

Round Goby method for determining age and biological parameters.

Version 1.0

L. Rozenfelde

Translated by M. Plikss

1. Biological sampling

The biological data of the round goby (*Neogobius melanostomus*) is collected during industrial control fishing in the Gulf of Riga and the open parts of the Baltic Sea, conducted in cooperation with Latvian coastal fishermen (Figure 1). The material is collected from April to September using various kinds of fishing gear, including round goby pots (mesh size 24-36 mm), eelpout pot (mesh size 36-40 mm), small fish pots (mesh size 36-60 mm), gillnets (net mesh size 32-64 mm), and specialized round goby nets (mesh size 24-36 mm). In addition, independent fishing assessments have been carried out since 2014. Scientific fishing is conducted in the coastal area 1-3 km south of the Southern pier, near Liepaja (Figure 1).

Samples are collected using different types of research net sets, such as nylon, capron, and "Nordic". In the coastal zone, the nets are placed in one or two stations, reaching depths of 4-5m. The nets are placed parallel or perpendicular to the coastline and are put in the sea in the evening and retrieved the next morning. From 2014 to 2016, nets with different net mesh sizes (17, 22, 25, 30, 33, 38, 50 and 60 mm) were used. One net station consists of six to eight connected individual nets. Each net is 1.8m high and 30m long, with the total station length varying from 180m to 240m.

From 2015 to 2020, "Nordic" nets with different net mesh size panels (10-60 mm) were also used. One "Nordic" net consists of nine different mesh sizes, each mesh size is 5m long and 1.8m high, hence the total length of one "Nordic" net is 45 meters, additionally in stations 6-8 "Nordic" nets are connected.

The round goby biological samples are collected during the benthic fish inventory scientific cruises in the Gulf of Riga, in May and October. Four stations were located here - Salacgrīva, Lielupe, Mērsrags, Roja (Figure 1). Fish inventory is carried out using MRTK ("Baltica") type fishing vessels. Material is collected using a bottom trawl, the trawl opening is 17m, liner net mesh size in the wings is 17mm, mesh size 6-8mm. The trawling duration is 30 minutes, excluding the time for deploying and retrieving the trawl. The trawling speed is 3 knots (5.6 km/h). In one hour, a 50,000 square meter area is fished.

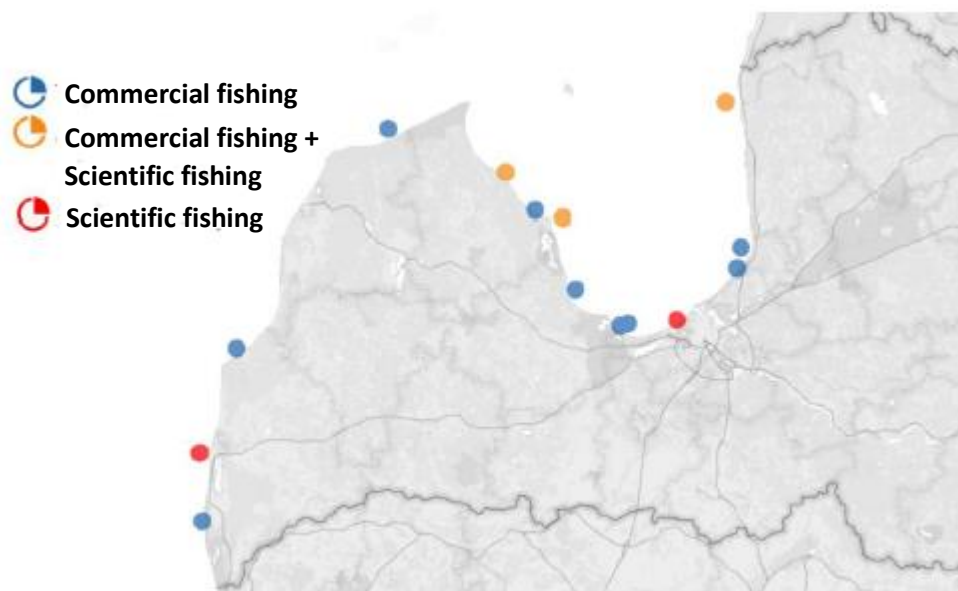


Figure 1. Locations where round goby sampling is conducted

Then, all samples obtained from both commercial and scientific fishing are brought ashore for further processing. The fish caught are divided into species, determining the total mass and catch. In addition, the exact coordinates of the sample collection, wind direction, water depth and total mass of the catch are also determined.

