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Acoustic Target Strength Measurements for Biomass Estimation of Aquaculture Fish, Redlip Mullet (*Chelon haematocheilus*)

Hansoo Kim [1,2], Donhyug Kang [1,*], Sungho Cho [1], Mira Kim [1], Jisung Park [1] and Kiseon Kim [3]

1 Maritime Security and Safety Research Center, Korea Institute of Ocean Science & Technology (KIOST), Busan 49111, Korea; hskim@kiost.ac.kr (H.K.); shcho@kiost.ac.kr (S.C.); mrkim0825@kiost.ac.kr (M.K.); jspark@kiost.ac.kr (J.P.)

2 Department of Ocean System Engineering, Jeju National University, Jeju 63243, Korea

3 MT-IT Collaborations Center, Gwangju Institute of Science & Technology (GIST), Gwangju 61005, Korea; kskim@gist.ac.kr

* Correspondence: dhkang@kiost.ac.kr; Tel: +82-51-664-3650

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Abstract: Redlip mullet (*Chelon haematocheilus*) is distributed in coastal waters of the North-Western Pacific Ocean and is a cultured fish in Korea. A hydroacoustic technique constitutes a useful method to assess the biomass and spatial distribution of mullet in sea cages or in coastal waters, and acoustic target strength (*TS*) information of the target fish is an essential parameter in using this method. In this study, *ex situ TS* measurements of 16 live mullets were made in an aquaculture sea cage in Korea. The split-beam scientific echo-sounder used for measurements was comprised of 38, 120, 200, and 420 kHz frequencies. An underwater video camera was simultaneously used to observe the mullets' behavior during the *TS* measurements. The mullet *TS* data was analyzed from a wide range of total fish length (*FL*: 14.3–40.3 cm). As results for all frequencies, the frequency dependence of the mean *TS* values were relatively low, and the difference in mean *TS* was within 2.5 dB. When the slope of the least-squares regression line was forced to 20 into the *TS* equation, the resulting value for the constant term (b_{20}) at each frequency was −67.0 dB, −68.3 dB, −66.3 dB, and −68.5 dB, respectively. The data tended to be frequency dependent. Additionally, the maximum *TS* appeared between tilt angles of 0° and 10°. These results indicate that *TS* measurements can be applied to estimate the biomass of the mullet in sea cages or in coastal waters.

Keywords: Target strength (*TS*); Redlip mullet (*Chelon haematocheilus*); *ex situ* measurement; *TS* function

1. Introduction

Redlip mullet (*Chelon haematocheilus*) is a euryhaline species that inhabits both marine coastal water and freshwater environments [1,2]. The species is a commercial aquaculture fish in Korea and has an ecological niche in the marine ecosystem [2,3]. It is widely distributed around the coastal waters of Korea, Japan, Taiwan, and China in the North-Western Pacific Ocean [4]. The young mullet feeds on phytoplankton or small organic sediments in coastal waters and the adult mullet becomes omnivorous [5].

The traditional netting method has been used to measure the abundance of mullet in coastal waters [6]. In addition, the biomass of mullet in aquaculture nets has been estimated by the total wet weight (*W*) using a scale. As these methods are labor intensive and costly, it is necessary to consider more efficient methods. Since 2000, a hydroacoustic method using the acoustic equipment of fisheries has been implemented to estimate the distribution and abundance of fish in marine and freshwater environments [7]. Hydroacoustic methods are considered to be an efficient way to overcome the limitations of traditional methods [8,9].

The acoustic target strength (*TS*) of individual fish and volume-backscattering strength (S_v) from fish schools must be obtained using well-calibrated acoustic equipment to determine an accurate biomass of a target fish. The *TS* information is required in order to convert S_v to absolute fish biomass. The accuracy of the *TS* information is a key parameter that determines the accuracy of the biomass estimate. Previous studies have reported that the *TS* information can cause up to 40% of the error in the biomass calculation [7,10].

TS is generally measured directly in in situ or *ex situ* experiments and indirectly use an acoustic scattering model [11]. The in situ method has the advantage of obtaining *TS* data in the natural state for the target fish, but it is difficult to distinguish the *TS* data due to other fish or unwanted fish [12]. The *ex situ* method uses tethered or caged live fish and has some advantages, such as species identification, tilt angle, and fish information (length and weight), but there is a possibility for error in the *TS* measurements due to unnatural movements from the artificial control [13]. The acoustic scattering model, based on physical morphology, estimates *TS* information at various frequencies and fish lengths, but it depends heavily on internal physical characteristic data (body or swim bladder shape, density gradient, and sound speed gradient) of the target fish [14]. There has been a tendency to use all three of these methods together to obtain more accurate *TS* data.

