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2016-17 Southern Salish Sea Acoustic Mid-Water Trawl Survey

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Findings of the 2016-17 Southern Salish Sea Acoustic Mid-Water Trawl Survey

Final Report to the Washington State Legislature
(Funded via Senate Bill No. 5166)

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Abstract

The mission of the Washington Department of Fish and Wildlife (WDFW) is to preserve, protect, and perpetuate fauna and flora occurring within the territorial boundaries of Washington State. Periodic assessments of the distribution and abundance of these species is fundamental to accomplishing this mission. When funding allows, and the need warrants it, these periodic evaluations develop into long-term monitoring programs that support adaptive and proactive monitoring and management strategies.

In the inland marine waters of Washington, referred to here as the southern Salish Sea, long-term monitoring programs of various spatiotemporal scales exist for: benthic fishes occurring on deep, relatively flat, muddy bottoms (via benthic trawl); benthic fishes occurring on deep (via remotely operated vehicles) and shallow (via scuba diving), high relief, rocky bottoms; and benthic to semi-pelagic fishes occurring in the nearshore (via beach seine and lampara net). Offshore pelagic fishes, however, have not been directly sampled in any systematic way since acoustic/trawl surveys focused on pre-spawning Pacific Herring were discontinued in 2009. During the three plus decades that these acoustic/trawl surveys occurred their geographic scope was limited and their catch, by design, consisted almost entirely of herring and, secondarily, Northern Anchovy.

Recognizing the substantial knowledge gap surrounding use of the pelagic environment by diverse fish species, and leveraging increased interest in management of marine resources, in 2015 the WDFW advocated for a systematic assessment of offshore waters of the southern Salish Sea. Through collaboration with members of the Senate, most notably Senator Christine Rolfes, Substitute Senate Bill 5166 was signed into law on May 7th, 2015. The bill directed the WDFW to “conduct a mid-water trawl survey at various depths throughout Puget Sound to evaluate the prevalence of adults of all species of forage [and pelagic] fish,” and to use the information obtained to improve conservation practices.

Between February 2016 and February 2017, the WDFW surveyed 18 index reaches throughout the southern Salish Sea (i.e., greater Puget Sound) using hydroacoustics and a mid-water trawl. Sampling occurred every other month, providing seven independent evaluations of fish presence and species composition, and vertical plankton tows were paired with each station to evaluate makeup of the prey base. Though over one hundred species of fishes and invertebrates were encountered, Pacific Herring dominated the acoustic signature and the catch across most sampling reaches and time periods, with Pacific Hake prevalent in the Whidbey Basin and Hood Canal, and Northern Anchovy prevalent south of the Tacoma Narrows. Seasonal shifts in distribution were apparent for some species, however, as demonstrated by the lack of herring in sampled waters during the late winter and early spring when individuals were spawning in the nearshore. Jellyfishes were common in several regions and tended to become more so during the late spring and through the summer.

The information gained from this survey will guide forage fish conservation and fishery practices and inform resource management policy moving forward. It will also be used as a baseline against which to compare future surveys as broad-based ecosystem level management of the marine environment continue to develop. A companion report focused on biological attributes of trawl-caught fishes is currently in development.

Introduction

Recognizing the inherent value of native species and the vital role that forage fishes play in sustaining the food web of the southern Salish Sea, in 2015 the Washington state legislature directed the Washington Department of Fish and Wildlife (WDFW) to conduct a study of pelagic fish abundance and distribution in this region using a mid-water trawl/hydroacoustic survey design (Senate Bill No. 5166). In prior consultation with the WDFW it was determined that this survey would use two vessels to sample index locations on a fixed schedule over the course of a one-year period, with the first vessel collecting hydroacoustic data and the second vessel following immediately behind to verify the species composition of the ensonified biological community. The purpose of this report is to provide information on the distribution and abundance of post-spawn and young-of-the-year forage fish in the southern Salish Sea, as well as a variety of other co-occurring species (e.g., squids, jellyfishes). Primary finfish and molluscan species of interest include: Pacific Herring (*Clupea pallasii* Valenciennes, 1847); various smelt (family Osmeridae), including Eulachon (*Thaleichthys pacificus*); Pacific Sand Lance (*Ammodytes personatus*); Northern Anchovy (*Engraulis mordax*); California Market Squid (*Doryteuthis opalescens*); and Pacific Hake (a.k.a. whiting, *Merluccius productus*).

In marine environments throughout the world forage fish are critically important to the strength and stability of the marine food web and to the fisheries that exploit species within these webs (Cury, Boyd et al. 2011, Pikitch, Boersma et al. 2012, Pikitch, Rountos et al. 2014, Robinson, Ruzicka et al. 2014, Szoboszlai, Thayer et al. 2015). Within the southern Salish Sea, all life stages of these fish serve as a vital food source for sea birds, marine mammals, and finfish, including several threatened and endangered species (e.g., Yelloweye Rockfish *Sebastes ruberrimus*, Chinook Salmon *Oncorhynchus tshawytscha*) (Drake, Emmett et al. 2010, Harvey, Williams et al. 2012, Lance, Chang et al. 2012). Sea birds, including gulls and diving ducks, feed on forage fish spawn deposited in the nearshore areas of the Salish Sea. Juvenile and adult forage fish are consumed by upper trophic levels and, because they are lipid-rich, offer an effective means of energy transfer to these upper trophic levels. For these reasons, the population status of southern Salish Sea herring was selected as a vital sign indicator of the overall health of Puget Sound by the Puget Sound Partnership in 2010 (http://www.psp.wa.gov/vitalsigns/). Efforts to document the distribution of spawning grounds for Surf Smelt *Hypomesus pretiosus*, Pacific Sand Lance, and Pacific Herring, and the pre-spawn holding and active spawning biomass for Pacific Herring, have occurred for decades in the southern Salish Sea (e.g., Lemberg 1978, Penttila 2007, Stick, Lindquist et al. 2014). The study documented here, however, represents the first ever effort to comprehensively characterize the geographic and seasonal distribution and abundance of various life stages of forage species in this region when they are not engaged in pre-spawn holding or active spawning behavior.

Methods

Survey Locations

After thorough review of fisheries data, known locations of herring and smelt spawning areas (Stick, Lindquist et al. 2014), previous acoustic sampling efforts, fish community sampling information, and bathymetric maps, 18 sampling reaches were identified throughout the southern Salish Sea (Figure 1), from the Nisqually Delta to the Gulf of Georgia. We intentionally avoided sampling in locations where mature herring congregate prior to spawning (i.e., pre-spawn holding areas), in order to limit survey impacts on the overall population of herring. Within each sampling reach, standardized stations were defined by drawing transect lines for the acoustic survey that avoided areas less than 15 meters deep to accommodate the trawl net. A minimum of 10 linear kilometers of transect were selected for each station, although the confined nature of some areas (e.g., Port Madison, Yukon Harbor) limited transect length to less than 10 km. Table 1 summarizes the physical description of each station, including the final transect lengths selected. The South Cypress Island station (C) was only sampled in February 2016. During that sampling effort, it was determined that currents were too strong in the region to effectively trawl and the station was replaced in all subsequent surveys with the Presidents Channel station (17).

Surveys were conducted every other month from February 2016 through February 2017. Due to logistical constraints associated with vessel transit time between stations, the sequence of sampling had to be structured rather than random. The 18 stations were divided into three geographically linked groups, which were surveyed on consecutive weeks. In general, stations from NW Nisqually north to Port Madison were surveyed one week; stations from Saratoga Passage into Hood Canal were sampled a second week; and stations from the Strait of Juan de Fuca up to the Gulf of Georgia were sampled a third week. Within each three-week sampling period, the Strait of Juan de Fuca to Gulf of Georgia ("Fraser River plume") stations were always surveyed during the period of lowest tidal currents to minimize impacts on trawling efficiency and vessel transit speed. Because of extensive transit time among stations and an inability to adequately predict catch processing time, factors such as time of day, tide stage, and current intensity were recorded, but not expressly included as survey design constraints.

The protocol for the survey called for the acoustic vessel (*R/V Molluscan* or *R/V Caurinus*) to begin surveying along the station transect. Acoustic data collection was monitored continuously and when an acoustic target of interest was observed, the latitude, longitude, and target depth was communicated to the trawl vessel. The trawl vessel (*F/V Chasina*) would then move into position to fish the trawl net at the specified location and depth. The depth of the trawl net was monitored in real time with a net sounder (see below) to ensure that fish collected by the trawl vessel were as consistent as practicable with the hydroacoustic signature obtained by the acoustic vessel.

Acoustics

Acoustic data were collected using a BioSonics DT-X echosounder equipped with 38 kHz and 120 kHz transducers. The transducers were mounted on a custom-built jig located amidships, with the face of each transducer resting 1 meter below the surface of the water. The data collection threshold was set to -130 dB, with a ping rate of 1.4 pps, and a pulse duration of 0.4 ms. Prior to commencing the acoustic survey at each station, temperature and salinity measurements were taken at approximately 10 m depth. Vessel speed was maintained at 4 knots during the acoustic survey.

Acoustic system calibration tests were conducted three times during the course of the project according to the instructions provided by the manufacturer. The acoustic transducers were placed into the water along with the appropriate frequency specific calibration sphere and allowed to equilibrate with ambient temperatures for 10 minutes. After equilibration, the calibration sphere was lowered to a depth of 5-10 m below the transducer face and data collection was begun. The calibration sphere was slowly moved throughout the acoustic beam. Data collected was processed using BioSonics Visual Acquisition software and the resulting target strength measurements of the calibration sphere were compared to theoretical values provided by BioSonics.

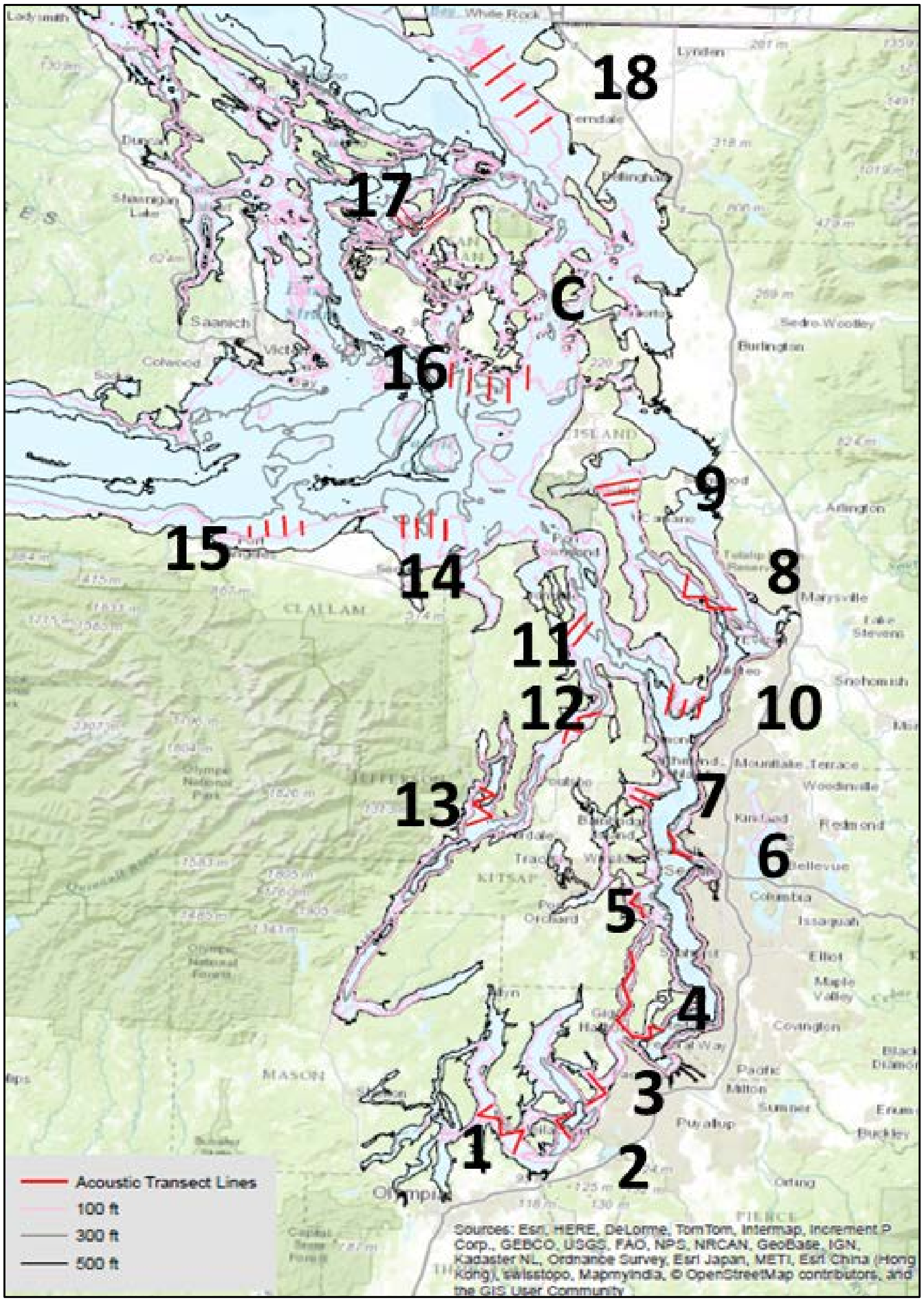

Figure 1. Map of the 2016-17 southern Salish Sea acoustic mid-water trawl sampling stations. Red lines indicate acoustic transects at each station. Station numbers correspond to values in Table 1, where additional attributes of the station are provided.

Table 1. Physical description and location of the 18 sampling stations.

