Quantitative Estimation of Zooplankton and Micronekton Biomass Using A High-Frequency Acoustic Method

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ABSTRACT

A 420 KHz echo sounding system was used to conduct acoustic surveys in the coastal waters of northeastern Taiwan. A two-step echo-integration method, that included the echo signal image processing method developed for this study as the second integration, was used to obtain the average adjusted volume backscattering strength for each echo-integration unit. In the acoustic surveys, IKMT and Norpac samplers were used to collect biological samples simultaneously to identify the species and size compositions and to estimate the biomass of zooplankton and micronekton. The acoustic data were compared to the biological data to determine the feasibility of using the two-step echo-integration method in estimating the biomass of zooplankton and micronekton. The results indicated that the echo signal image processing method developed in this study removed most signals oringinated from fish, as well as all those other than from zooplankton and micronekton. When the scattering layer of zooplankton and micronekton was obscured by fish echo signals, echo-integration without the image processing method caused 7.39 times overestimation of the zooplankton biomass. In the sound scattering layer composed of copepods or euphausiids as dominant species group, its average target strength differed significantly, and its response to sound waves was in different geometric, transition or Rayleigh scattering region.

Key words: Acoustic quick assessment method, Echo signal image processing, Zooplankton, Micronekton.

INTRODUCTION

Many species of zooplankton and micronekton are negatively phototactic organisms. They make vertical migration to the euphotic zone near the water's surface during the night and move to the deep hypophotic zone in the daytime. In both zones they act as food sources for many species of pelagic fish. Therefore, zooplankton and micronekton play an

important role in the food chain and biological production processes in the marine ecosystem. Some species, such as those belonging to Families Myctophidea and Euphasia, are used directly by humans. For the fisheries management, the estimation of biomass and distribution of these low trophic organisms are as important as the assessment of fish stocks.

The standing stock assessment of zooplankton and micronekton is tradi-

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tionally conducted by the net sampling method. However, this method have some disadvantages, including small sampling power, large variance, high labour costs and large bias. In recent years, the rapid development of electronic and computer sciences has enabled us to use an echo sounder to estimate the standing stocks of fisheries resources (Lee et al., 1990; Bondreau et al., 1992; Pedersen et al., 1992), and of zooplankton and micronekton (Pieper, 1979; Suzuki et al., 1984; Lee et al., 1989; Crawford et al., 1992). In the past, echo signals from fish were mixed with those of zooplankton and micronekton. We have developed an echo signal image processing method to filter out most of signals that do not belong to zooplankton and micronekton. This study compared the biomass of zooplankton and micronekton estimated by this echo signal image processing method with that from the net sampling method to determine the feasibility of using the acoustic assessment method in estimating the standing stocks of zooplankton and micronekton.

There is no clear boundary between zooplankton and micronekton. Generally, the organisms of intermediate body size and swimming ability between zooplankton and nekton are called micronekton (Nemoto, 1983). In this study most of the zooplankton and micronekton are mesoplankton (1-5 mm), macroplankton (5-10 mm), and magaplankton (>1 cm) (Jeng et al., 1991).

MATERIALS AND METHODS

I. Acoustic survey

The acoustic and net sampling surveys were conducted in the coastal waters of northeastern Taiwan, during September 10-12, 1991 and July 26-29, 1993, from the research vessel "Haifu" of the Taiwan Fisheries Reseach Institute. The water

depth in the survey area was less than 120 m. For the acoustic surveys, a 420 KHz Biosonic quantitative echo sounder (Model 102) was used. Each of the surveys was conducted both during sailing and drifting of the vessel (Fig. 1). When the vessel was sailing, the transducer was mounted on a tow body. It was towed by the derrick on the bulwark at a constant depth of 1-2 m to reduce the effect of surface reverberation caused by air bubbles. When the vessel was drifting, the transducer was set on the bulwark. The survey was conducted for 24 hours.

