Validation of acoustic echo counting for studies of zooplankton behavior

Alex De Robertis

Acoustic methods can be employed to census zooplankton in large volumes of water rapidly, but ecological interpretations of the results are often complicated by ambiguities concerning the identity of acoustic backscatters. Here, a multi-step approach is developed to evaluate the potential of acoustic echo counting for behavioral studies of zooplankton using OASIS (Optical–Acoustic Submersible Imaging System), an instrument designed for concurrent optical and acoustic imaging of zooplankton. A combination of field and laboratory analyses (1) demonstrate general agreement between echo counts and depth-stratified net sampling, (2) show that fish and zooplankton can be distinguished on the basis of target strength (TS), (3) indicate that zooplankton taxa (euphausiids and gammarid amphipods) could not be differentiated on the basis of TS, and (4) demonstrate a positive relationship between body size and TS in euphausid crustaceans. Simulation of the system’s performance suggests that at high densities of reflective targets, echo counting increasingly underestimates the true number of targets present. These results confirm that echo counting is a valuable method for behavioral studies and underscore the importance of independent verification of the identities of acoustic backscatters.

Key words: acoustics, echo counting, optical verification, target strength, TS, zooplankton.

Received 19 June 2000; accepted 13 November 2000.

A. De Robertis: Scripps Institution of Oceanography, La Jolla, CA 92093-0208, USA.
Tel: +1 858 534 0738; fax: +1 858 822 0562; e-mail: aderobertis@ucsd.edu

Introduction

A central aim of biological oceanography is to comprehend mechanisms regulating populations of planktonic animals. Much of the field study of population processes has been made in the context of regional surveys which integrate spatially over large ocean areas. Studies conducted at these scales may overlook important events occurring at smaller spatial scales. Population trajectories through time reflect the integrated experiences of all members of the population, and are based on processes occurring at spatial scales much smaller than the extent of the population. Behavior mediates how organisms interact with the environment, and can have profound demographic consequences at the population level (Hassell and May, 1990). For instance, by regulating their vertical position, mobile plankters can experience different advective regimes, temperatures, light intensities, food concentrations, and predator abundances. These changes can translate to altered mortality and birth rates (Ohman, 1990) as well as increased retention within favorable habitats (Wroblewski, 1982; Lynch et al., 1998; Cowen et al., 2000).

Although the importance of zooplankton behavior in heterogeneous environments has long been recognized (Mullin and Brooks, 1976), the study of zooplankton in situ at spatial scales relevant to behavior has been limited by conventional sampling techniques. Since the late 1980s, there has been an increasing consensus that new methods with increased temporal and spatial resolution should be developed to study zooplankton distributions and behavior (Price et al., 1988; Marine Zooplankton Colloquium, 1989). This impetus coupled with recent advances in electronics and computing has led to the development of new sensors for use in the study of zooplankton. Major approaches include video-based instruments such as the video plankton recorder (Davis et al., 1992; Schulze et al., 1992), non-video optical methods such as the optical plankton counter,
still cameras, and holography (Sprules et al., 1992; Malkiel et al., 1999), and high frequency acoustics (Smith et al., 1992; Holliday and Pieper, 1995).

Acoustic techniques are attractive for behavioral studies as they are capable of non-invasively sampling large volumes of water using automated data processing techniques. However, the primary limitation of acoustic techniques is that they provide very little taxonomic information. On the other hand, optical methods offer high resolution taxonomic information but are generally limited to small sampling volumes near the sensor due to the rapid attenuation of light in water. Additionally, optical methods are prone to altering animal behavior due to the use of artificial illumination and short sampling distances.

One solution to such problems is to validate a subset of acoustic observations using optical methods. In this manner, accurate taxonomic identification and rapid, non-invasive sampling of large volumes of water is possible. Despite the strengths of this approach it has not been widely employed although there are some notable exceptions (Backus and Barnes, 1957; Hershey, 1967; Benfield et al., 1998). Recently, a new instrument package, OASIS (Optical-Acoustic Submersible Imaging System; Jaffe et al., 1998) was developed by coupling a 445 kHz multibeam sonar (Jaffe et al., 1995) with a digital camera. OASIS enables acoustic localization of individual zooplankton with concurrent optical identification of a subset of the acoustic targets that trigger a digital camera. Echo counting, in which echoes from individual animals are identified, is better suited for behavioral studies than the echo-integration techniques typically employed by conventional echo sounders which measure volume backscatter by integrating the energy scattered from animal assemblages.

The objective of this study is to evaluate the utility of the echo-counting approach employed by OASIS for behavioral studies of zooplankton. Validation of acoustic backscatter is essential for correct interpretation of field data. To this end, several distinct approaches have been employed: (1) simulation modeling of the acoustic target strength (TS) of animals identified in optical images, (3) laboratory determinations of euphausiid TS, (4) the use of models to predict zooplankton scattering properties, and (5) comparison of acoustic echo counts with predictions derived from depth-stratified net sampling. Although this analysis was conducted with reference to a specific instrument, the combination of several different aspects of validation is equally appropriate for other bioacoustic studies.

