GEF BALTIC SEA REGIONAL PROJECT
PHASE 1: 2003 – 2005

FLOUNDER AGE READING WORKSHOP

Copenhagen, Denmark
7-10 March, 2006

EDITED BY

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International Council for the Exploration of the Sea

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1 INTRODUCTION

1.1 Terms of Reference

The Lead Laboratories in Baltic Sea Regional Project „On Age Determination and Fish Stomach Analysis" and „On Coastal Activities" decided to hold a Flounder Age Reading Workshop in Rostock, Germany 7-10 March 2006 to:
1. Describe national sampling and age determination of flounder in their country including the following:
a) short overview on the biology of local stocks and its implications on the formation of otolith structure – annual rings,
b) description of the stated problems in age determination,
c) ability to folllow cohorts.

2. Prepare a set of otoliths (matrices) that could be used for demonstration of ring formation for stock and characteristic seasons (prespawning, postspawning or feeding) with the aim to demonstrate regularity in the deposition of rings. Some of these sets could be used for comparative reading at the Workshop.

3. Perform age determination of flounder from otolith sets prepared by the participants. Compare, analyse and discuss the results of this age determination:
a) intensive bilateral cross-readings by means of a double-tube microscope
b) multilateral discussions in front of a video/computer screen.

4. Elaborate recomendations (decisions) for future cooperation:
a) otolith sample exchange (if relevant) on bilateral or multirateral basis,
b) necessity to plan similar workshops in future on regular basis (if relevant)
c) management of future cooperation (chairman)

1.2 Participation

The meeting was attended by:

Carin Ångström	Sweden
Ulrich Berth	Germany
Ann-Britt Florin	Sweden
Christian Friess	Germany
Kristiina Jürgens	Estonia
Georgs Kornilovs	Latvia (chair)
Tomasz Nermer	Poland
Sandra Pabrinkyté	Lithuania
Kordian Trella	Poland
Didzis Ustups	Latvia
Dace Zilniece	Latvia

2 Review of national sampling programmes of flounder

2.1 Estonia

Flounder lives along the whole Estonian coast. It is not so abundant in the eastern part of the Gulf of Finland and of the Gulf of Riga. The eastern limit of abundant flounder concentrations in the Gulf of Finland is 26° E. To the east from that degree flounder spawning has not been not detected.

There are at least two different races (infraspecies) of flounder in Estonian waters. The flounder, that spawns pelagic eggs in deeper areas, is known as deepflounder (*Platichthys flesus trachurus* natio *baltica* infranatio *pelagicus*), and the population, that spawns demersal eggs in the coastal waters, is known as bankflounder (*Pl. fl. tr.* natio *baltica* infranatio *sublitoralis)*. There are several areas near the northern Estonian coast, where the eggs of bankflounder were found. It, probably, spawns in the Gulf of Finland near the northern and northwestern coast of island Hiiumaa and near the southwestern coast of Saaremaa, also. Possibility that the deepflounder could spawn in the Gulf of Finland during the last 15 years is very small because of unfavorable hydrological conditions.

In coastal spawners the bulk of males mature at the age of 3 years, females at 4 years. In gonad development the variation is wide. In Estonian waters flounder transfers into the III maturity stage in August, in the Central Baltic in October. In deep areas flounder spawns in April-May, in coastal areas in May-June.

Estonian flounder catches have varied between 2731 tons in 1965 and 102 t in 1995. After the peak yield in 1983 (1611 t) the catches diminished to about 400-500 t in the recent years.

In 2001-2003, the total commercial flounder landings (including by-catch of turbot) were 480 tonnes in average; 92% of landings originate from the coastal gill-net and trap-net fisheries (in SD 29 mainly seine catches). Data from coastal fish survey indicate increased CPUE values for flounder in most of coastal areas since the mid-1990s. Directed fishery is closed from February to June, and the minimum legal size for landings is 21 cm.

Most of flounder in coastal fishery is landed from ICES SD 32 (74% in 2003), and the landings are the biggest in the IV quarter (55%) followed by III, II and I quarter (21, 18 and 5%, respectively).

Sampling is weighted according to fishing method and expected landings in each subdivision. The number of samples based on the extended program D3 (50 specimens per 200 tonnes, which corresponds to two samples, 100 individuals) is not adequate to cover sampling of all gear types and catch areas. In recent years, the total number of flounder aged from commercial landings in Estonia has been > 1000. The annual sampling schedule for 2005 consists of 28 samples (1400 fish) in ICES subdivisions 28, 29, and 32. Simple random sampling is applied. Each individual will be measured for length and aged. The sampling scheme will be modified annually, depending on catch rates and fishing activities (Table 2.1.1).

Additional (fishery independent) data will be gathered during the coastal fish gill-net sampling by MI.

Table 2.1.1.

The number of flounder samples to be collected by ICES Sub-division, gear type and year quarter weighted by catch rates in 2003 (one sample corresponds to 50 individuals):

Gear type	Year, quarter	ICES Sub-division			Sampling per gear and year quarter
		28	29	32	
Gill-net	1	0	0	0	0
	2	0	1	1	2
	3	1	1	2	4
	4	1	1	3	5
	Total	**2**	**3**	**6**	**11**

Trap net	1 2 3 4	0 1 1 1	0 1 1 1	0 2 2 3	0 4 4 5
	Total	**3**	**3**	**7**	**13**
Seine	1	0	0	0	0
	Total	**0**	**2**	**0**	**2**
	Grand total	**5**	**6**	**13**	**26**

For the age determination of flounder both sagital otoliths are removed by tweezers and stored in paper bags. Whole otoliths are cleaned with water, sunk into glycerine and studied using a binocular microscope with reflected light in 16 x magnification.

2.2. Germany

Landings

With some 90% flounder is the dominating flatfish species in the Sub-division 24. During the last 10 years, the German total catch varied there between 1100 and 1800 metric tonnes.
During this period, the total landing share of fishery directed to flounder and conducted mainly by 17,5 m cutters decreased to about 30% of total flounder landings. The main share was taken as a by-catch to the cod fishery. The amount landed was correlated to the effort in cod fishery as well as to demand and price for flounder. Considering the reported landings, the stock appeared to be under-fished. But the discard of flounder in the cod fishery remains an important issue but is not well estimated.
The minimum landing size is 25 cm. Main fishing areas are the Oderbank area and the fishing grounds north-easterly of Ruegen.

Fishing Season

The fishery directed to flounder is conducted during the feeding period (3rd and 4th quarter). Landings in December and January are nearly exclusively by-catch to the cod fishery. The flounder in the Sub-division 24 spawns between mid February and end of March. The landings between February and June are negligible (below 150 metric tonnes). Between mid of February and end of April the flounder is protected by a closed season for the females.

Stock and Recruitment Situation

The presently comparably high and stable stock size level between 21 and 28 thousand metric tonnes biomass (sub-divisions 24+25) bases on a series of good to very good year classes in the last ten years. In the catch the age groups 3 to 5 are dominating.
The average weight in the landing varies between 280 and 300 grams, having in mind that the samples are taken from landings of sorted fish.

Fishery Research

The sampling of commercial landings from the flounder directed fishery is concentrated in the period between July and November. An average of 15 to 20 samples of unsorted but minimum sized flounder are analysed for length distribution (ca. 400 individuals) and age/biological parameters (ca. 250 individuals).

During the BITS ground trawl surveys in February and November at about 45 stations an average total of 1500 to 2000 fish are sampled for length distribution and 500 for age/biological parameters.
In July, a young fish survey is conducted for the Oderbank flounder mainly aimed on the age class 2.

Historical Time Series

The ground trawl surveys were started in 1979 with the research vessel EISBAER and after the changes in 1992 continued by the research cutter SOLEA. Until the year 2000 the HG20/25 bottom trawl was used and since 2001 the TV3/520.
The Oderbank local population young fish survey was started in 1983 and since then continuously conducted by the research cutter CLUPEA with always the same trawl (the so called Warnemuender Dorschzeese).
The changes of vessels or gear were accompanied by comparative fishing experiments.

