METHODOLOGY FOR COLLECTION, ANALYSIS AND PROCESSING OF ZOOPLANKTON SAMPLES

1. Objective and application of the method

This method has been developed to document in detail the methods for the quantitative and qualitative determination of zooplankton in the Gulf of Riga and the Baltic Sea. The method described below has been developed to characterise zooplankton sampling, data processing, and storage in the Marine Division of the Fish Resources Research Department of BIOR.

2. Zooplankton sampling in the Gulf of Riga and the Baltic Sea

2.1. Equipment

- Juday-type zooplankton net (upper ring diameter 36 cm, middle ring diameter 50 cm, mesh size 160 µm);
- Flow-meter fixed on the upper ring of the net;
- Formalin or alcohol to fix the samples;
- Wash bottle for alcohol (500-1000 ml);
- Bottles for sample storage (500 ml);
- Measuring beaker (1000 ml);
- Mesh sieve for sample concentration (mesh size 100 µm);
- Funnel.

2.2. Zooplankton sampling

Samples are taken using a Juday net: it is first lowered to the required depth and towed vertically to the surface. The net is rinsed, and its contents are poured into a measuring beaker. If the sample contains a lot of water, it is filtered on a sieve (mesh size 100 µm). The resulting sample is rinsed into a storage bottle using a wash bottle filled with alcohol. In the Gulf of Riga, samples are collected in two water layers: the entire layer from the bottom to the surface and the upper 0-20 m of water. At stations less than 20 m deep, the entire water column shall be sampled.

In the Baltic Sea, the depth of the sampling layer depends on the depth of the station. At shallower stations (depths up to 100 m) the whole water column from bottom to surface is

sampled, whereas at deeper stations (depths > 100 m) the water columns 0-50 m and 0-100 m are sampled. The sampling sites are shown in Figure 1.

The sampling record shall record the following information: date, month, year, time, station, station coordinates, depth (m), collection layer, flow meter reading before collection, flow meter reading after collection, and bottle number.

