Title
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Journal
Deep-Sea Research Part II: Topical Studies in Oceanography, 45(7)

ISSN
0967-0645

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Publication Date
1998-07-01

DOI
10.1016/S0967-0645(98)00030-7

Peer reviewed

PERGAMON

Deep-Sea Research II 45 (1998) 1239–1253

DEEP-SEA RESEARCH
PART II

OASIS in the sea: measurement of the acoustic reflectivity of zooplankton with concurrent optical imaging

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Received 17 April 1997; received in revised form 13 February 1998; accepted 1 March 1998

Abstract

A new instrument Optical-Acoustic Submersible Imaging System (OASIS) has been developed for three-dimensional acoustic tracking of zooplankton with concurrent optical imaging to verify the identity of the insonified organisms. OASIS also measures *in situ* target strengths (TS) of freely swimming zooplankton and nekton of known identity and 3-D orientation. The system consists of a three-dimensional acoustic imaging system (FishTV), a sensitive optical CCD camera with red-filtered strobe illumination, and ancillary oceanographic sensors. The sonar triggers the acquisition of an optical image when it detects the presence of a significant target in the precise location where the camera, strobe and sonar are co-registered. Acoustic TS can then be related to the optical image, which permits identification of the animal and its 3-D aspect.

The system was recently deployed (August 1996) in Saanich Inlet, B.C., Canada. Motile zooplankton and nekton were imaged with no evidence of reaction to or avoidance of the OASIS instrument package. Target strengths of many acoustic reflectors were recorded in parallel with the optical images, triggered by the presence of an animal in the correct location of the sonar system. Inspection of the optical images, corroborated with zooplankton sampling with a MOCNESS net, revealed that the joint optically and acoustically sensed taxa at the site were the euphausiid *Euphausia pacifica*, the gammarid amphipod *Orchomene obtusa*, and a gadid fish. The simultaneous optical and acoustic images permitted an exact correlation of TS and taxa. Computer simulations from a model of the backscattered strength from euphausiids are in good agreement with the observed data.

1. Introduction

In recent years, there has been an expansion of the use of acoustic techniques for measuring abundance and biomass of zooplankton in pelagic ecosystems (Holliday

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0967-0645/98/$ - see front matter
PII: S0967-0645(98)00030-7

and Pieper, 1995; Simmonds and MacLennan, 1996; Wiebe et al., 1996). When compared with pumps and nets, acoustic techniques offer several advantages. These include the ability to assess zooplankton and nekton *in situ* at ranges of meters to hundreds of meters without perturbing the animals' behavior or spatial distribution; the capability for real-time data display and evaluation; and the sampling of organisms in considerably larger volumes of seawater. An additional advantage of the acoustic technology used here is that the FishTV sonar (Jaffe et al., 1995) permits three-dimensional localization of animal positions, beyond the simple abundance estimates usually made with echo integration techniques. With such measurements we can assess spatial dispersion patterns, as well as swimming velocities (McGehee and Jaffe, 1996), animal trajectories, and other aspects of animal behavior. On the other hand, a limitation of acoustic techniques taken alone is that they do not permit unique identification of the type of organisms responsible for the acoustic backscattering.

Optical imaging is an increasingly useful approach for identifying and enumerating pelagic animals *in situ*. High resolution video (e.g. Davis et al., 1996) and digital still cameras (e.g. Kils, 1992) have the capability to resolve the morphological details necessary to make satisfactory identifications of animals. Unfortunately, due to both resolution requirements and propagation loss (attenuation and spreading), optical techniques do not permit imaging at long ranges. They are thus restricted to sensing organisms in relatively small volumes of seawater at a limited distance from the optical sensors. In contrast, sonars with frequencies between 100 kHz and several MHz can easily be designed to record single reflections from small animals (0.5–40 mm) at ranges of tens of meters.

Due to the different advantages of acoustic and optical approaches for sensing zooplankton and other pelagic organisms, it seemed natural to combine the two techniques. Thus, optical imaging could be used to identify zooplankton and determine their three-dimensional orientation, while sonar could be used to assess the animals' abundance and behavior in a larger volume of seawater at greater range. The complication is creating an instrument that simultaneously has the ability to image animals optically and record the acoustically backscattered wave (BW) from the identical animal. This goal is particularly challenging because the BW from the animal should be recorded in the far field of the sonar. Fortunately, for our purpose, a very sensitive CCD camera will provide acceptable images at ranges that are far enough from the sonar source to obtain a good estimate of target strength. Although the conventional far field of the FishTV system begins at a range of 2.5 m, in practice we have been able to obtain reasonable values of target strength at 2 m. The essential trick (as illustrated schematically in Fig. 1 and described fully below) is to make the path length from the sonar to the target greater than from the camera to the target.