2.3. Latvia

Fishery

Latvian landings (ICES Subdivisions 26 and 28) are based on the by-catch in cod trawl and gillnets fishery. Direct flounder trawl fishery in open sea is carried out mostly in the 4th quarter. In coastal area, a seine fishery directed at flounder takes place in the 2nd and 3rd quarters.
Latvian catches of flounder are taken in ICES Subdivision 26 and 28. Main landings are realized in SD 28. Last 10-15 years landings of flounder were on stable level. In comparison to middle of 70-ies, landings were on low level. However, in the last two years catches has significantly increased. In 2005 sharp increase of landings was observed in all flounder fishing sectors (coastal area, open sea area – trawl and gill-net fishery)

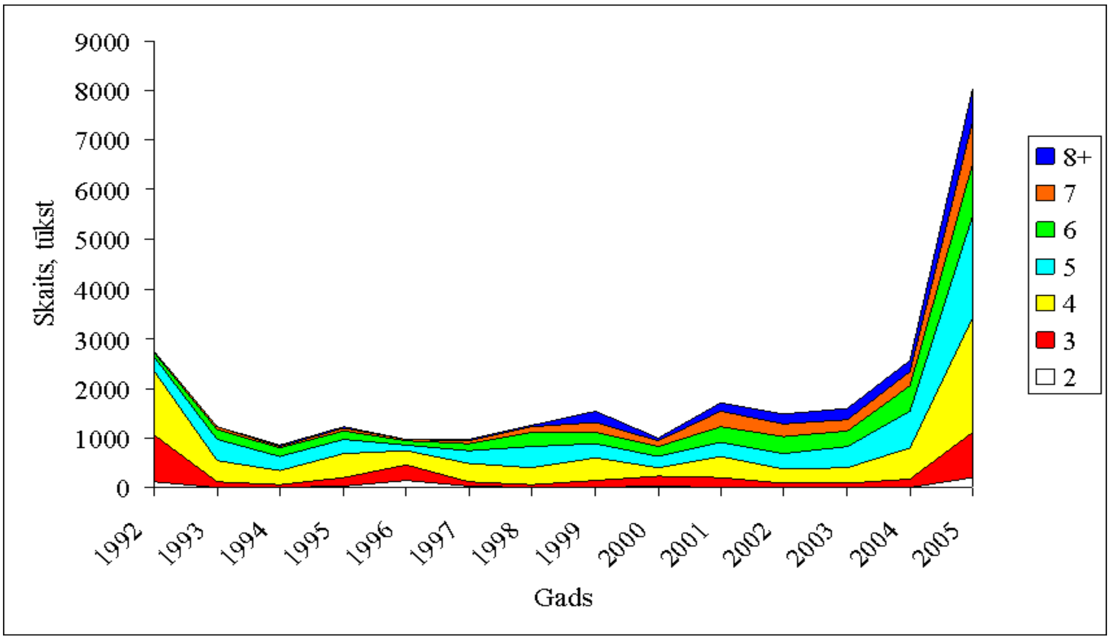

Figure 2.3.1. Catch in numbers of flounder (CANUM) in Latvian landings by age group

Biology of local stock

There are two stocks in SD 28. Stock in SD 28, including the Gulf of Riga, is greatly confined to the coastal areas, have some connections with the stocks in SD26 and 32. Spawning time for flounder is March- June in the depth of 40-80 m and the spawning migration takes place to eastern coast of Gothland.

Flounder males mature at age 3 and females at age 4.

Other flounder stock is greatly confined to the coastal areas, for this stock the spawning time is May- July and, contrary to southern stocks, it occurs in the littoral and coastal zones, in depths of 2- 20 m.

Juvenile feeding grounds are located in the coastal area of Latvia and the highest concentrations are usually observed in Kolka area in the Irben Strait, where juvenile samples are collected since 1989.

Sampling programme

Sampling of otoliths is carried out from two types of fisheries- commercial and scientific. Collection programme from commercial fishery is under National Fisheries Data Collection Programme. Programme requires 20 samples per year, which are divided by ICES Subdivision (26 and 28), fishery location (open sea, coastal area) and fishing gear (bottom trawl, gillnet, flounder seine). Each sample consists of 50 flounder. Data are collected from landings and discards. Biological analysis of flounder (length, weight, sex, maturity stage, age) is carried out for approximately 1000 specimens yearly.

Second part of sampling programme is takes place in two scientific surveys: Baltic International Trawl Survey (BITS) and Latvian Young Fish Survey. Data from BITS are collected twice a year (March and November). Otoliths are sampled from ICES Subdivision 28, according to sampling programme from each haul. From each centimeter length group 5 otoliths are taken.

Data from Young Fish Survey are collected from May to November, usually 5 surveys per year. Surveys take place in Latvian coastal area of the Irben Strait and the Baltic Sea area, close to Latvian-Lithuanian border. On average 50 samples of flounder are collected. From each sample 20 otoliths (from different length groups) are taken.

Table 2.3.1.

Number of collected otoliths in 2005

Collection format	Number
Commercial fishery	884
BITS surveys	284
Young fish survey	1010
Total	2178

Age determination

For flounder age determination *Sagitta* otoliths are used.

Age determination is carried out with binocular *Leica WILD M3Z* at magnification x16 in reflected light. In the first year nucleus, nucleus ring, metamorphosis ring, first year increment and winter zone is formed in otolith. Age is determined by counting winter rings.

2.4. Lithuania

The most abundant flatfish species, in Lithuanian economical zone of the Baltic Sea, is river flounder or flounder (*Platichthys flesus*), while sea flounder is very rare. Flounder is important for Lithuanian fishery, since it makes a greater part of landed fish by

Lithuania fishermen at the Baltic Sea. Usually, fishermen catch flounder as by-catch in cod trawls, flounder trawls as well as gill nets. Flounders can live at different salinities and that is why this species is common in all the Baltic Sea; however, their spread and density depend on some other environmental factors. The landings of the flounder in Lithuania varied during the last thirty years. In 1973 there were 1300 tones landed in the Baltic Sea. The catch of the flounder raised in 1975 and reached maximum (nearly 18000 tones). After 1975 there was period of decline and it lasted for about 15 years. In 1995 landings started to grow up again and reached maximum 18100 t.

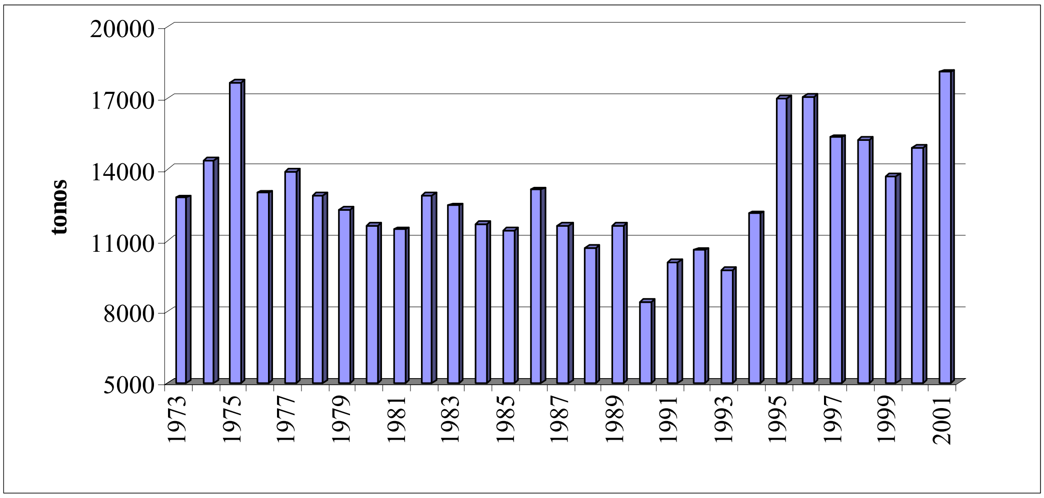

Figure 2.4.1. Lithuanian commercial flounder landings in the Baltic Sea during 1973-2001

In Lithuanian zone of the Baltic Sea, river flounder is one of the most abundant fish species in the fisheries. Commercial landings of the flounder started grow up since 1993 after coastal fishery was started. In 2001-2005 maximum catches were reported during the period 1993-2005 (Figure 2.4.2). Landings increased ten times since 1993 to the 2001-2005 period. It seems that the increase is caused by raised fishing intensity.

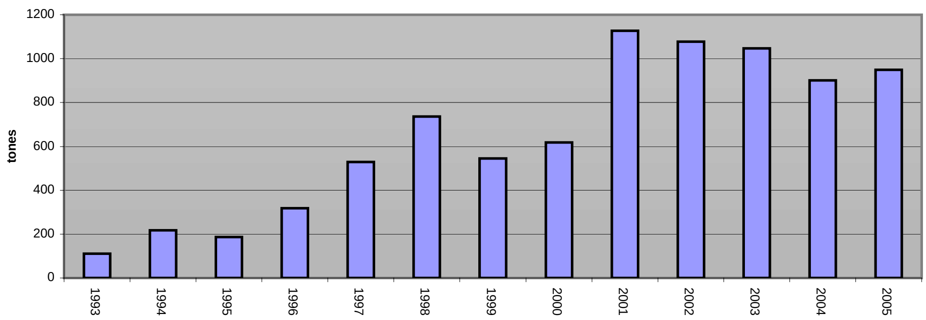

Figure 2.4.2. Commercial flounder landings in the Baltic Sea economical zone of Lithuania during 1993-2005

Scientific surveys demonstrate decreasing trends in CPUE. According to the data of coastal fish monitoring it is concluded that the stock of the river flounder declines (Figure 2.4.3).

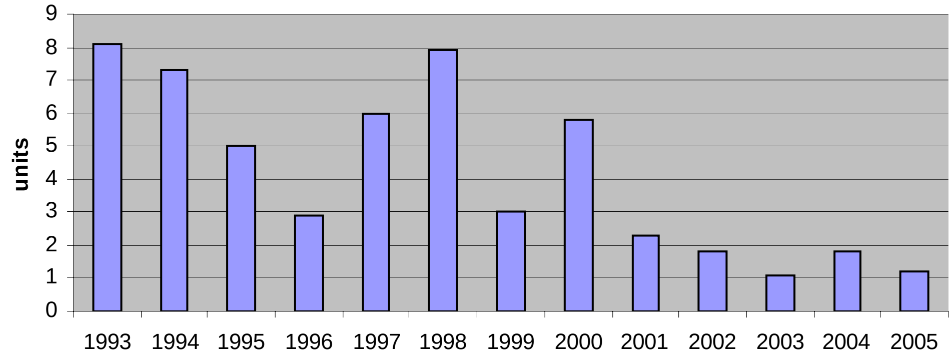

Figure 2.4.3. Shift of flounder abundance (CPUE – catch in numbers per unit effort; 30 m of 17-70 mm nets per 8 hours) according to the monitoring data in the coastal waters of the Baltic Sea, Lithuania, during 1993-2005.

