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Short communication

Acoustic classification of coexisting taxa in a coastal ecosystem

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ABSTRACT

Classifying coexisting taxa in a coastal ecosystem remains an analytic challenge due to the difficulty in verifying species compositions within backscatter data. Multifrequency measurements (38, 70, 120, 200 kHz) were combined with midwater trawls and zooplankton MultiNet tows in Hood Canal, WA, to classify backscatter dominated by single fish species (Pacific Herring, Pacific Hake) or major zooplankton taxa (euphausiids, copepods). Backscatter was categorized into aggregations, single targets, and layers based on morphology. Aggregations and single targets were identified in raw volume backscattering strength (S_v), while layers were classified using differences in mean volume backscattering strength ($\Delta MVBS_{i\text{-}j} = MVBS_i - MVBS_j$, where i and j denote frequency in kHz). Based on a subset of trawl-validated *in situ* acoustic measurements, backscatter with $-16\,dB < \Delta MVBS_{200\text{-}38} \leq 2\,dB$ were classified as fish, and $2\,dB < \Delta MVBS_{200\text{-}38} < 30\,dB$ as zooplankton. Backscatter identified as fish were further classified to hake when $\Delta MVBS_{120\text{-}38} < -4.8\,dB$, and herring when $\Delta MVBS_{120\text{-}38} \geq -4.8\,dB$. The classification method was evaluated using a second set of trawl-validated acoustic data, resulting in classification accuracy of fish or zooplankton ranging from 95% to 100%. At the species level, misclassifications of herring and hake were both ~13%. Removal of aggregations and single targets before calculating ΔMVBS values minimized the possibility of mixed species backscatter within layers. This classification technique provides an approach to separate coexisting aggregations of dominant taxa which are common in mid- and low-latitude coastal ecosystems.

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1. Introduction

Active acoustics is increasingly used to study the abundance, distribution, and behavior of fish and zooplankton communities (e.g., Greenlaw, 1979; MacLennan and Holliday, 1996; Sato et al., 2013). Using ships, moorings, or cabled observatories, acoustics provides continuous measurements of organism densities at high spatial and temporal resolutions. Despite these advantages over traditional net samples, species identification remains a major challenge when using acoustics (Horne, 2000). Multiple frequencies are commonly used to separate gas-bearing from non-gas-bearing organisms, utilizing frequency-dependent backscatter characteristics (e.g., Foote, 1980; Holliday and Pieper, 1980). Among various multifrequency classification methods, differencing the mean volume backscattering strength (ΔMVBS) of two frequencies (Kang et al., 2002; Korneliussen and Ona, 2003; Madureira et al., 1993) has been the most widely used.

Using ΔMVBS to categorize and identify aquatic organisms appears simple, yet its utility and efficacy depend on the species composition, relative abundance, frequency-dependent backscatter characteristics, and spatial overlap of species present. In mid- and low-latitude coastal ecosystems, where coexisting aggregations of different taxa are common, classification of acoustic data is challenging due to the difficulty in verifying species composition of various backscatter types such as aggregations and layers. A combination of sampling gears is essential to characterize mixed aggregations of fish and zooplankton, but logistical constraints often make concurrent sampling difficult. As a result, ΔMVBS values necessary for classification are often not estimated due to the lack of ground-truthing. Alternatively, backscatter models could be used to estimate ΔMVBS values if species compositions and length distributions are known, as well as suitable backscatter models for the species of interest are available (e.g., Kang et al., 2002). Due to the difficulties in satisfying all of these requirements, many studies acquiring multifrequency acoustic data utilize single frequencies to estimate distributions and densities of target species (e.g., Mackas

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et al., 1997; Parker-Stetter et al., 2008). Even though ΔMVBS is a well-established classification method, the sequence of steps to classify coexisting species has not been described in detail.

This study demonstrates an approach to classify coexisting dominant taxa, using data collected in a temperate fjord within existing analytic techniques of fish school and single target detections. The objective was to establish an acoustic classification technique that separates fish from zooplankton, and then separates dominant fish species. In this case, zooplankton, Pacific Herring (*Clupea pallasi*), and Pacific Hake (*Merluccius productus*).

2. Material and methods

2.1. Data collection

Data collected in Hood Canal, WA, were used as a case study for the development of the classification technique. Surveys were conducted during June, July, August, September, and October in 2012 and 2013. CTD (conductivity, temperature, depth) profiles, acoustic measurements, and net samples were collected by two vessels during day and night over four days at four locations within Hood Canal to characterize densities and distributions of dominant taxa and oceanographic properties. Acoustic measurements and midwater trawls were conducted from the *R/V Centennial*. Zooplankton sampling and CTD profiles were conducted from the *R/V Clifford A. Barnes*.

