The effects of scattering-layer composition, animal size, and numerical density on the frequency response of volume backscatter

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Land-associated, sound-scattering layers of mesopelagic micronekton surround the Hawaiian Islands. These animals undergo diel migrations during which they split into multiple, distinct layers that have differences in animal density, taxonomic composition, and size. A video-camera system capable of quantitatively estimating the biological constituency of the layers was combined with a four-frequency, vessel-mounted, echosounder system (38, 70, 120, and 200 kHz) to examine the effects of layer features on the frequency response of volume backscatter. Volume scattering was correlated with animal density at all frequencies, but the effects of animal length and layer composition were frequency-specific. Only scattering at 70 kHz matched the predictions of volume scattering based on the mean echo strengths and densities estimated from camera profiles, suggesting different scattering mechanisms at other frequencies. Differences in volume scattering between pairs of frequencies, however, did strongly correlate with animal length and layer composition and could be used as measures of the biological properties of layers. Applying this technique to the data shows strong partitioning of habitat by taxa and animal size in space and time, indicating the importance of competition in structuring the community.

Keywords: acoustics, fisheries, multifrequency, myctophids, scattering layer, volume backscatter.

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Introduction

The use of multifrequency, acoustic-volume backscatter (e.g. measurements at a number of discrete single frequencies) to estimate the biomass and size distribution of organisms ranging from zooplankton to swimbladder-bearing fish has been proposed for several decades (McNaught, 1968; Holliday, 1977; Johnson, 1977). Holliday (1977) set out equations to describe the scattering from mixed aggregations of scatterers. Simply put, volume scattering is related to the number of individuals with a given backscatter cross section multiplied by that cross section, plus the number of individuals of another size class or different species with a different cross section multiplied by that cross section, etc., for each frequency used. Different major classes of acoustic scatterers, e.g. small, roughly spherical zooplankton, larger zooplankton shaped more like a bent cylinder, fish with air-filled swimbladders, and fish without swimbladders, present different scattering cross sections and scattering spectra (see synthesis in Medwin, 2005). Therefore, volume scattering measured in the ocean depends on the type of scatterers present, their numerical density, and their size distribution. Other characteristics of the animals' behaviour can also come into play: the orientation of individual targets, for example, can affect an individual animal's acoustic cross section (MacLennan *et al.*, 1990), and hence the measured volume-scattering strength. The distribution, or spacing, of insonified individuals can also impact volume scattering (Jech and Horne, 2001).

Previous studies of multifrequency volume scattering from biology have taken on three forms. In the first approach, a forward method, biological samples are used to predict the volume scattering based on the densities of different scattering types, their known individual acoustic-backscatter strengths, and the size distribution of scatterers of each type (e.g. Love, 1975). These predictions of volume-scattering strength can then be compared with the measured volume scattering averaged over the same area sampled biologically. That approach requires a good understanding of the scattering processes involved for each individual scattering type, or experimental measurements to predict accurately the expected acoustic cross section for each size of each type of scatterer.

The second approach, an inverse method, also uses measured volume scattering and biological samples. The samples are used to calculate relative densities of scattering types, to analyse the size distribution of individual scatters, and to determine relevant acoustic-backscattering cross sections. Acoustic cross sections can be measured experimentally (e.g. Greenlaw, 1977; Stanton *et al.*, 1996, 1998b), but are often derived from models of the scattering type (Arnaya and Sano, 1990; Clay and Horne, 1994; Stanton *et al.*, 1998a). A statistical or another data-fitting approach is then used to find the best combination of the number of

scatterers of each type and size to produce the observed measurements of volume backscatter at each frequency (Holliday, 1977; Greenlaw and Johnson, 1983). The measurements of the biology, and often the acoustic-backscatter models, are used to constrain the solution to realistic parameters. To perform inversions from volume-backscatter data, the frequencies used must span the transition from Rayleigh to geometric (Holliday and Pieper, 1995), which is rarely practical for animals larger than zooplankton. Moreover, like the forward approach, this technique requires good estimates of the frequency spectrum of the acoustic cross section of targets.

The third approach involves the comparison of scattering at multiple frequencies, either pairwise (Madureira *et al.*, 1993; Korneliussen and Ona, 2002) or by a composite of all frequencies using a classification approach (Korneliussen and Ona, 2002; Jech and Michaels, 2006). Differences in frequency spectra are used to create categories of scattering types or to separate a target of interest from other scatterers. For example, Korneliussen and Ona (2003) separated scatterers into five categories, two fish and three zooplankton groups, which were then compared with net tows to confirm their identities with ~95% accuracy. The categorization presented is based on extreme differences in size and identity, with little resolution for observing more subtle shifts in composition at the spatial resolution they sampled. The advantage of this approach is that, at least for categorization if not for biomass estimation, it requires little *a priori* knowledge of the target's backscatter characteristics.

A common feature of all of these approaches to interpreting multifrequency volume backscatter is the collection of biological data. Most studies use net samples, or occasionally pumping, to capture potential targets. Horizontal net tows can sometimes be stratified into a few depth categories, but are typically collected over periods of the order of 30 min or longer, which can equate to distances of 3–6 km at typical trawl speeds. Vertical tows limit horizontal spatial averaging and potential time integration, but still provide limited depth characterization. The biological samples then have integration volumes both in depth and horizontal space that are much larger than is possible for analysis of volume backscatter. The resolution of the ground-truthing necessary for interpretation of the backscatter is often not capable of observing small-scale features or those that happen quickly, e.g. rapid changes as a result of diel migration.

The mesopelagic sound-scattering layers surrounding the Hawaiian Islands make up a land-associated community of micronekton (2–10 cm long animals) that undergoes diel migrations with both a vertical component of 500–600 m and a horizontal component of 5.5 km both inshore and then again offshore (Benoit-Bird *et al.*, 2001; Benoit-Bird and Au, 2006). During these migrations, the scattering community splits into multiple layers with differences in micronekton density, composition, and size. The biological characteristics of these layers change on timescales of <1 h and horizontal spatial scales of a few hundred metres (Benoit-Bird and Au, 2004, 2006). This presents a natural experiment for examining the effects of layer composition in terms of taxonomy, size, and animal numerical density on volume backscatter. However, the dynamics of these layers have also presented a problem for collecting the biological samples necessary for understanding the volume-scattering measurements.

In this study, a video-camera system developed by Benoit-Bird and Au (2006) was used to examine quantitatively the numerical density, size, and taxonomic composition of micronekton in the scattering layers associated with the Hawaiian Islands. The camera system was combined with a four-frequency, vessel-mounted echosounder system (38, 70, 120, and 200 kHz) to measure the volume backscatter of micronekton layers, with changes in the distribution of layers occurring as a result of temporal and spatial dynamics. The camera can be used to obtain biological data over periods of tens of minutes and, depending on target density, resolution as a function of depth of up to 1 m. The variety of conditions set up throughout the mesopelagic community's dynamic migration period made it possible to investigate the effects of changes in taxonomic composition, animal size, and numerical density on the frequency response of volume backscatter, as well as the interaction of these factors. The changes in these biological characteristics are relatively subtle compared with those from many other studies. For example, all animals in the layers are micronektonic without air-filled enclosures, rather than small plankton vs. swimbladdered fish. This presents a greater challenge for separating target types, but also presents an opportunity to understand the volume-backscatter process in greater detail.

