Title: Measurements of density contrast and sound-speed contrast for target strength estimation of *Neocalanus* copepods (*Neocalanus cristatus* and *Neocalanus plumchrus*) in North Pacific Ocean

Running Title: Density, sound-speed contrasts, and target strength of *Neocalanus* copepods

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Abstract: The mass density and sound-speed contrasts against surrounding seawater (*g* and *h*, respectively) of *Neocalanus* copepods (*N. cristatus* and *N. plumchrus*) were measured in 2006 and 2007 to compute theoretical target strength (TS). The values of *g* ranged 0.997-1.009 in *N. cristatus* and 0.995-1.009 in *N. plumchrus*. There were no correlations in prosome length (PL) and *g*. The values of *h* ranged 1.006-1.021 in *N. cristatus* and 1.013-1.025 in *N. plumchrus*, and varied by change in temperature. TS were estimated by theoretical sound scattering model with the values of *g* and *h* that computed by temperature, salinity, and depth where specimens were collected. Regressions of the tilt averaged TS vs. PL were obtained at 38, 120, and 200 kHz. The averaged TS of *N. cristatus* and *N. plumchrus* at 120 kHz, which is widely used as high frequency, were ranged from -110.0 to -103.1 dB and from -121.4 to -109.7 dB. The higher the frequency was, the higher the value of averaged TS. The differences of TS at 120 and 38 kHz varied from 14.8 to 16.4 dB in *N. cristatus*, from 17.9 to 18.7 dB in *N. plumchrus*. The differences of TS at 200 and 120 kHz varied from 2.9 to 5.5 dB in *N. cristatus*, from 5.3 to 6.5 dB in *N. plumchrus*.

Key Words: Copepod, Density contrast, *Neocalanus cristatus*, *Neocalanus plumchrus*, Signal-to-noise ratio,
Sound-speed contrast, Target strength

Title:

北太平洋における*Neocalanus*属カイアシ類（*N. cristatus*および*N. plumchrus*）の理論ターゲットストレングス推定に必要な密度比，音速比の測定

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Abstract:

Neocalanus 属カイアシ類の理論ターゲットストレングス（TS）を推定するため，海水との密度比 *g*，音速比 *h* を測定した。*N. cristatus* と *N. plumchrus* の *g* の範囲は 0.997～1.009，0.995～1.009 で体長との相関はみられなかった。一方 *h* は 1.006～1.021，1.013～1.025 の範囲で水温による変化がみられた。採集深度における水温，塩分，深度から計算した *g*，*h* を用い，DWBA モデルにより周波数 38，120，200kHz における理論 TS と体長の回帰式を得た。

INTRODUCTION

Copepod is one of the most dominant groups of zooplankton communities in the ocean. In the subarctic Pacific and its marginal seas, *Neocalanus* species are the dominant large grazing copepods [1]. For example in Oyashio region, *N. cristatus* and *N. plumchrus* are important prey for fishes and whales the basis of the energy channel [2-3] in the surface layer during their growth from spring to summer. Their life cycles are annual or biennial, which included vertical migration down to 500 m or more following their growth in the surface layer during spring to summer [4, 5].

The collections of zooplankton are made with closing net to explain the vertical distribution. On the other hand, investigating the patch size and abundance in horizontal plane continuously, electric plankton counters [6], laser optical plankton counter [7], and video plankton recorder [8] are developed. These sampling methods are mainly purposed to collect specimens and/or to identify particles. Investigating more widely and easily, acoustic survey with quantitative echosounder [9] is proposed.

In acoustic survey, acoustic backscattering strength is measured and translated into the abundance by target strength (TS) of individual target. Thus, we need to know the TS of target species. For fish which have swimbladder filled with gas [10] and no swimbladder (e.g. *Todarodes pacificus* [11]), *in situ* or *ex situ* TS measurements are made with split or dual beam echosounder [9]. However, theoretical sound scattering models are generally used to obtain the TS such as small zooplankton [12-15]. The mass density contrast *g* and sound-speed contrast *h* against surrounding seawater are one of the most important factors to compute TS of fluid like zooplankton. For instance pairs of euphausiids' *g* and *h* are reported that the values of them are different from each species, even though the same species the values change seasonally [14-16]. In copepods, few pairs of *g* and *h* are reported. Greenlaw and Johnson [17] reported *g* and *h* of *N. plumchrus*, which was preserved, and Køgeler *et al*. [16] reported the value of *Calanus finmarchicus* but the stage of them are not specified. The sound-speed of copepods is thought to change as well as body-mass density, because the body-mass density of copepods changes in season and their life stage [18]. TS should be computed with *g* and *h* that appropriate for the season and the stage when and where the acoustic survey is occurred, because change the values of *g* and *h* affect the variation of theoretical TS. In addition, avoiding preserved specimen, live specimen should be used for measuring *g* and *h*, because the preserved samples lost their volume or weight and it would be caused an error of TS estimation [12, 14, 19]. In this study, we purposed (i) to measure the mass density contrast *g* and sound-speed contrast *h* of live *N. cristatus* and *N. plumchrus*, and (ii) to estimate TS by theoretical sound scattering model with the value range of *g* and *h*.

MATERIALS AND METHODS

Specimens were collected with a 2 × 2 m opening (mesh size 8 mm) framed midwater trawl (FMT) [20] aboard the R/V *Kaiyou Maru No.7* (499 ton) under the project "Research on the Assessment of Fisheries Stocks in the Waters around Japan" in June 2006 off shore Hiroo, Hokkaido, Japan (42°8.3'N, 143°37.7E). And with a NORPAC net (mouth diameter 45 cm, mesh size 0.35 mm) and an Isaacs-Kidd midwater trawl (mesh size 13 mm) aboard the R/V *Kaikou Maru* (860 ton) during a cruise of the Japanese Whale Research Program under Special Permit in the Western North Pacific-Phase II (JARPN II) in August 2007 in the Western North Pacific (44°56.9'N, 165°54.7'E) (Fig. 1). The net tows were conducted to target at a strong scattering layer observed on the echograms. Before or after net tow at each sampling point, oceanographic observations were conducted with CTD (Sea-Bird, SBE19) or XCTD (Tsurumi-Seiki, XCTD-1) to obtain the temperature and salinity. After towing of nets, following measurements were made with live copepods on board immediately.