2.1.1. Acoustic data collection

Acoustic backscatter data were collected using Simrad EK60 split-beam echosounders operating at 38, 70, 120, and 200 kHz. The transducers were deployed 2 m below the surface on a rigid pole mounted to the vessel's starboard side. The centers of each transducer were no more than 47 cm apart to maximize spatial overlap of the beams. The 38-kHz transducer had a beam width of 12° (between half power points), while the 70, 120, and 200 kHz transducers all had beam widths of 7°. Echosounders operated at 0.5–2 Hz with a pulse duration of 512 μs. Backscattered acoustic signals were digitized into 10-cm depth bins. All echosounders were calibrated using a 38.1-mm diameter tungsten carbide sphere (Demer et al., 2015). Vessel speed during acoustic surveys was 5–6 knots, decreasing to 2–3 knots during midwater trawling.

2.1.2. Biological samples

Acoustic backscatter including layers and fish aggregations detected by the echosounders were sampled with a midwater trawl or zooplankton nets to estimate species composition and length distributions. The sampling gear used depended on the suspected identity of the organisms.

Midwater trawling: Aggregations and layers of fish and large invertebrates were sampled with a Marinovich midwater trawl (6 × 6 m opening) fitted with a 3.2-mm knotless liner in the codend. The fishing depth of each trawl was targeted to sample regions of high acoustic backscatter, with trawl depth being monitored and directed using a real-time pressure sensor (PI50; Kongsberg Maritime) attached to the headrope. Trawl duration was typically ~8 min, but varied from 3 to 33 min depending on the observed density of backscatter. Since the trawl did not have a closing mechanism, the catch could result from the depths shallower than the target fishing depth. Catches were identified, enumerated, weighed, and a sample of each species was measured for length.

Zooplankton sampling: Zooplankton were sampled with a MultiNet system (Hydro-Bios) configured with five, opening-closing 335-μm mesh nets with a 0.25-m^2 mouth opening, double flowmeters, and a CTD sensor. Depth-stratified oblique tows were conducted at a vessel speed of 2–4 knots to target backscatter layers observed by the echosounders. All MultiNet tows were conducted within the acoustic survey areas less than 2 h from the acoustic measurements to minimize temporal mismatch between net sampling and acoustic measurements. MultiNet tow duration within a targeted layer varied from 1 to 7 min depending on the vertical thickness of the layers. Samples were fixed in 5% formalin buffered with sodium borate. In the laboratory, a subsample from each zooplankton tow was identified to taxa, counted, and measured for length using the silhouette method (WHOI Silhouette DIGITIZER v 1.1; Davis and Wiebe, 1985; Little and Copley, 2003). Euphausiid length was measured from the posterior base of the eye stalk to the end of the sixth abdominal segment [Standard Length 3 in Mauchline (1980), as cited by Ashjian et al. (2004)]. Total body length was measured for all other zooplankton taxa. Biomass (wet weight) was estimated based on length measurements using equations adapted from previous studies (Lavaniegos and Ohman, 2007; Webber and Roff, 1995; Williams and Robins, 1979).

Table 1
Parameters used for the school detection module in Echoview. Different values were used for detecting herring ($S_{v\ \text{herring aggregation}}$) and other aggregations ($S_{v\ \text{other aggregation}}$). Values for maximum horizontal linking distance were adjusted based on sampling frequencies, with 3 m for data sampled at 2 Hz and 10 m at 0.5 Hz.

Parameters	Values	
	herring aggregation	other aggregation
Minimum threshold (dB re 1 m^{-1})	−60.0	−70.0
Minimum total school length (m)	5.0	4.5
Minimum total school height (m)	2.0	0.5
Minimum candidate length (m)	5.0	4.5
Minimum candidate height (m)	2.0	0.5
Maximum vertical linking distance (m)	0.2	0.2
Maximum horizontal linking distance (m)	3.0 (10.0)	3.0 (10.0)

2.1.3. Oceanographic data

To characterize vertical and seasonal variability of water properties, at least 8 CTD (SBE 911plus; Sea-Bird Electronics) profiles were collected during each monthly field survey. Monthly averages of CTD downcasts through the water column were used to estimate sound speed and absorption coefficients, which were in the calculation of volume backscattering strength (S_v; dB re 1 m^{-1}) based on acoustic measurements.

2.2. Data analysis

2.2.1. Acoustic data processing

Acoustic data were processed using Echoview (version 5.4; Echoview Software Pty Ltd.). Vessel noise estimated during passive acoustic measurements (38 kHz: −152 dB, 70 kHz: −160 dB, 120 kHz: −149 dB, 200 kHz: −145 dB) was removed by linear subtraction. Data shallower than 5 m were removed from analyses to eliminate near-field transducer effects and reduce backscatter from surface bubbles. The sounder-detected bottom was visually inspected, corrected if necessary, and data within 0.5 m of the bottom were removed from analyses.