Comparing commercial landings of the river flounder in Lithuanian zone of the Baltic Sea and CPUE during monitoring surveys it is evident that intensive fishery have influenced the abundance of the river flounder stock (Figure 2.4.4.).

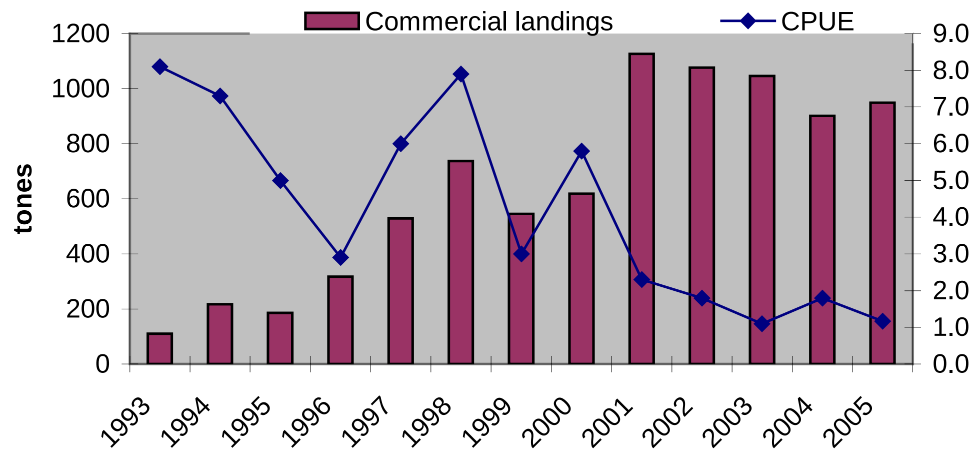

Figure 2.4.4. Commercial flounder landings in the Baltic Sea economical zone of Lithuania and flounder abundance (CPUE) dynamics according to the monitoring data in the coastal waters of the Baltic Sea, Lithuania, during 1993-2005.

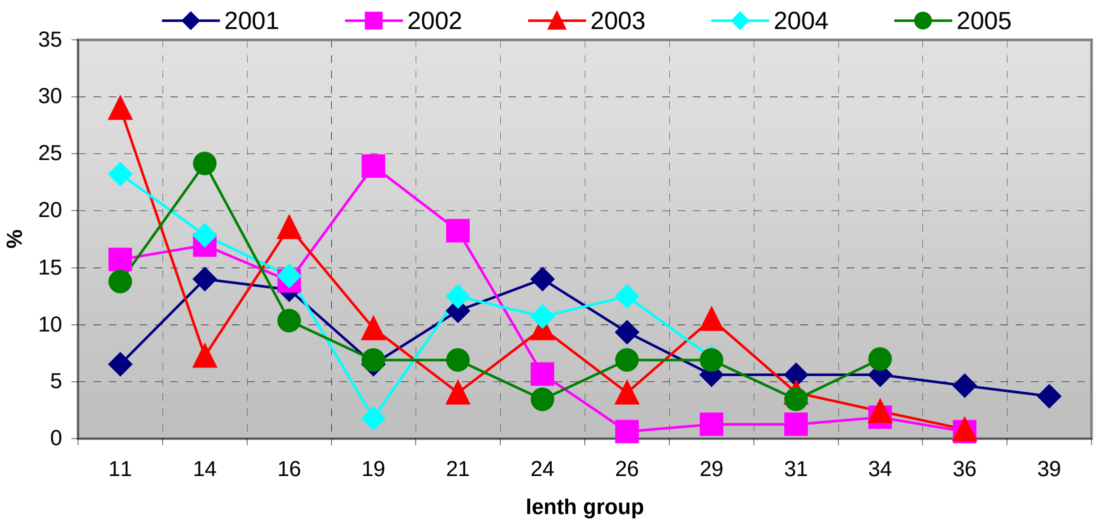

Figure 2.4.5. Flounder size structure in catches obtained during scientific surveys in 2001-2005.

There was investigated the age of the river flounder in 2001-2005. The average age of caught river flounder is about 2+ - 4+ years, while 5+ - 7+ years and older individuals are much less abundant.

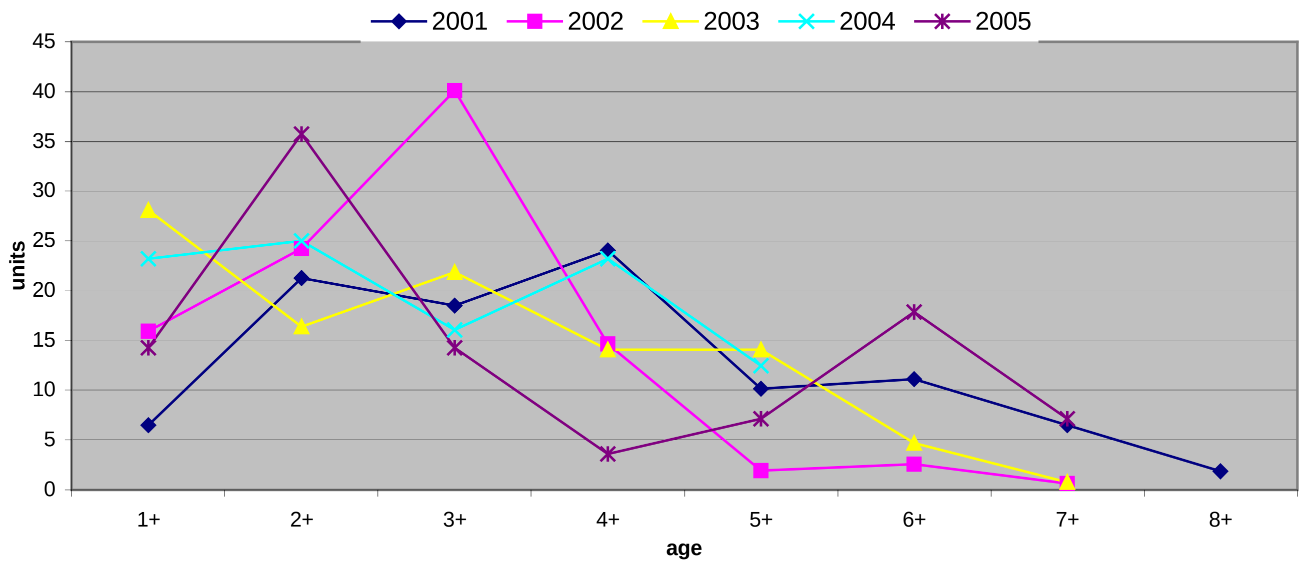

Figure 2.4.6. Age structure of flounders caught during 2001-2005

Surveys data on the flounder juveniles abundance during the recent years demonstrate, that the abundance of young river flounder declines in the Lithuania costal zone of the Baltic Sea. This can be influenced by intensive fishery on most fecund adult flounder.

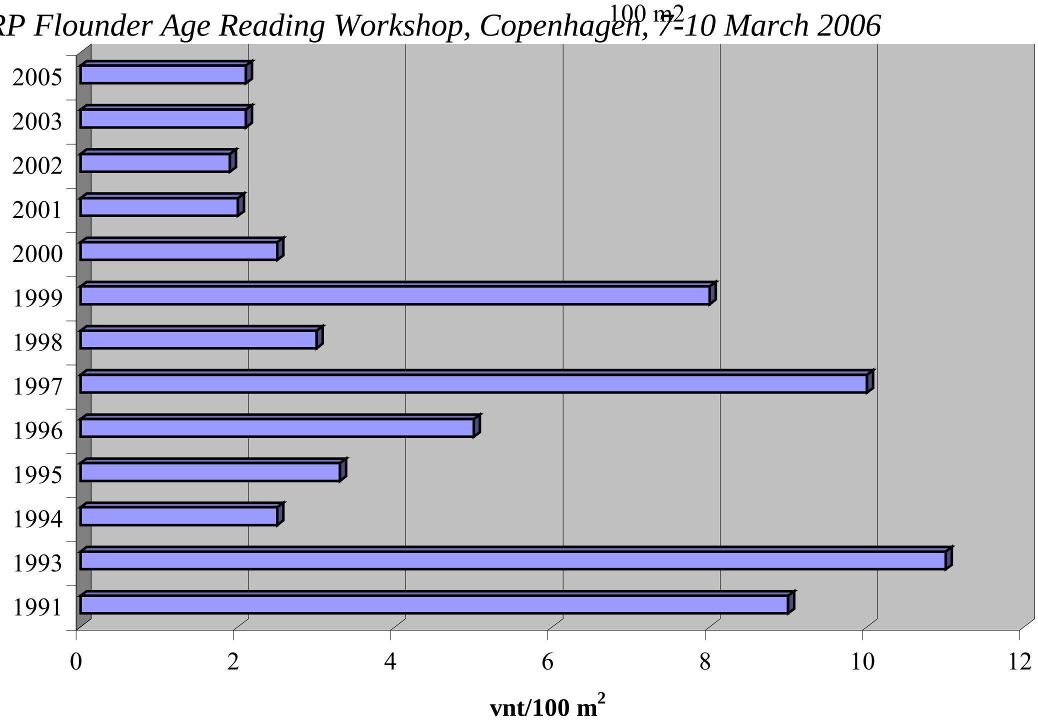

Figure 4.2.6. Flounder juveniles abundance in the coastal waters of the Baltic Sea, Lithuania.

