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Acoustic classification of zooplankton

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Accurate acoustic characterization of zooplankton species is essential if reliable estimates of zooplankton biomass are to be made from acoustic backscatter measurements of the water column. Much work has recently been done on the forward problem, where scattering predictions have been made based on animal morphology. Three categories of scatterers, represented by theoretical scattering models, have been identified by Stanton *et al.* (1994): gas-bearing (e.g. siphonophores), fluid-like (e.g. euphausiids) and hard elastic-shelled (e.g. pteropods). If there are consistent differences in the characteristic acoustic signatures of each of these classes of zooplankton, it should be possible to solve the inverse problem by using acoustic backscatter data to infer mathematically the class of scatterer. In this investigation of the feasibility of inverting acoustic data for scatterer-type, two different inversion techniques are applied to hundreds of pings of data collected from broadband ensonifications ($\sim$350 kHz–750 kHz) of individual, live zooplankton tethered and suspended in a large tank filled with filtered sea water. In the Model Parameterization Classifier (MPC), the theoretical models for each scatterer-type are represented as either straight lines with slope and intercept parameters or rectified sinusoids with frequency and phase parameters. Individual pings are classified by comparison with these model parameterizations. The Empirical Orthogonal Function-based Classifier (EOFC) exploits the basic structure of the frequency response (e.g. presence of a resonance structure) through decomposition of the response into empirical orthogonal functions. Small groups of pings are classified by comparing their dominant modes with the dominant modes representative of the three scatterer-types. Preliminary results indicate that the acoustic classification of zooplankton ensonifications into categories representing distinct scatterer-types is feasible. Ultimately, it may be possible to develop *in situ* acoustic systems that are capable of inverting for the types of organisms sampled, thereby bridging the gap between acoustic backscatter measurements and estimates of zooplankton biomass.

Key words: acoustics, biomass, classification, inversion, scattering, zooplankton.

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Introduction

The acoustic characterization of various species of zooplankton is essential if biologists wish to use volume backscatter measurements of the ocean as indicators of zooplankton type, size, and biomass. Traditional acoustic biomass estimation methods have employed single-frequency acoustic measures in conjunction with either theoretical models (e.g. Greenlaw, 1979) or empirical regression relationships between the acoustic backscatter data and the biomass collected in simultaneous net samples (e.g. Flagg and Smith, 1989). However, biomass estimates based on simple regression curves or on single-frequency echo energy measurements may be subject to large errors. For example, Wiebe *et al.* (in press) found that although volume scattering at 420 kHz was 4–7 times higher at two stratified sites versus a mixed site on Georges Bank, MOCNESS-collected biovolumes at these sites were not significantly different. Greenlaw (1979) noted that the volume scattering from a region containing a single 22 mm fish was the same as that from a region containing 260 similar-sized euphausiids. In fact, Stanton *et al.* (1994) observed that