To relate acoustic backscattering to the abundance or biomass of animals in the ocean, shipboard or other enclosed systems have been utilized for measuring the backscatter characteristics of captured animals as a function of frequency and animal orientation (Wiebe et al., 1990; McGehee et al., 1998). Extrapolation of these tank measurements have been valuable in interpreting the survey results. Although many

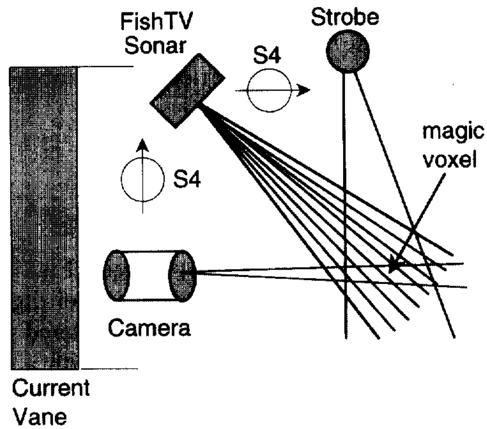

Fig. 1. A schematic diagram of the OASIS system illustrating the underwater sonar (FishTV), strobe (Nordelite 400), digital still camera (Benthos, Inc.), and ancillary sensors (InterOcean S-4 current meters, with temperature and salinity sensors). Distance from magic voxel to sonar is 1.9 m and to camera is 1.5 m.

of these tank measurements probably can be directly extrapolated to the field, the more rigorous way to measure animal backscatter characteristics would be to perform such a measurement *in situ* on unencumbered animals. In addition, tank measurements are particularly difficult to interpret for animals that use air to regulate their vertical position in the water column and thus change their acoustic impedance as a function of depth. Such animals include those fish with swim bladders and siphonophores. In the case of crustacean and other zooplankters, *in situ* confirmation of the tank measurements is needed.

Despite the need for the *in situ* measurement of backscatter characteristics of zooplankton, there has been little work in this field. One notable exception concerns the euphausiid *Euphausia superba* (Hewitt and Demer, 1991; Hewitt and Demer, 1996), where the monospecific nature of large krill swarms has permitted the *in situ* estimation of target strength, although multiple echoes were used for this purpose. Since the orientation of single animals was not measured, euphausiids were assumed to be randomly distributed when measured in lateral view. In ecosystems populated by a diverse taxa, the measurement of *in situ* target strengths would be vastly easier to interpret if the exact animal from which the sound wave was backscattered were known. The OASIS technology that we describe here is valuable for such identifications, in addition to its more general use as a tool for *in situ* behavioral studies.

In this article, we report the first successful optical imaging of underwater animals with concurrent measurement of the acoustic backscattered wave (BW) from the identical animal. Here we focus on animal target strength, which, to date, has been the primary way that most investigators have characterized these backscattered waves. Our study site was Saanich Inlet, a fjord on the eastern side of Vancouver Island, British Columbia, whose bottom waters experience seasonal anoxia. Euphausiids aggregate in well-defined vertical layers in Saanich Inlet (Bary and Pieper, 1970;

Greenlaw, 1979; Mackie and Mills, 1983) and are often associated with the oxycline, especially in the daytime. These layers, combined with the relatively low species diversity of zooplankton, made this fjord particularly suitable for our initial OASIS deployments.

Methods

The four main components of the OASIS package (Fig. 1) are an underwater multibeam sonar imaging system (FishTV), an underwater digital camera (Benthos, Inc.), a pair of Interocean S-4 current meters, and a red-filtered strobe light (Nordelite 400). All instruments are mounted on an alloy frame with a vane to maintain the sensors oriented into the flow. The sonar system is described in Jaffe et al. (1995), however, a brief description is presented here.

In order to collect a three-dimensional backscattered image, FishTV uses a set of 16 rectangular transducers of size 9 cm × 1 cm, 8 to transmit and the other 8 to receive. Operating at a frequency of 445 kHz $\pm$ 12.5 kHz, experimental evidence indicates that the system can collect backscattered sound waves from single animals as small as 1 cm (euphausiids) at a range of approximately 10 m. A three-dimensional image is formed by sequentially pulsing the transmitting transducers and receiving on all of the 8 receivers simultaneously. The half power points of the transducers is approximately $2^\circ \times 20^\circ$, and, using the product theorem for imaging systems, the approximate angular resolution of the system is $2^\circ \times 2^\circ$. The range sampling of the system is 7.5 mm, and the actual range resolution of the system is 2.5 cm. FishTV records an $8 \times 8 \times 512$ image at frame rates as high as 4 frames/s, which is the time varying response of the BW on the 8×8 grid of transducers. A single element within this volume is referred to as a voxel. For the purposes of the experiments described here, the FishTV system was used to image a volume of approximately 4.6 m^3 from a range of 1.9 to 5.7 m from the sonar.

