1

## Supplementary Information

2

## **3 Materials and Methods**

# 4 **Pump-CTD and nutrient measurements**

The pump-CTD system had an approximate water flow of 1.5 1 min<sup>-1</sup> in a FALMAT-hose 5 cable with an outer diameter of 18 and an inner diameter of 6 mm. The pump tube was 6 7 directed into the lab and split into a nutrient and a gas line. Measurements with custom-made STOX-sensors (Unisense, Denmark) revealed a background concentration of 25 nmol/l 8 oxygen originating from the pump-CTD system. Pumping volume was constantly controlled 9 via precision flow-through devices, and delay time between pump inlet and sample outlet was 10 continuously calculated and converted to sampling depth. All samples were measured during 11 lowering of the system with a speed of 1 cm s<sup>-1</sup> (further details in the cruise report: DOI: 12 10.2323/cr msm33). To construct depth profiles from successive casts, data were aligned 13 according to density; for all these profiles the depths showing the respective densities during 14 15 the first cast were used.

16

For continuous nutrient measurements in the outflow of the pump-CTD, the auto-analyzer 17 18 was connected to the water outlet of the pump-CTD system. The injection syringe of the autosampler dipped directly into the core pool of the outflowing water and thereby avoided any 19 20 contact of the samples with laboratory air prior to analysis. This procedure considerably reduced contamination, particularly of ammonium. Sampling time was set to 26 s and 21 washing time to 7 s. As the lowering speed of the pump-CTD was set to 1 cm/s, the vertical 22 resolution of sampling was 33 cm throughout the observed water column, of which 26 cm 23 24 were included in successive analytical pulses and thereby integrated, and 7 cm were lost due to rinsing and blank measurements between the injections. 25

## 27 Nucleic acid extractions

For metagenome analysis 6 l of water were filtered on Sterivex-GV 0.22 µm, PVDF. DNA 28 from frozen Sterivex-filters was extracted using a QIAmp DNA Mini kit (Qiagen, Hilden, 29 30 Germany) after removing the filter from the plastic support. For physical cell disruption, the filter pieces were added to a tube containing lysis buffer and low-binding zirconium beads 31 (200 µm, OPS diagnostics, Lebanon, NJ, USA) and vortexed for 5 min before proceeding 32 with the manufacturer's instructions. The extracted DNA was sent for library preparation 33 (BS80: Illumina TruSeq PCR free library; BS90, BS102, BS115: Rubicon Thruplex library 34 prep) and Illumina HiSeq sequencing to the SciLife Lab (Stockholm, Sweden). For 35 metatranscriptomic analyses from six selected depths, 1.5 l of fixed water samples from the 36 AFIS system were filtered on 0.2 µm polycarbonate filter and stored at -80 °C. RNA from 37 frozen filters was extracted as described elsewhere (1). For downstream absolute 38 quantification of RNA transcripts, standards were added to the lysis buffer before the start of 39 RNA extraction (2). Due to a lysis buffer spill during the extraction of the sample from 111 m 40 water depth, absolute transcript numbers will be slightly overestimated in this sample. DNA 41 leftovers in the RNA extracts were removed using a Turbo DNA-free Kit (Thermo-Fisher 42 Scientific, Waltham, MA USA) and subsequently concentrated and purified using the RNA 43 Clean & Concentrator<sup>TM</sup>-5 Kit (Zymo Research, Irvine, CA, USA). Because positive PCR 44 amplifications with the 16S rRNA specific primers Com1f/Comr2r-Ph (3) indicated the 45 46 presence of DNA traces in the RNA extracts, DNase digestion and subsequent purification was repeated as described above. The purified RNA was sent to Fasteris (Plan-les-Ouates, 47 Switzerland) for Illumina-HiSeq sequencing after rRNA depletion with the RiboZero kit for 48 bacteria (Epicentre, Madison, WI, USA). Library preparation for single reads was performed 49 using the TruSeq stranded mRNA kit (Illumina, San Diego, CA, USA). 50

