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Realistic measurement uncertainties for marine macronutrient measurements conducted using gas segmented flow and Lab-on-Chip techniques

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Abstract: Accurate and precise measurements of marine macronutrient concentrations are fundamental to our understanding of biogeochemical cycles in the ocean. Quantifying the measurement uncertainty associated with macronutrient measurements remains a challenge. Large systematic biases (up to 10 %) have been identified between datasets, restricting the ability of marine biogeochemists to distinguish between the effects of environmental processes and analytical uncertainty. In this study we combine the routine analyses of certified reference materials (CRMs) with the application of a simple statistical technique to quantify the combined (random + systematic) measurement uncertainty associated with marine macronutrient measurements using gas segmented flow techniques. We demonstrate that it is realistic to achieve combined uncertainties of ~1-4 % for nitrate + nitrite (ΣNOx), phosphate (PO_4^{3-}) and silicic acid ($\text{Si}(\text{OH})_4$) measurements. This approach requires only the routine analyses of CRMs (i.e. it does not require inter-comparison exercises). As CRMs for marine macronutrients are now commercially available, it is advocated that this simple approach can improve the comparability of marine macronutrient datasets and therefore should be adopted as 'best practice'.

Novel autonomous Lab-on-Chip (LoC) technology is currently maturing to a point where it will soon become part of the marine chemist's standard analytical toolkit used to determine marine macronutrient concentrations. Therefore, it is critical that a complete understanding of the measurement uncertainty of data produced by LoC analysers is achieved. In this study we analysed CRMs using 7 different LoC ΣNOx analysers to estimate a combined measurement uncertainty of < 5%. This demonstrates that with high quality manufacturing and laboratory practices, LoC

analysers routinely produce high quality measurements of marine macronutrient concentrations.

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Dear Editor,

Re: Realistic measurement uncertainties for marine macronutrient measurements conducted using gas segmented flow and Lab-on-Chip techniques

We submit the above named manuscript for your consideration for publication in virtual special issue on Marine Analysis in the journal Talanta. The manuscript presents new and previously unpublished data and is not submitted for consideration for publication elsewhere.

Our manuscript details a novel statistical approach for the quantification of analytical uncertainty associated with measurements of marine macronutrient concentrations. Quantifying realistic analytical uncertainties for marine macronutrient measurements remains a significant challenge for marine chemists, and underestimation has previously led to errors in the interpretation of global macronutrient datasets [1]. In our approach we utilize commercially available CRMs and a simple statistical method to incorporate both systematic and random sources of uncertainty into our estimates of analytical uncertainty. In doing so we demonstrate that it is possible to achieve combined uncertainties as small as 1.2 %. We recommend that this simple, cost effect approach should be adopted as best practice by the oceanographic community and that combined uncertainties should be reported alongside marine macronutrient concentrations.

Our manuscript includes data generated by traditional gas segmented flow techniques and novel Lab-on-Chip analyzers. Lab-on-Chip (LoC) technology can be installed on moorings and autonomous vehicles, thus greatly enhancing our capability to measure marine macronutrient concentrations. LoC technology is currently maturing to a point that it is routinely being used to elucidate environmental processes [e.g. 2, 3]. It is therefore imperative that we assess the measurement quality of data produced by LoC analyzers. Here we conducted an inter-comparison and demonstrate that LoC measurements are directly comparable with those made using gas segmented flow techniques. Moreover, we apply our novel statistical approach to data produced by 7 individual nitrate + nitrite analyzers. In doing so we demonstrate, that with trained analysts and high quality manufacturing, it is possible to achieve combined uncertainties of < 5%.

The following suggested reviewers are experts in the field of marine biogeochemistry and particularly in the determination of macronutrient concentrations, and thus would be well placed to provide a critical assessment of our manuscript.