There are at least two different subspecies of river flounder in Baltic Sea: the flounder, that spawns in coastal waters and flounder that spawns in deep water. River flounder is frequent in the Lithuanian zone of the Baltic Sea, and in the northern part of the Curonian lagoon. Flounders inhabit the depth up to 50-60 meters, and prefer sandy bottom. Juveniles of the river flounders live near coast, but the adults prefer deeper water. The biggest known length of the river flounder in the Baltic Sea is about 45 centimeters, and weight 1,2 kg. In the economical zone of the Lithuania the biggest caught flounders were 44-45 centimeters, the weight was 1,1-1,2 kg. The growth is quite slow. The growth is slower in the bays, comparing with growth in the open sea. Flounders mature at the age of 3-4 years, and 16-20 centimeters length. Flounder spawns in March and May, when water temperature reach +2/4 degrees above zero. Usually spawns in the coastal zone, in the bigger distance from the river delta. Prolificacy varies from 0,4 to 2,0 millions eggs. The embryos development lasts about 2-6 days. The juveniles of the river flounders feed on small crustaceans, invertebrate, mollusks and shrimps. Large flounders feed additionally on other fishes, especially smelt juveniles.

Year	Coastal water	Open sea	Total
1995			186,3
1996			316,4
1997			553,9
1998	30,18	706,52	736,70
1999	49,70	495,60	545,30
2000	28,13	573,00	601,13
2001	25,46	1101,28	1126,74
2002	36,31	1040,96	1077,27
2003	22,42	1021,39	1043,81
2004	21,391	833,591	854,982
2005	21,82	927,137	948,955

Table 2.4.1. Commercial landings of the river flounder in the Baltic Sea economical zone of Lithuania (tones)

Sampling is implemented according to the State monitoring. The number of samples is based on this program (100 samples). Each individual is measured for length, weight and aged. The juveniles are caught using beach seine.

Both sagital otoliths are removed, cleaned and stored in Ependorph tubes. Whole otoliths are sunk into the water and are studied using a binocular microscope with reflected light in 16 x magnification.

2.5. Poland

There is a constant sampling programme of flounder dating back to 90`s which covers subdivisions 24, 25 and 26. Nowadays the sampling of flounder is included in the Polish National Programme for Collection of Fisheries Data. Samples are taken mainly from scientific surveys and commercial vessels (Table 2.5.1.).

Sub-division	Quarter	Commercial catches				Research vessel Baltica		
		Catches	No. of samples	No. of flounder		No. of samples	No. of flounder	
				Measured	Analysed		Measured	Analysed
26	I	377	5	614	200	12	1262	96
	II	444	1	172	50			
	III	685	4	625	207			
	IV	394	2	316	107	14	1385	100
	Total	1900	12	1727	564	26	2647	196
24+25	I	2546	7	711	259	28	1636	115
	II	653	1	167	61			
	III	1611	6	620	273			
	IV	1554	9	1041	353		396	85
	Total	6364	23	2539	946	13	2032	200
Total		8264	35	4266	1510	39	4679	396

Table 2.5.1. Materials collected in 2004 for the flounder assessment.

Polish catches of flounder fluctuated from 3.700 tons to 8.919 tons (1991-2004) (Fig.2.5.1)

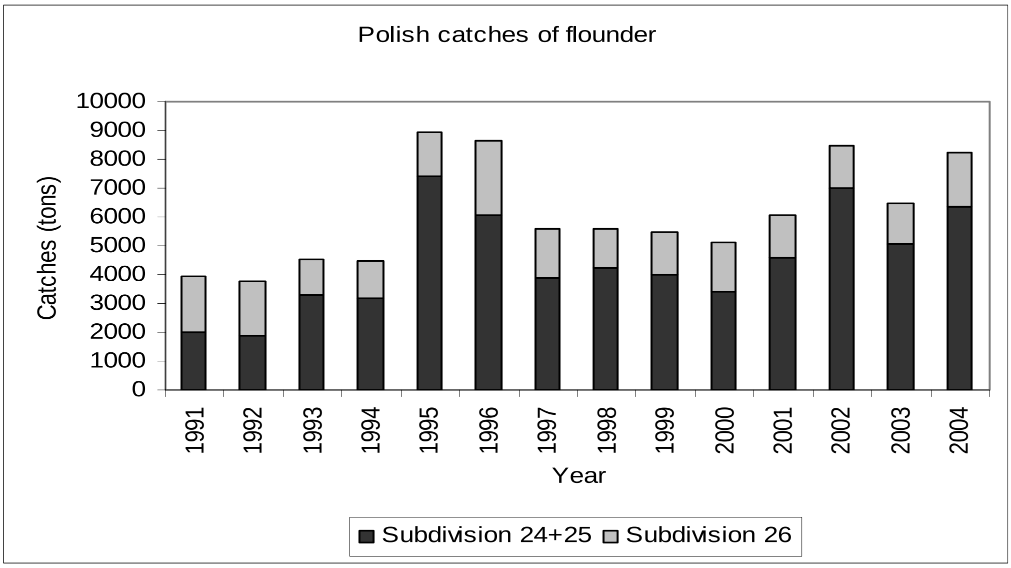

Figure 2.5.1. Polish catches of flounder in Sub-divisions 24-26.

The age group structure of the flounder catches in 2004 ranged from 2 to 10 age classes with dominant 3-5 years of age. (Fig. 2.5.2.).

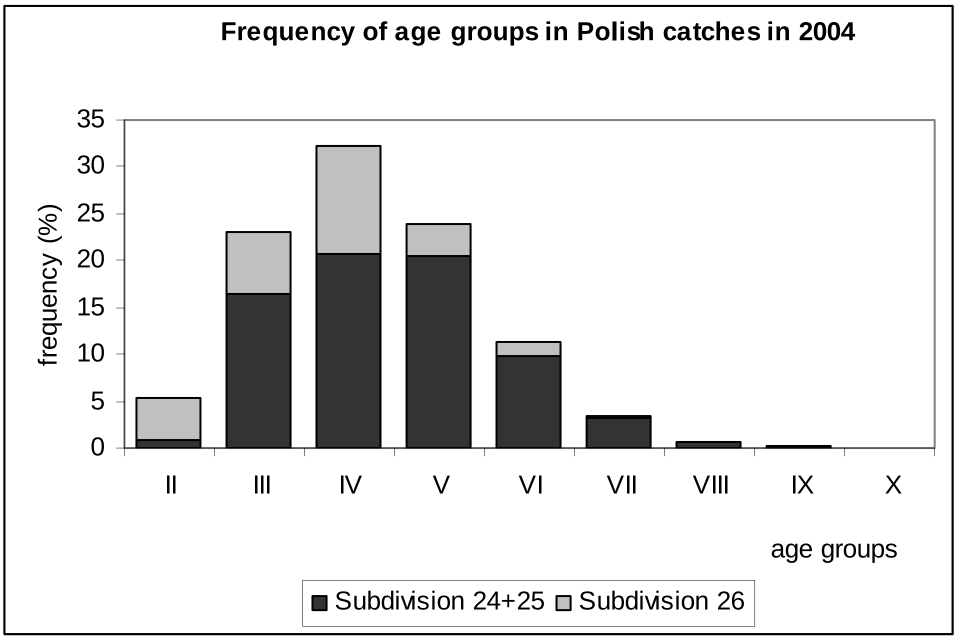

Figure 2.5.2. Age composition of flounder catches in 2004.

After the removal both otoliths are stored in plastic hole plate with 96 holes, filled with water. Otholits are placed horizontally in Petri dish filled with water. The age is analysed using a binocular microscope with reflected light (16x magnification)

The age of flounder is determined by one person all the time (J.Kuczyński). In the investigations implications between idea of local stocks and the formation of otolith structure – annual rings are discounted.

2.6. Sweden

Fishery

Flounder is mainly taken as by-catch in cod fishery and directed fishery by demersal trawls and gillnets. In total 311 tonnes were landed in Swedish fishery in 2005 whereof 95% were caught in the Baltic Sea. Most of the fish is taken in the southern Baltic Sea ICES SD 23-25, but a lot is also taken along the Swedish east coast (SD 27) and east of Gotland (SD 28). Disregarding the period 1996-1998 when other fish were misreported as flounder the catches has been stable during the last decades.

Sampling/Surveys

According to the EU Data Collection program samples are taken from commercial landings and data collected on age, length, weight, sex and maturity stage. For 2006 in total 800 individuals will be sampled during the third quarter of the year from the gillnet fishery in SD 23, 27 and 28. An additional 200 individuals will be sampled during the fourth quarter from the demersal trawl fishery in SD 25. Length samples are taken from the gillnet fishery in SD 23 and 25 during the first and third quarter and from SD 27 and 28 during the third quarter. Each sample consists of at least 200 fishes. From the trawl fishery length samples are taken during the first and fourth quarter in SD 25.