The in situ measurements of the acoustic target strength (TS) of animals identified in optical images, (3) laboratory determinations of euphausiid TS, (4) the use of models to predict zooplankton scattering properties, and (5) comparison of acoustic echo counts with predictions derived from depth-stratified net sampling. Although this analysis was conducted with reference to a specific instrument, the combination of several different aspects of validation is equally appropriate for other bioacoustic studies.

Materials and methods

Study site

OASIS was deployed during two midsummer cruises in 1996 and 1997 in Saanich Inlet, BC, an environment in which the macrozooplankton is known to be dominated by dense populations of the euphausiid Euphausia pacifica and the lysianassid amphipod Orcheston obtusus (Bary et al., 1962). This site was selected because the low diversity of large-bodied animals greatly simplifies the ecological interpretation of acoustic records. The inlet has a narrow sill which restricts deep-water circulation (Herlineux, 1962), and in both years waters deeper than 125 m were anoxic at the station at which OASIS was deployed (48°34.4′N 123°30.4′W).

Acoustic processing

Acoustic targets in the field of view were identified with an algorithm that searched each of the 64 acoustic beams for an acoustic signal exceeding a threshold value in three consecutive range samples. For the deployments reported here, each beam was sampled in 442 range bins, 0.75-cm thick, starting at a distance of 2.3 m from the transducer. The acoustic beams overlap slightly to ensure that a contiguous volume is sampled. As result of this overlap a highly reflective target will be detected in multiple beams (receiver, transmitter pairs) at a given range. To avoid identifying a single animal as multiple acoustic targets in adjacent beams, a procedure has been developed (hereafter referred to as trimming) in which a portion of the volume surrounding a region of intense acoustic backscatter is eliminated from further consideration in identifying further acoustic targets. Voxels (volumetric pixels) surrounding the target in the azimuth and elevation plane are eliminated on a TS-dependent basis using the following relation:

\[
\text{trim}_\text{distance} = | (\text{trim}_\text{rate}(\text{TS} \text{- threshold} + 1)) |
\]  

where \( \text{trim}_\text{distance} \) is a measure of how many beams to delete, \( \text{TS} \) is the target strength of the target, \( \text{trim}_\text{rate} \) is a constant, and \( \text{threshold} \) is the target detection threshold. All targets for \( \pm 3 \) range samples that are \( \leq \text{trim}_\text{distance} \) away from the target are deleted from future consideration in identifying additional targets (Figure 1). This particular trimming geometry was selected because it corresponds to the beam pattern produced by OASIS' perpendicularly aligned rectangular transmit and receive transducers. Trimming is applied sequentially from the most to the least reflective target identified in the sampled volume. A threshold of \(-95\) dB and a \( \text{trim}_\text{rate} \) of 0.35 were used to process all in situ data reported here.
Simulation of processing algorithms

At high animal densities, echo-counting systems become unable to resolve echoes from individual animals, and the true number of animals will be underestimated. The TS-dependent trimming algorithm used to eliminate false targets exacerbates this effect as it eliminates weak targets adjacent to more reflective targets. Additionally, the TS distribution reported by OASIS after trimming may be biased towards higher TS targets, as the trimming algorithm is applied sequentially from the most reflective target to the weakest.

To characterize the potential biases in both numerical counts and TS distributions introduced by the trimming procedure, the consequences of the trimming algorithms were simulated using a Monte Carlo approach (Manly, 1991). The simulations were based on the assumption that the acoustic scatterers are randomly distributed in space. In the simulations, a specified number of targets were randomly assigned a TS based on a pre-determined TS distribution. The targets were then assigned a uniformly distributed random spatial position within a volume larger than the field-of-view. Targets within the field-of-view were then identified, their position was translated to the corresponding voxel location and the trimming algorithm was applied. The number of targets remaining after trimming and their TS were then recorded.

Two model runs using TS distributions representing the extremes observed in situ (Figure 2) are presented here. In each instance, a series of target densities exceeding the range of macrozooplankton abundances observed in net samples was simulated. For a given initial TS distribution and target density, the mean and standard deviation of the number of targets remaining after trimming was computed from 500 iterations of the

Figure 1. Example of trimming algorithm applied at a single range slice in the azimuth and elevation plane for a target 7 dB above threshold (black circle). Each of the cells represents a 2"x2" acoustic beam formed by a transmitter-receiver pair. For a reflection of this intensity, all signals detected within two beams (indicated by the shading) and three range samples from the target location are removed from further consideration in finding new targets.

Figure 2. TS distributions used in the Monte Carlo simulation of the consequences of the trimming algorithm. TS distributions are from OASIS echo counting profiles. Case 1 (filled) represents weak scatterers recorded at 50–70 m on 11 August 1996 at 16:38, and case 2 (open) is an assemblage of highly reflective scatterers recorded at 20–40 m on 31 July 1997 at 01:38. These two cases represent extremes of the range of the TS distributions observed in situ.
In situ TS determinations

To characterize the acoustic signatures of the organisms detected by OASIS, the TS of animals triggering the in situ optical images was compared with body size measured from the images. The detailed methodology relating to the in situ TS measurements has been reported elsewhere (Jaffe et al., 1998) and will only be briefly discussed here. Where part of an organism was observed, a corrected length was estimated, but only when more than half of the organism was visible. The TS of the organism was determined by searching a subset of the backscatter matrix ± 5 range bins from the exact location triggering the camera system for a backscatter maximum. The acoustic reflections in this 0.007 m³ volume were inspected to ensure that there was only a single target in the area. Cases in which the backscatter maximum was located at the periphery of the array were excluded, as in these locations it is not possible to distinguish between a weak reflector in the center of a beam and a reflective target at the edge of a beam.

