Fisheries Technology Committee **ICES CM 2002/B:03**

Report of the

Study Group on Target Strength Estimation in the Baltic Sea

Sète, France
7–8 June 2002

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1 EXECUTIVE SUMMARY

The Study Group evaluated the results of calculating backscatter properties of a group of herring and a group of sprat collected in October in the Baltic proper using x-rays to obtain the swimbladder shape and KRM (Kirchhoff-ray mode) modelling. Calculated target strength increases monotonically as a function of length for both the herring group (27 specimens) and the sprat group (25 specimens) at 38 kHz. Calculated mean target strengths varied from approximately –42 to –38.5 dB over a length range extending from 75 to 150 mm caudal (i.e. standard length). Mean target strengths of sprat were consistently higher than those of herring of the same size. Mean target strength-length curves differed noticeably in amplitude and pattern between the two species at 120 kHz. Mean target strengths of herring and sprat at 38 and 120 kHz both peak dorsally at approximately 85^{o} relative to the caudal extension of the fish body length axis, but vary over a broad range for both species. This corresponds well to the mean tilt angle of approximately 5˚ tilt (front part up) relative to the body axis of the swimbladder for both species. Target strengths decrease as tilt angles deviate from horizontal (i.e. head up or head down). Calculated mean backscattering response surfaces showing the variations of the backscattering response with fish length/sound wavelength ratio (L/λ) and tilt angle contain the same general features for both herring and sprat. They peak at an angle of 85 ^{o} across the range modelled. Target strength is not consistently greater for one species over the other over the range of frequencies examined (38 – 200 kHz). Sprat target strength exceeds that of herring at low (38 – 90 kHz) and intermediate frequencies (130 – 160 kHz) and vice versa at other frequencies, but the difference does not exceed two decibels at any frequency.

Reviews of the latest literature of TS of herring and sprat and current information on the diel cycle of stomach fullness and seasonal cycle of fat content and in different part of the Baltic area are included.

A revised protocol for TS measurements on the Baltic herring and sprat is suggested.

2 INTRODUCTION

2.1 Participation

The meeting was attended by:

Fredrik Arrhenius	(Chair)	Sweden	
John Horne		USA	*Observer*
Michael Jech		USA	*Observer*
Bo Lundgren		Denmark	
Ingvald Svellingen		Norway	
Karl-Johan Stæhr		Denmark	*Observer*
Faust Shvetsov		Latvia	
Vladimir Severin		Russia	

2.2 Terms of Reference

According to the Annual Science Conference Resolution (2001/2B02) in Brugge, the Study Group of Target Strength Estimation in the Baltic Sea [SGTSEB] (Chairperson: F. Arrhenius, Sweden) will meet at Sète, France from 7–8 June 2002 to:

a) Discuss the results of the biological properties that affect backscattering of Baltic fish i.e. swim bladder volume and shape, fat content and stomach content and fullness;
b) Discuss the results of backscattering models especially change in biological and physiological factors affecting the TS;
c) Evaluate the single target TS measurements on herring and sprat during the surveys 2001 in the Baltic;
d) Apply modelling methods on the case of the herring and compare their results to the existing information;
e) Review the latest literature of TS of herring and sprat;
f) Review current information of seasonal cycles of fat content and diel cycles of stomach fullness in different part of the Baltic area.

The Study Group makes its report available to Working Group on Fisheries Acoustics Science and Technology and will report by 30 June 2002 for the attention of the Fisheries Technology and the three-year duration of the study group, projects will be conducted and reviewed to improve understanding of biological and physical effects on Baltic Sea herring and sprat target strengths (TS). At the conclusion of the effort, the study group will propose guidelines for the development of better-parameterized herring and sprat-TS relationships.

2.3 Background

When using echointegrator data for fish abundance estimation, the target strength (TS) of the fish is an important parameter for the converting integrated acoustic energy to absolute fish abundance. Errors in acoustic estimates can be ascribed to several causes. The problem is generally not the accuracy of the echosounders as such, since high precision and comparability of acoustic measurements on isotropic standard targets is well documented and verified. The main problem is the quantitative interpretation of the acoustic echoes received from targets of unknown scattering characteristics. The same fish or school can produce very different acoustic echoes. This may be due to intensity variations caused by interference between echoes from different parts of a fish or a school which can be considered a purely stochastic process. Other intensity variations are due to fish behaviour caused by for example the presence of the survey vessel or predators or controlled by basic biological rhythms and functions in connection with feeding, migration or reproduction.

One of the most important factors influencing the final biomass and abundance estimates in stock assessments is the choice of TS to biomass conversion formula. By convention until now, the TS of a fish in the formulas is an accepted mean value for normal survey conditions varying linearly as a function of fish length. The actual TS constants in this formula applied since 1983 for Baltic Sea acoustic surveys are the same as those used for North Sea herring. However, recent findings have shown that herring TS at the surface is generally much higher than the applied TS (Zhao, 1996, Ona *et al*., 2000, 2001). During the FAST Working Group meeting in Haarlem, Netherlands, 2000 (ICES, 2000) it was concluded that:

- There is evidence of cycles and trends in the main ecological characteristics of the Baltic herring that lead to changes in the anatomical, physical and behavioural parameters influencing the TS values. There is a consensus that the TS equation used until now should be revised;
- Mean target strength depends on two types of components. Some of them are rather easy to measure, and a good relationship can be found with the TS values. Others present a high variability that no method can help to reduce. Therefore it is important to recognize those factors where knowledge and measurements would significantly improve the estimation of abundance;
- A significant number of data already exist which could help to measure the effect of the main factors and their importance;
- Some new models could greatly help to evaluate the magnitude of the effects of the main factors;
- There is need for experiments to better understand the TS values and their variability.

Based on these conclusions it was recommended that a study group should be created to review these problems in more detail, with herring as a target species. A full list of the acronyms used in this report can be found in Appendix A.

3 BIOLOGICAL PROPERTIES THAT AFFECT BACKSCATTERING

Herring and sprat are physostomes, with open swimbladders, and it is believed, without a gas secretion gland (Blaxter and Batty, 1990). The swimbladder normally reflects 90% or more of the backscattered energy (Foote, 1980). However, there is considerable variation in measured TS among individual fish, even those of the same size and species. This is due to the dependence of the echo on the internal anatomy, especially the shape of the swimbladder, which can be very different among fish, even though they are similar in size and external appearance.

During the 2002-meeting the Study Group of Target Strength Estimation in the Baltic Sea (SGTSEB) decided to use a generalized TS equation of the form:

$$TS = f(\text{frequency}) + f(\text{length}) + f(\text{pressure}) + f(\text{Temp., Sal.}) + f(\text{orientation}) + f(\text{activity}) + f(\text{lipid}) + f(\text{gut fullness}) + f(\text{gonad})$$

in order to explicitly specify which acoustic and biological parameters are considered important and should be recorded or sampled during annual herring surveys and other times of the year. The equation includes the physical and biological factors that are believed to affect acoustic TS of a single fish. The symbol "*f*" denotes a function that can be linear or non-linear, empirically or theoretically derived, and is not of the same magnitude for all variables in the formula.

