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TARGET STRENGTH STUDIES ON ANTARCTIC SILVERFISH (*PLEURAGRAMMA ANTARCTICUM*) IN THE ROSS SEA

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Abstract

Target strength (TS) of preserved (frozen and defrosted) Antarctic silverfish (*Pleuragramma antarcticum*) of total length (TL) from 110 to 202.5 mm were measured *ex situ* (Ancona Bay) at 38, 120 and 200 kHz in May 2007 and in February 2009. Overall *ex situ* TS–TL relationships were: TS_{38} = 36.83Log TL(cm) –103.62 (*N* = 18); TS_{120} = 34.75Log TL(cm) –84.20 (*N* = 19); TS_{200} = 26.71Log TL(cm) –73.74; (*N* = 16). Nine *in situ* acoustic trawl experiments targeted at juveniles and post-larvae of *P. antarcticum* with a total length from 13 to 70 mm, were conducted in the southeast of the continental slope and shelf of the Ross Sea at depths from 30 to 120 m, during the Italian expeditions of 1997/98, 2000 and 2004. The regression lines for estimating TS from nine experiments were: TS_{38} = 57.58Log TL(cm) –123.77; TS_{120} = 51.94Log TL(cm) –105.92; TS_{200} = 46.61Log TL(cm) –95.29. These results indicate that the TS–TL relationships at 38, 120 and 200 kHz of adults of *P. antarcticum* differ consistently from those of post-larvae and juveniles. However, a simple model derived from the geometrical and acoustic characteristics of different parts of adult *P. antarcticum* and designed to fit fish life stages from adults to post-larvae and frequencies from 38 to 200 kHz, can predict the experimental TS measured both *ex situ* and *in situ* with a mean difference <1.5 dB, although the differences between the predicted and measured TS at some fish lengths were high (from 7.16 to –3.14 dB). The model shows that the main contribution to the scattering for small fish is from soft tissues and for larger sizes from hard tissues (e.g. skeletal structures). This may explain why small and large fish have different TS–TL relationships. In the authors' opinion the model could be used to give a first indication of the absolute abundance of *P. antarcticum* in the Ross Sea. A critical point is the decision rule to be used to differentiate *P. antarcticum* from *Euphausia superba* and *E. crystallorophias* that are present in the same region of the Ross Sea. Following the three-frequency decision criterion described in Azzali et al. (2004), some sizes of *P. antarcticum* could be confused acoustically with *E. crystallorophias*, but not with *E. superba*.

Résumé

L'indice de réflexion (TS) de calandres antarctiques (*Pleuragramma antarcticum*) conservées (congelées et décongelées) d'une longueur totale (TL) de 110 à 202,5 mm a été mesuré *ex situ* (baie d'Ancône) à 38, 120 et 200 kHz en mai 2007 et en février 2009. Dans l'ensemble, les relations TS–TL *ex situ* étaient : TS_{38} = 36,83Log TL(cm) –103,62 (*N* = 18) ; TS_{120} = 34,75Log TL(cm) –84,20 (*N* = 19) ; TS_{200} = 26,71Log TL(cm) –73,74 ; (*N* = 16). Neuf expériences acoustiques *in situ* de chalutage visant des juvéniles et des post-larves de *P. antarcticum* d'une longueur totale de 13 à 70 mm ont été réalisées au sud-est de la pente continentale et du plateau de la mer de Ross à des profondeurs de 30 à 120 m, au cours des campagnes d'évaluation italiennes de 1997/98, 2000 et 2004. Les droites de régression pour l'estimation de la TS des neuf expériences étaient : TS_{38} = 57,58Log TL(cm) –123,77 ; TS_{120} = 51,94Log TL(cm) –105,92; TS_{200} = 46,61Log TL(cm) –95,29. Ces résultats indiquent que les relations TS–TL à 38, 120 et 200 kHz pour les adultes de *P. antarcticum* diffèrent systématiquement de celles des post-larves et des juvéniles. Toutefois, un modèle simple dérivé des caractéristiques géométriques et acoustiques de différentes parties de *P. antarcticum* adulte, conçu pour reproduire les stades vitaux des post-larves jusqu'aux adultes et les fréquences de 38 à 200 kHz, peut prédire le TS expérimental, mesuré tant *ex situ* qu'*in situ*, avec une différence moyenne de <1,5 dB bien que, pour certaines longueurs de poissons, les différences entre les TS prévus et les TS mesurés soient élevées (de 7,16 à –3,14 dB). Le modèle indique que la principale contribution à la rétro-diffusion provient, pour les poissons de petite taille, des tissus mous et pour ceux de grande taille, des tissus durs (structures du squelette, par ex.), ce qui peut expliquer pourquoi les poissons de petite taille et ceux de grande taille ont des relations TS–TL différentes. Selon les auteurs, le modèle pourrait donner un premier indice de l'abondance absolue de *P. antarcticum* dans la mer de Ross. Un point critique est de décider quel critère employer pour différencier *P. antarcticum* de *Euphausia superba* et de *E. crystallorophias* qui fréquentent la même région de la mer de Ross. En suivant le critère de décision à trois fréquences décrit dans Azzali et al. (2004), certaines tailles de *P. antarcticum* risquent d'être confondues, lors d'un échantillonnage acoustique, avec celles de *E. crystallorophias*, mais non pas avec celles de *E. superba*.

Резюме

Сила цели (TS) сохраненной (замороженной и размороженной) антарктической серебрянки (*Pleuragramma antarcticum*) общей длиной (TL) от 110 до 202.5 мм измерялась *ex situ* (залив Анкона) на частотах 38, 120 и 200 кГц в мае 2007 г. и в феврале 2009 г. В целом, соотношения TS–TL *ex situ* составляли: TS_{38} = 36.83Log TL(см) –103.62 (N = 18); TS_{120} = 34.75Log TL(см) –84.20 (N = 19); TS_{200} = 26.71Log TL(см) –73.74; (N = 16). В ходе итальянских экспедиций 1997/98, 2000 и 2004 гг. на юго-востоке континентального склона и шельфа моря Росса на глубинах от 30 до 120 м было выполнено девять акустических траловых экспериментов *in situ*, направленных на изучение молоди и постличиночных особей *P. antarcticum* с общей длиной от 13 до 70 мм. Графики регрессии для оценки TS по данным этих девяти экспериментов были следующими: TS_{38} = 57.58Log TL(см) –123.77; TS_{120} = 51.94Log TL(см) –105.92; TS_{200} = 46.61Log TL(см) –95.29. Эти результаты говорят о том, что соотношения TS–TL на частотах 38, 120 и 200 кГц для взрослых особей *P. antarcticum* систематически отличались от соотношений для постличиночных особей и молоди. Однако простая модель, полученная по геометрическим и акустическим характеристикам различных частей тела в случае взрослых особей *P. antarcticum* и предназначенная для аппроксимации стадий жизненного цикла рыбы (от взрослых до постличиночных) и частот от 38 до 200 кГц, может прогнозировать экспериментальную TS, измеренную как *ex situ*, так и *in situ* со средним расхождением <1.5 дБ, хотя расхождение между прогнозируемой и измеренной TS при некоторых длинах рыбы было высоким (от 7.16 до –3.14 дБ). Модель показывает, что основное рассеяние в случае мелкой рыбы происходит от мягких тканей, а в случае рыбы более крупного размера – от твердых тканей (напр., скелетной структуры). Этим можно объяснить, почему мелкая и крупная рыба имеют различные соотношения TS–TL. По мнению авторов, эта модель может использоваться для получения исходных показателей абсолютной численности *P. antarcticum* в море Росса. Решающее значение имеет правило принятия решений, используемое для того, чтобы отличать *P. antarcticum* от *Euphausia superba* и *E. crystallorophias*, которые встречаются в том же регионе моря Росса. Если следовать трехчастотному критерию принятия решений, описанному в работе Azzali et al. (2004), некоторые размеры *P. antarcticum* могут акустически отождествляться с *E. crystallorophias*, но не с *E. superba*.

Resumen

El índice de reverberación (TS) de muestras conservadas (congeladas y descongeladas) de diablillo antártico (*Pleuragramma antarcticum*) de longitud total (TL) entre 110 y 202.5 mm fue medido *ex situ* (Bahía Ancona) a frecuencias de 38, 120 y 200 kHz en mayo de 2007 y febrero de 2009. En general, las relaciones *ex situ* TS–TL fueron: TS_{38} = 36.83Log TL(cm) –103.62 (N = 18); TS_{120} = 34.75Log TL(cm) –84.20 (N = 19); TS_{200} = 26.71Log TL(cm) –73.74; (N = 16). Durante las expediciones italianas de 1997/98, 2000 y 2004 se efectuaron nueve experimentos acústicos *in situ* con redes de arrastre dirigidos a *P. antarcticum* juveniles y postlarvales de 13 a 70 mm de longitud total, al sureste del talud continental y en la plataforma del Mar de Ross, a profundidades de 30 a 120 m. Las líneas de regresión para la estimación del TS de los nueve experimentos fueron: TS_{38} = 57.58Log TL(cm) –123.77; TS_{120} = 51.94Log TL(cm) –105.92; TS_{200} = 46.61Log TL(cm) –95.29. Estos resultados indican que las relaciones TS–TL a 38, 120 y 200 kHz para los adultos de *P. antarcticum* difieren sistemáticamente de las de los peces postlarvales y juveniles. No obstante, el TS experimental *ex situ* como *in situ* con una diferencia promedio de <1.5 dB se puede predecir con un modelo sencillo derivado de las características geométricas y acústicas de distintas partes de los peces adultos de *P. antarcticum*, diseñado para acomodar los estadios de vida de los peces desde adultos a postlarvales y frecuencias de 38 a 200 kHz; si bien hubo grandes diferencias (de 7.16 a –3.14 dB) entre el TS previsto y el TS medido en peces de cierta longitud. El modelo muestra que los tejidos blandos contribuyen más a la dispersión en los peces pequeños y los tejidos duros (v.g. esqueleto) en los peces más grandes. Esto podría explicar la causa de la diferencia entre la relación TS–TL de peces grandes y pequeños. Los autores estiman que el modelo podría ser utilizado para dar una indicación inicial de la abundancia absoluta de *P. antarcticum* en el Mar de Ross. Un elemento esencial es el criterio de decisión a ser utilizado para diferenciar *P. antarcticum* de *Euphausia superba* y *E. crystallorophias* presentes en la misma región del Mar de Ross. De acuerdo con el criterio de decisión de tres frecuencias descrito en Azzali et al. (2004), algunas tallas de *P. antarcticum* podrían confundirse acústicamente con *E. crystallorophias*, pero no con *E. superba*.

Keywords: Antarctic silverfish, *Pleuragramma antarcticum ex situ* target strength, *in situ* target strength, target strength models, CCAMLR

Introduction

Antarctic silverfish (*Pleuragramma antarcticum*) are an important component of the mid-trophic level and a key link between plankton and the community of top predators in the Ross Sea. Several studies have been published on its biology and ecology (De Witt, 1970; Hubold, 1985; Hubold and Hagen, 1997; Guglielmo et al., 1998; Vacchi et al., 2004). However, there is a lack of knowledge on the acoustic properties of *P. antarcticum* and thus there is no acoustic estimation of their distribution and abundance in the Ross Sea.

Pleuragramma antarcticum belongs to the category of non-swimbladder animals that, unlike swimbladdered fish, include species (crustaceans, molluscs, fish) whose physiology, shape, size and acoustic performance are very different. Acoustic studies of animals without a swimbladder have not been conducted over as large a range of species and sizes as that of fish with swimbladders. The main acoustic studies of non-swimbladders taxa are from mackerels, squids and euphausiids. It is unlikely that those results could be adequate to give even a rough indication of the target strength (TS) of *P. antarcticum*.