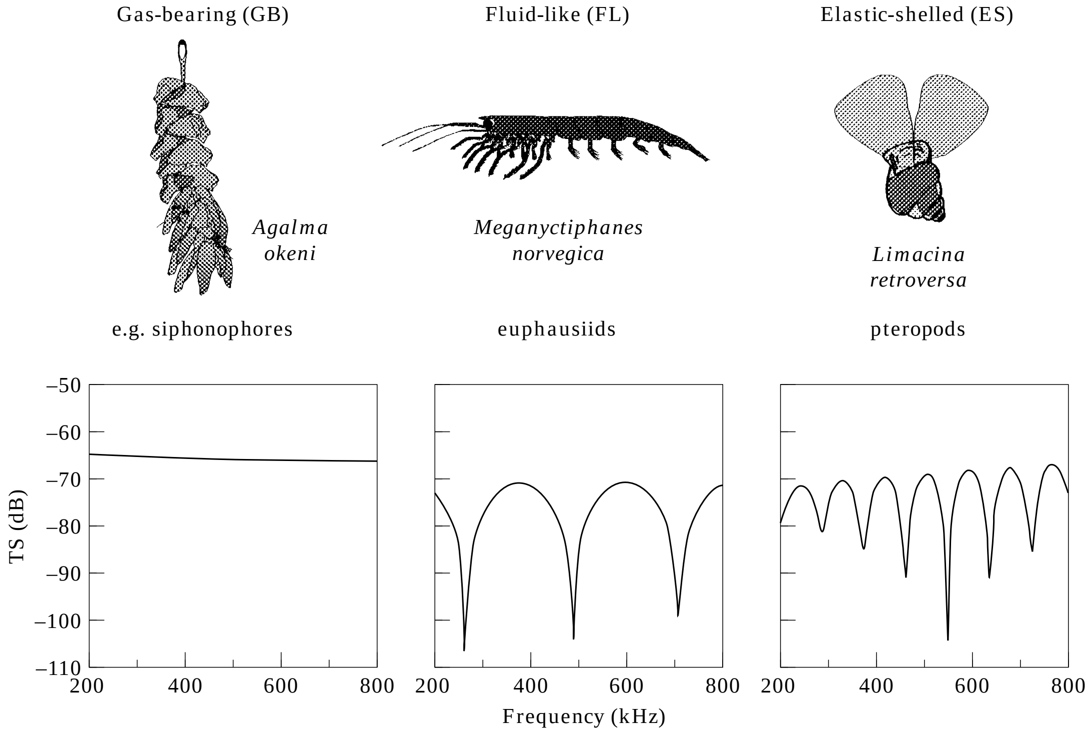

Figure 1. Examples of zooplankton from the three acoustic scattering classes. Plotted below are simplified theoretical models that describe the acoustic scattering from these three classes of animals under certain conditions. The details of these models are included in Stanton (1989) (gas-bearing) and Stanton *et al*. (1996) (fluid-like and elastic-shelled).

the relative echo energy per unit of biomass measured from animals ranging from elastic-shelled gastropods to fluid-like salps varies by a factor of $\approx$19 000 to 1. This huge species-dependent variability in echo energy per unit biomass has important implications for the interpretation of acoustic survey data. Attempts to equate larger acoustic returns to the presence of more or larger animals (and thereby conclude that the higher the echo energy, the greater the biomass in the ensonified region) could lead to gross errors in biomass estimates by several orders of magnitude (Stanton *et al*., 1994).

The solution to the forward problem involves predicting the properties of the acoustic return from a scatterer based on knowledge of the physical and geometric properties of the scatterer as well as the specifications of the sonar system used to ensonify it. Various theoretical models have been developed to predict acoustic scattering from zooplankton based on animal morphology (Greenlaw, 1979; Stanton, 1989; Chu *et al*., 1993; Stanton *et al*., 1994, 1996). To develop and corroborate scattering models, target-strength measurements (most at one or a few discrete frequencies) have been made of zooplankton, both experimentally constrained (e.g. Demer and Martin, 1995 – tethered; Foote *et al*., 1990 – encaged), and *in situ* (e.g. Hewitt and Demer, 1991). Recently, Stanton *et al*. (1994) made target-strength measurements of single organisms over a broad range of frequencies simultaneously by ensonifying tethered zooplankton representative of the species from Georges Bank with broadband chirps. Comparison of the data with theoretical scattering models has resulted in the division of these zooplankton into three acoustic types: (i) GB gas-bearing (e.g. siphonophores); (ii) FL fluid-like (e.g. euphausiids); (iii) ES elastic-shelled (e.g. pteropods). Examples of zooplankton from these scattering classes and theoretical models used to describe the acoustic scattering from these organisms are shown in Figure 1. The characteristic acoustic signature of each of these classes is unique. As a result, it should be possible to invert acoustic backscatter data for the class of scatterer.