In the formula TS is the target strength $10*\log10(ts) \equiv 10*\log10(\sigma/4\pi)$, where σ is the acoustic back scattering cross section of the fish. Frequency is the acoustic frequency used during the survey. Length is the fish length (*e.g.,* total or standard length). Pressure is the hydrostatic pressure at the depth of the fish and f(pressure) a measure of the depth effects mainly on the swimbladder. Temperature and salinity are the environmental conditions at the depth of the fish which may have both direct and indirect effects on TS. Direct effects include variation in the density and sound speed (i.e. acoustic impedance) contrasts between the fish body, swimbladder, and surrounding water. Indirect effects include larger or smaller swimbladder volumes needed for buoyancy compensation in different water densities. Orientation is the tilt, roll, and yaw of a fish relative to the sound beam transmitted by the echosounder transducer. Activity is a measure for the movement of a fish (*e.g.*, swimming or resting). In this model, fish behaviour is thus a combination of orientation and activity. Lipid is the fat content in the fish body, gut fullness is the standard index for the amount of stomach content, and gonad is the selected standard index for the development stage of the gonads. The condition of the fish described by these parameters has a direct effect on the density and sound speed contrasts between the fish body and water, and indirect effects on the swimbladder volume to compensate for the buoyancy change. In addition, gut and gonads may press on the swimbladder and directly influence its shape.

Several measurements during the past few years have shown that TS is depth dependent. This depth dependency may also include differences in fish behaviour with depth (Ona *et al*., 2000, 2001, 2002). This has led to the development of a TS formula of the type suggested above. They propose that preliminary mean target strength of herring should be expressed as:

$$\langle TS \rangle = 20\log L - 65.4 - 2.3\log(1+z/10) + 0.24(GSI)$$

where L is total length in cm, z is depth in meters and GSI is the gonadosomatic index.

4 BALTIC SEA HERRING AND SPRAT KRM MODELING

In the 2001 study group meeting a summary of reported TS values for herring and sardine was produced (Appendix C), which indicates that TS measurements are highly variable for herring in different areas.

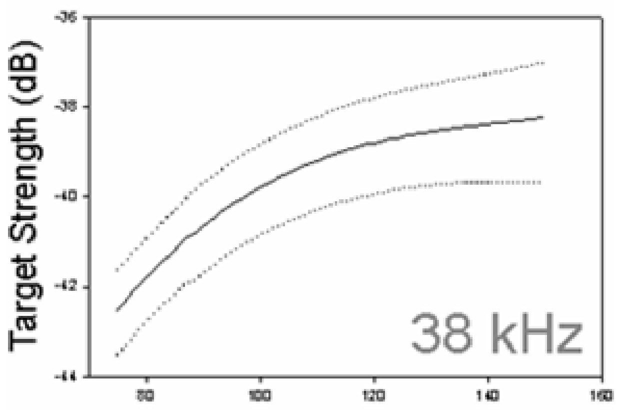
Salinity in the Baltic Sea is very low compared to the open sea, and it is likely that this would have a definite impact on the TS of the fish. To obtain neutral buoyancy in water of low salinity the fish will have to compensate with an increased swimbladder volume. It is assumed that this will increase the back scattering cross section of the fish and hence the TS. Another effect of the lower salinity will be lower acoustic impedance of the water relative to the fish, which will also affect the acoustic TS. It is also possible that that fish living in low salinity are different with respect to for example, condition factor, behaviour etc., but this is not known and needs to be investigated.

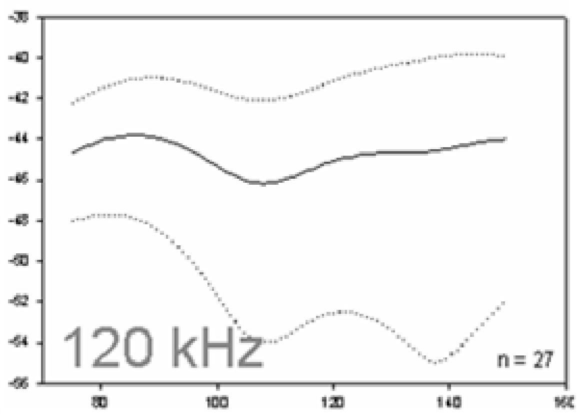
Members of the Study Group of Target Strength of Baltic Sea (SGTSEB) have used insights from previous backscatter modelling analyses and empirical studies to guide a combined modelling and measuring investigation on backscatter properties and range of TS from herring and sprat. The sampling procedure is described in ICES (2000) and Appendix B. Radiographs have been digitized and used in Kirchhoff Ray Mode (KRM) backscatter models to estimate backscatter amplitude and variance from individuals of herring and sprat.

Previous modelling and empirical studies suggest that several biological and physical factors potentially influence TS of individual fish: swimbladder presence, orientation (i.e. tilt, roll, direction), length, acoustic frequency, depth, fish activity, gut content, lipid content, maturity state, and surrounding water conditions (i.e. temperature, salinity). KRM modelling runs and empirical data will be used to quantify the range and distributions of factor values, and the resulting effect on TS. A comparison of the magnitude of the effects should enable an ordinal ranking of the relative influence of biological and physical factors on TS. KRM TS estimates will be compared to *in situ* TS measurements of herring and sprat during assessment survey cruises.

4.1 TS *vs* Length

Herring





Sprat

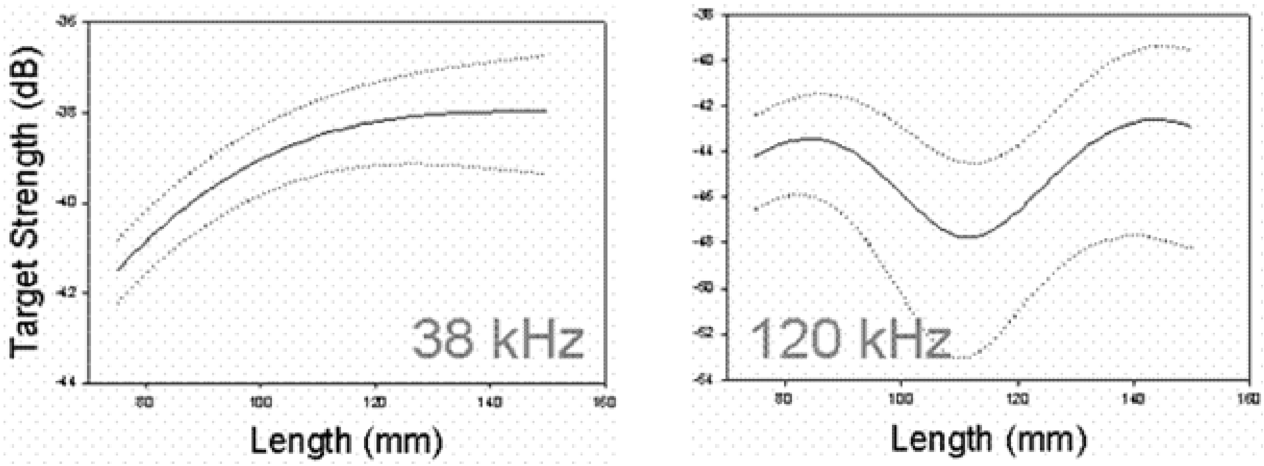


Figure 3.1.1. Calculated mean target strength versus length for groups of radiographed herring and sprat. Dotted lines indicate one standard deviation of the mean for 27 herring and 25 sprat.

