Title: Density and sound-speed contrasts, and target strength of Japanese sandeel *Ammodytes personatus*

Running Title: Target strength of Japanese sandeel

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ABSTRACT

Sound-speed and density contrasts (*h* and *g*, respectively), important acoustic material properties, of Japanese sandeel *Ammodytes personatus* were measured to estimate theoretical target strength (TS). The measured sound-speed contrast of adult fish varied between 1.016 and 1.023 (mean, 1.020), which showed temperature dependence. The measured density contrast differed significantly between juvenile and adult. The density contrast of juvenile varied between 1.017 and 1.024 (1.021), and that of adult varied between 1.026 and 1.038 (1.032). Using these results, TS at 38 and 120 kHz in the fishing season were estimated by an empirical sound scattering model. TS of an individual fish varied significantly with change of tilt angle. TS of near dorsal aspect (TS_{max}) and tilt-averaged TS (TS_{ave}) differed up to 7 dB. At both frequencies, two different TS_{ave}-length relationships ($TS_{ave} = a \log L + b$) were obtained for adult and juvenile. The coefficients of log *L* of adult were close to 20, suggested that backscattering strength was proportional to the square of body length. These values were larger in juvenile (34.0 at 120 kHz, 56.5 at 38 kHz), suggested that backscattering strength varied drastically with the cube or fifth power of body length.

Key Words: *Ammodytes personatus*, density contrast, Japanese sandeel, sound-speed contrast, target strength, theoretical scattering model

INTRODUCTION

Japanese sandeel *Ammodytes personatus* is one of the most important commercial fish species in the Inland Sea, Sendai bay, and Ise Bay, Japan. Larval and juvenile fishes (3.5–5.0 cm in standard length, SL) are mainly caught in the main fishing season from February to April. In this season, fishing pressure on Japanese sandeel is extremely high and about 90% of recruited stock is caught. Therefore, stock assessments are performed in real time and the end of fishing season is determined during the season to keep sustainable spawning adult stock (e.g. Two billion individuals in Ise Bay).[1,2]

Stock assessments of Japanese sandeel have been made by indirect methods using CPUE data (e.g. the DeLury in Ise Bay). However, estimated results of abundance based on CPUE data frequently differ from real abundance when CPUE and abundance don't have a proportional relationship owing to various factors. Against this background, quantitative monitoring using acoustic method has been thought to be better to estimate abundance of Japanese sandeel directly.

Acoustic surveys measure the amount of acoustic backscattering energy in the water column, so we need to know the target strength (TS) of individual fish to convert the measured backscattering into fish biomass. TS has been measured directly with split- or dual-beam echosounders (*in situ* measurement) for many fish species.[3,4] However, sandeels are difficult to detect as a single target in the field, because they don't have a gas-contained swimbladder that provides high acoustic reflection.[5] There are a few reports on experimental TS measurements (*ex situ* measurement) of other sandeel species, such as *A. hexapterus*.[6,7] However, also *ex situ* TS measurements of swimbladderless organisms has many technical problems and only limited frequency and size ranges are available.[8]

On the other hand, TS can also be computed using theoretical sound scattering models. Theoretical models represent a target shape by an approximate geometric configuration, and several models for swimbladderless species, such as DWBA model[9] and Liquid deformed cylinder model[10-12], have been developed to date. The use of theoretical models for estimating TS has several merits; they can estimate, for example, both the frequency characteristics of backscatter and the backscatter pattern related to fish orientation. These models need the body material properties, such as density contrast g and sound-speed contrast h against surrounding water, in TS calculations. However, these material properties of sandeels have not been reported, and thus theoretical TS of sandeels have not been computed. The objective of this study was to provide the density and sound-speed contrasts of Japanese sandeel for estimating effective TS. Then, theoretical values of TS were calculated using an empirical sound-scattering model.

In many studies, mass density of marine organisms has been measured by suspending them in the graded density solutions.[13] On the other hand, for estimating sound-speed of small organisms, the "time-average method" that use the travel time difference of the sound between a compartment with and without the presence of organisms have been developed, though there are very few reports on sound-speed of small fishes.[14,15] By applying these methods, Yasuma[16] estimated the value of g and h of juvenile and adult swimbladderless mesopelagic fishes (1.5–15.0 cm SL), and estimated their TS using the deformed cylinder model. In a subsequent study, the estimated values of TS were fitted well with the TS values measured in water tank experiments.[17] Based on these results, Yasuma[17] suggested that those methodologies are available for the TS estimation of other small swimbladderless fish species.