Station Number	Station Name	Average Depth (m)	Transect Length (km)	Latitude	Longitude	Basin Designation
1	NW Nisqually Drayton Passage	64.7	18.4	47.165	-122.794	South of Narrows
2	South Toliva Shoal	103.4	7.1	47.177	-122.657	South of Narrows
3	Hale Passage	58.0	8.6	47.246	-122.589	South of Narrows
4	Colvos and Dalco Passages	81.0	25.9	47.385	-122.528	Main Basin
5	Yukon Harbor	83.2	6.4	47.541	-122.525	Main Basin
6	North Elliott Bay	86.7	7.5	47.644	-122.439	Main Basin
7	Port Madison	57.9	8.1	47.725	-122.504	Main Basin
8	South Saratoga	122.6	14.3	48.041	-122.386	Whidbey Basin
9	North Saratoga	50.8	17.1	48.223	-122.573	Whidbey Basin
10	Possession Bar	132.1	13.6	47.876	-122.425	Main Basin
11	Oak Bay	64.1	10.9	47.998	-122.659	Main Basin
12	Squamish Harbor	54.4	12.2	47.831	-122.671	Hood Canal
13	Dabob Bay	125.8	16.1	47.687	-122.858	Hood Canal
14	Dallas Bank	57.4	16.9	48.156	-123.007	Juan de Fuca
15	East Port Angeles	46.4	11.9	48.159	-123.323	Juan de Fuca
16	South Lopez Island	76.6	23.6	48.403	-122.865	Juan de Fuca
17	President Channel	113.1	21.3	48.669	-123.055	Fraser Plume
18	Gulf of Georgia	38.5	27.7	48.884	-122.842	Fraser Plume
C	South Cypress Island	46.4	11.2	48.535	-122.705	Fraser Plume

In general, at least one trawl tow (usually two) was completed at each station during, or shortly after, each acoustic survey. The acoustics operator determined the location and depth of an acoustic target while collecting acoustic data and communicated the coordinates and depth to the trawl vessel, which then proceeded to deploy the net. In cases where all or a large portion of the acoustic transects did not contain practical acoustic targets (see Appendix A), at least one "blind tow" was conducted on a transect line to evaluate "background" biomass present in the water column but not detected by the echosounder using the predetermined data collection configuration.

Trawl Deployment

A contracted 17.7-m (58-ft) commercial fishing vessel, the *F/V Chasina*, performed the trawls using a mid-water Polish rope trawl with a maximum 7.3 m x 7.3 m (24 ft) opening held agape by pelagic metal doors. The cod end was fitted with a 1-cm^2 knotless mesh liner. The net was deployed with an amount of warp (i.e., cable) estimated to set the net at the desired depth (roughly a 2:1 ratio). Beginning in April, 2016, the net depth and net opening were monitored in real time with a net sounder (Marport Trawl Explorer®), which also provided a 360-400 kHz echogram 5 m above and below the head rope. Net fishing depth could be altered by adjusting the warp or by altering vessel speed. Once the net was determined to be fishing the desired depth the trawl set was considered to have begun and the coordinates and time marked. Prior to and during the tow the trawl vessel crew monitored the trawl vessel's echosounder and the Marport net sounder and remained in communication with the acoustics vessel to determine if the desired acoustic target was being effectively fished, making adjustments as needed. Prior to this (February 2016 only), tow depth was recorded using a Reef Net Ultra® Sensor attached to the head rope; this provided data only on the maximum depth of tow and was available only after the trawl concluded and the device was connected to a computer.

In general, tows were conducted along the acoustic transect lines, except where currents, logistical factors (e.g., submerged hazards, commercial traffic), or bathymetry dictated otherwise, in which case tows intersected acoustic transects at or near a specified target. Target tow duration was 15 minutes and tows were considered complete at the initiation of haul back. Start and stop locations and times, bottom depth, net depth, and vessel speed were recorded, as well as a variety of environmental factors including weather conditions, moon phase, tide height, wind speed and direction, air temperatures, water temperature at the depth of tow, barometric pressure, and degree of cloud cover. Target tow speed was 3.0 knots (1.5 m/sec) and tows generally occurred in the direction of the current. When towing in crepuscular hours or after dark, all deck lights were extinguished prior to the tow to avoid altering fish behavior.

Catch Processing

Once the trawl net was hauled back and the catch emptied onto a sorting table, ESA-listed species (Chinook Salmon, *O. tshawytscha*; steelhead trout, *O. mykiss*; Hood Canal summer Chum Salmon, *O. keta*; Eulachon, *Thaleichthys pacificus*; Boccaccio, *Sebastes paucispinis*; and Yelloweye Rockfish *S. ruberrimus*), as well as sharks, skates and other rockfish species, were immediately placed in a recovery tank with fresh seawater and released unless they were intentionally, lethally collected for other researchers under permit (see section on Project Collaborations below). All organisms were then separated by species, counted, and weighed. If a species was too numerous to count, the entirety of individuals was bulk weighed and a subsample counted so that a ratio could be applied to the overall weight to obtain an approximate overall count. In large catches predominantly composed of two or three species that were impractical to separate, these species were bulk weighed together and subsample reference buckets sorted to species, counted, and weighed (allowing extrapolation to the entire catch). Length measurements were obtained for a subsample (~50 individuals) of all fish species and, when practical, invertebrates. Samples collected for further analysis by the WDFW or other researchers were placed in plastic bags, labeled with tow specifics, and frozen on board the research vessel.

Conductivity-Temperature-Depth Meter (CTD)

During the months of April, October, December (2016), and February 2017, vertical temperature and salinity profiles were obtained at a point on each acoustic transect for each station. For April, December, and February 2017, data was obtained by attaching a Valeport mini-CTD® to the zooplankton net during vertical plankton tows from 2 m off the bottom to surface (see below). Due to equipment failure, water column profiles in October were collected at each site using a more advanced unit, a SeaBird SEACAT® Profiler (SBE 19 plus V2) provided by the Northwest Fisheries Science Center (NOAA). The SeaBird profiler was equipped with an instrument package that measured temperature, density, conductivity, dissolved oxygen (SBE 43 oxygen sensor), depth, photosynthetically active radiation (PAR; Biospherical PAR sensor), fluorescence (WET Labs ECO-FL-NTU sensor®), and pH (SBE 18 pH sensor). Profile data were processed using Sea-Bird SBE Data Processing software (Version 7.23.1) binned by 0.5-m increments.

Plankton Tows

Vertical plankton tows were conducted at each sampling station, each month. Sample protocol was that of Keister (2015; SSMSP Zooplankton Sampling Protocol, available at http://faculty.washington.edu/jkeister/SSMSP%20Zooplankton%20sampling%20protocol_v.7.pdf).

The plankton net was a 60-cm diameter ring net with 200 µm mesh and a 4:1 filtering ratio. The plankton net was weighted, equipped with a flow meter, and accompanied by either a Valeport MiniCTD® or a Reef Net Ultra® Sensor to record maximum depth. The net was deployed to within 10 m of the bottom, but no deeper than 200 m. Deployment and retrieval rates were maintained at a constant rate of 30 m/min. After retrieval, the flow meter information was recorded and the contents of the net were rinsed into the cod end. Zooplankton samples were preserved in 5% buffered formalin and stored in labeled jars for later processing. When zooplankton samples were collected during crepuscular hours or in darkness, deck lights were extinguished 0.5 km prior to reaching the sampling location and kept off until the net had been fully retrieved to avoid influencing plankton behavior.

In August, a neuston net was deployed to assess larval fish (ichthyoplankton) abundances at each station. A 0.5 m x 1 m rectangular net frame equipped with a 330-µm mesh net was deployed. The net was placed abeam of the trawl vessel, ahead of the ship wake. The net frame was equipped with a torpedo flow meter and total revolutions were noted prior to and after deployment to estimate sample volume. During deployment the net frame was kept $^{2}/_{3}$ submerged, or as close as practical given sea conditions. Tow duration was 10 minutes and vessel speed was maintained at 2 knots. Neuston samples were preserved in 5% buffered formalin and stored in labeled jars for later processing. When neuston samples were collected during crepuscular hours or in darkness, deck lights were extinguished 0.5 km prior to reaching the sampling location and kept off until the net had been fully retrieved.

Laboratory Necropsy of Frozen Fish Samples

During regular operations, 100 Pacific Herring were saved and frozen, per station each sampling month, for assessment of the age composition, growth patterns, sex ratio, maturation schedule, fecundity, and genetic stock structure of herring ("biometrics") in the southern Salish Sea. When available, up to 100 individuals of other forage fish species were also retained and frozen at each station. Necropsies were conducted in the WDFW Marine Resources Laboratory (Olympia, WA); fish were thawed, measured for standard length (to the nearest millimeter), weighed (whole body to nearest gram), and a portion of caudal fin clipped and fixed in 70% ethanol. Three ctenoid scales were taken from the pectoral fin region per individual, mounted on microscope slides with a light adhesive solution, and retained for later ageing by WDFW staff. Sagittal otoliths were excised from 10 individuals per cm size class via a transverse cut through the cranium and stored in 70% ethanol for later age determination. Gonads were excised and assigned a sex based on gross anatomical features exhibited. If sex was not macroscopically apparent, a small portion of gonad was removed, squashed between two microscope slides, and assessed for sex via light microscopy. Maturation condition was assessed macroscopically and scored utilizing the seven stage gonad maturity scale (Hay 1985). Detailed results of these evaluations of biological and population parameters will be provided in a companion report as time allows.

Acoustic Data Analysis

Acoustic data files were processed in EchoView (Version 8). All acoustic files from each station-month combination were merged into a single EchoView file for processing. Bottom lines were created using the best bottom candidate algorithm and edited by hand as necessary. Acoustic transects were divided into 100-m intervals and 25-m depth bins for analysis, resulting in 100 m x 25 m cell resolution. Single target detection variables were created for each frequency. The target strengths of the single targets were exported into a spreadsheet and a frequency histogram was generated to determine the appropriate value to use for echo integration analysis. Single target detection parameters were -60 dB threshold, 0.70 to 1.5 normalized pulse length measured at 6 dB, 4 dB maximum beam compensations, and a maximum standard deviation of 0.6 degrees.

Echo integration was used to obtain density and biomass estimates from the acoustic data. Variables selected for output from EchoView included: Thickness_mean (m), Wedge_Volume_Sampled (m^3), and Density_Number ($fish/nmi^2$). The scaling factor (expected target strength) for echo integration was obtained for each station-month file from the single target detections analysis described above.

The post processing calculation for total fish density is[1]:

$Fish/m^3_{Total} = (Density\_Number/1582^2)/Thickness\_Mean$

[1] http://support.echoview.com/WebHelp/Reference/Algorithms/Analysis_variables/Density_number.htm
$1\ nmi^2 = 1{,}582^2\ m$

Combining Acoustic Data and Trawl Data

Abundance estimates obtained from the acoustic data were combined with the trawl catch information to partition species-specific abundance and biomass estimates from the aggregate acoustic signal. The total species composition of the mid-water trawl for all station-month combinations was reviewed to identify non-acoustically relevant species, meaning those species that cannot reliably be detected acoustically or the acoustic properties of the species are not well understood. Abundance and biomass estimates generated from the acoustic data were limited to species in the trawl catch that were deemed acoustically relevant (see Appendix A). This process eliminated all invertebrate species with the exception of California market squid. Acoustic data was also culled to eliminate 100 m x 25 m cells that were identified as anomalous. These anomalous cells were identified as having too few acoustic pings, limiting the ability to acoustically characterize the cell. This occasionally occurred at the end of transects when cells were truncated or if the vessel speed was slowed for some reason (marine traffic, mechanical issues) and the cell over sampled (cells containing fewer than 40 pings or greater than 110 pings were eliminated from the analysis). This culling process eliminated only 3% of the >350,000 total cells.

For report clarity, any species that failed to exceed 5% of the composition of any single trawl were grouped together as "Other". This filtering did not eliminate any forage fish species (herring, smelt, sand lance, or anchovy) and served mainly to eliminate clutter introduced to the analysis and presentation by rarely caught species (e.g., several species of flatfish, Longnose Skate *Raja rhina*).

Equations for the species-specific estimates are given below where P_s is the proportion of specimens of species *S* in the trawl catch and N_s is the number of species captured in the trawl for each station-month combination:

$$\sum_{s=0}^{N_S-1} P_S = 1$$

Fish Densities: $Fish/m^3_{Species\_S} = Fish/m^3_{Total} * P_s$

Fish Abundance: $Total\ Fish_{Species\_S} = Fish/m^3_{Species\_S} * Wedge\_Volume\_Sampled$

Species Biomass: $Biomass_{Species\_S} = Total\ Fish_{Species\_S} * Average\ Fish\ Weight_{Species\_S}$ (from trawl sample(s))

Normalized Biomass: $Biomass_{Species\_S}/m^3 = Biomass_{Species\_S} / Wedge\_Volume\_Sampled$

Age Estimation

Herring length-at-age information from historical WDFW data was used to estimate the ages of the trawl-caught herring. Data from 2000-2009 was used to minimize the effect of decreasing size-at-age observed in southern Salish Sea herring over a period of decades. Herring age for the WDFW dataset was determined by counting scale annuli under magnification and standard length was used as the measure of fish size.

Herring length-at-age was plotted in a frequency table in 1-mm size bins. Visual inspection of the table showed greater than expected length overlap among the age classes. To resolve this, length bins for each age class were selected by an iterative optimization approach until each length bin contained approximately 70% of the appropriate age class. The length-at-age matrix and the percentage of appropriately aged fish using these size bins is shown in Table 2.

Table 2. Pacific Herring length frequency age estimations ("True Age" from scale readings).

	Estimated Age Based on Standard Length				
True Age	**Age 2**	**Age 2-3**	**Age 3**	**Age 3-4**	**Age >4**
2	85%	49%	21%	9%	1%
3	15%	48%	68%	63%	34%
4	0%	3%	10%	25%	45%
5	0%	0%	1%	3%	16%
6	0%	0%	0%	0%	4%
7	0%	0%	0%	0%	1%
8	0%	0%	0%	0%	0%
Size Bin (mm)	130-145	146-155	156-165	166-178%	>178

Statistical Analysis of Biodiversity

Patterns in trawl catch composition within and among stations, and over time, were evaluated via non-metric multidimensional scaling (nMDS) analysis based on the Bray-Curtis similarity index using PRIMER-E version 7.0.13 (Quest Research Limited). This method quantifies species-specific abundance patterns across the whole of the data set and identifies suites of species with correlated patterns that drive overall variation in observed biodiversity. The resulting multidimensional scores are then used in a permutational multivariate analysis of variance (PERMANOVA) to evaluate statistical significance of these relationships. Environmental variables can also be assessed in a correlational context as potential explanatory factors driving biodiversity patterns.

There were several qualifications for data from a given tow to be included in the nMDS analysis of patterns over time and space. A station must have been sampled during all months of the survey, which means two tows were dropped: one at Cypress Island in February of 2016 and a Hood Canal "exploratory" set. Stations where gear failure occurred (e.g., the doors crossed on deployment, the stabilizing chains detached while fishing) were also eliminated, resulting in removal of seven sets. Sets which caught zero (6 sets) or nearly zero (<5 individuals total, 22 sets) fish were also removed due to a lack of species diversity information for the analysis to act upon.