Laboratory TS determinations

To arrive at an independent measure of the acoustic signatures of euphausiids of different body sizes, a series of laboratory TS measurements was made on tethered live euphausiids (primarily Euphausia gibboides) ranging from 21 mm body length in a 20 m³ tank. Experimental animals were lightly anesthetized with MS-222 to reduce the probability that they would perform an escape response and tail-flip out of the shallow container in which they were being tethered. The euphausiids were tethered by tying a human hair around the abdomen anterior to the first pleopod. Great care was taken to ensure that the euphausiids were submerged at all times in order to avoid introducing acoustically reflective air bubbles under the carapace. The hair was tied to a 30-μm diameter nylon fiber that was kept taught by a weight attached to the terminal end. The tethered animals were suspended in the tank in a known location in OASIS’ field of view and allowed to recover for 20 minutes. After this recovery period the euphausiids swam actively in circles around the tether in the horizontal plane. The hair maintained the euphausiid within a maximum radius of ~ 5 cm from the point of tether attachment. Subsequently, 500 determinations of TS were made for each individual over a 15 minute period. The tether was acoustically undetectable above the experimental −101 dB (corrected value; see below) target recognition threshold. The tether did not constrain the orientation of euphausiids relative to the sonar, which was inclined at an angle of 27 degrees relative to the surface of the water. This position was selected to mimic the position of the sonar relative to the horizontal during field deployments.

Unfortunately reverberation near the periphery of the tank required placing the tethered euphausiids in an acoustically quiet area at a range of 1.15 m, which is in the near-field (<2.4 m) of the acoustic beam for this system. In the near-field, the acoustic pressure field is complex and does not decay smoothly with distance (Lockwood and Willette, 1973), as assumed by the theoretical corrections for the spreading of acoustic beams. To determine the magnitude of the effect of making measurements in the near-field, a series of TS determinations was made on a 2-mm diameter spherical glass standard target at a series of ranges along and angles across the acoustic beam.

Acoustic scattering models

Scattering models were employed to estimate backscattering characteristics of euphausiids and thecosome pteropods based on their shapes and material properties using model formulations and parameter values given by Stanton et al. (1994). Euphausiids were modeled using a randomly-oriented fluid, bent-cylinder model which estimates average TS. Thecosome pteropods were modeled as spherical elastic-shelled bodies assuming that the acoustic reflection stems from the front interface of the body. Models were used to compute backscattering at 445 kHz for a range of body sizes.

Scattering from euphausiids was also modeled using a distorted-wave Born approximation (DWBA) method (McGehee et al., 1998) that allows scattering to be computed for any animal orientation. The model was parameterized for scattering at 445 kHz using the “generic” euphausiid parameters of McGehee et al. (1998). To account for the geometry of the OASIS sonar, scattering was computed for sound impinging at −27 degrees from the horizontal. The model was run for vertical swimming angles of orientations of 30.4° and 10.5° and 50.3° in the vertical plane to mimic laboratory measurements (mean, mean ± 1 s.d.) of E. pacifica swimming angles (Miyashita et al., 1996), assuming isotropic orientation in the horizontal plane. Model solutions for each of the three swimming angles were computed for animals rotated 360° in the horizontal plane in one degree increments.

Zooplankton sampling

Zooplankton were sampled in both 1996 (six vertical series) and 1997 (seven vertical series) with a 1 m² MOCNESS (Wiebe et al., 1985) in the immediate vicinity of the station where OASIS was deployed. Preliminary analysis confirmed that euphausiids and
Validation of acoustic echo counting for studies of zooplankton behavior

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Species</th>
<th>Body sizes enumerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphausiacea</td>
<td>see text</td>
<td>&gt;4 mm</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>Orcheston obtusus</td>
<td>all</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>Cyphocaris challengeri</td>
<td>&gt;4 mm</td>
</tr>
<tr>
<td>Decapoda</td>
<td>unidentified megalopae</td>
<td>&gt;4 mm</td>
</tr>
<tr>
<td>Decapoda</td>
<td>Munida quadrispina larvae</td>
<td>all</td>
</tr>
<tr>
<td>Mysidacea</td>
<td>Neomysis rayii</td>
<td>&gt;10 mm</td>
</tr>
<tr>
<td>Thecosomata</td>
<td>Limacina helicina</td>
<td>all</td>
</tr>
</tbody>
</table>