Target strengths increased monotonically as a function of length for both groups of herring (n=27) and sprat (n=25) at 38 kHz. Mean target strengths ranged from approximately –42 to –38.5 dB over a length range extending from 75 to 150 mm caudal (i.e. standard length). Mean sprat target strengths were consistently higher than those from herring.

Mean target strength curves differed in amplitude and pattern between the two species at 120 kHz. Herring mean target strengths varied little (i.e. approximately 1 dB) from the mean –44 dB over the entire length range. Sprat mean target strengths increased monotonically from –41.5 dB to –38 dB over the length range.

4.2 TS *vs* Frequency

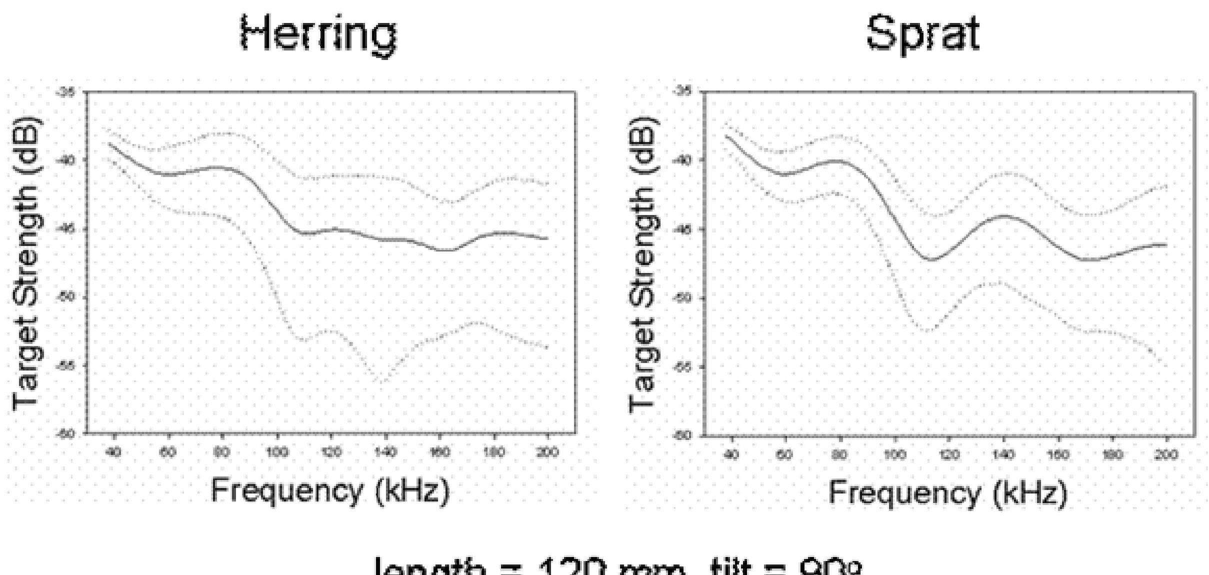
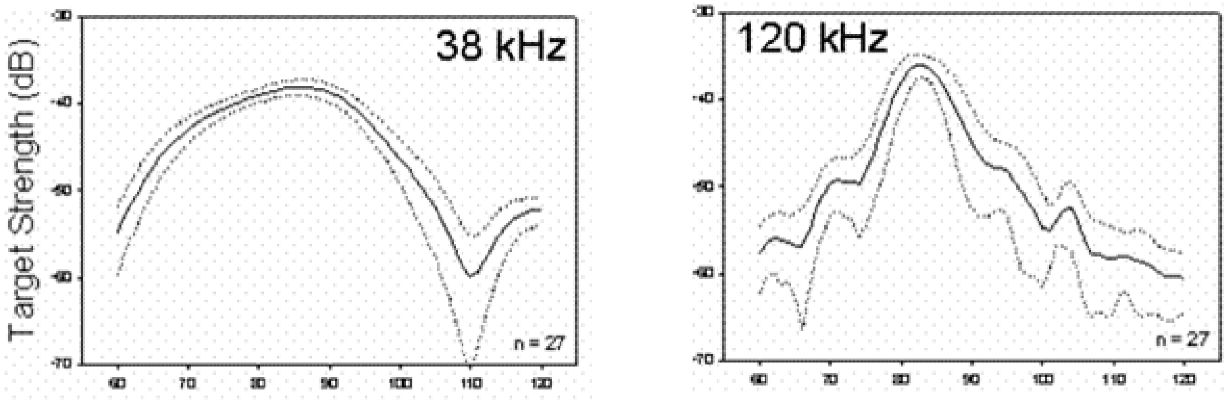


Figure 3.2.1. Calculated dorsal (tilt = 90°) mean target strength as a function of frequency of herring and sprat of length 120 mm, based on groups of radiographed herring and sprat. Dotted lines indicate one standard deviation.

Mean target strengths decreased as a function of frequency for both herring and sprat. Herring mean target strengths decreased at a slower rate and more consistently than sprat target strengths. A distinct reduction in sprat target strengths occurring at approximately 110 kHz did not occur in the herring predicted target strength curve. The variance in both target strength curves increased as a function of frequency.