The camera used for this study was a sensitive digital CCD camera manufactured by Benthos Inc. (Falmouth, MA). The camera incorporates a Kodak CCD chip with 1524 × 1024 resolution. The optics were adjusted so that at a range of approximately 1.5 m from the camera, the field of view would be 15 cm × 10 cm. Image resolution was thus 0.1 mm. The camera was also equipped with a remote iris and focus attachment which permitted remote adjustment of these parameters. The strobe light used was rated by the manufacturer at 400 Ws with an exposure time of approximately 2–4 ms, depending on charge. The strobe was filtered with a long pass filter with a half-power cutoff wavelength of approximately 660 nm. Red illumination was used because the spectral sensitivity of the eyes of *Euphausia pacifica* decreases sharply at wavelengths above 546 nm (Boden and Kampa, 1965). In addition, a set of S-4 current meters, equipped with current velocity, temperature, pressure, and conductivity sensors was deployed, with one rotated by 90° to the other so that the three-dimensional velocity vector could be measured.

A modification was made to the FishTV system software so that a trigger pulse was generated when an animal producing sufficient backscatter passed within the correct

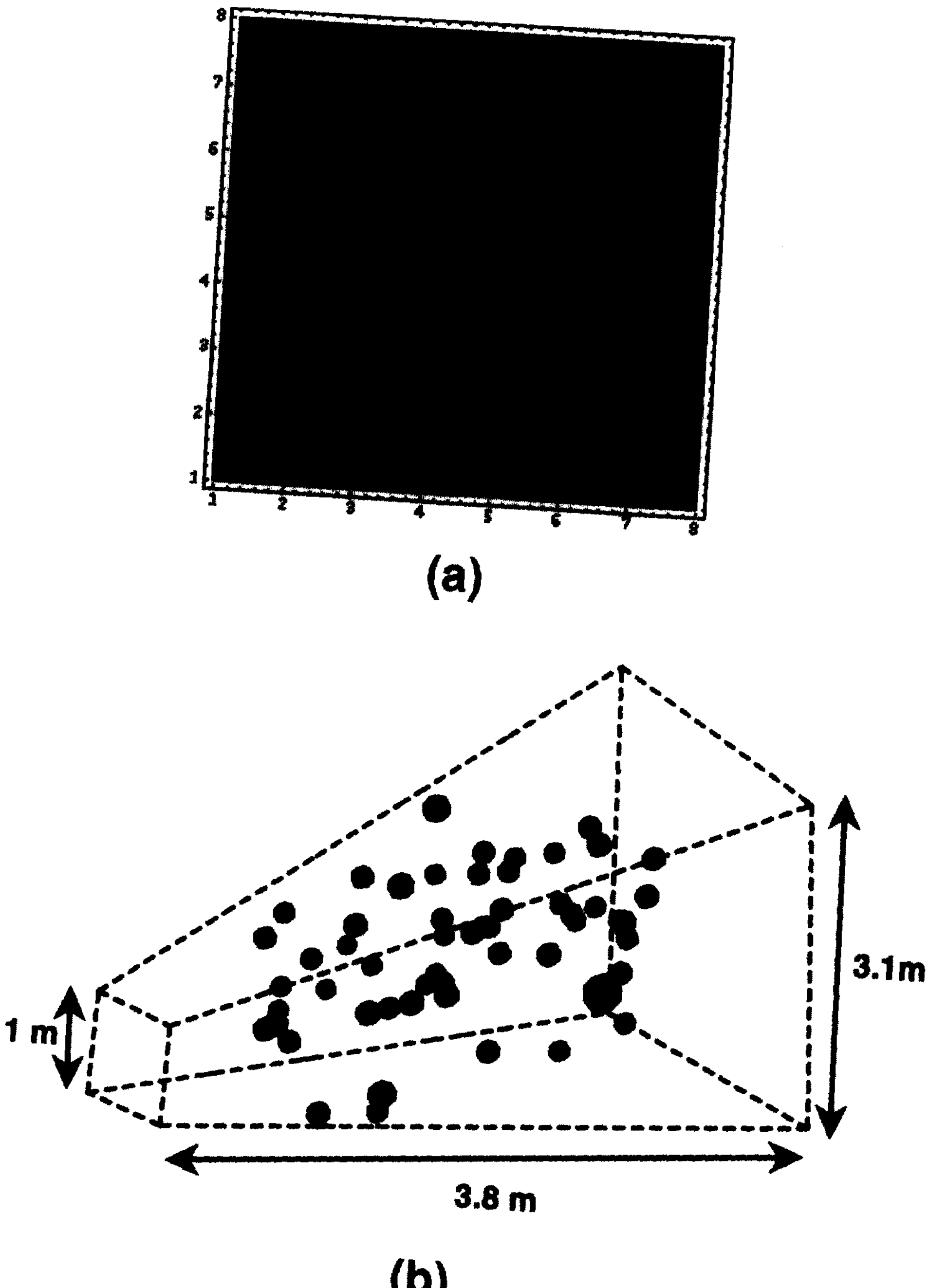

Fig. 2. (a) A contour plot illustrating acoustic backscatter intensity of a euphausiid at a single range slice. Contour levels are 0.99, 0.5, 0.15, 0.05, and 0.01 of the maximum value for the 8 × 8 matrix. (b) A three-dimensional representation of the field of view of the sonar system, illustrating the locations of targets as icosahedrons. Red indicates a fish target (− 54 dB), and blue indicates zooplankton targets (− 82 to − 70 dB).

three-dimensional location of the sonar where the camera, strobe lights and sonar were co-registered. We refer to this location as the "magic voxel". This pulse was used to trigger the simultaneous acquisition of an optical image by firing the strobe light, electronically activating the camera and opening the shutter. Using this technique, many optical images of single pelagic animals were obtained which could be uniquely associated with a sonar BW. The threshold of the BW was set sufficiently above the system noise level (effectively equivalent to a target strength at this range of -94 dB), so that noise would not trigger the camera. Post cruise analysis of the threshold indicated that animals whose target strengths exceeded -90 dB were triggering the acquisition of an optical image.

To estimate the target strength (TS) of the animals, the sonar system was calibrated using standard techniques. A USRD model E-8 transducer serial 67 was used to measure the acoustic energy radiated by the transducers in order to compute the system gains.