II. Biological sampling

During the acoustic survey, the IKMT and Norpac plankton samplers (nets) were used simultaneously at the depth of sound scatters in the water (Fig. 1). The IKMT sampler was 1.43 m \times 1.54 m at the net mouth, 7.6 m in total length, and 0.52 m in cod end diameter, with a 0.5 mm mesh size. The volume of water filtered through the sampler was calculated using a flowmeter attached at the mouth of its cod end. The IKMT sampler was used for horizontal towing. Each tow lasted 20-30 minutes at a speed of 3 knots. The towing depth was monitored by a SCAMMAR acoustic net condition monitoring system (Model 400-Trawl). Its sensor was attached to the mouth of the IKMT sampler. The net position was shown on a computer monitor and saved on a floppy disk at 10-second intervals. The Norpac sampler was 0.45 m in diameter at the mouth, 1.8 m in length, with a 0.35 mm mesh size. The volume of water filtered through the sampler was calculated using a flowmeter placed in the center of the mouth. The vertical sampling was conducted at a speed of 1.5 m per second. For each station, three samplings were conducted for the strata of 0-25 m, 0-50 m and 0-90 m. Changes

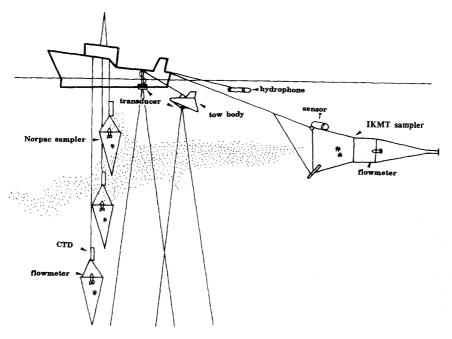
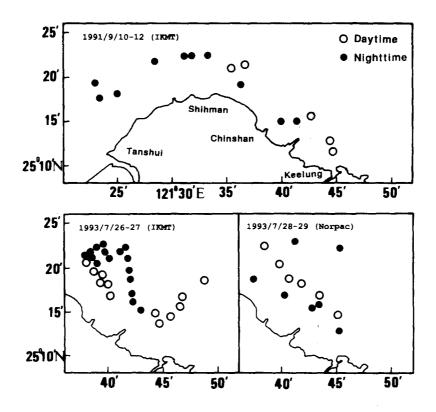


Fig. 1. A Sketch showing the instrumental arrangement in the acoustic survey and net sampling.



Phg: 2. Sampling stations for the IKMT and Norpac net sampling survey.



in the sampler depth during the sampling were recorded by the mini STD (SD200) attached at the mouth.

A total of 82 net samplings were conducted, including 43 IKMT and 39 Norpac tows (each station with three tows) (Fig. 2). The former had 20 tows in the daytime and 24 tows at night. The 39 Norpac tows were completed at 2-hours intervals for 24 hours. The samples collected were preserved in 5% formalin water solution and transferred to the laboratory within 72 hours after collection. For each sample, the weight (wet weight) was taken, the number of individual organisms were counted. The species of each individual was identified. The biomass (mg/m³) of each species was estimated.

III. Post processing of echo signal

As some fish were often mixed with zooplankton, their backscattering strength was larger than that of zooplankton and micronekton alone. Echo integrators, with fixed hardwares and softwares, available on the market today are unable to discriminate signals of zooplankton from those of fish, causing possible bias in the biomass estimation. Therefore, the echo signal image processing method was

developed in this study to remove the signals of fish.

The flow chart of the echo signal image processing method that converts original signals into sequential 640×480 \times 8 bit digital image data using an A/D converter is shown in Fig. 3. If fish echo traces were found in the digital image, the signals were removed. Plate 1A shows a section of an echogram obtained by the echo signal image processing method. Near the stratum of 40 m deep, there is a scattering layer of zooplankton and micronekton. Utilizing a digital image processing technique, we were able to filter out the images other than those of zooplankton and micronekton. Then, we used a linear transformation to convert the filtered images (Plate 1B) into voltage, and calculated the integrated voltage square (E) for each echo-integration unit (EIU) using the following equation:

$$E = \frac{1}{-} \sum_{i=1}^{I} \frac{1}{J} \sum_{j=1}^{J} V_{ij}^{2}$$
 (1)

Where I is the horizontal distance of EIU; J is the vertical depth of EIU; V_{ij} is the digital voltage value of internal positioning (i, j) in EIU. In this study, I