A density-bottle method [12, 14, 21] was used for measuring body-mass density. In this method, body-mass density was determined by evaluating the buoyancy of each specimen. We prepared a series of solutions which mixed seawater with glycerol by 0.002 g/cm^3 steps. The zooplankton was anesthetized (FA100, 4-allyl-2-methoxyphenol) before measurement, then a specimen was placed in each solution in turn. The density of the specimen was determined the mean density of the last sinking solution and first floating solution. The measurement was followed Mikami *et al*. [17] and Yasuma *et al.* [21]. The mass density of seawater (ρ_{sw}) was calculated as a function of mean temperature, salinity, and pressure [22] that observed depth range where the net tows were conducted. Density contrast g was given by

$$g = \frac{\rho_{\text{animal}}}{\rho_{\text{sw}}} \qquad (1)$$

where ρ_{animal} is the mass density of specimen. After measurement, each specimen was preserved in buffered formalin with seawater (10% v/v) in a micro tube individually.

The speed of sound through the animal body was estimated by a time-average approach. The underlying principle is based on the measurement of the time-of-flight method [23]. The measurements of sound-speed were performed using an acrylic 'T-tube' [23] with two transducers mounted at both ends of the horizontal tube. The 5μs sinus pulse at 400 kHz was transmitted from one of the transducers and received the other. The transmitting time of the pulse between transducers was measured with a digital oscilloscope (LeCroy, 9314AM in 2006; Agilent, DSO6012A in 2007). We measured the transmitting time of seawater-copepod mixture (T_{total}), only seawater (T_{sw}), and the volume fraction of copepod in the mixture (V). These relations are given as

$$T_{\mathrm{total}} = (1-V)T_{\mathrm{sw}} + VT_{\mathrm{animal}} \qquad (2)$$

where T_{animal} is the passing time through the part of copepods' body in T-tube. The sound-speed ratio h is given by

$$h = \frac{T_{\mathrm{sw}}}{T_{\mathrm{animal}}} = \frac{C_{\mathrm{animal}}}{C_{\mathrm{sw}}} \qquad (3)$$

where C_{sw} and C_{animal} are the speed of sound through seawater and body of copepods, respectively. Because C_{sw} is known from T_{sw}, the measurement distance (200 mm), C_{animal} can be yielded. The sound-speed measurements were performed that the T-tube was bathed in a temperature-controlled tank, in which temperature was varied by about 0.5°C per 10 minutes. After measurement, the copepods volume V was measured by the displaced volume submerging specimens in a graduated cylinder. After measurement, specimens were preserved in buffered formalin with seawater (10% v/v). Both specimens that mass density and sound-speed measured were scaled prosome length (PL) with a microscope.

The sound scattering from zooplankton was estimated by Distorted Wave Born Approximation (DWBA, [24, 25]) model. The algorithm of Matlab (MathWorks, version 6.1.0.450) codes to estimate the target strength of deformed cylinder [13] was modified and given as

$$f_{\mathrm{bs}} = \int_{r_{\mathrm{pos}}} \frac{k_{\mathrm{sw}}^2 a_{\mathrm{c}}}{4k_{\mathrm{animal}}} \left(\frac{1+h^2}{gh^2} - 2 \right) e^{2ik_{\mathrm{animal}} r_{\mathrm{pos}}} \frac{J_1(2k_{\mathrm{animal}} a_{\mathrm{c}} \cos\beta_{\mathrm{tilt}})}{\cos\beta_{\mathrm{tilt}}} \left| \mathrm{d}r_{\mathrm{pos}} \right| \qquad (4)$$

where f_{bs} is the complex backscattering amplitude, relation to σ_{bs} given by $\sigma_{\mathrm{bs}} = |f_{\mathrm{bs}}|^2$, r_{pos} is the position along the axis of deformed cylinder, k is the acoustic wavenumber given by $k = 2\pi/\lambda$, where λ is the acoustic wavelength. The subscript sw refers to the surrounding seawater while the subscript animal refers to zooplankton, J_1 is a Bessel function of the first kind of order 1, a_{c} is the cross-section radius of the cylinder, and β_{tilt} is the local angle between the cylinder and incident wave. We digitized and obtained at least 79 sets of r_{pos} and a_{c} from each image of specimens, which measured body-mass density or sound-speed. In this study, the swimming angle of copepods was assumed randomly. TS (dB) is defined as $TS = 10\log_{10}(\sigma_{\mathrm{bs}})$ and the averaged TS was defined as the mean TS of every 1° .

RESULTS

Obtaining the acoustical parameters of seawater (i.e. sound-speed and mass density), the temperature and salinity were measured with CTD or XCTD. The acoustical parameters were calculated from the temperature, salinity, and depth where the net tows were conducted (Table 1). The specimens collected in 2006 were distributed in layers where deeper and lower temperature than that in 2007. In C_{sw} and the mass density of seawater, the differences between in 2006 and 2007 were negligible.

In 2006 and 2007, both the body-mass densities of *N. cristatus* and *N. plumchrus* were measured by density bottle method, and then the PL was measured individually (Table 2). We calculated ρ_{sw} with each parameter listed in Table 1. The entire specimen was belonged to copepodite stage 5 (C5) [26]. In 2006, PL of *N. cristatus* ranged 6.94-7.95 mm with mean value 7.38 mm, and *N. plumchrus* ranged 3.68-4.61 mm with mean value 4.09 mm. In 2007, PL of *N. cristatus* ranged 6.30-7.41 mm with mean value 6.92 mm, and *N. plumchrus* ranged 3.77-4.35 mm with mean value 4.03 mm. PL of *N. cristatus* in 2006 was different from that in 2007 ($P < 0.05$, Mann-Whitney *U*-test). The PL of *N. plumchrus* in 2006 was not different from that in 2007 remarkably ($P > 0.05$). In body-mass density, *N. cristatus* ranged from 1.026-1.037 g/cm^3 with mean value 1.030 (g = 1.003), and *N. plumchrus* ranged 1.022-1.036 g/cm^3 with mean value 1.026 (g = 0.998) in 2006. In 2007, *N. cristatus* ranged from 1.023-1.035 g/cm^3 with mean value 1.026 (g = 1.000), and *N. plumchrus* ranged 1.023-1.032 g/cm^3 with mean value 1.024 (g = 0.998). The body-mass density were significant different between species or year ($P < 0.05$). Each specimen in 2006 and 2007, there was no significant correlation between body-mass density and PL.