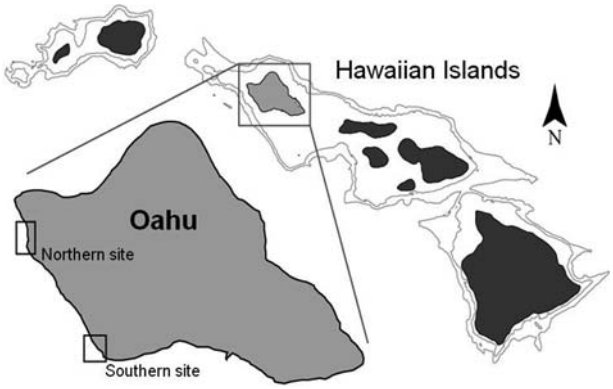

Figure 1. Map of Oahu showing the Hawaiian Islands (see text for coordinates). The northern study area sampled in February 2005 and April/May 2006, and the southern study area sampled in February and April/May 2005 are highlighted. The 1000- and 2000-m isobaths around the islands are shown.

The goal of the work was to observe the relationship between the frequency response of volume scattering and the biological features of scattering. The volume scattering was analysed as a function of the biological features of the layers in an approach similar to that of Korneliussen and Ona (2002, 2003). However, the substantially greater sample size and the graded nature of the biological variability permitted observation of the effects of continuous variation of single and combined features, rather than broad, categorical comparisons.

Methods

Study sites

The study area extended west of the leeward coast of Oahu, Hawaii (Figure 1). The study covered three periods, two during 2005 (17–26 February and 20 April–27 May) and one during 2006 (9 April–16 May). The April/May 2005 study site was located off the southern leeward coast of Oahu in the area 21°19.3′N 158°8.3′W, and the 2006 study site off the northern leeward coast of Oahu in the area 21°30.5′N 158°14.2′W. In February 2005, sampling was conducted over seven nights, with three of them covering the northern site and the other four the southern

site. The order of sampling between sites was random. During each of the April/May 2005 and 2006 sampling periods, a series of shipboard surveys three or four days long was conducted, with sampling between 20:00 and 08:00 local time. The shipboard surveys were designed to coincide with spring (full and new moons) and neap tides (first- and third-quarter moons), spanning both ebb and flood of each tidal cycle. Surveys were conducted only when weather permitted small-boat operations, which were limited to swells of ~2–3 feet.

Survey methods

All surveys were conducted from the 9 m FV "Alyce C" at a speed of 9.26 km h^{-1} (5 knots). Shipboard echosounders were used to measure the volume backscatter of the micronekton layers. A four-frequency, split-beam scientific echosounder system at 38, 70, 120, and 200 kHz (Simrad EK60) was mounted 1 m below the surface on a rigid mount attached to the vessel's gunwale, so that the centres of each transducer were no more than 35 cm apart, to maximize spatial comparability of the data (Korneliussen *et al.*, 2004). The 38 kHz echosounder used a 256 μs pulse, the 70 kHz echosounder a 128 μs pulse, and the 120 and 200 kHz echosounders a 64 μs pulse. The pulse lengths were chosen to allow the greatest vertical resolution possible for each instrument to meet other constraints of the experiment. However, for the purposes of this analysis, all data were binned into 1-m vertical-resolution samples to minimize the problem of different sampling volumes. The effects of increasing the signal-to-noise ratio caused by increasing the pulse lengths at lower frequencies are therefore counteracted by decreasing the signal variance with increased sample size in each vertical bin (~3 samples in 1 m at 38 kHz, ~5 at 70 kHz, and ~10 at 120 and 200 kHz). The 38 kHz system had a 12° and each of the higher frequencies a 7° conical split beam. Because of the nearly continuous nature of the scattering layers studied, differences in beam angle are unlikely to be important in comparing the mean volume backscatter per unit volume of water (Diner, 2001; Jech and Michaels, 2006), when averages are made across many measurements, as here where the period was 10 min or ~1200 echoes. Tests for systematic avoidance were carried out to verify this assumption.

All four echosounders were calibrated using an indirect procedure, incorporating a 38.1-mm diameter, tungsten-carbide reference sphere as prescribed by Foote *et al.* (1987), using the same arrangements as for this experiment. For calibration, the reference sphere was held between 10 and 12 m away from the transducers to ensure measurement in the far field. Calibration was carried out for all pulse lengths between 64 and 256 μs for each frequency, and from these pulses, it was possible to gain an understanding of the effect of pulse length on the measured responses. The effects of pulse length on s_a gain and target strength (TS) were less than 5% across the full range of pulse lengths for each frequency, so within the range typically considered acceptable for calibration error. This supports the use of comparisons within a frequency of measurements taken at different pulse lengths. Similarly, calibrations were carried out at a variety of transmit powers to minimize non-linear interactions at each frequency for the experiment (Korneliussen *et al.*, 2004).

A high-resolution, vertical-profiling package (Figure 2) was used to characterize micronekton at stations along the surveyed transects. The profiler was equipped with a SBE-25 CTD (temperature, salinity, pressure), a suite of instruments for measuring other physical and optical properties of the water column, an optical plankton counter (OPC), and a Tracor acoustic-profiling system (TAPS) for assessing zooplankton, as well as a low-light camera system for micronekton. The camera system uses only infrared lighting and can be used to identify micronekton and to measure animal size, as well as to estimate quantitatively the numerical density of animals, while causing no significant avoidance (Benoit-Bird and Au, 2006). Two low-light cameras were used in a stereo fashion to allow animals identified in both cameras to be localized precisely within the 575 l of the cameras' overlapping fields of view, permitting quantitative density estimates of mesopelagic animals to be made and measurements of their size to be taken. Measurements of size take into account the apparent length of the animal in both cameras, which allows their actual length to be determined using basic trigonometric relationships, so eliminating the problem of apparent foreshortening from one camera. At all stations, the profiling package was lowered from the surface to within 5 m of the seabed or 150 m, whichever was less, at a rate of 10–15 cm s^{-1}. The profiler was then raised to 3–5 m above the minimum depth of the shallowest observed scattering layer as fast as feasible, then lowered at 10–15 cm s^{-1} to 2–3 m below the deepest observed layer (Figure 3). This was repeated to provide three replicate casts in the video data.

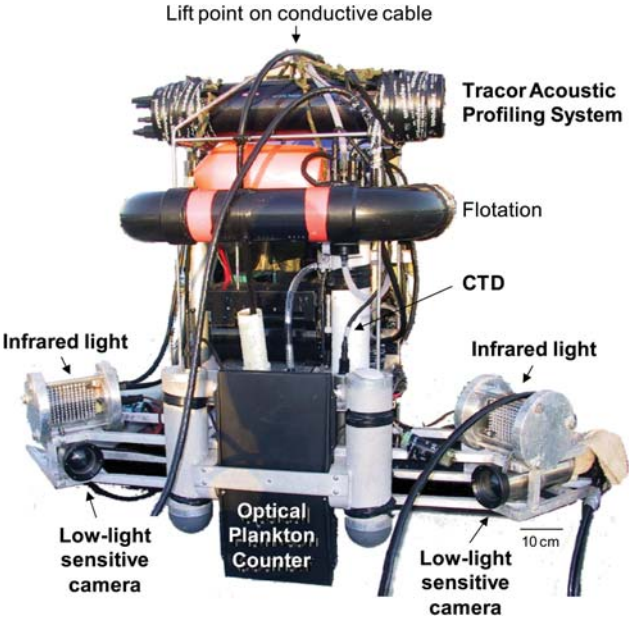

Figure 2. Photograph of the high-resolution, vertical-profiling package used here to characterize the physics, zooplankton, and micronekton. The system is designed to be nearly neutrally buoyant, to allow slow descent and high-resolution data collection. For these reasons, the flotation is indicated.