Considering labor and costs, acoustic methods that use high frequencies are a very efficient methods for obtaining rapid biomass estimates of cultured Redlip mullet (*Chelon haematocheilus*). Unfortunately, there is very little information about *TS* measurements and related information on this mullet species.

The purpose of this study was to measure the *TS* of cultured Redlip mullet, which is the key parameter for estimating biomass using the acoustic technique. As a result of this study, we derived the relationship between the *TS* function and the total fish length (*FL*, cm) or wet weight (*W*, g) at frequencies of 38, 120, 200, and 420 kHz. Furthermore, we tried to understand *TS* characteristics based on the swimming angle.

2. Materials and Methods

2.1. Fish Species and Experimental Apparatus

Redlip mullets were sampled on the commercial aquaculture farm around the coastal waters in the South Sea of Korea. The mullets were held in sea cages for 2 weeks during adaptation at the Marine Research Center, Korea Institute of Ocean Science & Technology (KIOST). *TS* was measured on a large floating fish farm (6 m width × 12 m length) platform. Total depth of the fish farm was about 14.0 m. To conduct *TS* measurements on fish of various lengths, we collected data from 16 fish among well-adapted fish in seawater. The maximum *FL* and *W* of the collected fish were about 40 cm and about 900 g (Figure 1).

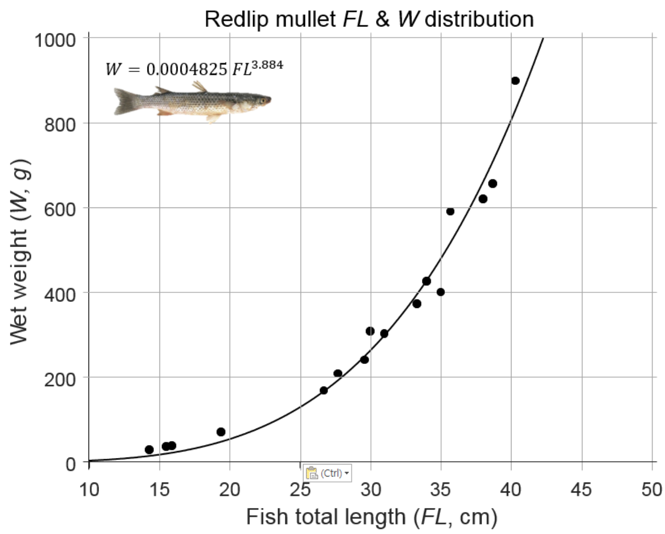

Figure 1. The relationship between total fish length (*FL*, cm) and wet weight (*W*, g) of 16 live Redlip mullets (*Chelon haematocheilus*).

Each live mullet was tethered with a very small monofilament line attached to its mouth after anesthetizing the fish (FA100, 4-allyl-2-methoxyphenol). When the mullet awoke from the anesthesia in the water, the line was tied to a vertical line that slowly descended to a depth of 4.5–5.5 m. The end of the vertical line was tied to a 2.0 kg weight. The distance between the tethered mullet and its weight was about 2.0 m, which was sufficient to separate the signal of the weight and the *TS* signal of the fish from all *TS* signals (Figure 2). The tethered mullet remained in a good condition throughout the experiment. After the *ex situ TS* measurements, the *FL* and *W* of individual mullets were measured with a scale and balance, and the fish were shock frozen immediately.