The acoustic data at each frequency were categorized into (i) aggregations (i.e., acoustic backscatter with discrete, closed edges), (ii) single targets (i.e., targets at densities lower than one per sampling volume), and (iii) layers (i.e., acoustic backscatter without discrete, closed edges), based on their morphologies (summarized in Fig. 1: 'Raw data' panel) as described below. Acoustic data categorized as aggregations and single targets were classified as 'fish'. Two sets of parameters (Table 1) were applied to detect different aggregation-size classes of fish in the 38 kHz raw data, using the school detection module in Echoview (Barange, 1994; Coetzee, 2000): 'herring aggregation' (Fig. 1; $S_{v\ \text{herring aggregation}}$ confirmed by midwater trawls), then 'other aggregation' ($S_{v\ \text{other aggregation}}$).

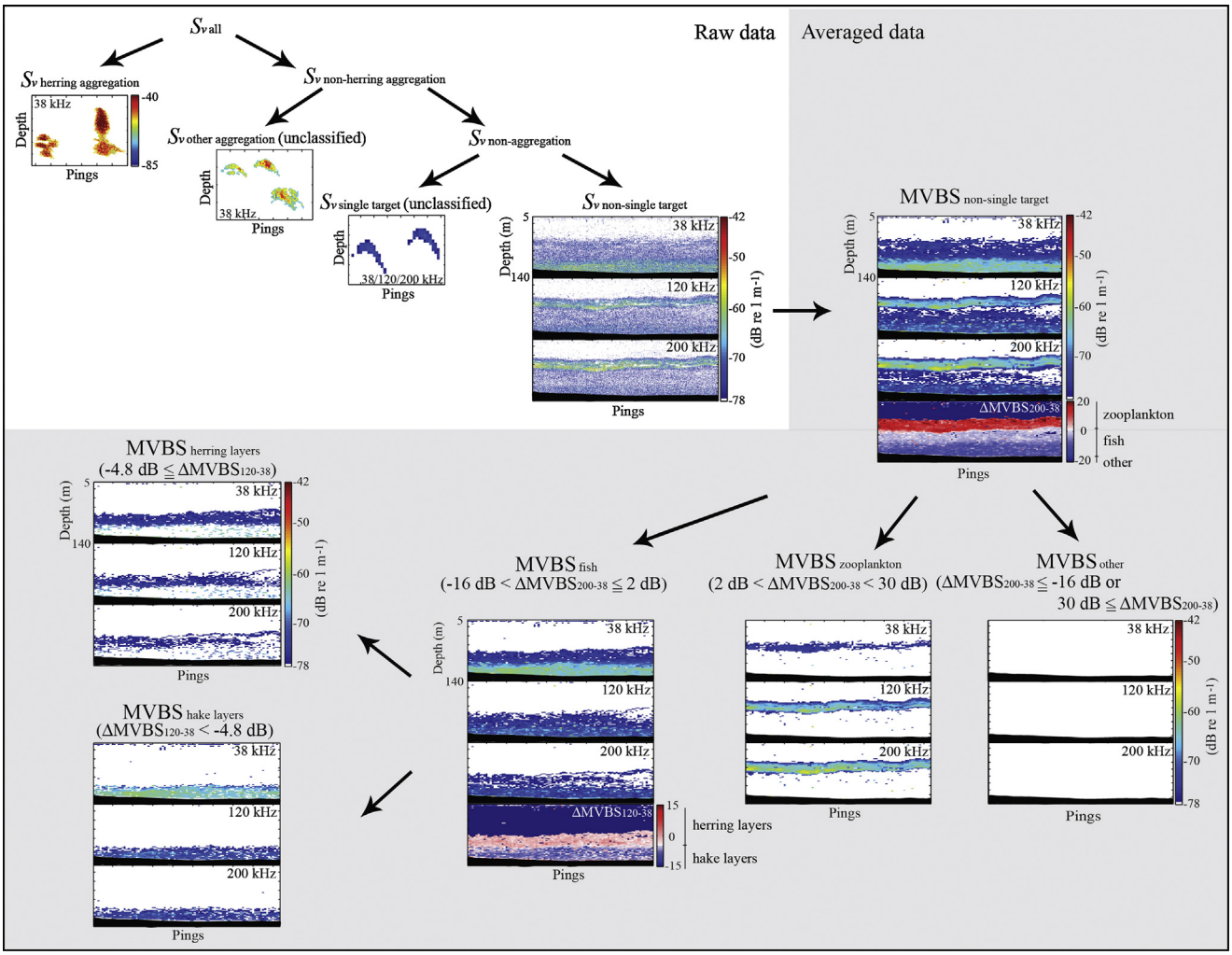

Fig. 1. Schematic illustrating the acoustic classification of coexisting taxa, including removal of aggregations and single targets in raw data (S_v) and then application of ΔMVBS on averaged data (MVBS).

Table 2
Parameters used for single target detection in Echoview.