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- 52

## 53 Bioinformatic processing of Illumina sequence data

Paired Illumina reads from 4 individual metagenome libraries were screened for rRNA-reads 54 using SortMeRNA 1.9 on the default rRNA-databases provided by the program. Remaining 55 non-rRNA-reads were adaptor-clipped using mira 4.0.2 (4) and quality clipped using sickle 56 1.33 (default settings) (5). Remaining intact read pairs from all four libraries were combined 57 and assembled using idba ud 1.1.1 (--mink 25 --maxk 97 --step 18) (6). Contigs shorter than 58 200 nt were discarded. Gene calling was performed using Prodigal's meta-procedure (v2.6.1) 59 (7). CDSs from the Prodigal output were functionally and taxonomically annotated using 60 NCBI's blastp (8) against KEGG and NCBI's NR. Genes of special interest were identified 61 using IHAT as described by Temperton et al. (2011) (9). Single-end metatranscriptome reads 62 were also adaptor- and quality clipped as described above and mapped onto the previously 63 generated metagenome using bowtie2 2.2.4 (10) with the very-sensitive settings. Mapped 64 reads were summarized using featureCounts 1.4.6 (11), requiring a minimum of 20 65 overlapped bases to assign a read to a feature. The number of reads coding for internal 66 standards of the metatranscriptome data were determined in every sample by a LAST search 67 (12) of the non-protein coding reads against a database containing sequence information of 68 the internal standards as well as representative rRNA and tRNA sequences (cutoff score: 69 500). The number of transcripts 1<sup>-1</sup> for Ppk1, Ppk2 and Ppx was estimated as detailed in 70 Satinsky et al. (2013) (2). In short, knowledge of the sequence of internal standards and the 71 72 exact amount of ng internal standard RNA added allowed us to calculate the number of RNA internal standard molecules that were added to each RNA extractions. The recovery rate of 73 the standard molecules in the sequencing data reads can be used to estimate fraction of RNA 74 molecules in the sample that were sequenced and deduce from this value the absolute number 75 of transcripts that were in the filtered water volume. 76

# Analysis of bacterial community composition, probe design and catalysed reporter deposition-fluorescence in situ hybridization

Of the Phenol/Chloroform extracted DNA/RNA mixture, DNA was digested using the Turbo 80 DNA free kit (Ambion). The RNA was transcribed in cDNA utilizing the iScript Select 81 cDNA synthesis kit (Bio-Rad Laboratories GmbH; Munich, Germany). After 16S rRNA gene 82 amplification the amplicons were purified using Agencourt© AMPure ® XP (Becker Coulter) 83 and sent for sequencing. The resulting sequences were analyzed using SILVA NGS 84 (Glöckner et al., 2017) with the settings Min. Align. Identity: 50%; Min. Align. Score: 40; 85 Min. Sequence Quality 30%; Min Length: 200, Max Ambiguties: 2%, Min OTU Identity: 86 97%, Min. Smilarity: 93%. SILVA NGS performs additional quality checks according to the 87 SINA-based alignments (Pruesse et al., 2012) with a curated seed database in which PCR 88 artifacts or non-SSU reads are excluded. The longest read serves as a reference for the 89 taxonomic classification in a BLAST (version 2.2.28+) search against the SILVA SSURef 90 dataset. The classification of the reference sequence of a cluster (97% sequence identity) is 91 then mapped to all members of the respective cluster and to their replicates. Best BLAST hits 92 were only accepted if they had a (sequence identity + alignment coverage)/ $2 \ge 93$  or 93 otherwise defined as unclassified. 94

95

96 CARD-FISH was carried out using the horseradish-peroxidase-labeled probe MaCo983. The 97 competitor probe MaCo983 without horseradish-peroxidase label was applied in parallel. For 98 signal amplification, tyramide labeled with the fluorescent dye carboxyfluoresceine was 99 incubated for 30 min at 37 °C. The filters were embedded in a Citifluor/Vetashield mix (5: 1) 100 containing 4,6-diamidino-2-phenylindole (1  $\mu$ g ml<sup>-1</sup> final concentration). Images were 101 acquired using a 100× Plan-Apochromat objective (Zeiss) and the Zeiss multi-band filter set 102 62HE.