The inverse problem is concerned with predicting the properties of the scatterer based on knowledge of the acoustic return from that object. In bioacoustical oceanography, some work has been done on identifying fish from their acoustic returns (e.g. Zakharia and Sessarego, 1982). Holliday *et al*. (1989) have estimated the size distribution of a zooplankton assemblage based on volume scattering data from a multi-frequency sonar system using 21 discrete frequencies. If the acoustic sampling includes a broadband signal with a continuous (or virtually continuous) range of frequencies and if the

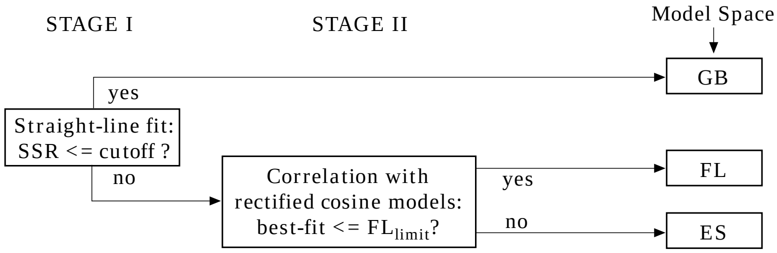

Figure 2. Summary of the MPC classification scheme. This classifier is based on a parameterization of the scattering models illustrated in Figure 1.

echoes from individual zooplankton are resolvable (e.g. Stanton *et al.*, 1994), a different type of inversion is possible. A spectral decomposition may be performed on the echo time series from each individual scatterer, and each zooplankter may be classified according to its frequency-dependent scattering characteristics. This type of classification inversion strives to identify individual scatterers based on their acoustic signatures, and can be carried out with or without relying on theoretical scattering models. The development of such a classification inversion of marine zooplankton based on single-ping broadband ensonifications is described in this paper.

Methods

Data collection and processing

The data used in the inversions were collected on a cruise to Georges Bank and the Gulf of Maine on RV ''Oceanus'' from 27 September to 6 October 1993. Organisms were captured in both vertical and oblique tows with a meter net (335 μm mesh) with a codend bucket (32 cm diameter by 46 cm long), and sorted into large containers for short-term storage under refrigeration to maintain seawater temperature. Prior to ensonification, a detailed sketch was made of each animal and measurements were made of animal length, width, size of shell (pteropods), and size of gas inclusion (siphonophores). Individual organisms were tethered with an acoustically transparent monofilament strand, and suspended in a 2.44 m diameter by 1.52 m high tank filled with filtered (through 64 μm mesh) sea water on-board the ship. Acoustic experiments included broadband ensonification (center frequency 500 kHz, ≈350 kHz–750 kHz) of each live animal: the return echoes from 50 acoustic transmissions were collected for each of nine siphonophores (*Agalma okeni*) and eight pteropods (*Limacina retroversa*), and 1000 returns were collected for a single euphausiid (*Meganyctiphanes norvegica*). An FFT was taken of the time series for each acoustic return; the ping was then represented by a 241-point sample of the echo spectrum.

Development of classification algorithms

Two types of classification algorithms were developed. The Model Parameterization Classifier (MPC) depends on comparison of the data with theoretical scattering models, whereas the feature-based Empirical Orthogonal Function-based Classifier (EOFC) is independent of the models, exploiting only the inherent characteristics of the acoustic returns. Both the MPC and the EOFC algorithms were applied to a data set of 1850 pings from 18 animals.