Parameters	Values
TS threshold (dB re 1 m^2)	−80
Pulse length determination level (dB)	12
Minimum normalized pulse length	0.8
Maximum normalized pulse length	2.0
Maximum beam compensation (dB)	12
Maximum standard deviation of minor-axis angles (°)	3.0
Maximum standard deviation of major-axis angles (°)	3.0

Detected aggregations were visually inspected to avoid false detections such as layers. If a suspected aggregation was consistent with the acoustic characteristics of a fish aggregation but not detected by the Echoview's school detection module, then it was manually defined as an aggregation using Echoview's region tool. Acoustic data not categorized as aggregations ($S_{v\ \text{non-aggregation}}$) were then used to identify echoes from single targets ($S_{v\ \text{single target}}$) using the single target detection parameters (Table 2) following Benoit-Bird et al. (2009). Single target detection was used as a tool to detect fish echoes, but target strengths (TS) were not estimated. The order of the categorization (i.e., detection of aggregations, then single target detection) avoided false detections of single targets within aggregations. Detected single targets were excluded from the data at each frequency, leaving layers ($S_{v\ \text{non-single target}}$). To decrease natural stochastic variability in acoustic backscatter, S_v data within each category were averaged into 20-ping horizontal by 2-m vertical bins (MVBS $_{\text{non-single target}}$), which corresponded to a horizontal distance of 15 m with 2 Hz sampling (62 m with 0.5 Hz sampling) at trawling speed of 3 knots, and 31 m with 2 Hz sampling (123 m with 0.5 Hz sampling) at acoustic survey speed of 6 knots. MVBS $_{\text{non-single target}}$ data were treated as layers and classified to dominant taxa.

2.2.2. Selection of representative samples for classification

To classify acoustic backscatter layers into taxa, we examined acoustic backscatter data collected during net sampling. When net catches indicated a dominance of a single taxon, regardless of the time of sampling, the corresponding periods of acoustic data were isolated, analyzed, and designated as representative samples. These representative samples were used to determine the frequency pairs and ΔMVBS values for separating taxa. An implicit assumption in this procedure is that each cell of the representative samples contains a single fish species (either herring or hake) or zooplankton.

Fish: A total of 149 trawls were screened to select representative samples. First, any trawl catch composition dominated by a single species (herring: > 80% by number and > 60% by weight, hake: > 60% by number and weight) was identified as a candidate trawl. These criteria were used to select trawl samples dominated by a single species, and ensured more than one sample for each species.

Second, acoustic data collected concurrently with candidate trawls were examined to select layers, and to exclude aggregations and single targets. Acoustic data corresponding to the area of the candidate trawl were defined horizontally by a time interval where the trawl had stabilized at the target fishing depth, and vertically by extending 12 m below the trawl headrope. For one trawl dominated by herring, the vertical depth was limited to 6 m below the headrope to exclude a non-herring acoustic layer having characteristics of low S_v values with diffuse aggregations. To account for the spatial difference between the transducers and the midwater trawl, horizontal offset between the transducers mounted on the vessel and the trawl was corrected based on deployed trawl wire lengths and trawled depth for each trawl. Effect of vessel speed on the horizontal offset is unlikely, because trawl wire lengths were fixed during the trawls and the headrope depth was maintained by adjusting ship speed. Third, echograms were visually examined to ensure that there were no obvious signs of another taxon, such as backscatter layers from zooplankton. Acoustic data passing these three criteria were designated as representative samples and assigned to the taxon dominating the trawl catch for further analysis.

Zooplankton: A total of 79 net samples were examined to select representative samples in which euphausiids and copepods dominated backscatter measurements. First, net samples dominated by euphausiids and copepods for more than 80% by wet weight were identified as candidate samples. Net samples were constrained to avoid including strong acoustic backscatterers such as thecosome pteropods (*Limacina helicina*) and gastropods whose densities were greater than 5 individuals (ind.) m^{-3}. Acoustic data corresponding to candidate samples were identified based on GPS locations and depths sampled by the MultiNet. Second, acoustic data collected near candidate samples were limited to contain only echoes from zooplankton by removing co-located fish aggregations and single targets from zooplankton backscatter layers (see Section 2.2.1). To ensure that only echoes from zooplankton were included, acoustic data dominated by fish aggregations and single targets, or diffuse layers of zooplankton were excluded from candidate samples based on visual inspections. Acoustic data passing these criteria were designated as representative zooplankton samples for further analysis.