In the FishTV system the backscattered energy from a single animal is distributed over many voxels in both range and azimuth. This is due to both the diffraction limitation of the system and the fact that the system oversamples the responses both spatially and temporally so as not to alias the data. Animal target strengths were estimated from the sonar images by taking the largest return from the group of voxels from the single animal which triggered image acquisition and converting the recorded voltage to decibels. A contour diagram of a single 8×8 range slice from the system is shown in Fig. 2a. Simulations of the bias introduced by this process indicate that the target strengths, judged in this manner will all be lower than or equal to the true target strengths with the bias being greatest when the animal is located between sonar beams. The resulting target strengths may be as much as 10 dB too low, but more commonly the underestimation of the target strength will be 3–5 dB. Although more advanced algorithms for estimation of TS and animal spatial location are under development, they were not used in the TS estimates presented here. In order to insure that the target strengths computed were not from side lobes reflected off an animal outside the field of view of the system, all of the sonar data were inspected to insure that the maximum value of the backscatter was at least one voxel away from all of the edges of the three-dimensional volume.

OASIS deployments and zooplankton sampling were conducted at station S9 (48°34′N, 123°30′W) in Saanich Inlet on 8–13 August 1996. The bottom depth was 226 m, and the depth where dissolved oxygen decreased below 0.5 ml/L in vertical profiles was 80 m. Target strengths and digital images presented here were acquired while the OASIS package was held at a fixed depth, with the research vessel R.G. Sproul tethered to a mooring. Zooplankton were sampled with a 10 net, 333 μm mesh, 1 m^2 MOCNESS (Wiebe et al., 1985) and preserved in 3.7% buffered formaldehyde.

Lengths of organisms in the digital images acquired by the OASIS camera were measured using Image Pro Plus 2.0. software (Media Cybernetics, 1996). The standard length of the fish was measured, euphausiids were measured from the center of the eye (rather than the rostrum, which was barely visible) to the end of the telson, and amphipods were measured from the base of the first antenna to the end of the telson. To account for variations in body curvature in amphipods and euphausiids as

Table 1
Regression relationships between body length and projected area or dry mass. Relation for fish is for juvenile walleye pollock from Harris et al. (1986)

Organism	X	Y	Relationship	r^2	n
Euphausia pacifica	L_4 (mm)	Dry mass (mg)	$y = 0.001x^{3.00}$	0.98	91
Euphausia pacifica	L_4 (mm)	Projected area (mm^2)	$y = 0.116x^2 + 0.02x$	0.96	399
Orchomene obtusa	L_4 (mm)	Dry mass (mg)	$y = 0.005x^{2.99}$	0.93	97
Orchomene obtusa	L_4 (mm)	Projected area (mm^2)	$y = 0.435x^{1.72}$	0.95	860
Fish	L_1 (cm)	Dry mass (g)	$y = 0.67e^{0.167x}$	0.76	68