4.3 TS *vs* Tilt

Herring



Sprat

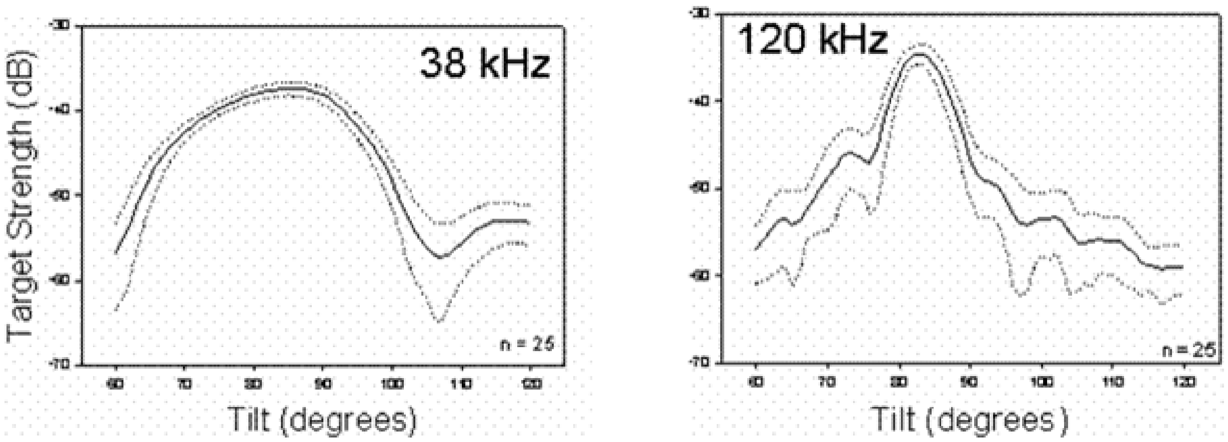


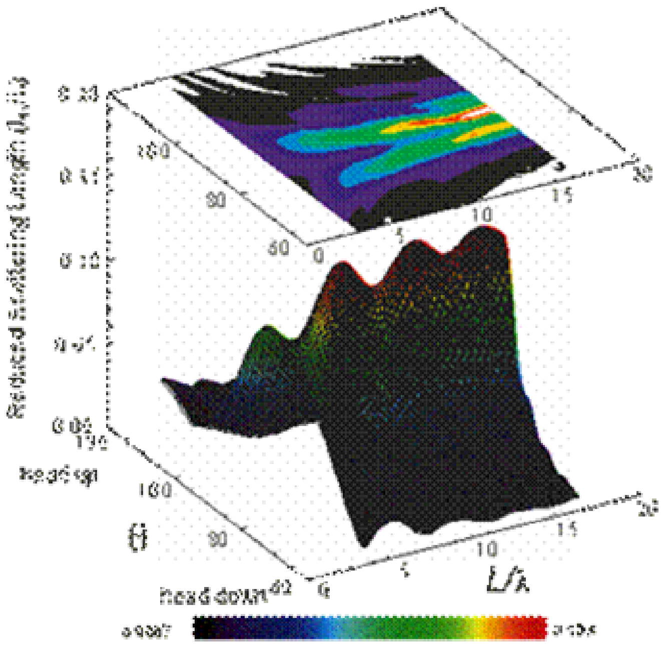
Figure 3.3.1. Calculated mean target strength as a function of tilt angle for herring and sprat of length 120 mm based on groups of radiographed herring and sprat. Tilt 90° dorsal direction, 0° tail and 180° head. Dotted lines indicate one standard deviation.

Mean target strengths of herring and sprat at 38 kHz peaked at approximately 85^o but spanned a broad range for both species. This angle corresponds to the mean tilt angle of the swimbladder for both species. Target strengths decreased as tilt angles deviated from horizontal (i.e. head up or head down). An additional increase in target strength curves occurred in tilt angles greater than 110^o for herring and greater than 105^o for sprat. Variance increased in both target strength curves as tilt angles deviated from horizontal.

Maximum target strengths also peaked at approximately 85^o for both species at 120 kHz. Echo amplitudes decreased at a greater rate as tilt angles increased from 0 (i.e. horizontal) relative to the decrease in echo amplitudes at 38 kHz. As observed at 38 kHz, target strength variance increased with tilt angle, irrespective of direction.

4.4 Backscattering Response Surfaces

Herring



Sprat

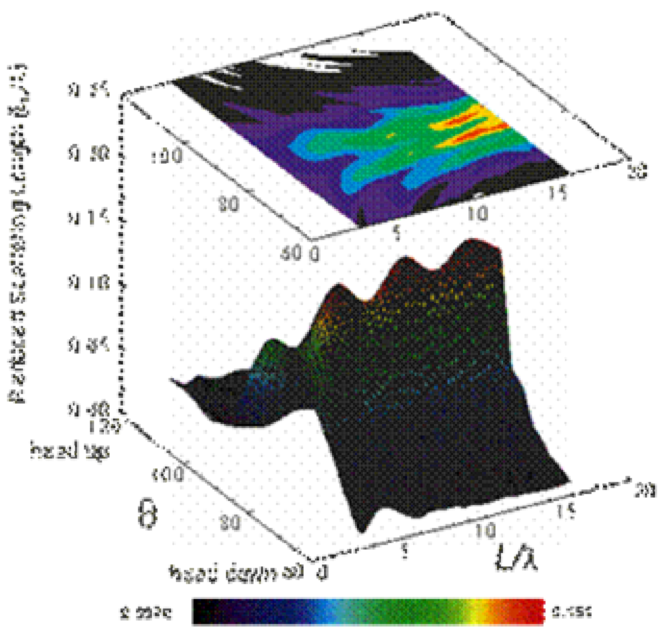


Fig 3.4.1. Calculated reduced scattering length as a function of tilt angle and fish length – acoustic wavelength ratio. The contour plot above each surface represents the standard deviation.

Mean backscattering response surfaces for herring and sprat contain the same general features. Reduced scattering lengths peaked at an angle of 85° across the range of length over acoustic wavelength (L/λ) modelled. Nodes and nulls in maximum echo amplitudes are attributed to constructive and destructive interference within the fishbody and between the fishbody and swimbladder. Echo amplitudes decreased in both species as tilt angles deviated from 5^{o} head down (i.e. 85^{o}).

Variance in echo amplitudes was not always proportionate to mean values. Large variances in echo amplitudes occurred at high L/λ values. Reduced scattering length variance was uniformly low at small tilt angles and low L/λ values.

4.5 Species Comparisons

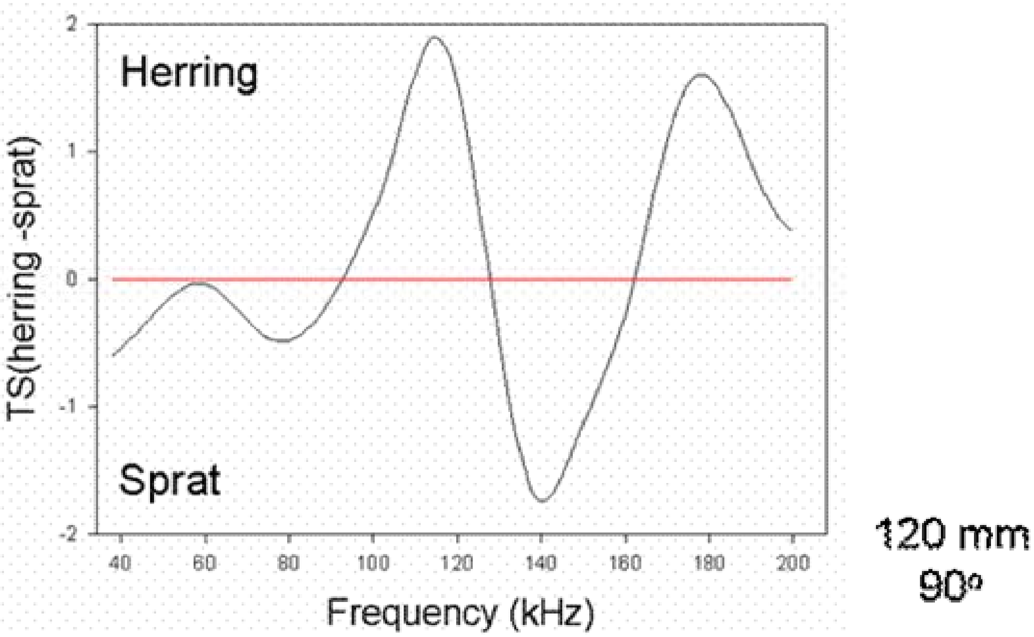


Figure 3.5.1. Difference between calculated dorsal mean target strength of herring and sprat of length 120 mm as a function of frequency.

Echo amplitudes were not consistently greater for one species over another over the range of frequencies examined (38 – 200 kHz) when fish length was assumed to be 120 mm. Sprat echo amplitudes exceed those of herring at low (38 – 90 kHz) and intermediate frequencies (130 – 160 kHz). Difference in echo amplitudes between the two species did not exceed two decibels at any frequency.