2.2.3. Classification of the dominant taxa

Acoustic backscatter frequency differences for all possible frequency pairs ($\Delta MVBS_{i\text{-}j} = MVBS_i - MVBS_j$, where i and j denote frequency in kHz) were calculated for each averaged cell of the representative samples. The difference in logarithmic domains is equivalent to the ratio of S_v in corresponding linear units (s_v; m^2 m^{-3}). Pairwise frequency difference values were compiled, and the mean and standard deviation at each frequency pair were calculated for each taxon. To minimize the effects of background noise in each cell, only cells where $MVBS_{\text{non-single target}}$ values greater than −78 dB and signal-to-noise ratios equal to or greater than 10 dB for at least one of the frequencies in the pair were used for further analysis. The threshold was determined by calculating nautical area scattering coefficient (NASC; m^2 nmi^{-2}) of the representative samples at thresholds ranging from −90 to −42 dB in 6 dB steps, following Jech and Michaels (2006). The final choice of the −78 dB threshold was chosen to maximize total NASC values, where no changes in NASC were observed when lower than −78 dB thresholds were applied.

Combinations of frequency pairs and values for separating representative samples of fish ($MVBS_{fish}$) vs. zooplankton ($MVBS_{zooplankton}$) vs. other ($MVBS_{other}$), and herring ($MVBS_{herring\ layers}$) vs. hake ($MVBS_{hake\ layers}$) were determined by selecting the frequency pairs having minimum overlap in ΔMVBS histograms. Total backscatter attributed to fish, including aggregations, single targets, and layers, was estimated using: $MVBS_{fish\ all} = MVBS_{all} - MVBS_{zooplankton} - MVBS_{other}$. Acoustic backscatter attributed to herring was estimated using: $MVBS_{herring} = MVBS_{herring\ layers} + MVBS_{herring\ aggregations}$. Other aggregations and single targets were not classified to species because of the difficulty in ground-truthing and applying the classification method to small aggregations.

2.2.4. Evaluation of the classification method

To evaluate accuracy of the classification method, net samples dominated by single fish species or major zooplankton taxa and not used as representative samples were selected as validation samples. Unlike representative samples, which were limited to layers, validation samples were selected regardless of acoustic morphology. For fish, trawl catches dominated by single fish species (herring: > 90% by number and > 60% by weight, hake: > 60% by number and > 40% by weight) were identified as candidate validation trawls. For zooplankton, net samples with more than 80% wet weight of euphausiids and copepods, and less than 5 ind. m^{-3} of thecosome pteropods and gastropods were identified as candidate validation trawls. Acoustic backscatter data corresponding to the candidate trawls were defined as described in Section 2.2.2 and echograms were visually examined to ensure that there were no obvious signs of another taxon, such as zooplankton backscatter layers in fish validations samples. For fish validation, echograms were also examined to ensure that no significant backscatter was present shallower than the trawled area so that trawl catches were representative of the region identified on the echogram. Acoustic data passing these criteria were designated as validation samples.

The classification method outlined in Fig. 1 was applied to the validation samples. We assumed that a single taxon dominated the validation samples and that any MVBS classified to a taxon other than the corresponding dominant trawl-caught taxon was a misclassification. For fish samples, percentages of NASC values classified within each category were estimated using 38 kHz data. For zooplankton samples, percentages of NASC values classified to fish, zooplankton, and other were estimated using 200 kHz data. NASC values of aggregations and single targets were not included, because fish could not be captured by the MultiNet and therefore catch compositions were not representative of fish.

3. Results and discussion

3.1. Species composition

Midwater trawling: Herring and hake were the dominant fish species and constituted 62% of midwater trawl catches by number in 2012 and 52% in 2013. Jellyfish (*Cyanea capillata*, *Phacellophora camtschatica, Aequorea victoria*, *Aurelia aurita*) were also abundant constituting 34% of the trawl catches by number in 2012 and 40% in 2013, with peak abundances in June and decreased through fall. Since the decline in jellyfish abundance corresponded to the disappearance of near-surface backscatter layers at 38 kHz which were present earlier in the season, we attributed the high density of jellyfish to incidental bycatch from surface layers.

Zooplankton sampling: Euphausiids, copepods, amphipods, decapods, chaetognaths, and crab zoea accounted for most of the zooplankton collected in the acoustic backscatter layers. Euphausiids (mostly *Euphausia pacifica*) and copepods were dominant acoustic backscatterers, with their abundance often exceeding 70% of the total zooplankton by wet weight (range: 4–100%). Major copepod species in Hood Canal are *Metridia pacifica, Paracalanus parvus*, *Pseudocalanus* spp., *Oithona similis,* and *Calanus pacificus* (Keister and Tuttle, 2013). High densities of thecosome pteropods (*Limacina helicina*) were observed in 3 out of 79 samples at densities greater than 80 ind. m^{-3}. Another acoustically important taxon is siphonophores that contain gas-filled pneumatophores

Table 3
Summary of ΔMVBS values of the representative samples.