Yours sincerely,

Dr Antony Birchill
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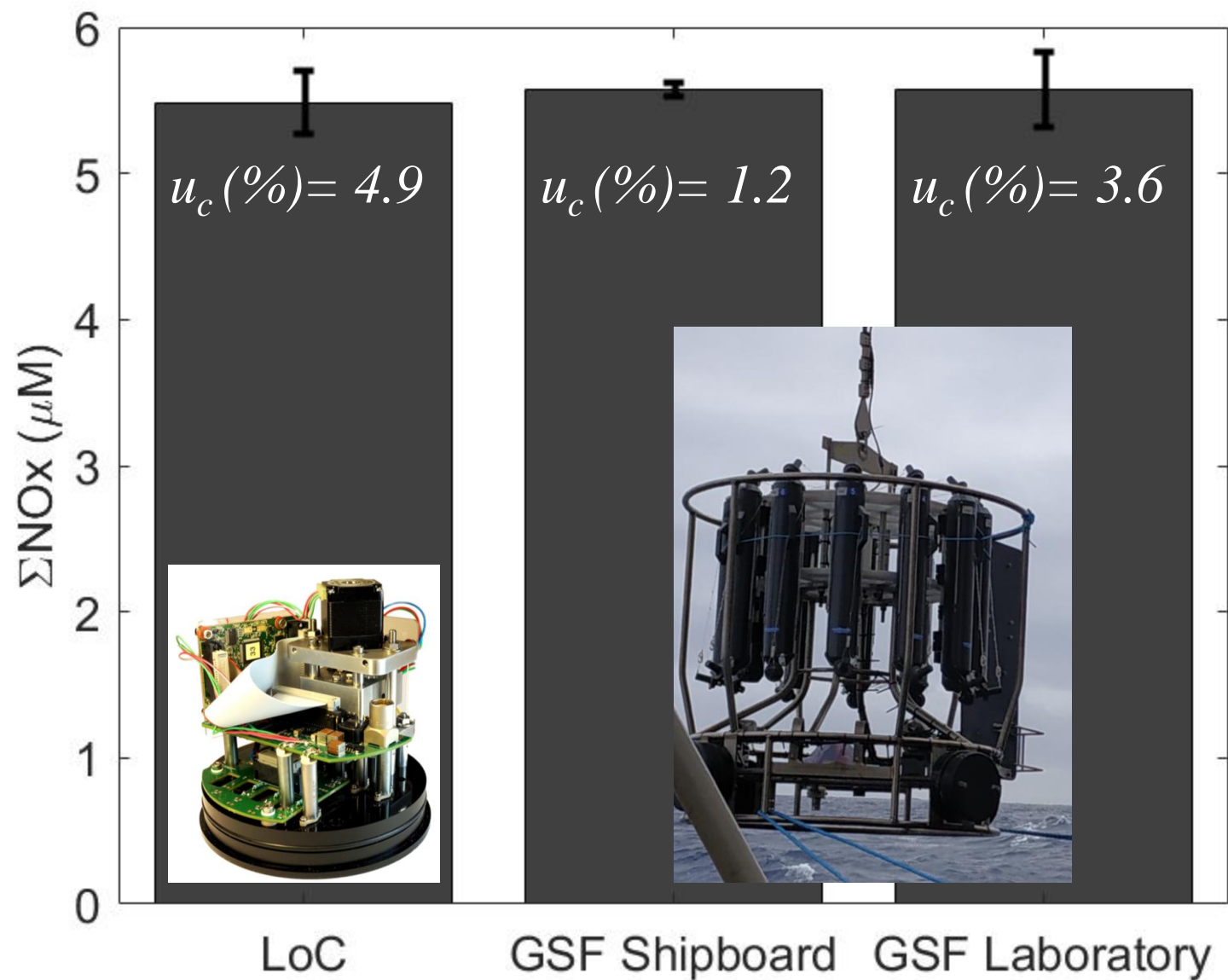
1. Gouretski, V.V. and K. Jancke, *Systematic errors as the cause for an apparent deep water property variability: global analysis of the WOCE and historical hydrographic data*. Progress in Oceanography, 2000. **48**(4): p. 337-402.

2. Yücel, M., et al., *Nitrate and nitrite variability at the seafloor of an oxygen minimum zone revealed by a novel microfluidic in-situ chemical sensor*. PloS one, 2015. **10**(7): p. e0132785.
3. Vincent, A.G., et al., *Nitrate drawdown during a shelf sea spring bloom revealed using a novel microfluidic in situ chemical sensor deployed within an autonomous underwater glider*. Marine Chemistry, 2018.

Quantifying realistic analytical uncertainties for marine macronutrient measurements remains a significant challenge for marine chemists, and underestimation has previously led to errors in the interpretation of global macronutrient datasets. We present a simple statistical approach for quantifying the combined measurement uncertainty associated with marine macronutrient measurements, we apply this to traditional gas segmented flow and novel Lab-on-Chip techniques.

*Highlights (for review)

1. Accounting for systematic bias is required for a realistic analytical uncertainty
2. Gas segmented flow techniques achieved a combined uncertainties of 1-4 %
3. Lab-on-Chip nitrate + nitrite analysers achieved a combined uncertainties < 5%



1 **Realistic measurement uncertainties for marine macronutrient** 2 **measurements conducted using gas segmented flow and Lab-on-Chip** 3 **techniques**

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17
18 Keywords: macronutrient, nitrate, phosphate, intercomparison, measurement uncertainty, Lab-on-Chip,
19 segmented flow.

20 **Abstract**

21 Accurate and precise measurements of marine macronutrient concentrations are fundamental to our
22 understanding of biogeochemical cycles in the ocean. Quantifying the measurement uncertainty associated
23 with macronutrient measurements remains a challenge. Large systematic biases (up to 10 %) have been
24 identified between datasets, restricting the ability of marine biogeochemists to distinguish between the
25 effects of environmental processes and analytical uncertainty. In this study we combine the routine analyses
26 of certified reference materials (CRMs) with the application of a simple statistical technique to quantify the
27 combined (random + systematic) measurement uncertainty associated with marine macronutrient
28 measurements using gas segmented flow techniques. We demonstrate that it is realistic to achieve combined
29 uncertainties of ~1-4 % for nitrate + nitrite (ΣNO_x), phosphate (PO_4^{3-}) and silicic acid ($\text{Si}(\text{OH})_4$)
30 measurements. This approach requires only the routine analyses of CRMs (i.e. it does not require inter-
31 comparison exercises). As CRMs for marine macronutrients are now commercially available, it is advocated
32 that this simple approach can improve the comparability of marine macronutrient datasets and therefore
33 should be adopted as ‘best practice’.

