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REPORT OF THE

STUDY GROUP ON TARGET STRENGTH ESTIMATION IN THE BALTIC SEA

Seattle, USA
22–23 April 2001

International Council for the Exploration of the Sea

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1 INTRODUCTION

1.1 Participation

The meeting was attended by:

Fredrik Arrhenius (Chair)	Sweden
Eberhard Götze	Germany
John Horne	USA
Michael Jech	USA
Bo Lundgren	Denmark
Ole Arve Misund	Norway
Kjell Olsen	Norway
Ingvald Svellingen	Norway
Karl-Johan Stæhr	Denmark
Elliott Hazen	USA

1.2 Terms of Reference

According to Annual Science Conference Resolution (2000/2B02) in Brugge last year, the Study Group of Target Strength Estimation in the Baltic Sea [SGTSEB] (Chair: F. Arrhenius, Sweden) will meet at Seattle, USA from 22–23 April 2001 to:

a) To prepare and disseminate as soon as possible a protocol for TS measurements on the Baltic herring, based upon the state of the art and especially the recommendations of the CRR (on TS measurements, 1999 (ICES, 1999)), adapting these recommendations to the special case of the Baltic Sea.

b) Establish a list of the main factors affecting the herring TS and study the effects through comparative analysis and measurements on various herring stocks (e.g., Baltic and Norwegian spring spawning herrings);

c) Collate the existing information and measurements on herring TS;

d) Apply modelling methods on the case of the herring and compare their results to the existing information;

e) From the databases available from the WGFAST members, measure the variability of TS *in situ* under various conditions (day-night, winter-summer, etc.);

f) Encourage experimental measurements through conventional and non-conventional methods.

The study group makes its report available to WGFAST and will report by 22 May 2001 for the attention of the Fisheries Technology and Baltic Committees. During 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-parameterised herring and sprat-TS relationships.

1.3 Background

In the application of acoustic data to fish abundance estimation, the target strength (TS) of the fish is an important parameter for the conversion of integrated acoustic energy to absolute fish abundance. Variability in acoustic estimates can be ascribed to several causes. High precision and comparability of acoustic measurements of isotropic standard targets are documented and verified. The main problem appears in the quantitative interpretation of acoustic echoes received from targets of unknown scattering characteristics. The same fish or school can produce very different acoustic echoes. These differences may be associated with pure stochastic processes combined with behavioural reactions that are controlled by basic biological rhythms and functions.

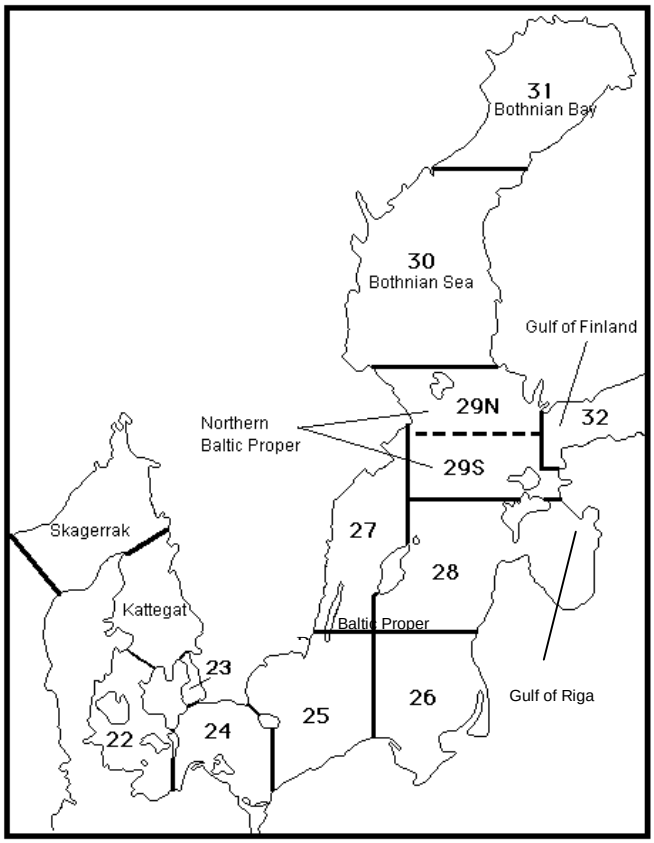
One of the most important factors influencing the final biomass and abundance estimates in stock assessments is related to TS conversion formulas. By convention, the TS conversion is expressed as the averaged function of fish length. The actual TS constants applied since 1983 for Baltic Sea acoustic surveys are 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 Working Group FAST 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 revisited;
- 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.