a consequence of their swimming motions, total length was obtained from the summation of 4 line segments (L_4) along the axis of the body. Most animals were oriented approximately at right angles to the camera, but in some cases they were not and a correction for parallax was therefore necessary. The angle of departure from the plane of focus (θ) was estimated with the on-screen angle tool in Image Pro. After measuring L_4, both θ and total length measured as a single line segment (L_1) were recorded. The length corrected for parallax (L_{par}) was calculated using the relationship: $L_{par} = L_4 + ((L_1/\cos(\theta)) - L_1)$. For fish, which are essentially uncurved, L_1 is substituted for L_4. The correction is very sensitive to large θ, and approaches infinity as an animal is imaged head on. The correction is only justifiable here because values of θ are small.

Where part of an organism was truncated at the edges of an image, a corrected length (L_{corr}) was estimated, but only when more than half of an animal was visible. L_{corr} was then computed from the proportionality between observable morphological features and total length. To express zooplankton dimensions in units of area and mass, preserved *Orchomene obtusa* and *Euphausia pacifica* from MOCNESS samples were imaged with a CCD camera system in lateral view and L_4 was measured as above. Lateral projected area also was measured with an on-screen tool. Organisms were dried at 55° for 24 h. and weighed with a Cahn 29 electrobalance. The resulting regressions are listed in Table 1. For fish, the projected images were measured on screen and the relation between length and mass were obtained from previously derived relationships for juvenile walleye pollock (Harris et al., 1986).

3. Results

Upon deployment of OASIS it was immediately apparent that large, motile zooplankton were being detected without any evident avoidance responses. We were able to watch a shipboard real-time display of the position of acoustic reflectors as they were slowly advected toward the OASIS package, which was oriented into the flow by the current vane. As animals approached from a range of 6 to 2 m from OASIS, there was no visible avoidance. In qualitative inspections of single acoustic frames, there was no tendency for animals to be less abundant at the leading edge of the acoustic field. We subsequently obtained numerous digital camera images of active zooplankton and fish when they entered the magic voxel at an acoustic range of 2 m and optical range of 1.5 m (e.g. Fig. 3–5). We infer from these results that these organisms were not responding to the presence of the instrument package in the water column.

Inspection of the optical images revealed three categories of organisms for which we have concurrent optical and acoustic data; euphausiids ($n = 11$), gammarid amphipods ($n = 21$) and fish ($n = 8$). Comparison of the images with zooplankton collected in vertically stratified net samples allowed the identification of the amphipods as *Orchomene obtusa* and the euphausiids as *Euphausia pacifica.* Figs. 3–5 show representative *in situ* images of *Orchomene*, *Euphausia* and fish. The amphipod and euphausiid figures include a reference specimen imaged in the laboratory. The animals in the images are clearly identifiable, although a diagonal pattern of camera noise is present due to the extremely low light level of the reflected images. This is partly due to the fact that all of the illumination is in a strongly attenuating part of the spectrum (> 660 nm). In addition, the planktonic organisms are easily differentiated, as euphausiids are transparent and have reflective photophores at the base of the pleopods and eyestalks, while *Orchomene* are opaque and have a distinctive curvature. The fish were identified as members of the family Gadidae (codfishes) due to the presence of three dorsal fins visible in some of the images. From mouth shape, 3 separate dorsal fins, and a lack of a chin barbel, they are provisionally identified

