Bacterial colonization and vertical distribution of marine gel particles (TEP and CSP) in the Arctic Fram Strait

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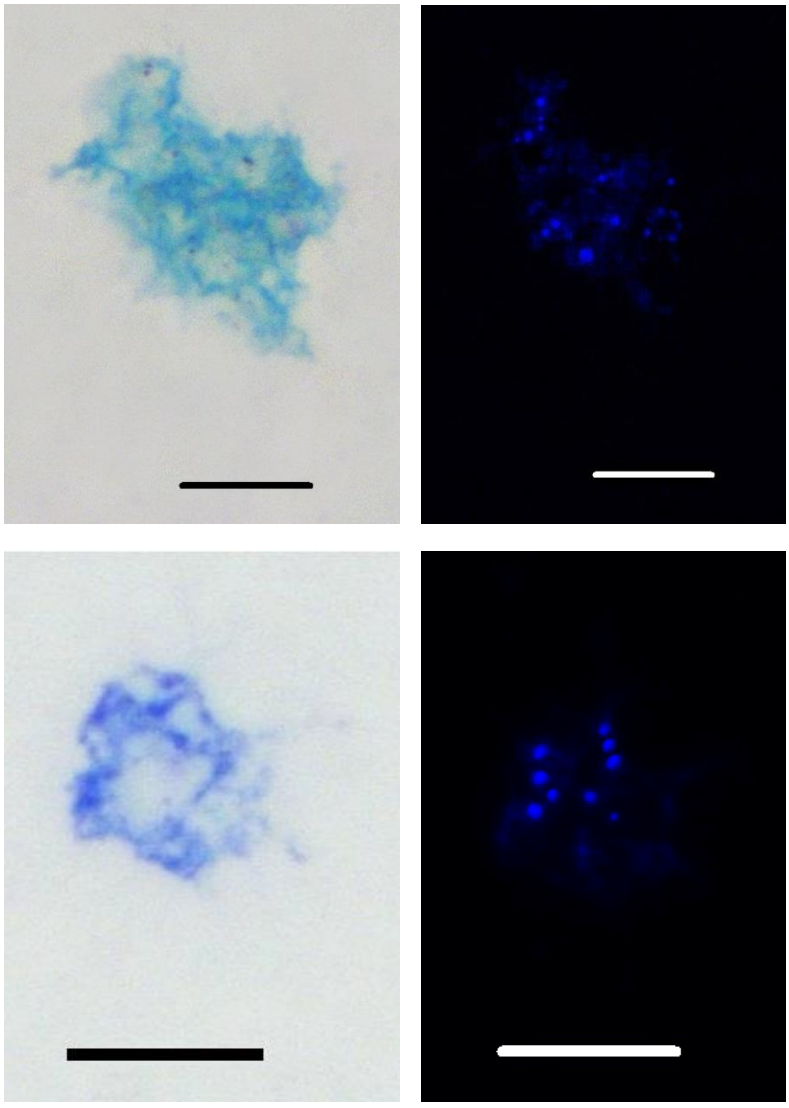
Supplementary material