Analysis of biodiversity patterns via nMDS is highly sensitive to rare species, but species may be rare in the catch simply because they are sampled ineffectively with the gear type being utilized rather than because they are truly rare in the ecosystem. To avoid undue influence from rare species deemed not to be targets of this survey (e.g., starry flounder, spot prawn) any species for which fewer than 50 total individuals were sampled over the course of the survey were eliminated from the analysis. This resulted in removal of 26 species from the dataset and also resulting the in the removal of one additional trawl set from the analysis because it became a zero-catch set. Together with the set validity criteria noted above, this resulted in a total of 188 sets deemed valid for analysis, and 28 species under consideration.

Once the final data set had been identified, all catch data were log(x+1) transformed to accommodate wide variation in individual counts. Quantitative environmental variables (e.g., temperature at depth) were normalized (i.e., the mean was subtracted from the coefficient of variation) and categorical variables (e.g., current intensity, light level) were transformed into coded integer inputs to be used as correlates to further understand biodiversity patterns.

Results

Hydroacoustic data paired with 225 pelagic trawls were obtained over the course of this study, and 127 vertical plankton tows and 73 CTD casts were completed. Mid-water trawl tow speed was typically 2.5 – 3.0 knots and generally in the direction of the current, although current or speed changes that impacted net depth varied tow speeds from 0.8 to 3.7 knots. Tows were conducted from as deep as 155 m to as shallow as 15 m. When towing near the bottom the net sounder was used to successfully keep the footrope off the bottom, although several tows impacted the seafloor at some point, typically when vessel forward motion ceased at the onset of haul back.

During the majority of tows high densities of fish were not encountered and the trawl vessel towed the net at the coordinates and depth supplied from the acoustic survey crew. On tows with greater concentrations of fish (see Appendix B) it was possible for the trawl vessel to more actively make adjustments in order to successfully sample the acoustic target. More commonly the target was a "layer" or "band" of more consistent but less concentrated acoustic signal (Appendix B) that was not as readily observable on the trawl vessel net sounder and, therefore, was assumed to persist at the coordinates and depth specified by the acoustics vessel. When light changes occurred (crepuscular hours) between the passage of the acoustic boat and trawl deployment it was sometimes necessary to alter target depth because of changes in fish behavior (e.g., rising in the water column during diel migration). The average time lag between measurement by the acoustics boat and the start point of the trawl was 52 minutes.

Trawl Catch Composition

Trawl catch was normalized by the length of tow and adjusted for currents as transects differed in length and, rarely, tows had to be ended early due to vessel traffic or sudden changes in bathymetry. A total of 96 different species were collected throughout the survey area, including 64 vertebrate species and 32 invertebrate species. Of these, nine species made up 96% of the overall catch (Figure 2). Trawl catches were dominated by Pacific Herring, which constituted 70% of the total vertebrate catch (Figure 2). Pacific Hake were the second most commonly encountered vertebrate, at 17% of overall catch. No other species of invertebrate or vertebrate exceeded 10% of the catch.

It should be noted that the trawl catches in this survey were not random samples but rather were directed samples based on the real-time observations of the acoustics boat. Additionally, due to the mesh size of the net (1 cm^2), it is unclear how effective the net was at collecting and quantifying certain species of invertebrates (especially gelatinous zooplankton) and narrow-bodied fish (i.e., Pacific Sand Lance). The resulting discussion will concentrate on the vertebrate species captured and will include California Market Squid (*Doryteuthis opalescens*, formerly *Loligo opalescens*) due to their potential as forage for culturally and economically important species in the Salish Sea.

In all, 11 of the 64 vertebrate species were collected in all six basins. California Market Squid, Fried Egg Jellyfish (*Phacellophora* spp.), and Moon Jellyfish (*Aurelia* spp.) were also captured in all basins (Table 3).

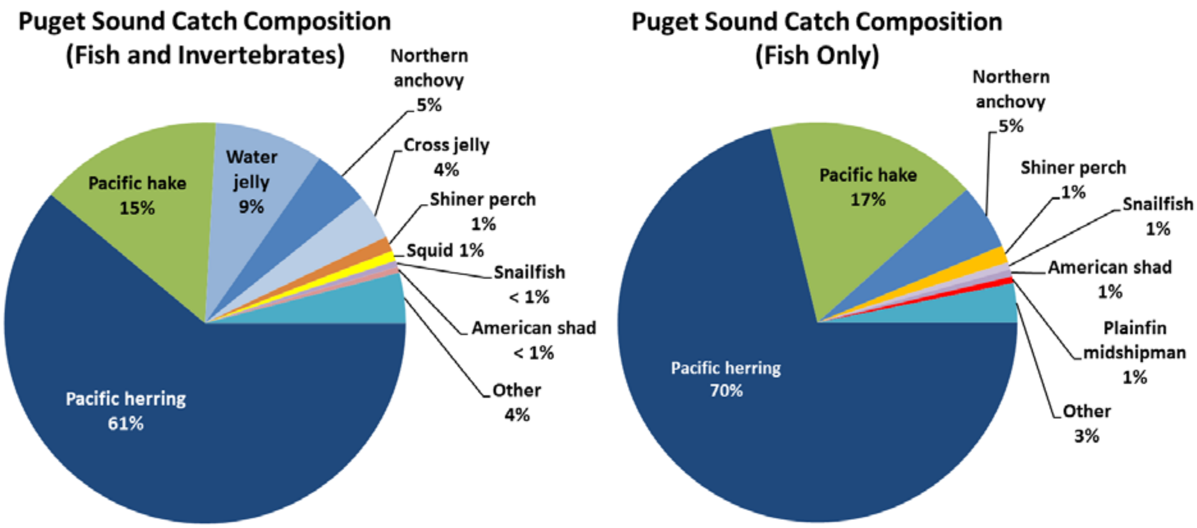

Figure 2. Total catch composition of fish and invertebrate species (left) and finfishes (right) collected during the 2016-17 Mid-Water Trawl Survey.

Table 3. Catch of the most abundant species by basin. Together these nine species made up 96% of the overall catch.

	Basin					
Common Name	**Fraser Plume**	**Hood Canal**	**Juan de Fuca**	**Main Basin**	**South of Narrows**	**Whidbey Basin**
Pacific Herring	7,752	17,324	4,665	23,182	9,835	14,202
Pacific Hake	103	1,053	8	6,501	628	12,377
Northern Anchovy	153	304	25	854	4,073	987
Shiner Perch	8	2	7	1,362	244	68
Market squid	31	142	305	349	326	7
American Shad	3	514	28	49	3	89
Spiny Dogfish	74	19	2	58	42	289
Northern lanternfish	157	17	80	6	1	1
Pacific Pompano	16	11	1	29	25	170
English Sole	105	19	1	71	23	5
Chinook Salmon	7	43	7	79	32	19

Pacific Herring was the dominate species in all basins, constituting 50% (Whidbey Basin) to 91% (Hood Canal) of the total catch (Figure 3). Pacific Hake (whiting) was common in the Main and Whidbey Basins, making up 20 and 44% of the catch, respectively. Northern Anchovy were captured in all basins and constituted 26% of the catch south of the Tacoma Narrows. Aside from Pacific Herring, no other species was captured in high numbers in all basins. For example, Pacific Hake were a significant component of the catch in the Main and Whidbey Basins, as noted above, but did not exceed 4% of the catch at any other basin. American Shad were only significant contributors to the catch in Hood Canal. Monthly total catches from the mid-water trawl (independent of basin) are shown in Figure 4 for total catch (top panel) and for fish only (bottom panel).

Total mid-water trawl catches were highest in the spring and fall sampling periods, and lowest in the winter and summer surveys. Herring were the most abundant fish caught in all survey months except during the summer, when hake became dominant, likely because many herring stocks migrate to the Pacific Ocean to feed in summer. The spike in hake abundance during the summer was driven primarily by large catches in the Whidbey and Main Basins and decreases in the overall abundance of herring and other fish species (Figure 5).

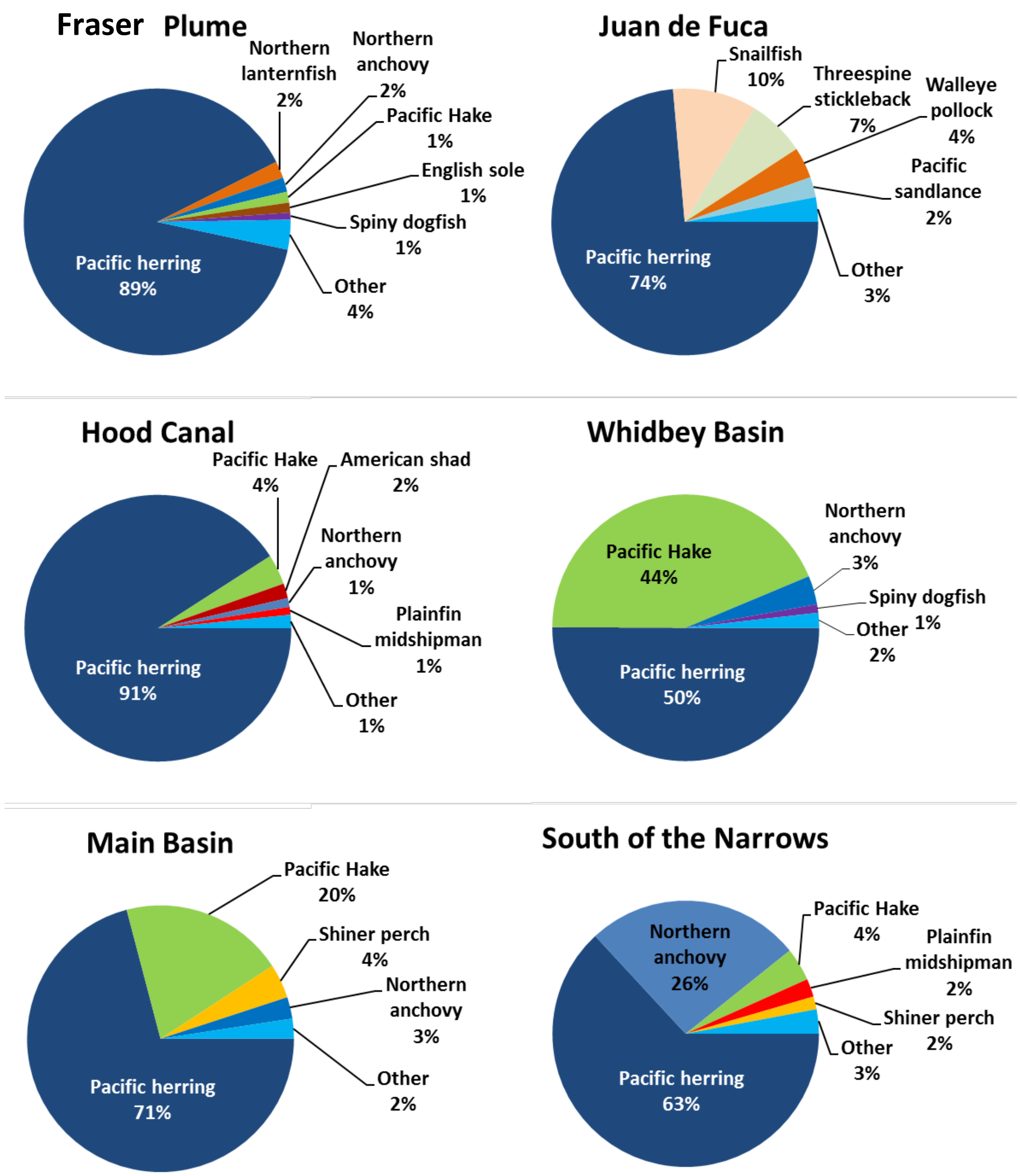

Figure 3. Relative abundance of fish in the mid-water trawl catch, by species, by basin.

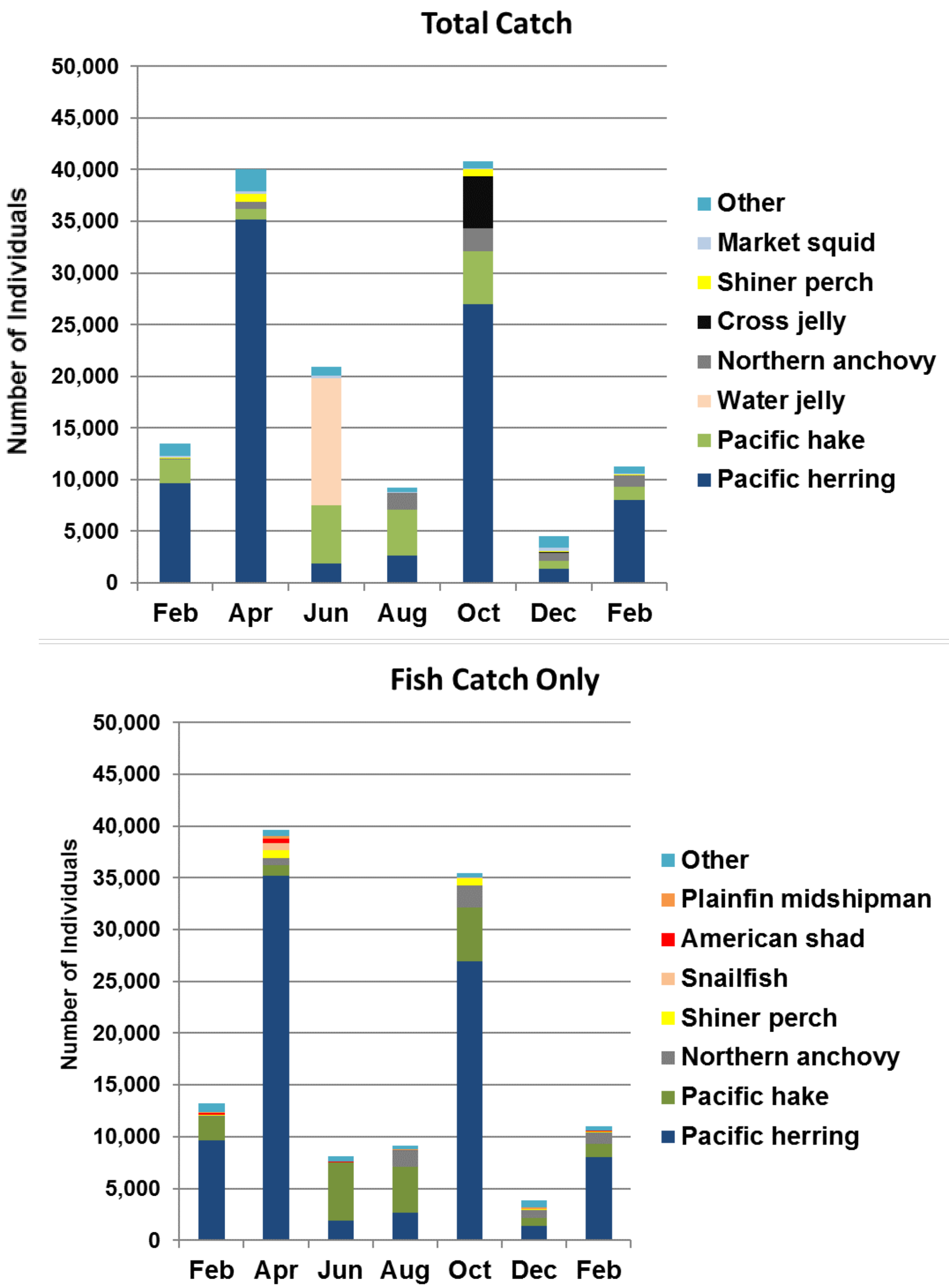

Figure 4. Total catch (top panel) and most abundant fish catch (bottom panel) by month, February 2016-February 2017.