34 Novel autonomous Lab-on-Chip (LoC) technology is currently maturing to a point where it will
35 soon become part of the marine chemist’s standard analytical toolkit used to determine marine macronutrient
36 concentrations. Therefore, it is critical that a complete understanding of the measurement uncertainty of data
37 produced by LoC analysers is achieved. In this study we analysed CRMs using 7 different LoC ΣNO_x
38 analysers to estimate a combined measurement uncertainty of < 5%. This demonstrates that with high
39 quality manufacturing and laboratory practices, LoC analysers routinely produce high quality measurements
40 of marine macronutrient concentrations.

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42 Introduction

43 Marine primary production sustains commercial fisheries [1] and influences atmospheric carbon
44 dioxide concentrations [2]. The biomass of marine primary producers is comprised of a suite of nutrients,
45 1 which must be acquired from surrounding seawaters. The regulatory role that the availability of nutrients in
46 2 seawater has upon marine primary production is well established [3]. In particular, the low availability of
47 3 nitrogen and phosphorus is known to limit primary production in much of the open ocean [4, 5]. In contrast,
48 4 anthropogenic perturbation of nitrogen and phosphorus cycles has resulted in eutrophic conditions in some
49 5 coastal waters, leading to an increase in the occurrence of harmful algal blooms [6] and regions of oxygen
50 6 deficiency termed 'dead zones' [7].

51 9 In order to understand and quantify the processes leading to oligotrophy and eutrophy, marine
52¹⁰ chemists routinely determine the concentration of nitrite + nitrate (hereafter ΣNO_x), soluble reactive
53¹¹ phosphorus (hereafter PO_4^{3-}) and silicic acid (hereafter $\text{Si}(\text{OH})_4$) in seawater. Collectively these inorganic
54¹² species are referred to as macronutrients and are considered an 'essential ocean variable' by The Global
55¹³ Ocean Observing System (<http://www.goosocean.org/>). The most common method of detection used to
56¹⁴ determine macronutrient concentrations in seawater is spectrophotometry. Spectrophotometry relies on the
57¹⁵ formation of a coloured dye, whereby the intensity of the dye is proportional to concentration of the analyte
58¹⁶ of interest. The 'Beer-Lambert-Bouguer' law is then used to relate the absorbance of light by the dye to the
59¹⁷ concentration of the analyte in solution. The Griess test is the most widely used technique for the
60¹⁸ determination of NO_3^- [8-10]. The Griess reagent contains two chemicals, sulphanilamide and N-(1
61¹⁹ naphthyl)ethylenediamine; under acidic conditions NO_2^- converts sulphanilamide to a diazonium ion that
62²⁰ readily couples with N-(1 naphthyl)ethylenediamine to form a coloured azo dye. The intense red/pink colour
63²¹ is measurable at ~520-550 nm. To detect ΣNO_x , any nitrate must first be reduced to nitrite. This is typically
64²² achieved by passing the sample through a copper-coated cadmium reduction column. 'Molybdenum blue' is
65²³ the most widely used technique for the determination of PO_4^{3-} and $\text{Si}(\text{OH})_4$ [10-15]. Orthophosphate and
66²⁴ molybdate react in an acidic medium to form 12-molybdophosphoric acid, which is then reduced to
67²⁵ phosphomolybdate blue by ascorbic acid. The intense blue colour formed is measurable at ~700 or ~880 nm.
68²⁶ A similar approach is typically adopted to measure $\text{Si}(\text{OH})_4$, whereby $\text{Si}(\text{OH})_4$ and molybdate react in an
69²⁷ acidic medium to form the silicomolybdic acid, which is then reduced to silicomolybdate blue. The intense
70²⁸ blue colour is measurable at ~810 nm, with a smaller peak observed at ~600-660 nm.

71²⁹ Traditionally, macronutrient concentrations are determined following manually sampling of
72³⁰ seawater samples; water is collected at known times and depths and then preserved for laboratory analysis
73³¹ on board ship or on land. Spectrophotometric detection has been combined with gas segmented continuous
74³² flow techniques to become the most common method of macronutrient analysis in seawater [13, 16]. This
75³³ allows for the analysis of large numbers (100s) of samples per day, which is typically required during
76³⁴ research cruises. The requirement for high sample throughput means that even short term analytical
77³⁵ uncertainties are often not reported for individual macronutrient measurements (i.e. the sample is analysed
78³⁶ once rather than in triplicate). Increased automation has led to a decrease in measurement quality [17] and
79³⁷ analyses of marine macronutrient concentrations reported at cross-over stations (i.e. a location at which two
80³⁸ research cruise tracks cross and seawater was sampled at the same geographic location) indicated that
81³⁹ systematic biases of up to 10% can exist between datasets [18, 19]. Systematic bias is the difference between
82⁴⁰ the estimated value and the 'true' value, and neglecting systematic bias can lead to an underestimation of
83⁴¹ analytical uncertainty [20-24].