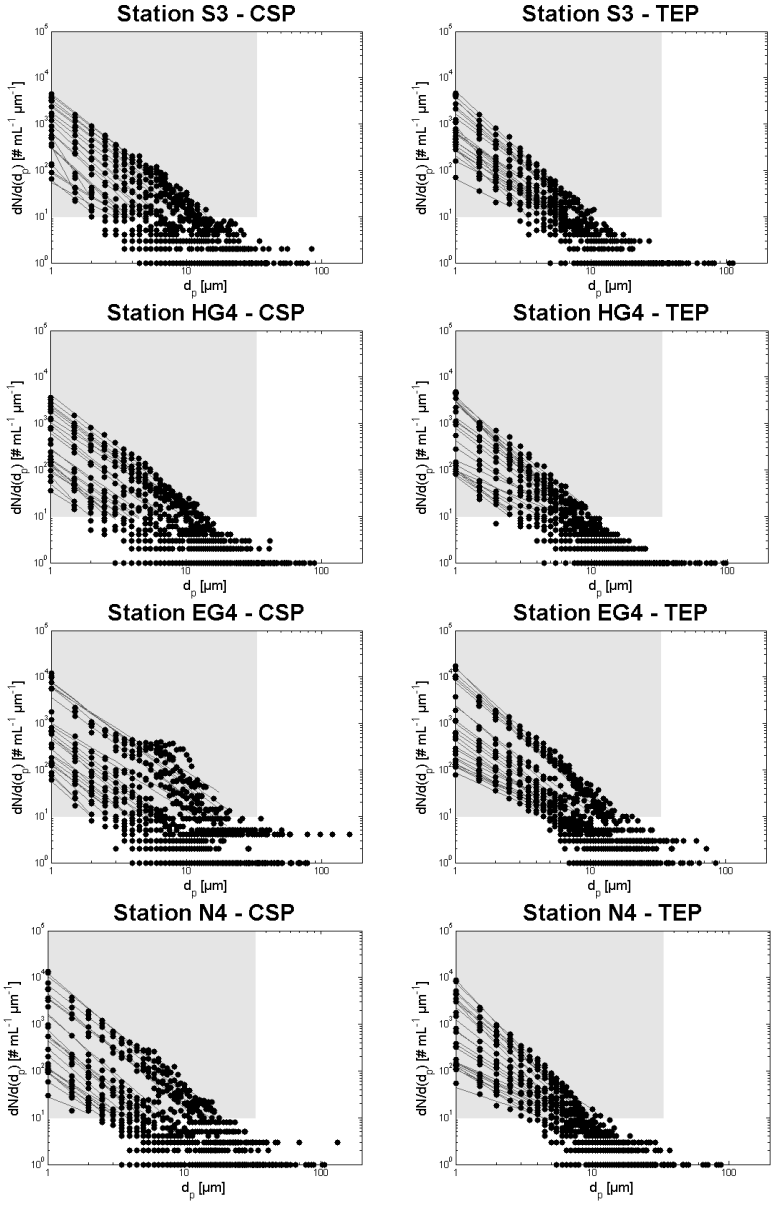
Table S1 Station list including the coordinate positions and exact sampling depths for all sampled stations.

Station Name	PS93.00	Date	Time [UTC+1]	Sampling Depths [m]	Position Lat	Position Lon
S3 (deep)	48-1	24.07.2015	08:05:00	250,500,1000,1500,2000,2284	78° 35.96' N	5° 04.13' E
S3 (shallow)	48-7	24.07.2015	15:59:00	5,15,30,50,75,100	78° 35.94' N	5° 04.10' E
HG4 (deep)	50-3	25.07.2015	22:20:00	250,500,1000,1500,2000,2409	79° 03.93' N	4° 10.72' E
HG4 (shallow)	50-7	26.07.2015	04:16:00	5,15,30,50,75,100	79° 03.91' N	4° 10.74' E
EG4 (deep)	58-1	30.07.2015	13:02:00	250,500,1000,1500,2000,2526	78° 50.07' N	2° 47.95' W
EG4 (shallow)	58-8	30.07.2015	20:41:00	5,10,20,30,50,100	78° 51.69' N	2° 42.56' W
N4 (deep)	63-1	04.08.2015	03:18:00	250,500,1000,1500,2000,2613	79° 44.18' N	4° 29.45' E
N4 (shallow)	64-4	04.08.2015	16:47:00	5,15,27,40,75,100	79° 49.30' N	4° 15.22' E

S2 Exemplary micrographs. Upper pannel : TEP stained with Alcian Blue (left) and associated bacteria stained with DAPI (right). Lower pannel: CSP stained with Coomassie Brilliant Blue G (left) and associated bacteria stained with DAPI (right). Scale bars in all micrographs represent 10µm (left micrographs: bright-field microscopy; right micrographs: wide-field fluorescence microscopy).



