Baltic cod otolith collection protocol
and
age determination method

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The biological data of Baltic cod are gathered during scientific survey (BITS - Baltic International trawl Surveys) or from commercial ships during trawl fishing. Up until 2018 the data was gathered from commercial trawl and gill-net fisheries. Biological analysis includes data like:

- Maximum length of the fish in cm
- Total weight of the fish
- Gutted weight (from scientific surveys)
- Sex (male – 1; female – 2)
- Gonadal maturity stage (according to a 7-stage scale). The 7-stage scale is a supplemented 6-stage scale. The descriptions of the stages are listed in the table below (Table 1.)

Table 1. The comparison of two Baltic cod maturity assessment scales

Gonadal state	7 stage scale (Nikolsky,1944, Powles,1958)	8 stage scale (Maier, 1908/Berner, 1960)
Immature	II	I,II
Maturing	III	III
Mature	IV	IV,V
Ripe and running	V	VI
Spent	VI	VII
Deformed	VII	VIII

- Berner, M., 1960. Untersuchungen uber den Dorschbestand der Bornholm- und Arkonasee 1953-1955. Z. Fischerei Hilfswiss. 9:481-602.
- Nikolsky G.V. 1944. Biology of fishes (in Russian).
- Powles, P.M. 1958. Studies of reproduction and feeding of Atlantic cod (Gadus callarias L.) in the south-western Gulf of ST. Lawrence. J. Fish. Res. Bd. Canada, 15(6)

From each acquired fish two otoliths (*sagitta*) are removed and wrapped in a paper fish scale-book. On each book information about the date, place, vessel is written. On each page there is the number of the fish and biological information (length, weight, sex, gonadal maturity stage) written down. In the laboratory all the information is transferred to biological analysis sheet and then transferred onto a computer.

Otolith placement in the fish skull:

In the inner ear of cod there are 3 chambers with fish ear bones – otoliths. In each of the chambers there is a pair of otoliths: lapilli (single-lapillus), *asterisci* (single - *asteriscus*) and *sagittae* (single -sagitta). The biggest of the otolith pairs are the *sagittae* otoliths which are also the ones being use for age determination. The placement of otoliths in the inner ear can be observed in Figure 1.

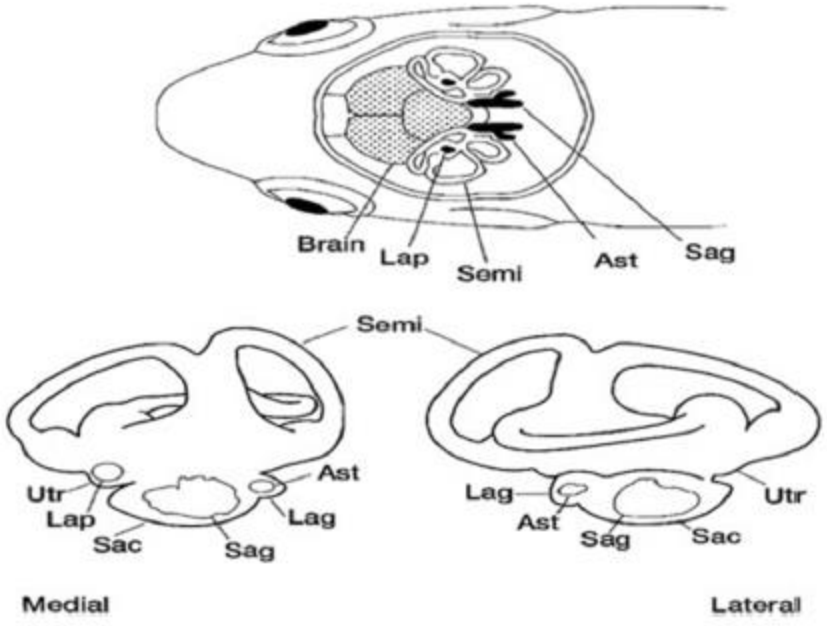

Figure 1. The placement of otoliths inside a head of a fish

Otolith extraction from fish skull and their preparation for age determination

Otoliths are extracted from fresh and not frozen fish straight after the trawled haul in the laboratory of the research vessel during biological analysis. On a commercial vessel extracted during the catch sorting process.