84⁴² An approach to account for systematic bias in marine macronutrient datasets is to use the observed
85⁴³ offset in concentrations reported at cross-over stations or reference climatology datasets to 'adjust'
86⁴⁴ macronutrient concentrations [17-19]. In surface waters, seasonal processes have large impacts on
87⁴⁵ macronutrient concentrations [e.g. 25], restricting this approach to deep waters where inorganic nutrient
88⁴⁶ concentrations are more stable and typically elevated due to the remineralisation of sinking organic matter.
89⁴⁷ In addition, this approach requires pre-existing data in the first instance, which can be problematical in
90⁴⁸ under-sampled remote ocean regions [17, 18], and if there is a pre-existing bias in the historic dataset then
91⁴⁹ the mean, and subsequent adjustments, are off-set from the true value. Moreover, there always exists a
92⁵⁰ danger of over correcting and removing features that result from environmental processes. For instance,
93⁵¹ comparisons are typically made between water masses; comparisons are made between seawater samples
94⁵² with a similar density rather than simply those collected at a similar depth. Hydrographic fluctuations can

95 introduce natural variability in deep water nutrient concentrations. The Atlantic Ocean for instance is
96 influenced by Antarctic Bottom Water containing high $\text{Si}(\text{OH})_4$ concentrations and by Mediterranean
97 Outflow Water that has different nutrient concentrations to other Atlantic water masses with a similar
98 density. Therefore applying an adjustment to regions where the prevalence of these waters masses varies
99 requires a larger tolerance for natural variation [18].

100 Producing realistic uncertainty estimates for marine macronutrient data remains a challenge for
101 marine chemists. The 4th Intergovernmental Panel on Climate Change report stated that ‘‘ *Uncertainties in*
102 *deep ocean nutrient observations may be responsible for the lack of coherence in the nutrient changes.*
103 *Sources of inaccuracy include the limited number of observations and the lack of compatibility between*
104 *measurements from different laboratories at different times’’ [26]. A current aim of The Scientific
105 Committee on Oceanic Research (SCOR) working group 147 (<https://scor-int.org/group/147/>) and
106 Optimising and Enhancing the Integrated Atlantic Ocean Observing Systems (AtlantOS;
107 <https://www.atlantos-h2020.eu/>) is to improve the comparability of global nutrient data. Efforts to achieve
108 this aim include the continuation of ongoing laboratory inter-comparison exercises [27-31] and updating the
109 best practice manuals for making marine nutrient measurements [e.g. 16]. A unifying recommendation of
110 the inter-comparison exercises was that seawater macronutrient certified reference materials (CRMs) be
111 developed and routinely analysed in order to improve nutrient data comparability. Stable CRMs for marine
112 nutrients are now commercially available [e.g. 32, 33], providing a powerful tool to assess systematic bias
113 [34].*

114 The oceanographic community is currently experiencing the development of novel Lab-on-Chip
115 (LoC) microfluidic analysers with the capability to measure ΣNO_x and PO_4^{3-} at nanomolar concentrations
116 [e.g. 35, 36, 37]. Microfluidic technology allows miniaturisation of existing chemical analytical methods,
117 thus LoC analysers can be deployed on moorings and mobile platforms [38, 39]. Consequently, LoC
118 analysers have the potential to greatly enhance our ability to sample the environment, and by measuring *in-*
119 *situ*, remove the need to preserve collected samples [40, 41]. LoC nutrient analysers have been deployed
120 with the aim of elucidating the environmental processes governing nutrient distributions [38, 39, 42],
121 moving them from developmental stages to routine scientific use. Consequently, it is critical that a concerted
122 effort is made to ensure that we understand the analytical uncertainty associated with data produced by LoC
123 analysers.

124 The aim of this communication is to present the application of a simple statistical approach for
125 quantifying the combined (random uncertainty + systematic bias) measurement uncertainty of marine
126 nutrient measurements made using gas segmented flow techniques and novel LoC platforms. This approach
127 utilises commercially available CRMs and requires no costly inter laboratory comparisons or cross-over
128 stations. Moreover, it accounts for short term and intermediate sources of random measurement uncertainty
129 (e.g. changing laboratory conditions, difference reagent batches, different analysts) and systematic bias.

130 **Materials and Methods**

131 A detailed description of analytical methods can be found in the supporting information. Standard gas
132 segmented flow techniques with spectrophotometric detection were used for the determination of ΣNO_x ,
133 PO_4^{3-} and $\text{Si}(\text{OH})_4$ [11, 13]. The spectrophotometric methods used in all techniques were the Griess (for
134 ΣNO_x) and molybdenum blue (for PO_4^{3-} & $\text{Si}(\text{OH})_4$) assays. ΣNO_x measurements for both standard gas
135 segmented flow techniques require that NO_3^- is reduced to NO_2^- by passing the solution through a copper
136 coated cadmium column.

137 The LoC analysers used in this study have been described in detail elsewhere [35-37, 43]. Briefly, LoC
138 analysers are composed of a three layer poly(methyl methacrylate) chip with precision milled micro
139 channels (150 μm wide, 300 μm deep), mixers and optical components consisting of Light Emitting Diodes
140 and photodiodes. Electronics, valves and syringe pumps are mounted on the chip, which is encased in a dark
141 water tight PVC tube. In addition, the ΣNO_x analyser has an off-chip copper coated cadmium-column for
142 the reduction of NO_3^- to NO_2^- . The analytical procedure used to determine ΣNO_x is as follows; 69 μl of
143 blank, sample or standard solution and 69 μl of imidazole buffer is injected into the chip via a serpentine