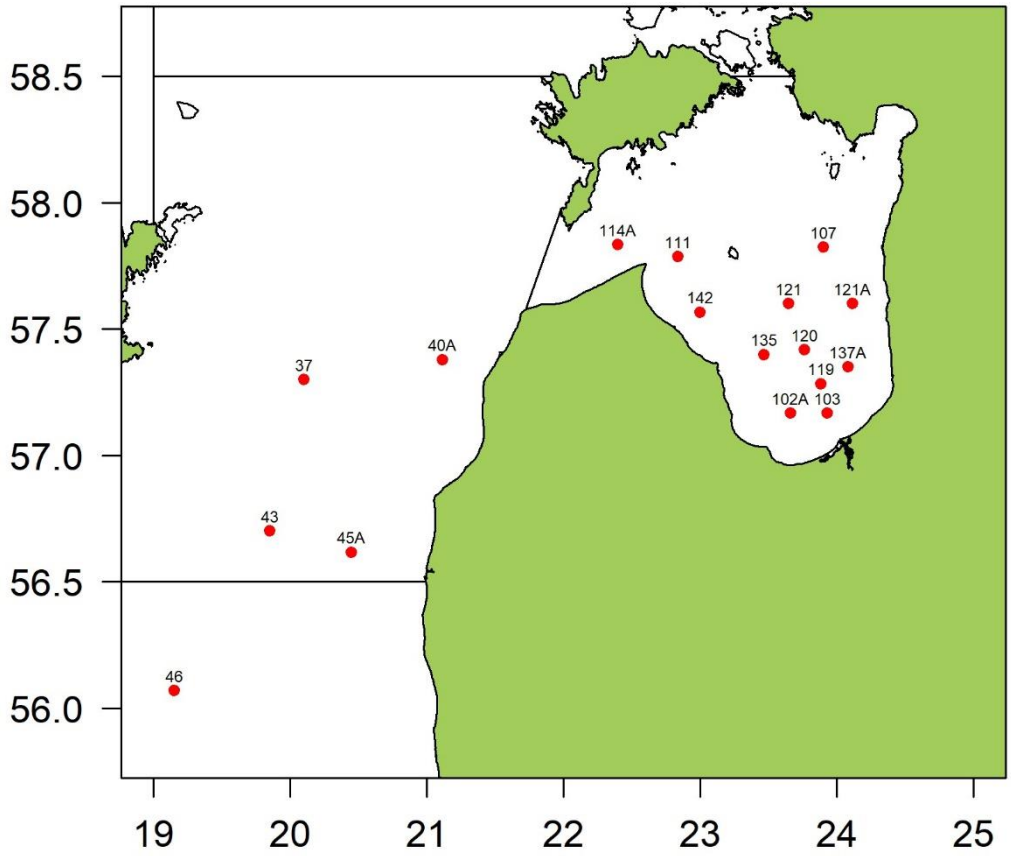

Figure 1. The locations of standard sampling stations in the Gulf of Riga and the central Baltic Sea.

3. Processing and analysis of zooplankton samples

3.1. Materials

- Measuring cups of 100 ml, 250 ml, 500 ml and 1000 ml capacity;
- Calibrated Stempel pipette (2 ml, 2.25 ml or 2.5 ml);

- Plankton counting chamber;
- Stereomicroscope equipped with a measuring scale and a light feed from below;
- A sieve for rinsing the sample, equipped with a mesh size of 100 µm or less;
- Wash bottle (500-1000 ml);
- Notebook to register the results of sample analysis.

3.2. Preparation of samples for analysis

Fixed zooplankton samples shall be rinsed through a rinsing sieve to remove formalin or alcohol from the sample. The rinsed sample is collected in a volumetric beaker using a graduated scale and concentrated or diluted to a specified volume. The volume of the prepared sample may be 50 ml, 100 ml, 200 ml, 300 ml or more. The dilution or concentration of the sample will depend on the total amount of zooplankton or the amount of individual countable organisms in the sample. For example, to accurately estimate the abundance of some of the rarer species (*Limnocalanus macrurus macrurus*, *Cercopagis (Cercopagis) pengoi*) in a sample, it is sometimes necessary to count them throughout the sample. In this case, the sample is concentrated to a volume of 100 ml or less and analysed in parts in a plankton chamber. The number of *Leptodora kindtii* is determined for the whole sample, and each individual is measured (Figure 2).