TS of non-swimbladder fish (e.g. mackerels) are from 16 dB (40 times) to 24 dB (250 times) lower than that of swimbladder-bearing fish of similar size (e.g. clupeids, gadoids) (MacLennan and Simmonds, 1992). On the other hand, TS of fish, such as *P. antarcticum*, that use lipid stores to control buoyancy, are expected to be much less depth dependent than those of fish that move up and down in the water column adjusting the volume of their swimbladder. The swimbladder accounts for over 90% of the echo energy (Foote, 1980a), and distributes the reflected sound more omnidirectionally than the flesh of a non-swimbladder fish. Therefore, TS of non-swimbladder fish is more influenced by the swimming angle. However, this effect could be less than expected for fish with scattering components (e.g. skeletal structure) distributed within their body. No *in situ* or *ex situ* measurements of tilt angle of *P. antarcticum* have ever been reported, and almost nothing is known of the acoustic and directional proprieties of their body and skeleton.

The ossification of *P. antarcticum* begins in the early juvenile stages (size: 45–48 mm; age: second year), coinciding with an increase in lipid content in the specific ventral sacs (from 12–15% of first year to 20–32% dry weight (DW) of second year; DeVries and Eastman, 1978; Eastman and DeVries, 1982; Wöhrmann et al., 1997). The ossification and lipid accumulation increase gradually up to the size of 65–70 mm (lipid content: 33–41% DW). Lipid content and skeleton structures have a crucial role both on the behaviour and acoustic scattering of *P. antarcticum*. Incorporation of low-density lipid (mainly triacylglycerols) prevents juvenile and adult *P. antarcticum* from sinking as the ossification process accumulates heavy materials in their bodies. In this way, *P. antarcticum* almost attain neutral buoyancy (density and speed contrast <1.020; Chu and Wiebe, 2005), a crucial factor for such a strong pelagic migrant (Wöhrmann et al., 1997). Lipid deposits are almost transparent to sound and so enhance the relative acoustic and directional properties of the skeletal structures and the surface of the body. Therefore, TS of *P. antarcticum* before the ossification (post-larvae, sub-juveniles: 15–45 mm length) can be considered as that of a weakly scattering fluid object, and at length >45–48 mm as that of an almost fluid object containing distributed scattering elements.

TS measurements can be grouped into three main categories (Foote, 1987), in accordance with the techniques and methods used.

Ex situ measurements on dead, stunned or living fish in cages

These measurements have been made over a wide range of frequencies, species and sizes of fish (Shibata, 1971; Haslett, 1970; Love, 1971; Greenlaw, 1977; Nakken and Olsen, 1977; Foote, 1980b; Fedotova and Shatoba, 1983; Sun et al., 1985; Arnaya et al., 1989; Mukai and Iida, 1996; Rose and Porter, 1996; Yasuma et al., 2006). The validity of the results has been questioned since they derived from fish whose acoustic and behavioural characteristics have been manipulated. For example, Sun et al. (1985) suggested that freezing the fish reduces the overall backscatter by 30%. However, the results of several *ex situ* experiments constituted a basis for calculating the expected echo from free-swimming fish, as did the observations on preserved euphausiids of Greenlaw and Foote's TS measurements on tethered and anesthetised fish. In general, the *ex situ* method should be considered as a first-approach method, useful when there is a lack of knowledge on acoustic performances of a fish species (e.g. *P. antarcticum*).

In situ measurements on free-swimming fish

These measurements include two methods: the direct method and the global method (also called comparison method; MacLennan and Simmonds, 1992). In the direct method, each fish is individually located in the beam and TS of each fish is determined. This method gives the best results with low bias and high accuracy, however, it may be of limited value where mixed species occur. In the global method the fish density, species and size determined by trawling are compared against echo integrator data; TS of a species is then given in terms of an 'average value'. The global method has been applied for measuring TS of several fish species (Pieper, 1979; Sameoto, 1980; Rose and Porter, 1996; Barange et al., 1996; Misund and Beltestad, 1996; Lillo et al., 1996), but does not allow the easy control of possible bias: catch composition, trawl efficiency, sampling selectivity, fishing volume, avoidance reaction and fitting echo-integrated layers to towed stratum (Barange and Hampton, 1994). Therefore, more discrepancies seem to occur in the TS estimations from the global method than in those from the direct method. However, faced with mixed populations with species providing very low and similar acoustic reflections and where it is very difficult to detect a single echo from the target species, the global approach is the only option. Where these circumstances are combined with unfavourable environmental conditions and large areas to be sampled, such as in the Ross Sea, the only practical approach is to determine an average TS using the global method. With an appropriate sampling regime it can be expected that, on average, the inaccuracies of the method can be accommodated.

Models

In the experimental models used in fishery science, TS, at a frequency, is assumed to be linearly related to the logarithm of the standard length (SL) or total length (TL) in the dorsal aspect ($TS = m \operatorname{Log} [\text{fish length}] + b$), with the two constants in the formula to be determined for each species by the best fit to *ex situ* or *in situ* data (TS–TL linear models). It is assumed that the variation in TS due to the orientation of the fish body in the acoustic beam is included in the two constants (Love, 1971; Nakken and Olsen, 1977; Foote, 1987). The regression coefficient m has been found to be close to 20 for a great many fish species (e.g. backscattering is proportional to length-squared). An alternate approach uses theoretical models. Simple theoretical models approximate the scattering components of a fish to geometric figures (spheres, ellipsoids, finite straight or bent cylinders) that make them amenable to calculations. The contribution of each part of the geometric components to the overall TS are calculated (Anderson, 1950; Haslett, 1962, 1970; Johnson, 1977; Stanton, 1988a, 1988b, 1989a, 1989b; Macaulay, 1994). Advanced theoretical models accurately measure and represent the morphology of the scattering components of fish using very-high-resolution instruments (phase contrast x-rays (PCX), computerised tomography (CT)). The acoustic properties of the different components are calculated from detailed laboratory acoustic measurements for a wide range of frequencies, angles of orientation and boundary conditions. Although these models have been developed mainly for fish with swimbladders (Foote, 1985; Francis and Foote, 1998; Clay and Horne, 1994; Horne et al., 2000; Macaulay, 2002), they are potentially applicable to other types of marine organisms (e.g. Antarctic krill (*Euphausia superba*), Demer and Conti, 2005; lantern fish Myctophidae, Yasuma et al., 2006). Theoretical models play a vital part in understanding and validating the results of *in situ* and *ex situ* experiments.

The aim of this paper is to determine TS of *P. antarcticum* at 38, 120 and 200 kHz using *in situ* and *ex situ* measurements and the usual TS–TL experimental models and then to design a theoretical model to explain and validate the experimental results. *In situ* measurements were performed on post-larvae and juvenile *P. antarcticum*, using the global method. The measurements on adult *P. antarcticum* were conducted on preserved (frozen and defrosted) fish, because the catches of adults were sporadic and insufficient to undertake any *in situ* calculations. The proposed theoretical model is based on the gross morphological and acoustic characteristics of the body and the vertebral column of adult *P. antarcticum*. It is a simple model,

Table 1: EK500 transceiver settings used during the two *ex situ* experiments.

Nominal frequency		38 kHz	120 kHz	200 kHz
Transducer type		ES38-B	ES120-7	ES200-7
Beam type		split	split	single
Trasmit power (kW)		2	1	1
Pulse duration (ms)		1	1	0.6
Band width (kHz)		wide	narrow	narrow
Ping interval (s)		0.1	0.1	0.1
Two-way beam angle (dB)	by manufacturer	–20.6	–20.5	–20.7
	by 2007, 2009 calibrations	–20.8	–20.1	
	standardised		–20.5	
s_V threshold (dB re m^{-1})			–80	
Noise margin (dB)		0	0	0

but adequate to understand and validate the *in situ* and *ex situ* results. The paper is not, in any sense, a complete investigation of the TS of *P. antarcticum*, rather it provides an overview of the current lack of acoustic knowledge on this important species, as well as on the Nototheniidae family in general, and attempts to address this situation using the best available data.

Methods

Ex situ measurements

Two experiments were conducted, the first in May 2007 and the second in February 2009 at a compound moored in Ancona Bay, Italy (43°37'N 13°28'E), near the RV *Dallaporta*, on which an acoustic laboratory was installed. Measurements of water temperature, density and salinity around the frame were obtained before each experiment from a Seabird Electronics SBE911 plus CTD. The trials of the experiments were performed:

- May 2007 – water temperature 16°C, salinity 36.50(psu), density 1 026.2 kg m^{-3} and pH = 8, corresponding to a sound speed of 1 512 m s^{-1};
- February 2009 – water temperature 9.1°C, salinity 34.74(psu), density 1 026.9 kg m^{-3} and pH = 8, corresponding to a sound speed of 1 486 m s^{-1}.

For acoustic measurements the Simrad EK500 echo sounder was used, with 38 kHz (type ES38-B) and 120 kHz (type ES120-7) split transducers and a single-beam 200 kHz (type ES200-7) transducer. The measurement data delivered with the three transducers indicated that their two-way beam angles, as well as their longitudinal and transversal –3 dB beam widths, were very similar. The transducers were bolted, with their centres as close as possible, to a floating platform, over the centre of the compound (Figure 1). Routine calibrations were carried out before each experiment with the copper spheres at a depth of around 10 m from transducers. In particular, the two-way beam angles of the split transducers were calculated from –3 dB points, measured according to the method described in the Simrad EK500 manual (1993). Results were close to the nominal values and constant in time (Table 1). In the two *ex situ* experiments, a standard value of Ψ obtained averaging the two-way beam angles and –3 dB beam widths (θ) for all three transducers, was used:

- two-way beam angle = –20.5 dB
- $\Psi = 8.9*10^{-3}$ str
- θ(transv.) = θ(longit.) = 7.2°.

The expected error due to beam-angle standardisation is <0.5 dB (6%).

The EK500 transceiver settings used in *ex situ* and *in situ* experiments were the same, except the s_V threshold that was set higher (–80 dB re m^{-1}) in *ex situ* experiments because the targets were suspended near the centre of the beam (Table 1). It should be noted that although equal pulse length and bandwidth at all frequencies, are the best settings (Korneliussen et al., 2004), this was not possible with the EK500 setup.

In 2007, 40 frozen *P. antarcticum*, ranging in size from 110 to 210 mm were used. They were divided into five length classes of eight individuals, as shown in Table 2(a). In 2009, only 16 frozen *P. antarcticum* were available. Their sizes ranged from 120 to 205 mm. They were divided into five classes as close as possible to those of 2007, containing from two to four individuals (Table 2b). Fish of the size class under examination were defrosted before each trial and their length measured (±5 mm). Then single fish were loosely tied in a dorsal position by their upper lip, fin and tail to monofilament lines (diameter = 0.1 mm; length = 3 m). These wires were

Table 2: Length classes of *Pleuragramma antarcticum* used in the experiment of May 2007 (a), and February 2009 (b). Small, medium and large fish used in one-fish trials are indicated in bold.

(a) May 2007 experiment: 40 individuals

Classes:	I	II	III	IV	V
Length (mm)	100	125	130	150	170
	105	125	135	150	170
	110	130	135	155	180
	110	130	140	155	180
	110	130	145	160	185
	115	130	145	160	190
	115	130	145	160	190
	115	135	150	165	210
Mean	110.0	129.4	140.6	156.9	184.4
SD	5.3	3.2	6.8	5.3	12.9
Number of trials	1	1	1	1	1

(b) February 2009 experiment: 16 individuals

Classes:	I	II	III	IV	V
Length (mm)	**120**	140	160	175	200
	125	145	**165**	**190**	205
	130	150	165	200	
		155	175		
Mean	125.0	147.5	166.3	188.3	202.5
SD	5.0	6.5	6.3	12.6	3.5
Number of trials	2	2	2	2	2

Table 3: Results of *ex situ* experiments carried out in May 2007 and in February 2009. Discarded measurements are in bold.