144 mixer upstream of an off-chip copper coated cadmium column, this solution is flushed through the chip to
145 waste. This process was repeated 4 times to fully flush the chip and prevent signal dilution or enhancement
146 due to carry-over from previous solutions. On the fifth flush, 69 μl of Griess reagent was mixed via an
147 1 additional serpentine mixer downstream of the copper coated cadmium column. The solution was then left in
148 2 the measurement cells for 110s to allow for colour development. Throughout the analytical cycle the voltage
149 3 output of the photodiodes was recorded at 1 second intervals. For each calibrated measurement, an analytical
150 4 cycle consisted of the analysis of a blank solution, sample (CRM) and then a standard solution. Thus a fully
151 5 calibrated measurement took 19 minutes and each sample has an associated blank and standard from which
152 6 to calculate the absorbance. The limit of detection of the ΣNO_x analyser, defined as 10 times the standard
153 7 deviation of a 0.05 μM nitrate standard, has been reported as 0.025 μM [35], two orders of magnitude below
154 8 the concentration of CRMs analysed in this study.

155 13 **Certified Reference Materials**

156 14 In order to quantify the accuracy of our analyses and to calculate our uncertainty, CRMs were
157 15 routinely analysed. All CRMs used in this study were sourced from KANSO CO., LTD.
158 16 (<http://www.kanso.co.jp/eng/index.html>). The CRMs were filtered (0.45 μm) natural seawater samples
159 17 collected from the Pacific Ocean, which were autoclaved and stored in 100 mL polypropylene bottles, which
160 18 were vacuum sealed in an aluminium-film bag. The concentrations were certified using the Griess and
161 19 Molybdenum blue colorimetric assays, the same techniques as used in this study. Certified reference
162 20 material CD comprised of 81% surface seawater from the Pacific Ocean (29.58°N, 149.15°E) and 19% of
163 21 seawater collected at 397 m depth in Suruga Bay, Japan. Certified reference material CJ comprised of 44%
164 22 surface seawater from the Pacific Ocean (32°N, 144°E) and 56% of seawater collected at 397 m depth in
165 23 Suruga Bay, Japan. Certified reference material CB comprised of 44% seawater collected from the Pacific
166 24 Ocean at 1187m (48.9°N, 166.6°E) and 56% of seawater collected at 397 m depth in Suruga Bay, Japan.
167 25 Certified reference material BW was collected at 270 m depth in Suruga Bay, Japan. Certified reference
168 26 material BZ was collected from the Pacific Ocean at 1187 m depth (48.9°N, 166.6°E).

169 34 **Statistical Methods**

170 35 Data were generated from the analyses of CRMs by two gas segmented flow analysers and seven
171 36 LoC analysers. A schematic of the experimental design is displayed in Figure 1. Analytical uncertainties
172 37 were calculated via the NordTest™ approach [44], which has recently been applied to marine trace metal
173 38 studies [21, 24]. The Nordtest™ approach combines random effects, including intermediate sources of
174 39 analytical uncertainty (e.g. different reagents and standards, different analysts, changing laboratory
175 40 conditions, different LoC analysers), and the uncertainty resulting from systematic bias. Systematic bias was
176 41 estimated via the analyses of CRMs. Consequently, the Nordtest™ approach accounts for both random and
177 42 systematic effects and will therefore produce a higher analytical uncertainty than the typically reported
178 43 standard deviation of replicate sample measurements, which only accounts for sources of short-term random
179 44 uncertainty. As this higher analytical uncertainty incorporates more of the possible sources of uncertainty, it
180 45 is considered a more realistic and reliable estimate. An example Microsoft Excel™ template can be found in
181 46 the supporting information. All uncertainties calculated in this study are presented as relative uncertainties.

182 53 The combined uncertainty (u_c) was estimated from the sum of the squares of two independent
183 54 uncertainty estimates:

$$184 \text{ } u_c = \sqrt{(u(\text{Rw}))^2 + u(\text{bias})^2} \quad (1)$$

185 59 Where $u(\text{Rw})$ represent within laboratory reproducibility and $u(\text{bias})$ represents method and laboratory
186 60 systematic bias. The laboratory reproducibility includes the pooled standard deviation of the measurements
187 61 of the same samples (or CRMs) over a period of several months. As nutrient samples are not stable for this
188 62

188 length of time once opened, fresh (within 1 week of opening) CRM samples were analysed and treated as
189 the same sample. Method and laboratory systematic bias was estimated after equation 2.

$$190 \quad u(\text{bias}) = \sqrt{(\text{RMS}_{\text{bias}}^2 + u(\text{Cref})^2)} \quad (2)$$

191 ² Where RMS_{bias} is the root mean square of the bias value (Eq.3) and $u(\text{Cref})$ is the uncertainty of the
192 ⁴ certified reference value (Eq.4).

$$193 \quad \text{RMS}_{\text{bias}} = \sqrt{(\sum(\text{bias}_i)^2/n)} \quad (3)$$

$$194 \quad U(\text{Cref})^2 = \sqrt{(\sum u(\text{Cref}_i)^2/n)} \quad (4)$$

195 ¹¹ Where bias_i is the percentage difference between the mean concentration value determined and the certified
196 ¹² value of a CRM, $u(\text{Cref}_i)$ being the uncertainty of the certified reference value and n being the number of
197 ¹³ CRMs used. Each estimate used the analyses of at least two different CRMs, each with different
198 ¹⁴ macronutrient concentrations. Final uncertainties were determined as u_c ($k=1$).