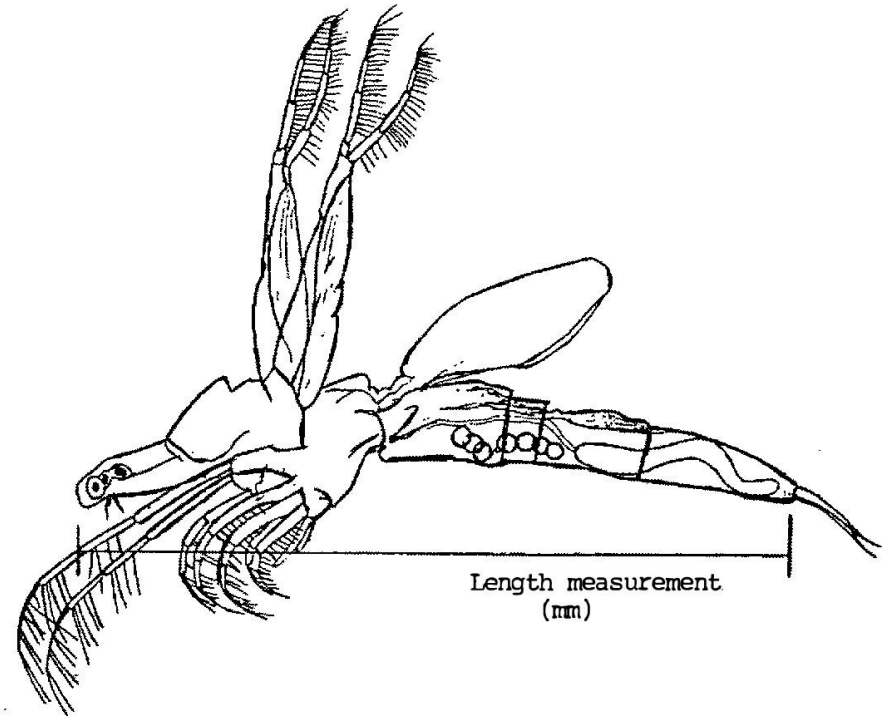

Figure 2. Length measurements of the cladoceran *Leptodora kindtii* (*Diplostraca*) (according to Koapach 1989).

3.3. Analysis of samples

The following information about the sample to be analysed shall be recorded in the notebook:

1) number of bottle;
2) date of sample collection;
3) time of sample collection;
4) station number (if available);
5) station coordinates;
6) sampling layer of samples;
7) station depth;
8) Flowmeter readings (start and end);
9) Volume of the sample dilution;
10) Volume of the Stempelpipette.

Mix the sample thoroughly so that the zooplankton organisms are evenly distributed throughout the sample. Immediately take a subsample with the Stempelpipette and transfer it to the plankton counting chamber. The number of subsamples transferred depends on the abundance of zooplankton in them. Data for each subsample shall be recorded separately.

In the subsample, determine the species or genus and their number. It is recommended to count to 100 individuals in the 3 dominant groups.

For the copepod species (*Acartia* spp., *Eurytemora affinis affinis*, *Limnocalanus macrurus macrurus*, *Pseudocalanus* sp., *Temora longicornis*, *Centropages hamatus*, *Cyclopidae*) the developmental stages (6 copepodite stages and nauplii) are determined. Limnocalanus macrurus macrurus shall be counted in a small volume throughout the sample if the number of individuals does not exceed 100. If there are more than 100 individuals, count to 100.

For cladocerans (*Diplostraca*), the species (*Evadne nordmanni*, *Cercopagis (Cercopagis) pengoi*) or genus (*Podon/Pleopis* spp., *Bosmina* spp.) should be identified. For *Evadne nordmanni*, *Bosmina* spp. and *Podon/Pleopis* spp. length measurements should be taken (see Figure 3) and the individuals should be counted separately for each size group. The size groups in millimetres are given in Table 1. Before starting work with the Stereomicroscope, it is necessary to calculate the number of sections per 1 mm of the measuring scale inserted in the Stereomicroscope. After measuring, the number of sections (from - to) corresponding to the size group in millimetres should be calcualted. *Cercopagis pengoi* is not measured and its number is determined for the whole sample.