From 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.

2 INFORMATION OF THE DIFFERENT STOCK COMPONENTS

Figure 1. Map of the Skagerrak Kattegat, and Baltic Sea. The Baltic Sea is divided into Baltic proper, Gulf of Finland, Gulf of Riga, Bothnian Sea and Bay. The different numbers indicate ICES Sub-divisions.



2.1 Gulf of Bothnia and Bothnian Bay

There are two different stocks of herring in the Bothnian Sea (SD 30) and the Bothnian Bay (SD 31) and they are assessed differently. Sprat is seldom found north of subdivision 29.

The salinity ranges from 6‰ in the southern part of the Bothnian Sea to almost freshwater at the northern part of the Bothnian Bay.

2.2 Baltic Proper

Fish stocks (assessment units) are defined according to both biological characteristics and practical aspects such as data availability. Fishery biologists have focused on how to estimate current abundance and biomass of herring to advise managers. The borders between assessment units/stocks of Baltic herring and sprat have changed several times. At present Baltic herring is assessed as one main stock in the central Baltic and one main stock in the southern Baltic, the latter with migrations to and from the Kattegat and Skagerrak. For stock assessment, ICES sub-divisions 25–29 and Gulf of Finland are considered to comprise one unit with "constant" growth characteristics for the individual fish. The issue of how to separate the herring into unit stocks has been discussed on many occasions during the last decades. As for herring, sprat is believed to consist of several populations in the Baltic proper. However, it is assessed as one management unit. The two different species aggregate together and cannot be separated by species in acoustics echograms. The splitting of species within acoustic data is accomplished using trawl catches.

This year's assessments used the same units as describes above i.e., all herring in Sub-divisions 25–29 and 32 were assessed as one stock. Furthermore the so-called "Gulf Herring" in the Gulf of Riga was assessed separately as requested (Figure 1). This herring stock is mainly fished in the Gulf, but is also caught along migration routes in the open sea. The Gulf Herring is included in the assessment of all herring in Sub-divisions 25–29 and 32 and the results of the separate assessment may be used for setting a regional TAC and/or quota allocation for the countries concerned.

Landings of herrings come from mixed fisheries for clupeoids (either fisheries targeting herring but with occasionally large by-catches of sprat or sprat fisheries — both industrial and for human consumption — with by-catches of herring) in Sub-divisions 25–29 and Gulf of Finland.

The surface salinity ranges from 6‰ in the northern part of the Baltic proper to 7–8 ‰ in the southern part. However salinity increases to 16–17 ‰ in the bottom water with a sharp halocline at 60–70 meter.

2.3 Skagerrak and Kattegat and Western Baltic

Herring in the Kattegat, Skagerrak and the Western Baltic can be separated into several autumn and spring-spawning stocks. The identification of spawning components is based on morphometric and meristic characteristics observed from samples taken at different spawning sites and seasons. There are three main components of herring stocks in the Skagerrak and Kattegat: North Sea autumn spawners, coastal Kattegat Winter spawners and spring spawners. The latter could be further subdivided into Skagerrak spring spawners and Baltic spring spawners. However the last two local spawners seem to be of minor importance for the fishery.

The herring in the western Baltic have been separated into three spring spawning and one autumn spawning components. The ICES Baltic Fisheries Assessment WG has taken a more holistic approach and recognizes currently only one spring spawning component in Sub-divisions 22 and 24; the Western Baltic spring spawning stock.

Knowledge of migration patterns and spatial distributions are schematic. The North Sea autumn spawners enter Skagerrak and Kattegat as larvae and migrate before maturing back to the North Sea after 2–3 years. The Western Baltic Spring spawners spawn around the Baltic Islands, Rügen (southern part of Sub-division 24) and are mixed with other Baltic spring spawners in the Southwest Baltic. Tag recaptures demonstrate that spent and some immature Western Baltic Spring spawners migrate northward after spawning in the Sound, the Belt Sea, the Kattegat, the Skagerrak and the North Sea. Unknown proportions of adult herring remain in the SW Baltic. In the 3rd quarter, herring begin a return migration back to the spawning areas around islands Rügen.

Catches of herring in Division IIIa (the Kattegat and the Skagerrak) have been assigned to two main assessment components:

- The Baltic/Division IIIa spring spawners
- The North Sea autumn spawners

Recent assessments of herring in Division IIIa and Southwest Baltic were based on the assumption that the influence of local stocks on the herring dynamics has minor importance.