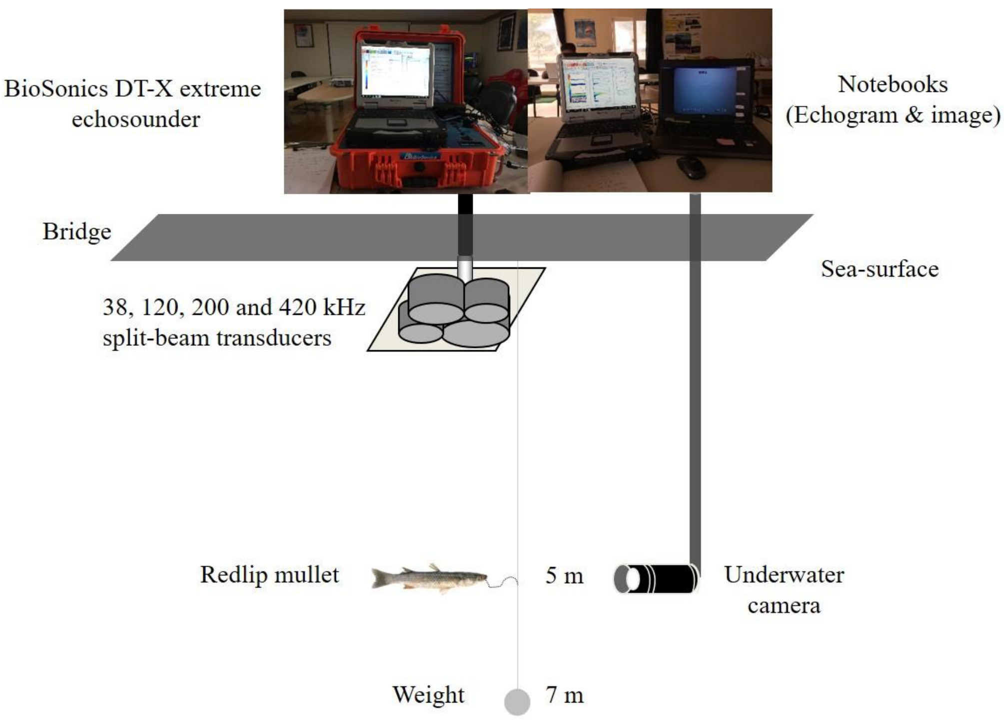

Figure 2. Experimental setup for the target strength (*TS*) measurements of Redlip mullet (*Chelon haematocheilus*) using a scientific echo-sounder (38, 120, 200, and 420 kHz).

2.2. TS Measurement

Ex situ TS measurements were collected from 16 live mullets at frequencies of 38, 120, 200, and 420 kHz with a split-beam transducer type echo-sounder (DT-X extreme; BioSonics, Inc., Seattle, WA, USA). The transducers were mounted about 0.5 m below the sea surface, facing in a downward vertical direction (Figure 2). Before the *TS* measurements, each transducer was calibrated using a tungsten-carbide calibration sphere. To reduce *TS* measurements from unwanted small fish, the minimum threshold level of the *TS* value was set to −60 dB for each frequency. When the fish was out of the acoustic-axis for an extended period, *TS* was measured by rotating the transducer body so that the fish was positioned near the acoustic axis.

The pulse duration and ping rate for all frequencies was set to 0.2 ms and 2 pings/s, respectively, considering the distance from the transducer's face to the fish. The detailed echo-sounder settings are listed in Table 1.

An underwater video camera (Water-7000DX; Tsukamoto Co. LTD., Suzuka, Japan) was installed at a depth of about 5.0 m in the horizontal direction to observe the behavior and tilt angle of the mullet during the *TS* measurements. The depth corresponded to the depth of the experimental fish used to measure *TS*. Both the transmitting time of the transducer and the recording time of the underwater video were synchronized before the *TS* measurements. The data from the underwater camera were used to analyze the influence of the swimming angle on the *TS* data. The measuring times of the *TS* data and the underwater video data were approximately 20–40 min, considering the number of *TS* data and the behavior of the fish.

Table 1. Several scientific echo-sounder parameters used for the target strength (*TS*) measurements of Redlip mullet (*Chelon haematocheilus*).

Parameters	38 kHz	120 kHz	200 kHz	420 kHz
Beam type	Split-beam			
Source level (dB)	212.6	219.6	220.5	219.0
−3 dB Beam width (°)	9.2	7.8	6.9	7.0
Absorption coefficient (dB/m)	0.00876	0.03916	0.05666	0.10118
Pulse duration (ms)	0.2			
Ping rate (pps)	2			
Temperature (°C)	13			
Salinity (psu)	32			

2.3. Data Analysis

The *TS* data was analyzed from the 16 live mullets. The measured *TS* data from the mullet among the total *TS* data within the water column were extracted using the specific *Target collection and TS distribution* module in Visual Analyzer software (Ver. 4.3) provided by the equipment manufacturer [15].

To remove unwanted *TS* data and to obtain the valid data, we used criteria that satisfied both conditions simultaneously; target depth and off-axis position. Considering that the depth of activity of the tethered mullet was 4.5–5.5 m, only the *TS* data that satisfied the depth range among all *TS* data was extracted. The half beam widths of the transducers used in the *TS* experiments were ±4.6° at 38 kHz, ±3.9° at 120 kHz, ±3.45° at 200 kHz, and ±3.5° at 420 kHz. Considering the compensated *TS* value with beam width, only the *TS* data to be positioned within the half beam-width angle at each frequency were extracted. We calculated the mean *TS* value using only the *TS* data that satisfied both of the above conditions simultaneously.