The surveys were conducted as parts of other studies and were constrained by the goals of the other programmes. Consequently, spatial analysis of the data was limited.

February 2005

During February 2005, 5-km transects running parallel to the coastline were located 1.5, 3.0, 5.0, and 7.0 km from the shore at both northern and southern sites. The order of transect surveying was random in a complete block design. Along each transect, two vertical casts with the high-resolution profiler were conducted.

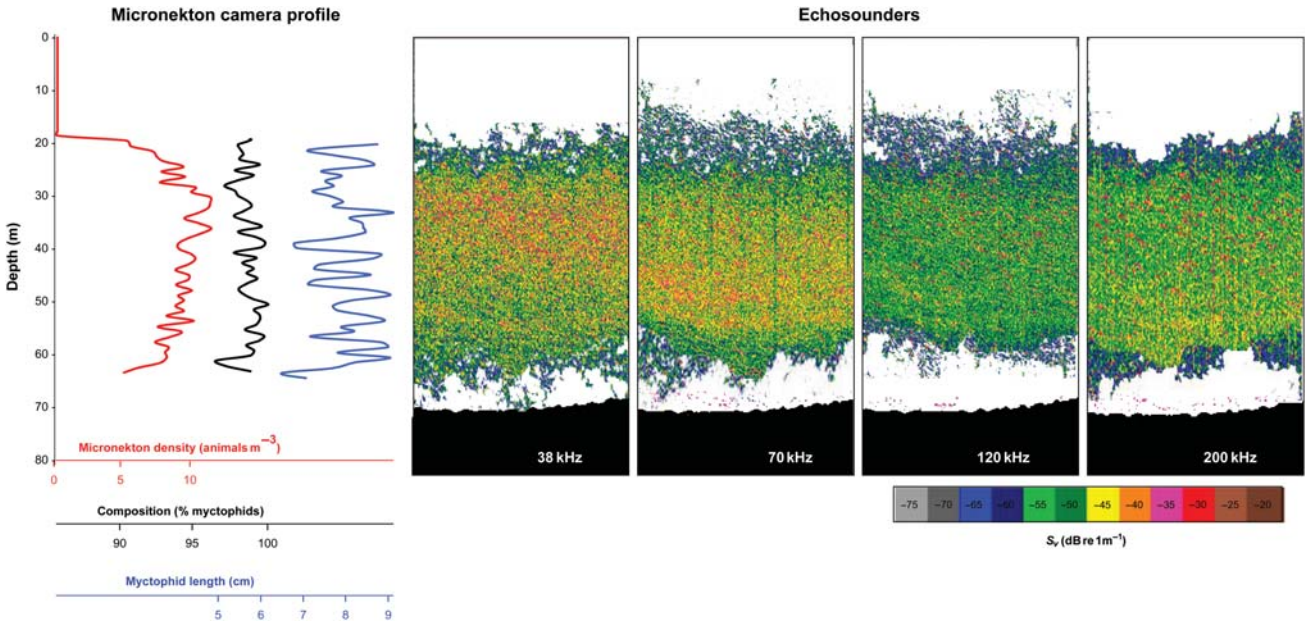

Figure 3. An example of data from the four echosounders and micronekton camera taken at ~21:40 local time, 2 km from the shore, in May 2006. The vessel was stationary during these echograms and for all data directly compared with the camera profiles.

The location of the first profile was selected randomly and the second was conducted 2.5 km from the first.

April/May 2005

During the April/May 2005 study period, four shipboard surveys were conducted along the southwest coast of Oahu. Transects 4 km long were located parallel to the coast, 1.0, 2.0, and 3.0 km offshore. Sampling in each three-night survey was conducted continuously from 20:00 to 03:00 local time. Vertical casts with the profiling package were carried out near the ends of each alongshore transect. The starting position along the survey grid, and the order of transects were randomized each night.

April/May 2006

During the 2006 study period, three shipboard surveys were conducted along the northwest coast of Oahu. During each three-day sampling effort in 2006, three transects 2 km long running onshore and offshore from ~1 km from the shore to 3 km from the shore were sampled constantly from ~20:00 to 03:00 local time. Casts with the profiler were conducted along the centre transect at the 10, 20, 25, and 40 m contours each time they were passed. Random selection was made as to the location of the first cast each night, as was whether the first transect was run inshore or offshore and whether the right- or left-side flanking transect was sampled first.

Data analysis

Echosounder data from the 5 min before and the 5 min after each camera profile when the vessel was stationary were averaged to provide volume-scattering estimates in 1-m vertical depth bins to a water depth of 150 m, the limit of the vertical casts with the profiling package, using the SonarData's Echoview software. A volume-scattering threshold of −75 dB was used for all measurements. This value was above the passively measured noise level at all frequencies by at least an order of magnitude down to 150 m, during both stationary sampling, and when the vessel was moving at typical survey speed in the maximum seas encountered, so eliminating the problem of noise removal (Korneliussen, 2000). The CTD casts showed a well-mixed water column, so a single value for sound speed was applied to each profile, based on the comparable CTD profile. The volume-scattering estimates were compared using one-tailed *t*-tests to determine any possible avoidance effects. The volume scattering for the periods just before and after profiles was then compared with the average volume scattering for the transect segment from that profile halfway to the next, using ANOVA to determine whether the casts were representative of transects, a second estimate of avoidance of the profiling package or the stationary vessel. Finally, the acoustic data were analysed visually for indications of avoidance. Volume-backscatter measures were also compared with estimates of zooplankton abundance from the OPC to eliminate variation in zooplankton abundance or biomass as a cause of variation in volume backscatter.

In situ measurements of TS values of individually insonified micronekton were made using the split-beam echosounder whenever target density and separation permitted. The number of targets per acoustic-reverberation volume for each frequency was determined for each pulse, and any values that exceeded 1 were not included in the analysis (Sawada *et al.*, 1993). This means that at a depth of 10 m, numerical densities could not exceed 2 animals m^{-3}. At 100 m, the maximum densities could not exceed 0.1 animals m^{-3}. These density values were within the ranges observed in the layers, although often far from the mean observed densities, meaning that the sample sizes were low. The frequency response of these individually identified animals was calculated for all targets identified simultaneously at all four frequencies.

Video data for the identification of shrimp and fish were analysed following the methods in Benoit-Bird and Au (2006). In summary, still views were extracted every 0.25 m from each of the two cameras to limit possible double counting of animals. Animals seen in still views from both cameras were identified to

the lowest taxonomic level possible. For the analyses presented here, all myctophid fish were grouped, because previous measurements of the TS of a variety of dominant myctophid species from the layers showed no species-specific effects (Benoit-Bird and Au, 2001). The coordinates of animals individually identified and their apparent length were used to determine animal length. As stated above, measurements of animal size take into account the apparent length of the animal in both cameras, allowing their real length to be determined using basic trigonometric relationships. Analysis of video for squid abundance could not be made from the extracted images owing to the rapid swimming speeds of these animals, causing blurring in still images. Around each extracted still frame, 15 s of moving video were observed. Squid could easily be seen in these moving images, and those identified simultaneously in both cameras were used to estimate squid density. Data were averaged in 1-m depth bins from each of the three replicate casts to provide adequate sample sizes, given the volume of water sampled.

Volume scattering averaged (in linear units) over the entire vertical range of the identified scattering layer was compared at each frequency and in pairwise frequency combinations (e.g. frequency differencing) to the individual biological measurements of layer characteristics averaged over the same depth range from the camera system, using regression techniques. In each case, the regression model with the best value of corrected fit was utilized. Statistical characteristics of the variability in the shape of the frequency spectra, including the variance about the mean (σ^2) and the skewness (μ_3), were also calculated, and their relationship with the biological characteristics of layers was analysed with regression techniques.