Fishery independent data are collected in BITS trawl survey in March and November in SD 25-28 and in coastal monitoring fishery in the autumn (August- October) using gillnets or trap-nets in SD 23, 27 and 28. Some of this series dates back to the 1960´s but age reading has only been done for a small part of the data. Additional data on length distribution and catch per unit effort in monitoring fishery is collected also in SD 20 and 21. No regular juvenile surveys are done.

Biology

There are two types of spawning behaviour in the Baltic Sea; spawning in the deep areas with pelagic eggs or spawning in shallow areas with demersal eggs. The spawning behaviour is related to the salinity level so that successful pelagic spawning is only possible in salinity of at least about 12 psu while demersal spawning can be successful at least down to 6 psu. The demersal spawners are distributed in the north of the Baltic Sea while the pelagic spawners occur in the south of the Baltic Sea and there is an overlap of the two different strategies in the area around Gotland. Spawning time also differs from north to south so that spawning takes place later in the year in the northern part. Spawning in SD 27 is from April- June while spawning in Skagerrak/Kattegat is from February-April.

Age determination

Otoliths are read whole and are read from sawed and stained samples giving different results (Fig. 2.6.1.), in general the reading from sliced otoliths give higher ages for the older fishes, supposedly because this technique reveal year rings that are hard to detect in older fishes because of the thickness of the otolith and the narrow growth zone of the rings.

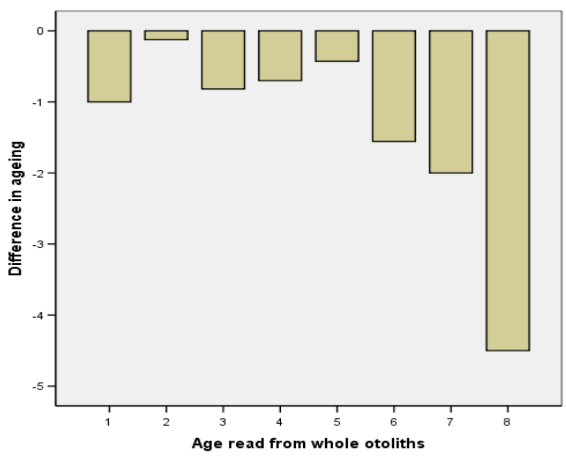

Figure 2.6.1. Mean difference in ageing of whole otoliths compared to cut otoliths. The whole otoliths give a lower age, especially in the older age classes.

The different techniques then results in different age distribution and different age-length relationships with the main conclusion that the cut otoliths shift the age distribution towards older individuals (Fig. 2.6.2.) and the age-length relationship is more of a diminishing curve than a straight line (Fig. 2.6.3.).

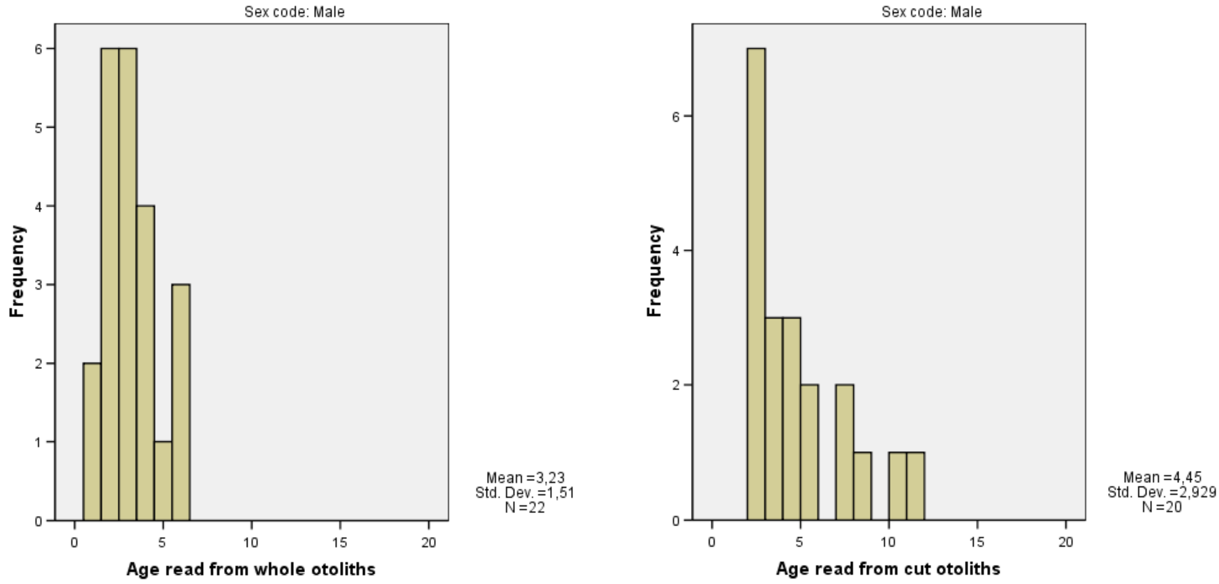

Figure 2.6.2. Age distribution in male flounder from SD 27 sampled with gillnets in October read from whole compared with cut otoliths.

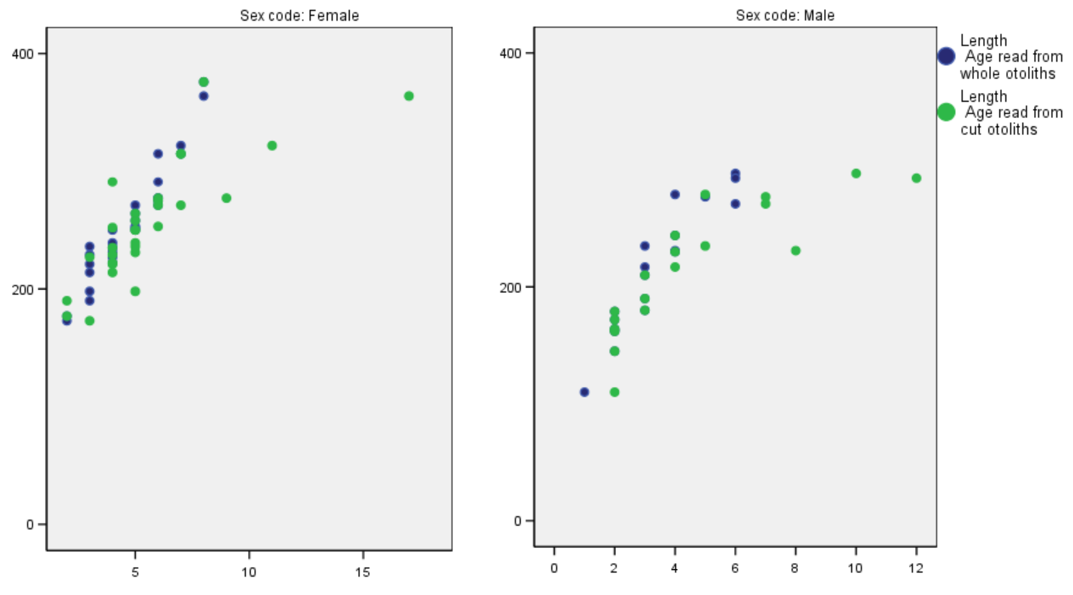

Figure 2.6.3. Age-length relationships in flounder from SD 27 sampled with gillnets in October read from whole compared with cut otoliths.

Using the cutting/staining technique flounder sampled from the commercial gillnet fishery in October in northern Öland (SD 27) was analysed during two consecutive years and there seems to be a possibility to follow cohorts; the dominating cohort was 7 years old in 2004 and, as expected, 8 years old in 2005 (Figure 2.6.4.).

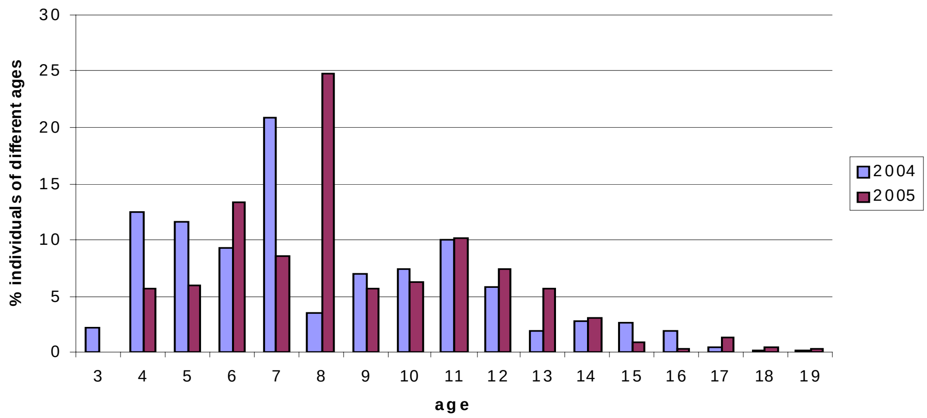

Figure 2.6.4. Age distribution of flounder sampled from commercial gillnet fishery in SD 27 in two consecutive years.