2. Assessment of biological parameters

During the processing of biological samples of round goby, length measurements are taken on randomly selected fish in each fishing act, determining the following parameters:

- Maximum length of fish in cm
- Full weight of fish in grams (not gutted)

The results of fish mass measurements are recorded on special cards (Figures 8 & 9). The information on these cards is duplicated with the corresponding information from the log cards. In addition, the full weight of the fish is also determined with a precision of 1 g. Then, based on the key principle (Figure 10), five fish from each length class of each gender (one centimeter interval) are subjected to biological analysis, additionally determining the following parameters:

- Gutted weight of fish in grams
- Gender of fish (codes: male - 1, female - 2)
- Stage of gonad development (according to Kiselevich's six-point scale). The decoding's of gonad stages can be seen in Table 1.
- Age of fish (in years)
- Food composition in stomachs (only from the scientific trips of the Gulf of Riga).

2.1 Maturity stages

Gonadal stage	Six-point scale (Koshelev, 1984)
Immature	I
Maturing I	II
Maturing II	III
Mature	IV
Ripe and running	V
Spent	VI

- Koshelev, B.V., *Ekologiya razmnozheniya r yb* (Ecology of Fish Reproduction), Moscow: Nauka, 1984

Fish gender is determined by the morphology of the gonads or by the presence of reproductive products, by making a cut through the fish's anal opening using surgical scissors. During the spawning season, gender can also be determined visually by the fish's external body coloration.

Reproductively active individuals correspond to the second to sixth scale, indicating that the gonads are mature and reproductive products such as oocytes, spermatogonia, or eggs can be seen. But immature or juvenile individuals correspond to the first scale, their gonads are thin, filamentous, and no reproductive products are visible.

2.2. Age determination

2.2.1. Removal and preservation of otoliths for subsequent age determination.

The age of the round goby is determined by analyzing the growth zones of the sagitta otolith pair. The otoliths are collected from defrosted fish, by firmly grasping the fish and making a transverse incision one centimeter above the eye line using a knife. The incision site is broken open, and the otolith pair is removed from the inner ear chamber of the fish using tweezers. These ear bones are located under the brain and are elongated with curved surfaces facing inward.

The otoliths are cleaned of tissue remnants. They are then consecutively placed in a scale notebook with the species or its abbreviation, the location and date of collection, and the fishing gear type, written on the first page (Figure 2). On the corner of each page of the notebook, the otolith numbering is noted, which corresponds to the individual number of the fish in the biological analysis protocols (Figures 2 & 8).



Figure 2. Example of a scale notebook with all the necessary

2.2.2. Processing of otoliths for age determination

2.2.2.1. "Burning and breaking" technique.

Up until 2018, the burning and breaking technique was used for otolith processing. Each otolith was briefly burned above a spirit lamp with the help of tweezers, depending on the size and intensity of the flame. The heat causes the organic materials and hyaline zones to become greatly darkened, but the opaque zone remains light in color. This results in a more pronounced color contrast between the two zones, allowing for more precise age determination.

When the otolith turns yellow-brown, it is carefully broken to obtain a cross-section (Figure 3). Then it is placed with the side down and the central part facing up against the binocular objective, using x40 magnification. The dark rings are counted from the core to the outer edge of the otolith for age determination.



Figure 3. Otolith of a round goby after applying the “burning and breaking” technique

2.2.2.2. "Thin section staining" technique.

To improve the precision of age determination, since 2019, otoliths are processed using the thin section staining technique. Initially, one otolith is removed from the otolith notebook. It is placed under the binocular and the core zone is marked with a pencil through which the cut will be made (Figure 4).

