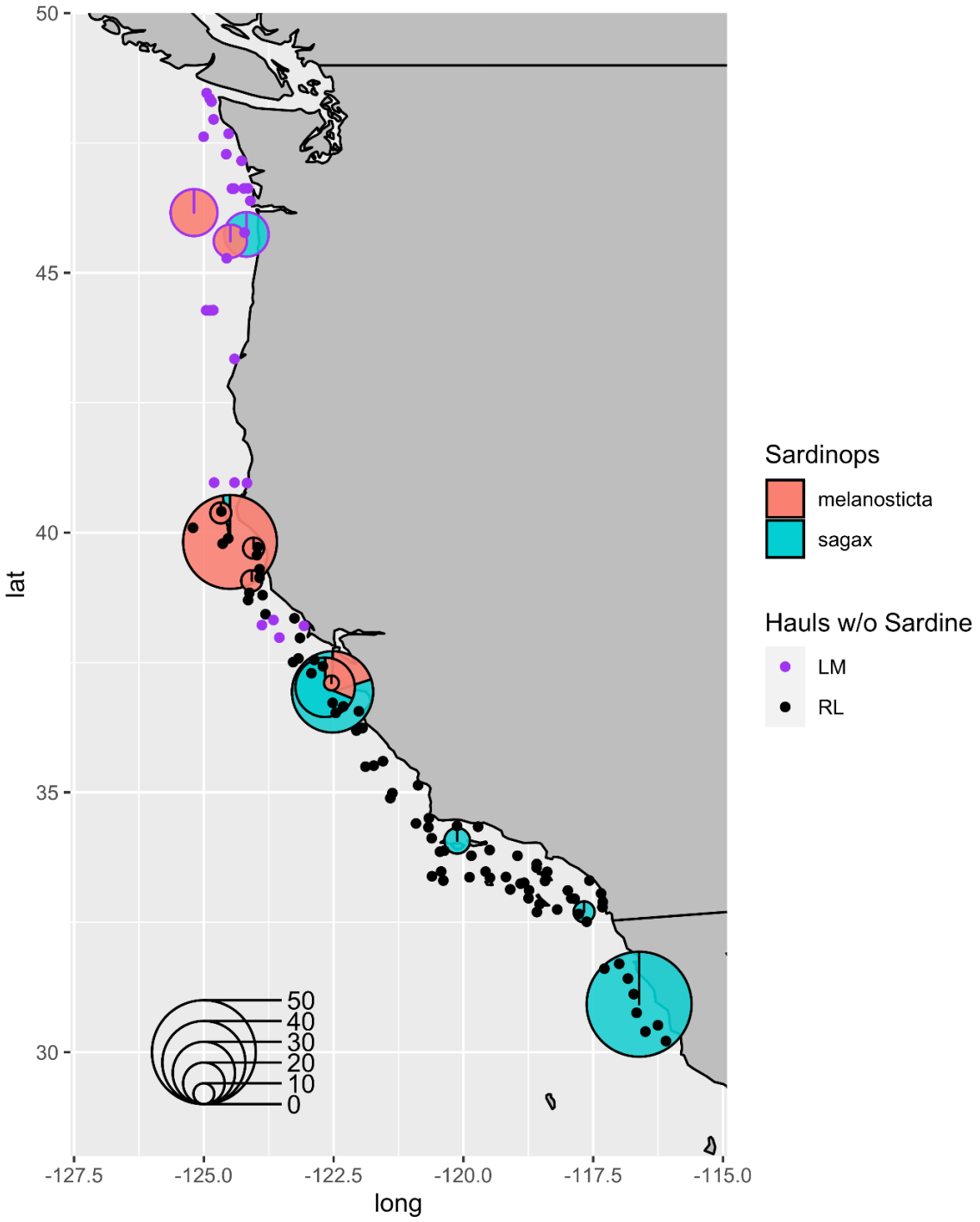

Figure 3. Trawl locations of Pacific and Japanese sardine taken during the 2022 CCES survey aboard the F/V Rueben Lasker (RL) = black and M/V Lisa Marie (LM) = purple. Dots indicate additional trawl locations where tissue samples for sardine were not collected but that may have taken sardine. Pies represent the relative proportion of species per trawl. Size of pie is relative to number of individuals.