104 Scanning electron microscopy (SEM) and energy dispersive x-ray micro analysis (EDX) Suspended particulate matter in 1 l seawater samples taken from the CTD rosette bottle was 105 filtered on 0.4 µm polycarbonate filters (Millipore) and rinsed with 50 ml purified water to 106 remove salt. After drying at 40 °C for 48 h, the filters were covered (vacuum sputter) with 107 elemental carbon for electrical conductivity. Analysis of carbon in these samples is thus not 108 possible, but this technique is optimal for analyzing all other elements. The identification of 109 minerals after automated particle element analyses is based on boundary values of 110 characteristic elements. These boundary values were defined by analyses of standard minerals 111 112 and adjusted by analyses of samples with known mineral composition. However, besides the additional information about the detected minerals or particle groups, the whole data set for 113 each sample was analyzed with a statistic software (xls; DataDesk) to verify the detected 114 clusters of particles in a "multi element space". Using this method, we were able to 115 characterize the general mineral and particle group composition of the samples and to search 116 for P in different binding forms. 117

118

### 119 Modeling

120 In our modeling approach the differential equation for diffusive transport (Fick's second law):

121 (1)  $\delta c / \delta t = D \cdot \delta^2 c / \delta x^2$ 

122 (D = diffusion coefficient, c = concentration, t = time; x = distance-coordinate), is solved by 123 using the explicit numerical solution:

124 (2) 
$$C_{PO_{d}(x,t+\Delta t)} = C_{PO_{d}(x,t)} + \Delta t \cdot D_{x} \cdot \left(C_{PO_{d}(x+\Delta x,t)} - 2 \cdot C_{PO_{d}(x,t)} + C_{PO_{d}(x-\Delta x,t)}\right) / \Delta x^{2} + \Delta t \cdot Prod_{x}$$

where  $C_{PO_4}$  is the concentration of phosphate at a given place *x* and time *t*,  $D_x$  is the diapycnal diffusivity at the depth *x*, as determined by Gregg and Yakushev (13), *x* is the depth and  $Prod_x$ is a change of concentration at a given depth per time step, caused by the release of phosphate from the particulate phosphorus pool, or visa verse a negative release is an incorporation into the particulate phosphorus pool. When the calculation of all concentrations of a time step is 130 completed, the next time step starts with the final concentrations of the preceding time step. By this approach, the concentration of phosphate with depth is calculated in cell E12-E172 for 131 depths between 80 and 120 m (cell C12-C172) with a  $\Delta x$  of 0.25 m (cell B12). Under starting 132 133 conditions (cell F12-F172), when the model is reset with the switch in cell B10, phosphate increases linearly with depth. This starting condition is without influence on the 134 concentrations in a final steady state. Only the concentration of the first cell (E12) and the 135 rates with which phosphate is released from the particulate pool or incorporated into it (cells 136 G12-G172) determine the final phosphate concentration. These latter rates were manually 137 138 adjusted until they resulted, under steady state, in a profile similar to the measured one. Steady state is reached after approximately 3 years (cell B26). 139

The modelled concentrations of particulate phosphorus originate only from the fluxes into and out of the dissolved pool. These fluxes are adjusted to produce the same shape of the phosphate profile as observed in nature. According to the depth-dependent rates  $Prod_x$ , with which phosphate is released from or incorporated into the particulate pool (cells G12-G172), the concentration of *PartP* (cells H12-H172) changes with each time step, which is expressed by:

 $PartP_{(x,t+\Delta t)} = PartP_{(x,t)} - \Delta t \cdot Prod_{(x)}$ 

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	A	В	С	D	E	F	G	н	1	J	K	L	M	N	0
															antatan Esta II
1	EXPLICIT numerica	i solution for adve	ctive, aispersi	ve/aimusive tra	ansport and s	imple reaction	s (as describ	ea by H.D. Sc	nuiz, în Chapt	er 15: Conce	otual models a	and Compute	r models. In: N	harine Geoche	mistry Eas. H
2															
3	How to use this spre	eadsheet:													
4	1. Set your EXCEL vi	a Tools – Options –	<ul> <li>Calculation to</li> </ul>	Calculation = N	fanual, to Itera	ation = 1 and to	Maximum Iter	ations = 1000							
5	2. Now each pressing	of the F9 key is one	e time step of tl	he explicit num	erical solution										
6	3. An input of '1' into i	position B10 and pre	essing F9 reset	s the spreadsh	eet to the start	conditions									
7	4 An input of '0' into	position B10 and rer	neated pressing	F9 performs t	ne model calc	lation In B26 th	he time since t	the model start	ed is given in v	ears					
8	5 Note the two diagra	ame towarde the rick	nt with A)measu	ured phoenhat	and particula	te P profilee (re	d) and profiles	regulting from	the model (are	and B) flu	v rates used fr	or production a	nd consumptio	n in the modes	
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9					0.004	0.004 -11	Dend		and Destant						
10	Reset = 1	1	X	D	C P04	C PO4_start	Prod	part P	part P start						
11			(m)	m2/s	(mmol/m3)	(mmol/m3)	mmol/m3*s	(mmol/m3)	(mmol/m3)						
12	delta_x =	0.25	80.00	4.00E-06	1.40	1.40	0.00E+00	0.02	0.02					files	
13	dt_max =	833	80.25	3.98E-06	1.43	1.43	0.00E+00	0.02	0.02		wode	ied and mea	asureu pro	nies	
14	delta t =	800	80.50	3.96E-06	1.45	1.45	0.00E+00	0.02	0.02			-	aanhala (um	-1/1	
15			80.75	3.94E-06	1.48	1.48	0.00E+00	0.02	0.02			Ph	osphate (µm	101/1)	
16			81.00	3 93E-06	1.51	1.51	0.00E+00	0.02	0.02		0		5	1	0
17	eink vol (m/e) =	0.000075	81.25	3 91E-06	1.53	1.53	0.00E+00	0.02	0.02		00 ĭ 0		,		°
10	sink_vel (m/d) =	0.000073	01.20	3.912-00	1.55	1.55	0.00E+00	0.02	0.02		80				
10	sink_ver (m/u) -	0.5	01.50	3.09E-00	1.50	1.00	0.00E+00	0.02	0.02		l ä				
19			01./5	3.872-06	1.59	1.59	0.00E+00	0.02	0.02		8				
20			82.00	3.85E-06	1.62	1.62	0.00E+00	0.02	0.02		8	8 8			
21	TIME COUNT:		82.25	3.83E-06	1.64	1.64	0.00E+00	0.02	0.02		N N	8 E	○ Ph	osphate mod	eled
22			82.50	3.81E-06	1.67	1.67	0.00E+00	0.02	0.02		85 - 8	8 E	° Ph	osphate mea	sured
23	t_total(s) =	0	82.75	3.79E-06	1.70	1.70	0.00E+00	0.02	0.02		8	8 H	□Pa	rt P modeler	1
24	t total(h) =	0.00	83.00	3.78E-06	1.72	1.72	0.00E+00	0.02	0.02		8	S 🖬			
25	t total(d) =	0.00	83.25	3.76E-06	1.75	1.75	0.00E+00	0.02	0.02		l 8	8 E	<b>u</b> Pa	n. P measure	a