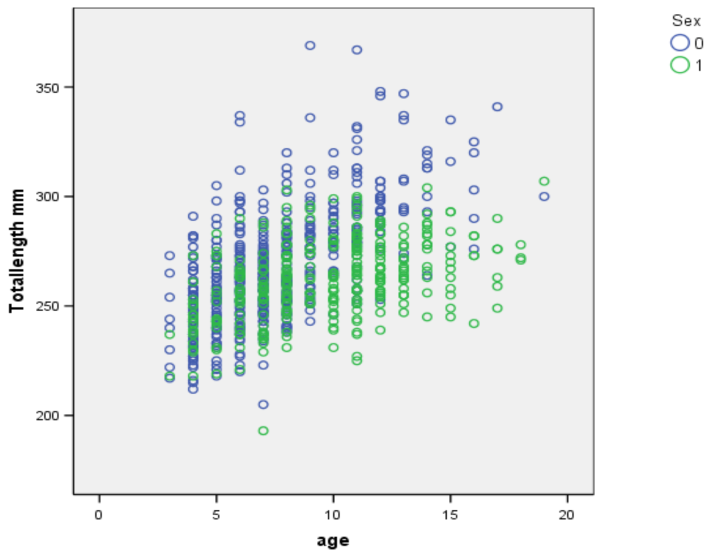

Figure 2.6.5. Flounder age/length relationship in SD 27.

3 Genetic differentiation in flounder in the Baltic Sea

In spring and early summer in 2003 tissue samples from adult flounders were collected through out the Baltic Sea with additional samples from Skaggerak, Kattegat and the North Sea. 12 of these samples have hitherto been analysed using microsatellite technique. The results show that there is an isolation-by-distance pattern, i.e. samples that are more spatially separated are also more genetically differentiated (Figure 3.1.). The most striking difference occurs between the sample from SD 25 (Bornholm basin, point 7 in Fig. 3.1) and 26 (central part of eastern Gotland basin, point 6 in Fig. 3.1), suggesting a division of flounder stocks into an eastern and western part.

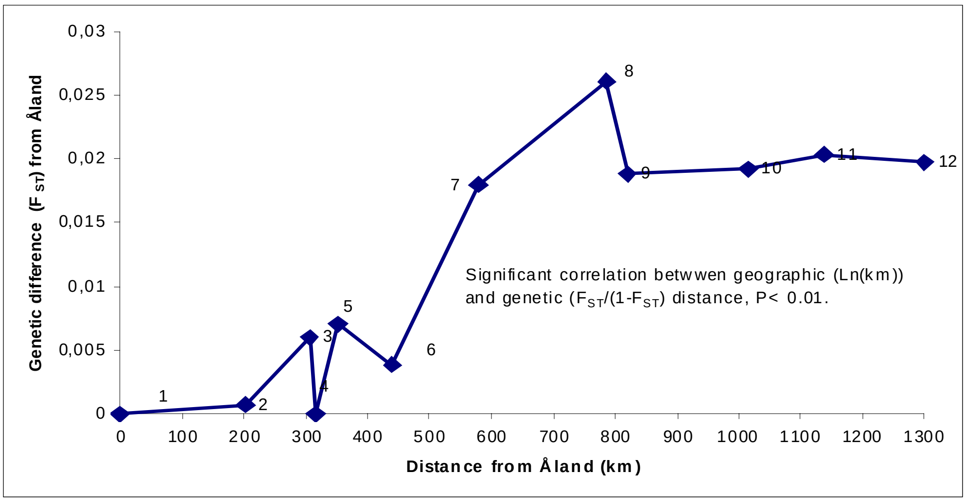

Figure 3.1. Relationship between geographic and genetic distance in flounder samples from: 1)Åland Sea, 2)Northern Gotland basin, 3)Western Gotland basin, 4)Gulf of Finland, 5)west part of Eastern Gotland basin, 6) central part of Eastern Gotland basin , 7) Bornholm basin, 8) Arcona basin, 9)Oresund straits, 10) Kattegat, 11) Skagerrak and 12) North Sea.

Another way to graphically view the genetic differentiation is to use a multi-dimensional scaling plot. An MDS plot of the flounder samples reveal that flounder genetically cluster in two groups in the Baltic– one in the north-eastern Baltic and one in the south-western Baltic. Also the Skagerrak/Kattegat/ North Sea sample are distinct from the others (Fig. 3.2.).

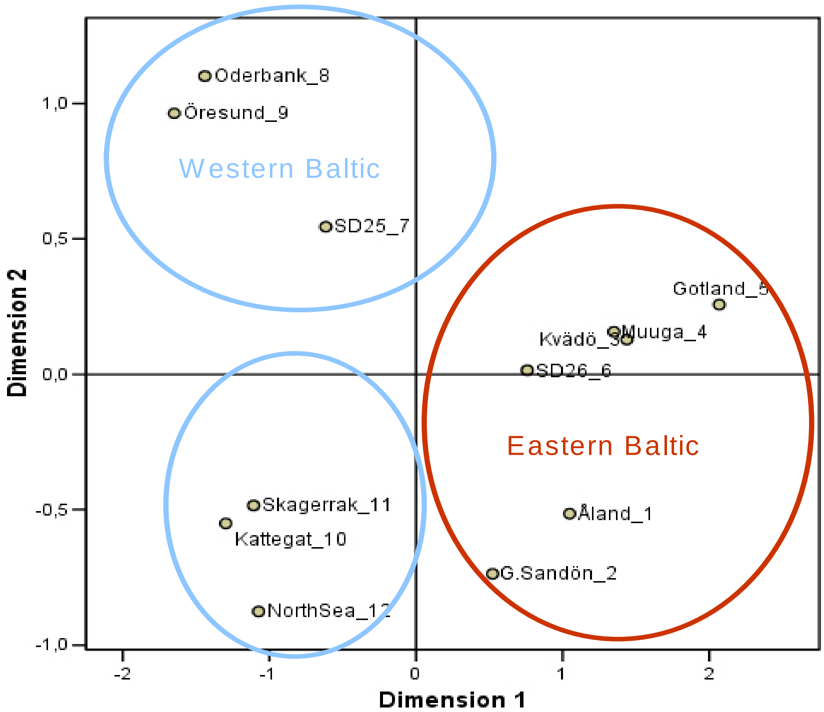

Figure 3.2. MDS plot of Nei's genetic distance. The first dimension explains 85% of the variance, while the second explains an additional 6%. This means that the difference between the eastern and western Baltic is much greater than the difference between the North Sea cluster and the western Baltic.

The biological reason for this subdivision of flounders could be that the different types of spawning behaviour- demersal or pelagic, can have resulted in genetic differentiation of stocks. Several flounder samples collected in Latvia and Poland will be analysed in the near future.

4 Age determination of flounder at the Workshop

4.1 Common age determination on the screen

Common age determination of flounder was performed viewing flounder otoliths on the screen from German and Latvian samples prepared for the Workshop. The age determination and joint discussion revealed several problems. It was considered that flounder otoliths have one or even two false rings before the first true winter ring. One of these false rings is regarded as metamorphosis ring. Distinction between metamorphosis ring and first winter ring was often dissimilar. It was also caused by the fact that the first visible ring had different size and form. In general it was agreed that the first winter ring should have form similar to the outside contour of the otoliths while false rings have form more close to ring or ellipse. The second problem was caused by the allocation of the last opaque zone to the growth of the previous or of the current year. It was caused by a lack of determined knowledge on the main growth season of flounder, when the summer increment of opaque zone appears on the otoliths. The current way of otolith collection in many countries are not able to reveal this phenomenon. Some of the otolith samples revealed rather surprising slow growth of flounder at age 1 that could be also connected with wrong interpretation of the age. It was also stated that age determination of older flounder is complicated due to thickening of the otoliths that burdens the distinction of winter rings. One common problem for most of the national laboratories was the absence of experienced flounder age readers and lack of international cooperation. Therefore the age determination of flounder is based on national school or even individual mastering.

To reveal the differences in age determination of flounder as well as to compare different methods it was decided to perform individual age determination of flounder otoliths. Flounder otoliths samples from Germany, Latvia and Sweden were available for age determination at the Workshop. Besides the Swedish otoliths were coupled by stained slices of the same otoliths and the age determination was accomplished also from the slices. It total the age determination was carried out for 68 otoliths from German sample, 32 otoliths from Latvian sample and 50 otolith slices and 26 accompanying whole otoliths from the Swedish sample. The results of age determination are presented in the following chapters.

The following methods were used for comparison of age determination results. The general agreement between readers was calculated as percentage of identically determined ages and inter-reader bias was examined using the Wilcoxon signed ranks test (Anon 1994). The results of agreement and inter-reader bias tests are presented separately for each national sample. Besides for the determined ages the modal age was defined which was considered as a true age. All age determinations were sorted in age groups in relation to modal age and inside each age for each reader the agreement with modal age, the standard deviation and coefficient of variation were calculated.

4.2. German sample

The German sample of 68 otoliths consisted of two parts collected in February and July. The results are shown in Tables 4.2.1. and 4.2.2. In general according to age determination the sample included relatively young flounder mainly belonging to age groups 1-4. The difference of average determined age varied from 2.24 till 3.24. The average percentage of identically determined age was 40.6%. The agreement with the modal age decreased with the age of the flounder but in general was rather low also for young flounder of age 1 – only 67%.