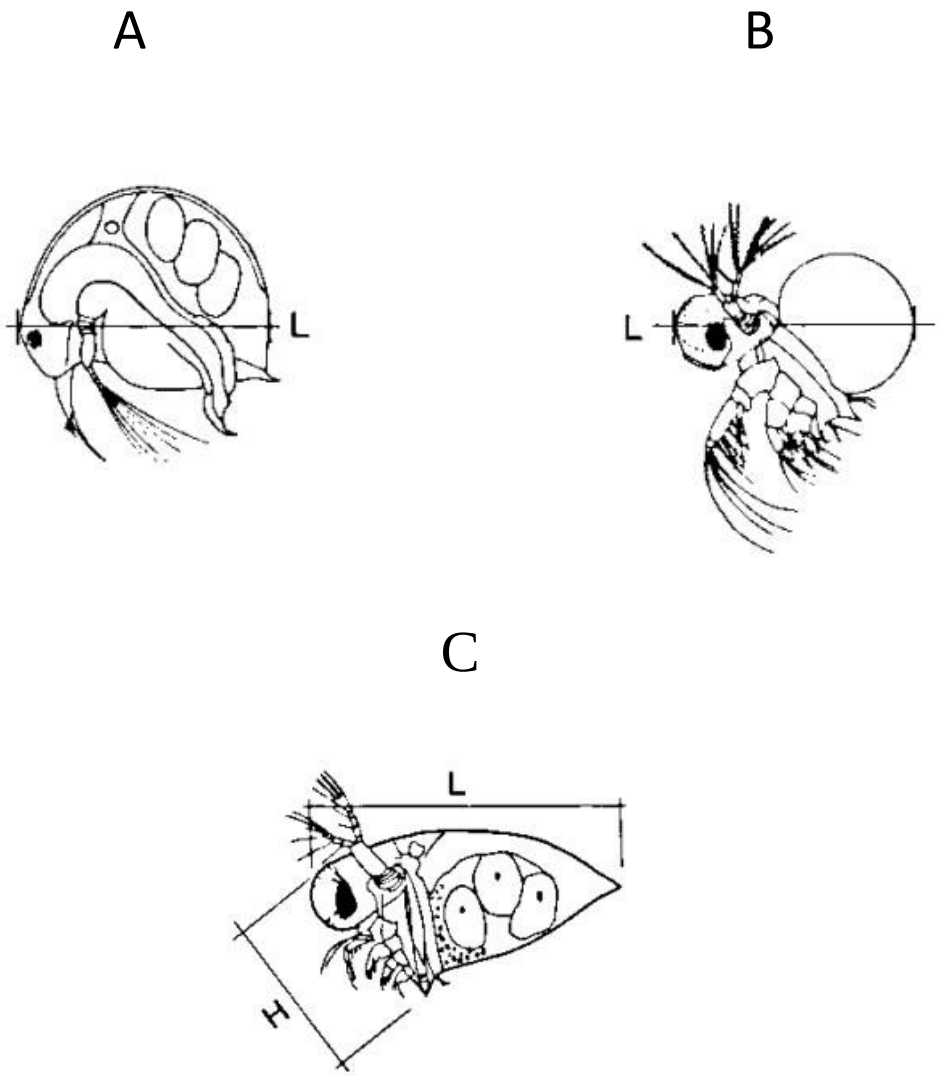

Figure 3. Length measurements of the cladocerans: (A*) Bosmina* spp.; (B) *Podon/Pleopis* spp.; (C) *Evadne nordmanni* (according to Hernroth 1985).

The rotifer (*Eurotatoria*) species (*Synchaeta baltica*, *Synchaeta monopus*, *Keratella quadrata quadrata*, *Keratella cochlearis*, *Keratella cruciformis*) and their number shall be determined.

The other zooplankton forms conditionally assigned to the group *Varia* should be identified to the lowest possible taxonomic level and their numbers determined.

4. Data Processing and Analysis

An MS EXCEL spreadsheet is used to summarise the sample information and sample count data for each month. The data spreadsheet will record the following information:

1) date of sample collection;
2) month of sample collection;
3) year of sample collection;
4) time of sampling
5) station number (if available);
6) coordinates of station (latitude/longitude and minutes);
7) station depth
8) upper and lower position of sampled water layer
9) start and end readings of water Flowmeter;
10) species name;
11) development stages/size groups.
12) number of individuals in the sample (N/x m^3);
13) number of individuals per cubic metre (N/m^3);
14) biomass of the taxonomic group (mg/m^3).

The data should be imported into the national database BIODATA and stored on the BIOR server.

5. The estimation of zooplankton abundance and biomass

The volume of water filtered (***V***) is determined from the readings of the water flow meter and the area of the plankton net ring. The net may be equipped with a Hydro-Bios water flow meter (model no. 438115 or 23.091 with back-run stop) or a General Oceanics flow meter (model 2030R6C with back-run stop and low-speed rotor).

The amount of water filtered shall be calculated according to the model of the flowmeter or according to the water column (if the network is not equipped with a flowmeter):

1) The volume of water filtered by the Flowmeter of **Hydro-Bios**, ***V*** (m^3), is calculated according to the following formula:

$$\boldsymbol{V = S \times (F_2 - F_1) \times 0,3,}$$

where ***S*** – is the area of the upper circle of the net (0.1 m^2 for a Juday net),
$\boldsymbol{F_2}$un $\boldsymbol{F_1}$ – is the end and start readings of the water Flowmeter of Hydro-Bios.