In this study, we measured the mass density and sound-speed and estimated the value of g and h, according to Yasuma[16] and Yasuma *et al.*[17] Based on the results, we estimated the TS values at 38 and 120 kHz, which are mainly used in coastal research vessels of Japan, using the deformed cylinder model. It has been suggested that mass density of juvenile (or larval) fish would differ from that of adult fish, because of the changes of water content, lipid content, and protein structure.[18,19] On the other hand, some study has reported that sound-speed of marine organisms would vary with temperature change.[16] Therefore, the measurements of g were made in different size classes, and the measurements of h were made in different temperature.

MATERIALS AND METHODS

Measurements of density and sound-speed contrasts

Fish samples were obtained on 20 January and 11 March 2003, in the fishing ground of Ise Bay (10–20 m water depth) using a commercial boat seine. Juvenile fishes (below 7.5 cm SL)[20] were caught in the January, and adult fishes (over 7.5 cm)[20] were caught in the March. These samples were immediately placed in plastic specimen bottles with seawater and frozen below –40°C on board. In the laboratory, the specimen bottles were thawed slowly in cold water.

Subsamples of 50 juveniles (3.3–7.4 cm) and 50 adults (7.5–13.2 cm) were used in the density measurements. We applied the density-bottle method[13,21], in which fish mass density, ρ, was determined by evaluating the buoyancy of each sample via a series of 1000 ml beakers containing seawater-glycerol solution of different density, ranging from 1.026 (seawater) to 1.100 g/cm^3 at 0.002 g/cm^3 steps. We defined the value of the bottle in which the fish was neutrally buoyant as fish mass density. If the specimen was not neutral in any solution, an average between the last sinking bottle and the first floating bottle was taken. The density contrast of fish body g was obtained by dividing ρ by the density of seawater (1.026 g/cm^3). The density of each bottle was confirmed using a glass areometer (15°C standard), and the solution in each bottle was kept at

15°C.

The sound-speed through the fish body was estimated by a time-average approach.[13,14] In this study, we used an acrylic 'T-tube' unit[16] which was improved the capacity of the tube and pulse settings, based on the units for zooplankton measurements (Fig. 1).[13,21] A continuous, sinusoidal-wave pulse of 170 kHz, 5 μs was radiated from one side of the tube to the other, and the time it took the pulse to pass through the tube containing seawater and fish was measured with a digital oscilloscope.

In the time-average approach, an empirical equation is used to relate passing time T_{total} through the mixture to the proportion of the volume filled by fishes V as

$$T_{total} = (1-V)\cdot T_{sw} + V\cdot T_{fish}\,, \tag{1}$$

where T_{sw} and T_{fish} are the passing time of sound through the seawater and through the fish body, respectively. The optimum range of the fish proportion, V, has been known to be 0.4–0.6.[16] In our experiment, 283 adult fishes (7.6–11.6 cm, 9.7 cm in average) were used, and the value of V was 0.47. The sound-speed contrast h is given by

$$h = \frac{T_{sw}}{T_{fish}} = \frac{C_{fish}}{C_{sw}}\,, \tag{2}$$

where C_{sw} and C_{fish} are the sound-speed through seawater and fish body, respectively. As C_{sw} is known from T_{sw} and the measurement distance (250 mm), C_{fish} can be deduced. The T-tube was sunk in a temperature-controlled tank and measured from 7°C to 23°C at 1°C steps. Details of the density and sound-speed measurements can be referred to

Yasuma.[16,17]

Sound scattering model

A total of 20 adults ranged from 7.5 to 11.5 cm (8.9±0.97, mean±SD) and 68 juveniles from 3.4 to 6.7 cm (4.5±0.82) were used in the TS calculation. We selected the liquid deformed cylinder model (liquid-DCM)[10-12] developed for non-swimbladder organisms to calculate the backscatter related to fish orientation from –50° to +50° (tilt angle: head-down to head-up position). The DCM describes a fish body shape as a series of adjacent, disc-like, cylindrical elements, based on the outlines of its dorsal and lateral aspects. We divided each outline into 20 equal parts, with 19 lines drawn perpendicular to the fish major axis (tail to snout line), following Sawada *et al.*[22] The density and sound-speed contrasts are the most important physical parameters in calculation, and these parameters were obtained from this study. The DCM has peculiar limitations; the aspect ratio of the major axis and minor axes (body height and body width) has to be large (approximately >5), and the fish tilt angle to be not too large (approximately <50°). Our calculations were within these limitations. Details of this model are described in Ye[10] and Ye *et al.*[12]

In the calculated results, we defined the maximum TS (TS_{max}) as the peak value in the plot of TS against fish tilt angle. The tilt angle distribution is required to calculate the tilt-averaged TS (TS_{ave}) according to Foote.[23] In this study, we applied a distribution within a mean of 0° (horizontal position) and a standard deviation of 15° in reference to the underwater observations of a relevant species *A. tobianus*.[24]

RESULTS

Density and sound-speed contrasts

The distribution of body mass density ρ versus fish standard length is shown in Fig. 2. The value of ρ ranged from 1.043 to 1.051 g/cm^3 (g was 1.017–1.024), with mean value1.048 (g was 1.021) in juvenile fish (3.3–7.4 cm), and ranged from 1.053 to 1.065 g/cm^3 (g was 1.026–1.038), with mean value 1.059 (g was 1.032) in adult fish (7.5–13.2 cm). These values showed significant difference between juvenile and adult ($p < 0.005$, Mann-Whitney *U-test*). There was no significant relationship between the ρ and standard length in each stage.