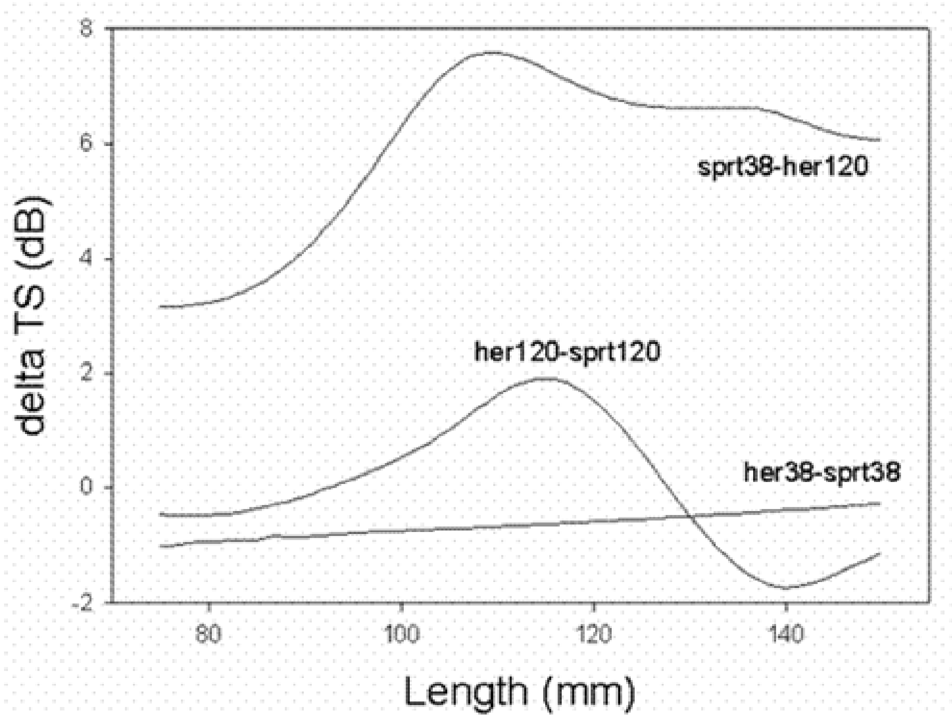


Figure 3.5.2. Difference between calculated dorsal mean target strength for herring and sprat as a function of length at 38 kHz and 120 kHz and between herring at 38 kHz and sprat at 120 kHz for a 120 mm herring and sprat.

Differences in frequency-dependent backscatter between the two species were dependent on the frequencies used as potential discriminating metrics. Target strengths differences at 38 kHz were less than 1 dB across the range of lengths modelled (75 – 150 mm). Target strength differences decreased as fish lengths increased. At 120 kHz, target strength differences were minimal at small lengths (75 – 100 mm), dominated by herring at intermediate lengths (100 – 110 mm), and then dominated by sprat at fish lengths greater than 130 mm. Maximum differences in target strengths occurred when comparing sprat at 38 kHz and herring at 120 kHz. Differences in target strengths between the two species increased to almost 8 dB at 110 mm and then decreased at larger lengths.

5 PROTOCOL FOR TS MEASUREMENTS ON BALTIC HERRING AND SPRAT

In order to evaluate the usefulness of the target strength relations presented in the previous chapters it would be of great value to obtain measurements of a range of target strengths during survey conditions. The study group suggests the following protocol:

- When running an acoustic survey run split-beam echo sounders with single target detection activated and look for areas with "good conditions", e.g. fish aggregations, which are reasonably large and disperse enough to give reliable single target detections. The definition of "dispersed" is outlined in Section 6 of the ICES Cooperative Research Report No. 235 (ICES, 1999), stating among other things that the s_a value for a 10 m layer should preferably be less than xxxx m^2/nm^2. It is an advantage if the fish aggregation comprises a single species or if the size distribution is such that different species groups can be clearly distinguished. Weather conditions should also reasonably quiet.
- Find a suitable position within such an area to take a trawl haul to cover the depth range of interest. Pelagic trawl should be preferred to bottom trawl, if the bottom depth is sufficiently large. Run TS data collection during the trawl operation. Obtain a CTD-profile immediately before or after the trawl haul.
- If time allows, do an additional small survey of the area and take an additional trawl haul with the same procedure as suggested above. Use a part of the time to collect TS-data while drifting, for example in connection with taking the CTD stations.
- Repeat this type of studies during surveys in as many seasons as available.
- Make sure that at least the following parameters from the echosounder are recorded: Acoustic frequency, transceiver menu parameters and echo detection menu parameters. Also store raw sample data from the study area

if storage capacity is available to make recalculations of the target strength data possible if special multiple target avoidance algorithms become available.

- Make sure that the complete set of standard parameters regarding the trawl catch, such as haul parameters, species composition and length-weight-age relations, is recorded and with respect to available resources take subsamples to determine as many of the following parameters as possible: Maturity stage distribution, lipid content and if resources are available, swimbladder dimensions according to the methods described in the Appendix B.

6 REVIEW OF THE LATEST LITERATURE ON TS OF HERRING AND SPRAT

Some new articles on this subject have appeared since last years report. Two papers were presented at 6th ICES Symposium in Acoustics in Fisheries and Aquatic Ecology in Montepellier, France, 10–14 June 2002.

Ona, E. 2002. An expanded target strength relation for herring. ICES J. Mar. Sc., 60: 493–499, 2003. Target strength experiments on adult herring (Norwegian Spring Spawning stock) in captivity have been conducted regularly over several years to investigate the acoustic effect of seasonal changes in fish physiology, as fat content, condition factor and gonadosomatic index (GSI). In particular, the dramatic effect of pressure on the herring target strength was established, but also the effect of herring swimming behaviour in deep water is described. The mean target strength of herring was found to be significantly dependent of pressure and GSI, which are also the parameters included in the new, expanded target strength relation.

Paper 75, Gorska, N and Ona, E. 2002. Modelling the acoustic effect of swimbladder compression in herring. ICES J. Mar. Sc., 60: 548–554, 2003. Presentation of how the swimbladder compression may influence herring target strength. Introducing swimbladder depth-compression in a Modal Based Deformed Cylinder Model, the analytical solutions have been obtained. One of them represents the backscattering cross section of fish oriented normally with respect to the echo sounder beam (normal dorsal incidence). The second solution accounts for the fish behaviour, size distribution and the variation of the fish flesh contrasts. The mean backscattering cross section was computed with selected orientation patterns, length distributions and contrast parameters. Further, the target strength depth dependence at different acoustical frequencies has been studied.

Two papers from the Herring 2000 symposia, Expectations for a New Millenium, Alaska Sea Grant College Program, AK-SG-01–04, in Anchorage, Alaska have been published.

Ona, E. 2001. Herring tilt angles, measured through target tracking (509–519 pp). The swimming angles at three frequencies were measured directly through split-beam target tracking with specialized split-beam hardware and software. In addition underwater video analysis was used to measure the relation between swimming angle and the actual tilt angle for herring at different depths.

Ona, E., Zhao, X., Svellingen, I. And Fosseidengen, J. E. 2001. Seasonal variation in herring target strength (461–487 pp). Target strength experiments on adult herring (Norwegian Spring Spawning stock) in captivity have been conducted regularly over several years to investigate the acoustic effect of seasonal changes in fish physiology, as fat content, condition factor and gonadosomatic index (GSI).

A paper dealing with the effect on acoustic measurements due to the change of behaviour and migration of fish during the diel cycle is also published.

A. Orlowski, Behavioural and physical effects on acoustic measurement of Baltic fish within a diel cycle. ICES J. Mar. Sc., 58: 1174 – 1183, 2001.

