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Comparison of two methods of measuring the depth of the redox potential discontinuity in intertidal mudflat sediments

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ABSTRACT: Measurements of the depth of the redox potential discontinuity (RPD) are becoming progressively more important as a promising variable to evaluate the ecological status of many benthic environments. Two methods are commonly used to measure depth of the RPD in intertidal habitats: (1) visually, often by digital imaging (aRPD); or (2) with redox potential electrodes coupled to a millivolt meter. However, since both measurements of RPD may focus on different attributes of the sediment, it remains uncertain whether they yield comparable results, especially in dynamic intertidal sediments. We used a paired design to compare RPD values derived from the 2 methods on 9 intertidal mudflats in the Bay of Fundy, Canada, over 2 yr. When all samples were examined together, there was a highly significant difference between the 2 methods. Furthermore, this difference was not consistent over space and time. Our findings clearly demonstrate that aRPD and RPD measurements cannot be compared directly, hampering between-study comparison.

KEY WORDS: Redox potential · Apparent redox potential discontinuity · Sediment colour change · Redox potential discontinuity · Intertidal mudflats · Bay of Fundy

INTRODUCTION

The transition from oxygenated to anoxic conditions in sediments is commonly termed the redox potential discontinuity (RPD). More accurately, the RPD marks the boundary between predominantly oxidizing and reducing conditions (Fenchel & Riedl 1970, Lyle 1983, Sturdivant et al. 2012). Depth of the RPD can vary extensively in time and space (Vorenhout et al. 2004, 2011, Hunting et al. 2012) and is influenced by particle size, temperature, wave action, organic matter input, photosynthesis, light intensity (Fenchel & Riedl 1970), dissolved oxygen, bacterial activity (Hunting et al. 2012, Hunting & Kampfraath 2013), and the presence of burrowing animals (Diaz & Cutter 2001, Solan & Kennedy 2002, Sturdivant et al. 2012). Measurements of depth of the RPD have recently garnered increased interest as they relate to the composition of benthic invertebrate communities, and ecosystem processes such as decomposition rates (Fenchel & Riedl 1970, McLachlan 1978, Valdemarsen et al. 2010b). Therefore, depth to the RPD may be a promising variable to evaluate the ecological status of many benthic environments (Birchenough et al. 2012).

Depth of the RPD is commonly measured using one of 2 methods: (1) visually, often by digital imaging (Munari et al. 2003, Trites et al. 2005, Westhead 2005); or (2) with redox potential electrodes coupled to a millivolt meter (Rosenberg et al. 2001, Wildish et al. 2001, Diaz & Trefry 2006). The visual or digital imaging method relies on the assumption that in the absence

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of oxygen, ferrous sulfides produced by microbial sulfate reduction precipitates as Fe-sulfides, which produces a grey/green or black coloration of the sediment (Lyle 1983, Bull & Taillefert 2001, Bull & Williamson 2001, Valdemarsen et al. 2009). The RPD is located where the sediment changes colour (Munari et al. 2003, Teal et al. 2009, Valdemarsen et al. 2009, Sturdivant et al. 2012), and when redox measurements (Eh) are not considered simultaneously, the RPD is termed the apparent RPD (aRPD) (Solan et al. 2004, Teal et al. 2009, Birchenough et al. 2012). This approach requires the visual examination of sediment *in situ*, in sediment cores (Trites et al. 2005, Valdemarsen et al. 2010a), or in the case of sediment profile imaging (SPI), insertion of cameras into the sediment (Nilsson & Rosenberg 2000, Teal et al. 2010, Birchenough et al. 2012). On the other hand, redox potential (Eh) measurements represent a bulk measurement that reflects the occurrence of multiple redox equilibria at the surface of the electrode (Teal et al. 2009, Teal et al. 2010). Eh is expressed in mV and reflects a system's tendency to receive or donate electrons. Electrodes are inserted either vertically or horizontally at different depths (Fenchel et al. 1975, Rosenberg et al. 2001, Diaz & Trefry 2006) into the sediment or sediment core. The depth of the RPD is identified as the zone where conditions change from oxidizing to reducing (Whitfield 1969, Fenchel & Riedl 1970, Wilding 2012). The majority of studies measuring Eh arbitrarily define the RPD as the transition from positive to negative mV readings (Fenchel & Riedl 1970, Revsbech et al. 1980, Birchenough et al. 2012, Hunting et al. 2012).