The sound-speed through seawater, C_{sw}, the sound-speed through the fish, C_{fish}, and the sound-speed contrast, h, are listed in Table 1. The sound-speed was higher in the fish body than in seawater within the temperature range examined. The h ranged from 1.028 (7°C) to 1.016 (15°C). In consideration of habitat temperature in main fishing season

(10–15°C), the *h* of Japanese sandeel was between 1.023 and 1.016.

Theoretical TS

Typical examples of TS-fish tilt angle relationship at 38 and 120 kHz obtained by the DCM are shown in Fig. 3. According to the average water temperature (12.9°C) of the main fishing season in Ise Bay, the values of the *g* and the *h* at 13°C (*g* in juvenile fish = 1.021, *g* in adult fish = 1.032, *h* = 1.018) were applied in the model calculations. TS_{max} were detected when the tilt angle was 0° in both stages and at both frequencies. In the adult fish, multi-peaked patterns were shown at both frequencies. The peaks were relatively sharp, especially in the higher frequency, suggesting that slight changes in fish tilt angle causes a major effect on TS variance. The values of TS_{max} at 38 kHz were higher than those of 120 kHz in most adult fish (Fig. 3). On the other hand, most of the juvenile fishes had a less (three- or four-) peaked pattern at 120 kHz and had a single-peaked pattern at 38 kHz. The values of TS_{max} at 120 kHz were higher than those of 38 kHz in all juvenile fish (Fig. 3).

The values of TS_{max} and TS_{ave} at the two frequencies are plotted in Fig. 4 as functions of logarithmic scale of fish standard length. Ranges of TS_{max} and TS_{ave}, and equations of the regression liens in Fig. 4 (the TS-length equation) are shown in Table 2. The coefficients of the log *L* of the regression lines were around 20 in adult fish. On the other hand, these values in juvenile fish were higher than those of adults, especially at 38 kHz (Table 2).

The difference of the values of TS_{ave} between 38 kHz and 120 kHz (ΔTS: $TS_{120kHz} - TS_{38kHz}$) is shown in Fig. 5. The values of TS_{ave} were higher at 120 kHz than at 38 kHz in all juvenile fish examined (<6.7 cm), and values of ΔTS were over 5 dB. On the other hand, the values of TS_{ave} were higher at 38 kHz than at 120 kHz in adult fish (>7.5 cm), and values of ΔTS were from 0 to –6 dB.

DISCUSSION

Density and sound-speed contrasts, and their influence on TS

Figure 6 shows the shift of the TS_{max} (at 38 kHz) with the change of the g on given values of the h (1.01–1.10 at 0.01 steps), calculated by the liquid-DCM. A 10.7 cm length, 1.0 cm height, 0.8 cm width prolate ellipsoid was used in the calculations, assuming a typical adult fish. Dark gray area in Fig. 6 is range of the g and h of adult fish in the temperature range of the main fishing season (Table 1), and light gray area is the whole range (including juvenile fish) measured in this study. The closed circle is the values of the g and h at 13°C used in the TS computation in this paper. Open circles in the figure are reference values of other fish species.[15,25-27] On each value of h, TS_{max} increases with increasing g. On each value of g, TS_{max} increases with increasing h. The reference values of g were from 1.010 to 1.041 and the values of h were from 1.007 to 1.020, and estimated TS_{max} of a prolate spheroid widely varied (maximum about 12dB) with the reference values of g and h. Thus, slight changes of g and h may lead to large difference in theoretical TS. This implies that potential 10 dB or more difference in estimated TS should be considered even in same shape if we simply applied reference value of other species. Applying our results, variation of TS in fishing season (dark gray area in Fig. 6) became within ±1.5 dB from the average value (closed circle in Fig. 6). This potential variation of TS would become larger (e.g. light gray area in Fig. 6), however, if the g of adult and juvenile fish were not discriminated, or if the larger change of water temperature (source of variation of the h) due to seasonal or regional difference was ignored. Therefore, in reference to the results of this study, adequate value of the g and h should be selected in the theoretical TS estimates of sandeel according to growth stage and surrounding water temperature.