All analyses assume that targets are horizontally distributed similarly throughout the entire volume sampled in a given depth bin (1 m). In other words, horizontal patchiness is at scales greater than those averaged over. At the deepest depths in this study, this equates to ~25 m horizontally over a 10-min periods. The volume sampled increases with depth in the acoustic measurements, so comparisons between depths needed to be made on an average-per-unit-volume basis, without taking into account the differences in sample size. Moreover, comparisons of the echosounder data with the camera system, OPC, and TAPS, all of which have a fixed sampling volume, necessarily assume that the small volume sampled is representative of the increasingly larger volume sampled by the echosounders with increasing depth.

Results

In all, 311 profiles, each of 10 min of acoustic-backscatter data at four frequencies and three replicate casts through volume-scattering layers, were made between February 2005 and May 2007 at the two sites off the leeward coast of Oahu, Hawaii. The mean density of micronekton on each cast, measured with the camera system, ranged from 1 to 20 animals m^{-3}. Camera data showed that layers were comprised primarily, >88% by number, myctophid fish ranging in length from ~1 to 9 cm. Density was weakly correlated with relative composition ($r = 0.19$, $p < 0.05$) and myctophid length ($r = -0.23$, $p < 0.05$). Relative composition was also weakly correlated with myctophid length ($r = 0.20$, $p < 0.05$). Despite these correlations, all combinations of density, relative composition, and myctophid length can be found in the dataset because of variation in the layers as a function of time, distance offshore, and site.

A one-tailed *t*-test was used to compare the volume-scattering measurements taken in the 5 min before and after a profile with the camera system. Post-profile volume scattering was not significantly lower than pre-profile volume scattering ($p > 0.05$ for all comparisons), suggesting no significant avoidance of the vessel or the profiler by micronekton. The volume scattering for the periods surrounding the profiles was then compared with the average volume scattering for the transect to the halfway point to the next profile, using ANOVA. No significant differences in volume scattering were detected between the profiles, and the surrounding transect ($p > 0.05$ for all comparisons), suggesting that stationary data acquisition does not affect micronekton behaviour significantly differently than underway sampling. The profiler was often observed in the acoustic data, permitting careful visual analysis of the scattering patterns surrounding the instrument package. No short-term behavioural patterns were observed in response to the profiler's movement, e.g. scatterers leaving the area and returning after the package passed.

The volume-backscatter measures from each cast were compared with the OPC measures of zooplankton abundance (counts per volume) and density (body volume per sampling volume). There was no significant correlation between zooplankton measures and volume scattering of layers identified as micronekton ($p < 0.05$ for each comparison). The levels of backscatter measured from zooplankton using TAPS were so low as to be completely swamped by the presence of even a single micronektonic scatterer.

The mean and standard deviation of volume backscatter over the entire depth range of the detected scattering layer from periods surrounding camera profiles are shown as a function of frequency in the left panel of Figure 4. The frequency response of the TS of individuals measured *in situ* is shown in the top right panel of Figure 4. These data showed a nearly linear frequency response of 1.52 log λ. The relationship between individual measures of layer characteristics made from the camera system and volume backscatter is shown in Figures 5–7. For those figures, the fraction of the variation in volume backscatter explained by the independent variable (R^2) is shown for each frequency, with significant relationships indicated by emboldening and an asterisk. Volume backscatter as a function of animal density (ind. m^{-3}) measured with the camera system is shown in Figure 5. Animal density and backscatter had a significant positive linear relationship at all four frequencies measured. Addition of a logarithmic calculation of animal density, as would be appropriate to compare density with the log form of volume backscattering, significantly reduced the "goodness" of the curve fit to the data, so is not shown. The slopes of the linear regressions were 20.6, 9.9, 14.8, and 17.4 at 38, 70, 120, and 200 kHz, respectively, and the intercepts for each line with a density of one individual were −50.7, −45.6, −48.9, and −60.6 at the same respective frequencies. These slopes and intercepts were not significantly different when the regression was broken into three parts: myctophids <3 cm, myctophids 2–5 cm long, and myctophids >5 cm. This analysis separates the possibly differing effects of density and animal size on volume scattering. The results show that the form of the relationships observed in Figure 5 is not affected by the mean size of fish within the scattering layer.

The relationship between layer composition identified from the video data and volume scattering is shown in Figure 6. Linear regressions again showed the best fit to the data. At 38 and 70 kHz, volume scattering significantly increased with increasing