All selected *TS* data was converted to the backscattering cross-section ($\sigma_{bs} = 10^{TS/10}$) for the linear value, which was used to calculate the mean *TS* values [16]. The mean *TS* was defined by

$$\text{mean } TS = 10 \log_{10}(\Sigma\sigma_{bs}/n_{\text{i}}) \quad (1)$$

The analysis was performed using three linear fitting models (Equations (2)–(4)) [17]. The *TS* functions were fitted to the mean *TS* data using the least-squares regression with *FL* and *W*:

$$TS = m \log_{10}(FL, \text{cm}) + b \quad (2)$$

$$TS = 20 \log_{10}(FL, \text{cm}) + b_{20} \quad (3)$$

$$TS = m \log_{10}(W, \text{g}) + b \quad (4)$$

where m is the slope (20 is the standard slope) and b and b_{20} are the intercepts, respectively [17].

The *TS* value is strongly affected by the tilt angle of the fish. In order to select the *TS* data that can judge the tilt angle, only the underwater images that can visually measure the angle were extracted. From the captured underwater image data, the tilt angle was estimated from the gradient of a straight line connecting the head to the tail. The tilt angle between the incident wave and the fish was defined as a positive angle (+) when the head was upward and a negative angle (−) when the head was downward.

3. Results

In this experiment, the seawater temperature was 13 °C, and salinity was 32 psu, so the speed of sound was 1496.7 m/s [7]. The 16 live mullets were judged to be in good condition during the experiments. Their total fish length (*FL*, cm) ranged from 14.3 cm to 40.3 cm (mean *FL* = 29.07 cm) and their wet weight (*W*) ranged from 28 g to 898 g (mean *W* = 335 g). The relationship between the *FL* and *W* is: $W = 0.0004825, FL^{3.884}$ ($r^2 = 0.97$).

Figure 3 shows a sample echogram of a mullets *TS* measurement (*FL*: 35.0 cm, 200 kHz). In the echogram, the *TS* data from the mullets were fully separated from unwanted surface targets, weight, and the bottom. The relatively low *TS* value (about −50 dB) at the beginning of the measurement was likely to be due to the unstable condition of the mullet at the tethered depth. The higher *TS* values (about −33 dB) show that the mullet was stable with an approximate ±5° tilt angle. The mean *TS* for mullet was calculated from the *TS* data obtained between 14:26 and 14:38.

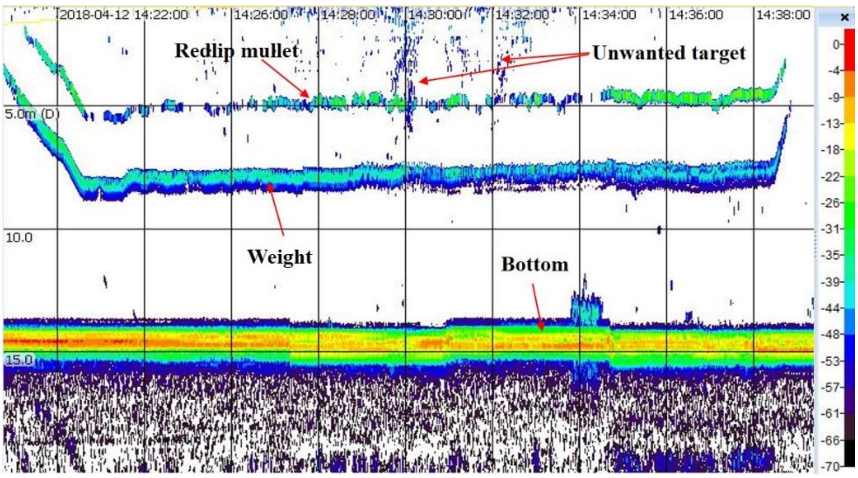

Figure 3. A sample echogram of the Redlip mullet (*FL*: 35.0 cm) from the *ex situ* target strength measurements.

Representative samples of the *TS* distributions at 38, 120, 200, and 420 kHz are shown in Figure 4. Generally, the *TS* of a fish with a large motion during *TS* measurements have a typical Rayleigh distribution, and thus the deviation of the mean *TS* tends to be large. However, the *TS* data measured in this study show similarity with the Gaussian distribution, and thus show relatively stable mean *TS* values. The numbers of valid *TS* data (745 at 38 kHz, 656 at 120 kHz, 454 at 200 kHz, and 460 at

420 kHz) were considered to be sufficient to calculate a mean *TS* value for mullet. The *TS* values for mullet, ranged from −52.4 dB to −30.6 dB at 38 kHz, −58.4 dB to −28.6 dB at 120 kHz, −57.0 dB to −26.4 dB at 200 kHz, and −59.2 dB to −27.0 dB at 420 kHz. The mean *TS* values from the data were −37.4 dB at 38 kHz, −37.4 dB at 120 kHz, −34.6 dB at 200 kHz, and −36.6 dB at 420 kHz.