S3 Size frequency distributions for both gel particle classes (CSP and TEP) at the different stations on a log-log scale. In each plot the frequencies (which were extrapolated to [mL^{-1} μm^{-1}]) of the different ESD size classes (d_p) are plotted for both replicates and all 12 depths. The first size class represents 1µm. Size classes covered always a range of 0.5µm each. Although the maximal particle sizes, which were only measured occasionally, are also depicted in the plot (white area), only the size range covered by the grey shaded area was taken to fit the regression. Resulting regression lines ($dN/d[d_p]=kd_p^{\delta}$) are indicated by grey lines and their calculated coefficients δ are shown in *Table S7*.



S4 Detailed description of the digital image processing to determine total abundances of bacteria per gel particle (area).

The workflow of the developed image analysis procedure to determine total abundances of bacteria per gel particle (area) will be described in the following for one exemplary filter spot (i.e. for one of the ten filter sectors captured per filter).

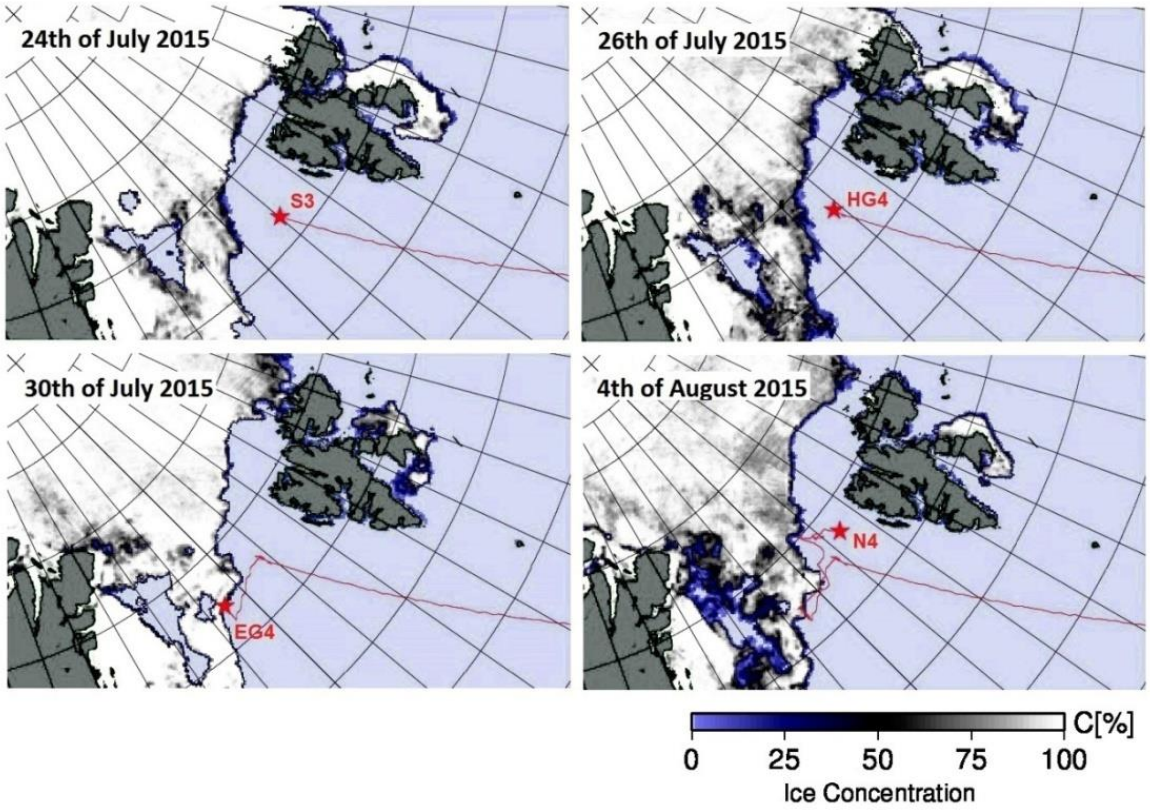
In a first step both micrographs -taken from the same filter spot- were simultaneously openend in the image analysis software *Image J (version 1.48)*. For the bright-field micrograph, depicting stained gel particles, the red color channel was chosen. For the wide-field fluorescence micrograph, depicting bacterial signals, the blue channel was chosen. On the bright-field micrograph the particles were thresholded and the particle parameters (routinously area, perimeter, major, minor and feret) were measured and recorded by the software. Afterwards, continuing in *Image J*, a bounding rectangle (i.e. the smallest rectangle enclosing the particle) was created around each thresholded particle in the bright-field micrographs. For these bounding rectangles the x- and y-coordinates of the upper left corner (x1 and y1), as well as the height and the width, were recorded into a text file (after applying a coordinate system with an inverse y-axis on the micrographs). Subsequently the perimeter(s) of all thresholded