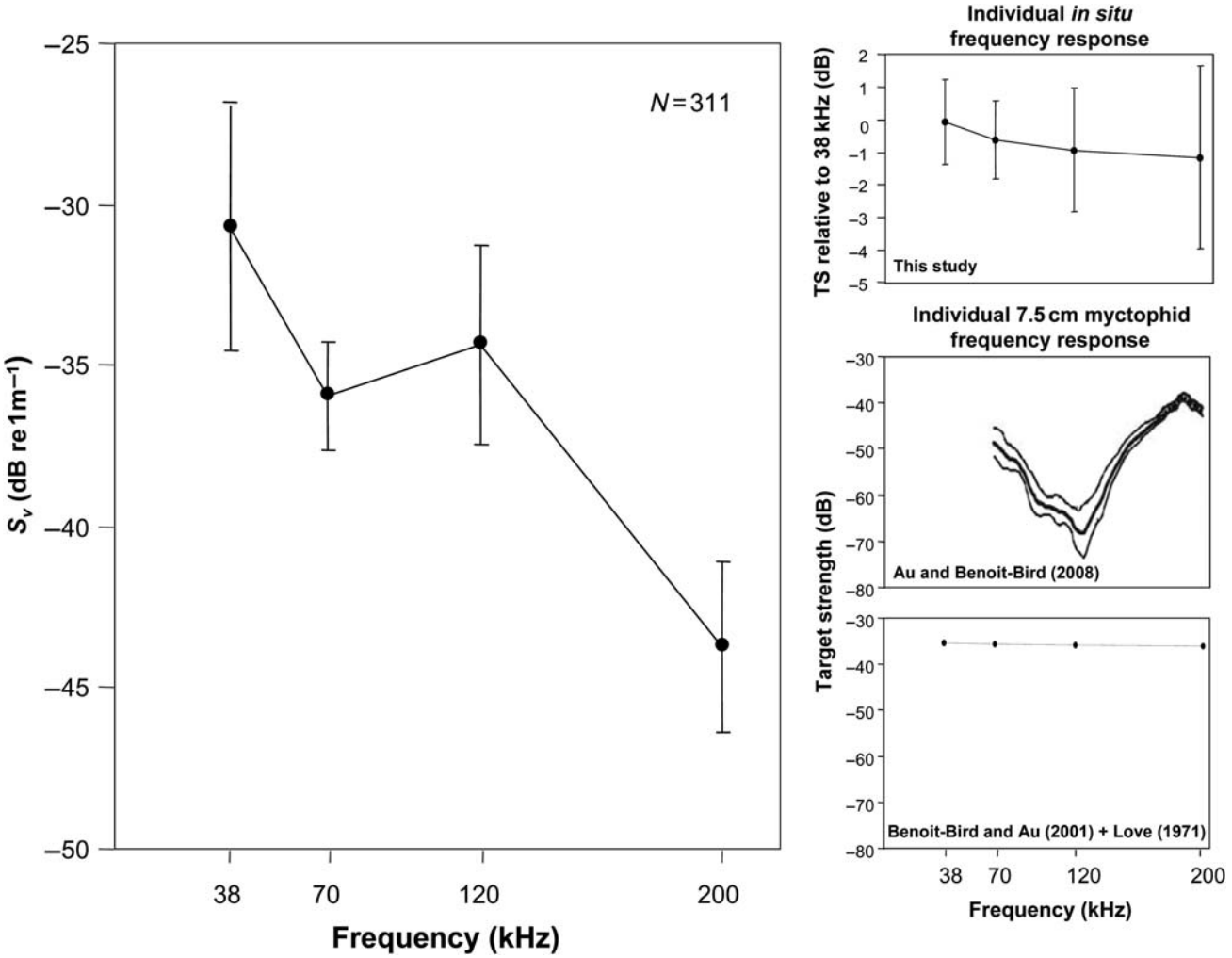

Figure 4. The left panel shows the frequency response of the mean volume backscatter from all profiles. Averages were calculated from the 10 min immediately surrounding each cast. Error bars show 1 s.d. The right panels show the frequency response of the target strength (TS) of individual targets. The top panel shows the TS relative to that measured at 38 kHz from individual targets measured *in situ* in this study at all four frequencies simultaneously. Error bars indicate 1 s.d. from the mean. The right middle and bottom panels show the TS of an individual myctophid ~7.5 cm long. The middle panel is from Au and Benoit-Bird (2008), who measured the response of individual myctophids with a broadband signal, and the bottom panel is the TS of a myctophid of 5 cm predicted by the 200 kHz measurements of Benoit-Bird and Au (2001). The values of TS at the other frequencies are predicted from the 200 kHz TS, using the offset established by Love (1971). Note that TS is in different units from volume backscatter, making comparison of absolute values inappropriate. However, comparison of the shape of the frequency response is possible.

relative abundance of the strongest scatters, myctophids, found in these layers (Benoit-Bird and Au, 2001). At 120 and 200 kHz, there is no significant relationship between the composition of layers and the volume backscatter.

Volume backscatter was significantly positively correlated with increasing length of the dominant scatterers in the layers only at 70 kHz (Figure 7). The best-fit regression models were linear rather than logarithmic. There was a significant negative trend in the relationship between myctophid length and volume scattering at 120 kHz, and no significant relationship between myctophid length and volume scattering at 38 or 200 kHz.

A summary of the correlations between individual parameters of scattering layers measured with the camera system and volume backscatter at each of four frequencies is provided in Table 1. Values indicate the *p*-value of the significance test for each relationship, with statistically significant values emboldened. Looking carefully at the patterns, it is evident that volume backscatter at all frequencies is correlated with the density of animals, so any differences in backscatter frequency would not be helpful in separating the effect of density. However, 200 kHz is only correlated with density, whereas 120 kHz is also affected by animal length. The difference in volume backscatter between these two frequencies is then related to differences in animal length (Figure 8). The fit of the linear regression relating myctophid length to the difference in volume backscatter between 120 and 200 kHz is extremely strong ($p < 0.0001$). Further, the difference in volume backscatter at 38 and 200 kHz should only be affected by layer composition. This positive relationship also has a good fit (Figure 9).

Another way to look at the effects of the layer variables measured with the camera system and the volume backscatter is to characterize the shape of the frequency response curve for all

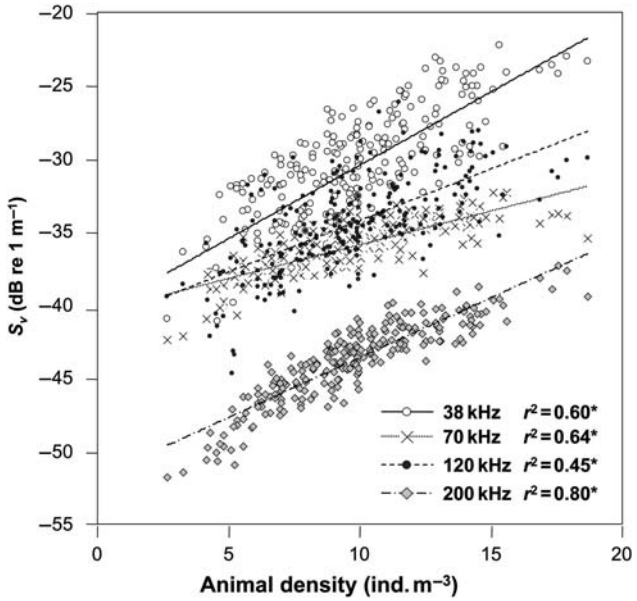

Figure 5. Volume backscatter measured by the echosounders as a function of animal density calculated from the camera system at each of the four frequencies measured. Relationships found to be significant are indicated by emboldened r^2 values and an asterisk.

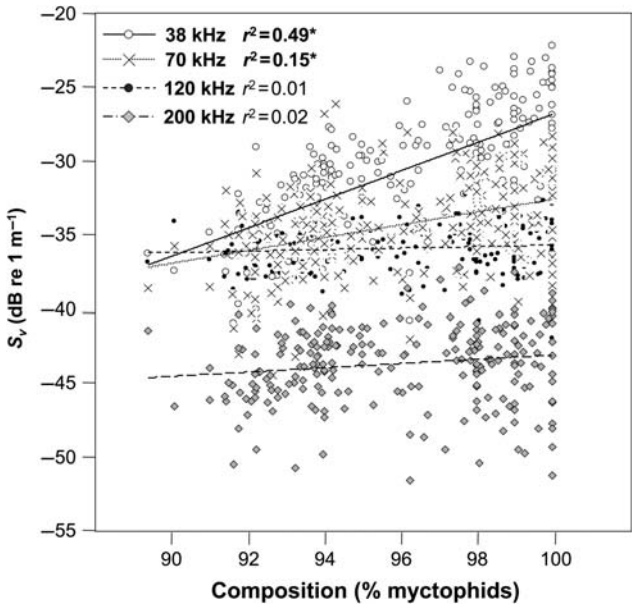

Figure 6. Volume backscatter measured by the echosounders as a function of animal composition calculated from the camera system at each of the four frequencies measured. Because the animals present were overwhelmingly myctophid fish, composition is represented as the percentage of animals identified as myctophids. Relationships found to be significant are indicated by emboldened r^2 values and an asterisk.

four frequencies. There was a significant increase in the variance of the frequency-response curve as a function of layer composition and myctophid length, but no significant change in variance as a function of animal numerical density (Figure 10). There was a significant effect of all three variables measured with the camera

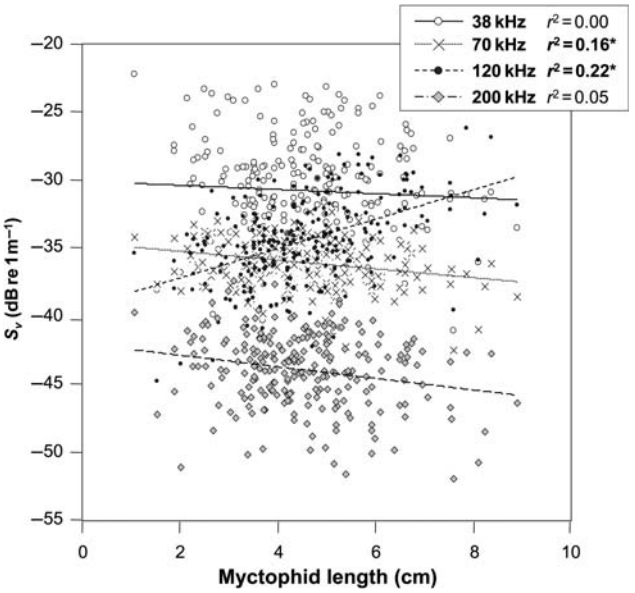

Figure 7. Volume backscatter measured by the echosounders as a function of myctophid length calculated from the camera system at each of the four frequencies measured. Because myctophid fish accounted for >88% of all the animals present, no other animal groups had a large enough sample size for similar analysis. Relationships found to be significant are indicated by emboldened r^2 values and an asterisk.