2) The volume of water filtered by the Flowmeter of **General Oceanics**, ***V*** (m^3), is calculated according to the following formula:

$$\boldsymbol{V = S \times (((F_2 - F_1) \times 57,56)/999,999),}$$

where ***S*** – is the area of the upper circle of the net (0.1 m^2 for a Juday net),
$\boldsymbol{F_2}$ and $\boldsymbol{F_1}$ - are the end and start readings of the water Flowmeter of General Oceanics,
57,56 – low speed rotor constant.

3) If the plankton net has not been equipped with a water Flowmeter, the volume of filtered water ***V*** (m^3) is calculated according to the formula:

$$\boldsymbol{V = (H_0 - H_1) \times S,}$$

where $\boldsymbol{H_0}$ – is the measurement of cable length (m) at the start of filtration,
$\boldsymbol{H_1}$ – is the measurement of cable length (m) at the end of filtration.

The number of individuals in the whole sample $\boldsymbol{N_p}$ is calculated:

$$\boldsymbol{N_p = n\,(V_p/a \times V_s)\,,}$$

where $\boldsymbol{n}$ – number of individuals in the subsample,

$\boldsymbol{V_p}$ – volume of the whole sample (dilution),

$\boldsymbol{a}$ – number of subsamples counted,

$\boldsymbol{V_s}$ – the volume of the subsample (volume of Stempelpipette).

Number of individuals per cubic meter $\boldsymbol{N_k}$ is calculated:

$$\boldsymbol{N_k = N_p/V,}$$

$\boldsymbol{N_p}$ – number of individuals in the whole sample,

$\boldsymbol{V}$ – volume of water filtered (m^3).

The biomass $\boldsymbol{B}$ of species or genus is calculated:

$$\boldsymbol{B = N_k \times m_{ind}\ ,}$$

where $\boldsymbol{N_k}$ – number of individuals per cubic meter (m^3),

$\boldsymbol{m_{ind}}$ – individual mass factor of species or genus.

Individual zooplankton mass factors documented by Hernroth (1985) for the Baltic Sea were used to calculate biomass, except for *Leptodora kindtii*. The individual mass factors given by Mordukhay-Boltovskiy F.D. (1954) were used to calculate the biomass of the cladoceran *Leptodora kindtii*.

6. Calculation of mean number and biomass

The mean number (ind./m^3) and biomass (mg/m^3) are calculated separately for each zooplankton species/genus. For the Gulf of Riga, it is necessary to calculate the average of the taxonomic groups in the two water layers:

1) upper 0-20 m;
2) the entire water layer from the surface to the bottom.

The Baltic Sea stations are divided into several groups depending on their depth and the sampling layer. Average biomass and abundance are calculated for each group separately. The distribution of the groups is as follows:

1) the entire central Baltic Sea (all stations regardless of depth);
2) shallow zone (stations <100 m depth);
3) deep zone (stations >100 m depth);
4) top layer 0-50 m.

7. Testing of the accuracy of the results

To check the accuracy of the results, 10 % of the samples need to be re-analysed.

8. Data storage

Data are stored in MS Excel format on the BIOR server and in the national database BIODATA.

9. References

Hernroth L. (ed.) 1985. Recommendations on methods for marine biological studies in the Baltic Sea Mesozooplankton Biomass Assessment, vol. 10, Baltic Marine Biological Publications. 32 lpp.

Koapaha, Joutje Ariel. 1989. Leptodora Kindtii (Focke): Seasonal Population Abundance and Food Web Interactions in Lake Ontario; 1984, 1986, and 1987. 84 lpp.