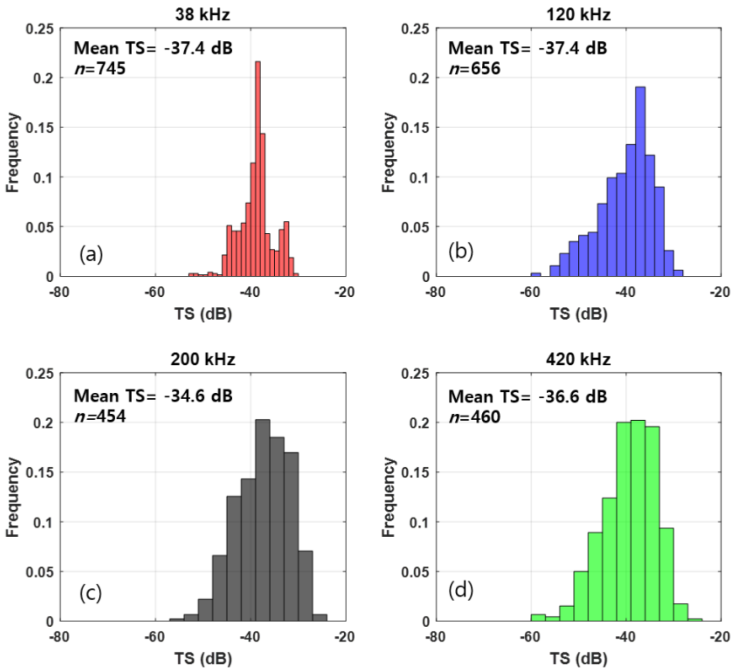

Figure 4. Representative target strength (*TS*) histograms of mullet (*FL*: 35.0 cm) at (**a**) 38, (**b**) 120, (**c**) 200, and (**d**) 420 kHz. Index, *n* is the sample size.

Figure 5 shows the relationships between the mean *TS* and the *FL* at each frequency. The black points are the mean *TS* values from the 16 mullet, which ranged from −47.6 dB to −34.4 dB at 38 kHz, −50.1 dB to −34.5 dB at 120 kHz, −49.4 dB to −33.8 dB at 200 kHz, and −49.9 dB to −34.4 dB at 420 kHz. Considering the mean *TS* values for all frequencies, the difference in the maximum mean *TS* was within 1 dB and the difference in minimum mean *TS* was 2.5 dB. These results indicate that the frequency dependence of the mean *TS* value was relatively low.

From Equation (2) using the mean *TS* data, it is determined by the least-squares regressions of mean *TS* with *FL*. The *TS* functions at all frequencies were fitted for Redlip mullet (Figure 5),

$$\begin{gathered} TS_{38\text{ kHz}} = 21.6 \cdot \log_{10}(FL) - 69.3 \\ (95\%\text{ CI}: 15.1\text{ to }28.1\text{ and } -78.8\text{ to } -59.8;\ r^2 = 0.78), \end{gathered} \tag{5}$$

$$\begin{gathered} TS_{120\text{ kHz}} = 22.9 \cdot \log_{10}(FL) - 72.4 \\ (95\%\text{ CI}: 13.0\text{ to }32.7\text{ and } -86.7\text{ to } -58.1;\ r^2 = 0.64), \end{gathered} \tag{6}$$

$$\begin{gathered} TS_{200\text{ kHz}} = 26.9 \cdot \log_{10}(FL) - 76.2 \\ (95\%\text{ CI}: 19.7\text{ to }34.0\text{ and } -86.6\text{ to } -65.8;\ r^2 = 0.82), \end{gathered} \tag{7}$$

$$\begin{gathered} TS_{420\text{ kHz}} = 26.2 \cdot \log_{10}(FL) - 77.5 \\ (95\%\text{ CI}: 20.0\text{ to }32.5\text{ and } -86.5\text{ to } -68.5;\ r^2 = 0.85), \end{gathered} \tag{8}$$

where r^2 is the coefficient of determination, and CI is the confidence interval for m and n. The mean TS values for each frequency were calculated from the standard fits of the TS function (see Equation (3)). The relationships between mean TS and FL were derived with the following equations (Figure 5):

$$TS_{38\text{ kHz}} = 20 \cdot \log_{10}(FL) - 67.0 \\ (95\%\text{ CI}: \; -67.9 \text{ to} - 66.1;\ r^2 = 0.78) \tag{9}$$

$$TS_{120\text{ kHz}} = 20 \cdot \log_{10}(FL) - 68.3 \\ (95\%\text{ CI}: \; -69.7 \text{ to} - 66.9;\ r^2 = 0.63) \tag{10}$$

$$TS_{200\text{ kHz}} = 20 \cdot \log_{10}(FL) - 66.3 \\ (95\%\text{ CI}: \; -67.5 \text{ to} - 65.2;\ r^2 = 0.77) \tag{11}$$

$$TS_{420\text{ kHz}} = 20 \cdot \log_{10}(FL) - 68.5 \\ (95\%\text{ CI}: \; -69.5 \text{ to} - 67.5;\ r^2 = 0.81) \tag{12}$$