Otolith extraction method:

The fish is placed on the measuring board with the head to the right side and make a cut behind the eyes slantwise towards the nose of the fish. Break open the cut and with tweezers remove the otoliths. The otoliths are in a clotted mass longitudinally inclined with the curved surfaces inwards (Figure 2.)

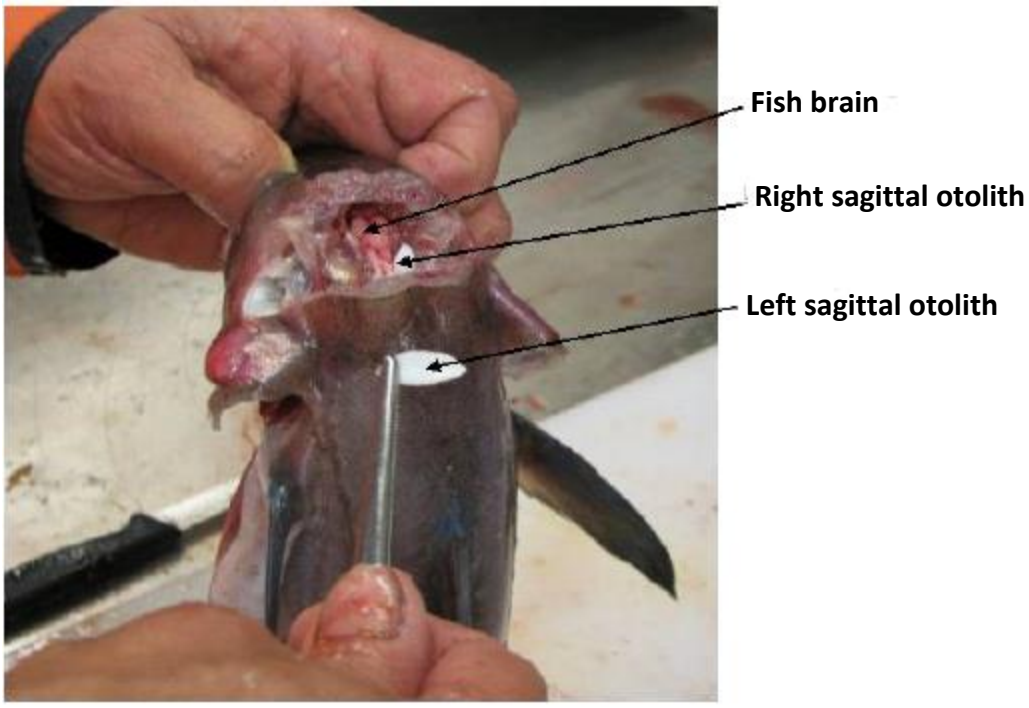

Figure 2. Otolith removal from the head of the cod

The removed sagittal otoliths get cleaned from tissue remains and are then wrapped in a paper book. In the laboratory prior to age determination the otoliths get washed, clean and weighed on “Kern” precision scales (precision to 1 mg). Afterwards, each otolith is cracked into two halves as shown in Figure 4. The cracking point should be on the curvature point of the *sulcus acusticus.* The two broken halves are placed in plastic plates that have multiple rows with 12 wells in each row. One plate can hold 72 otolith pairs (Figure 3).

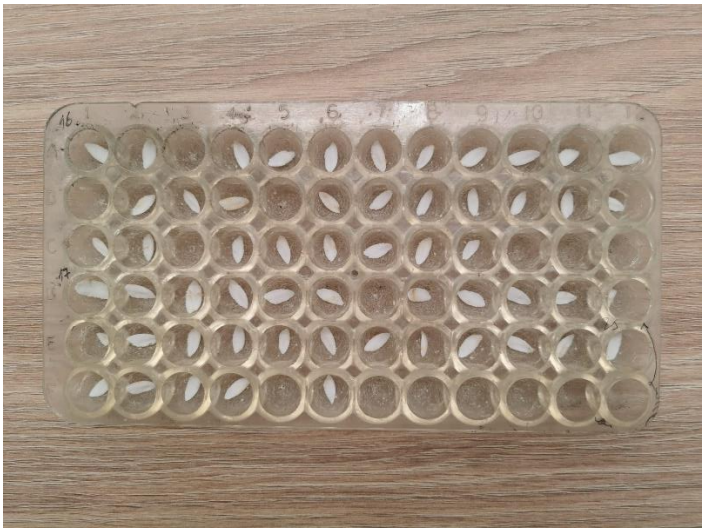

Figure 3. Plastic plate with wells for otoliths.