Mordukhay-Boltovskiy F.D. 1954. Materialy po srednemu vesu vodnykh bespozvonochnykh basseyna Dona. Problemy gidrobiologii vnutrennikh vod [Materials on the average weight of aquatic invertebrates of the Don River Basin. Problems of hydrobiology of inland waters]. In: Trudy problemnykh i tematicheskikh soveshhaniy. Vypusk 2 [Proceedings of the Thematic and Problem Workshops of the Zoological Institute. Issue 2]. Moscow-Leningrad: Zoological Institute of the AS of the USSR Publ., pp. 223–241. (In Russian).

Table 1. Individual mass factors of cladocerans (*Diplostraca*, formerly *Cladocera*) in the Baltic Sea in different size groups (Hernroth 1985).

Length group (mm)	*Bosmina spp.*	*Podon/Pleopis spp.*	*Evadne nordmanni*
<0,3	0,0025	0,002	0,002
0,3-0,4	0,007	0,006	0,006
0,4-0,5	0,015	0,013	0,01
0,5-0,6	0,025	0,025	0,02
0,6-0,7	0,045	0,04	0,03
0,7-0,9	0,08	0,07	0,05
0,9-1,1	-	0,14	0,09
>1,1	-	0,2	0,14

Table 2. Individual mass factors of zooplankton species for the central Baltic Sea in different seasons according to Hernroth (1985).

Species/genus	Development stage	Individual mass factor (mg)			
		Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec
Acartia spp.	F	0,020	0,020	0,020	0,020
	M	0,018	0,015	0,015	0,015
	5	0,012	0,012	0,011	0,009
	4	0,012	0,012	0,011	0,009
	3	0,005	0,005	0,005	0,005
	2	0,005	0,005	0,005	0,005
	1	0,005	0,005	0,005	0,005
	0	0,002	0,002	0,002	0,002
Temora longicornis	F	0,060	0,065	0,060	0,050
	M	0,055	0,040	0,045	0,050
	5	0,018	0,015	0,015	0,014
	4	0,018	0,015	0,015	0,014
	3	0,009	0,006	0,006	0,004
	2	0,009	0,006	0,006	0,004
	1	0,009	0,006	0,006	0,004
	0	0,003	0,003	0,003	0,003
Pseudocalanus sp.	F	0,050	0,045	0,050	0,055
	M	0,035	0,030	0,035	0,050
	5	0,020	0,020	0,020	0,020
	4	0,020	0,020	0,020	0,020
	3	0,007	0,008	0,010	0,010
	2	0,007	0,008	0,010	0,010
	1	0,007	0,008	0,010	0,010
	0	0,003	0,003	0,003	0,003
Centropages hamatus	F	0,055	0,050	0,045	0,050
	M	0,045	0,040	0,040	0,045
	5	0,017	0,015	0,015	0,017
	4	0,017	0,015	0,015	0,017
	3	0,006	0,006	0,006	0,006
	2	0,006	0,006	0,006	0,006
	1	0,006	0,006	0,006	0,006
	0	0,003	0,003	0,003	0,003
Eurytemora affinis affinis	F	0,025	0,050	0,025	0,025
	M	0,020	0,040	0,020	0,020
	5	0,013	0,014	0,014	0,014
	4	0,013	0,014	0,014	0,014
	3	0,005	0,005	0,005	0,005
	2	0,005	0,005	0,005	0,005
	1	0,005	0,005	0,005	0,005
	0	0,002	0,002	0,002	0,002
Limnocalanus macrurus macrurus	F	0,700	0,700	0,700	0,700
	M	0,500	0,500	0,500	0,500
	5	0,013	0,200	0,200	0,200