Table 4.2.1. Inter-reader bias test – Wilcoxon test (upper part) and the percentage of identically determined age between the readers of the German otoliths sample. Wilcoxon test: - agreement (28.6%), * possible disagreement (3.6%), ** disagreement (67.8%)

	1	2	3	4	5	6	7	8
1		-	**	-	**	-	**	**
2	52.2		**	*	**	-	**	**
3	36.8	34.3		**	**	**	**	**
4	49.3	52.9	41.8		**	-	**	**
5	29.9	13.2	56.7	33.8		**	-	-
6	39.4	41.8	31.8	35.8	25.4		**	**
7	20.9	10.3	61.2	30.9	70.6	22.4		-
8	34.3	16.2	64.2	39.7	91.2	22.4	76.5	
Average	37.5	31.6	46.7	40.6	45.8	31.3	41.8	49.2

Table 4.2.2. Comparison of the modal age with the age readings of the German otolith sample

Modal Age	Number	Parameters	Reader								Ave-rage
			1	2	3	4	5	6	7	8	
1	14	Agreement	57.1	35.7	64.3	85.7	92.9	14.3	85.7	100.0	67.0
		Stand.dev.	0.5	0.6	0.5	0.6	0.3	0.9	0.4	0.0	
		Coeff.of var.	35.9	35.7	36.6	47.7	28.8	36.5	39.2	0.0	32.5
2	18	Agreement	41.2	17.6	88.2	52.9	82.4	23.5	76.5	88.2	58.8
		Stand.dev.	0.8	0.7	0.3	0.7	0.4	1.0	0.4	0.3	
		Coeff.of var.	28.7	21.5	15.7	27.5	21.5	38.8	24.8	17.6	24.5
3	20	Agreement	65.0	55.0	80.0	60.0	55.0	60.0	40.0	70.0	60.6
		Stand.dev.	1.2	0.6	0.4	0.6	0.5	0.7	0.6	0.5	
		Coeff.of var.	33.0	17.3	15.4	17.5	20.0	23.7	24.3	17.4	21.1
4	14	Agreement	69.2	57.1	61.5	71.4	21.4	57.1	42.9	21.4	50.3
		Stand.dev.	1.0	0.5	0.7	0.6	0.8	0.9	0.5	0.5	
		Coeff.of var.	22.6	11.4	18.7	14.5	24.9	22.9	15.0	17.0	18.4
Average (age 1-4)		Agreement	58.1	41.4	73.5	67.5	62.9	38.7	61.3	69.9	59.2

4.3 Latvian sample

Latvian flounder otolith sample consisted of 32 otoliths collected in different seasons of the year. The results are shown in Tables 4.3.1. and 4.3.2. According to age determination the sample compared to German sample included older flounder mainly belonging to age groups 2-6. The difference of average determined age varied from 3.0 till 6.03. The average percentage of identically determined age was only 22.9%. The agreement with the modal age decreased with the age of the flounder and was below 50% for all age groups. The age determination results of the Latvian sample were the worst at the Workshop that is certainly connected with the older age of flounder and smaller annual zones of a slower growing fishes.

Table 4.3.1. Inter-reader bias test – Wilcoxon test (upper part) and the percentage of identically determined age between the readers of the Latvian otoliths sample. Wilcoxon test: - agreement (25%), * possible disagreement (3.6%), ** disagreement (71.4%)

	1	2	3	4	5	6	7	8
1		**	-	-	**	-	**	**
2	15.6		**	**	**	*	**	**
3	25.0	31.3		-	**	**	**	**
4	34.4	18.8	50.0		**	**	**	**
5	25.0	0.0	3.1	8.4		**	-	-
6	21.9	40.6	37.5	40.6	0.0		**	**
7	34.4	0.0	9.4	12.5	62.5	3.1		-
8	21.9	0.0	6.3	6.3	78.1	0.0	56.3	
Average	25.5	15.2	23.2	24.4	24.4	20.5	25.5	24.1

Table 4.3.2. Comparison of the modal age with the age readings of the Latvian otoliths sample

Modal Age	Number	Parameters	Reader								Ave-rage
			1	2	3	4	5	6	7	8	
2	3	Agreement	100.0	0.0	0.0	0.0	100.0	0.0	100.0	100.0	50.0
		Stand.dev.	0.0	0.6	0.6	0.0	0.0	0.0	0.0	0.0	
		Coeff.of var.	0.0	17.3	17.3	0.0	0.0	0.0	0.0	0.0	4.3
3	8	Agreement	62.5	0.0	37.5	75.0	50.0	25.0	75.0	37.5	45.3
		Stand.dev.	0.9	1.4	1.1	0.7	0.7	1.5	0.5	0.7	
		Coeff.of var.	27.3	28.3	27.3	22.0	31.3	31.3	16.8	31.4	27.0
4	5	Agreement	40.0	0.0	40.0	0.0	60.0	20.0	60.0	80.0	37.5
		Stand.dev.	3.1	1.7	1.7	1.1	0.5	1.6	0.5	0.4	
		Coeff.of var.	45.8	25.4	29.9	21.1	15.2	26.4	15.2	11.8	23.8
5	6	Agreement	66.7	33.3	66.7	50.0	16.7	50.0	0.0	0.0	35.4
		Stand.dev.	0.6	1.5	0.5	1.4	1.2	0.8	1.0	0.9	
		Coeff.of var.	12.6	25.2	11.1	25.1	36.9	14.4	34.7	29.8	23.7
6	6	Agreement	33.3	16.7	83.3	83.3	0.0	33.3	16.7	16.7	35.4
		Stand.dev.	1.8	1.5	0.4	0.4	0.5	1.3	1.5	1.2	
		Coeff.of var.	26.3	18.0	7.0	6.6	15.6	19.5	41.1	28.1	20.3
Average (age 2-6)		Agreement	60.5	10.0	45.5	41.7	45.3	25.7	50.3	46.8	40.7

4.4 Swedish otolith sample

The Swedish otolith sample consisted of 28 otoliths collected in October which were coupled with part of the stained otolith slices. The results are shown in Tables 4.4.1. and 4.4.2. According to age determination the sample compared to German sample included older flounder mainly belonging to age groups 2-5. The difference of average determined age varied from 3.42 till 5.27. However, for few otoliths with modal age of 6 and more the difference between individual determinations reached 8. The average percentage of identically determined age was only 29.7%. The agreement with the modal age decreased with the age of the flounder. It was 75% for age 2 and decreased to 40.5% for age group 5.

Table 4.4.1. Inter-reader bias test – Wilcoxon test (upper part) and the percentage of identically determined age between the readers of the Swedish otoliths sample. Wilcoxon test: - agreement (33.3%), * possible disagreement (9.5%), ** disagreement (57.1%)

Reader	2	3	4	5	6	7	8
2		*	-	**	-	**	**
3	19.2		-	**	*	**	**
4	8.0	48.0		**	-	**	**
5	3.8	26.9	36.0		**	-	-
6	38.5	34.6	28.0	7.7		**	**
7	7.7	34.6	44.0	38.5	15.4		-
8	19.2	34.6	44.0	80.8	7.7	46.2	
Average	16.1	33.0	34.7	32.3	22.0	31.1	38.8

Table 4.4.2. Comparison of the modal age with the age readings of the Swedish otoliths sample

Modal Age	Number	Parameters	Reader								Average
			1	2	3	4	5	6	7	8	
2	4	Agreement	0.0	100.0	100.0	100.0	25.0	100.0	100.0	75.0	75.0
		Stand.dev.	0.0	0.0	0.0	0.0	0.8	0.0	0.0		
		Coeff.of var.	0.0	0.0	0.0	0.0	27.2	0.0	0.0	3.9	3.9
3	3	Agreement	33.3	0.0	100.0	66.7	33.3	100.0	66.7	57.1	57.1
		Stand.dev.	1.0	1.2	0.0	0.6	1.5	0.0	0.6		
		Coeff.of var.	25.0	34.6	0.0	21.7	32.7	0.0	21.7	19.4	19.4
4	6	Agreement	33.3	66.7	60.0	50.0	33.3	50.0	66.7	51.4	51.4
		Stand.dev.	1.5	0.8	1.1	0.8	1.9	0.5	0.5		
		Coeff.of var.	28.2	18.6	26.1	24.5	34.0	15.6	14.1	23.0	23.0
5	6	Agreement	66.7	33.3	66.7	16.7	33.3	33.3	33.3	40.5	40.5
		Stand.dev.	0.8	1.4	0.8	0.8	1.4	0.8	0.9		
		Coeff.of var.	15.2	28.3	15.2	19.6	24.1	18.1	22.4	20.4	20.4
Average (age 2-5)		Agreement	33.3	50.0	81.7	58.4	31.2	70.8	66.7	56.0	56.0

4.5 Swedish sample of stained otolith slices

This Swedish sample consisted of 50 stained otolith slices part of which was coupled with Swedish otolith sample. The results are shown in Tables 4.5.1. and 4.5.2. According to age determination the sample compared to German sample included older flounder mainly belonging to age groups 2-7. The average determined age varied from 3.92 till 5.47 and the difference between extreme values was slightly lower than in the Swedish otolith sample. The average percentage of identically determined age was only 31.9%. The agreement with the modal age decreased with the age of the flounder. However, the agreement was rather low for the younger age groups – only 59.4% for age group 2. It could be connected with problems in distinction between metamorphosis ring and first winter ring. It should be noticed that only one expert had experience of flounder age determination from otolith slices. Therefore the obtained results should be regarded with care. In general it could be stated that difference between individual readings was lower than in otolith samples.