According to a comparative study with water tank experiments, Yasuma[16,17] reported that TS and its tilt angle shift of some swimbladderless species, estimated by the DCM model (same method as the present study), corresponded with the experimental measurements. The methodology and reliability of the results in the present study are supported by the previous reports. However, accuracy of estimated TS would be worse if g and h were not accurate. The measurements of g and h in the present study were conducted in a laboratory with some limitations, which might lead to different results from the accurate values in the habitat. Thus, problematic points in the present measurements should be discussed.

At first, we used frozen specimen to the experiments. Some authors have suggested that freezing and long preservation may cause significant changes in material conditions, such as water content and tissue composition, of marine organisms.[28,29] However, it is known that the effect of freezing on tissue compositions should be minimum if the organisms were frozen in a moment at low temperatures (e.g. <–40°C).[30] We made a kind of flash freezing so that the composition and condition of material would not change[30], and samples were moved for the measurements soon (few days) after the sampling. Though the effect of freezing on the g and h should be examined using live individuals in future, we think, at this time, that significant difference between live fishes and frozen fishes did not occur.

Secondly, fish mass density was measured at a constant temperature. However, the mass densities of seawater and lipid, a major component of fish flesh, change less than 0.03% between 4°C and 30°C[18,31]; such change is negligible.

Thirdly, the sound-speed contrast was obtained only in adult fish, though it was also applied to the model computations of juvenile fish. Though, there is no report that compares the sound-speed contrast between juvenile and adult swimbladderless fishes, there are some reports that sound-speed contrast of zooplankton, such as krill, showed the dependence on body size.[32] For example, sound speed of *Euphausia superba* (20-57 mm) ranged from 1.018 to 1.044 in relation to body size[32], which would lead to 2-4 dB differences in same body shape. Future work needs to measure the size dependence of sound-speed contrast to make our results of TS estimate in juvenile fish more accurate.

Target strength

Though this is the first report on target strength of Japanese sandeel *A. personatus*, only a few studies can be found in other related species. Armstrong[7] used cage experiments on groups of adult fish to estimate a TS-length equation for *A. hexapterus* in the Atlantic Ocean; the result was, $TS = 20 \log L - 93.7$ (at 38 kHz). On the other hand, Thomas *et al*.[6] measured tethered individual fishes of *A. hexapterus* in Prince William Sound, and got a equation, $TS = 20 \log L - 80.0$ (at 120kHz). Though these experiments were conducted at different frequencies, there was an appreciable difference between equivalent size of them, and our results in adult fish (TS_{ave} in Table 2) were similar to the former. The reason for the large difference is unclear. One possibility reason would be the distribution of fish tilt angle assumed. We estimated the averaged TS_{ave}, assuming the tilt angle distribution of the fish as a mean of 0° and a standard deviation of 15°.[23,24] On the other hand, Thomas *et al*.[6] measured near horizontal aspect only. It is apparent that distribution of fish tilt angle is critical for the estimates of TS_{ave}, because slight changes in tilt angle leaded to the large TS variance (Fig. 3), and because the TS_{ave} and TS_{max} indicated large differences (Table 2). Additionally, the equations of TS_{max} obtained in this study were rather similar to the equation by Thomas *et al*. (Table 2). We used the reference value of relevant species[24] related to tilt angle because of lacking information on swimming behavior, and direct observations are required for obtaining more reliable results in the future.

In this study, two different TS_{ave}-length equations for adult and juvenile fish were obtained at each frequency (Fig. 4, right panels; Table 2). In adult, coefficients of log L were close to 20 at 38 and 120 kHz, suggesting that backscattering cross section (σ_{bs}: $TS = 10 \log \sigma_{bs}$) is proportional to the square of body length. In juvenile, on the other hand, coefficients were much higher (56.5 at 38 kHz, 34.0 at 120 kHz), which suggest that the σ_{bs} varies drastically with the cube (at 120 kHz) or fifth power (at 38 kHz) of body length (Table 2). It is known that the σ_{bs} of marine animals without air bladder become proportional to the square of body length when the body length is sufficiently large relative to the wavelength λ (e.g. $L/\lambda > 2$).[17,33] On the other hand, the σ_{bs} varies in proportion to the cube to fifth power of body length when the body length is small (e.g. $L/\lambda < 2$).[17,33] Our results were fairly consistent with these reports.

TS of fish without swimbladder is considerably lower than that of fish with swimbladder of equivalent size.[8] Moreover, TS are much lower in the region where the coefficient of log L is larger than 20 (or where $L/\lambda < 2$). In this region, additionally, TS varies sensitively with body size. Therefore, if juvenile fish dominates in field survey, the use of 120 kHz would be preferable, so that higher acoustic reflection can be obtained and TS composition of target school will not vary widely. On the other hand, the use of the two frequencies in that situation would be useful for size or species identification using TS differences (Fig. 5).