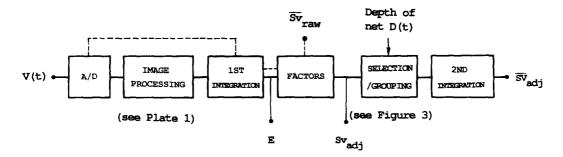


Fig. 3. A flow chart of the echo-integration method to transform original signals (V(t)) to average adjusted volume backscattering strength (\overline{Sv}_{adj}) (E, integrated voltage square; \overline{Sv}_{raw} , average volume backscattering strength calculated from original signals using a commercial echo integrator; D(t), track of net towing recorded by net recorder; dotted lines, processing steps for computing \overline{Sv}_{raw} value; solid lines, processing steps for computing \overline{Sv}_{adj} value).

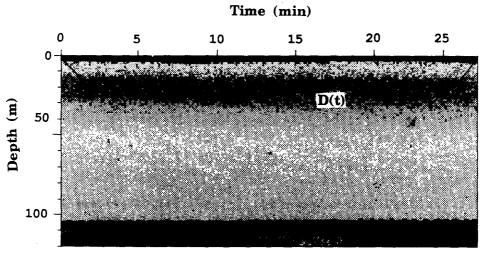
was set at 480 pulses (2 minutes) and J was at 5 meters.

Finally, E was transformed into volume backscattering strength Sv (dB/m³) for each EIU by calibrating parameters of echo sounder. The Sv inherits the density information of zooplankton and micronekton for UIV (Wu et al., 1989). As the value of Sv was calculated from filtered echo signals, it was considered the 1st stage integration and called adjusted volume backscattering strength, or Sv_{adi}.

IV. Comparison of acoustic data with biological data

In order to extract the acoustic signals

on the towing tracks of IKMT Norpac nets, the Svadj values from the 1st stage integration were used as input for the 2nd stage integration (Fig. 3). The paths of IKMT and Norpac towings were based on the recorded depth of the nets at the times of acoustic survey. Based on the echogram of the IKMT towing of July 28, 1993 (Fig. 4), the distribution of the scattering layer formed by zooplankton and micronekton was at a depth of 10-30 m. The track of net towing D(t) is shown by the solid line in the upper figure. The Sv_{adi} values along with the track of net towing were marked by the bold numerals in the lower table in the figure. They



Print-out of Svadi on the track of IKMT

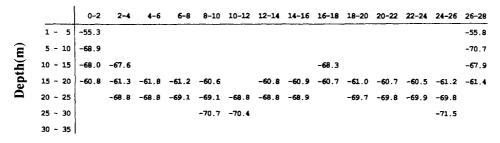


Fig. 4. An echogram of scattering layer along the survey line during the night of July 28, 1993 (D(t) showing the track of the towing IKMT sampler) (upper figure) and \overline{Sv}_{adj} values along the track (lower table).

were used for calculating the average adjusted volume backscattering strength (Sv_{adj}) by the following equation:

$$\overline{Sv}_{adj} = 10 \log \left[\Sigma \log^{-1} \left(0.1 \operatorname{Sv}_{adj} \right) / n \right]$$
 (2)

Where n is the number of EIU. The Sv_{adj} value was used as an index of the biomass of zooplankton and micronekton. In order to compare the Sv_{adj} value with the values obtained by the commonly used echo integrator, we also calculated the average volume backscattering strength (Sv_{raw}) from original signals using a commercial echo integrator without the echo image processing method and the second integration process. Since the biomass is proportional to integrated voltage square, the difference in estimated biomass of zooplankton by the two methods was expressed as a ratio (Rm) calculated by the following equation:

Rm =
$$\log^{-1} (0.1 \overline{Sv_{raw}}) / \log^{-1} (0.1 \overline{Sv_{adi}})$$
 (3)

According to the theory of Burczynski (1982), when the signals from uniform kind of scatters are randomly distributed, the (Sv_{adj}) is proportional to the reflected signals of the number of scatters. Consequently, the density of the scatters \underline{D} (inds/m³) and its relationship with Sv_{adj} are expressed by:

$$\overline{Sv}_{adj} = TS + 10 \log D$$
 (4)

where TS is target strength. When an echo sounder is used for estimating the biomass in the water, TS is an important scaling factor. When TS is unknown or from mixed species, Equation (4) is expressed by the following equation:

$$\overline{Sv}_{adj} = a + b \log \rho$$
 (5)

where ρ (mg/m³) is the biomass estimated by the net sampling.