Since both methods of measuring the RPD may focus on different attributes of the sediment, it is uncertain whether both methods yield comparable RPD depths. Two studies (Rosenberg et al. 2001, Diaz & Trefry 2006) compared *in situ* aRPD values derived from SPIs with Eh measurements, and demonstrated that both methods correlated fairly well. However, both studies were conducted in the relatively stable subtidal (Rosenberg et al. 2001) and deep sea sediments (Diaz & Trefry 2006). It remains uncertain whether both methods would yield similar results when studying the relatively dynamic intertidal sediments. A direct comparison between both methods in intertidal sediments over a large spatial and temporal scale is thus required. Therefore, using a paired design, we measured aRPD and redox potential (Eh) at several locations on 9 intertidal mudflats over a 2 yr period. We then evaluated whether the 2 methods yielded similar depths of the RPD and how the match changed over both space and time.

MATERIALS AND METHODS

This study was conducted on 9 intertidal mudflats in the upper Bay of Fundy, Canada, between May 2009 and March 2011, as part of a larger study on mudflat community dynamics. The sites were Mary's Point (MP), Daniels Flats (DF), Grande Anse (GA), Pecks Cove (PC), and Minudie (MN) in Chignecto Bay; as well as Moose Cove (MC), Noel Bay (NB), Avonport (AV), and Starr's Point (SP) in Minas Basin (see map in Einfeldt & Addison 2013). Each mudflat represented a unique combination of biotic and abiotic conditions (T. G. Gerwing unpubl. data). Mudflat sediments consisted mostly of silt. Volume-weighted mean particle size, measured in the top 5 cm of the sediment in July 2009, ranged from 13 to 55 µm depending on the mudflat and depth layer (T. G. Gerwing, J. Murray, M. A. Barbeau unpubl. data); except for one sampling location at Avonport (for a 2–3 cm depth layer), where the mean was 112 µm. At each mudflat, 2 transects (800 to 1800 m long depending on the intertidal width of the mudflat) were established perpendicular to the low water line. Transects were separated by 500 to 2000 m. Each transect was stratified into 4 equal zones based on transect length. We visited mudflats near low tide every 3 to 8 wk (for a total of 19 sampling rounds), and in each sampling round we randomly selected one location (a plot) within each zone for data collection. Thus, 8 data points were collected per site, 72 per sampling round, and 1368 in total. We visually measured (to the nearest 0.5 cm using a ruler) the depth of the aRPD in the void left behind by removing a 7 cm diameter sediment core (collected for a related study). The transition from light-coloured to darker (green/grey or black) sediments was defined as the aRPD. Over the course of this study, 6 different observers collected data using the visual method. Observers were trained in advance to ensure consistency. As close as possible (1 to 5 cm) to the void left by the corer, we took one redox profile. An 11 cm long silver/platinum electrode (Orion Combination Metal Electrodes, 9778-BNWP, ThermoFisher Scientific) was used with a field redox meter (EcoScan pH6, EC-PH6/02K, ThermoFisher Scientific). The electrode was inserted vertically into the sediment in 1 cm increments to a depth of 10 cm. Readings at each depth interval were allowed to stabilize (Whitfield 1969) before being recorded. Stabilization time varied from seconds (usually) to ~1 h (rarely). Inclusion or exclusion of the 6 data points which took over an hour to stabilize did not change our results. The electrodes were cleaned between profiles and sampling rounds (Whitfield