This is the first paper, which provides the two important acoustic material properties and theoretical TS of Japanese sandeel. The TS_{ave}-length equations given in Table 2 and frequency difference of TS_{ave} given in Fig. 5 are recommended for use in acoustic surveys. Additionally, the methods used here would be applicable to studies on other small swimbladderless fish species.

As mentioned above, measurements of sound-speed contrast of juvenile fish and swimming angle are the most important future works to make our results more reliable.

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FIGURE CAPTIONS

Fig. 1 Instrumentation for sound-speed measurement. PZT-transducers are mounted on each side of the T-tube. The bore of the tube is 80 mm and the measurement distance is 250 mm.

Fig. 2 Relationship between body mass density ρ and standard length of fish measured by the density-bottle method. The open circle is juvenile fish and closed circle is adult fish.

Fig. 3 Typical TS variations of juvenile (left) and adult (right) fishes as a function of fish tilt angle, obtained by the DCM. Solid line, at 38 kHz; broken line, at 120 kHz.

Fig. 4 Relationship between TS (left panels, TS_{max}; right panels, TS_{ave}) and log of standard length (L in cm). Upper panels are TS at 38 kHz and lowers are TS at 120 kHz. The open circle is juvenile fish and closed circle is adult fish. Equations of regression lines in each panel are given in Table 2.

Fig. 5 Difference of TS_{ave} values (ΔTS: $TS_{120kHz} - TS_{38kHz}$) between 38 kHz and 120 kHz plotted against standard length.

Fig. 6 Relationship between density contrast *g* and TS_{max} of a prolate ellipsoid (10.7 cm length, 1.0 cm height, 0.8 cm width) on given values of the *h* (0.01 steps), calculated by the liquid-DCM. Dark gray area is range of adult fish in the temperature range of the main fishing season (Table 1), and light gray area is the whole range (including juvenile fish) measured in this study. The closed circle is the values used in the TS computation in this paper. Open circles are reference values of other fish species: 1, Reference value by Furusawa (*g* = 1.040, *h* = 1.020).[25] 2, Herring (1.026, 1.018).[26] 3, Yellow tail (1.039, 1.011).[26] 4, Jack mackerel (1.041, 1.053).[26] 5, Chub mackerel (1.041, 1.026).[26] 6,White croaker (1.036, 1.027).[26] 7, Japanese bluefish (1.028, 1.030).[26] 8, Larval cod (1.017, 1.007).[26] 9, Dogfish (1.038, 1.045).[26] 10, Orange roughy (1.040, 1.040).[27] 11, Larval anchovy (1.029, 1.013).[16] 12, Northern lampfish (1.010, 1.036).[17]

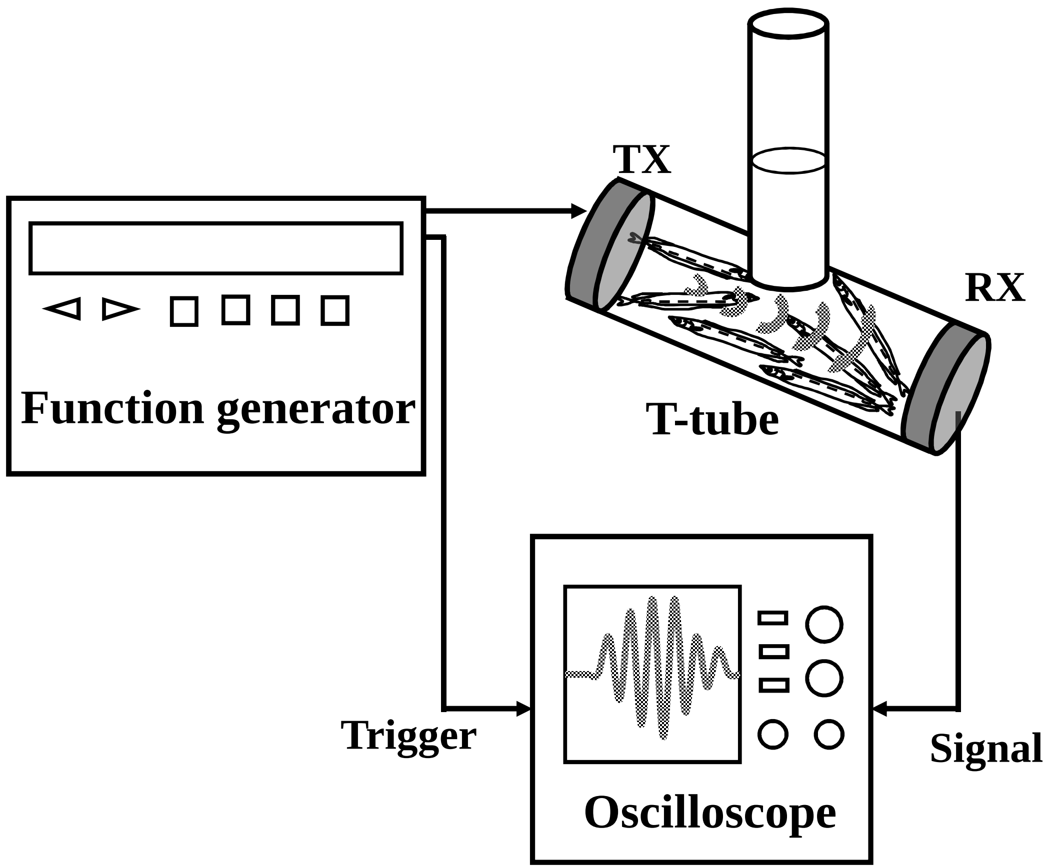
Table 1 The sound-speed through seawater, C_{sw}, and through the fish, C_{fish}, and the sound-speed contrast, h at each temperature. Values in temperature range of main fishing season are desclibed by boldface