The TS of zooplankton and micronekton may change with their size and sound frequency. The value of equivalent spherical radius (ka) describes the size of zooplankton acoustically (Greenlaw, 1979). It is calculated by the following formula (Johnson, 1987):

$$Ka = L \pi / 2 \lambda \tag{6}$$

where L is the total length (cm) of zooplankton and λ is wave length.

RESULTS

I. Identification of sound scatters

Two types of echo signals were identified in the survey area. The 1st type was the reflects of the scattering, the signals from a variety of fish (Plate 2A). The 2nd type was the reflects of zooplankton and/or micronekton mixed with fish (Plate 2B).

Ten species of zooplankton and micronekton were identified in the samples collected by the nets. They belonged to planktonic crustacean organisms, such as copepods and euphausiids, noncrustacean organisms, such as sagitta, pteropoda, tunicata, polychaeta, cndaria, fish larvae and fish eggs. Copepods constituted more than 50% of individuals in the samples (Fig. 5). They were the most important dominant group of zooplankton in the sound scatters. Euphausiids were the second most dominant group.

The frequency distribution of biomass of zooplankton and micronekton collected from the net samplings revealed that the nighttime catches were usually larger than the daytime catches (Fig. 6). The sound

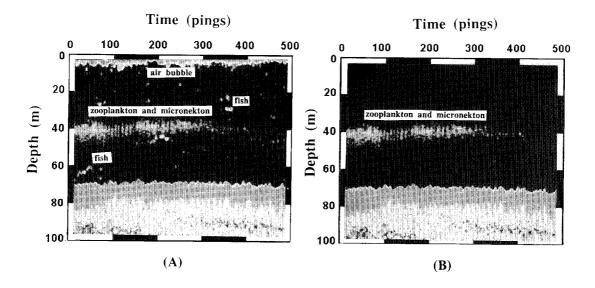


Plate 1. A): Acoustic signals were transformed to digital image data. Three kinds of echo traces are distinguished; air bubble, fish, and zooplankton and micronekton.
B): Image of scattering layer formed by zooplankton and micronekton after removing air bubble and fish by digital image processing.

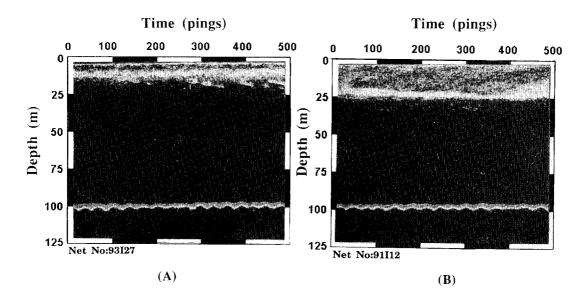


Plate 2. A): A typical echogram of Group 1 in which scattering layers are obscured by fish schools.

B): A typical echogram of Group 2 in which scattering layers were not obscured by fish schools.

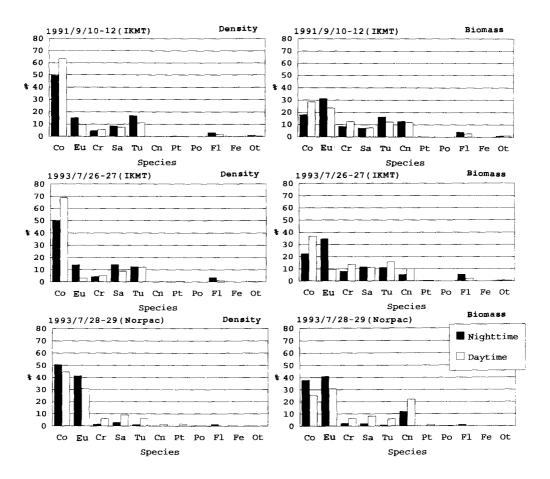


Fig. 5. The species composition by density and biomass estimated from biological samples (Co, Copepoda; Eu, Euphausiacea; Cr, Crustacea; Sa, Sagitta; Tu, Tunicata; Cn, Cndaria; Pt, pteropoda; Po, Polychaeta; Fl, Fish larvae; Fe, Fish egg; Ot, Others).

scatters show a more or less homogenous distribution during the daytime, with an aggregative distribution at night. The largest catches in the daytime were usually composed of copepods, while euphausiids were the major catch at night. The change in the biomass of species from daytime to nighttime indicated that the vertical distribution and migration of zooplankton differed greatly among species.