The MPC involves two stages of classification (Fig. 2). In Stage I, the gas-bearing model is parameterized as a straight line with slope (m) and intercept (b) parameters. A straight line is fit through each echo spectrum (SPEC) by linear regression ($y = mx + b$) and the SSR is computed for each return by taking the sum of squares of the residuals: $SSR = \Sigma(y(i) - SPEC(i))^2$. Pings are classed as GB (gas-bearing) if $SSR \leq t$, where t is an arbitrarily chosen threshold that gives the best classification of a sample data set. The GB model space consists of 25 bins that quantify the SSR associated with each ping. Pings not classed as GB enter the second stage of classification. In Stage II, the FL (fluid-like) and ES (elastic-shelled) models are simplified by parameterizing them as a rectified cosine with frequency (null spacing) and phase shift parameters. To construct the model spaces, several model realizations of rectified cosines, differing in null spacing and phase shift, were created. The ranges of these two parameters for FL and ES model realizations were determined by examining the appropriate theoretical model as well as several dozen pings for each type. To include the range observed in the data, nine different null spacings (linearly spaced from every 130 to every 370 kHz) were chosen for the FL model realizations. For ES, five different null spacings (linearly spaced from every 60 to every 100 kHz) were used. The possibility of phase-shifted returns was also accounted for in the model spaces, resulting in 81 different FL model realizations and 25 different ES model realizations. The echo spectrum of each ping was correlated to the model realizations; maximum correlation

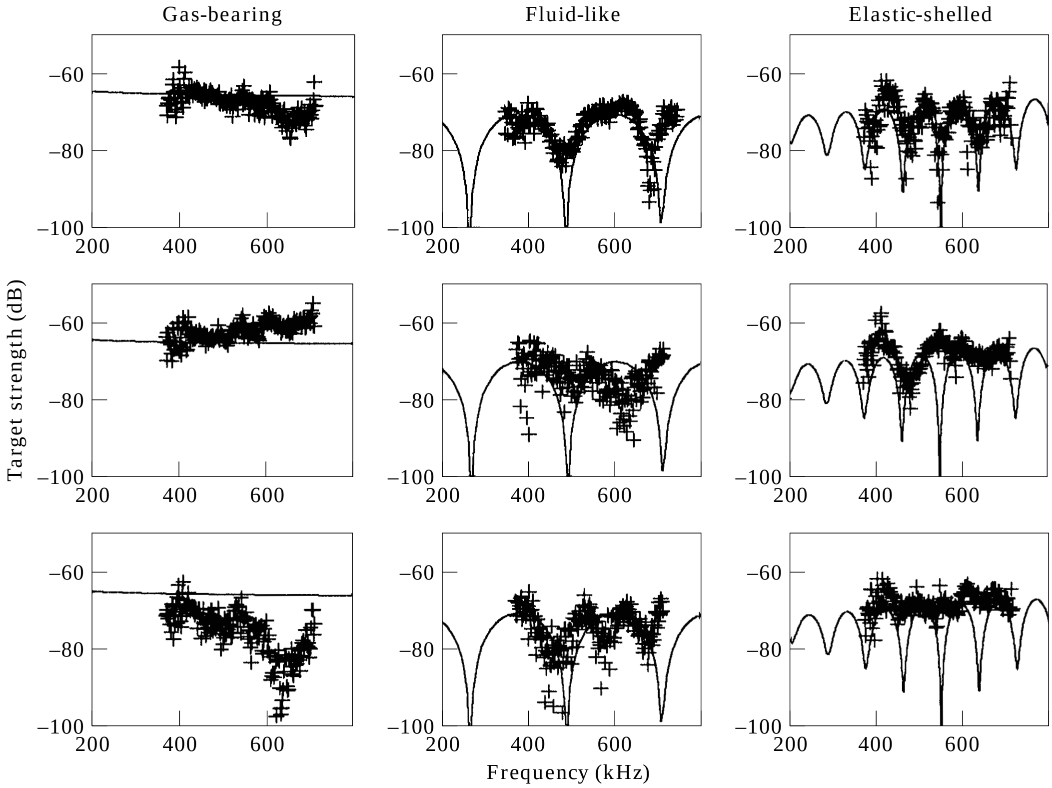

Figure 3. Target strength versus frequency for selected examples of the three scattering classes. Ping-to-ping variability in spectra from the same animal as well as animal-to-animal variability are illustrated. Pings in the gas-bearing category (GB) are from a single siphonophore (*Agalma okeni*), fluid-like (FL) pings are from a decapod shrimp (*Palaemonetes vulgaris*) (top), and a euphausiid (*Meganyctiphanes norvegica*) (middle and bottom), and the elastic-shelled class (ES) is represented by two pteropods (*Limacina retroversa*) (bottom 2 from same animal). The superimposed curves are based on the theoretical scattering models plotted in Figure 1. These data and associated models are presented and discussed in more detail in Stanton *et al.* (1996).

indicates best fit. Classification of a ping involves determining to which model space the best-fit model belongs.