Experiment	Length (mm)		38 kHz		120 kHz		200 kHz	
	Mean	SD	TS	SD	TS	SD	TS	SD
May 2007	110.0	5.3	–64.92	0.58	**–70.50**		**–72.12**	
	129.4	3.2	–63.31	0.22	–44.58	0.50	–42.39	0.90
	140.6	6.8	**–69.45**		–44.78	1.45	–44.77	1.33
	156.9	5.3	–58.11	0.25	–40.66	1.27	–40.72	0.57
	184.4	12.9	**–65.53**		**–69.25**		**–49.91**	
February 2009	125.0	5.0	–60.85	1.51	–45.51	0.88	–43.17	1.81
			–60.47	0.23	–48.10	3.23	–44.25	1.58
	147.5	6.5	–61.30	0.20	–42.64	1.88	–43.83	3.82
			–63.00	0.32	–43.71	2.31	–43.20	3.74
	166.3	6.3	–57.78	0.30	–42.36	0.22	–43.23	0.25
			–59.29	0.30	–45.36	0.23	–40.87	0.13
	188.3	12.6	–54.67	0.35	–39.71	2.00	–39.91	2.67
			–41.26		–38.60	2.69	–39.24	1.36
	202.5	3.5	–54.33	2.82	–37.74	2.09	–37.58	2.65
			–54.54	1.10	–36.26	1.09	–38.21	0.85
	120 small	single	–63.40	0.22	–45.86	0.87	–45.08	1.30
		split	–65.49	4.04	–46.78	4.41		
	165 medium	single	–62.68	0.13	–40.48	1.44	–41.29	2.86
		split	–61.69	3.40	–41.9	3.81		
	190 large	single	–55.88	0.17	–43.01	1.28	–40.20	5.55
		split	–56.74	4.50	–41.50	4.50		
Number of TS measurements			18		19		16	
Max. TS			–54.33		–36.26		–37.58	
Min. TS			–65.49		–48.10		–45.08	

stretched between two opposite bars of the compound so that fish were situated near the acoustic axis and from 5 to 7.4 m beneath the transducers. This distance is greater than the maximum extension of the near field (2 m at 38 kHz). The monofilament lines had a tilt of around 15° to simulate a fish swimming angle of +15° (head up) or –15° (head down). In order to check the calibration and acoustic axis orientation, the 60 mm standard sphere (TS = –33.5 dB at 38 kHz) was kept in place through the experiments at 10 m from the transducers.

In 2007, one trial was performed on each length class. In 2009, each length class was examined twice, through two non-consecutive trials, to estimate the changes in TS due to changes of fish position and aspect within the scattering volume. Moreover, three one-fish trials were carried out on a small, medium and large fish (Table 2b). A total of 18 trials were carried out in the two experiments. Each trial consisted of a block of 10 measurements of s_A ($m^2 nmi^{-2}$) at 38, 120 and 200 kHz taken every 0.2 n miles using a simulated sailing vessel travelling at 10 knots. The ambient noise and backscattering response from the underwater framework and wires were measured before and after each trial and subtracted from the measured data. However, this contribution was negligible in both experiments. The volume backscattering coefficient s_V (m^{-1}) was calculated from s_A values returned from the integration layer ($r_2 - r_1 = 7.4 - 5 = 2.4$ m), following the Simrad EK500 manual (1993). The expected backscattering cross-section (m^2) of the mean fish TL for each length class was calculated from the s_V values and the fish-number density ρ (equation 1[1]).

In the one-fish trials the values of target strengths at 38 and 120 kHz were calculated from both the σ_{bs} values and the TS tables produced by the EK500 echo sounder. A total of 21 TS measurements at 38 and 120 kHz and 18 at 200 kHz was obtained from all the trials. However, the TS measurements with the largest (and unexplained) deviations from the TS–TL least-squares regression line were discarded (Table 3).

The *ex situ* experiment in 2007 was carried out at a water temperature about 7°C higher than that of 2009 and in both experiments the water temperatures were much higher than that of the Ross Sea (which ranged from –0.5 to –1°C). There is currently no information regarding the dependence of fish acoustic performances on water temperature parameters on live fish, and very little information on dead fish (Yasuma et al., 2006). Therefore, no adjustment for these differences in water temperature was attempted.

In situ measurements

Data from 131 hauls conducted during three expeditions to the Ross Sea (1997/98, 2000, 2004) and analysed following the global method indicated that taxonomic composition of the catch accounts for the largest proportion of variation in mean *in situ* TS estimation. Other factors that produce either systematic variations (trawl efficiency, avoidance reaction) or stationary variations (factors attributable to fish behaviour) in TS, are either unknown or not significant if TS is measured as an average value. It was assumed that a variation of around 20% or less in mean TS arising from the taxonomic composition, added to a probable 10% variation for unknown non-stationary and non-systematic factors, was sufficient to estimate a correlation coefficient $|R|$ exceeding the critical value in the TS–TL regression line ($|R_{cr}|$ for the nine trials was 0.666; Table 7 in Crow et al., 1960). For this study, only catches in which *P. antarcticum* were similar in size and comprised around 80% or more by number were analysed.

These conditions on size and species composition were found only in nine of 131 examined hauls. They are:

- three hauls of 35 conducted in December and January 1997/98;
- three hauls of 63 conducted in January and February 2000;
- three hauls of 33 conducted in January 2004.

The nine hauls were distributed on the Ross Sea southeast continental slope (four hauls from 72°37'–73°36'S and 175°17'–171°12'E) and the continental shelf (five hauls from 74°–76°S and 165°–176°E) (see Figure 2). The trawls of 1997/98 and 2000 were carried out with a 5 m^2 Hamburg plankton net (HPN, 1 000 µm mesh size). A pelagic trawl with doors was used in the 2004 expedition. This net has the same mesh size as the HPN codends (1 000 µm), but a mouth opening of 12 m^2. The trawls used in each fishing experiment are shown in Figure 2. In all the expeditions the trawl depth was determined on the basis of echo sounder observations, monitoring only the upper 200 m of the water column. A Simrad ITI system was used for monitoring the position and vertical opening

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[1] In order to simplify the presentation of the paper, please refer to the appendix for all equations.

of the net. The filtered volume was measured by a flowmeter (General Oceanics, Inc.), attached to the centre of the mouth. The taxonomic composition and mean length of *P. antarcticum* was determined for each haul. The size (TL) in the nine selected hauls ranged from 13–70 mm. The composition (% *P. antarcticum* by number) is reported in Figure 2. The average towing speed during each haul was 3.4 knots (SD = 0.56 knots). The mean duration of a haul was 49.7 min (SD = 10.1 min). Immediately after every haul, a CTD station was performed. On average, the nine hauls were carried out at a water temperature of –0.7°C, salinity 34 (psu), relative density 1.028, corresponding to a sound speed of 1 445 m s^{-1}.

In the three expeditions to the Ross Sea, TS data were collected by the same EK500 echo sounder used in *ex situ* experiments, with the 38, 120 and 200 kHz transducers housed within an oil-filled recess in the ship's hull, behind a polycarbonate window. For logistic reasons the echo sounder was calibrated in Italy in November, a few days before departure of the RV *Italica* for Antarctica (with the three copper spheres placed at around 25 m depth, at a water temperature of around 9°C, salinity 35 (psu), relative density 1.027, corresponding to a sound speed of 1 490 m s^{-1}). Transceiver settings during the fishing experiments were as that used in *ex situ* experiments (Table 2), but S_V threshold was set at –90 dB re m^{-1} to reduce its impact on fish TS. The s_A coefficients were generally obtained from the echo sounder at 0.1 n mile intervals. The start and stop positions for each tow were marked on the echograms. The distributions of s_A coefficients at the three frequencies in cells corresponding to 0.1 n mile length by 2 m depth were recorded with date and time. The depth stratum, date and time sampled by the net and fish distribution were read from the echograms. The s_A values corresponding to the stratum sampled by the net were also recorded. The mean volume backscattering coefficient s_V was estimated from the s_A values averaged along the transect length and the thickness of the sampled stratum, following the Simrad EK500 manual (Simrad, 1993). The backscattering cross-section of the fish σ_{bs} was calculated by equation (2).

The echo sounder was calibrated at a temperature about 10°C higher than that at which hauls were conducted. According to laws of physics, this difference in sound speed should not affect the measurements of the mean volume backscattering coefficient s_V, because the changes of the equivalent beam angle Ψ and of gain G cancel each other, and it has a negligible effect (overestimation of <0.2 dB) on TS (Bodholt, 2002). However, changes in transducer materials (piezo-electrical ceramics, metals) and then in the electrical impedance Z with temperature can potentially produce significant changes in the echo sounder performance (Brierley et al., 1998). Demer and Renfree (2008) demonstrated that the effects of changes in Z with temperatures ranging from 1° to 18°C are different for different transducer types (ES38-B, ES120-7 and ES200-7) and potentially also for different transducers of the same type (depending on whether the operational frequency is higher or lower than the resonance frequency). According to Demer and Renfree (2008), it could have led to TS overestimation of ≤0.75 dB at 38 and 200 kHz and of ≤1.5 dB at 120 kHz. However, recess window material and oil type could influence these estimations. In the authors' opinion these variations in TS are within the range of the other systematic variations from other sources and therefore no adjustment for the effect of water temperature was attempted.

Models

Experimental models of TS–TL relationship at 38, 120 and 200 kHz are given as a best-fitting (e.g. least-squares) equation to the experimental data: $TS = m \, Log (TL_{cm}) + b$, for both *ex situ* measurements on adults (TL = 11.00–20.25 cm) and *in situ* measurements on post-larvae and juveniles (TL = 1.33–6.89 cm) of *P. antarcticum*.

The proposed theoretical TS model was calculated by examining in some detail the geometric and scattering characteristics of the body and the vertebral column of several adult *P. antarcticum*. X-rays of preserved fish, ranging in TL between 110 and 205 mm were examined transversally and longitudinally. The positions of the sectional views measured from the reference point (the nose) at different coordinate x and all dimensions (lateral and dorsal) were related to the overall length of the fish (TL in mm). The fish were also weighed and the volume and density of the body as a whole, and of the backbone in particular, were determined using the Archimedes principle. The average dimensions of the body and vertebral column were calculated at different positions along the axis of the fish. The bony spines and fins, as well as the light downward curvature of the vertebral column, were ignored, as they presumably do not contribute appreciably to the level of reflected sound. The dimensions of the ellipsoid that fits reasonably well for the fish body were: L = standard length = c_l (TL), H = body height at the base of the pectoral fin = c_h (TL) and W = width of the body at the base of the pectoral fin = c_w (TL), where c_l, c_h, c_w are morphological coefficients of the *P. antarcticum* body (Figure 3). This approximation was reasonably accurate, at least for the fish body volume, that was close (<10%)

to the volume determined experimentally. The approximate equivalent volume (V) and spherical radius (r_b) of the fish body at a function of the morphologic coefficients were calculated according to equations (3) and (4).

For high frequencies (i.e. $1 << (kr_b) \rightarrow 20$, ellipsoid; k = wave number = $2\pi/\lambda$), the asymptotic upper limit of the acoustic cross-section of an ellipsoid (σ_{body}), approximating the fish body (dorsal aspect), is expressed in equation (5) (Haslett, 1970).

At low frequencies (i.e. $1 >> (kr_b) \rightarrow 0.2$, spheroid), when the equivalent spherical radius of the fish body fall in the Rayleigh scattering region, the lower asymptotic limit of the acoustic cross-section can be represented by equation (6) (Haslett, 1970).

The parameter R_b in equations (5) and (6) is the reflection coefficient of the fish flesh. It depends on: (i) the mass density contrast g (density of *P. antarcticum* flesh/density of Ross Sea water); (ii) the sound speed contrast h (compression speed in *P. antarcticum* flesh/sound speed in Ross Sea water); (iii) the shape of the fish body (ellipsoidal for adults (equation 5), spherical for post-larvae and sub-juveniles (equation 6)). The following formula, obtained by interpolating those for ellipsoids and spheroids proposed by Stanton (1989b), was used for calculating R_b at all (kr_b) in equation (7).

The density and sound-speed contrast were taken from experiments on two sets of juvenile *P. antarcticum* with lengths of 60–75 mm (9 animals: $g = 1.018$; $h = 1.017$) and 69 mm (11 animals: $g = 1.007$; $h = 1.013$) (Chu and Wiebe (2005; Table 3)). The mean values of the two experiments: $g = 1.012$ and $h = 1.015$, produced $|R_b\ (kr_b)|$ values ranging from 1.7% (spheroid) to 2.6% (ellipsoid) (in good agreement with the mean characteristics of fish soft tissues: R_b = 1.9% (Haslett, 1970)). The frequency response of the backscattering cross-section σ_{body}, for (kr_b) ranging from 0.2 to 20 is similar to that of a high-pass filter, however, the non-linear models used in the past (e.g. Johnson, 1977) do not provide a convenient way to link the asymptotic responses at low and high frequencies. On the contrary, the non-linear high-pass model described by Cann (1980) (see equation (8)), allows the adjustment of the knee that links the lower and upper asymptotic limits through the parameter s (s can be adjusted from 2 (soft knee) to ∞). The formula is flexible and can be used in many ways, for example as band-pass, limiter and detector. Taking this function, and performing a minor algebraic manipulation, yields the acoustic cross-section of the *P. antarcticum* body (σ_{body}) valid in the range ($0.2 \leq kr_b \leq 20$) (see equation 9):

The sharpness parameter s used in this paper is: $s = 10$ (hard knee).