II. Estimation improved by echo signal image processing method

The solid line in Fig. 7 is the

estimated regression line between the $\overline{Sv_{adj}}$ values and biomass with the correlation coefficient (r-value) of 0.74 (P < 0.01), and the broken lines are the 95% confidence intervals. When the echo signals from a given water layer were processed with a direct stratified integration method, the distribution of $\overline{Sv_{raw}}$ was more or less random without significant correlation with the biomass (r=0.36, P>0.05). The distribution of $\overline{Sv_{raw}}$ values were divided into two groups; Group 1 included those values outside the 95% confidence intervals (21 samples) and Group 2 is those values inside these

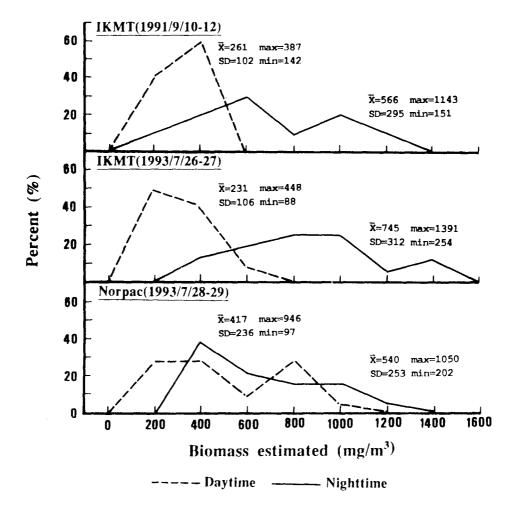


Fig. 6. Frequency distribution of biomass estimated by the IKMT and Norpac samplers.

intervals (61 samples). The former were found to be echo signals of fish in the scattering layer (Plate 2A) and the latter was from zooplankton and micronekton mixed with a small number of fish.

Table 1 shows the difference in the Rm values, estimated using Equation (3) between Group 1 and Group 2. The results show that the Rm value of Group 1 was 7.39, implying that when raw echo signals (Sv_{raw}) was used to estimate the biomass of zooplankton and micronekton, the result was 7.39 fold overestimation (Table 1). For Group 2, the Rm value was only 1.57 fold, much lower than that

of Group 1. The bias of the estimates for Group 2 was one fifth that in Group 1. Therefore, for estimating the biomass of zooplankton and micronekton by the echointegration method, it is necessary to remove fish echo signals. The echo signal image processing method developed in this study effectively removed most unwanted fish signals and greatly improved the relationships between echointegration outputs and the biomass of zooplankton and micronekton.

III. Estimation of single target strength of dominant species

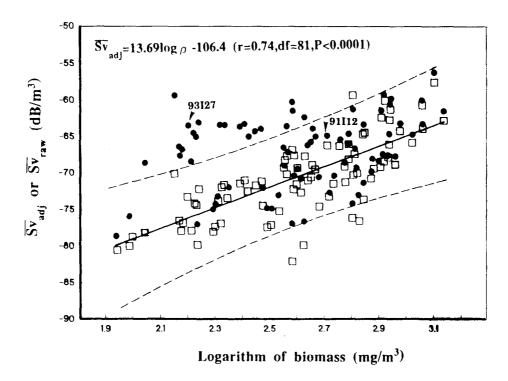


Fig. 7. The scattering plots of \overline{Sv}_{raw} and \overline{Sv}_{adj} against the logarithms of zooplankton biomass estimated by net sampler (solid line, the regression line; dash lines, 95% confident interval; \Box , the volume backscattering strength calculated by two-step echo integration process using echo signals obtained from image processing method (\overline{Sv}_{adj}); and \bullet , the volume backscattering strength calculated by the one-step echo integration using original echo signal (\overline{Sv}_{raw}).