The EOFC depends on properties of the frequency response that are independent of the theoretical scattering models. The EOFC matches the echo spectrum to the scattering class based on an empirical orthogonal function decomposition. The frequency spectra are decomposed into modes that represent the variation of the data from the mean value. This is accomplished by computing the eigenvalues λ_i and eigenvectors (EOFs) φ_i of the covariance matrix ($K = A^T A$), where A is a matrix in which each row represents a mean-subtracted echo spectrum. The modal decomposition hinges on the fact that $K\varphi_i = \lambda_i \varphi_i$. The eigenvector corresponding to the maximum eigenvalue is the dominant mode. The model space for a given scattering class is then represented by the dominant modes (based on 50-ping data sets) of each individual in that class. For classification, an EOF decomposition is performed on ensembles of five pings. The five ping-ensemble dominant mode is then correlated to the model space, which includes the modes for all scattering classes. Classification involves determining to which model space the best-fit dominant mode belongs.

Results

Examination of the acoustic returns from these animals revealed considerable variability in the spectra, both between separate ensonifications of a single zooplankter as well as between different individuals in the same scattering class. This is illustrated with examples of the frequency responses from each of the three scattering classes plotted together with the corresponding theoretical models (Fig. 3); some returns resemble the theoretical models more than others. In addition, noise contamination was more evident in returns from some animals than others. To assess the performance of