particle(s) were selected on the bright-field micrograph and the selection was transferred to the corresponding wide-field fluorescence micrograph. On the wide-field fluorescence micrograph, all maxima (i.e. the most intensely DAPI stained spots) were detected within the selection. Those maxima were counted and considered to represent the number of all bacterial cells attached to the thresholded particles, as the two pictures show exactly the same filter position. Before counting the maxima, an individual noise tolerance level was set to verify that the maxima supposed by the software correspond to real bacterial signals, and to furthermore account for varying signal intensities on the analysed micrographs. Besides counting the maxima, also the centroids (which are the center points of the DAPI signals) were determined and the coordinates of those points (X and Y) were recorded into a second text file. Then, using *MATLAB (version R2011a)*, the information of the two micrographs was combined. Both priorly produced text files were loaded and the second x- and y- coordinates (x2 and y2) of the bounding rectangles were calculated. Then it was tested for each bounding rectangle and all DAPI signal centroids, whether the X- and Y- coordinates of the DAPI signals' centroid were falling into a bounding rectangles coordinate range (i.e. if: $x1 < X > x2$ and $y1 < Y > y2$). If this was true, a 1 was written into a set up matrix. If the condition did not apply, a 0 was printed at the according place into the matrix. After checking all possible combinations, the columns of the matrix were summed up to a vector which represented the number of counted DAPI signals per bounding rectangle (i.e. the number of bacteria on a particle with a known area). The final output was summed up in an *EXCEL* sheet. As high amounts of micrographs required an automatisation of the image analysis procedure, a macro for *Image J* and and a *MATLAB* routine were developed. Those scripts provided a complete automatisation of all steps described priorly - except from the particle thresholding and the DAPI signal noise tolerance leveling, which has to be manually adjusted.