1969, Wildish et al. 2004), and were replaced every 1 to 4 mo. Temperature readings were also taken (using a digital meat thermometer) for each depth interval, and redox values were corrected to the standard hydrogen potential (Whitfield 1969, Hargrave 1972, Hargrave et al. 1997). The RPD was defined as the transition from positive to negative Eh values (Fenchel & Riedl 1970, Wildish et al. 1999, Wildish et al. 2004). Missing data (467 out of the 1368 total) resulted when the field redox meter was damp and ceased to work (372 points), or the RPD was below the depth measurable by our electrode (140 points). These cases were omitted from analysis. A paired *t*-test (with the remaining 901 data pairs) was used to test the null hypothesis that there was no difference between the 2 methods. An ANOVA using the difference between methods (Eh – aRPD) as the dependent variable was then used to determine if differences varied through space (Site) and time (Round). All factors were random, and a variance component analysis was performed (Searle et al. 1992). To ensure an orthogonal design for analysis, sampling rounds which did not include all sites were omitted from the ANOVA (there were 685 data points for this analysis).

RESULTS

Results from the visual and electrode methods of determining depth of the RPD differed significantly (t = 7.0, df = 900, $p < 0.0001$), with the electrode method producing a slightly greater average depth (4.0 cm) than the visual method (3.3 cm). The electrode method measured a deeper depth of the RPD in 55.9% of cases, while the visual method gave a deeper depth in 33.4% of cases. Absolute differences between the 2 methods varied from 0 to 14 cm, with an average of 2.4 cm. The most common difference was 1 cm, while 27% of samples differed by 3 cm or more, and 10% differed by 6 cm or more (Table 1, Fig. 1, Appendix 1). An absolute difference of 14 cm between methods was obtained once, when the Eh values indicated the RPD was at the surface, and the aRPD was observed 14 cm into the sediment.

The difference between the 2 methods varied inconsistently with site and round (significant Site × Round interaction; Table 2). Variation was greatest (66% of the random variation) at our smallest spatial scale (from one plot to another). Temporal variation, related to round and the interaction between round and site, was also substantial. Variation at our largest spatial scale, site, was less important (4% of the random variation).

Table 1. Frequency distribution (counts and percent) of the absolute differences between 2 methods (aRPD and Eh) of measuring the depth of the redox potential discontinuity

Difference (cm)	Frequency	%	Cumulative %
0	87	9.7	9.7
1	262	29.1	38.7
2	177	19.6	58.4
3	134	14.9	73.3
4	96	10.7	83.9
5	54	6.0	89.9
6	36	4.0	93.9
7	25	2.8	96.7
8	10	1.1	97.8
9	11	1.2	99.0
10	7	0.8	99.8
11	1	0.1	99.9
12	0	0.0	99.9
13	0	0.0	99.9
14	1	0.1	100.0

DISCUSSION

We compared 2 commonly used methods of determining the RPD depths in intertidal sediments. The methods yielded different results, and this difference varied over time and space (Tables 1 & 2, Fig. 1, Appendix 1). The differences often exceeded 5 cm, which is substantially greater than the µm to cm scales relevant to the biota residing in intertidal sediments (e.g. bacteria, algae, polychaetes, and amphipods).

The observed differences are likely due to the fact that the 2 methods measure different aspects of the aquatic environment. Redox (Eh) electrodes measure the instantaneous redox potential of the sediment, which can be very dynamic (Lyle 1983, Grenthe et al. 1992, Valdemarsen et al. 2010b). In contrast, the visual method represents an integrated long term average of redox conditions (aRPD). Reduction and oxidization of ferric sulfides, the primary reaction responsible for sediment color change, does not occur instantly (Jørgensen & Fenchel 1974, Lyle 1983, Grenthe et al. 1992, Valdemarsen et al. 2010b). Consequently, sediment colour change may lag behind the more dynamic redox potential (Lyle 1983, Teal et al. 2009, Teal et al. 2010), resulting in discord between the 2 methods. Thus, the aRPD does not reflect the RPD in the same way as determined by Eh values (Birchenough et al. 2012), suggesting that RPD values cannot be compared among studies when different methods are used.