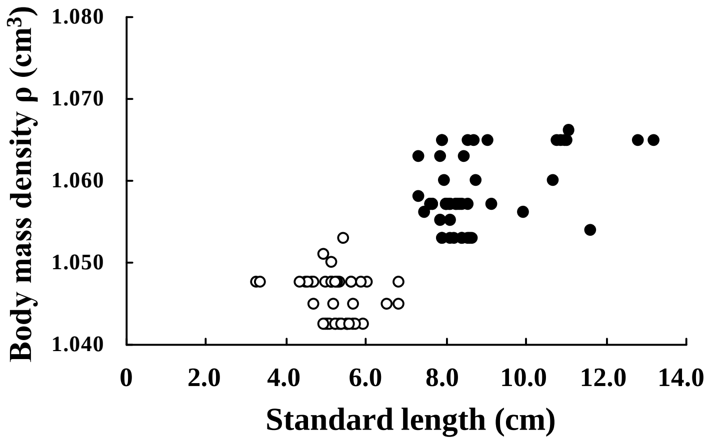
Temperature	C_{sw}	C_{fish}	h
7	1479	1520	1.028
8	1479	1518	1.026
9	1481	1519	1.025
10	**1487**	**1521**	**1.023**
11	**1491**	**1522**	**1.021**
12	**1495**	**1524**	**1.019**
13	**1498**	**1527**	**1.020**
14	**1501**	**1528**	**1.018**
15	**1504**	**1528**	**1.016**
16	1505	1531	1.017
17	1509	1534	1.017
18	1510	1536	1.017
19	1512	1540	1.018
20	1514	1541	1.018
21	1518	1547	1.019
22	1519	1549	1.019
23	1521	1551	1.020

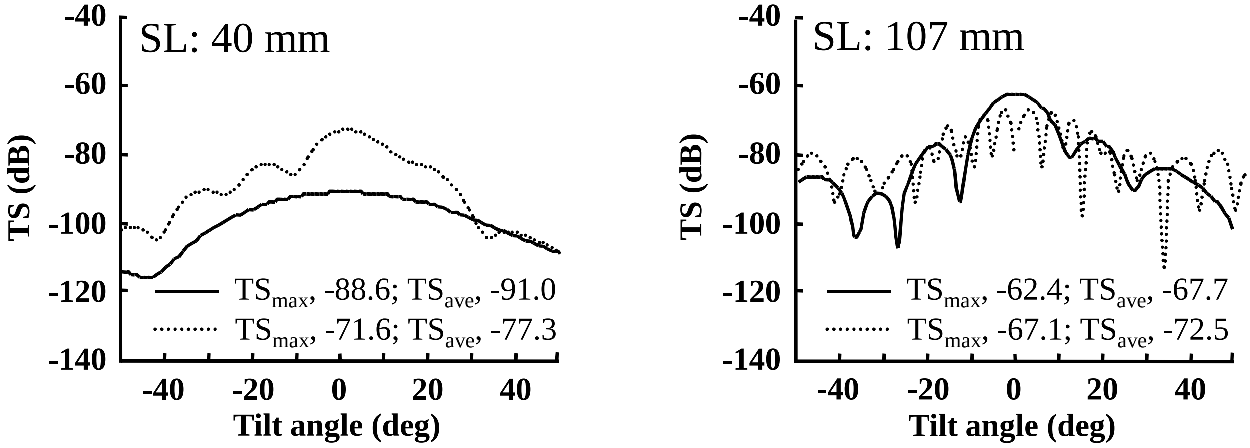
Table 2 Ranges of TS_{max} and TS_{ave}, and equations of the regression liens (TS-length equation) in Fig. 4