For $(kr_b) \rightarrow \infty$ and $(kr_b) \rightarrow 0$ the asymptotic formulas of the acoustic cross-section were obtained at high (equation 5) and low (equation 6) frequencies respectively. The backscattering cross-section of the fish body is: $\sigma_{body}/4\pi$.

The core of the vertebral column was approximated to a straight cylinder of length $L_v = c_v$ (TL) and radius $r_v = c_r$ (TL) where c_v and c_r are the morphological characteristics of the backbone. The backscattering cross-section of the vertebral column was calculated with a procedure similar to that followed for the body and using the formulas reported by Stanton (1989b, Table 1). In the geometric region (i.e. $1 << (kr_v)$, rigid cylinder with reflectivity R_v), the upper asymptotic limit of the acoustic cross-section is represented by equation (10).

In the Rayleigh scattering region (i.e. $1 >> (kr_v)$, fluid cylinder with reflectivity R_v) the lower asymptotic limit of the acoustic cross-section is represented by equation (11).

For all (kr_v) values (high-pass model), performing the same computations on equations (10) and (11) as those done for equations (5) and (6), is shown in equation (12).

The amplitude reflection coefficient of the bone R_v was calculated after Stanton (1989b) (see equation (13)).

As there are no measurements of g, h for a *P. antarcticum* skeleton, values of $g = 1.036$ (following the measurements on backbone density) and $h = 1.25$ were used, giving $|R_v| = 0.22$, which is in reasonable agreement with the mean characteristics of fish hard tissues ($R_v \approx 26\%$; Haslett, 1970).

Assuming the linearity of acoustics in the fish target and using the superposition of echoes, considering that over 98% of the incident energy passes through the soft tissue so that the value of R_v in water can be taken (although the bones are surrounded by fish flesh), the total backscattering cross-section and TS of *P. antarcticum* can be expressed as equations (14) and (15).

This model is designed to fit data to a range of frequencies and life stages as wide as those examined in this paper, i.e. frequencies from 38 to 200 kHz and lengths from 0.013 to 0.202 m.

Results

System performance

The acoustic system calibrations with standard spheres exhibited statistical stationarity (almost constant mean and variance for all frequencies) in TS and in beam-angle parameters both over days (during *ex situ* experiments) and between years (during the experiments *in situ*: 1997/98, 2000, 2004 and *ex situ*: 2007, 2009).

Ex situ results

The mean s_A coefficient from the underwater framework and wires was stationary and very low during both *ex situ* experiments: mean s_A from noise $<10^{-4}$ mean s_A from small single fish (120 mm).

The results of the two experiments are summarised in Table 3. The overall distribution of *P. antarcticum* TS (dB re 1 m^2) ranged:

- at 38 kHz from –65.49 (TL = 110 ± 5.4 mm) to –54.33 (TL = 202 ± 3.5 mm);
- at 120 kHz from –48.10 (TL = 125 ± 5 mm) to –36.26 (TL = 202 ± 3.5 mm);
- at 200 kHz from –45.08 (TL = 120 mm) to –37.58 (TL = 202 ± 3.5 mm).

In the repeated trials, TS from the same fish but with different aspects in the beam differed from –1.5 to 3 dB.

TS levels (dB re 1 m^2) were significantly ($P < 0.05$) related to fish total length at 38, 120 and 200 kHz (Figure 4). The best-fit (least-squares) linear model was:

- at 38 kHz: $TS_{38}(\text{adult}) = 36.83\text{Log}(TL_{cm}) - 103.62$; $N = 18$; $R^2 = 0.75$;
- at 120 kHz: $TS_{120}(\text{adult}) = 34.75\text{Log}(TL_{cm}) - 84.20$; $N = 19$; $R^2 = 0.73$;
- at 200 kHz: $TS_{200}(\text{adult}) = 26.71\text{Log}(TL_{cm}) - 73.74$; $N = 16$; $R^2 = 0.78$.

In situ results

Details of *in situ* fishing experiments are reported in Table 4. The maximum depth of the net in the nine hauls ranged from 30 to 119 m (mean value 69.4 ± 33.5 m). The mean water parameters averaged from 0 to 200 m depth during the nine hauls were: $T = -0.74° \pm 0.37°$C; $S = 34.20 \pm 0.12$ (psu); density: 1027.73 ± 0.22 kg m^{-3}. The overall distribution of *P. antarcticum* TL ranged from (13.3 ± 0.3) to (68.9 ± 11) mm, spanning life stages from post-larvae (TL = 10–30 mm; N = 104 070) to sub-juveniles and juveniles (TL = 30–70 mm; N = 2 899). The trawl catches in all hauls were dominated by *P. antarcticum* (mean value in % by numbers 87.0 ± 7.6 and by weight 89.2 ± 14.1). Other species present in the catch that could be present in the acoustic signals were ice krill (*E. crystallorophias*) (highest in % by numbers – 21.3%), *E. superba* (highest in % by numbers – 17.7%) and bigeye krill (*Thysanoessa macrura*) (highest in % by numbers – 10.5%). Figure 5 shows the relationship between TS and TL measured *in situ* at 38, 120 and 200 kHz. The overall distribution of *P. antarcticum* TS ranged:

- at 38 kHz from –115.02 (TL = 13.3 ± 0.3 mm) to –74.98 (TL = 68.9 ± 11 mm);
- at 120 kHz from –98.83 (TL = 13.3 ± 0.3 mm) to –64.07 (TL = 68.9 ± 11 mm);
- at 200 kHz from –90.36 (TL = 13.3 ± 0.3 mm) to –53.81 (TL = 68.9 ± 11 mm).

TS levels were very significantly ($P < 0.01$) related to total length at 38, 120 and 200 kHz. The line of best fit to data was:

- at 38 kHz: $TS_{38}(\text{juven.}) = 57.58\text{Log}(TL_{cm}) - 123.77$; $R^2 = 0.99$;
- at 120 kHz: $TS_{120}(\text{juven.}) = 51.94\text{Log}(TL_{cm}) - 105.92$; $R^2 = 0.98$;
- at 200 kHz: $TS_{200}(\text{juven.}) = 46.61\text{Log}(TL_{cm}) - 95.29$; $R^2 = 0.97$.

Results from the theoretical model

After measurements of the dimensions of 18 preserved fish, ranging in TL between 110 and 205 mm, the mean body of a 'standard silverfish' was approximated to an ellipsoid of axes L, H, W:

L = standard length SL = $c_l(TL) = (0.88 \pm 0.02)TL$;

H = body height at the base of the pectoral fin = $c_h(TL) = (0.118 \pm 0.01)TL$;

W = body width at the base of the pectoral fin = $c_w(TL) = (0.087 \pm 0.007)TL$.

Table 4: Details of *in situ* experiments on *Pleuragramma antarcticum* conducted in the 1997/98, 1999/2000 and 2003/04 Italian expeditions to the Ross Sea, showing start/end date and location of each haul, fish distribution during hauls, catch results and mean target strength estimates. Two types of fish distribution are considered: local, when fish are concentrated within a transect length shorter than one nautical mile, scattered if fish are spread out over two or more nautical miles. Within both distributions fish are classified as dispersed or schooled depending on the echogram shape.

Expedition		1997/1998			1999/2000			2003/2004		
Net model – haul number		PHN-08	PHN-14	PHN-33	PHN-07	PHN-57	PHN-59	HPRI-18	HPRI-19	HPRI-33
Date (local)	D/M/Y	19/12/97	21/12/97	02/01/98	22/01/00	04/02/00	05/02/00	15/01/04	16/01/04	27/01/04
	Start (h.min)	1.13	16.08	8.34	1.47	17.40	5.54	11.31	5.50	10.40
	End (h.min)	2.15	16.59	9.43	2.26	18.28	6.27	12.33	6.44	11.36
Location (start)	Lat. (S)	73.945°	73.592°	74.890°	75.900°	73.210°	74.560°	72.839°	72.622°	75.220°
	Long. (E)	175.229°	175.691°	164.640°	176.120°	171.200°	170.970°	174.510°	175.273°	167.480°
	Bottom depth (m)	594	500	558	628	573	354	347	443	385.6
Haul	Duration (min)	62	51	42	39	48	33	62	54	56
	Length (m)	5926	4646	5556	4634	5672	4028	6009	4546	4999
	Mean speed (kn)	3.1	3.0	4.3	3.8	3.8	4.0	3.1	2.7	2.9
	Max. depth (m)	72	30	45	34	62	47	119	110	100
	Filter volume (m^3)	22964	18890	19877	17968	25576	21404	64070	56289	58883
Forms of fish distribution	Type	scattered dispersed	local dispersed	scattered dispersed	scattered schooled	scattered schooled	local dispersed	local dispersed	local schooled	scattered schooled
	Vertical extent (m)	58–74	10–58	35–60	26–58	60–65	26–58	95–135	80–110	75–115
	Transect length (m)	234	96	35	354	68	108	57	45	96
Net catch (*P. antarcticum*)	Number	353	1321	418	453	209	56933	30	115	47137
	% by number	82.3%	82.9%	78.7%	96.8%	92.5%	99.7%	78.9%	84.6%	87.1%
	Mean TL (mm)	42.3	45.5	35.1	53.9	40.4	16.4	43.9	68.9	13.3
	Range (mm)	33.8–52.3	38.2–49.1	25.1–43.6	44.7–62.2	38–41.2	13.1–42.5	13.1–42.5	57.8–80.7	9.8–63.3
	Total weight (g)	99.4	496.9	63.1	285.9	50.2	672.1	38.0	214.0	773.4
	% by weight	78.6%	75.6%	98.9%	99.6%	97.3%	94.8%	99.6%	97.9%	60.6%
Net catch (*E. superba*)	Number	76	272						5	
	% by number	17.7%	17.1%						3.7%	
	Mean TL (mm)	38.0	41.0						45.5	
	Total weight (g)	27.0	160						3.9	
	% by weight	21.4%	24.4%						1.8%	

(continued)

Table 4 (continued)

Expedition		1997/1998			1999/2000			2003/2004		
Net model – haul number		PHN-08	PHN-14	PHN-33	PHN-07	PHN-57	PHN-59	HPRI-18	HPRI-19	HPRI-33
Net catch (*E. crystallorophias*)	Number			113	15		15	4	9	6981
	% by number			21.3%	3.2%		0.0%	10.53%	7%	12.9%
	Mean TL (mm)			11.2	23.5		23.1	17.9	22.6	23.6
	Total weight (g)			0.7	1.1		1.4	0.1	0.6	503.6
	% by weight			1.1%	0.4%		0.2%	0.30%	0.26%	39.4%
Net catch (other)	Number					17	177	4	7	
	% by number					7.5%	0.3%	10.5%	5.1%	
	Total weight (g)					1.40	35.40	0.03	0.10	
	% by weight					2.7%	5.0%	0.1%	0.0%	
Mean TS (38 kHz)		–86.83	–85.37	–92.70	–82.68	–89.13	–113.21	–86.34	–74.98	–115.02
Mean TS (120 kHz)	(dB re 1 m^2)	–74.47	–68.45	–76.93	–67.99	–74.69	–96.23	–71.55	–64.07	–98.83
Mean TS (200 kHz)		–67.62	–66.61	–71.78	–62.73	–65.46	–83.16	–63.25	–53.81	–90.36

Table 5: Comparison between data predicted by the theoretical model and data measured *in situ* and *ex situ*.