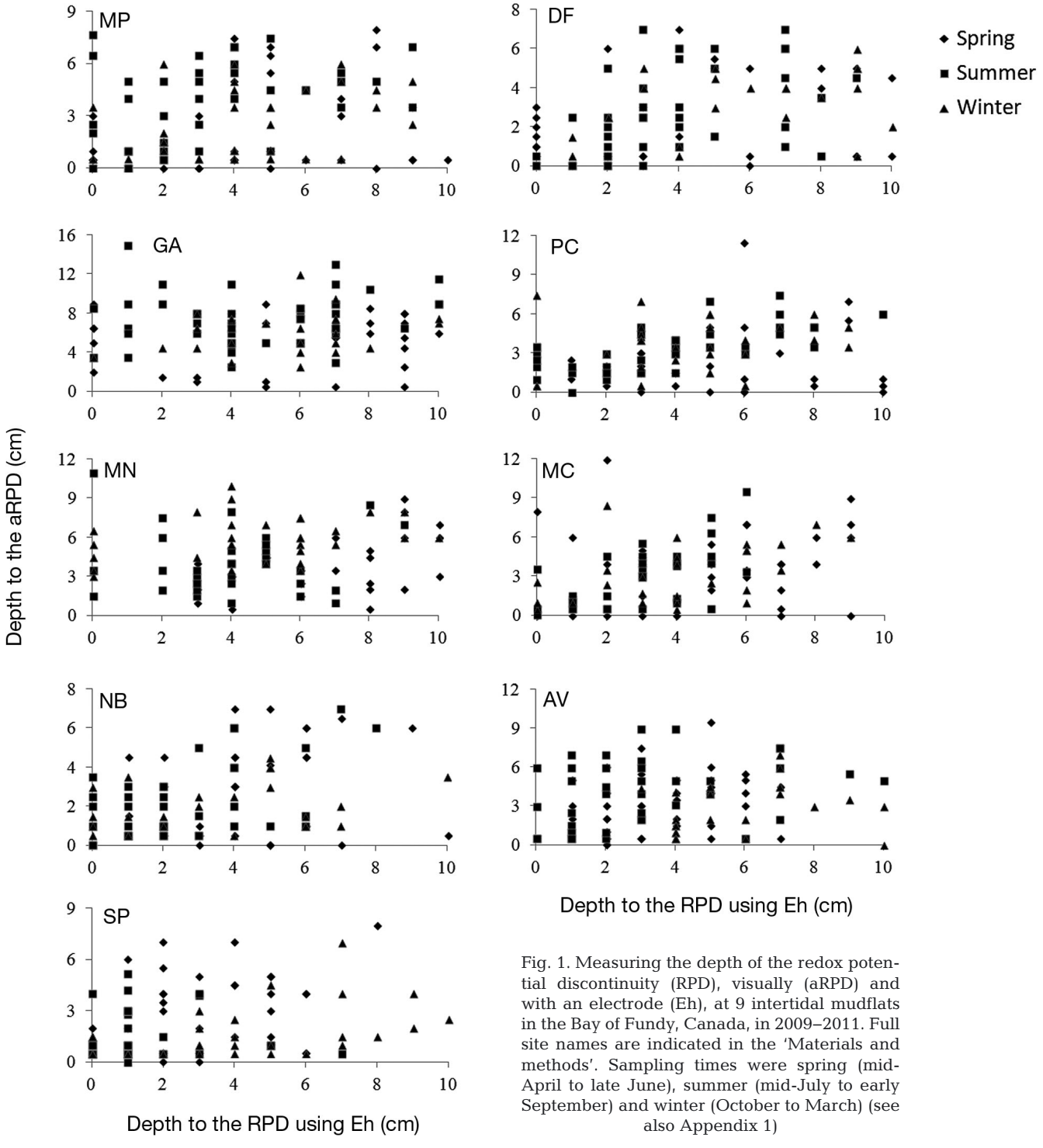

Fig. 1. Measuring the depth of the redox potential discontinuity (RPD), visually (aRPD) and with an electrode (Eh), at 9 intertidal mudflats in the Bay of Fundy, Canada, in 2009–2011. Full site names are indicated in the 'Materials and methods'. Sampling times were spring (mid-April to late June), summer (mid-July to early September) and winter (October to March) (see also Appendix 1)

Rosenberg et al. (2001) and Diaz & Trefry (2006) found good correlation between their aRPD depths as measured by SPI, and RPD depths obtained with electrodes inserted horizontally into sediment cores. SPI images are typically digitally enhanced and analyzed, allowing very accurate determination of the depth of the aRPD. The visual approach in our study, lacking image capture and enhancement, did not achieve the same level of accuracy. However, as the aRPD was striking, and the sediments (predomi-

Table 2. Random-factor ANOVA evaluating how the difference between the visual (aRPD) and electrode methods (Eh) of measuring depth of the redox potential discontinuity varied through space and time. Variance components indicate the contribution of each source of variation to the overall variation in the differences between methods

Source of variation	df	MS	*F*	p	Variance components Estimate	%
Round	10	116.1	8.40	<0.001	1.74	17.8
Site	8	43.7	3.17	0.003	0.42	4.3
Round × Site	80	14.2	2.19	<0.001	1.12	11.5
Error (i.e. plot)	586	6.5			6.48	66.4

nantly silt) were relatively stable in our study sites, we are confident that our methodology provided quality measures of the depth of the aRPD. Rosenberg et al. (2001) and Diaz & Trefry (2006) also performed their studies in relatively stable subtidal and deep sea environments, while our study was performed in an intertidal environment. Intertidal sediments are influenced by dynamic variables such as wave action, air and sunlight exposure, and tidal currents. Any or all of these variables could explain the inter- and intra-study differences. In addition, we used a single electrode pushed vertically into the sediment *in situ,* over a large spatio-temporal scale. Diaz & Trefry (2006) and Rosenberg et al. (2001), on the other hand, used several electrodes inserted horizontally into extracted sediment samples in short term experiments over a smaller spatial scale. These methodological differences may also have contributed to the observed inter-study differences. This highlights the need for greater standardization of methods to allow inter-study comparisons.