199 ¹⁷ Results and Discussion

200 ¹⁹ Gas Segmented Flow Analysis

201 ²¹ CRM analyses were conducted using gas segmented flow analysis during a research cruise in the
202 ²² South Atlantic Ocean in 2018 on board the *RRS. James Cook* over a period of 42 days (Table 1). During the
203 ²³ research cruise nutrient samples were analysed daily, thus these CRM analyses were conducted in a typical
204 ²⁴ research environment where the analysts were analysing 100s of samples per day on-board a ship. The
205 ²⁵ instrument was calibrated daily with six standards encompassing the expected concentration range of
206 ²⁶ collected samples. The limit of detection was defined as 3 times the standard deviation of 20 replicates of
207 ²⁷ the lowest concentration standard for each calibration during the research cruise. The limits of detection
208 ²⁸ limit varied throughout the cruise, but ranged from 0.04-0.1 μM , 0.02-0.035 μM and 0.04-0.11 μM for
209 ²⁹ ΣNO_x , PO_4^{3-} and $\text{Si}(\text{OH})_4$ respectively. The concentration of samples analysed ranged from <LoD-38.93
210 ³⁰ μM , <LoD-2.56 μM and 0.29- 131.25 μM for ΣNO_x , PO_4^{3-} and $\text{Si}(\text{OH})_4$ respectively.

211 ³¹ The combined uncertainties and the concentration range over which they were calculated are
212 ³² displayed in Table 2; the combined uncertainty for ΣNO_x analyses was determined as 1.2%, for PO_4^{3-}
213 ³³ analyses to be 3.4 % and for $\text{Si}(\text{OH})_4$ analyses to be 2.2 %. Systematic bias accounted for 51% (ΣNO_x),
214 ³⁴ 57% (PO_4^{3-}) and 42 % ($\text{Si}(\text{OH})_4$) of the combined uncertainty. Thus 58-43% of the analytical uncertainty is
215 ³⁵ not accounted for if systematic bias is excluded from the estimate. On 24 occasions during the research
216 ³⁶ cruise, two analysts collected 10 individual sample aliquots from the same Niskin water sampler that is used
217 ³⁷ to collect seawater at depth in the ocean. These aliquots were then analysed in sequence, and therefore the
218 ³⁸ variability in these results will be the outcome of uncertainties associated with the sampling procedure from
219 ³⁹ the Niskin sampler and uncertainties associated with short-term analytical reproducibility [45]. For the
220 ⁴⁰ concentration range over which the combined uncertainties were calculated (ΣNO_x 5.63-36.66 μM , PO_4^{3-}
221 ⁴¹ 0.446-2.58 μM and 14.27-111.85 μM $\text{Si}(\text{OH})_4$), the relative standard deviation resulting from analyses of 10
222 ⁴² samples was always less than the combined analytical uncertainty estimate (Fig. 2), confirming the necessity
223 ⁴³ to account for systematic bias to calculate a realistic analytical uncertainty. However, at lower
224 ⁴⁴ concentrations, close to the limit of detection, the analytical uncertainty increases and therefore may be
225 ⁴⁵ larger than the combined uncertainty calculated using CRMs with higher macronutrient concentrations [34].
226 ⁴⁶ It is therefore imperative that the range over which the combined uncertainty is calculated is reported
227 ⁴⁷ alongside the value itself.

228 ⁴⁸ We consider that the combined uncertainties presented here for ship board gas segmented flow
229 ⁴⁹ analysis (1.2-3.4 %) are remarkably small, particularly given the challenges associated with making high
230 ⁵⁰ quality nutrient measurements whilst at sea (e.g. reliance on pre-weighed salts and reagents, moving
231 ⁵¹

laboratory, analyst fatigue). Precision alone for marine nutrient measurements has been reported as typically ~2-3 % [18, 46]. In comparison, reported values for combined uncertainties associated with trace metal measurements, albeit at sub-nanomolar concentrations, range from 7.5-12 % [21, 24]. To establish whether our calculated measurement uncertainties can be considered typical, a smaller set of CRM analyses was conducted in a separate laboratory. These analyses yielded combined uncertainties of 3.6-3.8 % (Table 2), only marginally larger than our extensive ship board analyses, suggesting that such combined uncertainties values can be consistently achieved when the analysis is routinely conducted by trained analysts. The second set of analysis also highlighted an additional advantage of regularly analysing CRMs, which is the ability to identify outliers. Application of the International Organization for Standardization (ISO) approved Grubbs test for outliers identified an extreme analysed ΣNO_x concentration for the both CD –KANSO and BZ-KANSO CRM on 26th June 2018; hence these values were excluded from the uncertainty calculation (Table 3; see Supporting Information). On the same day the estimated PO_4^{3-} concentrations for both CD-KANSO and BZ-KANSO CRM were also the largest determined within the dataset. Together, these results indicate that there existed an additional source of systematic uncertainty common to both measurements on 26th June 2018. Therefore, although the PO_4^{3-} values did not fail the Grubbs test, they were still excluded from the calculation. Including the extreme values in the calculation resulted in much larger estimated combined uncertainties (8.1 % for ΣNO_x & 7.2 % for PO_4^{3-} , Table 2). If this information is reviewed in real time it would allow the analyst to recalibrate before analysing samples. Alternatively, the analyst can retrospectively flag any sample data generated on such a day as suspected of being of suspect quality.