7 REVIEW THE DIEL CYCLE CONTENT AND STOMACH FULLNESS AND SEASONAL CYCLE OF FAT CONTENT

There are relatively few papers on the diel cycle of stomach content and especially annual cycle of fat content for herring and sprat in the Baltic. Below are the papers that were considered by the study group members. The geographical areas and ICES Subdivisions referred to are shown in the map in Figure 6.1.

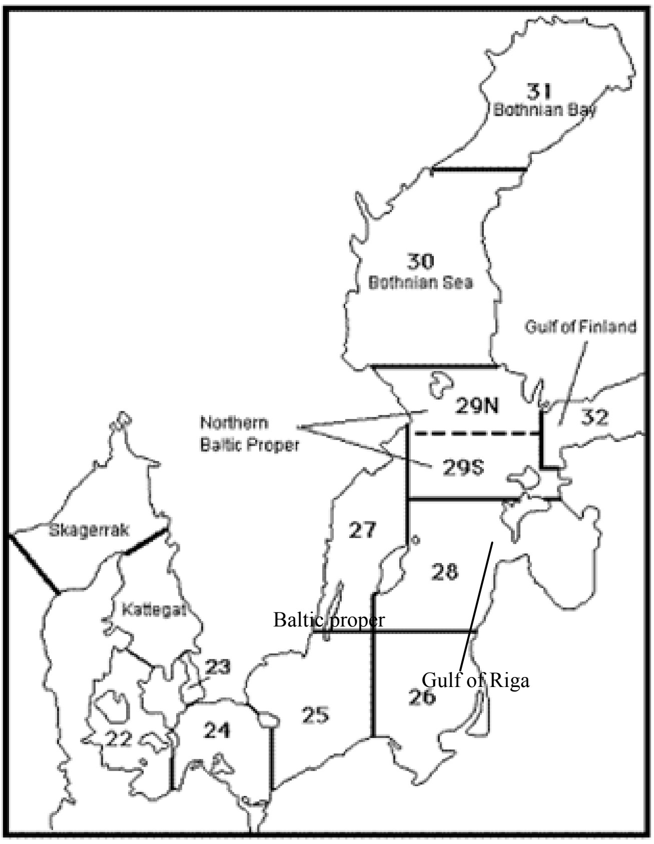


Figure 6.1. Map of Skagerrak, Kattegat and the Baltic Sea. The Baltic Sea is divided into Baltic proper, Gulf of Finland, Gulf of Riga, Bothnian Sea and Bothnian Bay. Numbers and thick lines indicate ICES Sub-divisions.

7.1 Eastern Baltic (SDs 26–28)

According to Fetter and Davidyuka (1996), herring showed a pronounced feeding peak (in the evening) during the summer period in shallow water areas, where the thermocline was absent, while in the deeper waters the period of continuous and intensive feeding lasted during the light hours. In the autumn two pronounced peaks of mysids consumption were observed during the morning and evening periods of vertical migration of the herring.

With regard to sprat, Starodub *et al*. (1992) noticed that the maximum of feeding and minimum number of empty stomachs changed from season to season but always occurred in the evening at the time of sunset.

7.2 Barther Bodden (north Germany, SD 24)

According to Franek (1988) herring does not feed at night, showing two peaks of maximum stomach fullness, one during the early morning period (7.00–10.00) and one during the evening period (18.00–20.00).

7.3 Gulf of Finland (SD 32)

Lankov (1986) and Raid (1985) performed some studies on juvenile herring. In spite of the different seasons of the two studies (autumn and June-July respectively), both the authors showed two maxima in the juvenile herring feeding intensity, one at 10.00 and one at 22.00 hours.

7.4 Baltic Sea (no specification)

Patokina (1996) showed that there were two peaks of herring feeding were: one in the first half of the day and one at sunset. Similar results were found both summer and autumn. On the other hand, sprat seemed to feed continuously during the daylight hours, especially in the summer.

7.5 Baltic proper

According to Arrhenius and Hansson (1993, 1994), herring in the northern Baltic proper showed two daily peaks in stomach fullness, one in the morning and one in the evening. The times for these maxima varied depending on the time for sunset and sunrise. Herring fed little or not at all during darkness. Similar results were also found by Cardinale *et al.* (2003) in the Bornholm basin (SD 25).

On the other hand, sprat seemed to feed during the daytime hours and not at all during darkness (Arrhenius, 1998; Cardinale *et al*., 2003).

8 RECOMMENDATIONS

8.1 Specific recommendations for future work

The Study Group recommends that each country that conducts acoustic surveys in the Baltic should store TS values of herring and sprat on all available frequencies (i.e. 38, 120 kHz).

The Study Group recommends that a cage experiment be conducted, organized by Russia, Latvia and Simrad in order to compare TS length distribution with *in situ* measurements of Gulf of Riga herring.

The group recommends that herring and sprat should be collected for X-ray studies from other areas and seasons to be included in the backscattering models, as they become available.

The Study Group recommends that the suggested protocol for TS measurements should be applied during all acoustic surveys, in 2002–2003, conducted in the Baltic Sea.

The study Group recommends that a ToR should be included in the ICES WG BIFS meeting 2003 to investigate the TS distributions and length frequency distribution from 2001–2002 surveys.

8.2 Next meeting in year 2003

8.2.1 Time and venue

The Study Group discussed its next meeting (to be decided at the Annual Science Conference in Copenhagen, Denmark). SGTSEB recommends that it will meet two days in June 2003 preceding the ICES FAST and ICES Symposium meeting in Bergen (Chairperson: B. Lundgren, Denmark). There will be also a meeting at the next ICES WG BIFS meeting in April 2003 to discuss this matter with Baltic acoustic colleagues and prepare the data for the Study Groups meeting in 2003.

8.2.2 Terms of reference

According to Annual Science Conference Resolution in Copenhagen, Denmark (C.Res.2002/x:xx) The Study Group of Target Strength Estimation in the Baltic Sea [SGTSEB] (Chairperson: B. Lundgren, Denmark) will meet in Bergen from 17–18 June 2003 to:

a) Evaluate the single target TS measurements on herring and sprat during the surveys in 2001–2002 and from cage experiments in the Baltic.
b) Apply the modelling methods on the case of the herring and sprat and compare their results to the existing information and single target TS measurements and cage experiments in the Baltic Sea
c) Recommend TS length relationships for herring and sprat in the Baltic Sea.
d) Prepare a final draft report.

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APPENDIX A: ACRONYMS

CTD	Conductivity-Temperature-Depth measuring instrument
BIFS	Baltic International Fish Survey
ICES	International Council for the Exploration of the Sea
KRM	Kirchhoff-ray mode model
SGTSEB	Study Group of Target Strength Estimation in the Baltic Sea
TS	Target Strength
WGFAST	Working Group on Fisheries Acoustics Science and Technology
WGNEPH	Working Group on *Nephrops* Stocks
SD	Sub-Divisions of sea areas in ICES

APPENDIX B: MANUAL FOR RADIOGRAPHING FISH

by John Horne and Michael Jech, Version 24 April 2001

Fish anaesthesia

An increasing number of government and institutions are now including fish in the range of vertebrate species. Investigators may be required to obtain "approved animal care protocols" before experimentation. Be sure to check with your institution and act accordingly before anaesthetizing and radiographing fish. Ranges of anaesthetic agents are available for use. Local opinion and regulation may dictate what you use but the goal remains the same - reduce fish movement during radiography to obtain clear images of the swimbladder in its natural state. We have used three methods with success. Each method has advantages and disadvantages.