Table 4.5.1. Inter-reader bias test – Wilcoxon test (upper part) and the percentage of identically determined age between the readers of the Swedish sample of stained otoliths slices. Wilcoxon test: - agreement (21.4%), * possible disagreement (14.3%), ** disagreement (64.3%)

Reader	1	2	3	4	5	6	7	8
1		*	-	*	**	-	**	**
2	46.0		-	**	**	**	**	**
3	46.0	54.0		**	**	*	**	**
4	24.0	16.0	24.0		-	-	**	**
5	32.0	10.0	14.0	54.0		**	*	**
6	38.0	16.0	18.0	52.0	44.0		**	**
7	28.0	8.0	16.0	40.0	40.0	44.0		-
8	22.0	6.0	6.0	42.0	56.0	44.0	62.0	
Average	33.7	22.3	25.4	33.3	35.7	36.6	34.0	34.0

Table 4.5.2. Comparison of the modal age with the age readings of the Swedish sample of stained otolith slices

Modal Age	Number	Parameters	Reader								Average
			1	2	3	4	5	6	7	8	
2	8	Agreement	75.0	25.0	0.0	75.0	62.5	75.0	75.0	87.5	59.4
		Stand.dev.	0.5	0.6	0.7	0.5	0.5	0.7	0.5	0.4	
		Coeff.of var.	20.6	22.3	20.5	26.7	21.8	31.3	26.5	18.9	23.6
3	7	Agreement	42.9	42.9	28.6	85.7	71.4	57.1	71.4	57.1	57.1
		Stand.dev.	0.8	0.8	0.7	0.4	0.5	0.7	0.5	0.5	
		Coeff.of var.	23.0	20.4	17.9	12.0	18.0	24.2	18.0	20.8	19.3
4	11	Agreement	54.5	18.2	27.3	63.6	81.8	72.7	36.4	54.5	51.1
		Stand.dev.	0.5	0.6	0.5	0.6	0.4	0.5	0.5	0.5	
		Coeff.of var.	11.7	12.6	9.9	15.8	10.6	13.2	15.0	14.7	12.9
5	8	Agreement	50.0	37.5	62.5	62.5	50.0	62.5	25.0	25.0	46.9
		Stand.dev.	0.7	0.9	0.8	0.9	0.5	0.9	1.2	0.8	
		Coeff.of var.	13.5	15.4	13.7	16.9	11.9	18.5	26.6	18.9	16.9
6	5	Agreement	20.0	0.0	40.0	40.0	40.0	40.0	80.0	60.0	40.0
		Stand.dev.	0.7	0.4	0.8	1.6	0.5	1.3	0.4	0.5	
		Coeff.of var.	10.1	6.2	12.3	24.2	10.1	18.1	7.7	9.8	12.3
7	3	Agreement	66.7	100.0	100.0	33.3	0.0	33.3	33.3	0.0	45.8
		Stand.dev.	1.2	0.0	0.0	0.6	0.0	0.6	1.5	1.0	
		Coeff.of var.	18.2	0.0	0.0	9.1	0.0	9.1	28.6	20.0	10.6
Average (age 2-7)		Agreement	51.5	37.3	43.1	60.0	51.0	56.8	53.5	47.4	50.1

4.6 Comparison of age determination from slices and otoliths

Since the second whole otolith from the otolith pair was available for 26 flounder otolith slices of the Swedish sample it was possible to compare the age determination from whole motoliths and otolith slices. The difference in age determination from whole otoliths and otolith slices was substantial and the average percentage of identically determined age was only 18.7%. The comparison did not revealed any definite pattern (Table 4.6.1), although it is considered that age determination from slices usually allows more precise age determination of older fishes thus on average giving higher ages than age determination from whole otoliths. Probably the obtained results are caused by lack of experience in work with otolith slices of the most of the readers as well as with the small number of the sample. However, the further observation of otolith slices on the screan revealed much easier agreement on age in comparison with whole otoliths.

Table 4.6.1. Number of differences in age determination of flounder from whole otoliths and the same otolith slices.

	Reader						
Difference	2	3	4	5	6	7	8
-5			1		2		
-4	1	2	3		3		
-3	3	1	2			1	1
-2	3	3		2	5	4	2
-1	5	3	3	4	3	6	5
0	6	2	7	4	4	4	7
1	2	6	2	8	2	2	5
2	2	3	3	2	4	6	4
3	2	3	2	2	1	1	
4		1	1	2		1	
5	3	2	2	2	2	1	2

4.7 General results of the age determination exercise

The results of comparison of flounder age determination revealed significant differences between age readers. Thus according to the Wilcoxon signed rank test the disagreement between all individual readings of the readers was stated from 57.1% in the Swedish otolith sample till 71.4% in the Latvian otolith sample (on average for all the samples in 65.1% of the cases). The percentage of identically determined age was on average from 22.9% in the Latvian sample till 40.6% in the German sample (on average 31.3% for all the samples). The agreement between readers decreased with the age of the flounder in the samples, however also for the youngest ages the agreement was rather low. The average determined age of readers differed significantly, and for the Latvian otolith sample the difference was as high as 3 years (Figure 4.7.1). It is obvious that readers 1, 2 and 6 give higher age than readers 5, 7 and 8. In samples with lower average age the percentage of identically determined age was higher (German sample) and seemingly there is more agreement between the readers determining the age of flounder from south-western Baltic in comparison with more slowly growing flounder from the Eastern Baltic (Latvian sample). It should be noticed once more that most of the age readers have little experience in age determination of flounder.

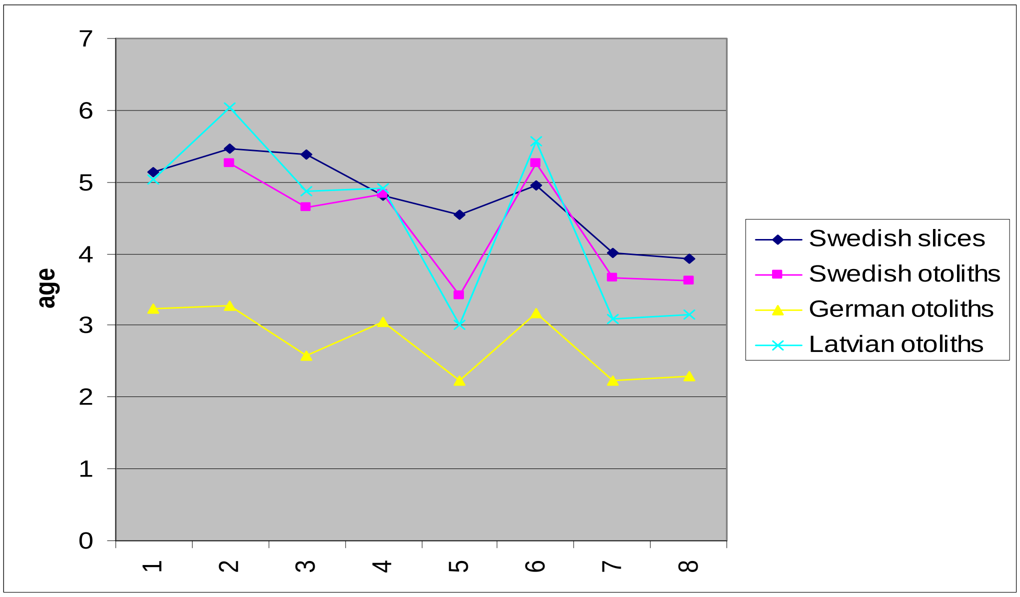

Figure 4.7.1. Average determined age by reader and sample.

4.8. Discussion of age determination results

The age deremination results of the otolith and otolith slices samples were presented to the participants and discussed. The otoliths and otolith slices were viewed on the screan and participants tried to explain why they have assigned the certain age. In general it could be concluded that the discussion confirmed the problems in age determination from otolith described in section 4.1. The age determination from otolith slices showed better concordance. Obviously the high differences in age determination exercise were caused by lack of experience in age determination from otolith slices. However, the winter zones were much better visible and countable than on whole otoliths. In some cases it was problematic to decide whether the first visible ring was the first winter ring or the metamorphosis ring. It could also be caused by wrong positioning of otolith during slicing. The fact that age determination from slices allows to follow year-class cohorts (see Section 2.6) that often is a problem in many national data sets, points on perspective use of flounder age determination from otolith slices. At least it could be a collateral method jointly with age determination from otoliths. It could be especially helpful for unexperienced readers, in age determination of difficult otoliths often belonging to older fishes. Unfortunately at present not all national laboratories have the necessary equipment for preparation of otolith slices, but this problem could be solved at least at present by cooperation between national institutes.

5 Future cooperation in flounder age determination

The results of age determination exercise revealed large differences between readers and substantial problems in interpretation of the visible otolith structure. There is also a lack of precise knowledge on biology of flounder that could be useful for age determination. A common problem for most of the national institutes is that flounder age is determined by unexperienced readers. Therefore it was concluded that cooperation between national institutes in this field is very necessary. The participants of the workshop consider that it is necessary to start the exchange of otolith samples that would be prepared in national institutes. It would be also desirable that both samples of otoliths and otolith slices are used in the exchange. The otolith slices will be prepared in the Swedish Institute of

Coastal Research. It was decided that samples will be prepared during the next 2-3 months and the exchange could be started. The age reading results will be sent to Latvian Fish Resources Agency. It was also proposed that sample exchange is followed by a Workshop that could be held in Swedish Institute of Coastal Research. The possible timing for the Workshop could be the second half of 2007 when the sample exchange could be completed.

References

ICES. 1994. Report of the Workshop on Sampling Strategies for Age and Maturity. ICES CM 1994/D:1.