Total length range (mm)	Difference	(TS predicted) – (TS measured) in dB re 1 m^2		
		38 kHz	120 kHz	200 kHz
From 205 to 110 (adult)	Max.	2.26	1.25	1.58
	Min.	0.25	–0.86	0.10
From 70 to 13 (post-larvae/juveniles)	Max.	5.07	7.61	4.30
	Min.	–3.14	0.34	–0.07
From 205 to 13 (all)	Mean	0.76	1.26	0.85
	SD	2.01	1.77	1.06
Number of data		27	28	25

Giving an equivalent spherical radius and volume:

$r_b = a_b(\mathrm{TL}) = (104 \pm 7)10^{-3}\ (\mathrm{TL})$
$V_b = (4/3)\pi\ (r_b)^3 = (4.74 \pm 0.95)10^{-3}\ (\mathrm{TL})^3$;

where TL = overall fish length in metre; $a_b = [((c_l\ c_h\ c_w)^{1/3})/2]$.

Overall, the standard silverfish volume calculated from ellipsoid approximation was 8% lower than the overall fish volume determined by the Archimedes' principle.

The square of the mean acoustic reflectivity of *P. antarcticum* flesh $[R_b\ (kr_b)]^2$, from equation (7), ranged from $(2.89)10^{-4}$ to $(6.76)10^{-4}$. Taking into account the values of c_h and a_b, the equation (9) of the backscattering cross-section of the fish body becomes as expressed in equation (16).

The core of the vertebral column of the standard silverfish approximates a cylinder having:

r_v = radius = $c_r(\mathrm{TL}) = (0.011 \pm 0.001)(\mathrm{TL})$
L_v = length = $c_v(\mathrm{TL}) = (0.65 \pm 0.02)(\mathrm{TL})$.

On average, the volume of the vertebral column calculated from these measurements was:

$V_v = (2.64 \pm 0.52)10^{-4}\ (\mathrm{TL})^3$.

The mean volume of the vertebral column was 5.1% of the overall measured volume of the fish, and 5.6% of the calculated volume (V_b). The mean density of fish bones has been found to be $(1.065 \pm 0.004)10^3$ kg m^{-3} (density contrast: $g = 1.036$). Assuming a sound speed contrast $h = 1.25$ (i.e. compression speed in the bones = 1 811 m s^{-1}) on the basis of the TS measured *in situ* and *ex situ*, the square of the mean acoustic reflectivity of wet *P. antarcticum* bones results would be $(R_v)^2 = (4.72)10^{-2}$. Taking into account the values of r_v, c_v and $(R_v)^2$, equation (12) becomes as expressed in equation 17. Figure 6 shows the TS–TL relationships at 38, 120 and 200 kHz, with TS calculated from equations (16) and (17): $\mathrm{TS} = 10\mathrm{Log}((\sigma_{body}/4\pi) + \sigma_v)$ where $(\sigma_{body}/4\pi) + \sigma_v = \sigma_{\mathrm{bs}}$ is the backscattering cross-section (m^2).

The differences between TS predicted from the model and those measured *in situ* and *ex situ* are reported in Table 5. Although for some small-sized fish (<70 mm) the differences between the predicted and measured TS are very high (up to 7.6 dB re 1 m^2), the difference averaged from 205 to 13 mm is <1.5 dB at all frequencies.

Defining the ratio between backbone and fish backscattering cross-sections as $\gamma = \sigma_{\mathrm{vert.}}/\sigma_{\mathrm{bs}}$ and averaging γ from TL = 15 to 205 mm, gives:

- at 38 kHz, $\gamma_{\mathrm{mean}} = 43\%$
- at 120 kHz, $\gamma_{\mathrm{mean}} = 81\%$
- at 200 kHz, $\gamma_{\mathrm{mean}} = 90\%$.

Therefore the mean backscattering cross-section of *P. antarcticum* σ_{bs}, within the bounds of the model (TL = 15–205 mm), is governed at low frequency mainly by the body flesh, and at higher frequencies by the material and dimensions of fish bone. Moreover, for sizes smaller than about three wavelengths, the main contribution to the scattering comes from the soft tissues of the fish ($\gamma < 50\%$), while for larger sizes it is formed by hard tissues ($\gamma > 50\%$). This gives, for example:

- at 38 kHz (λ = 38.1 mm) for TL < 130 mm, $\gamma < 48\%$;
- at 120 kHz (λ = 12.1 mm) for TL < 40 mm, $\gamma < 46\%$;
- at 200 kHz (λ = 7.2 mm) for TL < 25 mm, $\gamma < 49\%$.

The equation of TS–TL deduced from equations (16) and (17) is not linear and quite complex. However, it can be put into a more commonly used linear form with excellent approximation:

- at 38 kHz: $\mathrm{TS}_{38} = 53.1\mathrm{Log}(\mathrm{TL}_{\mathrm{cm}}) - 120.9$; $R^2 = 0.97$;
- at 120 kHz: $\mathrm{TS}_{120} = 48.3\mathrm{Log}(\mathrm{TL}_{\mathrm{cm}}) - 100.65$; $R^2 = 0.99$
- at 200 kHz: $\mathrm{TS}_{200} = 41.1\mathrm{Log}(\mathrm{TL}_{\mathrm{cm}}) - 89.8$; $R^2 = 0.97$.

These equations could be used as a first approach for estimating the absolute abundance of *P. antarcticum* in the Ross Sea. A critical point is the criteria to be used to discriminate *P. antarcticum* from *E. superba* and *E. crystallorophias* that co-occur in some areas. Following the three-frequency decision criterion described in Azzali et al. (2004), the results summarised in Table 6 were obtained. Some sizes of *P. antarcticum* (145–95 mm and 45–25 mm) could be misclassified as *E. crystallorophias*, however, it seems improbable to confuse *P. antarcticum* with *E. superba*.

Table 6: Results of the three-frequency decision criterion, described in Azzali et al. (2004), applied for discriminating *Pleuragramma antarcticum* from *Euphausia superba* (E.s.) and *E. crystallorophias* (E.c.); 'unknown' species class – (?). *Pleuragramma antarcticum* TS at 38, 120 and 200 kHz have been calculated from the theoretical model.

Decision criterion	Frequency ratio 120/38 *E. superba* (E.s.) Window: from 2 to 13.8 dB *P* (E.s. < 13.8 dB) = 93.3% mean: 10.16 ± 2.44 dB; *N* =130 *E. crystallorophias* (E.c.) Window: from 13.8 to 18.5 dB *P* (E.c. > 13.8 dB) = 97.8% mean: 16.33 ± 1.35 dB; *N* = 49			Frequency ratio 200/120 *E. superba* (E.s.) Window: from 1 to 4.3 dB *P* (E.s. < 4.3 dB) = 93.3% mean: 2.56 ± 1.15 dB; *N* =75 *E. crystallorophias* (E.c.) Window: from 4.3 to 8 dB *P* (E.c. > 4.3 dB) = 92.7% mean: 6.04 ± 1.20 dB; *N* = 52			Frequency ratio 200/38 *E. superba* (E.s.) Window: from 2 to 18.5 dB *P* (E.s. < 18.5 dB) = 95.5% mean: 12.97 ± 3.26 dB; *N* = 75 *E. crystallorophias* (E.c.) Window: from 18.5 to 27 dB *P* (E.c. > 18.5 dB) = 97.8% mean: 22.35 ± 1.9 dB; *N* = 49			First vote: $TS_{120/38}$	Second vote: $TS_{200/120}$	Third vote: $TS_{200/38}$	Majority vote rule
TL (mm)	TS_{120}	TS_{38}	$TS_{120/38}$	TS_{200}	TS_{120}	$TS_{200/120}$	TS_{200}	TS_{38}	$TS_{200/38}$				
205	–39.4	–53.0	13.6	–38.5	–39.4	0.8	–38.6	–53.3	14.8	E.s.	other	E.s.	?
195	–39.7	–54.1	14.5	–39.2	–39.7	0.5	–39.2	–54.5	15.4	E.c.	other	E.s.	?
185	–39.9	–55.3	15.4	–39.8	–39.9	0.1	–39.8	–55.8	16.0	E.c.	other	E.s.	?
175	–40.1	–56.5	16.4	–40.4	–40.1	–0.3	–40.5	–57.2	16.7	E.c.	other	E.s.	?
165	–40.7	–57.8	17.1	–41.1	–40.7	–0.4	–41.1	–58.5	17.4	E.c.	other	E.s.	?
155	–41.6	–59.1	17.5	–41.8	–41.6	–0.2	–41.8	–60.0	18.2	E.c.	other	E.s.	?
145	–42.8	–60.4	17.6	–42.5	–42.8	0.3	–42.5	–61.4	19.0	E.c.	other	E.c.	E.c. (?)
135	–44.5	–61.7	17.2	–43.1	–44.5	1.4	–43.1	–63.0	19.8	E.c.	E.s.	E.c.	E.c. (?)
125	–46.5	–63.0	16.5	–43.7	–46.5	2.8	–43.7	–64.5	20.8	E.c.	E.s.	E.c.	E.c. (?)
115	–48.7	–64.3	15.6	–44.2	–48.7	4.5	–44.2	–66.1	21.9	E.c.	E.c.	E.c.	E.c.
105	–51.0	–65.6	14.6	–44.5	–51.1	6.5	–44.6	–67.7	23.1	E.c.	E.c.	E.c.	E.c.
95	–53.6	–66.9	13.3	–45.7	–53.6	7.9	–45.7	–69.2	23.5	E.s.	E.c.	E.c.	E.c.
85	–56.4	–68.2	11.8	–47.8	–56.5	8.8	–47.8	–70.8	23.0	E.s.	other	E.c.	?
75	–59.5	–69.8	10.3	–50.9	–59.7	8.7	–51.0	–72.4	21.4	E.s.	other	E.c.	?
65	–62.9	–72.1	9.2	–54.6	–63.3	8.6	–54.7	–74.3	19.6	E.s.	other	E.c.	?
55	–66.7	–76.9	10.2	–58.9	–67.3	8.4	–59.0	–78.3	19.3	E.s.	other	E.c.	?
45	–70.7	–85.3	14.6	–63.9	–71.9	8.0	–64.1	–85.9	21.8	E.c.	E.c.	E.c.	E.c.
35	–74.8	–94.4	19.6	–69.8	–76.8	6.9	–70.4	–94.5	24.2	other	E.c.	E.c.	E.c. (?)
25	–79.1	–103.4	24.3	–76.5	–81.7	5.2	–77.9	–103.3	25.4	other	E.c.	E.c.	E.c. (?)
15	–82.7	–116.8	34.1	–83.5	–84.8	1.2	–86.2	–116.6	30.4	other	E.s.	other	?

Discussion

The strengths and weaknesses of this study can be elaborated by consideration of the following topics.

Appropriateness of methods used for TS measurements. The choice of methods for measuring TS (dead fish suspended in a cage, global method) was determined by the difficulty of detecting single *P. antarcticum* in the field. This is because adults that have a very low acoustic reflection, occurred in deep water (200–400 m), and post-larvae, sub-juveniles and juveniles that have a TS similar to that of Euphausiids, were often found associated with krill populations. Moreover, in the Ross Sea the opportunities to carry out net sampling where fish were detected were severely limited by the presence of ice. Therefore, *in situ* TS measurements using sophisticated (e.g. direct) methods were not available for this study.

Input power and its effect on TS measurements. Sound will always be generated non-linearly, but if the energy in the sound is low, the non-linear wave equation can be approximated by the usual linear equations (Korneliussen et al., 2004). In *ex situ* and *in situ* measurements, however, 1kW input power was used at 120 kHz and 200 kHz (Table 1). This leads to a non-linear generation of sound in the water column that cannot be ignored. For 200 kHz at 1kW, the non-linear effects would lead to a theoretical 6 dB loss at 100 m range (4 dB loss is within 10 m, 5 dB within 20 m). From 100 to 400 m, there would only be an additional 0.5 dB loss. There is a similar trend for 120 kHz: 2 dB loss within 100 m (0.75 dB loss within 10 m, 1.25 dB loss within 20 m), additional 0.75 db up to 600 m. For 38 kHz non-linear effects are negligible. As the calibration sphere was placed: (i) at 25 m depth before *in situ* TS measurements most of the loss (5 dB) was already accounted for; (ii) at 10 m depth before *ex situ* TS measurements (5–7.4 m) the loss was completely compensated.