Pacific Herring

Pacific Herring were the dominant component of the mid-water trawl catch; a total of 76,960 herring were captured. Herring were found throughout the survey region but were most numerous in the Main Basin and Hood Canal. The spring (April) and fall (October) surveys captured the greatest number of herring. Presumably this was a result of spawning stocks moving between winter spawning locations and summer feeding areas (Figures 4, 5), and younger fish becoming large enough to be captured in the trawl (Figure 6).

Estimating the age of herring based on their standard length indicates that the young-of-the-year (YOY) herring begin to appear in the trawl catches in June, when 41% captured in the Main Basin were YOY. YOY and Age 1 herring were captured in the greatest numbers in summer and fall in the Fraser Plume, Hood Canal, and the Strait of Juan de Fuca. Herring aged 2+, which represent potential spawners, were present throughout the year and made up 79% of the total herring captured, but only 57% of the herring captured during the summer and fall, after age 1 and YOY fish entered the population.

Figure 5. Catch of the most abundant species by basin and month (larger versions of these plots are available in Appendix C).

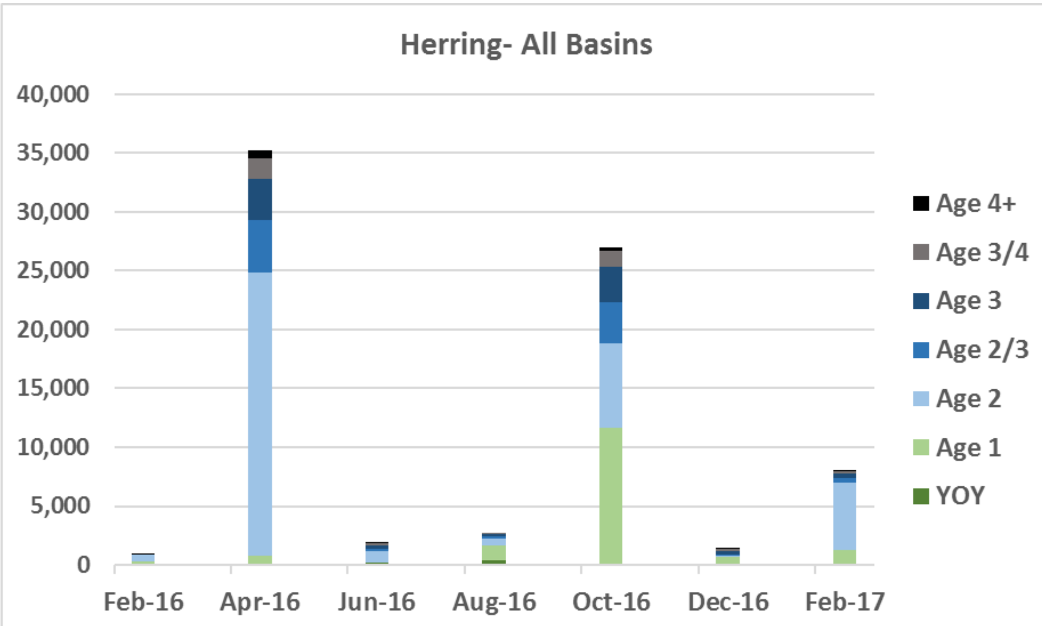

Figure 6. Age structure and number of captured Pacific Herring by survey date.

Other Forage Fish Species

Pacific Hake were the second most common species captured in the trawls. Though defined by Washington State Administrative Code as bottom fish (WAC 220-300-040), hake are considered forage fish here because of the role that small (<20 cm TL) individuals, like those captured in the trawl, play in the food web. Pacific Hake represented 17% of the total vertebrate catch and 15% overall (n=18,147). Hake were captured in all basins throughout the sampling year. However, 88% of the hake were collected at three sampling stations: Possession Bar (Main Basin) and North and South Saratoga (Whidbey Basin).

Northern Anchovy were anecdotally reported as very abundant in the Salish Sea during 2016-17 (Duguid et al. 2018). Anchovies were the third most abundant forage fish captured in the mid-water trawl, making up 5% of the total catch (n= 6,396). Anchovy were most abundant south of the Tacoma Narrows (South Basin), where 64% of the total anchovy catch was obtained. Anchovy typically inhabit the nearshore regions of the southern Salish Sea and the large schools reported in embayments in the region were not effectively sampled at many of our stations, which generally avoided water shallower than 15m to ensure proper trawl deployment and efficacy.

Smelt, including Eulachon, (Family Osmeridae) were relatively rare in the mid-water trawl catches, constituting <1% of the total catch (n=83). Smelts were exclusively caught in the northern areas of the survey region; no smelt were caught in Hood Canal or south of the Narrows. Eulachon were the most common, followed by Night Smelt, Longfin Smelt, and Whitebait Smelt. Surf Smelt, another nearshore species, were almost never captured in the midwater trawl as they inhabit shallow waters. As with most of the smelt species, anchovy also tend to inhabit nearshore areas and were likely under-represented by our sampling efforts.

Pacific Sand Lance (PSL) occurred in only 3 trawls (often retained by jellies in the net) and made up <1% of the total catch (n=234). Pacific Sand Lance were predominately captured in the Strait of Juan de Fuca (n=156) and the Main Basin (n=77); a single PSL was captured south of the Tacoma Narrows. In addition to inhabiting areas outside of our sampling areas, the mesh size of the trawl net was likely inefficient at capturing PSL. This inaccessibility to traditional sampling methods is one reason that an estimate of sand lance biomass in the southern Salish Sea has remained elusive for several decades.

Though not a forage fish species, also of note in the mid-water trawl catch was the capture of a juvenile sixgill shark (*Hexanchus griseus)* in February 2017. The 81 cm specimen was collected at the NW Nisqually station at a tow depth of approximately 80 meters. The shark was released alive and in good condition.

Acoustic Biomass Estimates

Acoustic biomass estimates were generated for each of the six basins, in total, by species, and by month. Herring were also the dominate contributor to biomass overall, with an estimated biomass of 115,834 kg for the entire survey (Table 4, Figure 7). Spiny Dogfish, due to their large size relative to other fish species captured in the trawl, were also large contributors to the estimated overall biomass.

Table 4. Acoustic biomass estimates (in kg) for the dominant species. "Other" is a grouping of all fish species that did not exceed 5% of the catch in any individual trawl sample.

Biomass Estimate for each Basin							
Common Name	**Fraser Plume**	**Hood Canal**	**Juan de Fuca**	**Main Basin**	**South of Narrows**	**Whidbey Basin**	**Total Biomass**
Pacific Herring	20,500	22,937	4,198	39,706	5,655	22,837	115,834
Spiny Dogfish	74,373		203	4,721	9,337	21,542	110,176
Pacific Hake	3,917	2,542		25,008	1,392	7,951	40,792
Chinook Slamon		48	4,698	18,742	4,672		28,159
Walleye Pollock	13,924		497	1,033			15,454
Northern Anchovy	2,234	462	36	1,448	4,486	131	8,797
CA Market Squid	50	51	172		1,027		1,299
Other	2,681	2,478	287	5,139	6,481	438	17,503

The acoustic estimate of Chinook Salmon biomass is likely an overestimate and is an artifact of low overall trawl catch at some sampling locations. When acoustic biomass estimates are produced for each species, the estimate is based on each species' proportion of the total catch. If the trawl catches few fish, the proportion of any one species increases. For example, fish tended to congregate tightly along the bottom at some sampling stations (Port Madison and North Elliott Bay), making them inaccessible to the mid-water trawl, and catch at these locations tended to be low. When the acoustic biomass estimate generated from the fish along the bottom is applied to the low overall catch, the species biomass estimates can become artificially inflated, as appears to have happened with Chinook Salmon in the Main Basin (due mainly to catches at North Elliot Bay; Table 4).

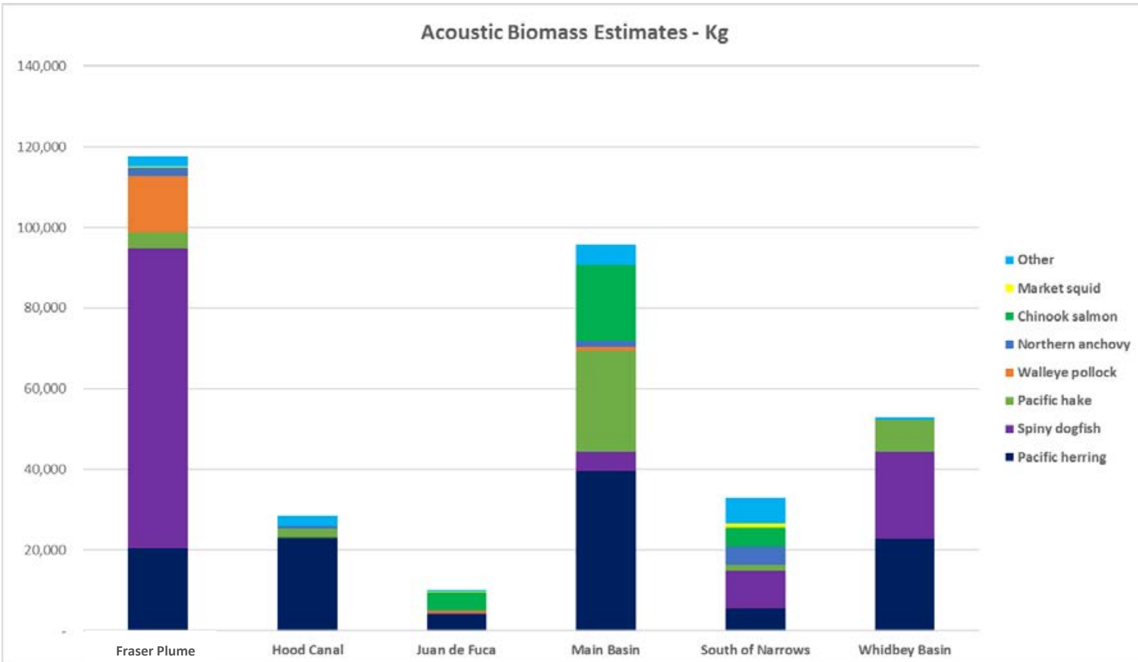

Figure 7. Acoustic biomass estimates (in kg) for the dominant fish species.

The sample volume of the acoustic beam is a function of water depth; in deeper water the sample volume increases. To minimize the effect of differing sample volumes among basins, biomass estimates were normalized using wedge volume to a standard volume (kg/m^3) to examine differences among basins.

The Fraser Plume and the Main Basin had the highest biomass estimates when normalized to sample volume while the Strait of Juan de Fuca had the lowest (Figure 8).

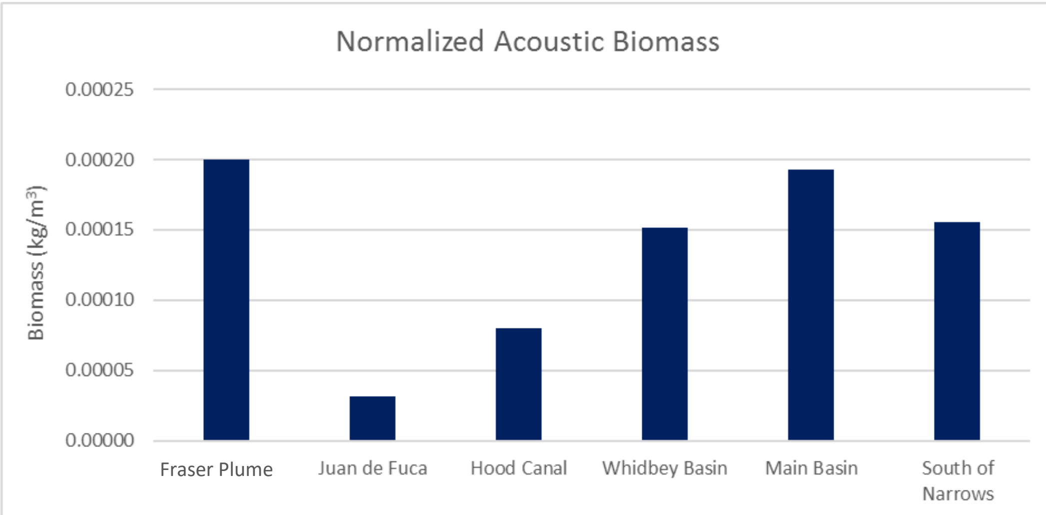

Figure 8. Total acoustic biomass estimates (normalized to kg/m^3) for each basin.

The total acoustic biomass estimate is greatest during the spring and into fall, with winter months having the lowest biomass estimates (Figure 9). This is in contrast to the mid-water trawl catch data, which had the lowest catch during the summer months. The difference is likely due to large aggregations of hake near the bottom that were not captured by the trawl, or schools of smaller fish that could not be fished effectively (e.g., Pacific Sand Lance).