A. MS-222. MS-222 is a trade name for tricaine methane sulfonate. Restrictions apply to distribution and usage depending on country. MS-222 essentially works by reducing efficiency of the gills to absorb oxygen from the water. Low doses will slow fish movements; high doses will kill the fish.

B. Clove Oil. Eugenol, the active ingredient in clove oil, is considered noncarcinogenic, nonmutagenic, and a GRAS substance by the FDA (Nagababu and Lakshmaiah 1992). Although not approved in the US for use in euthanasia, it has been effectively used as a fish anaesthetic for several fish species (e.g. Endo *et al*. 1972; Hikasa *et al*. 1986; Soto and Burhanuddin 1995; Anderson *et al*. 1997). The active ingredient in clove oil is eugenol. Fish are induced quicker and recover slower from exposure to clove oil than exposure to MS-222 (Munday and Wilson 1997). Two other favourable comparisons are that lower concentrations of clove oil are required to anaesthetize fish than MS-222 (Griffiths 2000) and MS-222 may influence olfactory capabilities of some fish (Lewis *et al*. 1985; Losey and Hugie 1994).

   a. Mix a 9:1 stock solution of 90–95% clove oil in ethanol (%?). Eugenol is insoluble in water and is mixed with alcohol to increase solubility.

   b. Depending on the size of the fish to be induced, a mixture of 40–60 ppm clove oil is used as an anaesthetic. To mix a 10 litre bath you will require:

Concentration (ppm)	Clove oil (ml)	Ethanol (ml)	9:1 Stock Mix (ml)	Water (l)
40	0.4	3.6	4	10
50	0.5	4.5	5	10
60	0.6	5.4	6	10

   c. Depending on the size of the fish and the concentration of clove oil used, fish will be anesthetized in as little as one minute or it may take up to 3–4 minutes. Watch for reduction and stopping of opercular pumping as a sign of activity. The goal is to eliminate movement but not to kill the fish.

   d. Using a small net or glove, transfer fish from holding tank to anaesthetic bath. Anesthetize only as many fish as are to be radiographed.

   e. Record the length of time required inducing the fish for future reference.

   f. Using a small net or glove, transfer fish from anaesthetic bath to radiographic cassette.

   g. After x-ray exposures (lateral and dorsal), transfer fish from radiography cassette to aerated recovery tank.

C. Non-aerated bucket. Use this method as a last resort. The fish is placed in bucket of water without anaesthesia and no aeration. As the fish uses up the oxygen, it will become anesthetized. Watch for reduction and stopping of opercular pumping as a sign of activity. The goal is to eliminate movement but not to kill the fish.

Freezing fish

Before the fish is frozen, care must be taken to avoid distorting, bending, crushing, or generally damaging the swimbladder. The fish should first be anesthetized, and then frozen. The fish can be frozen using one of two methods.

I. Flash-freeze in freezer (<-10^{o}C).

A. Anesthetize the fish (see Section I).

B. Label or mark the fish, preferably with a fish tag attached to the dorsal fin.

C. Measure and record the total length, standard length, depth, and width of the fish body. Depths and widths can be measured using calipers.

D. Lay the fish flat on a piece of wax-paper or other “non-stick” paper in the freezer.

E. Wait for the fish to freeze.

II. Super-cooled alcohol bath (Ona, Foote?). This will be updated.

Radiographing fish

Current Kirchhoff-ray mode model input is a digital file of length (x), height (y), and width (z) coordinates (Figure 1) that are obtained from lateral and digital radiographs of fish bodies and swimbladders. The goal is to image the body and swimbladder in their ‘natural’ shape and orientation. Capturing high contrasts around the perimeter of soft tissue structures is somewhat different than clearly imaging skeletal structures in diagnostic radiographs of injured bones (Figure 1).

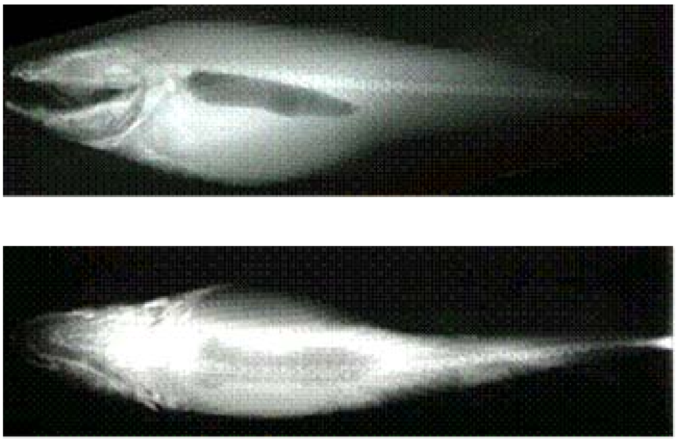

Figure 1. Lateral (upper) and dorsal (lower) radiograpghs of walleye pollock (*Theragra chalcogramma*). The swimbladder is the dark organ located below the vertebral column.

We recommend that you work with an experienced x-ray technician (veterinary or hospital) to maximize the probability of clear images. This approach may also reduce the amount of official paperwork and/or permits that have to be completed for your local animal care office.

Things to do before starting:

- Organize traffic flow and duties to minimize the amount of time that live fish will exposed to air. Have the aesthetic bath and recovery tank/pail in close proximity to the x-ray machine. Aerate water in the recovery tank.
- Prepare support wedges for dorsal exposures. The easiest method is to use chunks of rolled, wet paper towelling. Paper towel is fairly opaque and does not obscure the perimeter of the fish body. The length and diameter of the chunks depend on fish size. Fish are propped up using paper towel chunks on opposite sides of the body.
- Choose a numbering system and prepare identification numbers using radiograph tape, letters, or even pieces of paperclip.
- Decide how many fish to expose on a single plate. The number of fish depends on the size of the cassette relative to the size of the fish. It is best to get both dorsal and lateral exposures on the same plate. If doing both exposures of a single fish on the same plate, use lead blockers to cover unexposed area. Remember to block all exposed areas in subsequent exposures.

Radiograph procedure:

1) Adjust settings on x-ray machine. Approximate settings match those of small animal or human extremities (i.e. paw or hand). Record all settings (kVp, mA, exposure time, distance from object to x-ray head, machine manufacturer, model, film type). Take trial exposure(s) of dorsal and lateral surfaces, develop film, and visually inspect edge contrast on developed film. Adjust settings as needed.
2) Cover cassette with wax paper or freezer paper to prevent moisture and fish slime from contacting film cassette.
3) Mark radiograph cassette with identification number and date. Record all animal information on a summary sheet (provide example).
4) Block cassettes to cover unused or exposed sections of film. If radiographing more than one fish on a plate, make sure that EACH fish is clearly numbered on the cassette and that the fish order is the SAME on lateral and dorsal radiographs.
5) Transfer fish from anaesthetic bath or freezer to cassette. Do dorsal exposure first. Make sure that fish is straight and upright. Radiograph fish.
6) Change blocked area or cassette, transfer fish identification number/date.
7) Make sure fish is laying horizontal and straight, and take lateral exposure.
8) Transfer fish to recovery bath.
9) Develop film as soon as possible. Repeat any radiographs if you cannot visually trace the perimeter of the swimbladder and fish body.