Table 1. Ratios of estimated biomass (Rm values, mean and standard deviations (S.D.); N, number of samples) between Group 1 estimated with the image processing method and Group 2 without the image processing method.

Group	N	Mean	S.D. 2.31	
Group 1	21	7.39		
Group 2	61	1.57	0.35	
Total	82	3.02	2.86	

When the plankton species presents a uniform mode, Equation (4) is adequate for determining TS. Since the samples collected in this study were a mixture of species, only a few net samples contained copepods or euphausiids as

the dominant species group (>67.2%). Therefore, the two samples with these dominant species groups (15 samples) were used in the TS calculation (Table 2). For the body length of copepods, the metasome was measured, and the length of euphausiids was from occipital notch to telson (Johnson, 1977). The estimated TS were at $-84.5 \sim -74.2$ dB for euphausiids and $-101.7 \sim -93.6$ dB for copepods. Because the latter was smaller in size than the former, the difference between the two species groups was greater than 9.1 dB.

IV. Relationship between volume backscattering strength and dominant species biomass

The 15 net samples, with dominant

Sample No.	Dominant species group	Average body length (cm)	Sv _{adj} (dB)	Density (ind./m³)	TS (dB/ind.)	Ka
91101	euphausiacea (71.2%)	1.27	-69.6	8	-78.6	5.586
91102	euphausiacea (75.2%)	1.13	-67.6	11	-78.0	6.289
93102	euphausiacea (74.1%)	1.35	-58.8	35	-74.2	5.938
93109	euphausiacea (74.8%)	1.11	-73.1	12	-83.9	5.363
93110	euphausiacea (68.4%)	1.13	-72.5	16	-84.5	5.803
93112	euphausiacea (67.2%)	1.27	-69.6	8	-78.6	5.586
93N07	euphausiacea (70.9%)	0.66	-68.9	23	-82.5	2.903
93N14	euphausiacea (74.9%)	0.56	-69.0	24	-82.8	2.463
91106	copepoda (77.8%)	0.18	-73.2	432	-99.6	0.792
91109	copepoda (74.1%)	0.17	-69.8	467	-96.5	0.748
93105	copepoda (69.4%)	0.19	-67.6	453	-94.2	0.835
93119	copepoda (77.3%)	0.19	-76.7	210	-99.9	0.834
93120	copepoda (81.4%)	0.23	-80.5	134	-101.7	1.012
93121	copepoda (79.5%)	0.21	-78.7	120	-99.5	0.924
93123	copepoda (74.0%)	0.21	-69.2	278	-93.6	0.923

Table 2. Estimated TS and Ka values for copepods and euphausiids (percentages in parentheses) from \overline{Sv}_{adj} and biomass data of zooplankton.

species euphausiids and copepods, were used to determine the relationship between the \overline{Sv}_{adj} and the total biomass. The linear relationships are:

Copepods:

$$\overline{Sv}_{adj} = 16.5 \log \rho - 110.7 \text{ (r=0.86,} \\ \text{df=6, P<0.05)}$$
 (7)

Euphausiids:

$$\overline{Sv}_{adj} = 22.0 \log \rho - 129.6 \text{ (r=0.90,} \\ \text{df=7, P<0.05)}$$
 (8)

Analysis of variance indicated that the two lines have similar slopes (F-value = 0.285, P>0.25) but different elevations (F-value = 22.50, P<0.0005). Because the TS of these two dominant groups of species differs greatly, the two regression lines show a great difference of \overline{Sv}_{adj} at the same biomass (Fig. 8).

DISCUSSION

Generally, when TS differs 3 dB, the total biomass estimated by the echointegration method differs by 100% (Rose, 1992). Since fish have larger bodies and swimbladders with higher TS values than those of zooplankton and micronekton, 7.39 times overestimation may occur if the scattering layer of zooplankton and micronekton is obscured by fish echo signals. Therefore, fish echo signals need to be removed when the echo-integration method is used for computing the biomass of zooplankton and micronekton.