*O. obtusus* dominated the zooplankton >4 mm, and thus were likely to be important acoustic scatterers. In addition to these taxa several relatively abundant large-bodied crustaceans, as well as thecosome pteropods (pelagic snails), which are known to be very intense acoustic reflectors (*Stanton et al., 1994*), were enumerated from the vertically-stratified MOCNESS samples (Table 1). Preliminary analysis of four samples integrating the water column indicated that in 1996, a single species, *Euphausia pacifica*, dominated the euphausiid community (97.8% *E. pacifica*, 1.7% *Thysanoessa spinifera*, 0.7% *Thysanoessa raschii*, n=1093). Due to the dominance of *E. pacifica* in this year, the euphausiids were not identified to species. A similar inspection of samples from 1997 revealed that the euphausiid assemblage was more diverse. To characterize the community composition in 1997, euphausiids >6-mm total length were identified to species, while smaller stages of these species were pooled. When material permitted, enough of each plankton sample was enumerated so that the sum of euphausiids and *O. obtusus* exceeded 150–200 individuals. When necessary a Folsom splitter was used to split samples. Euphausiids and amphipods were measured to the nearest 0.4 mm with an ocular micrometer as defined in *Jaffe et al. (1998)*. Taxa other than euphausiids and *O. obtusus*, which were generally much less abundant, were counted in the splits used to determine euphausiid and *O. obtusus* abundance.

Comparison of enumerations and OASIS acoustic data

Intercomparisons of OASIS acoustic echo counts and MOCNESS-derived zooplankton abundances were conducted by computing the “forward-problem” (*Holliday and Peiper, 1995*). In this approach, acoustic backscattering is predicted on the basis of measurements of zooplankton abundance, taxonomic composition, and knowledge of the acoustic reflectivity of the abundant taxa. It has previously been employed to predict the intensity of the acoustic reflection from a volume of water, or volume backscattering (*Wiebe et al., 1996; Greene et al., 1998*), rather than the number of distinct targets detected by echo counting. The forward-problem for echo counting individual targets can be represented as:

\[ E_{\text{pred}} = \sum_{i=1}^{t} \sum_{j=1}^{s} n_{ij} < p_{ij} > \]  

(2)

where \( E_{\text{pred}} \) represents the predicted number of echoes in a given volume, \( n_{ij} \) is the numerical abundance of size class \( j \) of taxon \( i \), \( p_{ij} \) is the average probability of detection of taxon \( i \) and size \( j \), and \( s \) and \( t \) refer to the numbers of size classes and taxa.

In order to compare OASIS acoustic echo counts with MOCNESS-derived zooplankton abundances, the density of organisms estimated from a net sample was multiplied by \( p_{ij} \), the probability of acoustic detection. For euphausiids, \( p_{ij} \) was estimated from the laboratory TS measurements of tethered euphausiids (described below). *O. obtusus* >3-mm body size was assumed to be detected 100% of the time on the basis of the TS of animals in the in situ optical images. The other, much less abundant taxa, were assumed to be detected 100% of the time (i.e. \( p_{ij}=1 \)). The numerical abundance of a size class within a given taxon from the net samples was used to estimate \( n_{ij} \).

The expected number of acoustic targets derived by computing the forward-problem was compared with the number of acoustic targets detected by OASIS. Logistical constraints at sea required that the net tows were often not conducted on the same dates as the acoustic profiles with which they are compared (Table 2). For each comparison, the abundance of acoustic scatterers corresponding to zooplankton targets (\(-95 \text{ dB}<\text{TS}< -66 \text{ dB}\)) was averaged over the depth interval sampled by the net. Depending on the depth range sampled, 60–420 acoustic returns were used to compute the average number of echo counts in the stratum.

Results

Simulation of processing algorithms

Simulation of the trimming algorithm illustrates that the degree to which OASIS undercounts acoustic scatterers at high densities is highly dependent on the underlying TS distribution (Figure 3). When the TS distribution corresponds primarily to weak reflections from small zooplankton, the simulation predicts little undercounting, with \( \sim 90\% \) of targets identified at a density of 60 scatterers \( > -95 \text{ dB m}^{-3} \). However, when the target TS distribution corresponds to intense reflections from large zooplankters and fish, \( \sim 50\% \) of the targets are detected at the same target density. A density of 60...
scattered \( \geq -95 \text{ dB m}^{-3} \) corresponds to the upper range of acoustic scatterers predicted from the forward problem calculations. The standard deviations of the mean fraction of targets counted by OASIS are relatively small, suggesting that the bias introduced by trimming should be relatively consistent between acoustic returns.

These simulations also confirm the \textit{a priori} expectation that application of the trimming algorithm biases the TS distribution towards more reflective targets, particularly at high target densities. The magnitude of this bias depends on the underlying TS distribution: for weak scatterers the distortion of the TS distribution is small, even at high target densities [Figure 4(a)], while the bias is more pronounced for more reflective targets [Figure 4(b)]. Very few of the most reflective targets are deleted by trimming, as the algorithm is applied sequentially from the most to least reflective targets, disproportionately deleting low TS targets.

\section*{In situ TS determinations}

The optical images indicate that in both 1996 and 1997, the acoustic detection of fish, euphausiids, and \textit{O. obtusus}}
triggered the camera. The TS of euphausiids and amphipods overlapped broadly, but the TS of fish detected in the optical images was generally higher than that of the zooplankton (Figure 5). Fish were more abundant in 1997, and were more likely to be observed in schools. Although the fish could not be identified in all the images, several of the fish were identified as juvenile pacific herring, *Clupea harengus pallasi*, or juvenile walleye pollock, *Theragra chalcogramma*. Both species are planktivorous, and are abundant in this area (Hart, 1973).
Laboratory TS determinations

Translation of the standard target in range near the center of the acoustic beam suggests that TS measurements made at a range of 1.15 m are 3 dB too low compared to measurements made in the acoustic far field [Figure 6(a)]. Moving the target across the beam at ranges of 1.15 m and 3.00 m shows that this +3 dB correction is appropriate across the axis of the acoustic beam [Figure 6(b)]. Decreased variability between replicate measurements made across the beam suggests that the higher variability in the measurements made in range is largely due to differences in the position of the bead relative to the central axis of the beam. On the basis of these results, a +3 dB correction factor was applied to the data as a first-order correction for making the laboratory TS measurements at a range of 1.15 m.