The sound-speed measurements were conducted by a time-average approach with the T-tube. The measurements of the sound-speed through *N. cristatus* ($C_{\mathrm{cristatus}}$), *N. plumchrus* ($C_{\mathrm{plumchrus}}$), and seawater (C_{sw}) were made between about 1 and 10 $^{\circ}$C (Table 3). The entire specimen was belonged to C5. In 2006, PL (mean ± *S.D.*) of *N. cristatus* and *N. plumchrus* were 7.29 ± 0.27 mm and 4.01 ± 0.16 mm. And in 2007, they were 6.90 ± 0.26 mm and 3.96 ± 0.14 mm, relatively. The PL of specimen was different between species or year ($P < 0.05$). The value of $C_{\mathrm{cristatus}}$, $C_{\mathrm{plumchrus}}$, and C_{sw} relatively increased with temperature. In 2006, sound-speed contrast h of *N. cristatus* and *N. plumchrus* ranged 1.006-1.011 and 1.013-1.025. In 2007, they ranged 1.014-1.021 and 1.018-1.024, relatively. The values of sound-speed contrast h were determined that the nearest temperature where nets were towed, and highlighted in bold (Table 3).

Approximating to deformed cylinder, we digitized the shape of *N. cristatus* and of *N. plumchrus* in 2006 and 2007. TS were estimated by DWBA model with g and h given in Table 2 and Table 3. Typical examples of relationship between TS variation and body tilt angle at 38 kHz, 120 kHz, and 200 kHz computed with value of g and h in 2006, were shown in Fig. 2. The variation of TS vs. tilt angle showed peaks around 0° (dorsal) and 180° (abdominal) at broadside incidence at 120 kHz and 200 kHz. The difference of TS as a function of tilt angle was about 12 dB and 19 dB at 120 kHz and 200 kHz in *N. cristatus*. In *N. plumchrus*, the peaks also appeared at 120 kHz and 200 kHz, the difference of TS was about 3 dB and 10 dB. The values of TS vs. tilt angle at 38 kHz that varied smooth and did not show obvious peak. The values of TS at 38 kHz were about -119 dB and -131 dB in *N. cristatus* and *N. plumchrus*, respectively.

The relationship between PL and tilt averaged TS were shown in Fig. 3 and the equations of logarithmic regression were shown in Table 4. The PL range shown in Fig. 3 were as follows; 6.60-7.95 mm, *N. cristatus* in 2006 (a); 3.68-4.61 mm, *N. plumchrus* in 2006 (b); 6.26-7.69 mm, *N. cristatus* in 2007(c); 3.54-4.26 mm, *N. plumchrus* in 2007 (d). The higher the frequency was, the higher the value of averaged TS. The difference between the values of averaged TS at 38 kHz and 120 kHz ($\Delta TS_{120\text{-}38} = TS_{120} - TS_{38}$) in the entire *N. cristatus* were from 14.8 to 16.3 dB, on the other hand, from 17.9 to 18.6 dB in *N. plumchrus*. The values of $\Delta TS_{200\text{-}120}$ in the entire *N. cristatus* and *N. plumchrus* were from 2.9 to 5.5 dB and from 5.3 to 6.5 dB.

DISCUSSION

The focus of this study was to estimate the TS of *N. cristatus* and *N. plumchrus*. The value of TS was affected with change in value of *g*, *h*, and tilt angle of body at invariable frequency. We discussed the influence on TS that was caused by the value of *g* and *h* especially. Averaged TS at 200 kHz with a change in *g* was estimated by *h* (1.000-1.060 by 0.010 steps), and shown in Fig. 4. The same specimens as in Fig. 2 were used. In each *h*, averaged TS increased when the value of *g* increased, and in each *g*, averaged TS increased when the value of *h* increased. Areas enclosed with gray-dotted line correspond to the range of averaged TS estimated by the whole value range of *g* and *h* measured in this study. The sound scattering is occurred at the boundary between different elements of the acoustic impedance. The sound scattering does not occur when the product of *g* and *h* is one, because there is no difference of acoustic impedance between specimens and seawater. Therefore, the values of TS around enclosed area varied dynamically with a change in *g* and/or *h*. The value of averaged TS indicated that these areas had the difference of about 20 dB in *N. cristatus* and about 12 dB in *N. plumchrus* at the maximum, even if the same digitized shape was used respectively. Target strength differed by 20 dB that caused overestimate (more than 10^2 times) or underestimate (less than 10^{-2} times) to translate from acoustic backscattering strength to abundance. Measuring the local *g* and *h*, and estimating practical TS with theoretical model is important for fluid like zooplankton. However, the values of *g* and *h* of zooplankton were not described in the Oyashio region but *E. pacifica* [14, 15]. If the averaged TS of *Neocalanus* copepods were estimated by using the value of *g* and *h* in *E. pacifica* instead of their own value, the former (open circles numbered 3) were about 15 dB higher than the latter (filled circles numbered 1 and 2) in Fig. 4. Estimation of TS by using *g* and *h* might be distinguished the value in *E. pacifica* even if that inhabit in the same Oyashio region [27] from that in *Neocalanus* copepods. Target strength estimated by using *g* and *h* in *C. finmarchicus* (open circles numbered 4 and 5) and *C. hyperboreus* (open circles numbered 6 and 7) [16] are 1-6 dB higher than that in this study. The different genus might have different value of *g* and *h*, even if specimens were live copepod. Target strength estimated by using *g* and *h* in *N. plumchrus* (open circles numbered 8 and

9), which preserved in formalin, are given by Greenlaw and Johnson [17] are about 10-12 dB higher than that in this study. The value of body-mass density is different between live and preserved specimens, because the composition and condition of body would change by formalin [12, 14, 19]. Live specimens should be used for measurements. Estimating theoretical TS required the adequate values of g and h that derived from live specimens.

Although swimming angles of *Neocalanus* copepods were assumed randomly, the differences of TS as a function of tilt angle at 120 kHz and 200 kHz (Fig. 2) were not negligible. Thus, the validity of the hypothesis should be discussed. The values of g were almost one (Table 2), in other words, the *Neocalanus* copepods were neutrally buoyant in the layer where they were distributed. They need not swim upward or downward to keep their position against negative or positive buoyancy. In contrast, *E. pacifica* swim upward with a mean of 30.4^{o} and a standard deviation of 19.9^{o} [28], because body-mass density of them is higher than seawater [15]. *Neocalanus* copepods are known to carry out little or no dial vertical migration during growth in the surface layer from copepodite stage 1 to stage 5 [4, 5]. As they are plankton, they do not move like fish, so they would not exhibit constant distribution of swimming angle. Hence, swimming angle of *Neocalanus* copepods were assumed distributed randomly. On the other hand, we applied three sets of swimming angle distribution (mean ± standard deviation) -5 ± 10^{o} (head down like fish [19]), 0 ± 10^{o} (horizontal position), and 30.4 ± 19.9^{o} (head up like *E. pacifica* [28]) to *N. cristatus* whose difference of TS as a function of tilt angle at 200 kHz was the largest (Fig. 2). The value of averaged TS applied by three sets of swimming angles were -94.5 dB (-5 ± 10^{o}), -94.3 dB (0 ± 10^{o}), and -98.8 dB (30.4±19.9^{o}) though averaged TS (random) was -100.1 dB. The swimming angle distribution certainly had effect on TS estimation. Thus, direct observations are needed to obtain information about swimming angle as a future work.