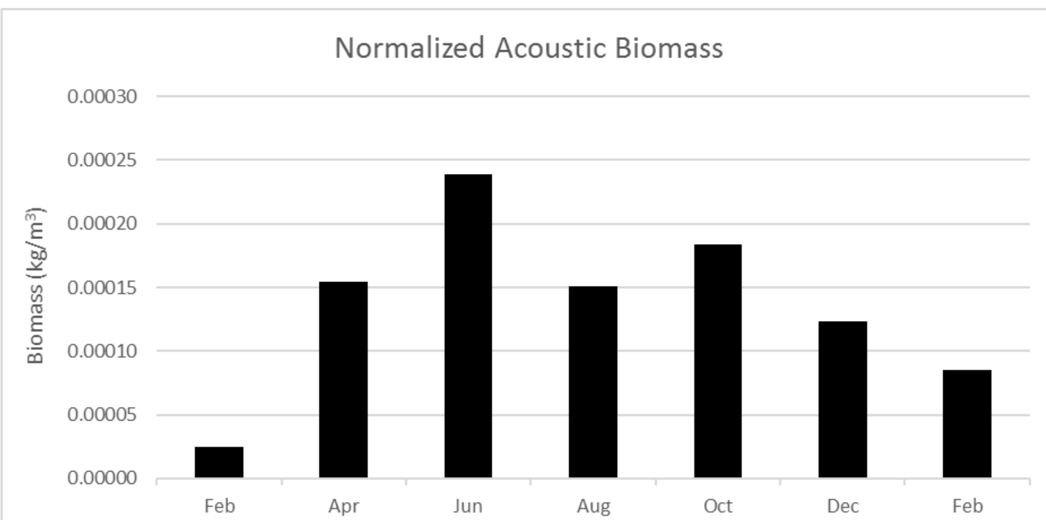

Figure 9. Total acoustic biomass estimates (in kg/m^3) for each month of survey, February 2016-17.

Acoustic Density Estimates

Fish density estimates for the dominant species of interest were produced for each basin. Both Pacific Herring and Pacific Hake had the greatest density in the Whidbey Basin and lowest in the Strait of Juan de Fuca (Figure 10). South Sound ("South of Narrows" Basin) had the highest density of Northern Anchovy.

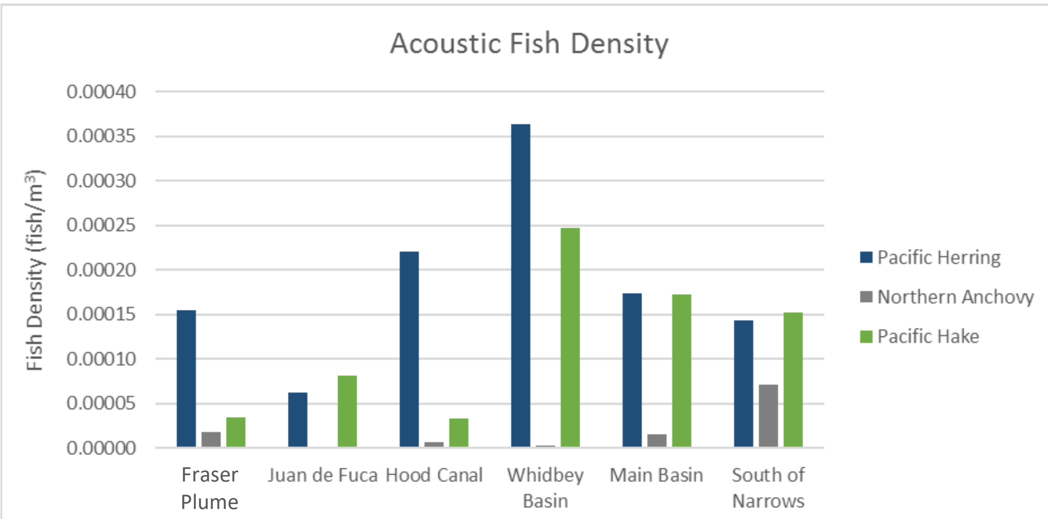

Figure 10. Acoustic fish density (fish/m3) for Pacific Herring, Northern Anchovy, and Pacific Hake.

Pacific Herring densities were generally consistent throughout the survey with moderate increases during the spring and fall as the fish moved to and from the spawning locations in the Sound (Figure 11). Low catches in February of 2016 likely underestimated the mid-water fish community because of delays in obtaining the Marport net sounding unit (installed for the April 2016 survey and all others); as a result, our trawl depths were less precise and likely resulted in reduced catches.

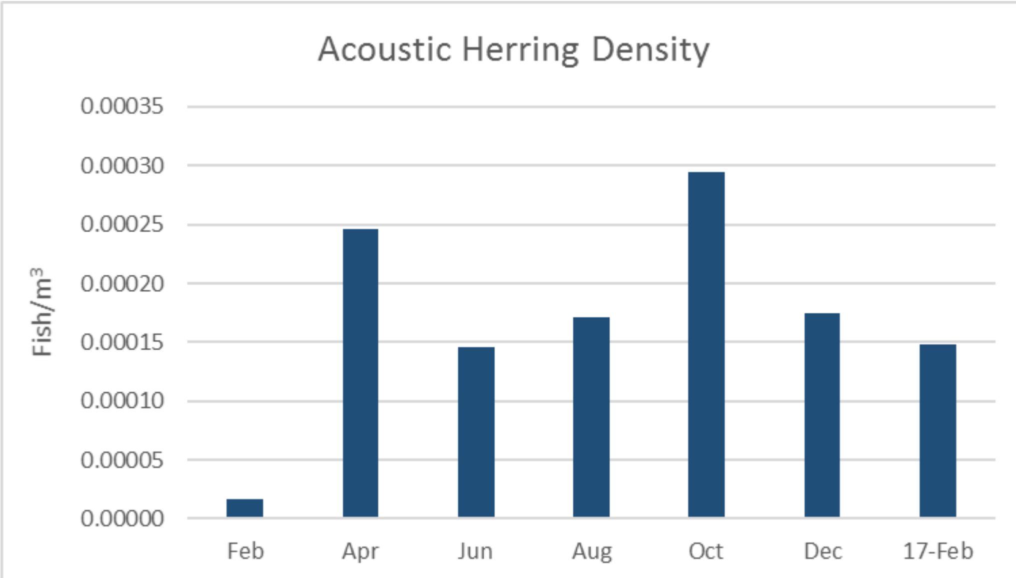

Figure 11. Acoustic estimates of herring density (fish/m3), February 2016-17.

Species density for herring was greatest, followed closely by hake (Figure 12). A maximum estimated density for herring was 8.45 fish/m^3 and occurred at the Colvos Passage station in the Main Basin during the February 2017 survey. Herring accounted for 6 of the 10 highest fish densities measured during the acoustic survey, California Market Squid accounted for two of the highest densities, and Chinook Salmon and Pacific Hake accounted for the remainder.

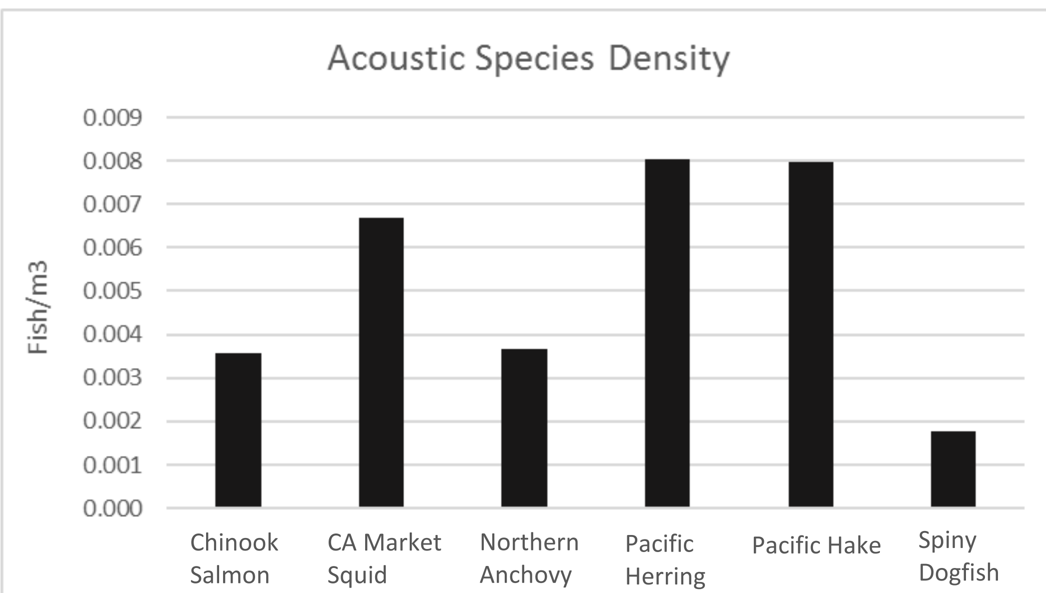

Figure 12. Overall acoustic fish density of the dominant fish species collected.

Herring occurred throughout the water column, but the greatest densities occurred at depths of 175-225 meters (Figure 13). Note that most sampling (100 tows) occurred during daylight hours, but trawls were also conducted during crepuscular periods and at night. Schooling pelagic forage fish like herring are expected to move up in the water column during periods of low light (diel migration) (e.g., Lemberg 1978).

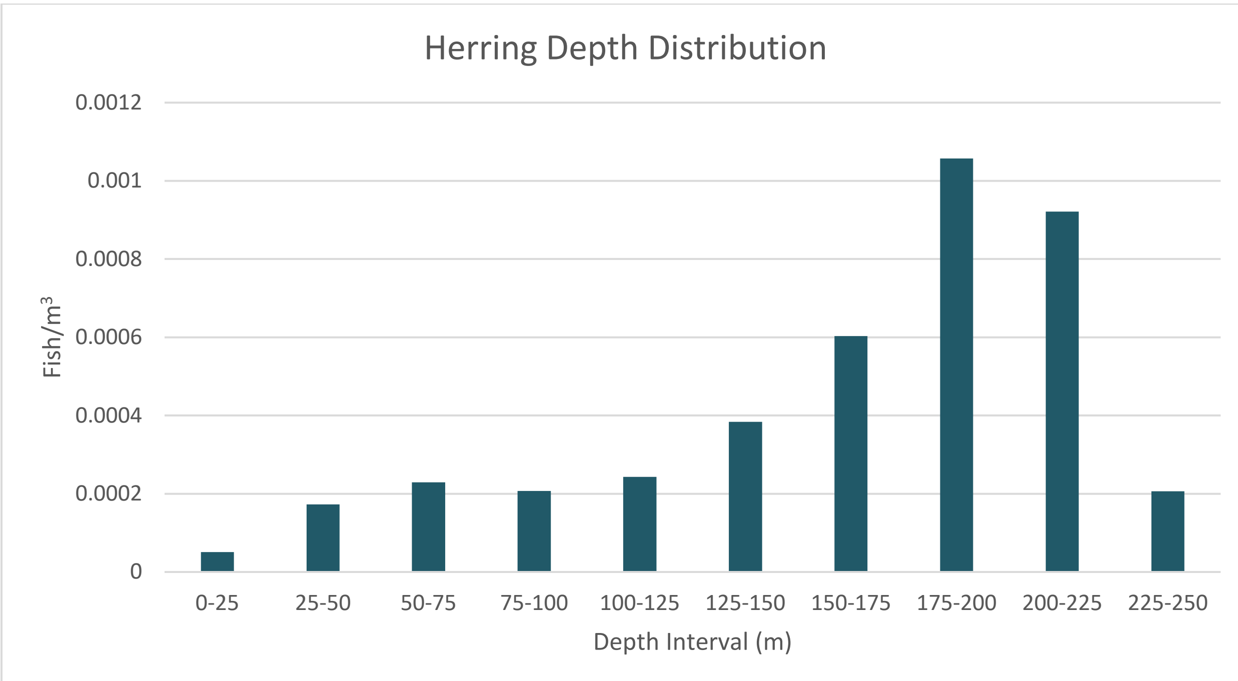

Figure 13. Average acoustic herring density (fish/m^3) at 25-meter depth intervals.

Spatiotemporal Patterns in Biodiversity

The most commonly encountered and abundant species in the trawl catch were Pacific Herring, Pacific Hake (Whiting), Water Jellies, and Northern Anchovy, and together these species drove seasonal and spatial patterns in biodiversity. Given that trawl sampling usually occurred after identifying areas of high acoustic biomass in an effort to verify species composition, it is no surprise that schooling/aggregating species dominated the catch.

Variation in biodiversity among basins was significant in many cases ($p<0.05$) (Figure 14), with Fraser Plume (FP) and Strait of Juan de Fuca (SJF) being most different from the remainder of the sampled basins. This was largely due to a lack of herring and hake in these two most northern basins, and an abundance of cross jellies in SJF (over 98% of all Cross Jellies encountered in the survey). South Sound (SS; aka South of Narrows) differentiated from the remaining basins due to high abundance of Northern Anchovy and Water Jellies, and Whidbey Basin (WB) and Hood Canal (HC) were highly similar with regard to overall biodiversity patterns (Figure 14 and Appendix A).

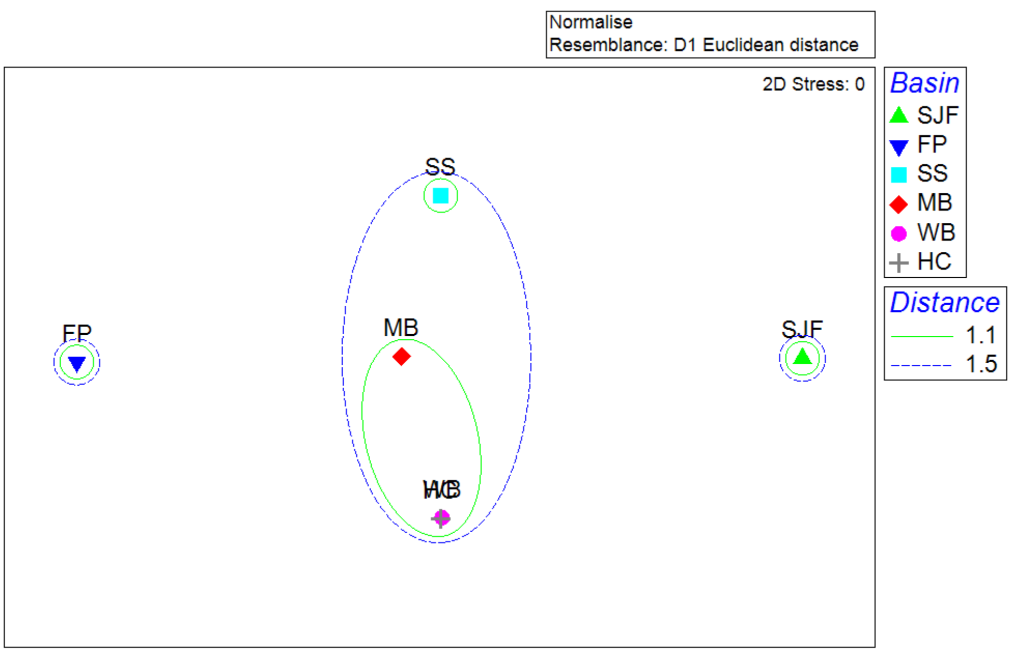

Figure 14. nMDS plot showing variation in biodiversity as a function of basin.