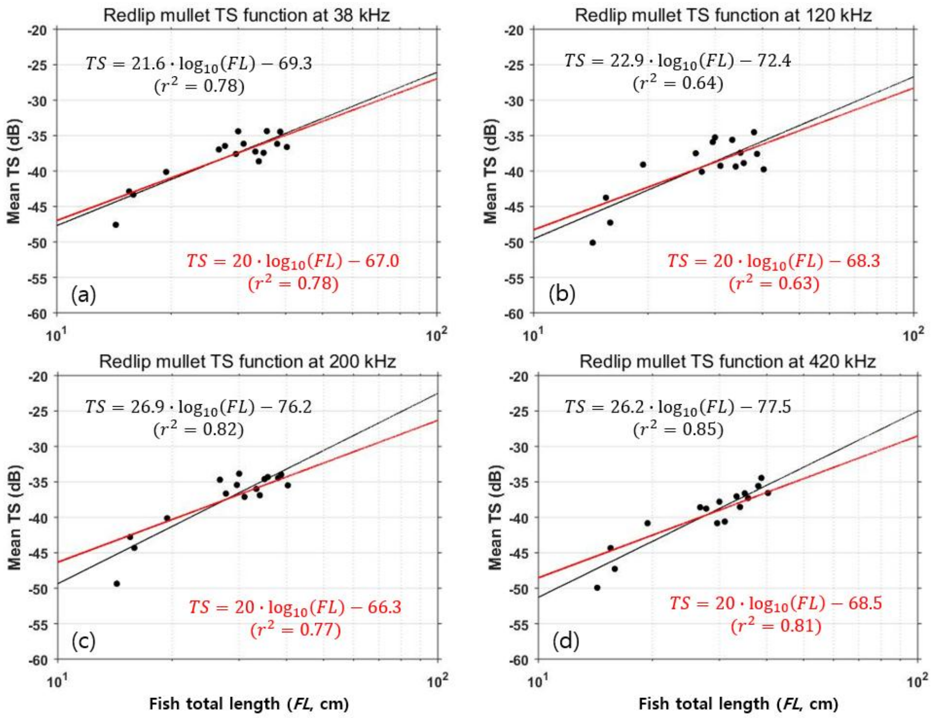

Figure 5. Relationships between target strength (TS) and total fish length (FL) at (**a**) 38, (**b**) 120, (**c**) 200, and (**d**) 420 kHz. Black points represent the mean TS values with FL. Black solid lines represent the least-squares regressions of mean TS, and the red solid line represents the standard fits of the TS functions.

The relationships between mean TS and W at each frequency were derived using Equation (4). The relationships between the mean TS and W were derived with the following equations (Figure 6):

$$TS_{38\text{ kHz}} = 6.7 \cdot \log_{10}(W) - 53.7 \\ (95\%\text{ CI}: 4.6 \text{ to } 8.7 \text{ and} - 58.7 \text{ to} - 48.8;\ r^2 = 0.77), \tag{13}$$

$$TS_{120\text{ kHz}} = 6.8 \cdot \log_{10}(W) - 55.5 \\ (95\%\text{ CI}: 3.6 \text{ to } 10.1 \text{ and} - 63.2 \text{ to} - 47.7;\ r^2 = 0.60), \tag{14}$$

$$TS_{200\ \text{kHz}} = 8.2 \cdot \log_{10}(W) - 56.6 \\ (95\%\ \text{CI}: 5.7\ \text{to}\ 10.6\ \text{and} - 62.4\ \text{to} - 50.8;\ r^2 = 0.79), \tag{15}$$

$$TS_{420\ \text{kHz}} = 8.1 \cdot \log_{10}(W) - 58.6 \\ (95\%\ \text{CI}: 6.0\ \text{to}\ 10.1\ \text{and} - 63.4\ \text{to} - 53.7;\ r^2 = 0.84), \tag{16}$$