	Mean ± standard deviation (dB)		
	Pacific Herring	Pacific Hake	Zooplankton
Number of trawls	5	2	10
Number of analysis cells	1074	270	2340
$\Delta MVBS_{70\text{-}38}$	−1.45 ± 1.84	−4.27 ± 2.12	3.61 ± 2.59
$\Delta MVBS_{120\text{-}38}$	−2.51 ± 2.21	−7.12 ± 2.22	9.93 ± 2.82
$\Delta MVBS_{200\text{-}38}$	−4.55 ± 2.04	−7.95 ± 2.09	12.21 ± 2.97
$\Delta MVBS_{120\text{-}70}$	−1.07 ± 1.09	−2.85 ± 1.83	6.32 ± 1.40
$\Delta MVBS_{200\text{-}70}$	−3.11 ± 1.19	−3.68 ± 2.01	8.60 ± 1.97
$\Delta MVBS_{200\text{-}120}$	−2.04 ± 1.02	−0.83 ± 1.44	2.28 ± 1.42

(e.g., *Nanomia bijuga*). Since intact siphonophores cannot be captured using MultiNet tows, Herrmann (2014) counted the number of nectophores (mouthless, pulsating swimming bell) of *N. bijuga* in the samples which resulted in densities of 0–0.3 m^{-3}.

3.2. Representative samples for classification

Acoustic data from 5 trawls were designated as representative samples of herring, with their catch composition ranging 91–99% by number and 63–96% by weight. For hake, acoustic data from 2 trawls were selected with their catch composition comprising 68–95% by number, and 61–80% by weight. The lower percentage of hake in catch composition by number was attributed to the presence of herring schools above the targeted layer, resulting in the contamination of trawl catches. Fork lengths of the fish caught in trawls selected as representative samples varied between 14.7 (mean) ± 4.0 cm (standard deviation) and 17.5 ± 1.1 cm for herring (n = 100–150 within trawls), and 19.0 ± 2.2 cm and 20.6 ± 2.9 cm for hake (n = 100–101).

Acoustic data from 10 net tows were selected as representative samples of zooplankton, with catch composition of euphausiids and copepods ranging 81–96% by wet weight. Lengths of zooplankton in the tows selected as representative samples ranged from 5.9 ± 1.9 mm to 10.5 ± 2.3 mm for euphausiids (n = 89–457 within tows), and 1.3 ± 0.4 mm to 1.6 ± 0.5 mm for copepods (n = 220–538).

3.3. Classification of the dominant taxa

ΔMVBS values of representative samples differed among taxa at some of the acoustic frequency pairs (Table 3). Backscatter layers dominated by zooplankton had consistently higher ΔMVBS values than those of herring and hake, allowing separation of zooplankton and fish. This separation was greatest at $\Delta MVBS_{200\text{-}38}$ (Fig. 2a), with $-16\,dB < \Delta MVBS_{200\text{-}38} \leq 2\,dB$ used to classify fish, $2\,dB < \Delta MVBS_{200\text{-}38} < 30\,dB$ used to classify zooplankton, and $\Delta MVBS_{200\text{-}38} \leq -16\,dB$ or $\Delta MVBS_{200\text{-}38} \geq 30\,dB$ used to classify other taxa (Fig. 1). Using these thresholds, zooplankton would be misclassified as fish in 0.3% of the samples, and there was no misclassification of fish as zooplankton. ΔMVBS values of herring and hake overlapped in most of the frequency pairs. Overlap of the two distributions was minimized at $\Delta MVBS_{120\text{-}38}$ (Fig. 2b), indicating the possibility to separate these taxa with minimal misclassification in the overlap zone. By taking the mid-point of the $\Delta MVBS_{120\text{-}38}$ mean values, acoustic backscatter with $\Delta MVBS_{120\text{-}38} < -4.8\,dB$ was classified as hake layers, and $\Delta MVBS_{120\text{-}38} \geq -4.8\,dB$ as herring layers (Fig. 1). This threshold would result in a misclassification of herring as hake in 12.5% of the samples, and hake as herring in 13.7% of the samples. Misclassification could also be due to the size of layers relative to the cell size. When layers extend over the cell and partially cover an adjacent cells, there is equal probability of the portion of a layer to be misclassified as either species. This alternate misclassification should average out. In addition, layer edges typically have weak backscatter values and are not expected to heavily contribute to the misclassification.

Acoustic classification using two frequencies has been widely used to distinguish zooplankton from fish. In Hood Canal, zooplankton exhibited a strong increase in backscatter strength with frequency, distinct from fish taxa, and consistent with previous studies (e.g., De Robertis et al., 2010; Madureira et al., 1993). Using $\Delta MVBS_{120\text{-}38}$ to separate hake from euphausiids was previously done by McKelvey and Wilson (2006) for the same species off the west coasts of U.S. and Canada. Their mean $\Delta MVBS_{120\text{-}38}$ for hake matched the value independently estimated in this study. The mean $\Delta MVBS_{120\text{-}38}$ value for herring was similar to the one reported by Edwards et al. (1984) for mixed herring and sprat aggregations, which resulted in the mean $\Delta MVBS_{120\text{-}38}$ of −2.05 dB based on TS values.