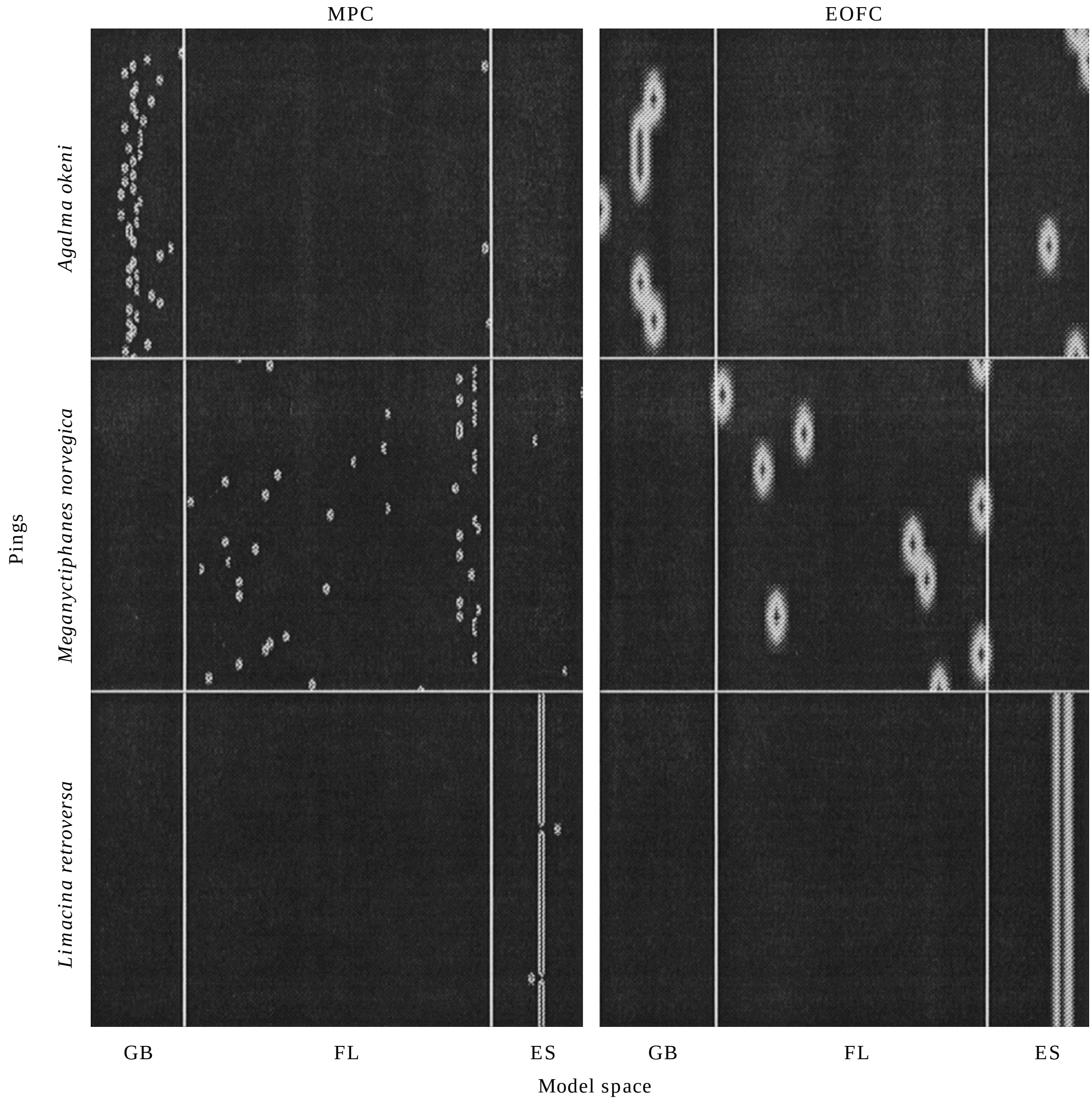

Figure 4. Classification results for the MPC and the EOFC for a selected subset of the highest quality data. The data set consisted of 50 pings each of a siphonophore (*Agalma okeni*) (Animal 18), a euphausiid (*Meganyctiphanes norvegica*) (Animal 33), and a pteropod (*Limacina retroversa*) (Animal 29). The MPC classifies each acoustic return separately, whereas the EOFC classifies based on 5-ping ensembles.

the classifiers with the best quality data, a 150-ping sample, consisting of 50 acoustic returns each of a siphonophore *Agalma okeni* (Animal 18), a euphausiid *Meganyctiphanes norvegica* (animal 33, run 5), and a pteropod *Limacina retroversa* (Animal 29), was extracted from the entire 1850-ping data set. This subset represents the highest signal-to-noise ratio (SNR) data, and the classification results for the MPC and EOFC for this 150-ping sample are illustrated in Figure 4. The MPC correctly classified about 95% of these pings, and the EOFC correctly classified about 87%. Table 1 summarizes the classification results for both classifiers for the complete data set.

Discussion

Signature variability

The observed variability in the frequency spectra of the acoustic returns within and between individuals in a scattering class (Fig. 3) can be attributed to differences in the behaviour and morphology of the animals.

Table 1. Summary of the classification results for the 18 animals ensonified during the experiment. For the MPC, n represents the number of pings, whereas for the EOFC, n is the number of 5-ping ensembles.

Species	Animal no.	Run no.	MPC results n	MPC results % correct	EOFC results n	EOFC results % correct
Agalma okeni	13	1	50	58	10	70
	14	1	50	50	10	80
	16	1	50	10	10	80
	17	1	50	66	10	80
	18	1	50	92	10	60
	19	1	50	10	10	20
	20	1	50	12	10	60
	21	1	50	48	10	40
	22	1	50	12	10	40
	Total		450	40	90	59
Limacina retroversa	23	1	50	20	10	100
	24	1	50	16	10	100
	26	1	50	2	10	90
	27	1	50	66	10	100
	28	1	50	40	10	50
	29	1	50	100	10	100
	30	1	50	0	10	70
	31	1	50	54	10	90
	Total		400	37	80	88
Meganyctiphanes norvegica	33	1	50	92	10	70
	33	2	300	86	60	77
	33	3	300	79	60	77
	33	4	300	86	60	88
	33	5	50	94	10	100
	Total		1000	85	200	81
All animals	Total		1850	64	370	77