Zooplankton and micronekton are usually smaller than fish in body size, but often have body lengths similar sound wave lengths. Their scattering characteristics vary by species and size, causing a wide variation in TS and having an immense impact on acoustic

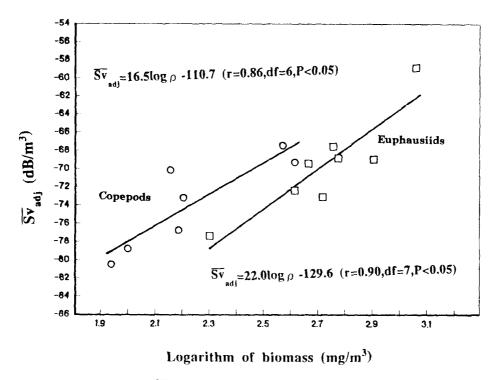


Fig. 8. Relationship between \overline{Sv}_{adj} (dB/m³) and the logarithms of zooplankton biomass (mg/m³) with dominant species groups of copepods and euphuasiids.

estimates. Fig. 9 shows the relationship between TS and Ka values for the biomass dominated by copepods and euphausiids, and the theoretical acoustic scattering characteristic of euphausiids derived from Johnson (1977). When the Ka value is equal to 1, the sound scatters in the transition region. When the Ka value is less than 1, the sound waves fall in the Rayleigh region. Within these two regions, TS varies with changes in sound frequency. When the Ka value is larger than 1, the sound waves fall in the geometric region, which indicates that the organism's TS has nothing to do with sound frequency, but is proportional to its body length (Greenlaw, 1977; Johnson, 1977; Macaulay, 1978; Greenlaw, 1979; Holliday and Pieper, 1980; Cochrane et al., 1991; Chu et al., 1992). In Table 2, the Ka values of euphausiid are between 2.463 and 6.289, while Ka values of copepods are mostly less than 1. As the 420 KHz sound scattered on the different regions (Fig. 9), two dominant species groups had different values of TS.

In this study, 15 of the total 82 net samples had copepods or eupdausiids as dominant species (Table 2). The others were mixtures of different species. Figure 10 shows the relationships between total biomass and \overline{Sv}_{adj} for daytime and night-time surveys in 1991 and 1993. Except for the 1991 relationships of daytime sample, which were not significant, the regression lines of the other five samples showed significant linear relationships. The surveys were conducted in the shallow waters where zooplankton and micronekton were more or less evenly distributed, suggesting that it is feasible

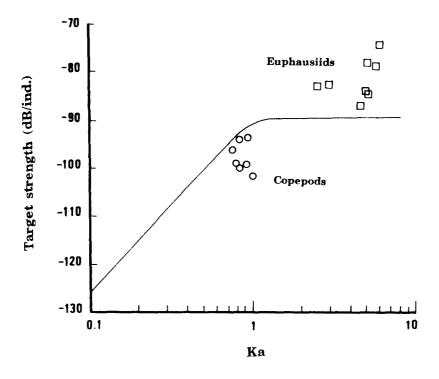


Fig. 9. Relationship between target strengths (TS) and Ka values for copepods and euphuasiids (solid curve, the theoretical acoustic signature of euphausiids from Johnson, 1977).

to apply the echo sounder to estimate biomass of unsorted plankton.

Of the five daytime net samples obtained in 1991 (Fig. 10), two samples (Nos. 91106 and 91109) had copepods as the dominant species and one sample (No. 91101) was dominated by euphausiids. This indicate that these five samples were obtained from a zone of an assemblage of the same species group. As the regression line was different from other five regression lines, the assamblages formed by different dominant species with great discrepancy of TS would cause bias of biomass estimation. As a result, the larger the size of aggregation of a group of related species in the distinct water layer, the easier it was to estimate the total biomass of the species group using the direct echo-integration method.

A comparison of the five regression

lines in Fig. 10 (excluding 1991 daytime samples) indicated that these five lines have similar slopes (analysis of variance, F-value = 2.51, P>0.25) but different elevations (F-value = 8.12, P< 0.0005). Accordingly, these five regression lines were rewritten as the following equation:

$$\overline{Sv}_{adj} = a + 15.1 \log \rho \tag{9}$$

Since euphausiids aggregate after evening, the increase in TS caused the decrease of the elevation (a) in Equation (9) for the nighttime surveys as compared to that of the daytime surveys.