A realistic estimate of analytical uncertainty becomes increasingly important with a higher number of data manipulations. For instance, observing changes and patterns in nutrient stoichiometry is a common approach used to investigate marine biogeochemical processes [e.g. 3, 4]. Taking the combined analytical uncertainty values for measurements made using gas segmented flow analysis on-board ship results in N:P, N:Si and P:Si ratios with uncertainties of 4.6 %, 3.4 % and 5.6 %, respectively. This information can be used to aid interpretation of the dataset, allowing the investigator to more accurately determine whether environmental processes drive observed changes in nutrient stoichiometry, or whether they may be artefacts resulting from analytical uncertainty.

The approach presented in this manuscript may be particularly useful for long term time series measurements. Changes to an analytical procedure over time, including changing analysts and analytical instrumentation, may contribute to measurement uncertainty. The primary function of a time series is to examine the temporal variability at a specific location. Therefore, applying an adjustment based on climatological average values risks removing the variability the scientist is aiming to observe. For instance, nutrient concentrations from the DYFAMED time-series station in the North West Mediterranean were pooled by month to generate monthly climatologies. Extreme values were then removed from these datasets; 13 %, 14 % and 10 % of ΣNO_x , PO_4^{3-} and $\text{Si}(\text{OH})_4$ data, respectively, were removed. Whilst this monthly climatology approach likely preserves the effect of seasonal to decadal processes, it risks removing the effect of processes occurring on shorter time scales [47]. Examples of such processes include downwelling and upwelling events driven by mesoscale and sub-mesoscale processes [e.g. 48, 49], phytoplankton blooms that can dramatically reduce inorganic nutrient concentrations on time scales of days [e.g. 25, 39, 50] and, to a lesser degree, atmospheric deposition that can release measureable quantities of inorganic nutrients to seawater [e.g. 51, 52]. In addition, at coastal time series, such as the L4 station of the Western Channel Observatory (Plymouth, U.K), variability in river discharge can influence nutrient concentrations over timescales of days [53]. The approach presented here would allow poor quality data to be identified without the risk of removing extreme data that result from such short term processes.

Lab-on-Chip analysers

The LoC analysers used in this study are designed and assembled at the National Oceanography Centre, Southampton. In this study, 7 individual LoC ΣNOx sensors were used to analyse CRMs during laboratory testing by two analysts over a period of two months (Table 4). The analysis of the CRM KANSO-CD was conducted using all methods described in this paper, therefore the results can be treated as an analytical inter-comparison (Fig. 3). There was excellent agreement between all three instruments (Gas segmented flow bench top system and LoC) with no statistically significant difference between mean ΣNOx values (1 way ANOVA, $p=0.05$). The results presented here provide further evidence that LoC platforms produce data that is directly comparable with traditional gas segmented flow techniques [36, 38, 39]. In doing so they provide a powerful tool with which to augment traditional sampling approaches.

A PO_4^{3-} analyser is being developed but is at a lower technology readiness level (TRL 7; Table S1) than the ΣNOx analyser (TRL 8) and therefore not at the developmental stage required for a study such as this; a more detailed combined uncertainty estimate for the PO_4^{3-} analyser will be reported in a subsequent study. To give an indication of the combined uncertainty associated with measurements made using early versions of the PO_4^{3-} analyser, CRM measurements made with two LoC PO_4^{3-} analysers during laboratory testing are taken from Grand et al. [37] (Table 4). An additional LoC sensor is in development for $\text{Si}(\text{OH})_4$ measurements; uncertainty data for this will be reported when the $\text{Si}(\text{OH})_4$ analyser technology is published.

For both ΣNOx and PO_4^{3-} LoC platforms, the combined uncertainty resulting from multiple platforms was calculated to be $< 5\%$ (Table 2). The Grubbs test was used to test for suspected extreme values. One value for ΣNOx was identified as a suspected outlier, and removed from the uncertainty calculation. It should be noted that variability between analysers as a source of uncertainty has been quantified for the LoC and not for the bench top gas segmented flow analysers used in this study. Future LoC sampling campaigns will include multiple sensors to increase spatial and temporal coverage. For instance, Vincent et al. [39] integrated a LoC ΣNOx platform into an autonomous underwater vehicle (AUV) to observe changes in ΣNOx distributions in the Celtic Sea. The AlterEco programme (<http://altereco.ac.uk/>) aims to expand on this approach to determine seasonal baseline characteristics of the North Sea. A key aspect is the deployment of multiple LoC ΣNOx analysers in AUVs over a period of > 1 year. The combined uncertainty values presented here provide confidence that high quality data will be generated during sampling campaigns such as that conducted as part of the AlterEco program. It is noted that additional sources of uncertainty will be present during deployments in the marine environment (e.g. temperature & pressure changes, biofouling). However, recent deployments indicate that the LoC platforms compare well with traditional benchtop techniques in glacial [42], riverine [36] and marine environments [37-39] and are not adversely affected by variations in environmental parameters. For instance, a comparison of data generated from a 21 day deployment of a LoC ΣNOx analyser in an AUV, with coincident measurements made using traditional water sampling and gas-segmented flow analyses yielded an uncertainty estimate of 1.2-4.9 % for the concentration range 1.42-5.74 μM [39].