Now, *N. cristatus* and *N. plumchrus* are known to migrate from near sea-surface to bathypelagic zone [4, 5] where undetectable by echosounders at high frequencies like 120 kHz, 200 kHz or higher. Although the acoustic survey of zooplankton required high frequencies, the higher the frequency is, the larger the absorption loss of echo intensity is, and the detectable distance decreases. Thus, we discussed the relationship between detectable range derived from the signal-to-noise ratio (SNR) [30] and minimum number of *N. cristatus* and of *N. plumchrus* per cubic meter. SNR was expressed by Furusawa *et al.* [30] as

$$S_N = \frac{4\pi^3 \rho_{\mathrm{sw}} \eta W a_{\mathrm{t}}^{\,4} f^{5.8} \exp\left[-2\left(\pi a_{\mathrm{t}} f / C_{\mathrm{sw}}\right)^2 \theta^2\right] 10^{-0.2\alpha r}\, T_S}{C_{\mathrm{sw}}^{\,3} r^4 N_{P0}^{\,2} \Delta f} \qquad (5)$$

where S_N is linear variable of SNR (i.e. SNR = 10log(S_N)), ρ_{sw} is mass density kg per cubic meter of seawater only in Eq. (5), η is electro-acoustic efficiency, W is maximum electrical power, a_{t} is radius of transducer, f is frequency, θ is the angle measured from the beam axis, α is absorption coefficient, r is the detectable depth corresponds to distance between transducer and target species, T_S is the product of the number of target species and the linear scale of the averaged TS, N_{P0} is the noise spectrum extrapolated to 1 Hz, and Δf is the bandwidth of transducer. Restricting on beam axis where the SNR is the largest, θ was substituted zero. The averaged TS in 2006 shown in Fig. 4 were used. N_{P0} is an eigenvalue which was assumed to 145 dB [31]. Parameters used for computing of detectable depth with echosounder, which is EK60 (Simrad) equipped R/V *Kaiyou Maru No.7*, are listed in Table 5. R/V *Kaikou Maru*, which used in 2007, was equipped EK500 echosounder (Simrad) that was not equipped 200 kHz split-beam transducer. Therefore we used EK60 for estimating the detectable depth (Fig. 5). The detectable depth increased when the frequency increased. The gray-solid vertical line numbered 1 is the number of *N. cristatus* in C5 corresponded to 2 ind./m^3 cited from Kobari *et al*. [32]. In this case, the detectable depth was spread to 10 m at 38 kHz, 34 m at 120 kHz, and 52 m at 200 kHz, respectively. In the case of Nagasawa *et al.* [33] (gray-solid vertical line numbered 2), the number of *N. cristatus* in C5 was 11 ind./m^3, and the detectable depth was 15 m, 50 m, and 73 m. In the case of Barraclough *et al*. [34] (gray-solid vertical line numbered 3), the number of *N. cristatus* in C5 is 93 ind./m^3, and the detectable depth was 26 m, 79 m, and 106 m. In the case of Kobari *et al*. [32] (gray-solid vertical line numbered 4), the number of *N. plumchrus* in C5 is 50 ind./m^3, the detectable depth was 10 m, 41 m, and 67 m. In the case of Kawamura and Hirano [35] (gray-solid vertical line numbered 5), the number of *N. plumchrus* in C5 was 745 ind./m^3, and the detectable depth was 20 m, 73 m, and 108 m, respectively.

N. cristatus and *N. plumchrus* are distributed patchily on horizontal plane near sea-surface during the foraging period. There are the remarkable contrasts of abundance between inside and outside of patches [34, 35]. Assuming the size of patches was smaller than the resolution of net tow, the number of copepods per cubic meter obtained from net tow was smaller than that in patches, because the number of copepods caught with net was divided by the volume filtered by net towing, regardless of the presence of the patches. This could be one of the reasons why the values reported by Nagasawa *et al.* [33] and by Kobari *et al*. [32] are smaller than the values reported by Barraclough *et al*. [34] and Kawamura and Hirano [33]. The former estimated the abundance of copepods by vertical net tow, on the other hand, the latter did with Longhurst-Hardy plankton recorder [6] or horizontal towing of net. However, the numbers of *N. cristatus* and *N. plumchrus* per cubic meter obtained by horizontal sampling that mention above are less than other copepods. For example, Kawamura [36] reported in *N. tonsus* from 3.3×10^2 to 2.4×10^4 ind./m^3, and Wiborg [37] reported in *C. finmarchicus* 4×10^7 ind./m^3. Although the number of *N. cristatus* and *N. plumchrus* per cubic meter in their patch might be more than reported by Barraclough *et al*. [34] and Kawamura and Hirano [35], we presumed them as 93 ind./m^3 and 745 ind./m^3, respectively.

Next, we discussed the influence of TS on detectable depth. Figure 6 shows the difference between detectable depth calculated by the maximum and the minimum TS at 200 kHz where enclosed area with gray-dotted line in Fig. 4. In *N. cristatus*, there was 83 m difference between the detectable depth that calculated from the maximum TS which indicated 131 m, and the minimum TS which indicated 47 m. In *N. plumchrus*, there was 56 m difference between the maximum TS indicated 123 m and minimum TS indicated 67 m. The difference between detectable depths could not be neglected that calculated by typical TS and by minimum TS or maximum TS. Therefore, acoustical survey is needed the adequate TS of the target species inhabiting in survey area.

As a result, the patches of *N. cristatus* and of *N. plumchrus* were able to be detected within 79 m and 73 m at 120 kHz, and 106 m and 108 m at 200 kHz with the EK60. During the foraging period of *N. cristatus* and *N. plumchrus* were distributed near sea-surface, and if their patches were distributed shallower than 106 m, they could be detected at 200 kHz with echosounder equipped on ship-bottom. Moreover *N. cristatus* and *N. plumchrus* can be identified using two-frequency method [38] within 73 m from transducer except acoustical dead zone near sea-surface. However, the detectable depth varied according to TS of target species, components in patches (*e.g.* other copepodite stage except C5, other zooplankton), noise level, and marine environments.