Substantial and significant ($p<0.05$) variation in biodiversity across months was also readily apparent (Figure 15), with August being characterized by a lack of Shiner Perch and English Sole, but high (relative) numbers of Pacific Sand Lance. Shiner Perch were also not encountered during June, although this may indicate an inshore migration for mating during summer months as opposed to a lack of abundance in the southern Salish Sea at large. February 2017 was unique from all other months in that the catch included relatively high proportions of herring, hake, and anchovy but very little else.

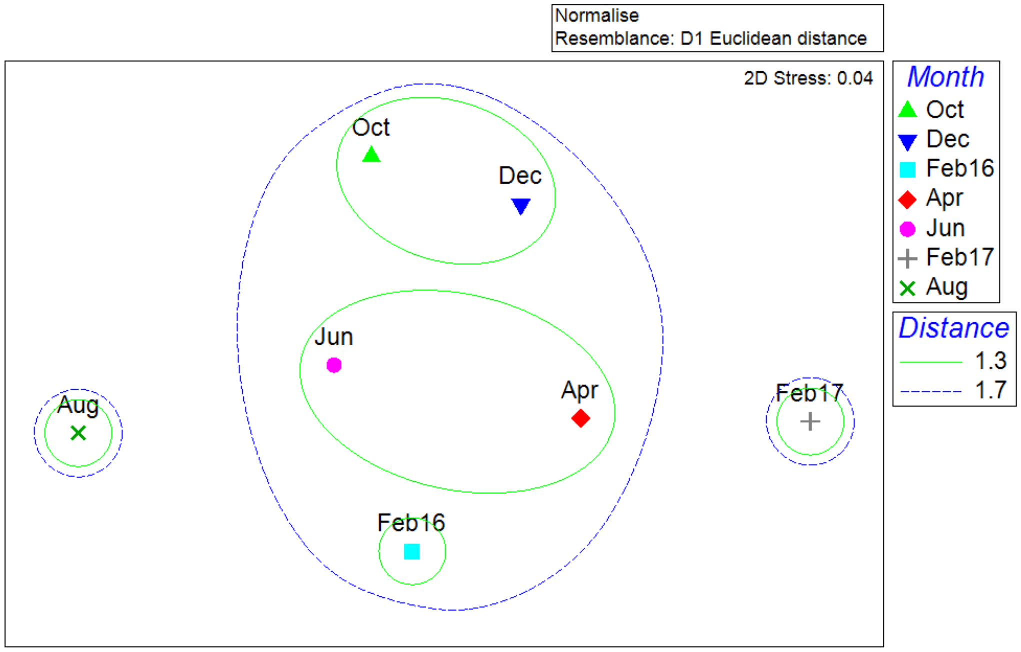

Figure 15. nMDS plot showing variation in biodiversity as a function of sampling month.

When basin and sampling month are considered in a two-factor analysis, random noise and a lack of consistent, representative samples begin to obfuscate patterns (Figure 16). With only a few trawl sets in any given basin in a given month, statistical significance among samples sometimes exists, but the biological significance of these “unique” encounters is questionable. Across months within FP, WB, and SJF biodiversity tended to be less variable, while biodiversity in more southern and interior basins (HC, MB, and SS) was driven by dominate species that fluctuated substantially in abundance from month to month (e.g., Northern Anchovy, Figure 17). In some cases these fluctuations coincided with known species-specific regional feeding and/or mating migrations, but in other cases the reasons for these differences remain elusive. In this study trawling occurred primarily as a mechanism to verify the nature of ensonified biomass targets and estimate species-specific biomass, with spatiotemporal catch composition analysis as a secondary goal. Clarification of observed biodiversity patterns is unlikely to be possible given the observed signal-to-noise ratio without regular annual and seasonal replication of mid-water surveys with substantially increased sampling effort.

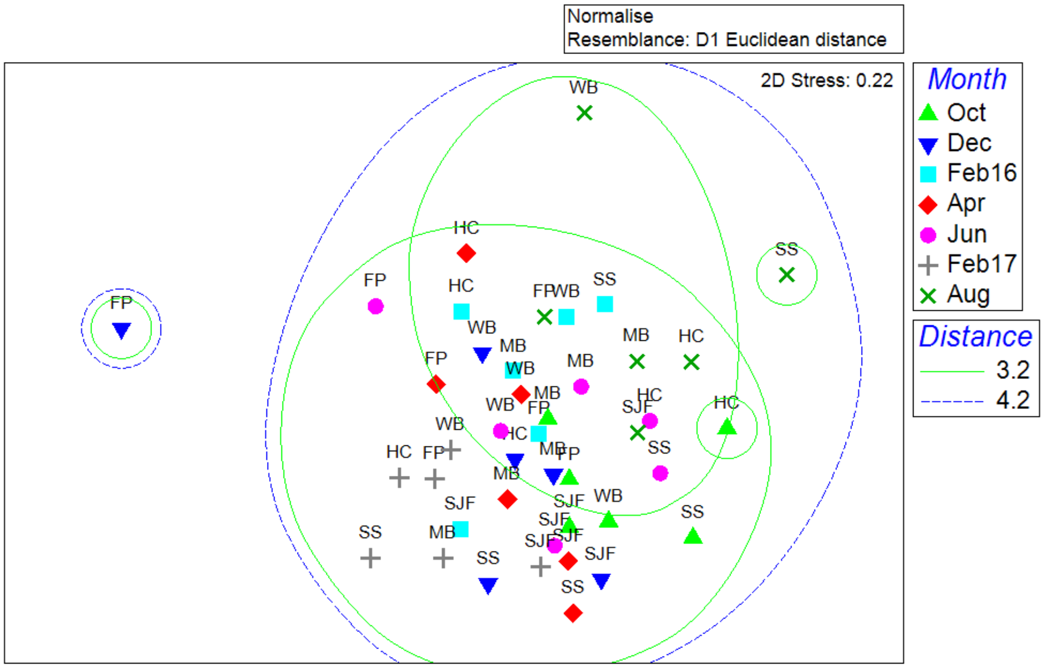

Figure 16. nMDS plot showing variation in biodiversity as a function of both basin and sampling month. Clear basin-specific seasonal trends are not apparent.

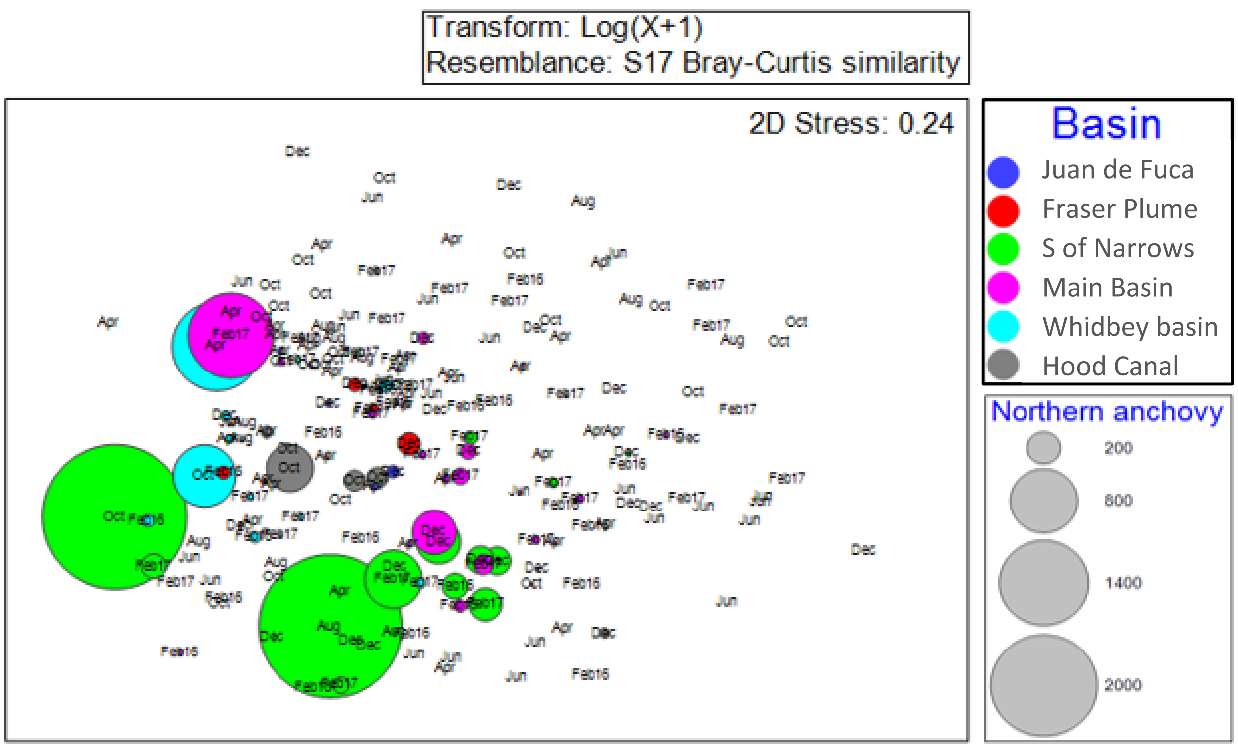

Figure 17. nMDS plot showing variation in anchovy abundance by basin across sampling month. High variability in catch was the rule, with most large catches occurring in southern basins.

A variety of environmental factors were measured during the survey with the intent of conducting correlation analysis with biodiversity patterns. These variables included weather conditions, moon phase, tide height, wind speed and direction, air temperature, water temperature at the depth of tow, barometric pressure, and the degree of cloud cover. Because trawling effort was focused on acoustic target validation and evaluation of species composition, however, sampling generally occurred under rather limiting logistical constraints. As a result, seasonal patterns in the environmental variables led to a high degree of autocorrelation among variables, and taken together this suite of parameters did not facilitate interpretation of biodiversity patterns beyond the level that sampling month had already made apparent.

Project Collaborations

As part of this project, we collaborated with and provided data and samples to many regional scientists working in the Salish Sea. Given the rarity of research trawling programs, we endeavored to utilize this effort well beyond forage fish, although several of these collaborations targeted Pacific Herring. Our collaborators, their affiliations, and a brief description of the projects are listed below:

- **Toxics-focused Biological Observation System (T-BiOS) for the Salish Sea (WDFW/NOAA):** During the mid-water trawl, four species of salmon were collected, including 187 juvenile and sub-adult Chinook, 71 Chum, 16 Coho (*O. kisutch*), and 51 Pink salmon (*O. gorbuscha*). These fish were processed to investigate the levels of toxic contaminants in juvenile and subadult salmon, which may affect their growth, reproduction, and survival, as well as causing similar effects in their predators. A subset of American Shad, an introduced species along the U.S. West coast, was also retained for contaminant and stable isotopes analysis to determine their trophic interactions in the Salish Sea.
- **Joshua Chamberlain (NWFSC), Dave Beauchamp (USGS Western Fisheries Research Center):** Chinook Salmon and adult Pacific Herring captured in trawls as bycatch in the San Juan Islands were frozen for a comparative diet analysis to determine the degree of diet overlap, competition, and predation between the two species during winter, when lower trophic level production is restricted by limited daylight.
- **Julie Keister (Oceanography, University of Washington):** Puget Sound Ecosystem Indicators-Zooplankton. Monitoring zooplankton communities in Puget Sound sub-basins allows researchers to develop robust metrics of ecosystem health. These metrics are used to evaluate impacts of physical change on the Puget Sound food web, understand the impact of global or local stressors on Puget Sound recovery indicators (e.g., forage fish and marine bird abundances), and provide guidance toward improved salmon harvest management and Puget Sound stewardship. Vertical net samples were taken at each station, typically between trawls, during the mid-water trawl project and preserved for future analysis of the zooplankton community in the hope of allowing seasonal and geographic comparisons. Sorting and analysis of these samples awaits funding.
- **Virginia Butler (Portland State University):** Potential for reconstructing body size of Pacific Herring from an archaeological site in Port Angeles, WA. The archaeological site records provide a *really* long history (going back ~2000 years) of fishes in this part of the Salish Sea (Strait of Juan de Fuca) that is especially interesting in light of the recent restoration project on the Elwha. WDFW mid-water trawl staff supplied a geographically and temporally diverse sample of modern herring of all size classes to be used to develop a regression model from vertebra size-body size relationships.
- **Paul Hershberger (USGS Western Fisheries Research Center, Marrowstone Field Station):** Study PS-16-1 is investigating the seasonality of *Ichthyophonus* in Pacific Herring from different locations throughout the Salish Sea. *Ichthyophonus* is a protistan parasite capable of causing population-level declines during disease outbreaks in the ocean and is suspected of contributing

to the loss of adult herring in Puget Sound (Hershberger et al. 2002). Although the percentage of infection (prevalence) increases with herring age, juvenile cohorts (age 0, YOY) sometimes have the heaviest infection intensities (# parasites/fish or tissue sampled) (Hershberger et al. 2016). Samples of adult herring (age 2+) taken during the mid-water trawl allowed a comparison of *Ichthyophonus* presence among the basins of Puget Sound, as well as seasonally. Results indicate that infection rates are higher in Hood Canal, perhaps as a result of increased infection pressures given the large, recent increase in the estimated herring biomass.

- **Katherine Maslenikov (Burke Museum, University of Washington):** Samples of unusual fish, including eelpout, smelt, Pacific Pompano *(Perrilus simillimus*), Northern Lampfish (*Stenobrachius leucopsarus*), clingfish, Longspine Combfish (*Zaniolepis latipinnis*), snailfish and others captured aboard the mid-water trawl were submitted for species identification and/or to add to the Burke Museum's fish collection.
- **James Losee (WDFW):** Resident Coho Salmon project. An unknown proportion of the Coho Salmon population resides in the southern Salish Sea until maturity, where these fish contribute to recreational and commercial fisheries the year following release. Results from coded-wire tag studies suggest that fish released at a larger size and/or released later than is typical may contribute to recreational fisheries at a higher rate than those released under traditional stocking approaches. In an effort to grow Coho Salmon to a larger size and encourage them to remain in Puget Sound for their entire life, the WDFW and the Squaxin Island Indian Tribe have delayed release of a proportion of juvenile Coho Salmon. To monitor the contribution these fish make to fisheries in Puget Sound, hatchery managers clip a portion of the ventral fin from delayed-release group. Catches of salmon from the mid-water trawl, particularly in the Main and South Basins, were inspected to detect ventral clips and frozen samples retained.
- **Paul Chittaro (NOAA, NWFSC):** Investigation of Whidbey Basin Pacific Hake populations. A once robust Pacific Hake population in Puget Sound, and particularly in the Whidbey Basin/Port Susan area, used to support a commercial fishery. After the stock declined in the 1970s, likely as a result of overfishing the spawning aggregations, it has failed to rebound for reasons that are only partially understood (Chittaro, Zabel et al. 2013). Pacific Hake captured during the mid-water trawl study are being processed to provide information on diet, growth rate, size-at-age, and age structure to better understand impediments to recovery.
- **Barry Berejikian and Megan Moore (NOAA/NWFSC**, **Manchester Field Station):** Puget Sound juvenile Steelhead Trout *(O. mykiss)* survival studies. One of the interesting trends captured, in part, by the mid-water trawl study was the re-emergence of Northern Anchovy as an abundant forage fish, particularly in South Sound. This may help explain the recent, dramatic increase in the survival rate of juvenile steelhead originating in South Sound through Central Puget Sound, which has increased from 26% in 2014 to 59% in 2016 (personal communication). The presence of large numbers of anchovy may benefit juvenile steelhead and other salmon by acting as a buffer prey from predation (particularly by harbor seals and/or harbor porpoise), i.e., "predator swamping".