S5 Sea ice maps calculated from AMSR2 data using the ARTIST sea ice algorithm (ASI 5.2), which has been validated in several studies (Spreen et al., 2008). The figure presented here is modified after http://www.iup.uni-bremen.de:8084/amsr2/. Red lines show course plot of RV Polarstern, red stars indicate the ship's position on the respective sampling day at 1:00 pm (UTC).

Thanks to...

Spreen G, Kaleschke L, Heygster G. Sea ice remote sensing using AMSR-E 89 GHz channels. *J Geophys Res* (2008) **113**:C02S03, doi:10.1029/2005JC003384.

...for providing the sea ice data.

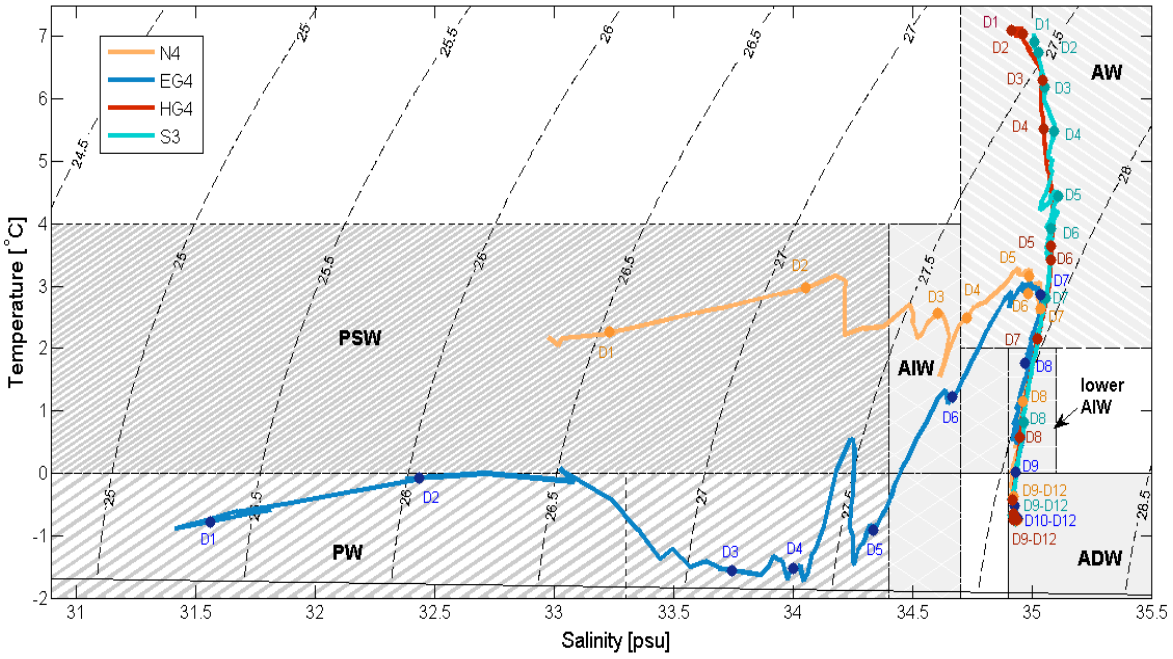


S6 TS-diagram. Colored lines show data at the four stations from CTD casts over the whole water column, dots indicate sampled depths (D1-D12). Filled boxes indicate watermasses after Aksenov et al. (2010): PW (Polar water), PSW (Polar surface water), AW (Atlantic water), AIW (Arctic Intermediate water), ADW (Arctic Deep water). In the plot density lines (sigma theta, σ_θ = [kg/m³]) and a freezing point line are indicated.

Thanks to...

Tippenhauer S, Torres-Valdes S, Wisotzki A. Physical oceanography measured on water bottle samples during POLARSTERN cruise PS93.2 (ARK-XXIX/2.2). *Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Bremerhaven* (2016), doi:10.1594/PANGAEA.863808

...for providing the T-S data.



Reference:

Aksenov Y, Bacon S, Coward AC, Holliday NP. Polar outflow from the Arctic Ocean: A high resolution model study. *J Mar Sys* (2010) **83**:14–37.

Table S7 Size frequency distribution coefficients δ for both gel particle types, at all stations and all depths. Numbers represent means of the two replicate samples, standard deviation is indicated after ±. NA means that standard deviation was not calculable due to one missing replicate. Depths for each station are shown in meters.