The value of *g* and *h* influenced on TS of copepods remarkably, same as other fluid like zooplankton. The local value of *g* and *h* should be measured for theoretical TS estimation, which is the scale factor of acoustic survey, because the value of *g* and *h* was different from species and life stage. In addition, evaluating the abundance of weak sound scattering organisms like copepods, we should consider the detectable depth derived from SNR of echosounder. In this study, the detectable depth was restricted on beam axis. Revealing the number of copepods that were detected in the entire acoustical beam of echosounder was required as a future work.

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Table 1 Mean oceanographic conditions and acoustical parameters of seawater where the net tows were conducted

	Targeted specimen	Temp. (^{o}C)	Sal. (PSU)	Depth (m)	C_{sw}† (m/s)	ρ_{sw}‡ (g/cm^3)
2006	*Neocalanus cristatus*	2.9	33.3	152.5	1462.0	1.027
	Neocalanus plumchrus	2.9	33.3	161.0	1462.3	1.027
2007	*Neocalanus cristatus*	7.5	32.9	40.0	1478.3	1.026
	Neocalanus plumchrus	9.1	32.9	25.0	1484.3	1.026

†Sound-speed through seawater

‡Mass density of seawater

Table 2 Prosome length and body-mass density of *Neocalanus cristatus* and *Neocalanus plumchrus*. Density contrasts g is calculated from ρ_{sw} listed in Table 1

	Targeted specimen	n[†]	PL (mm)		Body-mass density (g/cm^3)		g
			Range	Mean ± *S.D.*	Range	Mean ±*S.D.*	
2006	*Neocalanus cristatus*	37	6.94-7.95	7.38 ± 0.23	1.026-1.037	1.030 ± 0.003	1.003
	Neocalanus plumchrus	49	3.68-4.61	4.09 ± 0.19	1.022-1.036	1.026 ± 0.003	0.998
2007	*Neocalanus cristatus*	52	6.30-7.41	6.92 ± 0.22	1.023-1.035	1.026 ± 0.004	1.000
	Neocalanus plumchrus	37	3.77-4.35	4.03 ± 0.12	1.023-1.032	1.024 ± 0.002	0.998

[†]Number of specimens

Table 3 Sound-speed through *Neocalanus cristatus*, *Neocalanus plumchrus*, and seawater measured by T-tube. The values of sound-speed contrast *h* were determined that the nearest temperature where nets were towed, and highlighted in bold

(2006) *Neocalanus cristatus* V = 35 % PL = 7.29 ± 0.27 mm				(2006) *Neocalanus plumchrus* V = 16 % PL = 4.01 ± 0.16 mm				(2007) *Neocalanus cristatus* V = 34 % PL = 6.90 ± 0.26 mm				(2007) *Neocalanus plumchrus* V = 34 % PL = 3.96 ± 0.14 mm			
Temp. (°C)	$C_{cristatus}$ (m/s)	C_{sw} (m/s)	h	Temp. (°C)	$C_{plumchru}$ (m/s)	C_{sw} (m/s)	h	Temp. (°C)	$C_{cristatus}$ (m/s)	C_{sw} (m/s)		Temp. (°C)	$C_{plumchru}$ (m/s)	C_{sw} (m/s)	h
10.0	1496.4	1487.3	1.006	10.0	1509.8	1487.3	1.015	10.0	1511.9	1486.8	1.017	10.1	1514.6	1487.1	1.018
9.5	1497.3	1485.2	1.008	9.5	1506.4	1485.2	1.014	9.5	1507.0	1484.8	1.015	9.7	1516.6	1485.6	1.021
9.0	1494.9	1483.1	1.008	9.0	1503.0	1483.1	1.013	9.0	1503.9	1482.9	1.014	**9.3**	**1510.3**	**1484.0**	**1.018**
8.5	1492.6	1480.9	1.008	8.5	1507.2	1480.9	1.018	8.5	1504.9	1481.0	1.016	8.5	1510.0	1481.0	1.020
8.0	1490.2	1478.8	1.008	8.0	1503.8	1478.8	1.017	8.0	1502.5	1479.0	1.016	7.9	1511.3	1478.6	1.022
7.5	1487.8	1476.7	1.008	7.5	1508.0	1476.7	1.021	**7.7**	**1502.1**	**1477.9**	**1.016**	7.5	1509.4	1477.1	1.022
7.0	1485.4	1474.6	1.007	7.0	1504.6	1474.6	1.020	7.0	1502.9	1475.2	1.019	7.0	1507.3	1475.2	1.022
6.5	1486.4	1472.5	1.009	6.5	1501.2	1472.5	1.019	6.7	1496.3	1474.1	1.015	6.6	1504.3	1473.7	1.021
6.0	1484.1	1470.4	1.009	6.0	1497.8	1470.4	1.019	6.0	1496.0	1471.4	1.017	6.1	1506.5	1471.8	1.024
5.5	1481.7	1468.3	1.009	5.5	1501.9	1468.3	1.023	5.6	1497.2	1469.9	1.019	5.7	1501.3	1470.2	1.021
5.0	1479.4	1466.2	1.009	5.0	1491.0	1466.2	1.017	5.0	1495.4	1467.6	1.019	5.1	1498.8	1468.0	1.021
4.5	1477.0	1464.1	1.009	4.5	1495.2	1464.1	1.021	4.5	1496.1	1465.7	1.021	4.5	1500.1	1465.7	1.023
4.0	1474.7	1462.0	1.009	4.0	1491.8	1462.0	1.020	4.0	1492.0	1463.8	1.019	4.0	1496.3	1463.8	1.022
3.5	1475.6	1460.0	1.011	3.5	1495.9	1460.0	1.025	3.5	1490.6	1461.9	1.020	3.7	1491.0	1462.7	1.019
3.0	**1473.3**	**1457.9**	**1.011**	**3.0**	**1485.1**	**1457.9**	**1.019**	3.0	1491.3	1460.1	1.021	3.0	1487.7	1460.1	1.019
2.5	1471.0	1455.8	1.010	2.5	1489.2	1455.8	1.023	2.5	1489.3	1458.2	1.021	2.1	1484.4	1456.7	1.019
2.0	1468.7	1453.8	1.010	2.0	1485.9	1453.8	1.022					1.5	1482.0	1454.5	1.019
1.5	1466.4	1451.7	1.010	1.5	1482.5	1451.7	1.021								
				1.0	1486.6	1449.7	1.025								

Table 4 Logarithmic regression of prosome length and target strength of *Neocalanus cristatus* and *Neocalanus plumchrus* given in Fig. 3