X-ray to trace

The conversion of x-ray images to digital files is achieved by:

1) The outline of the fish body and swimbladder from dorsal and lateral radiographs are hand traced onto acetate transparencies. We have found that the eye is better at detecting edges than are current algorithms, especially when the edges are not well defined.
2) Fish body and swimbladder measurements that are required for the modelling estimates are described in Figure 2. The following table corresponds to the measurements.

X-ray Fish Measurements

Species: ______________________ Date: _________________

Source: ______________________ Measured By: __________________

Fish Number	Body Lat X-Min	Body Lat X-Max	Body Lat Y-Min	Body Lat Y-Max	SB Lat X-Min	SB Lat X-Max	SB Lat Y-Min	SB Lat Y-Max	BodyDor Y-Min	BodyDor Y-Max	SB Dor Y-Min	SB Dor Y-Max

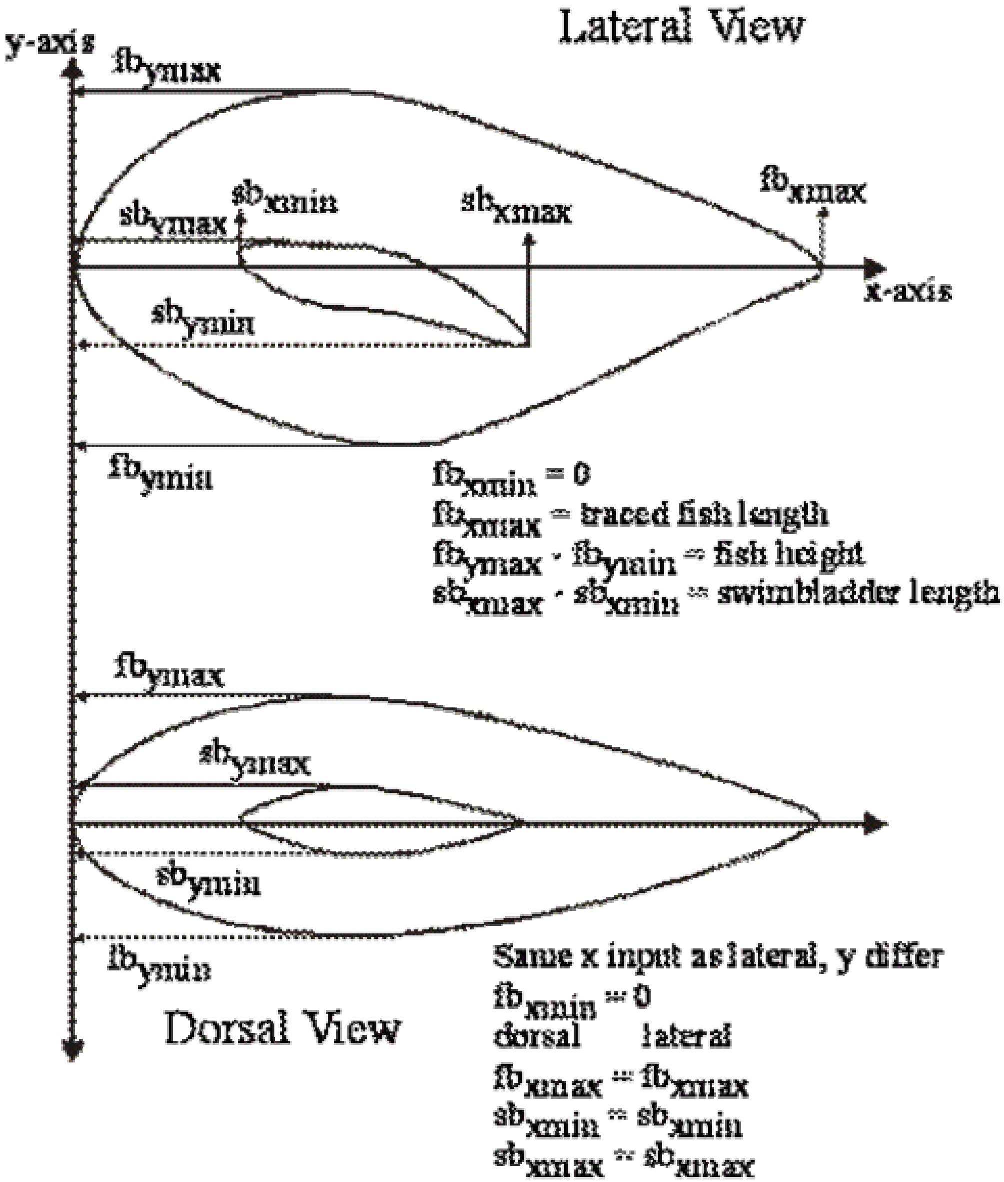

Figure 2. Schematic displaying locations of fish body (fb) and swimbladder (sb) lengths (x-axis), heights (Lateral view y-axis), and widths (Dorsal view y-axis). These measurements are used to scale the digital images to actual fish morphometry.

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APPENDIX C: SUMMARY OF REPORTED TARGET STRENGTH OF HERRING AND SARDINE

by Svellingen

References Author	 Year, publication	her/sard.	Results b20	 TS(30 cm) [dB]	Method	Fish depth [m]	Area
• Olsen	1976	h	-66.5	-37.0	Tethered fish	(8 – 35)	Norway
• Nakken and Olsen	1977	h	-65.2	-35.7			
• Hagström and Røttingen,	1982	h	-73.5	-44.0	Comparison		Norway
• Halldorsson and Reynisson,	1983	h	-69.4	-39.9	*In situ*		Iceland
• Edwards *et al*.	1983	h	?				
• Edwards *et al*.	1984				Caged fish		Scotland
• Degnbol *et al*.	1985	h	-72.6	-43.1	*In situ*		
• Lassen and Stæhr,	1985	h	-70.8	-41.3	*In situ*		Baltic Sea
• Foote *et al*.	1986	h	-72.1	-42.6	*In situ*		Norway
• Foote	1987	h	-71.9	-42.3	Summary report		
• Rudstam *et al*.	1988	h	-69.9	-40.4	Comparison		Northern Baltic
• Kautsky *et al*.	1990	h	-67.0	-37.5			
• Reynisson,	1993	h	-67.1	-37.6	*In situ*		Iceland
• Carrera, Miguel and Iglesias	1993	s	-64.3	34.7	*In situ*		Mediterranean
• Olsen and Ahlquist	1996	h	Depth dependants		Experiment		Norway
• Barange, Hampton and Sole	1996	s	-70.5	41.0	*In situ*/empirical		South Africa
• Misund and Beltestad	1995/1996	h	-69.8	-40.2	Comparison		Norway
• Svellingen and Ona,	Berlin 1999	s	-66.4	-36.9	*In situ*	(10 – 25)	West Africa
• Vabø *et al*.		h	-67.6	-38.0	*In situ*	(40 – 300)	Norway
• (Depth dependent TS relation established).							
• Zhao, X.,	1996, Thesis M.phil. UIB, Bergen	h	-64 –69	–34.5 –39.6	Experiment	(5 – 20)	Norway
• Ona *et al*.	Herring 2000	h	-64 –69	–-34.5 –39.6	Experiment	(5 – 20)	Norway
• Ona and Svellingen	Seattle, April 2001	h	Depth dependant TS		Experiment	(15 – 115)	Norway