CSP

S3			HG4			EG4			N4		
Depth			Depth			Depth			Depth		
5	-2.37	± 0.10	5	-2.33	± 0.02	5	-2.59	± 0.01	5	-2.28	± 0.14
15	-2.22	± 0.05	15	-2.28	± 0.06	10	-2.52	± 0.23	15	-2.51	± 0.23
30	-2.32	± 0.17	30	-2.32	± 0.04	20	-2.30	± 0.44	27	-2.72	± 0.25
50	-2.32	± 0.07	50	-1.89	± 0.02	30	-2.03	± 0.42	40	-2.64	± 0.11
75	-2.60	± 0.09	75	-2.30	± 0.22	50	-2.22	± 0.15	75	-2.50	± 0.48
100	-2.70	± NA	100	-2.14	± 0.17	100	-2.26	± 0.01	100	-2.44	± 0.09
250	-2.53	± 0.20	250	-2.37	± 0.24	250	-2.28	± 0.42	250	-2.39	± 0.06
500	-2.66	± 0.03	500	-2.25	± 0.29	500	-2.14	± 0.18	500	-2.13	± 0.48
1000	-2.40	± 0.70	1000	-2.57	± 0.78	1000	-2.21	± 0.03	1000	-2.32	± 0.35
1500	-2.10	± 0.34	1500	-2.53	± 0.33	1500	-2.50	± 0.22	1500	-1.93	± 0.17
2000	-2.28	± 0.86	2000	-1.75	± 0.06	2000	-2.27	± 0.28	2000	-1.60	± 0.65
2284	-4.82	± 0.44	2409	-2.42	± 0.34	2526	-2.63	± 0.14	2613	-2.08	± 0.07

TEP

S3			HG4			EG4			N4		
Depth			Depth			Depth			Depth		
5	-2.75	± 0.09	5	-2.78	± 0.09	5	-2.63	± 0.02	5	-2.70	± 0.48
15	-2.71	± 0.08	15	-2.96	± 0.16	10	-2.77	± 0.00	15	-2.99	± 0.18
30	-2.56	± 0.00	30	-2.74	± 0.07	20	-2.97	± 0.04	27	-2.82	± 0.10
50	-2.16	± 0.11	50	-2.42	± 0.08	30	-2.57	± 0.01	40	-2.69	± 0.18
75	-2.13	± 0.25	75	-2.23	± 0.04	50	-2.12	± 0.07	75	-2.46	± 0.05
100	-2.01	± 0.04	100	-2.21	± 0.02	100	-2.32	± 0.17	100	-2.10	± 0.02
250	-2.12	± 0.11	250	-2.23	± 0.07	250	-2.13	± 0.12	250	-1.86	± 0.05
500	-2.21	± 0.12	500	-2.14	± 0.13	500	-1.82	± 0.18	500	-1.67	± 0.15
1000	-2.36	± 0.47	1000	-1.73	± 0.11	1000	-1.40	± 0.12	1000	-1.62	± 0.08
1500	-1.77	± 0.41	1500	-2.09	± 0.65	1500	-1.42	± 0.04	1500	-1.35	± 0.38
2000	-1.99	± 0.28	2000	-1.77	± 0.06	2000	-1.73	± 0.18	2000	-1.41	± 0.04
2284	-2.72	± 1.45	2409	-1.38	± 0.09	2526	-1.62	± 0.07	2613	-1.61	± 0.51

S8 Multi-focus stack (created from several pictures taken while focussing through the aggregate) of two aggregates derived from the Marine Snow Catcher, stained with Alcian Blue (left) or Coomassie Brilliant Blue G (right). The scale bar, which is only valid for each enlarged section, represents 10µm. The total aggregate length of the left aggregate was measured to be 410µm, the total aggregate length of the right aggregate was measured to be 520µm. Micrographs were taken by bright-field microscopy.