3 REVIEW THE EXISTING INFORMATION AND MEASUREMENTS ON HERRING TS

To estimate fish abundance in acoustic surveys, TS must be known as a function of fish length (L). The form of this function is commonly:

$$TS = 20 \text{ Log } (L) - b$$

where b is a constant (Foote, 1987). The value of b may be determined by comparing observed TS histograms with the length distribution of the ensonified fish, obtained by trawling or other means (MacLennan and Simmonds, 1992). The TS to fish length relation at frequency 38 kHz currently applied was developed by Foote (1987) for clupeoids;

$$TS = 20 \log (L) - 71.9$$

The TS to fish length relation at frequency 38 kHz currently applied for Baltic clupeids is the one recommended by ICES (ICES, 1997);

$$TS = 20 \log (L) - 71.2$$

The latter regression was based mainly on *in situ* estimates made in the mid 1980s (Degnbol *et al.*, 1985; Lassen and Stæhr, 1985; Foote *et al.*, 1986).

During the study group meeting a summary of reported TS of herring and sardine was produced and indicated the TS value for 30 cm fish (Table 1). These findings indicate that TS measurements are highly variable for herring in different areas.

A recent finding of the Norwegian spring spawning herring is that TS's at the surface are generally 3–5 dB higher than the TS applied to herring during surveys. TS increase with developed gonads by approximately 2 dB (Zhao, 1996). 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). The preliminary mean target strength of herring is proposed:

$$<TS>=20\log L-66.3-3.5\log(1+z/10)+0.24(GSI)$$

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

4 MAIN FACTORS AFFECTING HERRING TS

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. The problem is that the echo depends on the internal anatomy – the shape of the swimbladder, for example, which can be very different among fish, which are similar in sized external appearance.

The Study Group of Target Strength Estimation in the Baltic Sea (SGTSEB) will use a general TS equation;

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

to direct acoustic and biological sampling during annual herring surveys and other times of the year. This equation includes physical and biological factors that are believed to affect acoustic TS of an individual fish. The symbol "f" denotes that TS is a function of these variables. A function can be linear or non-linear, empirically or theoretically derived, and is not necessarily equivalent among all other variables in the formula.

Table 1 Summary of reported Target Strength of herring and sardine.

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
• 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 & Ahlquist	1996	h	Depth dependant TS		Experiment		Norway
• Barange, Hampton and Sole	1996	s	-70.5	-41.0	*In situ*/empirical		South Africa
• Misund and Beltestad	1995	h	-69.8	-40.2	Comparison		Norway
• Svellingen and Ona	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	h	-64 –69	-34.5 –39.6	Experiment	(5–20)	Norway
• Ona *et al.*	2000	h	-64 –69	-34.5 –39.6	Experiment	(5–20)	Norway
• Ona and Svellingen	2001	h	Depth dependant TS		Experiment	(15–115)	Norway

Frequency is the acoustic frequency used during a survey. Length is a measure of the fish length (*e.g.*, total or standard length). Pressure is a measure of the depth effects on the swimbladder. Temperature and salinity are measures of the environmental conditions. Temperature and salinity may have both direct and indirect effects on TS. Direct effects include variation in the density and sound speed contrasts between the fish body, swimbladder, and surrounding water. Indirect effects include larger or smaller swimbladder volumes for buoyancy compensation in changing water densities. Orientation is a function of the tilt, roll, and yaw of a fish relative to the pressure wave transmitted by a transducer. Activity is the movement of a fish (*e.g.*, swimming or resting). In this model, fish behaviour is a combination of orientation and activity. Lipids, gut fullness, and gonad development may also have direct effects on the density and sound speed contrasts between the fish body and water, and indirect effects on the swimbladder size due to buoyancy compensation. In addition, gut fullness and gonad development may directly influence the shape of the swimbladder.

TS of fish is mainly affected by anatomy, behaviour and hydrographic conditions. A factor when comparing the TS of herring in the Baltic Sea to other areas is likely to be the difference in salinity. Salinity in the Baltic Sea is very low compared to the open sea, and it is assumed that this low salinity has a rather strong impact on the TS of the fish. For 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 again 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.