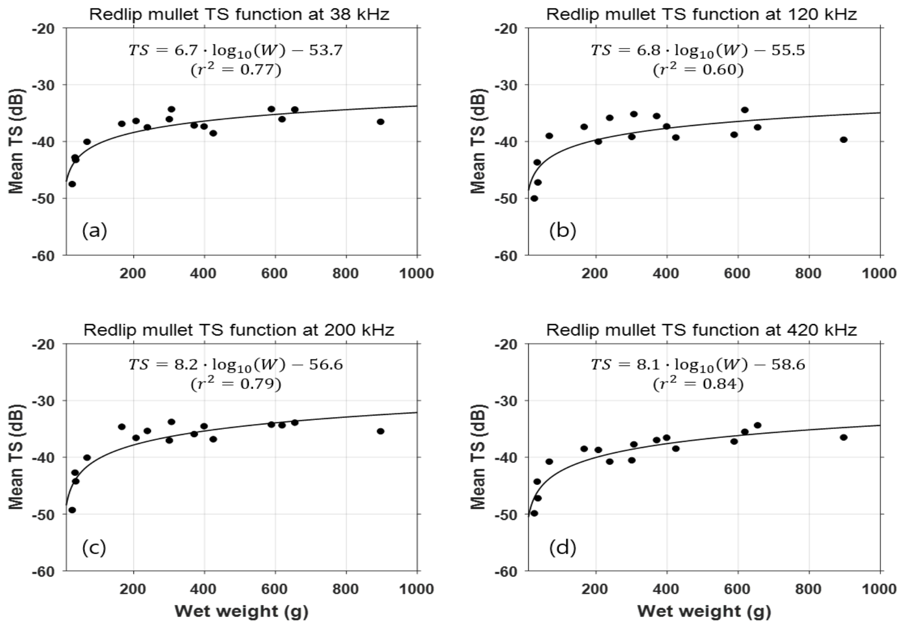

Figure 6. Relationship between the target strength (*TS*) and wet weight (*W*) at (**a**) 38, (**b**) 120, (**c**) 200, and (**d**) 420 kHz. Points represent the mean *TS* values, and solid lines represent least-squares regressions of mean *TS*.

4. Discussion

According to fisheries statistical data, about 5839 tons of mullet were cultured in 2012, which was 7.7% of the total amount of fish cultured in Korea [18]. Redlip mullet is a valuable aquaculture species in the northwest Pacific Ocean that is mainly cultured along the coast of the Yellow Sea and the South Sea of Korea [3,4,19]. Until now, the traditional method of weighing was used to calculate the biomass of mullet on a fish farm [6]. This method requires a high cost, time, and human labor to estimate biomass. Using a hydro-acoustic method is an alternative way to overcome these limitations [7].

Research and development of an automatic fish-counter system, based on the hydroacoustic technique, are underway in Korea. One of the most important parameters for applying this system to a fish farm is to understand the acoustic *TS* characteristics of the target fish. The *TS* information of Redlip mullet was unknown before this study.

In fisheries acoustics, the target strength (*TS*) functions are essentially required to convert volume backscattering strength (*Sv*) to absolute fish biomass. The accuracy of the *TS* information is a key parameter that determines the accuracy of the biomass estimate. In more detail, the *Sv* value within depth intervals can be changed to volume-backscattering area (S_A) for every one nautical mile of survey transect. By assuming that measurements of *Sv* represent the linear sum of echoes from individual fish within the sample volume, the numerical density of fish (number per unit area of sea surface) can be estimated by dividing S_A by the backscattering cross-section (σ_{bs}, m^2) of a single fish. Biomass density of fish (ϱ, g m^{-2}) can be estimated by multiplying the numerical density by the weight of a single fish [20].

The *TS* of individual fish is influenced by the tilt angle and the volume of the swim bladder. As the volume of the swim bladder in the body is difficult to calculate directly, the mean *TS* is expressed as a function of fish length or weight. Generally, the larger the fish length, the greater the swim bladder volume, and hence the larger the *TS*. The swim bladder is the most important factor affecting the *TS* of fish. In particular, it is known that more than 90% of the *TS* value is related to the size of the swim bladder [7]. Generally, a higher *TS* value is obtained when the tilt angle between the incident wave and the swim bladder area is perpendicular.

After the *TS* experiments, the swim bladder shapes were obtained using radiography (M-1005; Softex, Mumbai, India). The lateral and dorsal views were acquired from X-ray images of frozen mullet (Figure 7). The mullet's head and back are flat-shaped and the body is wide, unlike other fish in the dorsal direction. In the case of Redlip mullet, the swim bladder has a positive inclination relative to the body axis and the tilt angle of the swim bladder was about 8° from horizontal.