In our study, the difference between the methods varied through time and space (Table 2), and we were unable to identify any specific spatial or temporal trends in the difference between the 2 methods. As discussed above, the 2 methods are likely measuring processes operating at different spatio-temporal scales, and this precludes the use of a 'correction factor' that could be applied to facilitate comparisons among methods. We, therefore, suggest that such comparisons should be avoided. Future studies should carefully consider which method suits the study objective. Based on our findings, and considering the documented precision and accuracy issues with redox measurements (ZoBell 1946, Wildish et al. 2004), we suggest that measuring Eh may not be appropriate for all studies. However, if the values are not intended to be electrochemically exact, or the margin of error is acceptable for the given purpose, then Eh measurements may capture the dynamics of the instantaneous redox potential. Recently developed Eh data loggers and permanently installed electrodes may offer a considerable advance in obtaining these measurements (Vorenhout et al. 2004, van der Geest & León Paumen 2008, Vorenhout et al. 2011). However, their applicability is limited at large spatial scales. In contrast, as sediment color change may lag behind redox reactions, the visual method (aRPD) may represent a more integrated average of redox reactions. This method may be more applicable to situations in which a long term averaged measure of the redox conditions is required. However, the potentially large margin of error and inter-observer variation associated with the visual method (aRPD) suggests that caution should also be used when applying this method. The use of SPI, however, enables researchers to enhance images and reduce observer bias, thereby enhancing the ability to compare between studies.