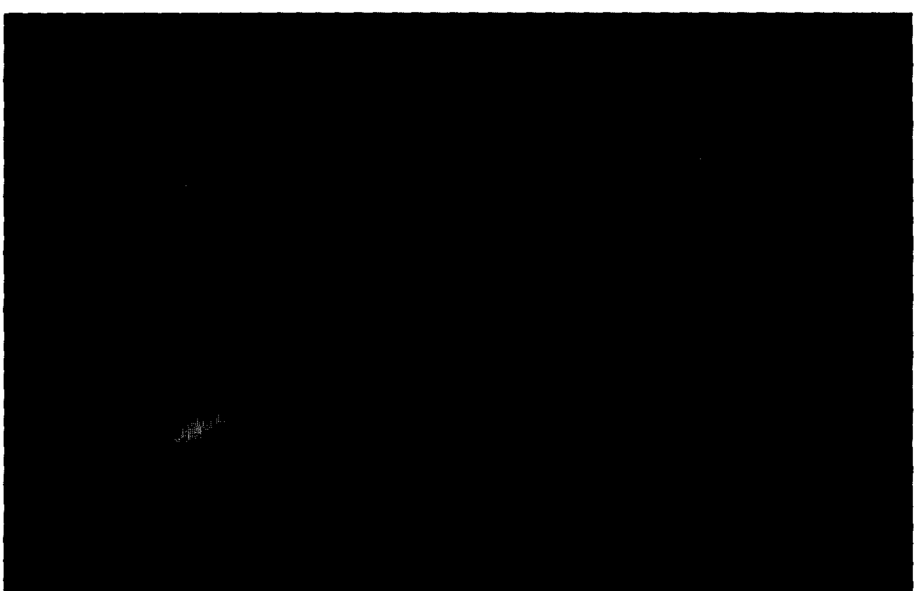

Fig. 3. Representative digital images of gammarid amphipods from Saanich Inlet. Panels A–C illustrate animals imaged at depths of 73–90 m between 1530 and 2137 on 9 Aug. 1996. Panel D illustrates an *Orchomene obtusa* imaged in the laboratory. Note that the body curvature in (D) is an artifact of preservation. Dimensions indicate total length.

Fig. 4. Representative digital images of euphausiids from Saanich Inlet. Panels A–C illustrate animals imaged at a depth of 40 m between 2056–2100 on 9 August 1996. Panel D illustrates an adult *Euphausia pacifica* imaged in the laboratory. Dimensions indicate total length.

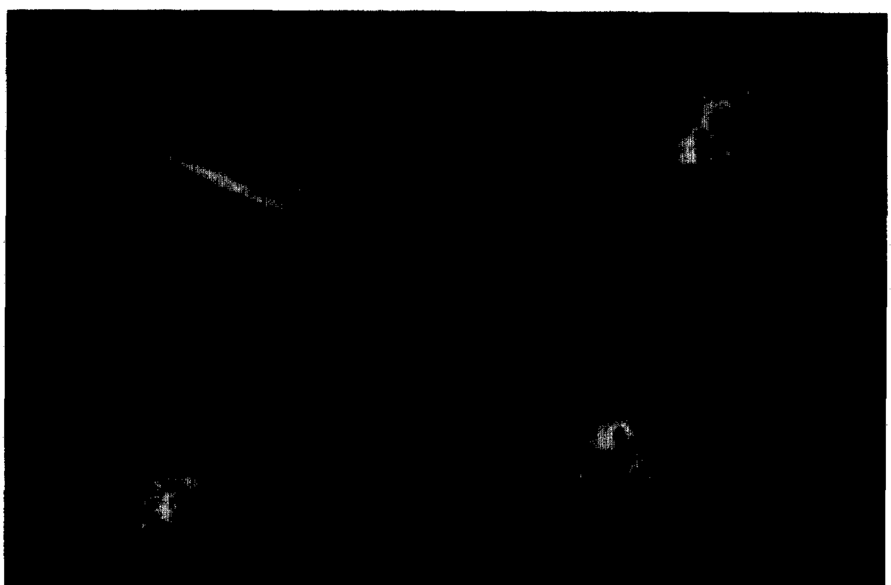

Fig. 5. Representative digital images of fish from Saanich Inlet (A–D), provisionally identified as *Theragra chalcogramma*. All fish were imaged at a depth of 40 m between 0210–0552 on 12 Aug. 1996. Dimensions indicate standard length, corrected for parallax.

(Hart, 1973) as juvenile walleye pollock, *Theragra chalcogramma*, which have been reported in Saanich Inlet (Bary and Pieper, 1970).

Fig. 6 illustrates the measured *in situ* target strengths as a function of animal type, dry mass, projected area and length. The results indicate that the target strengths for