Different location of data acquisition and acoustic system calibration. There is no doubt that calibration must be performed in waters having similar properties as that of the measurement area (MacLennan, 1990). For logistic reasons, however, the acoustic system had to be calibrated in waters (Adriatic Sea) at a temperature about 10°C higher than that of the data acquisition area. This could have led to a maximum overestimation of *in situ* TS at 38 and 200 kHz of ≈0.75 dB and at 120 kHz of ≈1.5 dB.

Acoustic proprieties of fish in relation to freezing and defrosting. It is very well known that the risk of deteriorating fish tissues by freezing and defrosting is very high. Following Sun et al. (1985), freezing the fish reduces the overall backscatter by around 30%. However, the variations may depend on the method of treatment and perhaps on fish species, leading to an unknown systematic error in the *ex situ* TS measurements.

Acoustic properties of dead fish in relation to water temperature. The water temperature during the 2007 *ex situ* experiment (16°C) was about 7°C higher than that of the 2009 experiment and about 17°C higher than that of the Ross Sea (from –0.5 to –1°C). TS is very sensitive to variation in relative density and sound speed that could be caused by water temperature. The densities of seawater and of lipids, an acoustically important component of the *P. antarcticum* body, change less than 0.03% between 4°C and 30°C (Neighbors and Nafpaktitis, 1982); such change has a negligible influence on the value of the relative density *g*. There is little information on the relative sound-speed parameter h in relation to water temperature. It seems to decrease as temperature increases (Yasuma et al., 2006). If that is so, comparison between TS measured in 2007 and 2009 experiments and between *ex situ* and *in situ* TS could be questionable. However, the possible variations of h with water temperature are not yet corroborated by sufficient experimental data.

Tilt-angle distribution. It is well known that tilt-angle distribution is required to calculate average TS. However, there is a lack of information on the *in situ* tilt angle of *P. antarcticum* which probably is different for animals of different size. In the *ex situ* experiments, a random distribution of ±15° was applied which was considered reasonable for adult *P. antarcticum*. The *in situ* experiments were performed on different sizes of *P. antarcticum* (post-larvae, sub-juveniles and juveniles), that probably swim at different tilt angles. Future studies need direct observations, with a video for example, on the actual mean swimming angle of *P. antarcticum* and on the relationship with age classes.

The theoretical model and its limitations. The theoretical model is based on few geometric parameters of the fish body and vertebral column, therefore it can provide only a general approximation of the scattering properties of fish. However, because it gives exact solutions of $\sigma_{bs}(f)$, it can provide guidance for other more detailed models. The morphological measurements were taken from only 18 frozen adults. In future, the body measurements must be validated by examining freshly caught fish of a greater range of TL.

Relative density and speed sound through the fish body. If the target species does not contain a swimbladder, the use of a theoretical model requires estimates of *g* and *h* in both the flesh and bone. In this study g and h of the flesh were taken from the experiments of Chu and Wiebe (2005) on juvenile *P. antarcticum* (60–75 mm), while *g* and *h* of adult fish bone were calculated theoretically (*g*) or deduced (*h*) from *in situ* and *ex situ* data. It is assumed that these parameters do not change with fish length. From the measurements of adult fish, no significant relationship was found between mass density (*g*) and TL. Additional research is required to improve the reliability of these parameters.

Overall *ex situ* and *in situ* TS–TL relationships compared to TS–TL equations from the model. The TS–TL relationships at 38, 120 and 200 kHz for adult *P. antarcticum* (*ex situ* measurements) differ consistently from those of post-larvae and juveniles (*in situ* measurements). However, the equations of the theoretical model are in good agreement with both *in situ* and *ex situ* data. The model can predict *in situ* and *ex situ* experimental data with a mean difference <1.5 dB at all frequencies, although for some small sizes (<70 mm) the discrepancies between the measured and predicted TS are high. The model shows that the main contribution to the scattering in fish smaller than three wavelengths is from soft tissues, while for larger sizes it is from hard tissues. This can explain why small and large fish have different TS–TL relationships.

Regression coefficient in the data. Experimental models used in fishery science to relate TS to TL often have a regression coefficient (slope) close to 20 dB. Data from *ex situ* and *in situ* measurements in this study, as well as from a theoretical model, indicate that the use of a slope of 20 is not appropriate for *P. antarcticum*. This result is in agreement with the reports of McClatchie et al. (2003) and Yasuma et al. (2003).

Discrimination of *P. antarcticum* from Euphausiids. Using the theoretical models for TS estimation and the three-frequency decision criteria for discrimination of Euphausiids (Azzali et al., 2004), indicates that in some length classes *P. antarcticum* could be confused with *E. crystallorophias*, but not with *E. superba*. It is of note that *g* and *h* of *P. antarcticum* are closer to those of *E. crystallorophias* than those of *E. superba* (Chu and Wiebe, 2005).

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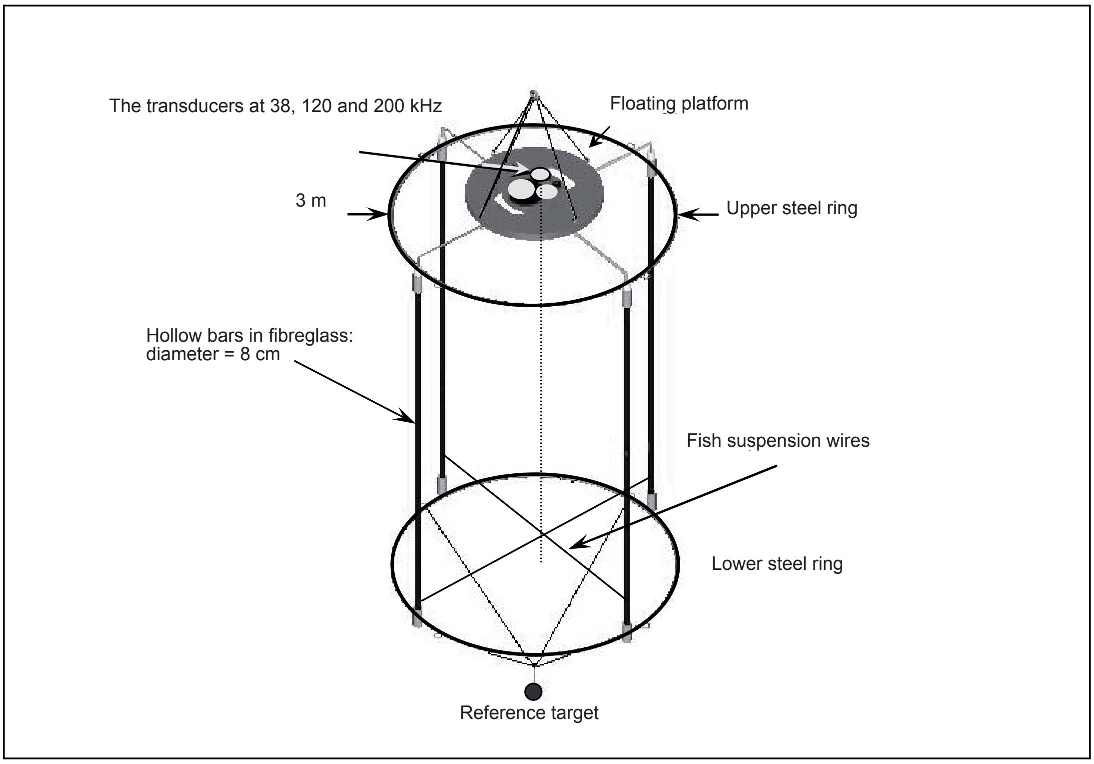


Figure 1: Apparatus used for *ex situ* target strength measurements on dead fish. The lower steel ring and the reference target are 7.5 and 10 m respectively below the transducers. The diameter of the two steel rings is 3 m. The fibreglass bars are 2 mm thick, 7 m long and filled with seawater. The suspension wires have a tilt angle of around 15°.

Location of fishing experiments and mean value in % by number of *Pleuragramma antarcticum* catches.			
Haul	Lat (S) Lon. (E)	% by number	Date
PHN-08 △	73° 56.70' 175° 13.74'	82.3%	1997 19 Dec.
PHN-14 △	73° 35.53' 175° 41.44'	82.9%	1997 21 Dec
PHN-33 △	74° 53.40' 164° 38.40'	78.7%	1998 02 Jan.
PHN-07 □	75° 54.00' 176° 07.20'	96.8%	2000 22 Jan.
PHN-57 □	73° 12.60' 171° 12.00'	92.5%	2000 04 Feb.
PHN-59 □	74° 33.60 170° 58.20'	99.7%	2000 05 Feb.
HPRI-18 ○	72° 50.36' 174° 30. 58'	78.9%	2004 15 Jan.
HPRI-19 ○	72° 37.30' 175° 16. 38'	84.6%	2004 16 Jan.
HPRI-33 ○	75° 13.20' 167° 28.80'	87.1%	2004 27 Jan.

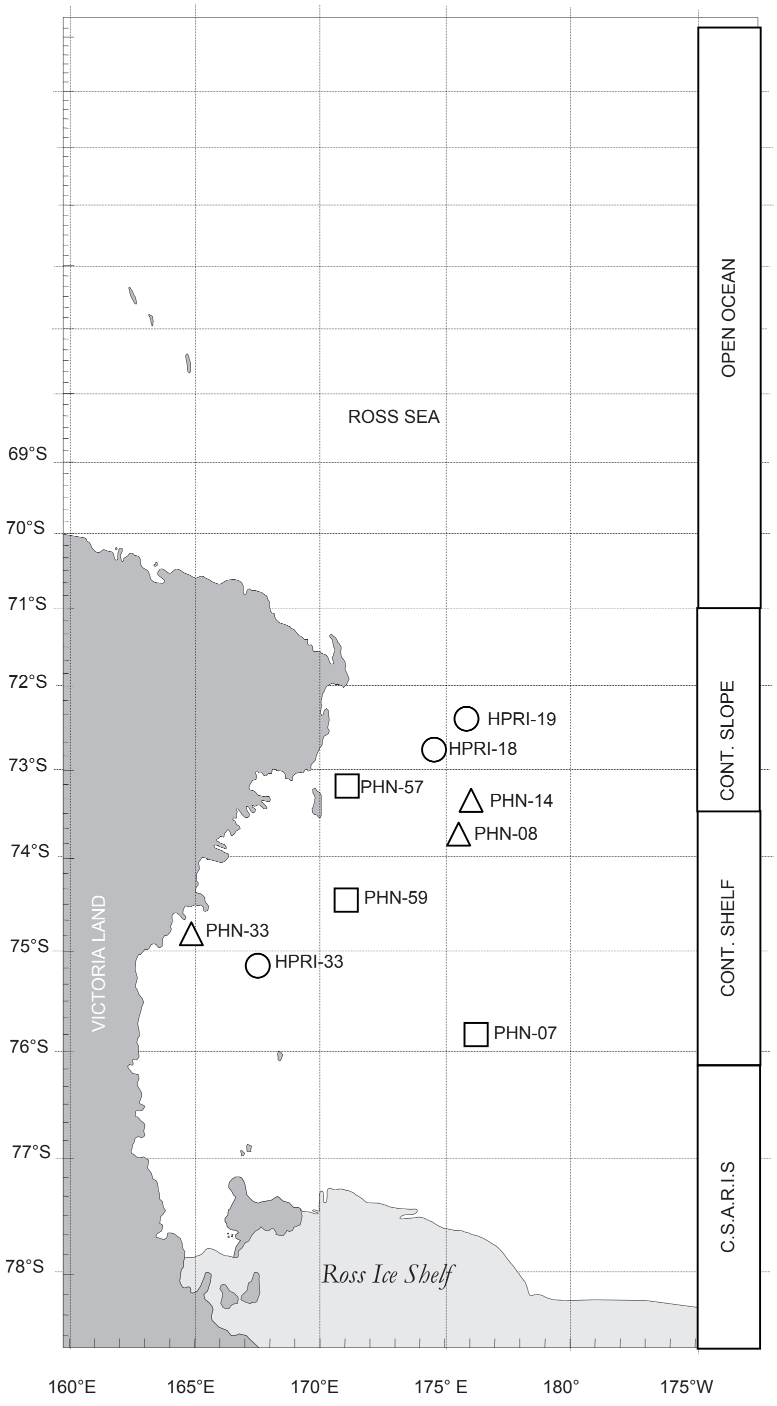
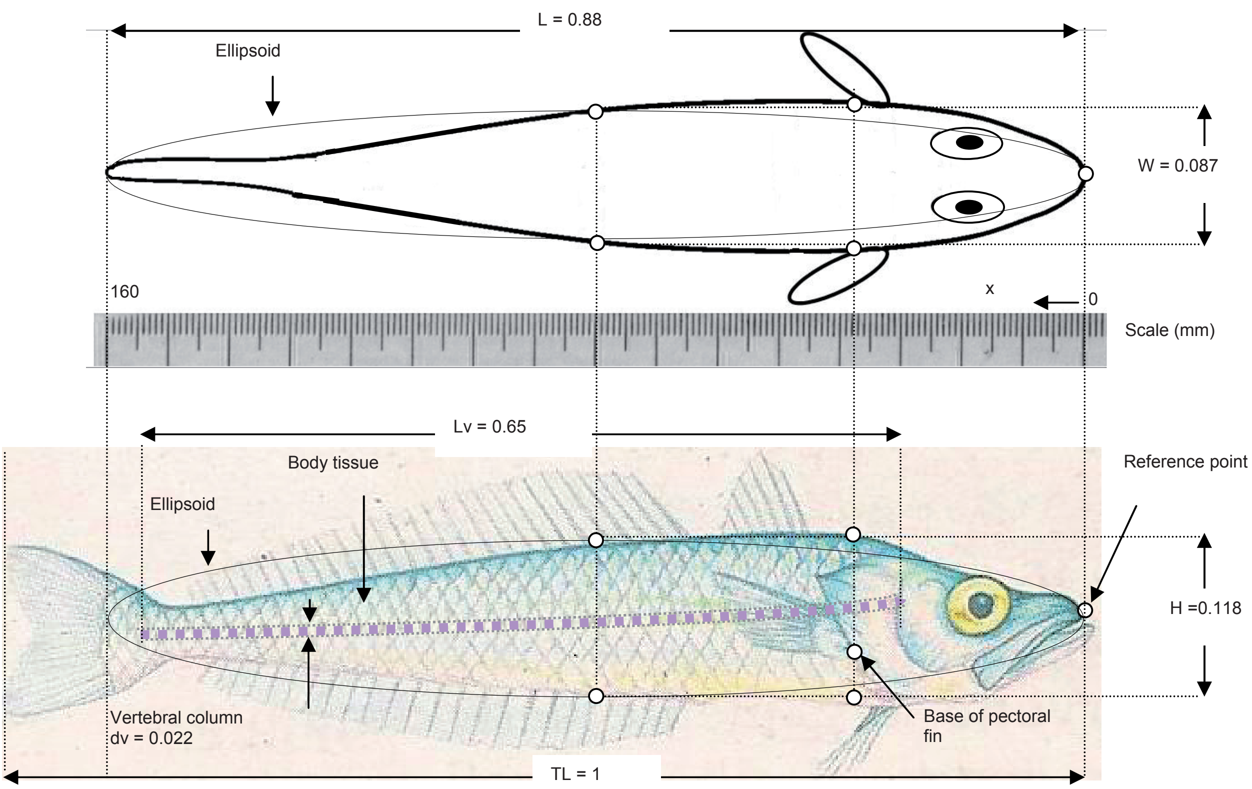