Conclusions

This report documents the abundance and occurrence of pelagic fishes, especially forage fishes, in Puget Sound. Pacific Herring were the most abundant species of fish in all basins examined (61% of the total catch), as evaluated using the sampling protocols described here. Pacific Hake was common in both the Main and Whidbey Basins and was the second most abundant forage species captured (15% of total catch). Northern Anchovies were the third most abundant fish species, but made up a large portion of the catch (26%) only in the South Sound. Pacific Sand Lance and smelt species (Family Osmeridae) were relatively rare. Overall eight fish species, plus California Market Squid, made up 96% of the total catch (Table 3). It is likely that larger aggregations of Northern Anchovy, Pacific Sand Lance, and smelt occur in the nearshore regions of the Sound in water too shallow for the mid-water trawl to operate, and thus these species are under-represented in this study.

By region, herring were also by far the most abundant species in the Fraser Plume (FP; 89% of total catch), Hood Canal (HC; 91%), Strait of Juan de Fuca (SJF; 74%) and Main Basin (MB; 71%). They also dominated the catch in South of Narrows (South Sound, SS; 63%) and made up 50% of the catch in the Whidbey Basin (WB), where hake were also abundant and made up 44% of the catch. When examined by the month of trawl, herring tended to be the most numerous species in all but the summer months, particularly in April (just after the spawning season for most stocks) and in October, as migratory herring returned to Puget Sound. Catches in December and February were still high but likely lower than in April and October because we intentionally avoided surveying the pre-spawn holding areas, where herring aggregate prior to spawning, to limit detrimental impacts to the spawning biomass. During June and August, when herring catches were lower, catches of Pacific Hake (Whiting) made up a largest portion of the fish catch.

The acoustic biomass estimate of herring, which also takes into consideration the size of the fish ensonified, for the entire survey was 115,834 kg, which is two orders of magnitude lower than the spawning biomass estimated by the annual WDFW herring spawn surveys in recent years (Stick et al. 2014, Sandell et al., 2019). This discrepancy is, at least in part, due to migratory patterns of habitat use by most herring stocks, seasonal variation in schooling and shoaling behavior, and sampling station placement well away from known holding and spawning grounds. Other significant contributors to Puget Sound biomass based on acoustics included North Pacific Spiny Dogfish and Pacific Hake (the larger size of Spiny Dogfish increases their contribution to the acoustic total). The maximum herring density was 8.44 fish/m^3 and the highest mean densities occurred in the 175-225 meter depth interval. Both Pacific Herring and Pacific Hake had their highest densities in the Whidbey Basin and lowest in the Strait of Juan de Fuca. The South of Narrows (South Sound) region had the largest density of Northern Anchovy.

Because trawling brings fish to hand for biometric analysis (length, weight, reproductive status, sex, etc.), we were able to determine the age of most of the herring captured by analyzing their scales. Young-of-the-year (YOY) Pacific Herring were first captured in June in the Main Basin (Oak Bay station). These fish were probably recently advected out of Hood Canal and most likely originated from the Quilcene Bay spawning population, where spawning has greatly increased since 2015 (Sandell et al.

2019). Later in the summer and into the fall, YOY herring and age 1 herring began to appear in the northern parts of Puget Sound. Herring aged 2+, which represent potential spawners, were present throughout the year and constituted 79% of the total herring captured, but made up only 57% of the herring captured during the summer and fall when age 1 and YOY herring entered the population.

An analysis of patterns in biodiversity (via nMDS) showed that there was significant variation between the regions, with the Fraser Plume and Strait of Juan de Fuca regions being the most divergent from those in Puget Sound. This was driven by lower relative catches of herring and hake in these regions, coupled with high catches of cross jellies in SJF during the summer months. South Sound ("South of Narrows") differed from the other regions due to the high abundance of water jellies (in the summer months) and northern anchovy, which appear to have expanded in many areas of the Salish Sea in recent years (Duguid et al., 2018). Whidbey basin and Hood Canal had very similar patterns in biodiversity. When analyzed by month, August and February (2017) were the most unique; August was differentiated by large catches of Pacific Sand Lance and low catches of Shiner Perch and English Sole, while February 2017 had large catches of herring, hake and anchovy but little else. The other summer months, April and June, were similar, as were October and December (with large percentages of herring caught, even though the total catch of herring was much higher in October, as previously discussed). February 2016 was differentiated by a broad number of species captured, although our ability to trawl the acoustic targets was limited by not yet having the depth sounder equipment, which likely affected the catch. Our ability to analyze these data by both month and region was hampered by low numbers of trawls in any one region in any one month; without seasonal replication (i.e., more sampling), it was impossible to discern biologically meaningful patterns.

This survey was the first by the WDFW to target forage fish, and particularly herring, when they were *not* aggregating prior to spawning or on their spawning grounds. Instead, it investigated pelagic fish community composition over the course of the year in a broader, pelagic-focused manner. Overall, the study captured a "snapshot" of Puget Sound after an extended period (late 2013 through 2016) of anomalously warm surface waters in the northeastern Pacific Ocean (nicknamed "the Blob") and will serve as a baseline for future comparisons. The value of this study will be amplified by future efforts to emulate this work, building a time series that will be an invaluable reference as the human population around the southern Salish Sea increases and the climate shifts over coming decades. This report is the first step in presenting these data; WDFW staff continue to work through the backlog of frozen samples that were captured to age fish and analyze samples for genetics, toxics, etc., and generate a technical report to make this information publicly available. These data will be further investigated to enhance understanding of the physical and biological determinants of forage fish abundance and aggregation in the southern Salish Sea and the dynamics between forage fish and the other trophic levels in the ecosystem.

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Appendix A

Mid-Water Trawl Total Catch Composition

An organism captured in the mid-water trawl was deemed acoustically relevant if its acoustic properties were known, well studied, or could be inferred from the scientific literature. Non-acoustically relevant organisms included gelatinous zooplankton, crabs, and other invertebrates, whose acoustic characteristics are unknown or poorly documented. Organisms were grouped as 'other' in the acoustic estimation of biomass if the species never exceeded 5% of the total catch of any single trawl sample.

Common Name	Basin								
	Fraser Plume	Hood Canal	Juan de Fuca	Main Basin	South of Narrows	Whidbey Basin	Total Catch	Acoustically Relevant	Grouped as Other
Pacific Herring	7,753	26,098	4,665	23,182	9,835	14,202	85,736	Yes	No
Pacific Hake	114	1,053	8	6,501	628	12,377	20,681	Yes	No
Aequora spp. (Crystal Jelly)	5	5	1	1,652	10,630		12,292	No	N/A
Northern Anchovy	153	305	25	854	4,073	987	6,397	Yes	No
Cross Jelly		15	5,065	46	19		5,145	No	N/A
Shiner Perch	8	2	7	1,362	244	68	1,691	Yes	No
California Market Squid	34	144	305	349	326	7	1,165	Yes	No
Snailfish spp.	3	54	646	7			710	Yes	No
American Shad	5	515	28	49	3	89	689	Yes	No
Plainfin Midshipman		272		36	342	12	662	Yes	No
Threespine Stickleback	45	5	444	12		4	510	Yes	No
North Pacific Spiny Dogfish	74	19	2	58	42	289	484	Yes	No
Walleye Pollock	60	6	239	55			360	Yes	No
Eelpout spp.		3		17	182	109	311	Yes	No
Pacific Tomcod	1	156		49	96	1	303	Yes	No
Northern Lampfish	177	17	80	6	1	1	282	Yes	No
Pacific Pompano	16	39	1	29	25	170	280	Yes	No
Coonstriped Shrimp	2		152	10	100		264	No	N/A
Pacific Sand Sance			156	77	1		234	Yes	No
English Sole	105	19	1	71	23	5	224	Yes	No
Sea Gooseberries		86	5	36	65	22	214	No	N/A
Chinook Salmon	7	44	7	79	32	19	188	Yes	No
Fried Egg Jellyfish	1	122	4	22	3	10	162	No	N/A
Spotted Ratfish		62		79	1	6	148	Yes	No
Alaskan Pink Shrimp	1	16		33	57	30	137	No	N/A
Crab spp.				100			100	No	N/A
Glass Shrimp	31	16		40	9		96	No	N/A

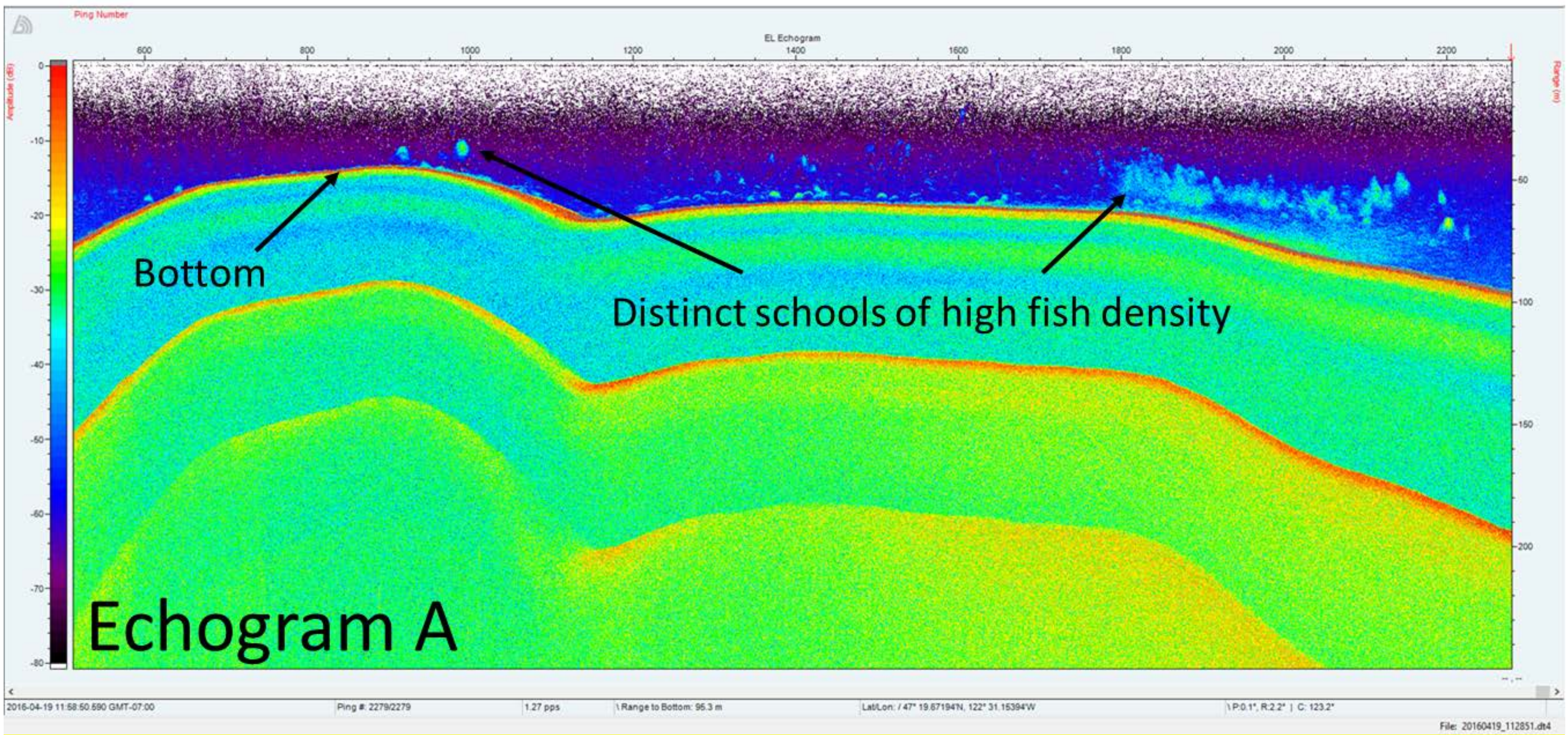
	Basin								
Common Name	**Fraser Plume**	**Hood Canal**	**Juan de Fuca**	**Main Basin**	**South of Narrows**	**Whidbey Basin**	**Total Catch**	**Acoustically Relevant**	**Grouped as Other**
Chum Salmon		1		62	8		71	Yes	No
Plumose Anemone				51			51	No	N/A
Pink Salmon	37		1	13			51	Yes	No
Spot Prawn	15		7	2	8	12	44	Yes	No
Northern Smoothtongue	42						42	No	N/A
Sidestriped Shrimp	27				4	1	32	No	N/A
Unidentified Invertebrates		13	12			7	32	No	N/A
Eulachon	22		7	1			30	Yes	No
Moon Jelly	3	1	1	15	6	2	28	No	N/A
Speckled Sanddab				27			27	Yes	Yes
Slender Sole		13		7	5	1	26	Yes	Yes
Starry Flounder	13	1		1	8	1	24	Yes	Yes
Night Smelt	23						23	Yes	No
Pile Perch				4		17	21	Yes	Yes
Dock Shrimp					19		19	No	N/A
Longfin Smelt	18		1				19	Yes	No
Coho Salmon	1	4		11			16	Yes	No
Longnose Skate				8	5	1	14	Yes	Yes
Humpy Shrimp					12		12	No	N/A
Pacific Spiny Lumpsucker	1		9	2			12	Yes	No
Whitebait Smelt			8	2		1	11	Yes	No
Sea Cucumber		9			1		10	No	N/A
Lion's Mane Jelly	4	1	1	2	1		9	No	N/A
Comb Jelly				8			8	No	N/A
Dungeness Crab	2			1		5	8	No	N/A
Sand Sole	1	5		1		1	8	Yes	Yes
Pallid Eelpout				7			7	Yes	Yes