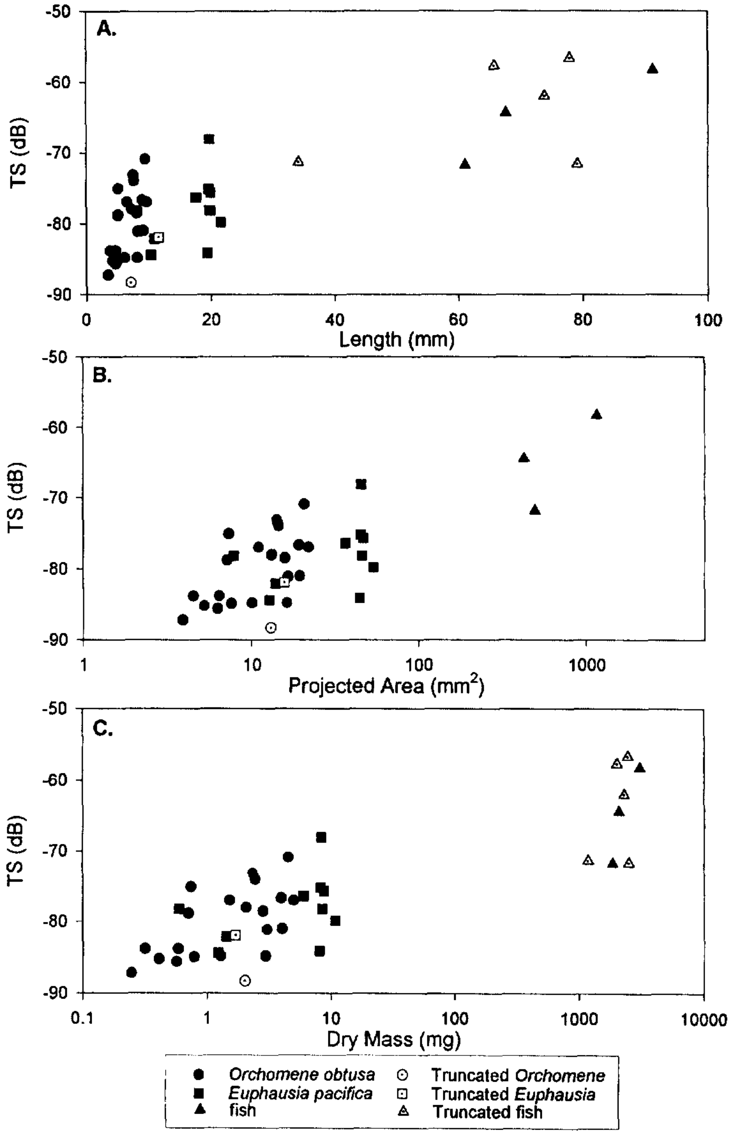

Fig. 6. Relationship between acoustic target strength (TS) and various measures of body size. (A) TS vs Length, (B) TS vs Projected area, and (C) TS vs Dry mass. Area and mass were obtained from the regressions in Table 1, except for fish, where projected areas were measured from *in situ* images of intact animals only. 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.

both zooplankters (*Orchomene* and euphausiids) were similar, while the fish target strengths were higher (on average). Some cautionary word should be mentioned in interpreting these fish target strengths, as the most intense returns were so strong as to come very close to saturating the system response.

In comparing the relationship between TS and the three different measures of animal size, the amount of variance explained by projected area ($r^2 = 0.57$) appeared similar to that explained by dry mass ($r^2 = 0.69$) or length ($r^2 = 0.67$). Despite considerable variability in TS at a given size (which is discussed further below), all 3 measures show that TS is not independent of body size ($p < 0.001$). We consider this first data set too limited to draw any conclusions regarding the properties of organisms that best account for target strengths as recorded by the FTV sonar. We also note that previous investigators (Demer and Martin, 1995; Holliday and Pieper, 1995) have documented the types of problems that can be encountered with narrow band sonar systems by assuming a fixed relationship between size and TS over a large size range of animals.

Since FishTV provides target strength as a function of position, the data also can be displayed in a volumetric representation. (Fig. 2b). The target strengths in Fig. 2 have been color coded from blue (− 82 dB) to red (− 54 dB). Based on the above correspondences between animal type and target strength, the more intense reflectors can be uniquely identified as fish. A temporal sequence of such images gives us the ability to enumerate both predator and prey, analyze spatial dispersion patterns, and examine the behavioral responses of the organisms to one another.

4. Discussion

Our first deployments of OASIS establish that the instrument is capable of imaging zooplankton and nektonic organisms at an optical range of 1.5 m and minimum acoustic range of 2 m, with no apparent alteration of the organisms' behavior. We are hopeful that refinements of the illumination system and camera will enable us to further increase the contrast ratio in optical images. With OASIS, optical images are collected intermittently to verify the identity of the organisms present in the field of view, while acoustic returns are obtained continuously at a frequency up to 4 Hz to permit continuous echo counting and tracking of animal behavior. While optical images provide very useful information concerning the types and orientation of organisms present, acoustic tracking can be done at a much greater range and in a much larger volume of seawater. The OASIS technology thus combines many of the best elements of both types of sensor.

In almost all cases, the zooplankton that triggered an identifiable optical image were oriented directly broadside (i.e. at right angles to the camera). Whether this was due an inability to see triggering animals in the head on view or simply to the dependence of target strength upon animal orientation was unclear. To explore the second possibility we ran a computer model of euphausiid scattering as a function of orientation (McGehee et al., 1998). The model uses a distorted wave Born approximation and was written to simulate sound backscatter from euphausiids in the 3–5 cm