			38kHz			120kHz			200kHz		
		n	Approximate expression	R^2	*S.E.*	Approximate expression	R^2	*S.E.*	Approximate expression	R^2	*S.E.*
2006	*Neocalanus cristatus*	79	TS = 50.5Log(PL) - 164.3	0.38	1.07	TS = 35.2Log(PL) - 135.8	0.25	1.00	TS = 28.8Log(PL) - 126.3	0.36	0.63
	Neocalanus plumchrus	90	TS = 49.0Log(PL) - 162.9	0.38	1.09	TS = 41.8Log(PL) - 140.2	0.30	1.15	TS = 32.4Log(PL) - 128.5	0.22	1.06
2007	*Neocalanus cristatus*	146	TS = 39.8Log(PL) - 157.3	0.44	0.70	TS = 26.2Log(PL) - 130.0	0.25	0.72	TS = 24.4Log(PL) - 123.9	0.38	0.49
	Neocalanus plumchrus	136	TS = 47.7Log(PL) - 163.3	0.43	0.82	TS = 40.9Log(PL) - 140.9	0.35	0.84	TS = 32.7Log(PL) - 129.8	0.25	0.84

Table 5 Parameters for calculating detectable depth by EK60

frequency	f	(kHz)	38	120	200
Electro-acoustic efficiency	η	-	0.7	0.75	0.75
Maximum electrical power	W	(W)	2000	1000	1000
Radius of transducer	a_t	(m)	0.212	0.076	0.050
Band width	Δf	(Hz)	2425	3026	3088
Absorption coefficient	α	(dB/m)	0.010	0.028	0.041
individual TS of *Neocalanus cristatus*	-	(dB)	-119.5	-104.2	-100.2
individual TS of *Neocalanus plumchrus*	-	(dB)	-133.1	-114.8	-108.7

Fig. 1 Sampling location of *Neocalanus cristatus* and *Neocalanus plumchrus*. The crosses correspond to where the samplings were made in 2006 and 2007

Fig. 2 Typical TS variations of *Neocalanus cristatus* (left) and *Neocalanus plumchrus* (right) as a function of tilt angle, estimated by DWBA model at 38 kHz as dotted line, 120 kHz as dashed line, and 200 kHz as solid line, respectively. Positive value of tilt angle denotes head up and negative value denotes head down

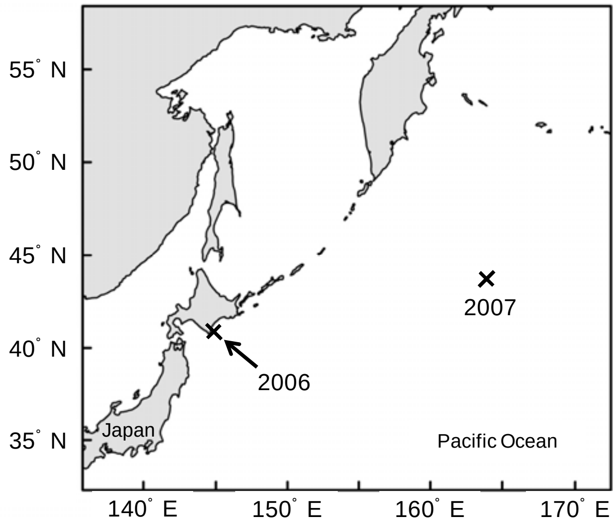
Fig. 3 Correlation between PL and averaged TS estimated by DWBA model of *Neocalanus cristatus* and *Neocalanus plumchrus* in 2006 and 2007. TS and logarithmic regression are indicated as follows; triangle and solid line at 38 kHz; square and broken line at 120 kHz; and diamond and dotted line at 200 kHz. Equations of regression line are given in Table 4. (a) *Neocalanus cristatus* in 2006, (b) *Neocalanus plumchrus* in 2006, (c) *Neocalanus cristatus* in 2007, (d) *Neocalanus plumchrus* in 2007

Fig. 4 Relationship between density contrast *g* and averaged TS at 200 kHz estimated in the same specimens as in Fig. 2 (*Neocalanus cristatus* in left and *Neocalanus plumchrus* in right). The values of sound-speed contrast *h* are given 1.000-1.060 by 0.010 steps. Areas enclosed with gray-dotted line are the range of averaged TS estimated by *g* and *h* measured in this study. The filled circles numbered 1 and 2 in both figures correspond to TS estimated by the values of *g* and *h* obtained in 2006 and 2007, respectively. Open circles are the referenced values of other zooplanktons; *Euphausia pacifica* caught in June (g = 1.046, h = 1.043) [27] as 3; *Calanus finmarchicus* caught in winter (g = 1.000, h = 1.029) and in May (g = 0.999, h = 1.021) [29] as 4 and 5; *Calanus hyperboreus* caught in winter (g = 0.999, h = 1.029) and in May (g = 0.997, h = 1.021) [29] as 6 and 7; *Neocalanus plumchrus* caught in summer (g = 1.047, h = 1.012) and (g = 1.047, h = 1.006) [31] as 8 and 9

Fig. 5 Relationship between detectable depth and number of copepods per cubic meter when SNR = 10 dB in *Neocalanus cristatus* (left) and *Neocalanus plumchrus* (right). The black lines (dotted line as 38 kHz, dashed line as 120 kHz, and solid line as 200 kHz) correspond to the minimum number of copepods that can be detected by EK60 at each depth. The gray-solid vertical lines numbered 1, 2, 3, 4, and 5 are the number of copepods (ind./m^3) referenced from follows; 2 ind./m^3 [46] as 1; 11 ind./m^3 [47] as 2; 93 ind./m^3 [48] as 3; 50 ind./m^3 [46] as 4; 745 ind./m^3 [49] as 5