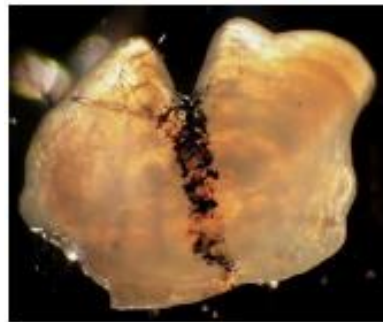


Figure 4. Unprocessed round goby otolith with marked cutting line through the otolith core.

Afterwards, special aluminium molds are prepared, consisting of two squares (7.5 x 7.5 cm) (Figure 5). These square molds will be used to cast otoliths. Initially, the aluminium molds are coated with silicone to prevent epoxy from adhering to the metal. Next, epoxy resin is mixed with the epoxy hardener (Struers A/S, Ballerup, Denmark) in a ratio of 6.6:55 ml, and the mixture is slowly stirred for two minutes. Once the mixture is homogeneous and reaches half its volume (~0.5 cm), it is poured into the prepared aluminium molds. After 24 hours, the epoxy mass fully cures, and using a special needle, eight parallel lines are drawn in the epoxy mass. Using tweezers, five to eight circular otoliths are sequentially placed in each line. The otoliths are positioned in the molds so that the pencil-drawn line precisely aligns with the line drawn in the epoxy. Then, a top layer of epoxy (~0.5 cm) is poured into the mold. After 24 hours, the epoxy mass cures completely. Using a chisel and a hammer, the epoxy molds are removed from the aluminium molds.

As a result, two square-shaped molds with embedded otoliths are obtained. On these molds, parallel lines are again drawn using a needle to prepare epoxy blocks for cutting.

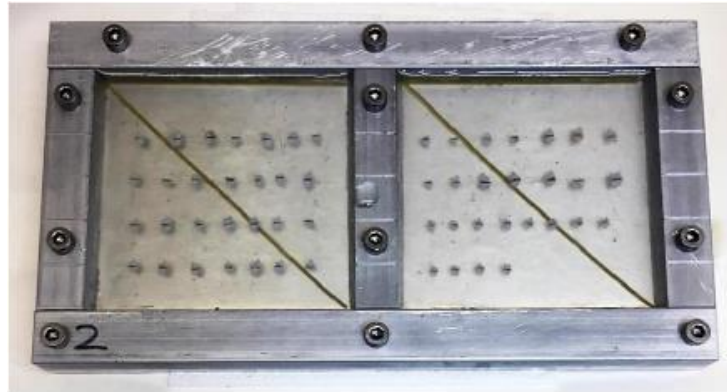


Image 5. Aluminium mold with circular seafloor otoliths embedded in epoxy.

The cross-sections of the otoliths are obtained using the sample preparation equipment "Struers Accutom-100." The epoxy blocks are cut using a diamond blade saw (thickness 0.4 mm) with a rotational speed of 300-5000 revolutions per minute and a cutting speed of the epoxy of 0.1 mm/sec. To initiate the cutting process, the epoxy block is securely fastened against the saw using special clamping screws. Then, the manually positioned line drawn on the epoxy mold is aligned with respect to the blade of the saw, and the cover of the "Struers Accutom-100" apparatus is closed, initiating the automatic cutting of the epoxy blocks. Initially, excess epoxy is trimmed, but upon the second cut, a 0.4 mm thick cross-section is obtained with a precision of 0.05 mm (Figure 6). These steps are repeated for the remaining lines. The cutting process for a single block takes approximately 20 minutes.

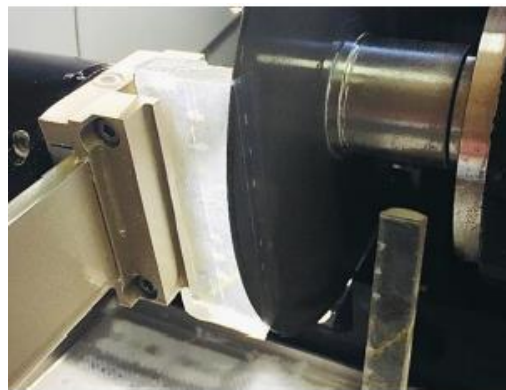


Figure 6. Cutting of epoxy blocks using the "Struers Accutom-100" diamond blade saw.