Table 1. Summary of significance values for correlations between each individual variable measured with the camera system and volume backscatter at each frequency.

Parameter	38 kHz	70 kHz	120 kHz	200 kHz
Numerical density	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Composition	**<0.0001**	**<0.005**	0.48	0.39
Animal length	0.97	**<0.001**	**<0.0005**	0.98

Statistically significant values at a value of $\alpha = 0.05$ are emboldened.

system and the skew of the frequency-response curve in volume backscatter (Figure 11). Significant relationships are again shown emboldened with asterisks.

Discussion

In this study, a large number of profiles (311) taken to measure the biological features of layers was compared with concurrent, relatively short-duration acoustic samples (~10 min; Figure 3). The sampling design did not cause significant changes in the behaviour of layers or avoidance of the profiler. Based on the results from TAPS and the OPC, layers identified as micronekton did not have significant contributions to volume scattering from zooplankton. Sampling permitted a range and a variety of combinations of biological parameters of micronekton layers to be observed and compared with acoustic measures. Ecosystems with multiple species and a large scattering layer of mixed composition present a challenge to the acoustic determination of taxonomic structure. Figures 5–7 show the continuous nature of all biological parameters, rather than the presence of distinct scattering groups. It is also clear that the extent of variation is relatively limited, with layers consisting of at least 88% by number of

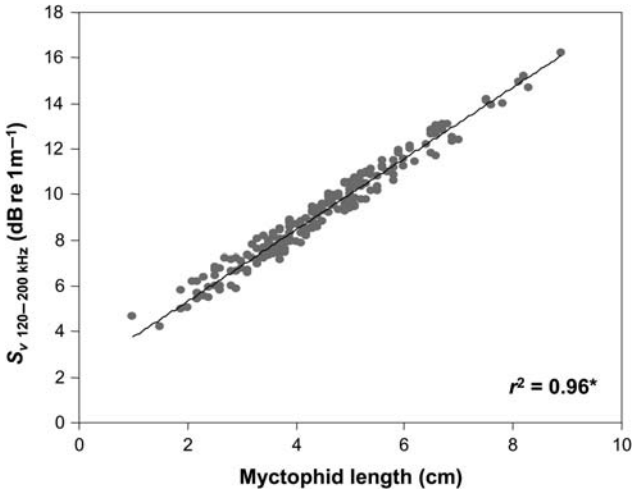

Figure 8. The difference in 120 and 200 kHz volume backscatter as a function of myctophid length calculated from the camera system.

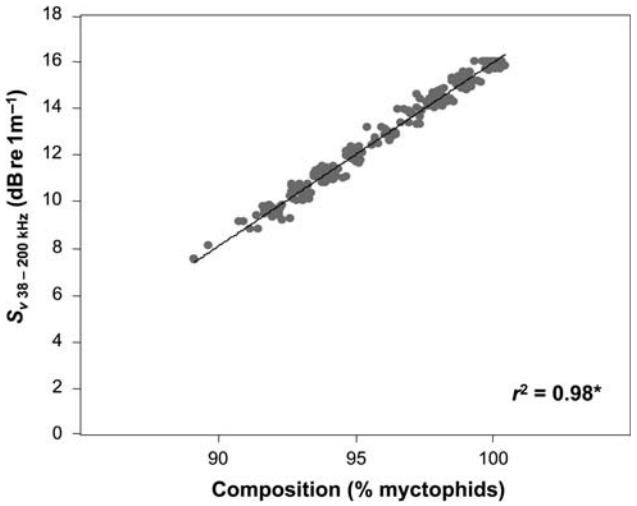

Figure 9. The difference in 38 and 200 kHz volume backscatter as a function of layer taxonomic composition calculated from the camera system.

myctophids of a relatively narrow size range, at mean densities of 1–20 fish m^{-3}.

The mean spectral response of volume scattering by the nearshore scattering layers around Oahu, Hawaii, showed the highest scattering levels at the lowest frequency measured, 38 kHz, with an apparent local minimum at 70 kHz, an increase at 120 kHz, and a substantial (e.g. 10 dB) decrease at 200 kHz (Figure 4, left panel). However, *in situ* measurements of the frequency response of individual values of TS showed a simple linear decrease in TS over the frequency ranged measured, with a relationship of 1.52 log λ (Figure 4, top right panel). Other work has shown the TS of individual fish over this size range to have a slight decrease of 0.9 log λ (Love, 1971) or increasing, ~6 dB over roughly the same frequency range covered here, with increasing frequency (Gorska *et al.*, 2007), unless there is resonance. Earlier studies showed volume-scattering peaks at 38 kHz (and lower frequencies) in fish and fish larvae with air-filled swimbladders (e.g. Korneliussen and Ona, 2003). The myctophids in the layers observed here do not appear to have air-filled swimbladders (Benoit-Bird and Au, 2001), so resonance is not a likely mechanism for the relatively high volume scattering at 38 kHz. However, the pattern observed is quite similar to that observed in cod (*Gadus morhua*), a large species with an air-filled swimbladder, which showed a relatively linear decrease in TS of ~7 dB, and a decrease in volume scattering of ~8 dB over the frequency range used here, even without clear resonance (Pederson *et al.*, 2004). Broadband measurements of individual animals from these scattering layers show that dorsal aspect values of TS can vary by as much as 35 dB between 50 and 200 kHz (Figure 4, middle right panel; Au and Benoit-Bird, 2008). For example, myctophids ~7.5 cm long had high TS at 50–70 kHz, decreasing to a sharp null between 100 and 125 kHz, then ascending to 200 kHz, where another decrease began. The position of this null was negatively correlated with length within a single species of myctophid, but this relationship was not maintained between species. A similar frequency response was observed in the squid of ~4–8 cm long measured. The pattern in shrimp of length 4.5–8.3 cm was not as clear. Their TS had multiple nulls between 100 and 170 kHz, with high values below and above these frequencies, and the hint of another drop in TS >200 kHz. Variance in the position of nulls and peaks observed in these measurements could explain the pattern of decreasing volume scattering from 38 to 120 kHz. However, the continued decrease in the volume backscatter at 200 kHz does not fit the individual tank measurements of TS of any of the micronekton taxonomic groups.

Another explanation for the observed frequency response is the presence of other scatterers. Likely candidates would be siphonophores and salps, gelatinous animals that often contain a gas inclusion and that can be important sources of lower frequency scattering, especially near resonance even at low densities (Lavery *et al.*, 2007). Salps and siphonophores have previously been captured during net tows targeting micronekton in scattering layers, primarily with a net not capable of opening or closing (Benoit-Bird and Au, 2001). These fragile animals are difficult to sample quantitatively with nets, but were quite visible in the micronekton video system because the species found in Hawaiian waters are typically in the size range 2–15 cm. They were sometimes common in vertical profiles (e.g. 2 m^{-3}), although they were detected only twice at depths >5 m. Micronekton were found at such shallow depths only in the samples nearest to shore around midnight. This extremely limited overlap with the observed distribution of micronekton suggests that these animals can be ruled out as causes for the relatively high scattering at 38 kHz observed over all samples.