5 MODELLING METHODS

The Study Group of Target Strength of Baltic Sea (SGTSEB) will use 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. Since herring and sprat mix in different proportions over a range of environmental conditions, collections of herring and sprat radiographs will be used to characterize anatomical and acoustical characteristics of these species throughout the Baltic Sea, Skagerrak and Kattegat. Radiographs will be digitised and used in Kirchhoff-ray mode (KRM) backscatter models to estimate backscatter amplitude and variance from individuals and groups 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 affect on TS. Comparison of affects among factors should enable an ordinal ranking of the relative influence of biological and physical factors on TS. KRM TS estimates will be compared to TS measurements of herring and sprat during assessment survey cruises.

A radiograph sampling guide will be written and distributed to groups that are able to sample herring and sprat. An initial draft of that guide is appended to this report (Annex I).

6 DEVELOP PROTOCOLS FOR TS MEASUREMENTS ON THE BALTIC HERRING AND SPRAT

6.1 General Sampling Protocols

To exemplify the different factors that might affect the TS, a table was drawn to describe measurements and samples and suggested protocols for those factors in the Baltic (Table 2). Protocols describe ideal sampling, number and type of samples can vary with availability.

Table 2. Factors affecting the target strength (TS) of a fish, measurements and samples, and protocols.

TS Factor	Measurements and Samples	Protocol
Acoustic frequency		
Length	Total Length and/or Standard Length	10 fish per length-class fish collected in different areas of the Baltic Sea.
Pressure/Depth	TS and Depth of *in situ* target	*In situ* TS protocol
Environmental Factors (*e.g.*, temperature, salinity)	Vertical Conductivity-Temperature-Depth (CTD) casts	CTD casts at regular intervals CTD casts corresponding to *in situ* TS measurements CTD casts corresponding to trawl Hauls
Orientation: roll, pitch, yaw	Underwater video	
Activity (*e.g.*, swimming)	Underwater video	
Lipid content	Laboratory analysis and/or Fish processing plant	10 fish per length-class for laboratory analysis or to validate lipid indices from fish processing plants.
Gut fullness	Stomach volume or Stomach content	10 fish per length-class
Gonad development	Sex Maturity Index or Gonad weight	10 fish per length-class
Radiographs	Lateral and Dorsal views	10 fish per length-class fish collected in different areas of the Baltic Sea

6.2 Biological Sampling for Length

It was recommended that live herring from three areas should be sampled (10 individuals from three size classes) during 2001–2002.

The following areas will be targeted:

- Trapnet-fishery in the Rügen area from March-May
- Trapnet-fishery in Gulf of Riga by Estonia/Latvia between April-June
- Trapnet-fishery in Gulf of Bothnian between May-July

Additional samples should be obtained from other areas and seasons to be included in the backscattering models, as they become available.

It was also recommended that live sprat should be sampled during the acoustic survey in the autumn sampled (minimum 10 individuals per length-group per area).

6.3 TS Measurements During Surveys

TS measurements on backscatter properties and range of target strengths (TS) from herring and sprat should be measured during the surveys. This should be done during “good conditions”, e.g., fish must be dispersed enough to be measured as single targets in the echosounder and high seas can make the results questionable. They must be accessible to trawls and be comprised of size distributions that clearly distinguish different species groups (Section 6 in ICES Cooperative Research Report No. 235 (ICES, 1999).

The first approach should be to take a trawl haul and CTD in the dedicated depth range. The next step should dedicate more time to TS data collection in the same area. The next step should be to take an additional trawl haul.

7 TS VARIABILITY MEASUREMENTS

The group recommends that it is vital to investigate and incorporate present results that measure the variability of TS *in situ* under various conditions (day-night, winter-summer, etc.) from other WGFAST members. However, this will be

evaluated by the study group when new insights from previous backscatter modelling analyses and empirical studies together with a combined modelling and measuring investigation on backscatter properties and range of target strengths (TS) from herring and sprat.

8 SUGGESTED EXPERIMENTAL MEASUREMENTS

The participants suggested that it is important to encourage studies to be carried out in the future, after analysis radiographs and other biological factors have been done. Experimental set-ups should include measurements on tethered individuals, free swimming individuals and free swimming aggregations.

9 RECOMMENDATIONS

9.1 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.

The Study Group recommends that additional biological information (e.g., fat content and stomach fullness) that potentially affect backscatter should be sampled during acoustic surveys together with measurements of temperature and salinity.

The group recommends that herring and sprat should be collected for radiography, and additional biological information from different areas in the Baltic Sea. A draft guide for which parameters to be sampled is attached to this report (Annex I).

It was recommended that live herring from three areas should be sampled (10 individuals from three size classes).