Stage	Standard length (cm)		38 kHz		120 kHz	
			Range	TS - length equation (R^2, S.E.)	Range	TS - length equation (R^2, S.E.)
Juvenile	3.4 - 6.7 (n = 68)	TS_{max} (dB)	–94.1 - –70.8	$TS_{max} = 62.2 \log L - 126.2$ (0.80, 2.45)	–75.5 - –63.1	$TS_{max} = 35.6 \log L - 93.0$ (0.75, 1.71)
		TS_{ave} (dB)	–96.8 - –73.7	$TS_{ave} = 56.5 \log L - 125.1$ (0.80, 2.45)	–81.7 - –71.2	$TS_{ave} = 34.0 \log L - 98.2$ (0.69, 2.00)
Adult	7.5 - 11.5 (n = 20)	TS_{max} (dB)	–68.5 - –61.3	$TS_{max} = 25.8 \log L - 90.3$ (0.75, 0.91)	–67.9 - –61.3	$TS_{max} = 18.4 \log L - 83.0$ (0.49, 1.47)
		TS_{ave} (dB)	–72.8 - –66.9	$TS_{ave} = 20.0 \log L - 89.2$ (0.68, 0.94)	–74.3 - –67.5	$TS_{ave} = 20.7 \log L - 92.1$ (0.48, 1.76)