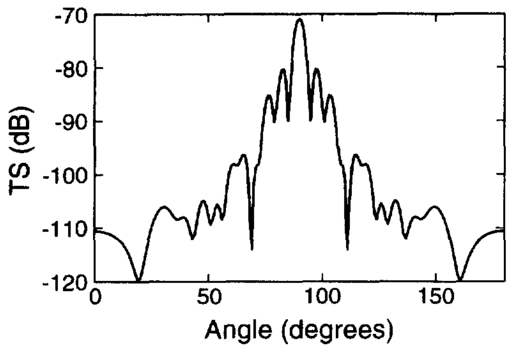

Fig. 7. TS as a function of orientation of a euphausiid to the transducer. 90° is broadside to the sonar. Modified from McGehee et al. (1997) for a 2 cm euphausiid at 435 kHz.

range at an acoustic frequency of 120 kHz. Since our animals are half the size (2 cm), but our insonification frequency is almost a factor of four greater (435 kHz), some caution must be used in interpreting the exact application of this algorithm to our data. Nevertheless, the simulations were in excellent agreement with our measured data and readily explain why animals were only observed broadside to the beam.

Fig. 7 shows the results generated from the model. When the 2 cm euphausiid is broadside to the beam (90°), a maximum target strength of − 70 dB is predicted. For fairly small rotations of the animal about the vertical axis, the predicted target strength of the animal decreases rapidly. When the animal is oriented at approximately 15° from a broadside view, the target strength of this adult euphausiid would drop below our acoustical target strength trigger of − 90 dB. It therefore is clear that almost all of the zooplankton were imaged in broadside view because of the steep dependence of target strength on angle. Presumably a model for the *Orchomene* would yield similar qualitative results.

One feature of the TS data is that observed target strengths are only weakly dependent on animal size. As pointed out by Demer and Martin (1995), when using narrow band systems it is not a entirely correct to use a linear inversion to obtain estimates of animal size from measured target strengths (cf. Greene et al., 1989; Greene and Wiebe, 1990). This is because the relationship between animal size and target strength is complex and nonlinear. In particular, there are deep nulls present in the backscatter spectrum whose location is a function of animal size (Demer and Martin, 1995). An additional cause of variation is the simple target strength estimation procedure used. The estimates of target strength using the procedure employed here are only accurate when animals are in the precise middle of the transmitting and receiving beams. The bias resulting from this procedure was estimated by simulating the measured target strengths from randomly distributed animals with identical target strengths. The results indicate that a spread of 3–5 dB would exist, due to our estimation procedure alone. It will be interesting to explore the effects of applying more sophisticated procedures for estimation of position and target strength to

OASIS data. However, the sensitivity of target strengths to several factors – especially orientation of the animals, as well as zooplankton taxonomic composition (Stanton et al., 1996) – suggests limitations to the achievable accuracy of acoustically determined estimates of zooplankton size distributions and biomass.

Several other observations may be useful to investigators who are contemplating using similar techniques. Large animals outside the optical field of view triggered the acquisition of an image because they reflected sound from the transducers' side lobes, resulting in a fair number of blank optical images. In a small number of the optical images multiple targets were imaged. These frames were discarded from consideration in estimating target strength. We note that the problem of multiple targets in the same range bin, which has plagued many target strength estimation procedures (Barange et al., 1996), can be easily remedied in our method by inspection of the optical images. Clearly, in high concentrations of animals, the probability of occurrence of these multiple images will be high, while in very low concentrations the frequency of multiple appearances of animals in the exact magic voxel location will be low. Therefore, the dimensions of the optical and acoustically imaged volumes should be optimized according to the expected densities of animals in different oceanic conditions.

In summary, we have introduced a combined optical and acoustic approach for *in situ* studies of zooplankton and nekton. The 3-D imaging sonar (FishTV) confers the ability to localize and track the positions of individual animals over time. The general advantages of this, as well as other acoustic methods, include the ability to insonify relatively large volumes of seawater at considerable distances from the sensor. Optical methods provide the ability to identify organisms, but in small volumes of seawater at very restricted ranges. The combined technology enables us to identify organisms, measure target strengths and orientations of freely swimming animals in their natural environment, and assess the behavioral characteristics of pelagic organisms in the absence of observer effects.

Acknowledgements

We thank the National Science Foundation (OCE94-21876) for providing the core funding to develop the OASIS system. In addition, the Office of Naval Research provided funds to purchase some of the equipment. A. D. R. was supported by an NSF predoctoral fellowship. We express our thanks to D. Van Holliday, Charles Greenlaw and Duncan McGehee of Tracor, Inc. for loan of the calibrated transducer and to Duncan McGehee for providing us a copy of his sonar backscatter/target strength model. We are grateful to all participants in the OASIS 1 cruise and the members of our engineering group (Greg Adelman, Kathy Lieberman, Mary Sisti and Ben Ochoa) for their efforts, to Annie Townsend for help with euphausiid identifications, H.J. Walker and C. Klepadlo for assistance with fish identifications, and the scientists and staff at the Institute of Ocean Sciences, Sidney, B.C. for their collegial assistance.

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