The final stage of this method involves staining the cross-sections of the otoliths. Initially, a 1% hydrochloric acid (HCl) solution is prepared, and the otolith cross-sections are immersed in it for one minute. They are then rinsed with water. For the preparation of the staining solution, 100 ml of neutral red dye (0.33%, Sigma-Aldrich, United Kingdom) is added to a staining cylinder, along with one gram of NaCl and 0.5 ml of acetic acid (95%, Sigma-Aldrich, United Kingdom). The mixture is gently stirred three to four times. The resulting solution is poured into a special staining container, which accommodates approximately 50 otolith cross-sections. The staining process lasts for 20 minutes. Afterwards, the cross-

sections are rinsed with water and dried. As a result of staining, the hyaline zones become darker, creating a pronounced color contrast between the hyaline and opaque zones, enabling the identification of individual hyaline zones and determining their count (Figure 7).

2.2.3. Age determination based on the otolith structure of Round Goby

Fish age is determined by counting the hyaline zones from the central part to the outer edge of the otolith (Figure 7). One opaque and one hyaline zone are formed within a year, which are considered as one annual zone. They are formed during the spring and summer seasons when the fish growth rate is accelerated. As a result, opaque bands are formed, while during the winter season, when growth slows down, translucent bands or hyaline zones are formed. In incident light, the opaque zone appears optically denser and appears light, while in transmitted light, the hyaline zone appears dark, forming age rings used to determine the age of the fish. For otoliths with observed crystallization, age cannot be determined accurately since annual bands cannot be properly distinguished. For age determination, otolith cross-sections are rotated under a binocular microscope (LEICA S6Z) at a magnification of x40. Motic MLC-150C or Leica GLS 100X illuminators are used for lighting.

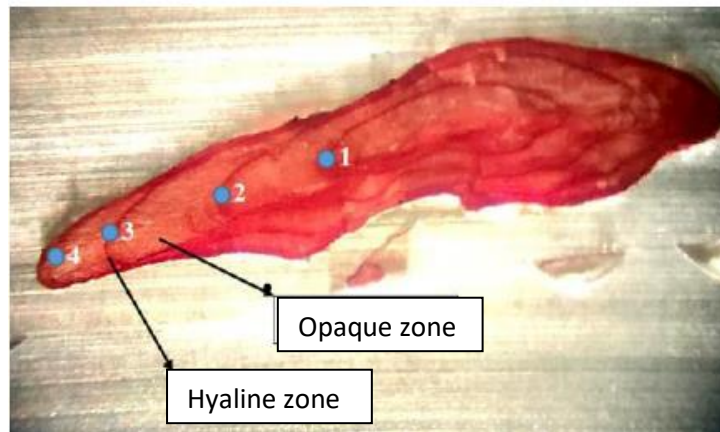


Image 7. Cross-section of a four-year-old Round Goby using the cutting and staining technique.

2.3. Determination of stomach contents composition

Samples for the analysis of stomach contents are collected during scientific surveys of benthic fish in the Gulf of Riga in May and October. The analysis is performed on the entire sample without using length-based subsampling. Initially, length measurements and a complete biological analysis are conducted for each fish. Then, using surgical scissors, an incision is made from the fish's anus to the upper body. The stomach contents are determined exclusively from the stomach. Therefore, with the aid of forceps and a scalpel, the organ is carefully dissected, and its contents are squeezed onto a glass petri dish. The dissected sample is observed under a binocular microscope (LEICA S6Z) at magnifications ranging from x16 to x40. All identified food items are recorded individually for each fish, including their total mass in grams (using electronic scales KERN EW 150 - 3M with a precision of 1 g), degree of digestion on a three-point scale (0 - fully digested, 3 - undigested), and the corresponding taxonomic classification in Latin.

Objects that cannot be assigned to a specific taxon are marked as "Un." or unknown. Additionally, if other relevant information such as parasites or fish anomalies is observed, it is noted in the "Notes" section. In subsequent data analysis, information on both the total mass and the proportionate abundance of species in the analysed sample is used. The collected data are summarized in stomach analysis protocols, which include information on the fish's biological parameters and food items (Image 11).