The corrected laboratory measurements of the TS of tethered euphausiids exhibited substantial variability [Figure 7(a)], with the range of values for a single animal often exceeding 25 dB. In many cases, particularly for smaller animals, the acoustic return was below the experimental noise floor of −101 dB. The laboratory and in situ determinations of euphausiid TS are qualitatively similar [Figure 7(b)], but statistical analysis reveals a marginally significant difference in the slopes determined with each of the two methods (ANCOVA: $F_{1,94}=2.96, p=0.089$). Hence, separate regressions have been computed for each measurement technique. Regression analysis using the median values of laboratory measurements ($y=0.86x−101.16, r^2=0.61, F_{1,57}=89.6, p<0.001$) and in situ TS determinations ($y=0.56x−94.3, r^2=0.23, F_{1,37}=11.3, p<0.005$) both indicate a positive relationship between body size and TS for euphausiids in the 6–22 mm size range. In combination with the in situ TS determinations, the measurements on tethered euphausiids suggest that for this particular acoustic frequency and animal assemblage, a TS criterion of −66 dB can be used to distinguish between backscattering from fish and zooplankton. None of the 32 500 corrected laboratory TS determinations on the 65 tethered euphausiids exceeded the −66 dB fish detection threshold. This criterion will underestimate the number of fish present, as it will erroneously identify fish as euphausiids, but is unlikely to identify any euphausiids as fish. Together, the in situ and laboratory measurements indicate that OASIS reliably detects euphausiids of body sizes >~7 mm using a field target threshold of −95 dB, that euphausiid TS increases with body size, and that zooplankton and fish can be distinguished using a −66 dB threshold.

The probability of acoustically detecting a given-sized euphausiid ($p_0$) was estimated from the laboratory TS determinations by determining the fraction of acoustic returns in which each euphausiid was detected above the −95 dB detection criterion used to process field data. These values were then fit with a nonlinear regression to estimate $p_0$ as a function of body size (Figure 8). This analysis indicates that the probability of detecting a euphausiid increases with body size. Computing this relation for ±1dB of the detection threshold resulted in similar regressions (Figure 8), suggesting that the relationship between body size and probability of

Figure 5. In situ TS measurements for taxa triggering the acquisition of an optical image in situ in Saanich Inlet. Animals were identified as fish (n=23), euphausiids (n=39), and Orchomene obtusus (n=25). Symbols distinguish between organisms that were imaged completely in the optical field and those whose bodies were truncated, for which the standard length was estimated if >50% of the body was in view. The horizontal line demarcates the −66 dB criterion used to distinguish fish and zooplankton.

Figure 6. (a) Median TS for taxa across range of 1.15 m (black) and 3.00 m (red). (b) TS across range of 1.15 m and 3.00 m for taxa showing possible range of monitoring in the form of a +3 dB correction.

Figure 7. (a) Laboratory TS measurements of tethered euphausiids showing substantial variability. The horizontal line shows the −66 dB TS criterion used to distinguish fish and zooplankton. (b) In situ TS measurements for taxa triggering the acquisition of an optical image in situ in Saanich Inlet. Animals were identified as fish (n=23), euphausiids (n=39), and Orchomene obtusus (n=25). Symbols distinguish between organisms that were imaged completely in the optical field and those whose bodies were truncated, for which the standard length was estimated if >50% of the body was in view. The horizontal line demarcates the −95 dB criterion used to distinguish fish and zooplankton.

Figure 8. Probability of detecting a given-sized euphausiid ($p_0$) as a function of body size. The analysis indicates that the probability of detecting a euphausiid increases with body size. Computing this relation for ±1 dB of the detection threshold resulted in similar regressions, suggesting that the relationship between body size and probability of
detection is robust to errors of this magnitude in the experimentally derived near-field correction.

Acoustic scattering models
The TS determinations reported here for larger-bodied individuals fall within the TS range predicted by the two euphausiid scattering models and an empirically derived regression for scattering from crustaceans at 420 kHz *(Figure 9)*. The empirical relation *(Wiebe *et al*., 1990)* overestimates the experimental data at all body sizes. Neither scattering model predicts a monotonic increase of TS with euphausiid body size, but rather, a more complex relation due to interference of backscattering from different surfaces of the target. The bent-cylinder model predictions of acoustic scattering are in qualitative agreement with measurements of euphausiid TS for body sizes >15 mm. The median values of DWBA model TS calculations for the mean vertical swimming angle of 30.4° are only in general agreement with the experimental data for euphausiids between 10–15 mm but do not predict an increase in TS with body size. However, the maximum TS value predicted by the model increases with body size, and is in agreement with the maximum TS values observed for the tethered euphausiids *(Figure 10)*. DWBA TS computed for