Knowledge about the depth of the RPD is important in many aquatic studies. By influencing the chemistry of aquatic sediments, the RPD greatly influences composition and density of benthic communities, as well as ecosystem processes such as decomposition rates (Fenchel & Riedl 1970, McLachlan 1978, Valdemarsen et al. 2010b). Information on the depth of the RPD is useful for understanding and evaluating not only the current state of aquatic sites, but also how and why sites vary in time and space. We showed that 2 different methods commonly used to derive RPD depths in intertidal habitats produce substantially different results. This difference varied in both time and space, and likely results from the different mechanisms that underlie the measurements. While RPD/aRPD measurements may offer valuable information, our results clearly demonstrate that aRPD and Eh RPD measurements cannot be compared directly, especially in dynamic sediment situations, hampering between-study comparisons.

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Appendix 1. Mean ± SE (n = 3 to 8) depth of the redox potential discontinuity (RPD, cm) measured using 2 methods: visual identification of the apparent RPD depth (aRPD), and depth measured using a field redox meter coupled to a platinum silver electrode. These data, for 9 intertidal mudflats in the Bay of Fundy over 2 yr, are those used in our ANOVA (Table 2), which represents an orthogonal subset of the total data set used in the paired *t*-test (Fig. 1). A sampling round took 4 to 5 d to complete (since only 2 randomly chosen mudflats could be sampled per day, one per sampling team); the date in the table is the start of the sampling round. See 'Materials and methods' for full site names