Changes in the orientation of the animal during ensonification may lead to ping-to-ping variability in the acoustic returns from a single target. For example, the spectra of fluid-like zooplankton can exhibit different null-spacings depending on the animal's orientation relative to the acoustic beam. Differences in orientation may explain why echoes from some elastic-shelled individuals contain several tightly spaced nulls, whereas echoes from others exhibit a flat spectrum. For certain orientations, Lamb (circumferential) waves may propagate and scatter back toward the receiver, yielding an oscillatory spectrum as a result of the interference between the direct return (from the front interface of the shell) and the Lamb wave. For other orientations, attenuation of the Lamb waves by the opercular opening may eliminate the interference pattern, and the spectrum may be flat (Stanton *et al.*, 1996). Variability in the frequency response between different animals of the same species or in the same scattering class can also be attributed to differences in apparent animal size (which may change as orientation changes). For fluid-like and elastic-shelled scatterers there is an inverse relationship between apparent animal size and null spacing in the frequency response. Although gas-bearing animals appear to exhibit predominantly flat spectra, some structure has been observed which may be attributable to interference between returns from the small gas inclusion and the large fleshy body, depending on the relative size of the inclusion (unpubl. data). Development of a successful acoustic classification scheme for zooplankton relies on the design of classification algorithms that are robust to the potentially confounding variability in the frequency spectra of the acoustic returns.

In order to better characterize the variability in the acoustic returns of animals in the three scattering classes, some statistics were compiled on various features of the spectra from the three high-quality 50-ping data sets. A mean level (mean TS, averaged in dB) was computed for each ping. This feature did not appear to be a good discriminator between *M. norvegica* (−73.4 dB) and *L. retroversa* (−71.7 dB), but may be a good way to distinguish *A. okeni* (−64.9 dB), since average levels for this organism seem to be higher. The distribution of mean TS may be a good discriminator, since it appears to be much tighter for *L. retroversa* (s.d. = 0.29) than for the other two data sets (s.d. = 3.33, *A. okeni*; s.d. = 2.71, *M. norvegica*). Similarly, the distributions of null spacing and phase shift appear much tighter for *L. retroversa* than for *M. norvegica*. This type of feature is a promising discriminator; since it is based on statistical analysis of several echoes from the same animal, its utility is dependent on the feasibility of collecting these data.

MPC performance

Those acoustic returns that fit the theory poorly will be more difficult to invert correctly with a theoretical model-based inversion scheme. Although the MPC performed remarkably well with the high-quality subsample, it was less successful in classifying some of the other data sets in which many of the pings did not closely resemble the theoretical models. In particular, the MPC was less successful with the *Agalma okeni* and *Limacina retroversa* data, and it is likely that this is a direct result of the variability in the frequency responses for these two scatterer types, as well as the presence of noise contamination.

Stage I of the MPC relies on the fact that spectra from scatterers that are well represented by a straight-line model will have considerably smaller SSR than spectra exhibiting deep nulls. The variability in *A. okeni* returns from animal to animal could be due to the presence of multiple-bubble gas inclusions in some of the experimental animals; individuals with multiple closely spaced inclusions, as can result from embolism upon being removed from depth too quickly (Pugh and Youngbluth, 1988), may exhibit a multiple-bubble interference pattern and a spectrum with nulls. Pre-ensonification visual inspection of the siphonophores in this experiment revealed multiple bubbles in all but two individuals (Animals 17 and 20), whereas the majority of the specimens contained only one bubble after ensonification. It is uncertain how many bubbles were present during ensonification, or whether the presence of multiple bubbles is the sole mechanism for the observed oscillatory spectra, since interference between returns from the body and a single bubble may also introduce spectral oscillations (unpubl. data). It is believed that these animals may exhibit both flat and oscillatory spectra. Most of the misclassified *A. okeni* were classed as FL by the MPC.