Figure 6. Measurements made to estimate the error associated with making TS determinations at a range of 1.15 m, which is in the acoustic near-field. Each point represents the mean of 100 determinations of the TS of a 2-mm diameter glass sphere. (a) Target is translated in range near the center of a beam. Each of the different symbols indicates a series (n=5) of measurements made by moving the target away from the sonar. The standard deviations are indicated, although they are often obscured by the symbols. Data are fit with a non-linear regression. (b) Translation of the target across the two degree wide acoustic beam in at ranges of 1.15 m and 3.00 m (n=5 series).
euphausiids with vertical swimming angles of 10.5° and 50.3° and rotated in the horizontal plane was similar to TS at the assumed swimming angle of 30.4° (mean deviation ± 1 s.d. was 1.4 ± 0.9 dB), indicating that the calculation is not sensitive to small biases in the vertical swimming angle.

Simulation of acoustic scattering from the shells of thecosome pteropods at 445 kHz predicts that pteropods are more acoustically reflective than euphausiids. TS is predicted to increase steeply with size for animals <1.5 mm (Figure 11). Thecosomes with a diameter >4 mm are predicted to have TS’s greater than the −66 dB criterion proposed to distinguish fish and zooplankton targets. The model suggests that the thecosome pteropods present in Saanich Inlet, which are <2 mm in body size will have a TS in the range defined for zooplankton targets.

**Zooplankton sampling**

Euphausiids, followed by *Orchomene*, made the largest contribution to the forward problem predictions of acoustic scatterers derived from the zooplankton enumerations. In shallow strata euphausiids are predicted to be the primary scatterers, whilst in deeper waters *Orchomene* become more important (Figure 12). Overall, when predictions are integrated over the upper 125 m sampled by OASIS, euphausiids and *Orchomene* accounted for 79% of predicted targets in 1996 (45% euphausiids, 34% *Orchomene*), and 86% of
predicted scatterers in 1997 (52% euphausiids, 34% Orchomene).

In both years an aggregation of euphausiids was present between 80–100 m during the day, and the majority of the population exhibited a “normal” (cf. Ohman, 1990) pattern of diel vertical migration towards the surface at night. The size-frequency distribution of euphausiids differed between 1996 and 1997. In 1996, euphausiids tended to be smaller in body size (mode at 5 mm), whilst in 1997 the modal body size was slightly larger, and there was a greater representation of animals >12-mm body size [Figure 13(a) and (b)]. In 1997, the euphausiid community was more diverse than in 1996 (85.1% E. pacifica, 10.9 % T. raschii, 3.3% T. spinifera, and 0.07% Nematoscelis difficilis, n=5824). Although the euphausiid community was more diverse than in 1996, two species, E. pacifica and T. raschii, accounted for 96% of individuals. Orchomene obtusus, the other dominant macrozooplankter, was vertically separated from the euphausiids, as it was abundant deeper in the water column, generally between 100–125 m. O. obtusus did not ascend into surface waters at night.

Comparison of nets and acoustics
To test whether reflections from thecosome pteropods contribute to the >-66 dB targets corresponding to fish, the abundance of pteropods from net samples and acoustic targets >-66 dB was compared (Figure 14). Pteropod abundance does not correlate significantly with the abundance of acoustic targets >-66 dB in 1996 (n=37, r=0.05, p>0.5), but exhibits a significant correlation in 1997 (n=46, r=0.44, p<0.005). The significance of the 1997 correlation depends on a single instance of high thecosome and high >-66 dB target
abundance. However, it should be noted that although thecosomes were more abundant in 1996, the abundance of strong acoustic reflectors was much lower in that year. Both thecosome pteropods and fish were abundant in near-surface waters at night, and the significant 1997 correlation is not an indication of causality, but rather is simply a reflection of this spatio-temporal overlap. Based on this evidence and the scattering model calculations it was concluded that thecosomes did not contribute significantly to echoes $> -66$ dB in this environment, and thecosome pteropods in the net samples were enumerated as “zooplankton” targets $<-66$ dB.

Comparisons of the predicted and observed number of acoustic scatterers exhibit substantial variability, particularly at the highest predicted densities. At the highest densities, OASIS tends to underestimate the predicted number of targets. The predicted and observed densities of acoustic targets were log transformed to reduce heteroscedasticity [Figure 15(a) and (b)], and were compared using a functional regression (Ricker, 1973, 1975). A functional regression is preferable to least-squares linear regression for this application due to the expectation of substantial variability in both the predicted and observed densities of acoustic targets.
Functional regression lines fit to the log-transformed data for 1996 [Figure 15(a), logy=0.90*logx+0.05, n=37] and 1997 [Figure 15(b), logy=0.93*logx+0.24, n=46] indicate significant positive associations between the predicted and observed densities of acoustic targets, as illustrated by the correlation coefficients (r=0.47, p<0.005, and r=0.78, p<0.001, respectively). The correlation between the two log-transformed variables accounts for 22 and 61% of the total variance in the data (r²=0.22 for 1996 and 0.61 for 1997). In both years, the significant positive correlations also hold without log transformation (r=0.56, p<0.001 for 1996, and r=0.43, p<0.005 for 1997). As illustrated in Figure 15(a) and (b), and by the y-intercepts of the log-transformed regression lines, the 1996 comparisons fall close to the 1:1 line, while in 1997, the observations tend to be higher than the predictions. The 95% confidence intervals for the slopes of the lines (0.61–1.31 for 1996 and 0.69–1.13 for 1997) indicate that both regression lines do not differ significantly from the expected slope of one.
Discussion