Date	Electrode	aRPD	Electrode	aRPD	Electrode	aRPD
	MP		DF		GA	
13 Jul 2009	4.13 ± 0.89	5.56 ± 0.41	5.13 ± 0.83	5.25 ± 0.27	6.43 ± 1.13	8.29 ± 0.57
30 Aug 2009	5.00 ± 0.71	4.80 ± 0.34	6.50 ± 0.87	3.00 ± 0.61	5.57 ± 0.78	6.79 ± 0.36
14 Apr 2010	4.50 ± 1.12	0.38 ± 0.13	1.43 ± 0.57	0.86 ± 0.40	5.50 ± 2.10	6.25 ± 0.32
11 May 2010	4.00 ± 0.76	0.21 ± 0.15	3.00 ± 0.58	0.50 ± 0.19	8.75 ±0.25	6.50 ± 1.37
31 May 2010	6.57 ± 0.90	2.29 ± 0.88	6.25 ± 1.15	2.38 ± 1.01	5.60 ± 1.54	3.50 ± 1.86
22 Jun 2010	0.75 ± 0.49	1.13 ± 0.42	0.00 ± 0.00	1.38 ± 0.35	4.57 ± 0.90	2.71 ± 0.70
14 Jul 2010	0.88 ± 0.35	1.50 ± 0.53	1.00 ± 0.33	0.19 ± 0.13	2.86 ± 0.83	5.71 ± 0.70
03 Aug 2010	1.63 ± 0.18	1.06 ± 0.32	2.75 ± 0.45	1.25 ± 0.33	3.88 ± 0.81	7.00 ± 1.31
26 Aug 2010	5.00 ± 1.05	4.13 ± 0.83	4.00 ± 0.80	3.18 ± 0.88	5.13 ± 1.01	7.56 ± 1.14
15 Oct 2010	3.00 ± 0.38	2.19 ± 0.70	4.25 ± 1.01	2.75 ± 0.68	5.25 ± 1.25	6.25 ± 0.59
4 Dec 2010	3.75 ± 0.53	1.56 ± 0.55	4.71 ± 1.02	2.43 ± 0.51	5.29 ± 0.52	7.36 ± 1.11
	PC		MN		MC	
13 Jul 2009	5.43 ± 0.48	4.50 ± 0.49	5.00 ± 3.00	8.00 ± 0.50	3.57 ± 0.72	3.31 ± 0.73
30 Aug 2009	5.17 ± 0.91	3.75 ± 0.42	5.00 ± 0.41	4.13 ± 0.72	3.00 ± 1.15	2.67 ± 0.88
14 Apr 2010	4.50 ± 1.09	2.38 ± 0.55	6.50 ± 1.55	4.75 ± 0.43	3.00 ± 0.82	5.57 ± 0.58
11 May 2010	6.14 ± 1.06	0.57 ± 0.28	7.25 ± 1.55	2.38 ± 0.55	6.29 ± 0.78	1.43 ± 0.69
31 May 2010	7.75 ± 1.03	1.75 ± 1.75	7.50 ± 0.72	2.75 ± 0.94	5.57 ± 0.72	2.64 ± 0.54
22 Jun 2010	3.88 ± 0.77	1.56 ± 0.27	5.17 ± 0.54	2.50 ± 0.30	3.63 ± 1.03	3.41 ± 1.44
14 Jul 2010	0.88 ± 0.40	1.63 ± 0.38	3.00 ± 0.89	2.13 ± 0.33	1.75 ± 0.49	1.00 ± 0.39
03 Aug 2010	2.75 ± 0.56	2.38 ± 0.38	3.63 ± 0.42	2.81 ± 0.34	4.00 ± 0.54	5.50 ± 0.85
26 Aug 2010	6.00 ± 0.85	4.50 ± 0.67	4.38 ± 0.50	4.75 ± 0.68	2.29 ± 0.64	1.89 ± 0.63
15 Oct 2010	3.63 ± 0.65	4.13 ± 0.65	3.88 ± 1.27	4.44 ± 0.40	4.86 ± 0.71	3.79 ± 0.70
4 Dec 2010	3.75 ± 0.41	4.56 ± 0.61	4.50 ± 0.54	6.63 ± 0.59	6.80 ± 0.86	5.00 ± 0.94
	NB		AV		SP	
13 Jul 2009	2.25 ± 0.65	1.75 ±0.63	3.67 ± 0.33	7.03 ± 1.97	1.43 ± 0.43	2.87 ± 0.68
30 Aug 2009	1.80 ± 0.48	1.67 ± 0.33	3.63 ± 0.87	4.44 ± 0.76	1.88 ± 0.93	1.31 ± 0.42
14 Apr 2010	5.40 ± 0.98	4.50 ± 0.47	4.63 ± 0.57	4.13 ± 0.21	4.86 ± 0.34	4.71 ± 0.41
11 May 2010	5.80 ± 1.24	2.20 ± 1.19	4.13 ± 0.64	2.00 ± 0.46	3.00 ± 0.93	2.06 ± 1.10
31 May 2010	4.50 ± 0.62	0.17 ± 0.11	2.29 ± 0.47	1.14 ± 0.42	4.00 ± 0.50	0.81 ± 0.25
22 Jun 2010	3.29 ± 0.68	2.73 ± 0.95	2.38 ± 0.38	2.25 ± 0.75	1.63 ± 0.42	1.31 ± 0.41
14 Jul 2010	0.50 ± 0.29	0.88 ± 0.72	1.75 ± 0.62	4.50 ± 0.91	0.88 ± 0.23	0.94 ± 0.33
03 Aug 2010	3.63 ± 0.78	1.44 ± 0.33	1.75 ± 0.37	2.75 ± 0.79	1.88 ± 0.55	1.38 ± 0.48
26 Aug 2010	1.50 ± 0.33	1.19 ± 0.25	2.00 ± 0.66	1.50 ± 0.49	0.63 ± 0.18	0.94 ± 0.06
15 Oct 2010	5.25 ± 1.65	3.00 ± 0.46	5.38 ± 0.53	2.56 ± 0.41	5.25 ± 0.84	1.38 ± 0.25
4 Dec 2010	5.71 ± 0.42	2.36 ± 0.53	7.50 ± 0.50	4.50 ± 0.54	6.67 ± 0.62	3.58 ± 0.94

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