Classification methods to observe the relationship between biology and volume scattering utilized in other studies (Korneliussen and Ona, 2002; Jech and Michaels, 2006) were not appropriate for the graded variation observed and the limited scattering types present here. Instead, a correlative approach was used to investigate the differences in volume scattering as a function of biological differences. Volume-scattering strength was strongly correlated with the density of micronekton at all frequencies measured, with higher densities correlated with higher scattering, as expected. However, density explained as little as 45% or as much as 80% of the variation in volume scattering, depending on frequency (Figure 5).

The intercepts of the regressions of volume scattering as a function of density with a value of one animal m^{-3} can be interpreted

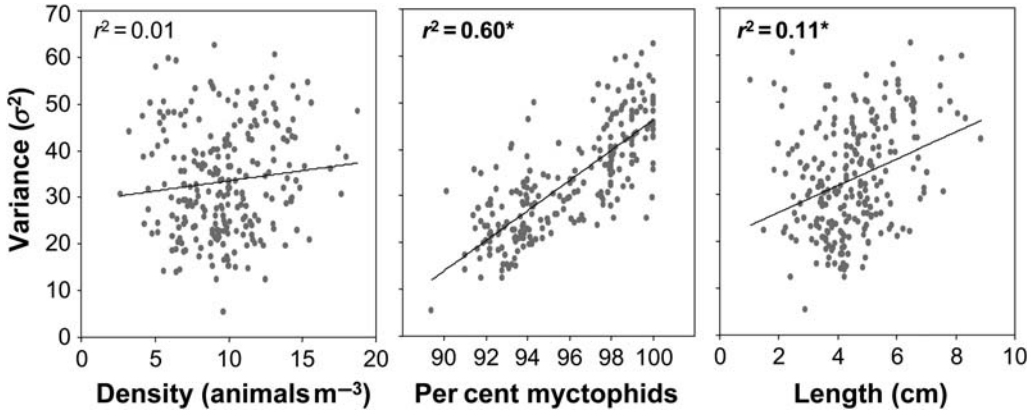

Figure 10. Variance in volume-backscatter strength between frequencies as a function of characteristics of the layers measured with the camera system.

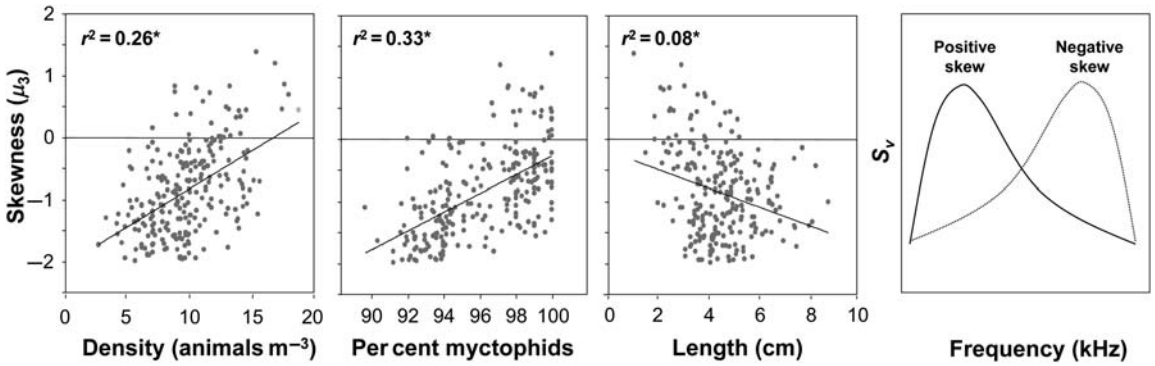

Figure 11. Skew in the frequency distribution of volume-backscatter strength as a function of the characteristics of the layers measured with the camera system (left three panels). The right panel shows an idealized example of a positively and a negatively skewed distribution.

as the mean individual TS of all samples at each frequency. Based on measurements of the TS of the myctophid species found in these layers (Benoit-Bird and Au, 2001) with frequency adjustments based on Love (1971), these values of TS would equate to myctophids with lengths of 2.35, 4.35, 3.05, and 0.8 cm at 38, 70, 120, and 200 kHz, respectively. The mean length of myctophids measured with the camera system over the entire dataset was ~4.5 cm. Only the 70 kHz estimate approaches the measured length value. Given the extremely close placement of the transducers, all four frequencies are measuring a nearly identical volume of water that, when integrated, should provide similar average values. A relatively flat frequency response in the values of *in situ* TS was measured (Figure 4, top right panel). It is clear that the volume-scattering response is either based on more than just the mean TS and the number of individuals in those layers or that the frequency response of TS varies with size. The only available measures of the frequency response of TS are for myctophids larger than the mean size observed here (Au and Benoit-Bird, 2008). Although the pattern observed in TS as a function of frequency in Au and Benoit-Bird (2008) does not fit this pattern, perhaps the individual spectra for smaller animals would not be inconsistent with the variability in frequency response observed.

Layer composition, measured as the percentage of animals identified as myctophids, was related to changes in volume scattering only at 38 kHz, where it explained $<$50% of the variability in volume scattering, and at 70 kHz, where it explained just 15% of the same parameter (Figure 6). Myctophids are the strongest scatterers identified in these layers, at least at 200 kHz, where individual TS measurements are available as a function of size (Benoit-Bird and Au, 2001). For frequencies that were significantly correlated with myctophid percentage, the relationship was positive, as would be expected from the relative individual scattering strengths of taxa in the layers. It is important for the interpretation of this result to note that the myctophids present in these scattering layers do not have air-filled swimbladders (Benoit-Bird and Au, 2001). The depth of layers would not be expected to be a confounding variable in the assessment because deformations of a solid swimbladder would not change individual scattering. Further, the well-mixed water column consistently present in the area shows no changes in sound speed with depth which could influence the analysis. Indeed, an ANOVA revealed no significant effect of mean layer depth on the volume scattering.

The length of myctophids was significantly correlated with volume scattering only at 70 and 120 kHz (Figure 7). In both cases, the percentage variability explained was extremely low, $<$25% in each case. However, the trend of the relationship was different at the two frequencies. Volume scattering was higher at 120 kHz when myctophids were larger, as would be expected based on the positive length–TS typical in fish, and has been verified in these myctophids (Benoit-Bird and Au, 2001). Volume

scattering was negatively related to myctophid length at 70 kHz. The reason for this relationship is not evident.

It is common when collecting multifrequency backscatter to compare pairs of frequencies. Often, all frequencies are compared with a reference frequency, most commonly 38 kHz because of its common use in fishery acoustics (Korneliussen and Ona, 2002). In this study, it was possible to select the pairs of frequencies for comparison based on what biological variables each did or did not explain. Because the 200 kHz volume-scattering strength was only related to the density of targets and not their identity or size, it was a good frequency for comparison. Because 70 kHz volume scattering was significantly affected by all three parameters measured, it was a poor choice for comparison. Two frequencies, 38 and 120 kHz, were only affected by two of the three variables measured, density and one other. By subtracting the scattering at 200 kHz which was related only to density, it is, in theory, possible to examine the effects of only composition in the 38 kHz data or only animal length in the 120 kHz data. When this is done, the predictive value of the individual parameters is extremely high. In fact, $>95\%$ of the variability in the backscatter difference in these frequency pairs can be predicted by a single biological parameter. Therefore, the differences in these pairs of frequencies can be used to predict the composition of insonified layers (38 and 200 kHz; Figure 9), as well as the average length of the myctophids (120 and 200 kHz; Figure 8) over the range of values measured (88–100% myctophids 1–9 cm long).