Figure 2: Map of the fishing experiments conducted in three expeditions to the Ross Sea: December and January 1997/98 (△), January and February 2000 (□), and January 2004 (○). The nine trials are distributed on the continental slope (four hauls: HPRI-19, HPRI-18, PHN-57 and PHN-14) and continental shelf (five hauls: PHN-08, PHN-59, PHN-33, HPI-33, PHN-07). The trawl catches in all hauls were dominated by *Pleuragramma antarcticum* (mean value in % by numbers 87.0 ± 7.6 and by weight 89.2 ± 14.1).



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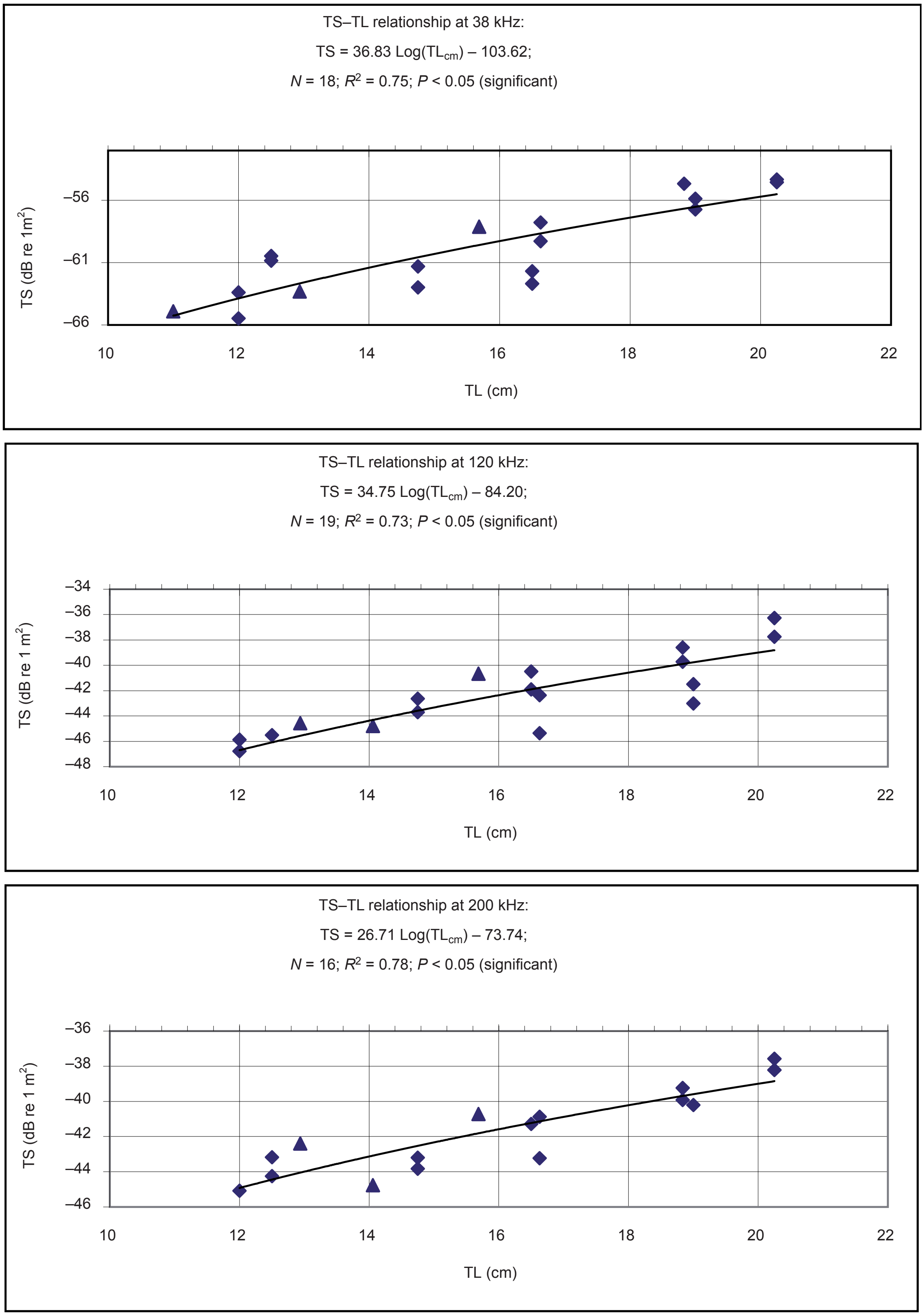

TS–TL relationship at 38 kHz:

$TS = 36.83\ \mathrm{Log}(TL_{cm}) - 103.62$;

$N = 18$; $R^2 = 0.75$; $P < 0.05$ (significant)

TS–TL relationship at 120 kHz:

$TS = 34.75\ \mathrm{Log}(TL_{cm}) - 84.20$;

$N = 19$; $R^2 = 0.73$; $P < 0.05$ (significant)

TS–TL relationship at 200 kHz:

$TS = 26.71\ \mathrm{Log}(TL_{cm}) - 73.74$;

$N = 16$; $R^2 = 0.78$; $P < 0.05$ (significant)

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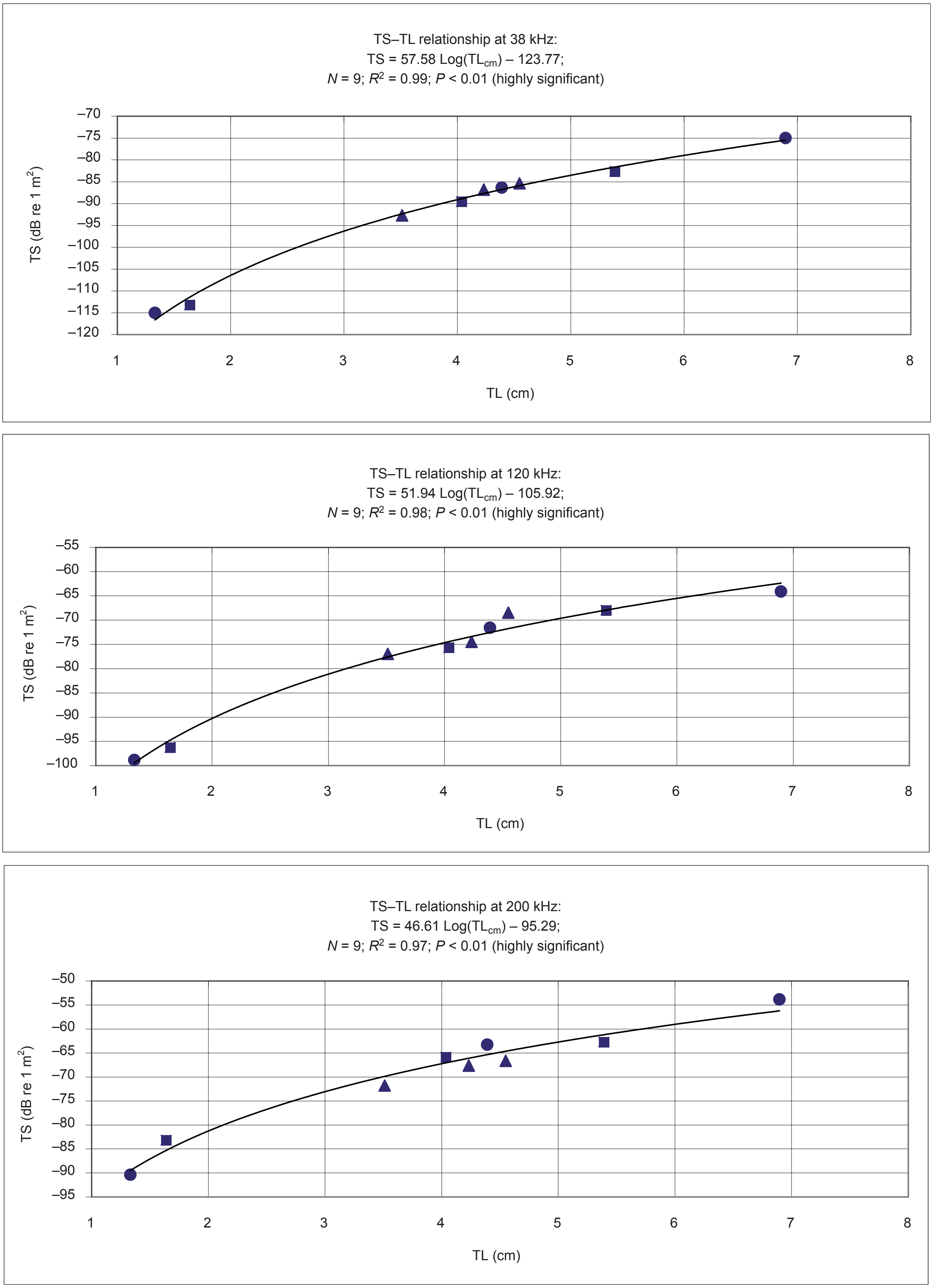