Zivju bioloģiskās analīzes kartiņa

Lapu skaits Lapa nr.

Zivju suga	AJG
Reisa No	1
Zvejotāja akta No	14
Apakšrajons	28
Zona	
LVK vecācis	408
Loma, kg	1.8

Datums	11.03.2005
Kuģa No	0837
Zvejotāja nos.	TVS
Asi izmērs, mm	8
Zvejotāja ilgums minūtēs	30

Perauga veids:

Rūpnieciskā zvejā jūrā

Pētnieciskā zvejā

Celta Bez celtas

Izmētums Ar atlasīti

Parauga svars, kg

Izmēru kategorija

No.	Garums, cm	Svars, g				Dziļums	Mātristāne	Mugļa paplūpums	Tauku sat.	Vaiņums	Populācija	Izse.
		Pinās	Ikdēlis	Aknes	Conādas							
1	22.5	200.3	155.2			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		
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												
26												
27												
28												
29												
30												
31												
32												
33												
34												
35												
36												
37												
38												
39												
40												
41												
42												
43												
44												

Figure 8: Example of a filled-out biological analysis form

Zivju masu mērijuma kartiņa

Lapušu skaits Lapas nr.

Zivju suga	AJG	Datums	28.05.2014
Releā No	1001	Kuģa No	0831
Zvejas akta No	1	Zvejas rīks	GNS
Apakšrajons	26	Acs izmērs, mm	110
Zona		Zvejas ilgums minūtēs	800
LV kvadrāts	501		
Loms, kg	5		

Parauga veids:

Rūpnieciskā zveja jūrā
 Pētnieciskā zveja BMS
 Osta Bez atlases
 Izmetums Ar atlasi

Parauga svars, kg	5
Izmēra kategorija	0

No.	Garums, cm	Dzīmi	S tac	No.	Garums, cm	Dzīmi	S tac	No.	Garums, cm	Dzīmi	S tac	No.	Garums, cm	Dzīmi	S tac	No.	Garums, cm	Dzīmi	S tac
1	13,6			51				101				151				201			
2	20,1			52				102				152				202			
3	13,2			53				103				153				203			
4	16,3			54				104				154				204			
5				55				105				155				205			
6				56				106				156				206			
7				57				107				157				207			
8				58				108				158				208			
9				59				109				159				209			
10				60				110				160				210			
11				61				111				161				211			
12				62				112				162				212			
13				63				113				163				213			
14				64				114				164				214			
15				65				115				165				215			
16				66				116				166				216			
17				67				117				167				217			
18				68				118				168				218			
19				69				119				169				219			
20				70				120				170				220			
21				71				121				171				221			
22				72				122				172				222			
23				73				123				173				223			
24				74				124				174				224			
25				75				125				175				225			
26				76				126				176				226			
27				77				127				177				227			
28				78				128				178				228			
29				79				129				179				229			
30				80				130				180				230			
31				81				131				181				231			
32				82				132				182				232			
33				83				133				183				233			
34				84				134				184				234			
35				85				135				185				235			
36				86				136				186				236			
37				87				137				187				237			
38				88				138				188				238			
39				89				139				189				239			
40				90				140				190				240			
41				91				141				191				241			
42				92				142				192				242			
43				93				143				193				243			
44				94				144				194				244			
45				95				145				195				245			
46				96				146				196				246			
47				97				147				197				247			
48				98				148				198				248			
49				99				149				199				249			
50				100				150				200				250			

Figure 9: Example of a filled-out mass measurement form

Apalā jūrasgrunduļa bioloģiskās analīzes "atslēga"

Vieta - Ķesterciems

Datums – 15.06.2021

Zvejas rīks – luču murds

Tēviņi																					
5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
				X	X	X	x	x	x	x	x	x	x	x	x		x				
				X	x	X		x	x			X	x		x						
				x		X		x	x			x	x								
						X		X				x									
						x		x				x									

Mātītes																					
5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
	X	x	x	x	X	X	x	x	x		x										
	X			x	X	X	X	x													
					X	X	x														
					x	X															
					x	x															

Figure 10: Example of a filled-out "key" form for biological analyses