Methods to analyse simultaneously all the frequencies used to obtain backscatter measurements in previous studies have been primarily categorical. An example is the development of a simple code to describe which frequencies measured exceeded a threshold value and comparing data based on these categories (Jech and Michaels, 2006). Another approach is to apply statistical descriptors to the response curve. The mean and median are simple descriptors of the distribution of data, but other descriptors can compress the volume scattering at four (or more) frequencies into a single value that can then be compared with independent biological variables. One descriptor is variance. This is a measure of how spread out the frequency response is. It includes not only the absolute range in scattering values, but also how often the response changes. For example, the volume scattering could have values of -40, -42, -44, and -55 dB, or -40, -40, -55, and -55 dB, at the four frequencies measured. In both cases, the minimum, maximum, and mean values are the same. However, the variance in the second case is half that of the first. Calculations of the mean and variance in the data were made on the linear form of the volume-scattering values to eliminate the log-transform effects. The results showed that a greater abundance of myctophids strongly increased the variability in the volume-scattering measurements between frequencies (Figure 10). The increase in the variability of volume-scattering strength with an increasingly homogenous layer composition is unexpected. An increase in myctophid length showed a weak positive relationship and animal density no relationship with variance.

Another measure of a distribution's shape is skew, or how symmetrical the curve is. Negative skew values indicate that lower frequencies have lower measures of volume scattering, whereas positive skew values indicate the opposite. Skew measurements were also calculated on linearized volume scattering. All three biological-layer characteristics measured had a significant relationship with skew. Skew values indicate that at the lowest animal densities measured, frequency curves shift to lower frequencies, whereas at the highest mean densities, the frequency response is nearly symmetrical, representing an equal importance of scattering at high and low frequencies (Figure 11). The decrease in the relative importance of lower frequency scattering at high numerical densities may explain the unusual frequency response curve observed for the mean data. A similar pattern is observed with layer composition. When the layers have the lowest relative composition of myctophids, low frequencies are disproportionately high, but at 100% myctophids, the frequency response is nearly symmetrical, though much more variable. A weak but opposite trend is observed with increasing myctophid length. These statistical descriptors of the entire scattering spectra compress the data into simple measures that may provide additional insight into the scattering process and can be used to predict the characteristics of the scattering layers observed.

The TS at 200 kHz of individual scatterers from the mesopelagic scattering layer near Hawaii was well characterized by Benoit-Bird and Au (2001). The TS for each taxonomic group was strongly related to length, with the slope approximating the square of the length, similar to the length–TS relationship observed in many other species (for a review, see McClatchie *et al*., 2003). However, the volume-scattering strength measured in this study at 200 kHz was not significantly related to target length. Similar mismatches were observed at 38 and 120 kHz, where measured volume scattering was not correlated with one of the measurements of layer features that impacts mean individual scattering. Only volume scattering at 70 kHz was significantly correlated with all measured layer features that are known to affect individual scattering. If individual mean values of TS are affected by animal length and identity as shown by controlled measurements, how can volume scattering not be related to all these parameters? A corollary to this question is how does the frequency response of the *in situ* volume scattering differ so greatly from the frequency response of targets measured individually *in situ* (Figure 4)?

Camera measurements show that the position of individual measurements in the field of view is random both in terms of spacing and horizontal swimming direction. This suggests that the relatively high volume-scattering strengths at 38 and 120 kHz are not caused by animal polarization or other layer distributional characteristics. The volume scattering at 200 kHz, in contrast to that at 38 and 120 kHz, is relatively low. This difference is unlikely to be caused by unknown variability in individual mean backscatter values, because this is the frequency for which the best TS data exist. To explain the volume scattering at 200 kHz, TS would have to be independent of target length. However, the length^2–TS relationship is well established in many species, including those measured here (McClatchie *et al*., 2003). Another possible explanation for the low scattering at 200 kHz could be "shadowing" caused by extremely high densities of individuals that decrease volume scattering relative to the sum of individual scattering strengths. However, the 200 kHz frequency has the smallest sampling volume and the highest resolution and would be least affected by this problem. Further, the volume scattering at 200 kHz shows a very strong positive correlation with density. No manifestations of extinction were observed at any frequency measured. The observed frequency effects are not likely to be caused by the presence of other, unknown scatterers. Smaller scatterers (zooplankton) and those with air inclusions (siphonophores and salps) were not abundant at the same depth range and showed only weak scattering. More important, any unknown scatterer

would need to show strong scattering at 38 kHz and 200 kHz and almost no scattering at 70 kHz to account for the pattern observed. These data may suggest that the dominant source of scattering in individual animals is not geometric at all frequencies. Perhaps some fish have enough air in their swimbladders to cause resonance, although if so, such fish were not measured in the limited *in situ* TS measurements made in this study (Figure 4). Perhaps, though, and as observed in other fish species, the dominant scattering source in a single fish changes with frequency (Gorska *et al*., 2005, 2007). Alternatively, constructive and destructive interference in the acoustic signals may play a more significant role in this mixed assemblage (in terms of both taxonomic composition including variability in species composition that cannot be assessed with the methods used, and animal size, with different interference patterns at each wavelength) than in the much more homogeneous groups in which linearity has been demonstrated (Foote, 1983).

The reasons for these frequency differences in volume backscatter are not clear, but the results underscore the importance of careful interpretation of echo-integration estimates of density even with good knowledge of individual scattering strengths and the identity of animals in scattering aggregations. The effects observed would have been difficult to measure in the field with a smaller number of paired samples and without the type of gradations in independent biological variables measured with relevant resolution to the acoustics that was possible here. However, these effects have important implications for the applications of acoustics to biological problems, including estimates of size, density, total abundance, and animal identity.

The differences observed in volume-scattering response as a function of frequency can be advantageous for the application of acoustics in this ecosystem. Variation in density explains 85% of the volume scattering at 200 kHz with no knowledge of individual target size over nearly the entire size range of animals present in these layers or information on subtle differences in layer composition. Therefore, the volume scattering at 200 kHz can be used as a simple metric of numerical density of these layers. Subtracting this effect from the volume scattering at other frequencies allows examination of other effects. Figures 8 and 9 clearly demonstrate the utility of the approach. Myctophid length can be predicted from the difference in volume scattering between 120 and 200 kHz with a fit value $>95\%$, as can layer composition with comparison of volume scattering at 38 and 200 kHz.

The approach of using scattering values and combinations of relative scattering as indicators of the biology is very useful in this ecosystem where significant variability in volume backscatter can be observed on vertical scales of as little as 1 m (see figures in Benoit-Bird and Au, 2003a, 2003b, 2006). These strong scattering features are difficult to ground-truth. No capture-based sampling methods can quantify mobile organisms with the necessary resolution, and even with the camera system used here, it is difficult to obtain the sample size necessary to observe statistically the changes in biology predicted by the strong volume-scattering changes with depth. Although the scattering at 200 kHz does vary significantly within these small-scale, vertical features, changes in the relative scattering between 120 and 200 kHz and between 38 and 200 kHz suggest that the primary biological cause for these strong, thin, horizontal strata in the scattering layers is the differences in animal length and the relative abundance of myctophids. This suggests strong, vertical partitioning of habitat by these animals in space and time by taxonomy and size class. Along with the high densities of animals observed in these layers, this partitioning indicates that competition is important in driving the behaviour and structuring the community of the scattering layers surrounding the Hawaiian Islands.

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