A separation of peaks in $\Delta MVBS_{120\text{-}38}$ was observed between herring and hake. Differences in their ΔMVBS values could be attributed to differences in animal length distributions, swimbladder structure and morphology, swimming angles, or combinations of these factors. Since length distributions of herring and hake overlapped greatly, it is unlikely that lengths contribute to the observed ΔMVBS differences of the two species. Swimbladders have been identified as the primary cause of acoustic backscatter in fish, accounting for as much as 90–95% of the reflected energy (Clay and Horne, 1994; Foote, 1980). Herring are physostomous (Blaxter et al., 1979) inflating swimbladders by 'gulping' air at the sea surface (Brawn, 1962), while hake are physoclistous regulating swimbladder volume through blood capillaries. Pressure effects on physoclists are less important than on physostomes, because swimbladder volumes can be adjusted as fish alter depth. Swimbladder morphology also differs between these two species. For individu-

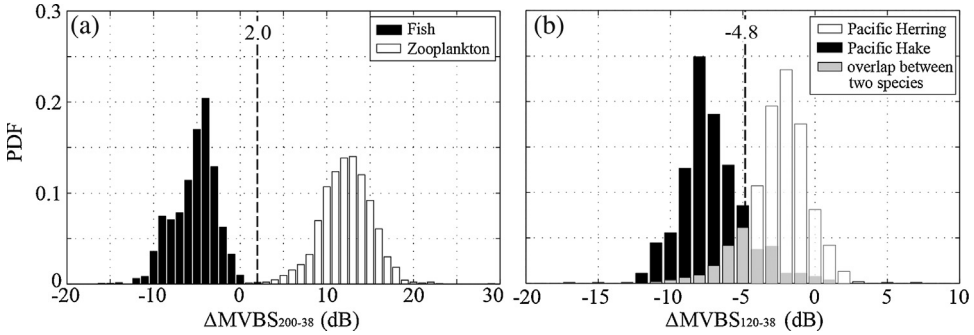

Fig. 2. Histograms of (a) $\Delta MVBS_{200\text{-}38}$ identified as fish (herring and hake combined) and zooplankton, and (b) $\Delta MVBS_{120\text{-}38}$ identified as herring and hake for 20-ping × 2-m analysis cells. Dotted lines indicate the values used to separate fish and zooplankton at $\Delta MVBS_{200\text{-}38}$ = 2.0 dB, and herring and hake at $\Delta MVBS_{120\text{-}38}$ = −4.8 dB.

als with similar lengths, swimbladder area and volume of herring are more than double that of hake (Gauthier and Horne, 2004; Henderson and Horne, 2007). Since acoustic backscatter intensity is a function of swimbladder cross-sectional area and shape at high frequencies (Ona, 1990), differences in morphological characteristics can result in a difference in ΔMVBS. Orientation of the fish also affects the amount of energy reflected by fish (Horne and Clay, 1998; Ona, 2001), causing subsequent changes in ΔMVBS values due to differences in backscatter between two frequencies (Kang et al., 2002).

3.4. Evaluation of the classification method

Classification of hake- and zooplankton-dominated acoustic backscatter data, confirmed by midwater trawl and MultiNet samples, was illustrated in Fig. 1. In the example, a thick layer near the bottom resulted in high backscatter at 38 kHz, while strong backscatter layers at mid-depths were observed at 120 and 200 kHz. Using $\Delta MVBS_{200\text{-}38}$, the backscatter layer located near the bottom was classified as fish, and the one in the middle of the water column zooplankton. No cells were classified as the other group in this example. Acoustic backscatter classified as fish was further divided into herring and hake using $\Delta MVBS_{120\text{-}38}$. Visual assessment of the classified echograms indicated that the identification of hake and zooplankton was consistent with expectations based on net samples. There was some misclassification of the near-bottom hake layer as herring. Weak backscatter layers above the hake layer were classified as herring, but we did not have trawl samples to verify them.

To quantitatively evaluate the classification method, validation samples were chosen (separately from representative samples) based on midwater trawl and MultiNet samples. Acoustic backscatter data from 6 trawls were selected as validation samples for herring, with catch composition ranging 95–100% by number and 65–94% by weight. For hake, acoustic data from 2 trawls were selected with catch composition of 77–87% by number and 46–85% by weight. Fork lengths of fish in validation trawls varied between 9.6 ± 0.9 cm and 17.8 ± 1.5 cm for herring ($n = 100$–101), and 16.5 ± 12.5 cm and 23.8 ± 8.9 cm for hake ($n = 115$–200). Acoustic backscatter data from 3 net samples were selected as validation samples of zooplankton, with catch composition of euphausiids and copepods ranging 84–100% by wet weight. Lengths of zooplankton in the tows selected as validation samples ranged from 8.2 ± 1.4 mm to 11.6 ± 2.9 mm for euphausiids ($n = 208$–315), and 1.4 ± 0.4 mm to 1.4 ± 0.5 mm for copepods ($n = 67$–123).