26	t total(vrs) =	0.0	83.50	3.74E-06	1.78	1.78	0.00E+00	0.02	0.02		8	8 E			
27	,		83.75	3.72E-06	1.80	1.80	0.00E+00	0.02	0.02		de la compañía de la comp	8 H	_		
28			84.00	3 70E-06	1.93	1.92	0.005+00	0.02	0.02		90 - ~	ă 🗄			
20			04.00	3.702-00	1.05	1.00	0.00E+00	0.02	0.02		Š	8 E			
29			04.25	3.002-00	1.00	1.00	0.00E+00	0.02	0.02		8	8 E			
30			84.50	3.00E-00	1.88	1.88	0.00E+00	0.02	0.02		S.	8 H			
31			84.75	3.64E-06	1.91	1.91	0.00E+00	0.02	0.02		18	8 8			
32			85.00	3.63E-06	1.94	1.94	0.00E+00	0.02	0.02		00	8 H			
33			85.25	3.61E-06	1.96	1.96	0.00E+00	0.02	0.02		95 - %	o. 8 🗏			
34			85.50	3.59E-06	1.99	1.99	0.00E+00	0.02	0.02			°0, 8 E			
35			85.75	3.57E-06	2.02	2.02	0.00E+00	0.02	0.02	Ê		~ <u>~</u> & =			
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38			86 50	3 51E-06	2 10	2 10	5.45E-08	0.02	0.02	6		8 H	°•		-
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40			97.00	2 495 06	2.10	2.10	1 405 07	0.02	0.02	ē		월드	്ട		
40			07.00	3.462-00	2.15	2.10	1.42E-07	0.02	0.02	ja ja		1	8		
41			87.25	3.46E-06	2.18	2.18	1.42E-07	0.02	0.02	5		8	<b>– –</b> %		
42			87.50	3.44E-06	2.21	2.21	2.29E-07	0.02	0.02			8	9	<b>k</b>	
43			87.75	3.42E-06	2.23	2.23	3.27E-07	0.02	0.02	·	05 -	18.	8	Ŷ	
44			88.00	3.40E-06	2.26	2.26	4.92E-07	0.02	0.02			B	, ଟ		
45			88.25	3.38E-06	2.29	2.29	5.74E-07	0.02	0.02				ă X		
46			88.50	3.36E-06	2.31	2.31	6.05E-07	0.02	0.02			目	8		
47			88.75	3.34E-06	2.34	2.34	6.05E-07	0.02	0.02				<u>ຊ</u> ຊ <sup>ອ</sup>		
48			89.00	3.33E-06	2.37	2.37	6.05E-07	0.02	0.02		140	目	8 - 8		
49			89.25	3.31E-06	2.39	2.39	5.74E-07	0.02	0.02			E	β <mark>⊔</mark> β		
50			89.50	3.29E-06	2.42	2.42	4.92E-07	0.02	0.02				<u>8</u> 8		
51			89.75	3.27E-06	2 45	2 45	2 29E-07	0.02	0.02			E	<u>8</u> 8		
52			00.00	2 255 06	2.49	2.49	2 805 07	0.02	0.02				S Ø		
50			50.00	3.232-00	2.40	2.40	-2.092-07	0.02	0.02				X X		
03			90.25	3.23E-06	2.50	2.50	-2.09E-07	0.02	0.02	⊢	115 -	日	E ø		
54			90.50	3.21E-06	2.53	2.53	-4.82E-07	0.02	0.02				88		
55			90.75	3.19E-06	2.56	2.56	-5.50E-07	0.02	0.02				1 g		
56			91.00	3.18E-06	2.58	2.58	-7.15E-07	0.02	0.02			目	- <u>8</u> 8		
57			91.25	3.16E-06	2.61	2.61	-7.15E-07	0.02	0.02			目	L 88		
58			91.50	3.14E-06	2.64	2.64	-7.70E-07	0.02	0.02			E	3		
59			91.75	3.12E-06	2.66	2.66	-7.70E-07	0.02	0.02		20				
60			92.00	3.10E-06	2.69	2.69	-8.25E-07	0.02	0.02		0	20		40	60
61			92.25	3.08E-06	2.72	2.72	-8.25E-07	0.02	0.02		v	20		-10	00
62			92.50	3.06E-06	2.74	2.72	-8 25E-07	0.02	0.02			Pa	rticulate P (n	mol/l)	
62			02.50	3.04E 00	2.74	2.74	9.255-07	0.02	0.02						
03			92.15	3.04E-06	2.11	2.11	-0.25E-07	0.02	0.02						
04			93.00	3.03E-06	2.80	2.80	-0.25E-07	0.02	0.02		-				
1 65			93.25	3.01E-06	2 82	2.82	-8 25E-07	0.02	0.02						