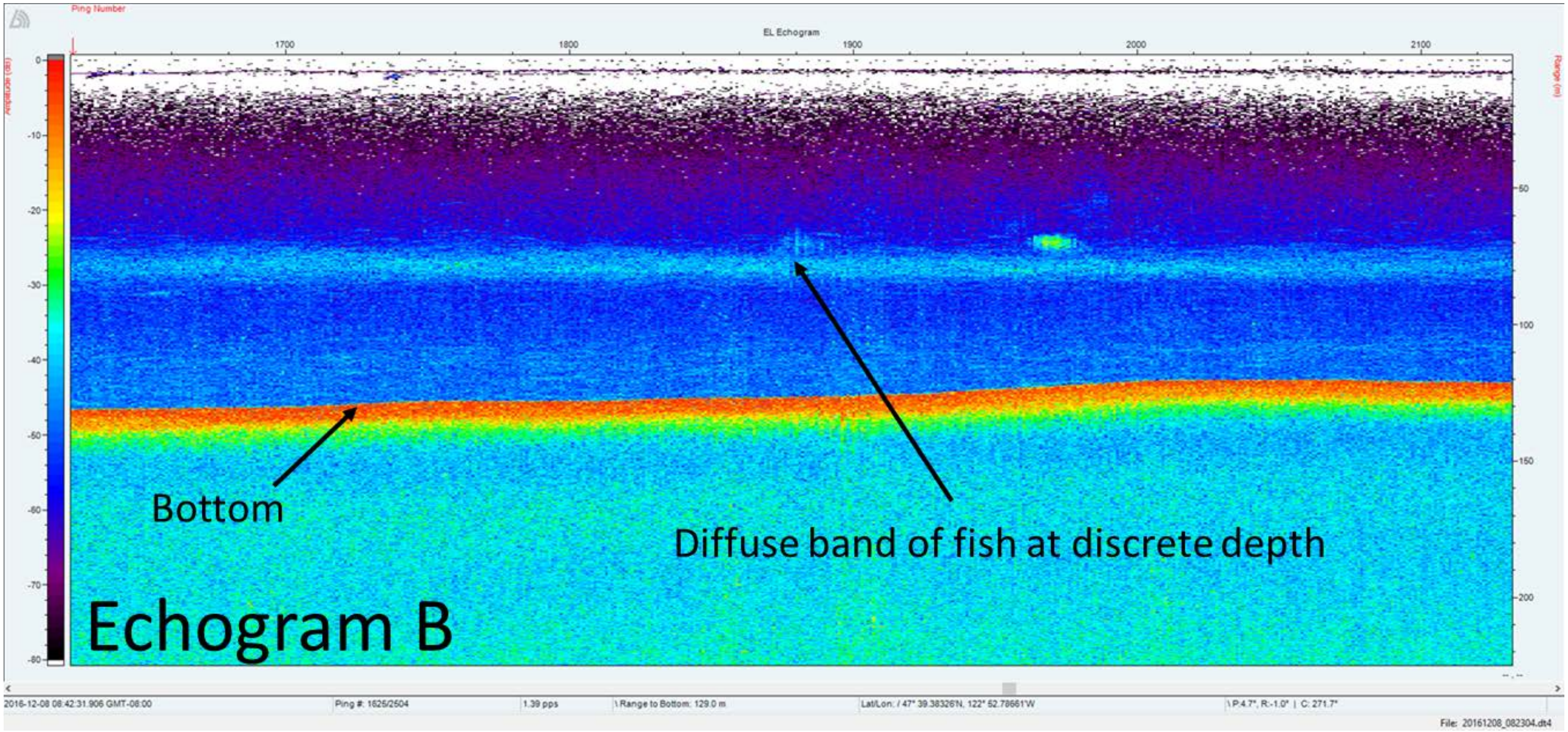
Common Name	Basin								
	Fraser Plume	Hood Canal	Juan de Fuca	Main Basin	South of Narrows	Whidbey Basin	Total Catch	Acoustically Relevant	Grouped as Other
Big Skate		1			5		6	Yes	Yes
Unidentified Jellyfish			5		1		6	No	N/A
Rex Sole		1		2	3		6	Yes	Yes
Splitnose Rockfish		6					6	Yes	Yes
Brown Rockfish				5			5	Yes	Yes
Unidentified Flatfish	2		2	1			5	Yes	Yes
Unidentified Codfish		3	2				5	Yes	No
Rock Sole		1		1		3	5	Yes	Yes
Flathead Sole	1	1		2			4	Yes	Yes
Rosy Tritonia					4		4	No	N/A
Staghorn Sculpin	1	1			1	1	4	Yes	Yes
Pacific Cod				3			3	Yes	Yes
Pacific Sanddab		1		1	1		3	Yes	Yes
Pacific Sandfish	2					1	3	Yes	Yes
River Lamprey					3		3	Yes	Yes
California Sea Cucumber				1	1		2	No	N/A
Northern Rock Sole					2		2	Yes	Yes
Quillback Rockfish					2		2	Yes	Yes
Unidentified Sculpin				2			2	Yes	Yes
Snake Prickleback				1	1		2	Yes	Yes
Surf Smelt	2						2	Yes	No
Arrow Goby					1		1	Yes	Yes
Bay Goby					1		1	Yes	Yes
Blackbelly Eelpout					1		1	Yes	Yes
Buffalo Sculpin					1		1	Yes	Yes
Canary Rockfish				1			1	Yes	Yes
Clubhook Squid				1			1	Yes	Yes

Common Name	Basin: Fraser Plume	Hood Canal	Juan de Fuca	Main Basin	South of Narrows	Whidbey Basin	Total Catch	Acoustically Relevant	Grouped as Other
C-O Sole					1		1	Yes	No
Giant Nudibranch					1		1	No	N/A
Giant Pacific Octopus				1			1	No	N/A
Unidentified Lamprey					1		1	Yes	Yes
Northern Clingfish	1						1	Yes	Yes
Unidentified Nudibranch		1					1	No	N/A
Pacific Lamprey				1			1	Yes	Yes
Puget Sound Rockfish						1	1	Yes	Yes
Roughhead Sculpin					1		1	Yes	Yes
Unidentified Sanddab		1					1	Yes	Yes
Sharpnose Crab			1				1	No	N/A
Showy Snailfish					1		1	Yes	Yes
Sixgill Shark					1		1	Yes	Yes
Unidentified Snail	1						1	No	N/A
Southern Rock Sole					1		1	Yes	Yes
Unidentified Squid			1				1	Yes	Yes
Stubby Squid				1			1	Yes	Yes
Sturgeon Poacher	1						1	Yes	Yes
Yellowtail Rockfish				1			1	Yes	Yes

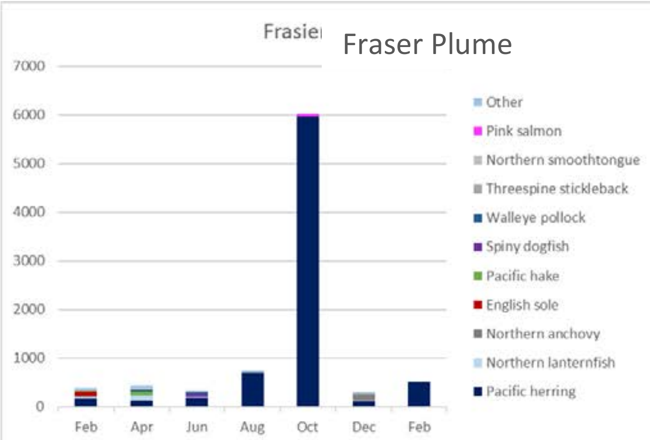
Appendix B

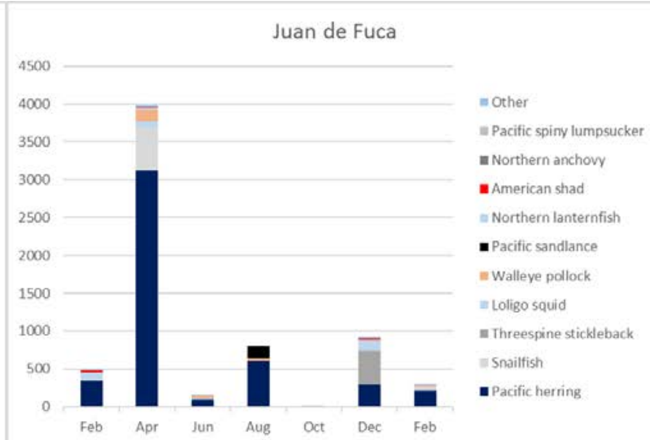
Screenshots of acoustic echograms illustrating the most common occurrences of acoustic targets observed during the survey. Echogram A depicts discrete schools of fish at high concentrations that were temporally and spatially distinct. Echogram B depicts a diffuse band of fish at a discrete depth, where the band was generally continuous for the entire length of the transect.

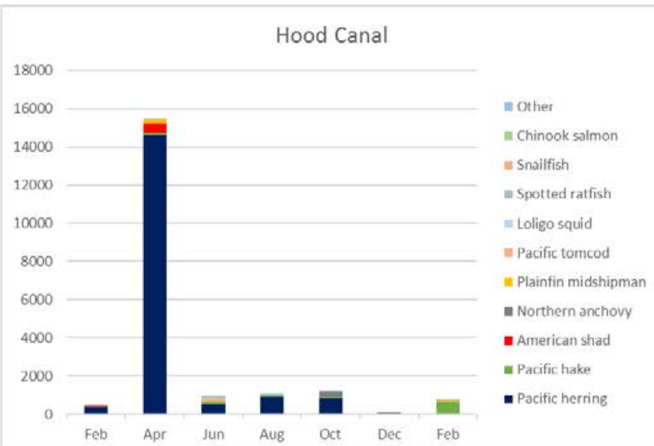


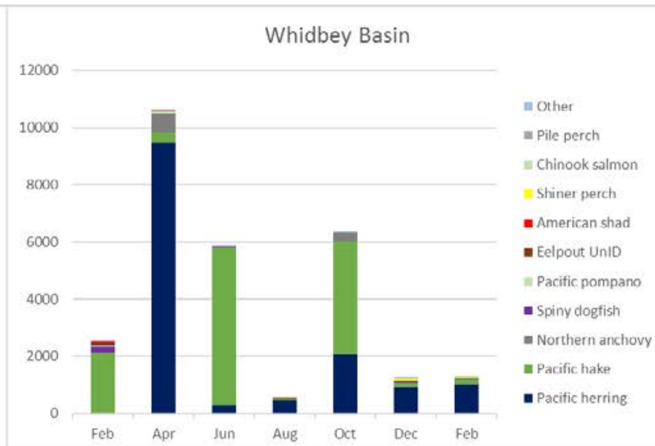

Ping Number
EL Echogram
1700
1800
1900
2000
2100
Bottom
Diffuse band of fish at discrete depth
Echogram B

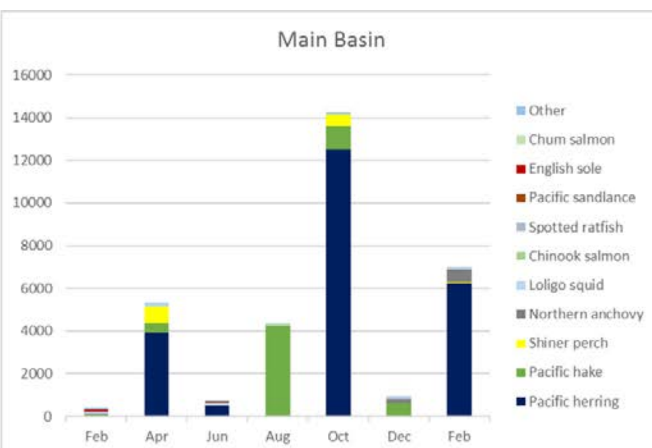
Appendix C

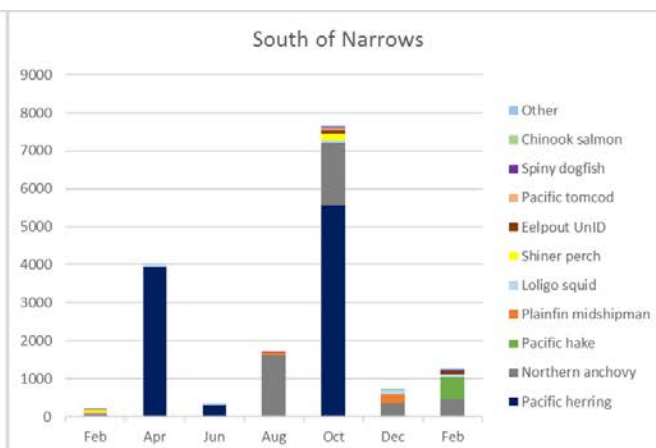

Frasie
Fraser Plume
7000
6000
5000
4000
3000
2000
1000
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Other
Pink salmon
Northern smoothtongue
Threespine stickleback
Walleye pollock
Spiny dogfish
Pacific hake
English sole
Northern anchovy
Northern lanternfish
Pacific herring


Juan de Fuca
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Other
Pacific spiny lumpsucker
Northern anchovy
American shad
Northern lanternfish
Pacific sandlance
Walleye pollock
Loligo squid
Threespine stickleback
Snailfish
Pacific herring


Hood Canal
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Other
Chinook salmon
Snailfish
Spotted ratfish
Loligo squid
Pacific tomcod
Plainfin midshipman
Northern anchovy
American shad
Pacific hake
Pacific herring


Whidbey Basin
12000
10000
8000
6000
4000
2000
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Other
Pile perch
Chinook salmon
Shiner perch
American shad
Eelpout UnID
Pacific pompano
Spiny dogfish
Northern anchovy
Pacific hake
Pacific herring


Main Basin
16000
14000
12000
10000
8000
6000
4000
2000
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Other
Chum salmon
English sole
Pacific sandlance
Spotted ratfish
Chinook salmon
Loligo squid
Northern anchovy
Shiner perch
Pacific hake
Pacific herring


South of Narrows
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Feb
Apr
Jun
Aug
Oct
Dec
Feb
Other
Chinook salmon
Spiny dogfish
Pacific tomcod
Eelpout UnID
Shiner perch
Loligo squid
Plainfin midshipman
Pacific hake
Northern anchovy
Pacific herring