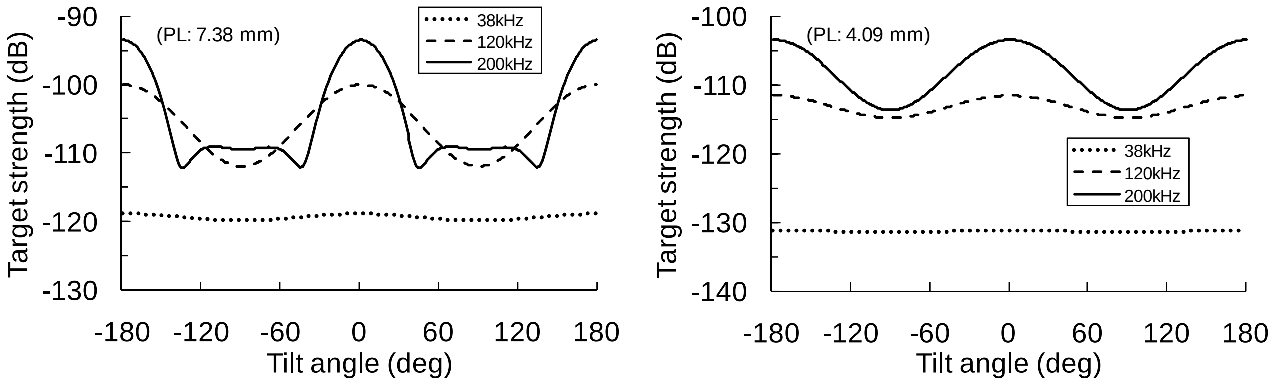
Fig. 6 Relationship between detectable depth and number of copepods per cubic meter when SNR = 10 dB in *Neocalanus cristatus* (left) and *Neocalanus plumchrus* (right) at 200 kHz. The black-solid lines are typical TS correspond to detectable depth at 200 kHz shown in Fig. 5, long-dashed lines and dash-dotted lines correspond to the detectable depth that calculated with the maximum and the minimum TS within enclosed area with gray-dotted line in Fig. 4. Gray-solid vertical lines are the number of copepods per cubic meter assumed 93 ind./m^3 in *Neocalanus cristatus* and 745 ind./m^3 in *Neocalanus plumchrus*, respectively

Fig. 1



55° N
50° N
45° N
40° N
35° N
2007
2006
Japan
Pacific Ocean
140° E
150° E
160° E
170° E

Fig. 2



Target strength (dB)
(PL: 7.38 mm)
38kHz
120kHz
200kHz
-90
-100
-110
-120
-130
-180 -120 -60 0 60 120 180
Tilt angle (deg)
Target strength (dB)
(PL: 4.09 mm)
38kHz
120kHz
200kHz
-100
-110
-120
-130
-140
-180 -120 -60 0 60 120 180
Tilt angle (deg)

Fig. 3

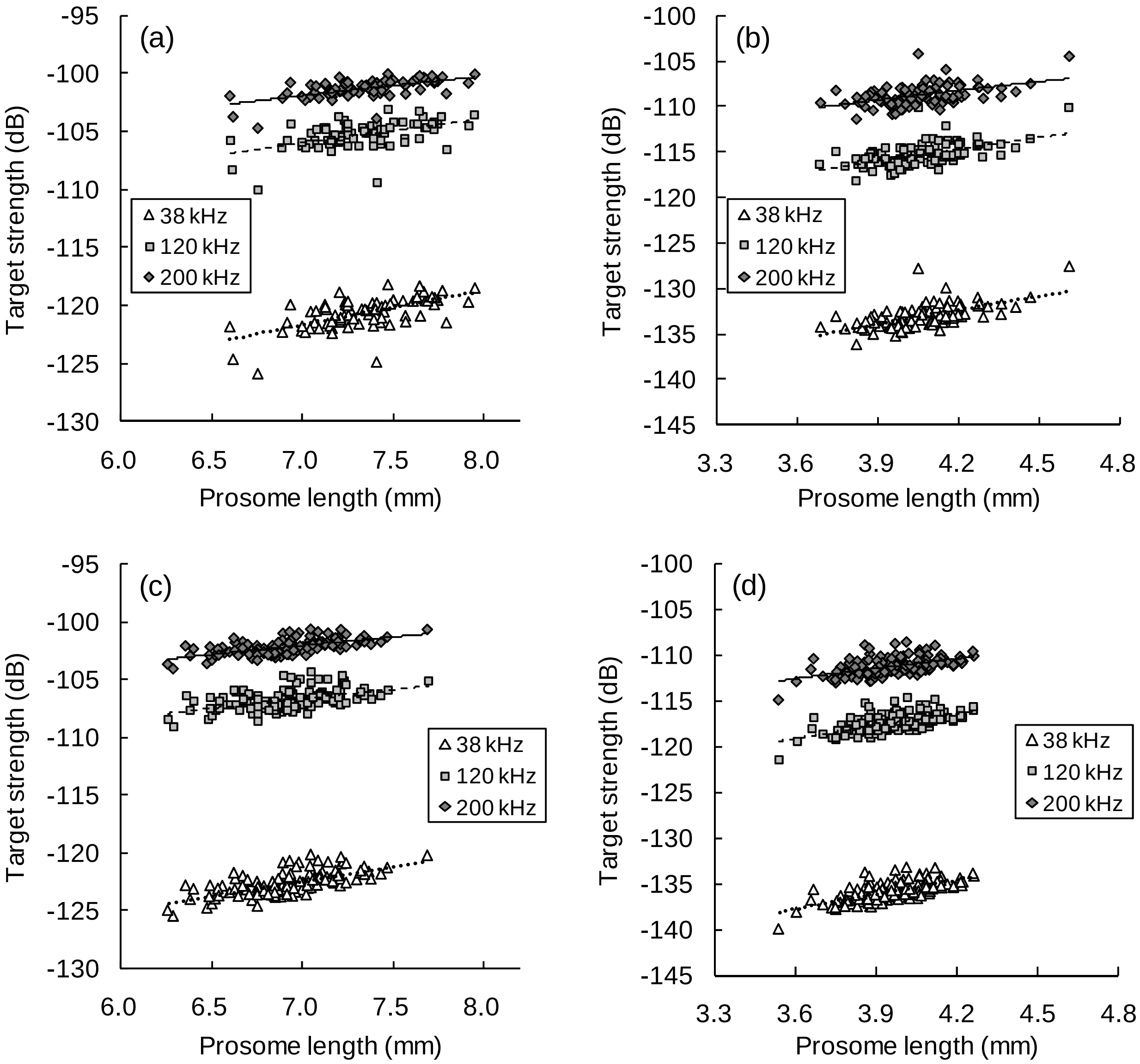
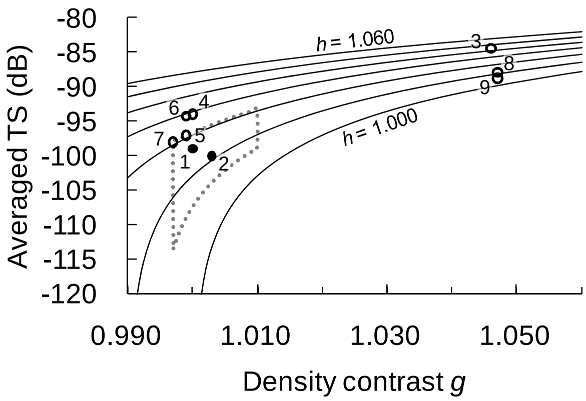
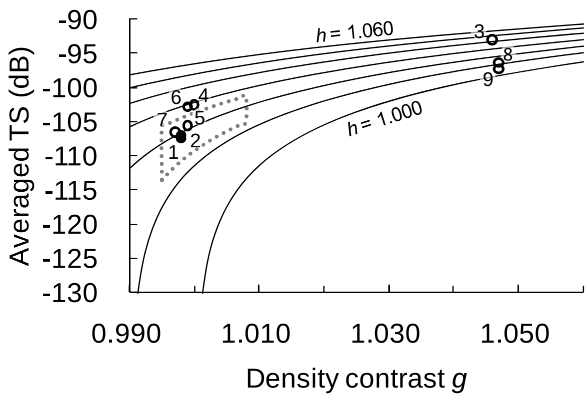