150 Screenshot of the model after reset:

151

When the model is reset to time zero (as explained in the head of the spreadsheet) the 152 particulate phosphorus pool *PartP* is set to a concentration of 20 nmol l<sup>-1</sup> at all depths, which 153 is the concentration measured directly above the suboxic zone. Over time the transport of 154 phosphorus from the dissolved into the particulate pool alone, without a removal of 155 particulate phosphorus, would result in *PartP* concentrations orders of magnitude higher than 156 observed in nature. Therefore, the model contains a function, which removes particulate 157 phosphorus by sinking of particles. For this, a certain amount of PartP in each cell oh H12-158 H172 is moved to the cell below with a sinking velocity, *sinkvel* (entered in cell B17 in m s<sup>-1</sup>) 159 by adding the term: 160

161 (4) + 
$$(PartP_{(x-\Delta x,t)} \cdot sinkvel \cdot \Delta t / \Delta x - PartP_{(x,t)}) \cdot sinkvel \cdot \Delta t / \Delta x$$

- 162 The sinking velocity in the model was adjusted to result in similar particulate phosphorus
- 163 levels as observed in nature.

## 164 Screenshot of the model at steady state after 3 years:



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166

In this overall approach, the dissolved phosphate concentrations, which we measured 167 accurately and with high vertical resolution, is the starting point of the model and the 168 modelled values follow closely the measured concentrations. The modelled values for 169 particulate phosphorus, which we determined with much lower resolution and accuracy, only 170 roughly represent the measured concentrations. Principally, this problem could be addressed, 171 e.g., by applying changing sinking velocities with depth, but unless more precise data on the 172 distribution of particulate phosphorus are available, this would not improve our overall 173 understanding of the system. 174

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Further examples for phosphorus rich particles with chains of magnetite from the suboxic zone of the Black Sea. The approximate diameter of the spherical cells (4.6 - 6.6 µm) are given below.



- 221 Identification of magnetosomes from the suboxic zone of the Black Sea by EDX analysis. (A)
- A chain of magnetosomes within a cell. (**B**) Spectrum of a magnetite crystal showing the high iron content.

223 iron 224



- 229 Chains of magnetites detected in sediment traps from the redoxcline in the Gotland Basin of
- the Baltic Sea. Similar chains were also observed in samples taken from the redoxcline.



#### 

# **Figure 4**

Example for the specific staining of large spherical cells with the probe specific for *Magnetococcus* related bacteria in samples from the Black Sea. (A) All microbial cells stained with 4,6-Diamidin-2-phenylindol (DAPI). (B) Cells hybridized with the probe specific for *Magnetococcus* related bacteria.



Maximum likelihood tree of the abdundant sequences derived from the Black Sea samples 269 affiliated to the Magnetococcaceae and related sequences from the ARB Silva database. A 270 core tree based on 1274 unambiguously aligned sequence positions of the full-length 271 sequences from the database was calculated and short sequences added without changing the 272 tree toplogy. Magnetococcus marinus MC-1 was set as outgroup. Original sequence 273 definitions in the GenBank database were replaced with a consistent nomenclature including 274 accession number, name and for the sequences from this study the number of reads detected. 275 Bold sequences indicate the sequences with a 100% match to MaCo983. Short 16S rRNA 276 277 sequences that were used for the phylogenetic analysis are deposited in the European Nucleotide Archive (ENA) under accession numbers LT960764-LT960776. 278



# 282 Table 1

283 Concentration of total dissolved phosphorus in µmol/l determined by ICP-OES and phosphate

determined colorimetrically at different water depths.

Depth (m)	diss P (µM)	Phosphate (µM)
72	1.6	1.3
77	1.6	1.4
87	1.3	1.3
90	0.9	0.7
92	0.2	0.0
96	2.2	1.7
99	4.6	4.0
103	6.7	6.9

286

# 287 **Table 2**

288 Relative abundance of particles rich in iron (>15%), rich in manganese (>10%), and rich in P

289 (>20%) in % of all particles.

290

Depth (m)	Fe-rich (%)	Mn-rich (%)	P-rich(%)
72	3.3	57.7	0.0
87	6.8	41.9	1.1
90	3.3	60.3	0.2
95	17.8	29.3	13.5
100	28.6	6.8	2.9
103	25.1	40.3	18.1
110	34.2	0.7	0.2
118	15.8	0.2	0.1

291

292

# 293 **Table 3**

294 Concentration of phosphorus, iron and manganese in the particulate pool determined by ICP-

295 OES in nmol/l.

Depth (m)	P <sub>part</sub> (nM)	Fe <sub>part</sub> (nM)	Mn <sub>part</sub> (nM)
72	21.8	34.3	45.2
87	20.8	33.4	28.5
90	29.4	25.3	47.7
95	48.6	28.6	6.7
100	52.6	31,8	5.3
103	29.9	24.5	15.6
110	25.2	22.7	5.1
118	24.4	21.1	2.0