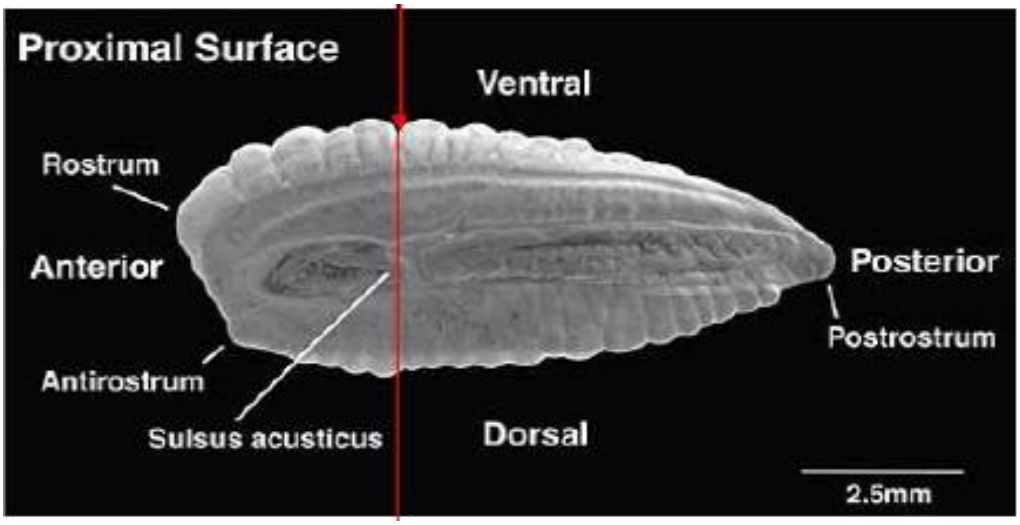

Figure 4. The external structure of a cod otolith and the otolith cracking line for age determination (marked in red).

Otolith burning:

For better visibility of the hyaline zones on the cracked otolith surface, the cracked surface of the otolith halves is burned over a flame from a spirit lamp for 4-18 seconds depending on otolith size. The otolith color after burning should be golden/coffee brown.

Age determination method

Age determination equipment:

Equipment: age determination of cod is carried out using a binocular (Motic SMZ-171 or LEICA S6Z). Usually a magnification of x16 is used. Otoliths are inserted into plastic with sharp ends, the surface is moistened with a water/alcohol mixture and viewed in reflected light. Motic MLC-150C or Leica GLS 100X illuminators are used for lighting.

Cod otolith internal structure:

During the year, cod otoliths form zones with different optical densities. During the growth of the fish, optically dense so-called "opaque" zones form; when the fish's growth decreases or stops, optically transparent, so-called "hyaline" zones form. During the year, one opaque and one hyaline zone form, forming one annual growth zone. In reflected light, the opaque zone is in a light color and the hyaline zone looks dark. In transmitted light, the opaque zone looks dark (black), the hyaline zone looks light.

Age determination criteria:

1. One opaque and one hyaline zone together form a **one-year zone**.

2. The theoretical fish birth date is assumed to be January 1st.

Zone formation in the Eastern Baltic cod otoliths and the age interpretation

Eastern Baltic cod differs from Baltic cod which inhabits the southern and western regions of the Baltic Sea (ICES subdivisions 22 and 24) in that only an opaque zone is formed during the first year of life of Eastern Baltic cod. The usual spawning period during the 1960-1990s was in the spring from March until May. After hatching from the eggs cod larvae live in pelagic water layer in summer and autumn and after transforming into fry they descend to the bottom water layers. The descent usually occurs in late November – December, in addition the fry length during this time is around 4-5cm. At this time, the otolith of the cod shows a dark-colored otolith core with a core ring, around it a narrow dark so-called settlement or metamorphosis ring, and a narrow light opaque zone (the first annual growth zone). Cod fry in the first year of life are assigned to the age group “0”. Such fish are referred to as “0+” when determining age. The formation of opaque zones continues in the spring of the following year, after January 1st the fish begins its second calendar year of life and is assigned to the age group "1" and referred to as "0+)1", even though only the first opaque zone is visible on the otolith. In March-April, larger fry (15-17cm in length) begin to form the first hyaline zone and its formation continues in the summer. Therefore, the first hyaline zone in Eastern Baltic cod forms only in the second year after birth. The opaque and hyaline zones together make up a fully formed annual zones. The age of the fish is usually determined by counting the hyaline zones. In the second half of the year, an opaque zone (in fact, the second annual growth zone) forms in the cod otoliths. In the first half of the year, the opaque zone on the edge of the otolith is considered an unfinished growth zone from the previous year and in the second half of the year it is considered a new growth zone of the current year.