	4	0,013	0,200	0,200	0,200
	3	0,028	0,028	0,028	0,028
	2	0,028	0,028	0,028	0,028
	1	0,028	0,028	0,028	0,028
	0	0,003	0,003	0,003	0,003
Cyclopoida	F	0,025	0,025	0,025	0,025
	M	0,020	0,020	0,020	0,020
	5	0,013	0,013	0,013	0,013
	4	0,013	0,013	0,013	0,013
	3	0,0035	0,0035	0,0035	0,0035
	2	0,0035	0,0035	0,0035	0,0035
	1	0,0035	0,0035	0,0035	0,0035
	0	0,0025	0,0025	0,0025	0,0025
Bosmina spp. (if not measured)		0,015	0,015	0,015	0,015
Podon/Pleopis spp. (if not measured)		0,050	0,050	0,050	0,050
Evadne nordmanni (if not measured)		0,040	0,040	0,040	0,040
Synchaeta spp.		0,006	0,006	0,006	0,006
Keratella spp		0,001	0,001	0,001	0,001
Bivalvia larvae		0,001	0,001	0,001	0,001
Fritillaria borealis		0,010	0,010	0,010	0,010
Polychaeta larvae		0,030	0,030	0,030	0,030
Pleurobrachia pileus		0,010	0,010	0,010	0,010
Parasagitta elegans		0,250	0,250	0,250	0,250
Oithona similis		0,006	0,006	0,006	0,006
Amphibalanus improvisus larvae		0,010	0,010	0,010	0,010
Cercopagis (Cercopagis) pengoi		0,400	0,400	0,400	0,400
Harpacticoida (Mesochra spp.)		0,010	0,010	0,010	0,010

Table 3. Individual mass factors of the cladoceran Leptodora kindtii (Diplostraca) in the Baltic Sea in different size groups according to Mordukhay-Boltovskiy F.D. (1954).

Length (mm)	Ind. mass factor (mg)	Length (mm)	Ind. mass factor (mg)	Length (mm)	Ind. mass factor (mg)	Length (mm)	Ind. mass factor (mg)	Length (mm)	Ind. mass factor (mg)
0,3	0,0002	2,3	0,0644	4,3	0,3833	6,3	1,1383	8,3	2,4976
0,4	0,0004	2,4	0,0727	4,4	0,4093	6,4	1,1906	8,4	2,5843
0,5	0,0008	2,5	0,0817	4,5	0,4363	6,5	1,2444	8,5	2,6730
0,6	0,0014	2,6	0,0914	4,6	0,4645	6,6	1,2997	8,6	2,7636
0,7	0,0022	2,7	0,1018	4,7	0,4939	6,7	1,3566	8,7	2,8561
0,8	0,0032	2,8	0,1129	4,8	0,5244	6,8	1,4151	8,8	2,9507
0,9	0,0044	2,9	0,1247	4,9	0,5562	6,9	1,4753	8,9	3,0473
1,0	0,0060	3,0	0,1374	5,0	0,5891	7,0	1,5370	9,0	3,1459
1,1	0,0079	3,1	0,1580	5,1	0,6233	7,1	1,6004	9,1	3,2465
1,2	0,0101	3,2	0,1651	5,2	0,6588	7,2	1,6655	9,2	3,3492
1,3	0,0127	3,3	0,1803	5,3	0,6956	7,3	1,7324	9,3	3,4540
1,4	0,0157	3,4	0,1963	5,4	0,7336	7,4	1,8008	9,4	3,5609
1,5	0,0191	3,5	0,2132	5,5	0,7730	7,5	1,8710	9,5	3,6700
1,6	0,0229	3,6	0,2310	5,6	0,8137	7,6	1,9430	9,6	3,7812
1,7	0,0272	3,7	0,2498	5,7	0,8558	7,7	2,0167	9,7	3,8945
1,8	0,0320	3,8	0,2695	5,8	0,8993	7,8	2,0923	9,8	4,0100
1,9	0,0374	3,9	0,2902	5,9	0,9442	7,9	2,1696	9,9	4,1277
2,0	0,0433	4,0	0,3119	6,0	0,9906	8,0	2,2488	10,0	4,2477
2,1	0,0497	4,1	0,3346	6,1	1,0383	8,1	2,3299		
2,2	0,0568	4,2	0,3584	6,2	1,0876	8,2	2,4128		