(a)
(b)
(c)
(d)
Target strength (dB)
Prosome length (mm)
38 kHz
120 kHz
200 kHz
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
6.0
6.5
7.0
7.5
8.0
3.3
3.6
3.9
4.2
4.5
4.8

Fig. 4



-80
-85
-90
-95
-100
-105
-110
-115
-120
Averaged TS (dB)
0.990
1.010
1.030
1.050
Density contrast g
h = 1.060
h = 1.000
1
2
3
4
5
6
7
8
9



-90
-95
-100
-105
-110
-115
-120
-125
-130
Averaged TS (dB)
0.990
1.010
1.030
1.050
Density contrast g
h = 1.060
h = 1.000
1
2
3
4
5
6
7
8
9

Fig. 5

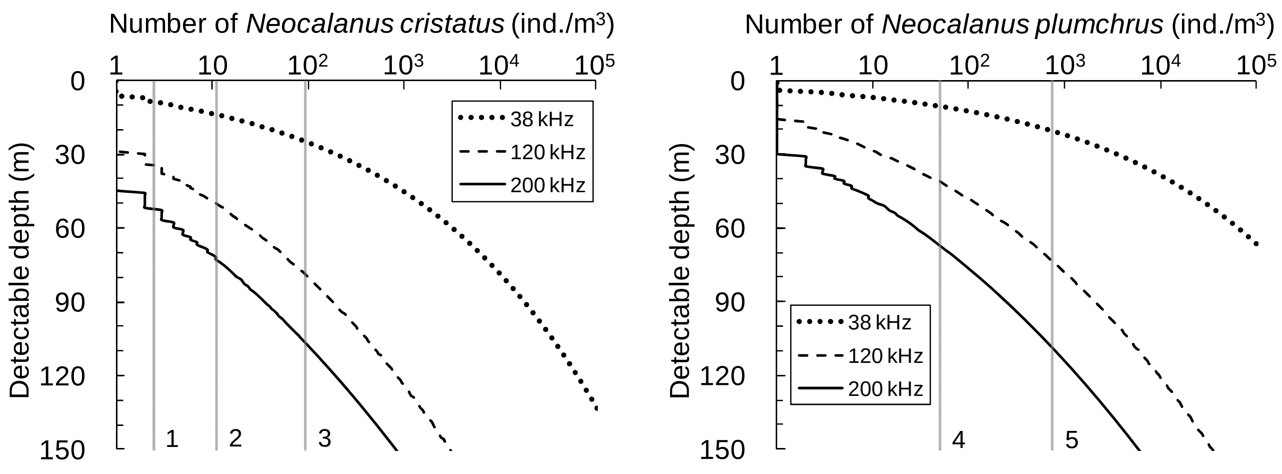
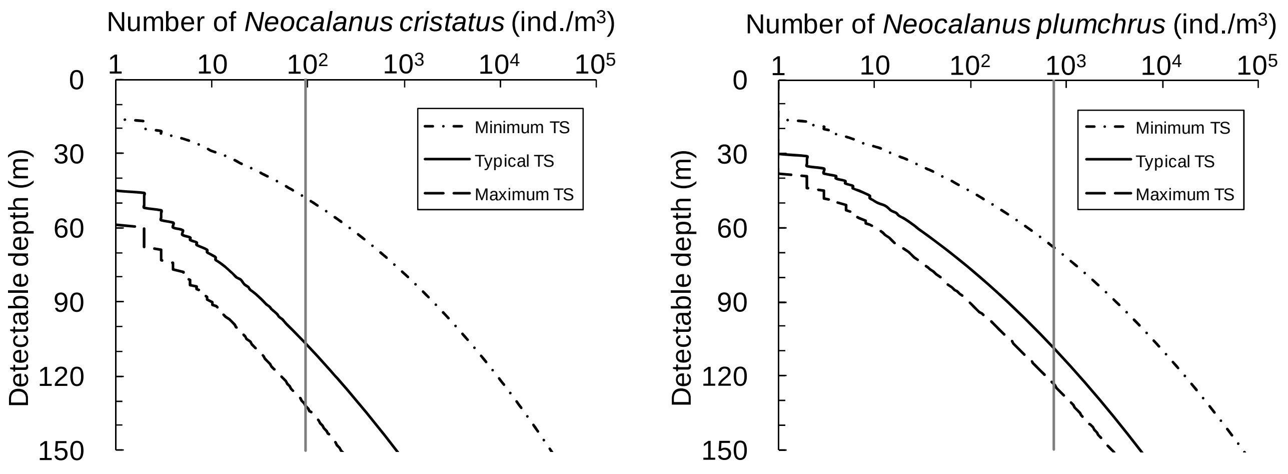

Number of *Neocalanus cristatus* (ind./m³)
1
10
10²
10³
10⁴
10⁵
0
30
60
90
120
150
Detectable depth (m)
38 kHz
120 kHz
200 kHz
1
2
3
Number of *Neocalanus plumchrus* (ind./m³)
1
10
10²
10³
10⁴
10⁵
0
30
60
90
120
150
Detectable depth (m)
38 kHz
120 kHz
200 kHz
4
5

Fig. 6



Number of *Neocalanus cristatus* (ind./m3)
1
10
10^2
10^3
10^4
10^5
Detectable depth (m)
0
30
60
90
120
150
Minimum TS
Typical TS
Maximum TS
Number of *Neocalanus plumchrus* (ind./m3)
1
10
10^2
10^3
10^4
10^5
Detectable depth (m)
0
30
60
90
120
150
Minimum TS
Typical TS
Maximum TS