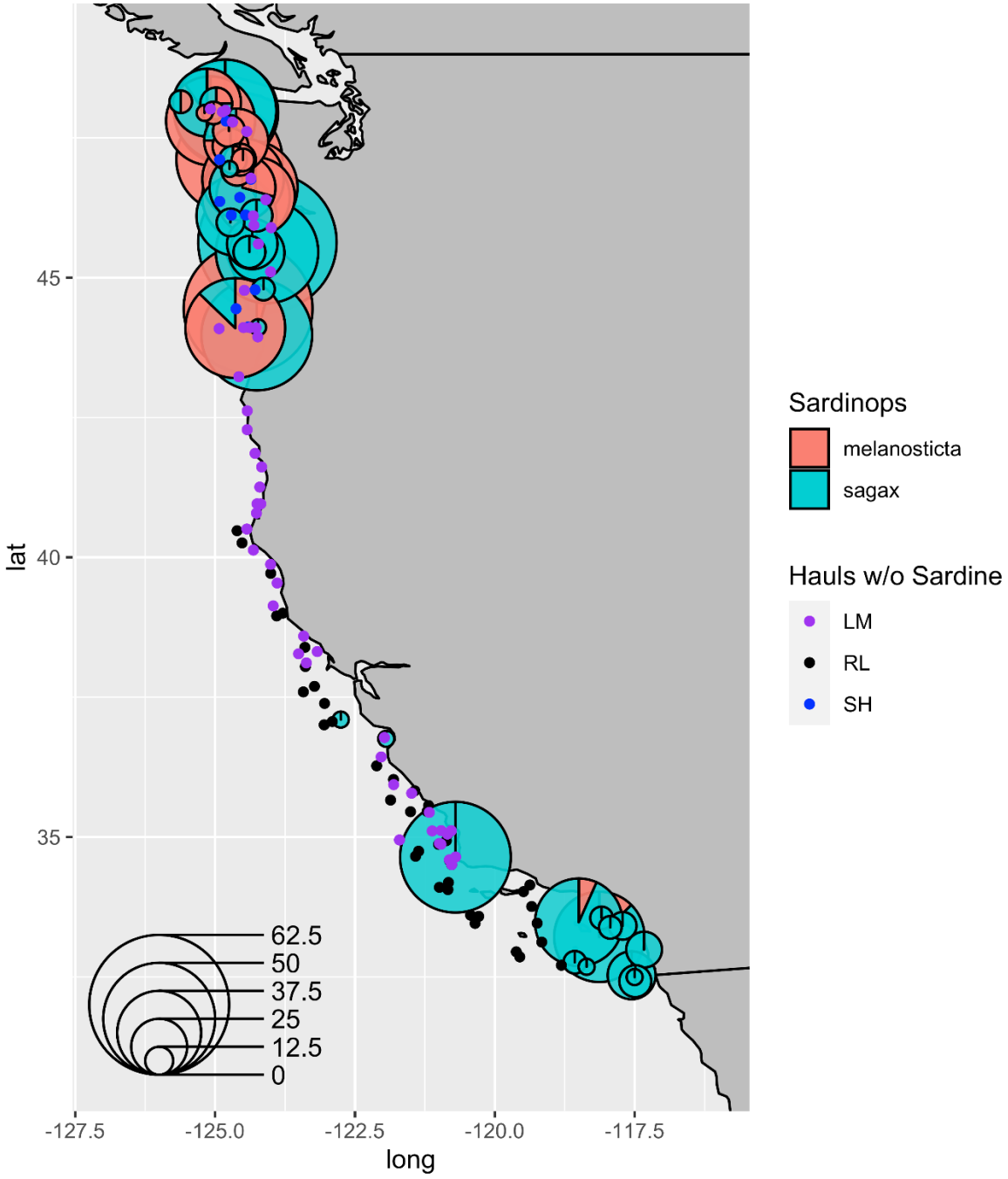

Figure 4. Trawl locations of Pacific and Japanese sardine taken during the 2023 CCES survey aboard the M/V Lisa Marie (LM) = purple, F/V Rueben Lasker (RL) = black, and F/V Bell M. Shimada (SH) = blue. Dots indicate additional trawl locations that did not catch sardine. Pies represent the relative proportion of species per trawl. Size of pie is relative to number of individuals.