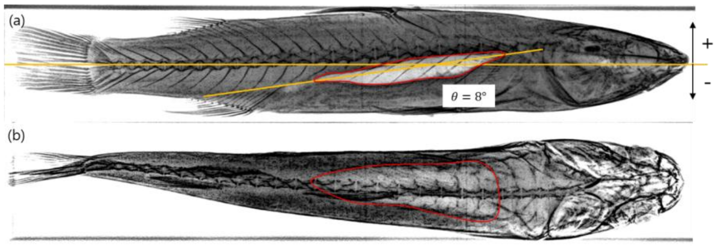

Figure 7. X-ray images of the mullet's swim bladder; (**a**) lateral aspect, and (**b**) dorsal aspect). The red lines is the boundary of the swim bladder.

Figure 8 shows the relationships between mean *TS* and the tilt angle at 200 kHz for 19.4 cm and 35.0 cm *FL* values. The tilt angle was calculated by extracting the time-synchronized capture images from an underwater camera. At that time, the *TS* values could not be obtained at all angles. As a result of the relationship between the tilt angle and mean *TS*, the *TS* values were about −35 dB and −32 dB for tilt angles of −10° to 0°, respectively. The closer the tilt angle of the swim bladder is to horizontal, the higher the *TS* value. The *TS* value tended to decrease for tilt angles less than −10° or more than 10°. The mean *TS* values were −41.4 dB and −38.0 dB when considering a tilt angle of ±20°, which is normal for fish swimming in a sea cage.

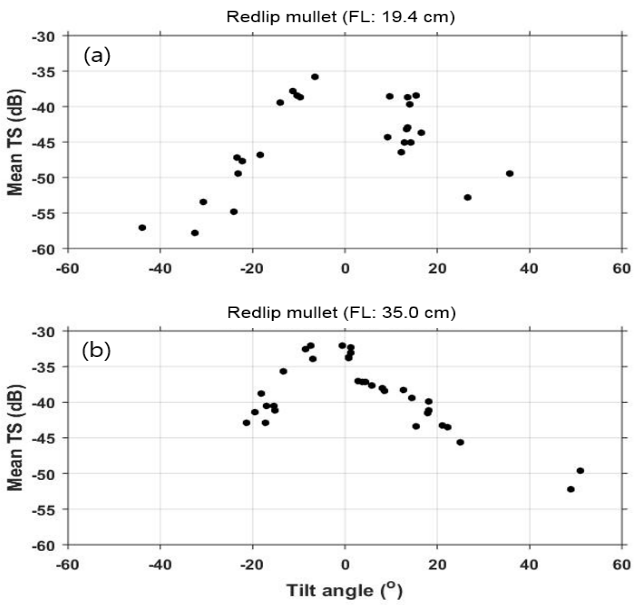

Figure 8. Variation between the tilt angle and mean target strength (*TS*; dB) of mullet at 200 kHz [(**a**) 19.4 cm fish total fish length (*FL*), and (**b**) 35.0 cm in *FL*].

The swim bladder is generally inclined +15°–30° in the positive direction from the horizontal compared to other fish [21]. Therefore, the highest *TS* value occurred when the tilt angle of the fish body was between −30° and −15°. Considering the characteristics of the acoustic signals transmitted in the vertical direction when the inclination angle of the swim bladder is high, the *TS* value of the fish also varied widely due to transmission and reflection fluctuations depending on the tilt angle at high frequency. In this study, the difference in the b_{20} value in the standard *TS* function at each frequency was within about 2 dB (Equations (9)–(12)). Generally, fish with large swim bladder angles in *TS* measurements have relatively high *TS* values at low frequencies. In the case of the mullet with small swim bladder angles, the difference of mean *TS*, with frequency, was relatively small because the slope of the swim bladder is almost parallel with a horizontal line. These results show that the *TS* functions are consistent at all frequencies (Figures 5 and 6). However, the reason why the result at 120 kHz is lower than that of the other frequencies is that the coefficient of determination for the accurate beam axis was relatively low at the time of the *TS* experiment.

Most fish in a sea-farm cage move predictably without sudden movement. The difference in the *TS* values between tilt angles of +20° and -20° was about 10 dB. Considering the relatively small fluctuation in the *TS* values at all frequencies in this study, the high frequency is valid to use when estimating fish biomass within a confined net at a fish farm.

The results of this study will provide *TS* information for Redlip mullet and can be applied to estimate biomass. In future studies, we will attempt to calculate the theoretical *TS* using morphological parameters, such as the three-dimensional swim bladder and the body shapes and the physical properties of Redlip mullet for a numerical simulation with a scattering model. In addition, we will compare and analyze the measured *TS* data with theoretical *TS* data to improve the accuracy of the *TS* information.

Author Contributions: H.K., S.C., M.K. and J.P. performed the acoustic target strength (*TS*) experiments and analyzed the data; K.K. and D.K. discussed the *TS* measurement results. H.K. and D.K. wrote the manuscript and offered useful suggestions.

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Conflicts of Interest: The authors declare no conflict of interest.

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