Thus, to divide the cod generation (fish born in the same year), in the first half of the year, even if there is no visible hyaline zone on the edge of the otolith, an age of +1 is added. In the second half of the year only the visible hyaline zones are counted. A schematic of zone formation and interpretation of age are shown in Figure 4.

		SPAWNING TIME					
	Zones on the	March-April		May-July		August-September	
Month	otolith	Interpretation	Scheme of	Interpretati	Scheme of	Interpretati	Scheme of
		of age	otolith	of age	otolith	of age	otolith
January							
February							
March							
April							
May							
June							
July							
August							
September							
October							
November							
December	one opaque zone	**0+**					
January							
February	one opaque zone	**0+)1**					
March							
April				**0+)1**			
May	one opaque zone and						
June	one hyaline zone	**1**					
July						**0+)1**	
August							
September							
October	first opaque zone +			**1**			
November	first hyaline zone +	**1+**					
December	opaque zone					**1**	
January	first annual zone +	**1+)2**		**1+)2**		**1+)2**	
February	opaque zone						
March							
April							
May	first annual zone +						
June	second annual zone	**2**					
July							
August							
September							
October	first annual zone +			**2**			
November	second annual zone+	**2+**					
December	opaque zone					**2**	
January	first annual zone +	2+)3		2+)3		**2+)3**	
February	second annual zone+						
March	opaque zone						

Figure 4. Schematic of zone formation in cod otoliths and interpretation of age.

Entering information into a database

When determining the age of catfish, on the cracked surface of the otolith under the microscope:

- count the opaque and hyaline zones and define the age according to the age interpretation method,
- measure the width of the broken surface of the otolith in ocular micrometer units (this corresponds to the thickness of the whole otolith) horizontally through the center of the otolith,
- measure the zone on the edge of the otolith and record the number in the protocol according to the "edge of the otolith codifiers" (Table 2).

Table 2. Otolith edge codifier for cod

Description of the zone on the otolith edge	Width of the zone, in ocular micrometer units	Code
Narrow opaque zone	<2	1
Wide opaque zone	>2	2
Narrow hyaline zone	<1	3
Wide hyaline zone	>1	4

Record the data in the relevant cells (columns) on the biological analysis form. Record the age of the catfish in the "Age" column, the width of the otolith in the "C" column, and the edge code of the otolith in the "otolith edge" column.

An example of filling out a biological analysis form is shown in Figure 5.

When conducting an expanded morphometric analysis, measure the width of each annual zone in the horizontal direction from the center of the otolith to the edge of the otolith.

Biological information from the form is entered into the computer in the relevant file and subsequently entered into the BIODATA database.

Zivju bioloģiskās analīzes kartiņa

Lapušu skaits 1 Lapas nr. 1

Zivju suga	COD	Datums	11.03.2005
Reisa No	1	Kuģa No	0837
Zvejas akta No	14	Zvejas rīks	TVS
Apakšrajons	28	Acs izmērs,mm	8
Zona		Zvejas ilgums minūtēs	30
LV kvadrāts	469		
Loms, kg	1.8		

Parauga veids:

- ☐ Rūpnieciskā zveja jūrā
- ■ Pētniecikā zveja
- ☐ Osta ☐ Bez atlases
- ☐ Izmetums ☐ Ar atlasi

Parauga svars, kg	
Izmēra kategorija	

No.	Garums, cm	Svars, g				Dzimums	Nobriešana	Kuņģa piepildījums	Tauku satt.	Vecums	Populācija	Rase
		Pilnais	Ķidātais	Aknas	Gonādas							
1	43	920	720			1	4	0		5		
2	30.1	276.8	218.1			1	4	1		3		
3	15.5	38.8	34.7			1	4	1		2		
4	17.1	44.8	40.6			1	3	2		2		
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Figure 5. Example of filling out a biological analysis form.