The ability of our method to accurately classify acoustic backscatter was evaluated by classifying validation samples. Within acoustic backscatter samples of herring confirmed by trawls, 99.9% of the NASC were classified as fish, and 0.1% as zooplankton. At the species level, 13.8% were misclassified as hake. Similarly, validation samples of hake showed that 99.7% of the NASC was classified as fish, with misclassification of 0.3% as zooplankton and 14.1% as herring. For zooplankton validation samples, 95.3% of the NASC was classified as zooplankton with 4.7% misclassified as fish. None of the acoustic backscatter was classified as other in any of the validation samples. These misclassification rates were similar to those estimated based on overlap in curves of the ΔMVBS histograms (Fig. 2). Backscatter contributions of unclassified categories (i.e., other aggregation and single target) varied from 5% to 48%, depending on the morphologies of fish backscatter contained in the validation samples. Due to their potentially high contributions to the total NASC values, classification of these categories to species level using nets will be necessary for accurately estimating herring and hake abundance.

3.5. Limitations of the classification method

The classification method proposed in this study makes several assumptions: (i) each analytic cell contains a single taxon, (ii) herring, hake, zooplankton, and other are the only categories of acoustic backscatter, and (iii) scattering properties and ΔMVBS of taxa do not change seasonally. These assumptions were also used in other ΔMVBS studies (e.g., De Robertis et al., 2010; Jech and Michaels, 2006; Kang et al., 2002).

Analytic cell size affects variability of ΔMVBS values and potentially violates the assumption that backscatter in each cell is dominated by a single taxon. The choice of cell size has varied among previous studies, depending on scales of aggregations, sampling rate of acoustics, and ship speed. The cell size used in this study was within the range used in previous studies [e.g., 7–24 m horizontal by 5 m vertical; De Robertis et al. (2010), 185 m horizontal by 5 m vertical; McKelvey and Wilson (2006)]. We also minimized the possibility of mixed species backscatter within a cell by removing aggregations and single targets before calculating ΔMVBS values. However, if a cell contained both zooplankton and fish within layers, then the cell was classified as fish, resulting in a lower abundance of zooplankton. Potential mixed-species cells are expected to occur at the edge of layers where zooplankton and fish can be co-located.

Identifying the appropriate frequency pairs for frequency differencing is challenging for successful classification of fish and zooplankton. Both the choice of frequency pairs and corresponding ΔMVBS values influence the effectiveness of the method. At our study site, jellyfish were a significant part of trawl catches during June and July, but were not included as classification categories. Acoustic backscatter features of jellyfish can be similar to those of fish (Purcell et al., 2000), and the reported $\Delta MVBS_{120\text{-}38}$ values of −3.3 dB to −2.2 dB (Brierley et al., 2001) are very similar to those used for herring in this study. Using the classification method proposed here, jellyfish would likely be misclassified as fish, specifically herring. Inclusion of additional information, such as location in the water column, may help improve classification, but there would still be vertical overlap with other categories due to diel vertical movement of some jellyfish (Moriarty et al., 2012).

Seasonal changes in fish length and body composition may affect backscatter properties of organisms, and resulting ΔMVBS values. In this study, herring and hake juveniles were caught in August through October, but potential changes in fish length due to growth were not reflected in the trawl catches due to selectivity of the trawl (McClatchie et al., 2000). TS generally increases with increasing length, but $\Delta MVBS_{120\text{-}38}$ of herring based on theoretical predictions varies between −3 dB and −9 dB without any correlation with fish length (Gauthier and Horne, 2004). Since the separation of peaks in ΔMVBS values is essential to classify taxa of interest, seasonal changes in ΔMVBS may potentially impact the efficacy of the classification. Body composition may change seasonally through growth and gonad production, which affects body density and consequently TS due to the changes in backscatter properties and/or swimbladder volume (Ona, 1990). These changes potentially affect ΔMVBS depending on relative changes in the backscatter of any frequency pair.

4. Conclusion

Species classification of acoustic data is an ongoing challenge in aquatic environments, including coastal ecosystems where several species may co-exist. Increased spatial overlap among taxa, which is a common feature in mid- and low-latitude ecosystems, makes classification more difficult. In this study, we demonstrated an empirical approach to classify acoustic backscatter of domi-

nant, coexisting taxa. Initial identification and separation of fish aggregations and single targets from the remaining backscatter was necessary to successfully separate fish from zooplankton. By filtering backscatter components and then frequency differencing in a defined sequence, the complexity of aquatic community compositions that can be categorized using multifrequency acoustic data increases. The procedure developed in this study focused on two particular fish species and dominant zooplankton taxa; adaptation to other ecosystems will likely require modification of the frequency pairs and ΔMVBS values used for separation.

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