The development of new techniques can lead to new ecological insights but new methods must be carefully validated in order to allow proper interpretation of results. The central goal of this study was to evaluate both the strengths and weaknesses of acoustic echo counting for investigations of zooplankton behavior. Several methods have been employed to characterize the utility of this approach. The insights afforded by these investigations demonstrate that, when used appropriately, echo-counting systems have the potential to be valuable tools for the study of zooplankton due to their ability to localize and detect individual animals.

Simulation of data-processing algorithms suggests that the trimming procedure results in up to a 50% decrease in the number of targets detected at the highest mean target densities predicted in Saanich Inlet, and distorts the TS distribution towards higher values. These results should be viewed as an upper bound to this bias, as in situ TS distributions which are biased towards more reflective targets by the trimming algorithm were used in the simulations. Undercounting by OASIS is evident in the comparisons of predicted and observed echo counts at the highest predicted densities. The

Figure 13. Size–frequency distributions of euphausiid total length from MOCNESS tows. Abundances were weighted by the depth range sampled to compute abundances per m² (a). Size–frequency distribution of euphausiids in 1996 based on six tows (n=4053). (b) Size–frequency distribution of euphausiids in 1997 based on seven tows (n=6646).
magnitude of the bias introduced by trimming is very sensitive to the underlying TS distribution, and will be highest when fish and acoustically reflective zooplankton are abundant. Large-bodied zooplankton and fish are abundant in Saanich Inlet, and this bias will be lower for many other environments. Most acoustic systems used for zooplankton studies have lower spatial resolution than OASIS, particularly in the plane parallel to the sonar, and will undercount animals at lower target densities. Undercounting at high target densities has also been documented for echo-counting procedures employed to enumerate migrating fish (Enzenhofer et al., 1998). Undercounting biases are potentially important for echo-counting sonars, and should be considered during phases of instrument development, sampling design, and data interpretation in order to avoid erroneous ecological inferences.

In situ optical imaging of acoustic targets indicates that, given the species assemblage in Saanich Inlet, fish can be distinguished from euphausiids and amphipods on the basis of a −66 dB TS cutoff, although the true number of fish will be underestimated and some will be counted as zooplankton. The TS determinations on tethered euphausiids and scattering model predictions support this conclusion. The target strengths of zooplankton targets overlap broadly, and taxa cannot be distinguished on the basis of their acoustic reflections using the present methodology. This result highlights the importance of acquiring ancillary taxonomic information using optical or conventional sampling devices when making acoustic measurements. For example, in the current study the interpretation of echo counts was facilitated by net sampling, which revealed that the macrozooplankton was dominated by two vertically separated taxa. In general, echo counting techniques will be most effective in environments with simple macrozooplankton assemblages.

Laboratory and in situ TS determinations indicate that OASIS consistently detects euphausiids >7 mm and that, despite substantial variability, on average, TS increases with body size. However, the regression lines derived from these two types of measurements are slightly different. Two factors could account for this discrepancy. The empirical correction for the near-field could be inadequate, or the euphausiids detected in the optical images might have a higher TS because they tend to be at a broadside orientation which leads to higher TS (Jaffe et al., 1998), while the laboratory determinations average many orientations. The TS of euphausiids is known to be highly dependent on orientation (McGehee et al., 1998; Stanton and Chu, 2000), and it is likely that the observed differences are due to a bias in animal orientation. The variability in the TS of a single euphausiid reported here (range often >25 dB) is consistent with the range of target strengths recorded in laboratory measurements of Euphausia superba backscattering at 120 kHz (McGehee et al., 1998). Although TS as a function of euphausiid orientation was not measured in this study previous work suggests that much of the variability observed in the laboratory TS

![Figure 14. Thecosome pteropod (Limacina helicina) abundance derived from depth-stratified net sampling vs. intense acoustic targets >−66 dB. The dotted line indicates the 1:1 relationship expected for exact correspondence.](image-url)
measurements can be attributed to changes in the orientations of the tethered euphausiids (McGehee et al., 1998; Stanton and Chu, 2000).

The use of the laboratory TS determinations in the forward-problem calculations assumes that the orientation of the tethered animals are representative of those in the field. The assumption of isotropic orientation in the horizontal plane seems reasonable, as there is no reason to expect preferred orientation in this plane in the in situ measurements, and the laboratory measurements were made over a period of 15 minutes while the animals swam in circles around the point of tether attachment. The DWBA model predicts that the median TS of animals rotated in the horizontal plane is not very sensitive to differences in vertical orientation, suggesting that applying the laboratory TS values in the forward problem calculations is a reasonable first approximation.