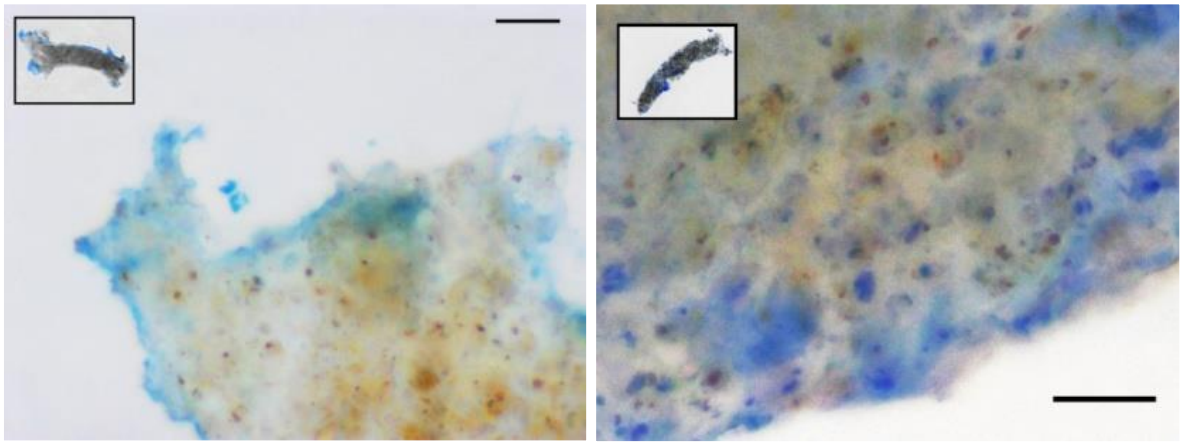


Table S9 Size (of particle) - frequency (of bacteria) distribution coefficients *a* and *b* for both gel particle types (TEP and CSP) over depth at all four stations (S3, HG4, EG4, N4). Numbers in brackets after 'TEP' or 'CSP' indicate water depths in meters. Depths indicated with '~' represent median between the four stations (as sampling depths in the euphotic zone were slightly adjusted at each station to sample the chlorophyll maximum and its gradient). '>2000m' refers to depths below 2000m.

	S 3		HG 4		EG 4		N 4	
Particle type	a	b	a	b	a	b	a	b
TEP (5m)	0.372	1.167	0.880	0.611	0.680	0.908	1.929	0.236
TEP (~15m)	1.345	0.401	1.236	0.354	0.724	0.720	0.407	1.046
TEP (~30m)	1.039	0.625	0.784	0.605	1.655	0.304	0.350	1.025
TEP (~45m)	0.838	0.821	0.557	0.846	0.801	0.740	0.567	0.741
TEP (~75m)	0.907	0.793	1.485	0.420	0.871	0.756	1.471	0.349
TEP (100m)	1.172	0.686	0.978	0.577	2.090	0.154	0.294	1.013
TEP (250m)	0.664	0.966	0.681	0.581	1.545	0.352	1.695	0.248
TEP (500m)	0.786	0.757	2.635	-0.066	0.791	0.798	0.887	0.614
TEP (1000m)	2.479	0.304	0.696	0.783	0.803	0.586	1.908	0.107
TEP (1500m)	1.921	0.110	1.168	0.356	0.875	0.632	0.837	0.616
TEP (2000m)	0.137	1.311	0.000	4.924	0.336	0.951	1.380	0.338
TEP (>2000m)	1.136	0.257	1.140	0.407	1.191	0.331	0.713	0.608
CSP (5m)	0.822	0.754	0.266	1.068	1.076	0.493	1.896	0.233
CSP (~15m)	0.908	0.808	0.341	0.966	0.888	0.474	0.648	0.659
CSP (~30m)	1.291	0.452	0.393	1.084	0.133	1.397	5.687	-0.141
CSP (~45m)	0.348	0.999	0.093	1.372	0.919	0.499	1.810	0.245
CSP (~75m)	2.829	-0.036	1.063	0.289	1.174	0.413	0.155	1.231
CSP (100m)	1.542	0.299	1.563	0.201	1.448	0.375	0.810	0.541
CSP (250m)	0.962	0.504	2.115	0.180	1.261	0.405	4.630	-0.158
CSP (500m)	1.810	0.203	2.000	0.000	1.000	0.377	0.097	1.269
CSP (1000m)	2.999	-0.005	0.649	0.620	1.901	0.181	2.000	0.000
CSP (1500m)	2.705	0.040	4.327	-0.221	2.740	-0.066	1.451	0.217
CSP (2000m)	0.907	0.645	0.005	2.490	1.854	0.135	2.000	0.000
CSP (>2000m)	7.545	-0.423	1.532	0.214	12.340	-0.477	1.249	0.288