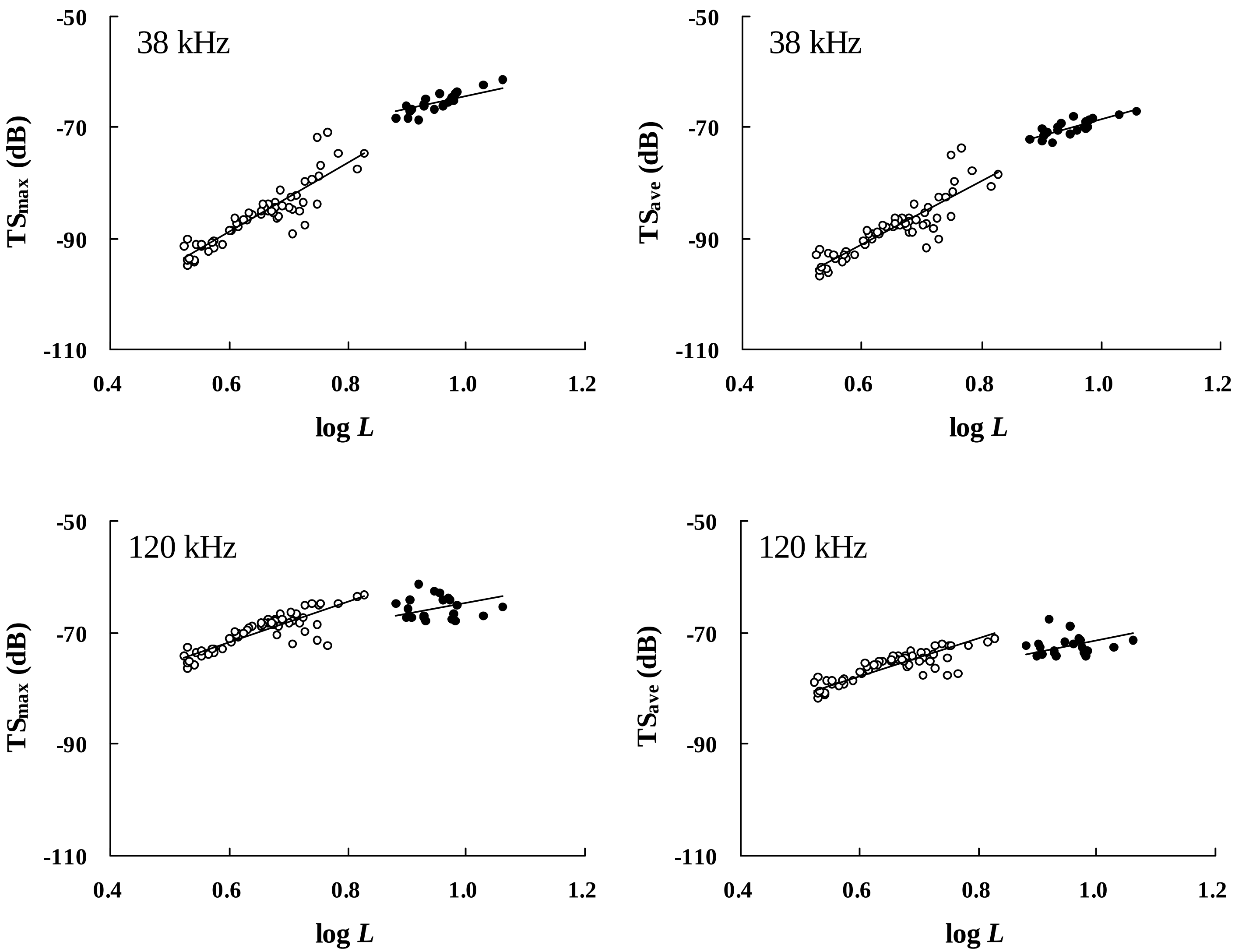
S.E., Standard error of estimate

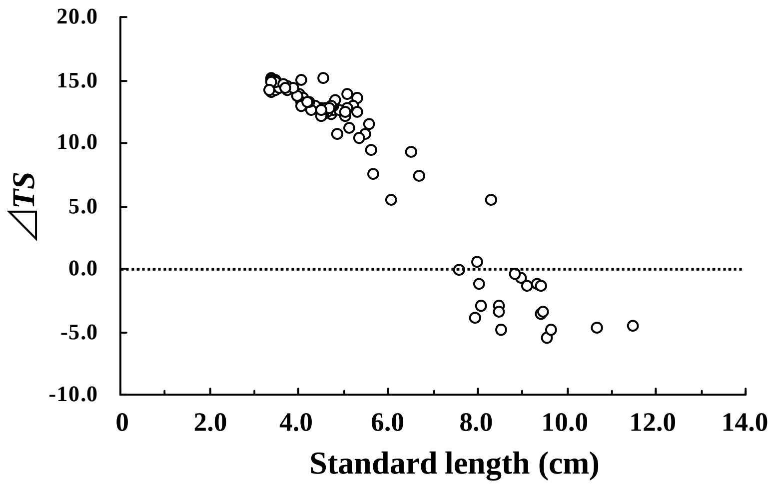

TX
RX
Function generator
T-tube
Trigger
Signal
Oscilloscope

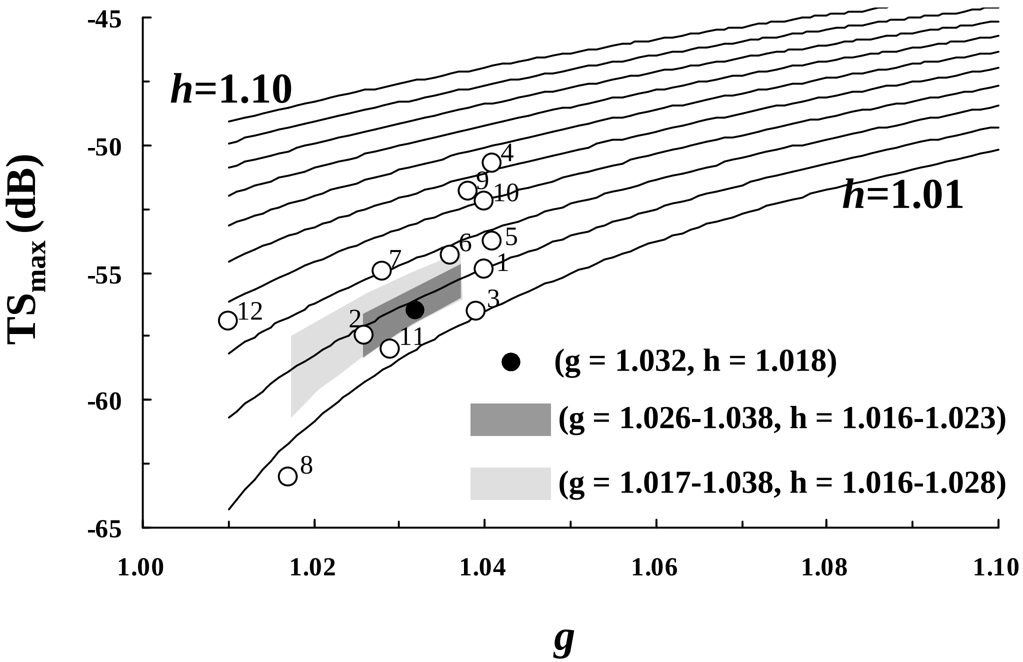

1.080
1.070
1.060
1.050
1.040
Body mass density ρ (cm3)
0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
Standard length (cm)



SL: 40 mm
TS (dB)
Tilt angle (deg)
TSmax, -88.6; TSave, -91.0
TSmax, -71.6; TSave, -77.3
SL: 107 mm
TS (dB)
Tilt angle (deg)
TSmax, -62.4; TSave, -67.7
TSmax, -67.1; TSave, -72.5


38 kHz
TSmax (dB)
TSave (dB)
120 kHz
log L
-50
-70
-90
-110
0.4
0.6
0.8
1.0
1.2


20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
△TS
0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
Standard length (cm)



-45
-50
-55
-60
-65
h=1.10
h=1.01
TSmax (dB)
1.00
1.02
1.04
1.06
1.08
1.10
g
1
2
3
4
5
6
7
8
9
10
11
12
(g = 1.032, h = 1.018)
(g = 1.026-1.038, h = 1.016-1.023)
(g = 1.017-1.038, h = 1.016-1.028)