Model predictions of euphausiid TS agree qualitatively with the measurements, but the scattering models...
do not predict the linear increase in TS with body size observed in the laboratory TS measurements. The relationship between TS and body size is complicated by deep nulls introduced by constructive and destructive interference of acoustic waves reflected from a single animal (Stanton et al., 1996), and a simple linear relation is thought to be inappropriate (Demer and Martin, 1995). The linear relationship between euphausioid body size and TS documented here may be a result of integrating over a wide range of animal orientations, and making measurements over a limited range of body sizes. The TS measurements reported here are lower than previous determinations made at a similar frequency (Wiebe et al., 1990), which showed a monotonic, but non-linear, increase in TS with body size in crustaceans. However, the measurements of Wiebe et al. (1990) may not be directly comparable as different species were used and most of the animals used to generate the regression were larger than the euphausioids used here. Additionally, the animals were at different orientations relative to the sonar in this study, with Wiebe et al.'s sonar being positioned directly under the animals rather than approximately to the side of the animals as in the experiments reported here.

Application of the forward-problem to vertically-stratified net sampling, and comparison with OASIS echo counts demonstrates a positive association between the results of net and acoustic sampling. In general, echo counts are higher than predictions at low target densities, while at high densities the trimming algorithm deletes large portions of the sampling volume resulting in undercounting. In 1997 the intercept of the functional regression illustrates that echo counts were higher than expected. This may be due to net avoidance by larger and more mobile euphausioids, which were more abundant in 1997. Interannual variations in the abundance of taxa not enumerated, such as copepods and the hyperiid amphipod *Parathemisto pacifica*, which may contribute to echo counts despite small body size (and presumably low probability of detection) by virtue of high abundance, may also be important. Furthermore, inadequacies in the relationship between body size and probability of detection, interannual differences in zooplankton acoustic properties due to changes in lipid composition, or differences in the sonar calibration between cruises could also contribute to the difference. A difference in sonar calibration is considered unlikely, however, as the sonar was calibrated prior to each cruise, and the *in situ* target strengths are consistent between the cruises.

Although the forward-problem predictions are in general agreement with the echo counts, a large proportion of the variability in both data sets remains unexplained by the functional regressions. The predictions are based on the results of net sampling, *in situ* TS determinations, experimental TS measurements, and assumptions regarding the scattering properties of several taxa. All of these factors can potentially contribute to the overall variability. There is a mismatch between time of day, date, and exact sampling location among the samples being compared. Zooplankton, and euphausiids in particular, can be extremely patchy, and variability between net tows suggests that this may be important in the present case. The depth of the oxycline in the fjord affects zooplankton vertical distribution (Bary et al., 1962), and the larger horizontal sampling scale of the MOCNESS may have resulted in mismatches with the environmental conditions sampled by the OASIS vertical profiles in a given depth stratum.

As detailed above, biases introduced by the trimming algorithm are potentially significant, particularly when highly reflective acoustic targets are abundant.

One of the weaknesses of the forward-problem approach to acoustic validation is that it is difficult to isolate the sources of error discussed above. However, previous comparisons of acoustic and conventional sampling with higher spatial and temporal coherence between measurements have shown higher correspondence between the two methods (Costello et al., 1989; Wiebe et al., 1996; Greene et al., 1998). This suggests that a substantial portion of the unexplained variance in the present comparisons is due to the spatial and temporal differences in MOCNESS and OASIS sampling. Despite the time-space offsets between the net samples and acoustic profiles, reasonably good correspondence between forward-model predictions and echo counts was observed. This may be in part due to the enclosed nature of Saanich Inlet, which reduces advective influences and increases local retention of zooplankton populations.

This study demonstrates that the high resolution echo-counting approach utilized by OASIS can be a valuable technique for zooplankton studies. Echo counting offers a distinct advantage over echo integration in that a single highly reflective scatterer cannot be misinterpreted as large numbers of weak scatterers (Stanton et al., 1994). The ability to detect changes in the size–frequency distribution of euphausioids from large numbers of acoustic reflections opens the possibility of identifying size-dependent behaviors (e.g. De Robertis et al., 2000) as long as distortions in the TS distribution due to the trimming algorithm are considered. Consistent detection of larger-bodied zooplankton indicates that is possible to track an animal’s movements by linking together consecutive acoustic positions (McGehee and Jaffe, 1996; Jaffe et al., 1999). The ability to distinguish fish and zooplankton allows interpretation of the potential for predator-prey interactions on the basis of fine-scale spatial distributions. It is important to recognize that the ability to distinguish between fish and zooplankton or to estimate zooplankton body size depends on factors such as acoustic frequency and plankton community composition, and that TS
relationships must thus be re-established for each investigation. Although these results demonstrate the utility of three-dimensional sonars such as OASIS for echo counting, they also emphasize the need for independent verification of the identities of acoustic targets.

**Acknowledgements**

D. McGehee kindly modified his scattering model for use in this paper. A. Townsend assisted with euphausiid identifications, and R. Rosenblatt assisted with fish identifications. I am indebted to M. D. Ohman and J. S. Jaffe for their guidance and their support of this work. C. Schell and E. Reuss developed a portion of the data processing methodology. This work was supported by NSF grant OCE94-21876 awarded to M. D. Ohman and J. S. Jaffe, and a NSF graduate fellowship to A. D. R.

**References**


Validation of acoustic echo counting for studies of zooplankton behavior