The following areas will be targeted:

- Trapnet-fishery in the Rügen area from March-May
- Trapnet-fishery in Gulf of Riga by Estonia/Latvia between April-June
- Trapnet-fishery in Gulf of Bothnian between May-July

Additional samples should be sampled from other areas and seasons to be included in the backscattering models, as they become available.

It was also recommended that live sprat should be sampled during the acoustic survey in the autumn sampled (minimum 10 individuals per length-group per area).

The Study Group recommends that the suggested protocol for TS measurements should be applied during all acoustic surveys, in 2001, conducted in the Baltic Sea.

The Study Group recommends that radiographs from all herring and sprat samples will be done at one national laboratory. We want to use an experienced x-ray technician (veterinary or hospital) to maximize the probability of clear images**.**

9.2 Next Meeting in Year 2002

9.2.1 Time and venue

The Study Group discussed its next meeting (to be decided at the Annual Science Conference in Oslo, Norway). SGTSEB recommends that it will meet two days in June 2002 in connection with the ICES Symposium meeting in Montpellier (Chair: F. Arrhenius, Sweden). There will be also a meeting at the next ICES WG BIFS meeting in April 2002 to discuss this matter with Baltic acoustic colleagues.

9.2.2 Terms of reference

The Study Group proposes that the following Draft Resolution for its 2002 meeting:

The Study Group of Target Strength Estimation in the Baltic Sea [SGTSEB] (Chair: F. Arrhenius, Sweden) will meet in Montpellier from 8-9 June 2002 to:

a) discuss the results of the biological properties that affect backscattering of Baltic fish i.e., swimbladder volume and shape, fat content and stomach content and fullness.

b) discuss the result of backscatter 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 in 2001 in the Baltic

d) review the latest literature of TS of herring and sprat

e) review current information of diel cycles of fat content and stomach fullness in different part of the Baltic area

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Acronyms

CTD	Conductivity-Temperature-Depth
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

APPENDIX 1 – FISH ANAESTHESIA AND RADIOGRAPHING PROTOCOLS

John Horne and Michael Jech

FISH ANAESTHESIA

An increasing number of governments 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 Methanesulfonate. Restrictions apply to distribution and usage depending on country. Care must be taken by the user of MS-222, and protective gloves must be worn when handling fish. 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. Manufacturer suggested dosages range from 10 to 1,000 mg/liter. It is suggested that you begin by anaesthetizing the fish at low dosages and determine anaesthetizing rates that are appropriate for your species.

B. Clove Oil. Clove oil is the preferred method to anaesthetize a fish. 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). 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 anaesthetized 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. Anaesthetize 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 anaesthetized. 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 anaesthetized, and then frozen. The fish can be frozen using one of two methods.

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

A. Anaesthetize the fish (see Section 11.1).

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 callipers.

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 ethanol bath (Ona 1982, 1990). The fish is "shock-frozen" using a super-cooled ethanol bath. The ethanol bath is cooled to temperatures <-50^{o}C using dry ice.

A. Prepare the ethanol bath.

B. Anaesthetize the fish (see Section 11.1).

C. Label of mark the fish, preferably with a fish tag attached to the dorsal fin.

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

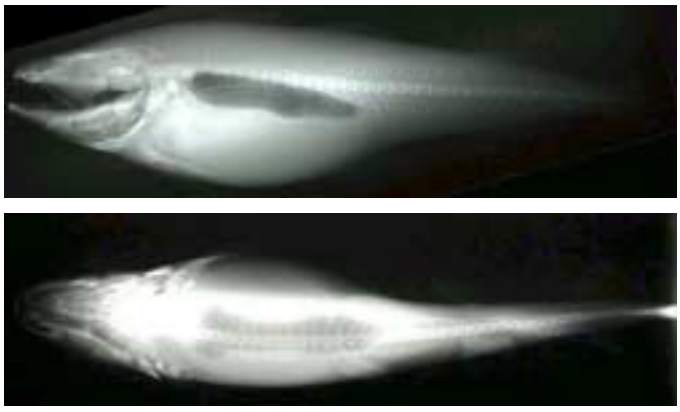
E. Using a pair of tongs, grasp the tail and head of the fish, and then place the fish in the ethanol bath while maintaining the fish in a natural, horizontal position.

F. The fish should be thoroughly frozen after a few minutes.

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 be 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.
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 lying 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 can not visually trace the perimeter of the swimbladder and fish body.

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