Because the surveys were conducted in the shallow water area along the coast, a group of related species assemblage of zooplankton is exceptional. In the study area, an even distribution of diverse

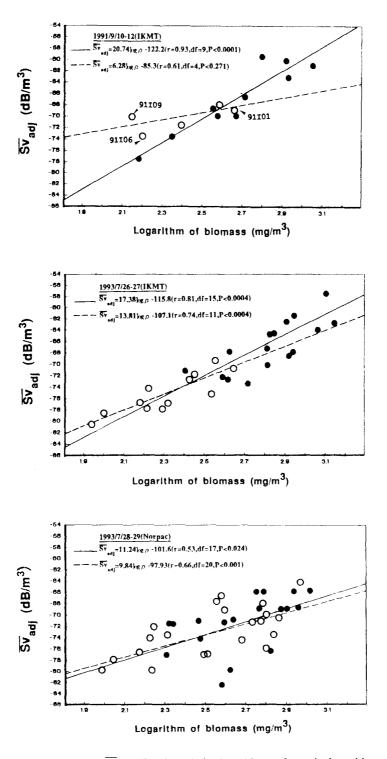


Fig. 10. Relationships between \overline{Sv}_{adj} (dB/m³) and the logarithms of zooplankton biomass (mg/m³) estimated by IKMT and Norpac samplers (solid circles and solid lines, nighttime samples; open circles and dash lines, daytime samples; numbers correspond to sample number in Table 2).

species is a common phenomenon. Therefore, Equations (7) and (8) are applicable to the water layer inhabited predominantly by either copepods or euphausiids. In order for the echo sounder to have a wider application in the biomass estimation of zooplankton and micronekton, it is recommended that the focus be placed on a water area with a distinct species group and scattering layer. To identify species by a single target scattering feature or by signal characteristics of the assemblage (Lee et al., 1990; Wu et al., 1992) through the echo signal image processing method, the biomass of single species or an assemblage of closely related species groups of zooplankton and micronekton can best be explored through direct use of the echo sounder system. However, there is still much room for further studies.

The sound frequency used in this study was 420 KHz. It has a high absorption coefficient (95 dB/km) and very low S/N ratio for deep waters. This could produce a poor quality of video images and errors generated from the deep water layers. If future exploration is to be carried out in deeper water, it is therefore recommended that a low frequency echo sounder be used. As for 420 KHz sound wave, the echo signals of 2 mm body length largely fall in the geometric region, where TS are proportional to the body length but not to the sound frequency. Below 2mm in body length, the echo signals fall in the transition region or Rayleigh region, where the TS vary with the sound frequency. In other words, the smaller target is sensitive to high sound frequency. Therefore, it is recommended that if net sampling in the water area or layer is difficult, multi-frequency echo sounders can be used for best results in determining plankton biomass and distribution (Holliday et al., 1989). This area also requires further studies.

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利用高頻聲探評估浮游動物及 微游泳動物生體量之研究

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本研究利用420KHz之聲探系統在本省東北部沿岸漁場進行探測,以影像處理法將浮游動物以及微游泳動物外之信號濾除,並利用二階段回訊積分法計算各水層修正後之平均體積散亂反射强度 \overline{Sv}_{adj} 值 (dB/m^3) 。聲探計測時並實施現場之浮游生物網採集,以採集所得之生物種類組成、豐度、密度等資料,與聲探回訊積分資料比對,探討聲探評估法直接推測浮游生物豐度的可行性。結果顯示本研究發展之聲探回訊信號處理法可去除魚類之反射回訊,擷取屬於浮游動物及微游泳動物之反射回訊。若浮游動物及微游泳動物的散亂層中之魚類信號未予以濾除,則聲探積分法所評估之生體量平均約高估7.39倍。網具採集顯示水層中之主要反射主體爲橈腳類及磷蝦類,其平均標物反射强度差異極大,且對420KHz聲波之反射座落在不同的反射特性區。

關鍵詞:超音波迅捷評估法,聲探回訊影像處理,浮游動物,微游泳動物。



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