The spectra of *L. retroversa* individuals were of two general types: those characterized by multiple, closely spaced nulls of more than 20 dB (e.g. Animal 29), and those with more or less flat spectra (e.g. Animal 30). Since the model parameterization used here does not account for possible attenuation of Lamb waves, the flat spectra are misclassified by the MPC. Much of the *L. retroversa* data also has lower SNR than data from the other two species. Because the MPC relies on matching the acoustic return to parameterizations of the theoretical models, noise contamination is particularly troublesome for this type of classifier.

EOFC performance

A feature-based classifier should be more robust in classifying returns that do not match the theoretical models, and if the signatures possess strong features, this type of classifier should also be less sensitive to noise contamination in the signal. The overall performance of the EOFC was considerably better than the MPC, particularly for *A. okeni* and *L. retroversa* returns. Because the model spaces are independent of the theoretical models, alternative orientations of *L. retroversa* resulting in different types of spectra are classified correctly as ES, resulting in a drastic improvement in performance over the MPC for these animals (MPC: 37% correct; EOFC: 88% correct). The modal feature, which represents the dominant variability in the signal, appears to be much stronger than the noise contamination in the *L. retroversa* data, contributing to the improved performance of the EOFC over the MPC. In fact, most of the five-ping ensembles for a given *L. retroversa* were assigned to the same dominant mode, indicating that returns from an individual share the same signature components. For *A. okeni* returns, the EOFC was able to discriminate the oscillatory spectra as GB even though the MPC classed them as FL, indicating that the dominant mode of variability for *A. okeni* was different than that for *M. norvegica*. Incorporation of higher order modes along with consideration of their energy content should further improve EOFC performance, particularly with scatterers that exhibit more than one type of characteristic acoustic return.

Field application

Successful implementation of a classification scheme which will result in an accurate estimate of animal biomass in the water column through the inversion of acoustic returns from zooplankton relies on the mode and quality of ocean sampling. Specific considerations are the type of acoustic data required to apply the classification scheme, including the minimum data set on which these inversions could be carried out, as well as the technological developments necessary to acquire this data set. Field application will require more than one single-target broadband ensonification per individual. Spatial resolution adequate to resolve individuals may be achieved by casting the echosounder through zooplankton aggregations. Technological challenges include variable beam width and SNR over the bandwidth of current broadband sources, although development of constant beam width broadband transducers is underway by others. These issues must be addressed to drive acoustic sampling technology in a direction that will facilitate the implementation of this acoustic classification approach for the purposes of increasing the accuracy of *in situ* zooplankton biomass estimates.

Summary and conclusions

Laboratory and theoretical investigations show that there are distinct differences in the spectral characteristics of broadband acoustic echoes from individual zooplankton in three scattering classes. This study outlines the application of two different classification algorithms (the MPC and the EOFC) in the inversion of 1850 broadband acoustic returns for scatterer class. Preliminary results for both classification algorithms with this limited data set are encouraging. For high SNR returns that correspond well to the theoretical models, the MPC correctly classifies the vast majority of data. In instances of degraded signal quality or noise contamination, the EOFC is more robust. There is potential for improving the results obtained with these algorithms by incorporating more sophisticated scattering models into the MPC and considering the information contained in the higher order, lower energy modes for the EOFC. Although this laboratory data set may not be fully representative of *in situ* acoustic data (e.g. *in situ* animal behaviour differs from tethered behaviour), the approach outlined in this paper illustrates the potential of exploiting class-specific differences in acoustic signatures for the purposes of automatic acoustic classification of zooplankton.

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