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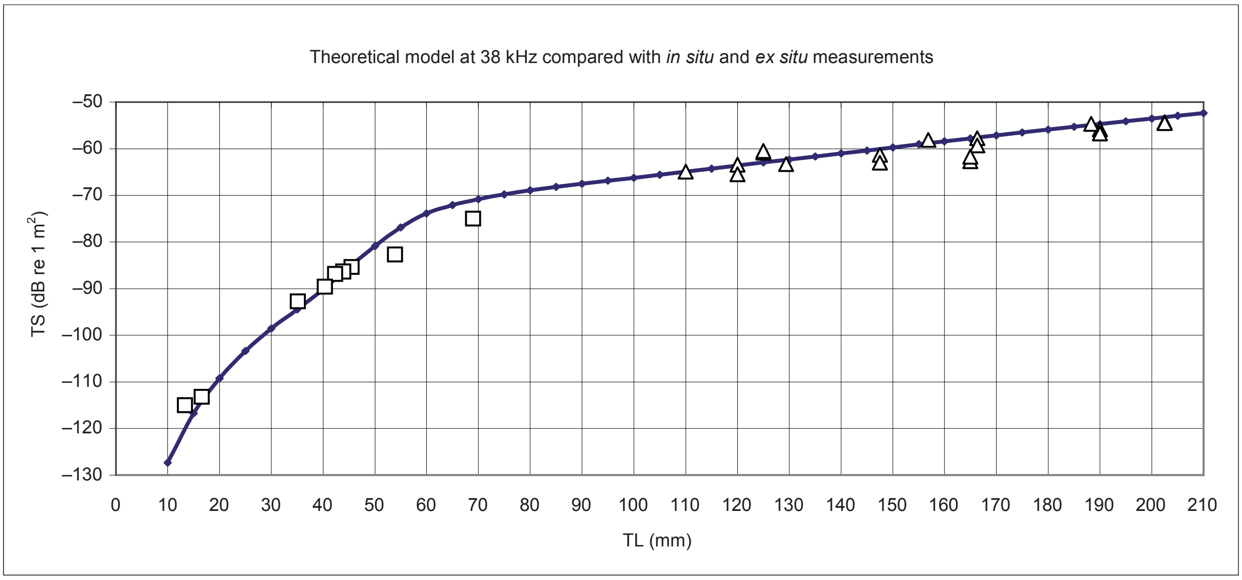

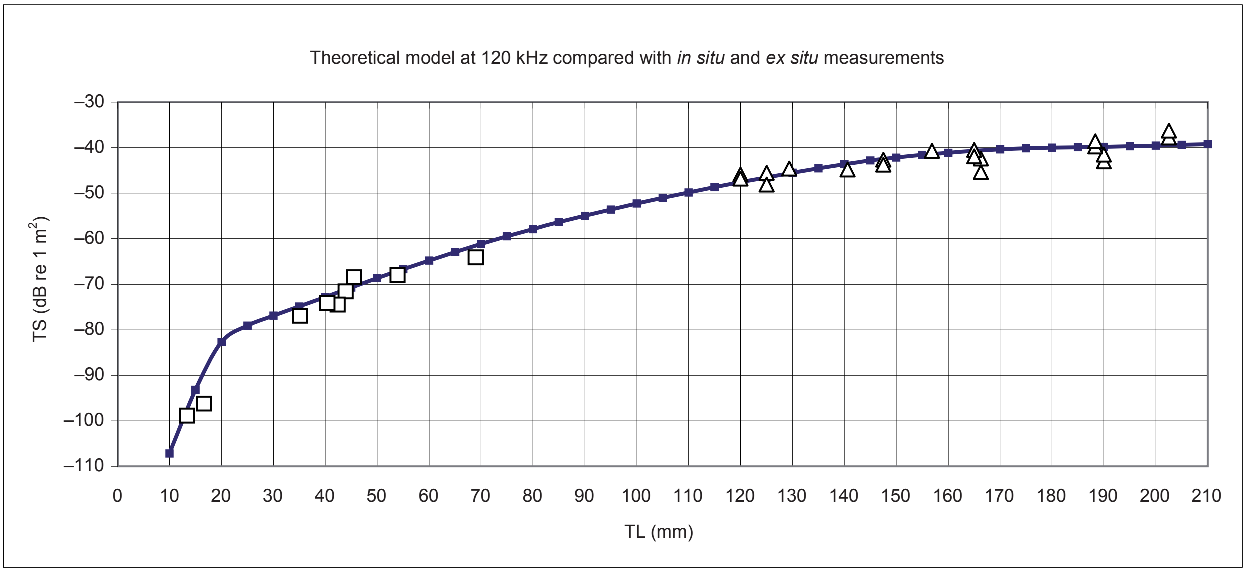

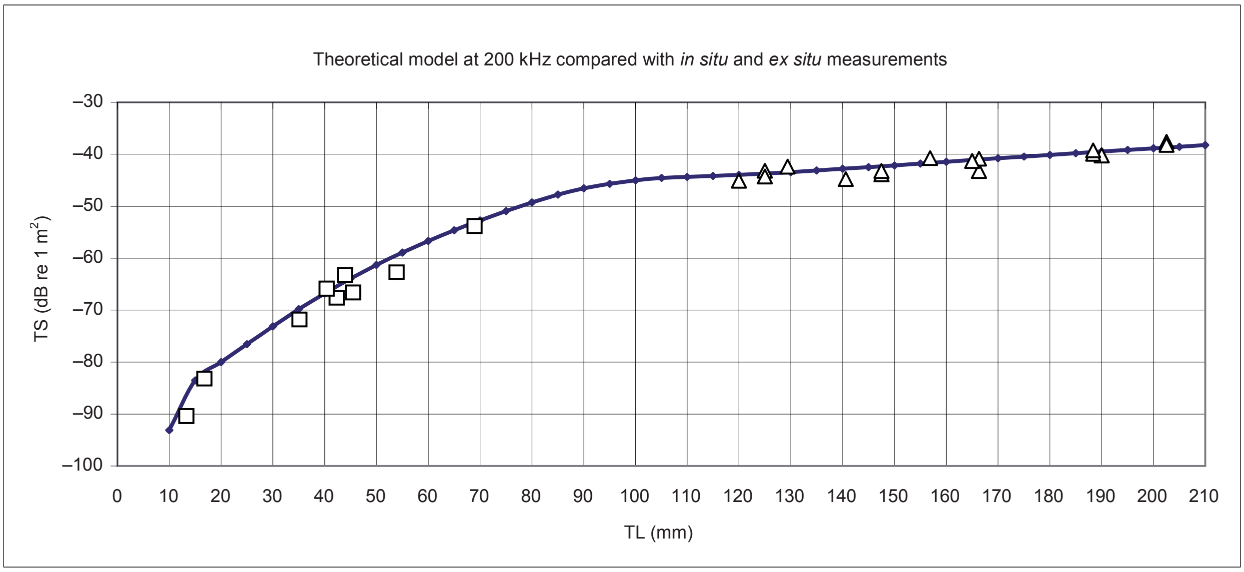

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APPENDIX

EQUATIONS

$$<\sigma_{\mathrm{bs}}(\mathrm{TL}_{\mathrm{mean}})> = s_V/\rho;\ (\mathrm{m}^2\ \mathrm{fish}^{-1}) \tag{1}$$

where $\rho = N/V$, $V = \Psi(r_2^3 - r_1^3)/3$ is the effectively insonified volume (m^3), and N is the number of fish within it.

$$<\sigma_{\mathrm{bs}}(\mathrm{TL}_{\mathrm{mean}})> = s_V/\rho;\ (\mathrm{m}^2\ \mathrm{fish}^{-1}) \tag{2}$$

where $\rho = N/V$ fish-number density, N = number of *P. antarcticum*, and V = filtered volume (m^3).

$$V = \frac{4}{3}\pi\left(\frac{LHW}{8}\right) = \frac{4}{3}\pi\left(\frac{c_l c_h c_w}{8}\right)(\mathrm{TL})^3;\ (\mathrm{m}^3) \tag{3}$$

$$r_b = \left(\frac{LHW}{8}\right)^{1/3} = \left(\frac{c_l c_h c_w}{8}\right)^{1/3}(\mathrm{TL}) = a_b(\mathrm{TL});\ (\mathrm{m}) \tag{4}$$

$$\sigma_{body}(1 << kr_b) = \pi\left(\frac{LW}{2H}\right)^2 R_b^2 = \left[\pi\left(\frac{c_l c_w}{2c_h}\right)^2 {R_b}^2\right](\mathrm{TL})^2 = 16\pi(a_b/c_h)^4[a_b(\mathrm{TL})]^2(R_b)^2;\ (\mathrm{m}^2) \tag{5}$$

$$\sigma_{body}(1 >> kr_b) = 16\pi^3\left(\frac{V^2}{\lambda^4}\right)R_b^2 = \frac{16\pi}{9}(kr_b)^4[a_b(\mathrm{TL})]^2(R_b)^2;\ (\mathrm{m}^2) \tag{6}$$

$$R_b(kr_b) = [(1-gh^2)/(2+\exp(-kr_b)^4)gh^2)] + [(1-g)/((1+\exp(-kr_b)^4)g)] \tag{7}$$

$$\sigma = l_{\lim}\Big/\sqrt[s]{1+[1/(kr_b)]^s} \tag{8}$$

where (kr_b) is the input, σ is the output, l_{lim} is the asymptotic limit of σ and s is the sharpness parameter, does provide such a link.

$$\sigma_{body} = \frac{16\pi}{9}\sqrt[s]{\frac{1+[(9(a_b/c_h)^4\tanh(kr_b)^4]^s}{1+[1/(kr_b)^4]^s}}[a_b(TL)]^2(R_b)^2;\ (\mathrm{m}^2) \tag{9}$$

$$\sigma_{vert.}(1 << kr_v) = \frac{1}{4\pi}(kr_v)[c_v(\mathrm{TL})]^2(R_v)^2;\ (\mathrm{m}^2) \tag{10}$$

$$\sigma_{vert.}(1 >> kr_v) = \frac{(kr_v)^4[c_v(\mathrm{TL})]^2}{4}(R_v)^2;\ (\mathrm{m}^2) \tag{11}$$

$$\sigma_{vert.} = \frac{1}{4\pi}\sqrt[s]{\frac{1+(kr_v)^s}{[1+(1/(kr_v)^4)^s][4(1+2arctg(kr_v)^4)^s]}}[c_v(\mathrm{TL})]^2(R_v)^2;\ (\mathrm{m}^2) \tag{12}$$

$$R_v = [(1-gh^2)/2gh^2] + [(1-g)/(1+g)] \tag{13}$$

$$\sigma_{\mathrm{bs}} = [(\sigma_{body}/4\pi) + \sigma_{vert.}];\ (\mathrm{m}^2\ \mathrm{fish}^{-1}) \tag{14}$$

$$\mathrm{TS} = 10\mathrm{Log}(\sigma_{bs})\ ;\ (\mathrm{dB\ re\ 1m^2}) \tag{15}$$

$$\frac{\sigma_{body}}{4\pi} = \frac{4}{9}\sqrt[s]{\frac{1+\left[5.49\tanh\left(0.104k\left(\mathrm{TL}\right)\right)^{4}\right]^{s}}{1+\left[1/\left(0.104k\left(\mathrm{TL}\right)\right)^{4}\right]^{s}}\left[0.104\left(\mathrm{TL}\right)\right]^{2}\left[R_{b}\left(kr_{b}\right)\right]^{2}}\ ;\ \mathrm{m}^{2} \tag{16}$$

where k_{38} = 164.66 m^{-1}; k_{120} = 519.99 m^{-1}; k_{200} = 866.65 m^{-1}; s = sharpness parameter = 10.

$$\sigma_{vert.} = \frac{1}{4\pi}\sqrt[s]{\frac{1+(0.011k(\mathrm{TL}))^{s}}{[1+(1/(0.011k(\mathrm{TL}))^{4})^{s}][4(1+2arctg(0.011k(\mathrm{TL}))^{4})^{s}]}}[(0.65)(\mathrm{TL})]^{2}(4.72)10^{-2}\ ;\ \mathrm{m}^{2} \tag{17}$$

where k, TL and s are the same as used in equation (16).