Conclusions and Future Recommendations

Analytical techniques used for the determination of marine nutrient concentrations are becoming increasingly automated, which will increase the quantity of data produced. Consequently, there is a need for simple statistical methods that produce realistic measurement uncertainties. It is clear from results presented here and elsewhere that accounting for systematic bias is necessary to produce realistic uncertainty values. Therefore, the NordTest™ approach is an ideal method for quantifying combined measurement uncertainty. A current objective of SCOR working group 147 is ‘*To promote the wider global use of reference materials by arranging workshops to actively encourage their use, and to provide training in analytical protocols and best practices, including sample preservation protocols, particularly targeted towards developing countries.*’ (<https://scor-int.org/group/147/>). Providing that CRMs are routinely analysed, the approach

321 presented here requires no additional laboratory analyses or costly inter-comparison efforts, and so there are
322 no additional costs incurred, which also makes this an attractive approach for scientists in developing
323 countries. Therefore, it is advocated that application of the NordTest™ approach presented in this study
324 1 becomes part of ‘best practice’ and that the combined uncertainty estimate (and the concentration range over
325 2 which it was calculated) should be reported alongside measurement data.
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326 4 The statistical approach described here offers advantages in determining analytical uncertainties for
327 5 the measurements undertaken by the LoC analysers. Combined uncertainties can be assessed as sensors are
328 6 manufactured, providing an objective method to assess between-analyser variability. It is recommended that,
329 7 as individual LoC technology matures to TRL 8, a rigorous assessment of measurement uncertainty is
330 8 conducted. The approach described here presents a simple method to achieve this. In this study we
331 9 calculated that the combined measurement uncertainty associated with data produced from multiple ΣNO_x
332 10 LoC analysers is $< 5\%$. This demonstrates the high quality and repeatability of the manufacturing process
333 11 and highlights the potential of autonomous LoC analysers to become routine measurement tools for
334 12 determining marine nutrient concentrations.
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341 33 **Author Contributions**

341 34

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343 36 Draft, Writing – Review & Editing, Visualization, Supervision, Project Administration. **Geraldine Clinton-**
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347 40 & Editing. **Thierry Cariou:** Investigation, Writing – Review & Editing. **Caroline White:** Investigation,
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349 42 Funding Acquisition. **Paul J. Worsfold:** Methodology, Writing – Review & Editing, Funding Acquisition,
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352 Table and figure captions

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354 1 **Table 1-** Measured and certified values for certified reference materials analysed using gas segmented flow
355 2 analysis during the research cruise JC159. Concentrations converted from $\mu\text{mol/kg}$ to $\mu\text{mol/l}$ assuming an
356 3 analysis temperature of $20\text{ }^\circ\text{C}$.

357 4 **Table 2-** The combined uncertainty estimate for each analytical technique. $u(\text{Rw})$ is the uncertainty resulting
358 5 from within laboratory reproducibility. $u(\text{bias})$ is the uncertainty resulting from systematic bias. u_c is the
359 6 resulting combined uncertainty. Conc Range is the concentration range of CRMs analysed.* estimate
360 7 calculated using previously published CRM data [37]. Values in brackets are the uncertainty estimates if
361 8 outliers (see text for details) are included in the calculation.

362 9 **Table 3-** Measured and certified values for certified reference materials analysed using using gas segmented
363 10 flow analysis during laboratory tests. Concentrations converted from $\mu\text{mol/kg}$ to $\mu\text{mol/l}$ assuming an
364 11 analysis temperature of $20\text{ }^\circ\text{C}$. Values in brackets are the uncertainty estimates if outliers (see text for
365 12 details) are included in the calculation.

366 13 **Table 4-** Measured and certified values for certified reference materials analysed using Lab-on-Chip
367 14 analysers during laboratory tests. Concentrations converted from $\mu\text{mol/kg}$ to $\mu\text{mol/l}$ assuming an analysis
368 15 temperature of $20\text{ }^\circ\text{C}$. * previously published CRM data [37]

369 16 **Figure 1-** The mean concentration and relative standard deviation (R.S.D) calculated from the analysis of 10
370 17 samples collected from the same Niskin water sampler (blue symbols). This approach incorporates
371 18 uncertainties associated with sampling and short term analytical reproducibility. One data point with a mean
372 19 phosphate concentration of $0.01\text{ }\mu\text{M}$ was removed as it was deemed to be below the limit of detection. The
373 20 dashed orange line denotes the combined uncertainty estimate calculated in this study ($k=1$). This approach
374 21 incorporates uncertainties associated within laboratory reproducibility and systematic bias.

375 22 **Figure 2-** The mean concentration and relative standard deviation (R.S.D) calculated from the analysis of 10
376 23 samples collected from the same Niskin water sampler (blue symbols). This approach incorporates
377 24 uncertainties associated with sampling and short term analytical reproducibility. One data point with a mean
378 25 phosphate concentration of $0.01\text{ }\mu\text{M}$ was removed as it was deemed to be below the limit of detection. The
379 26 dashed orange line denotes the combined uncertainty estimate calculated in this study ($k=1$). This approach
380 27 incorporates uncertainties associated within laboratory reproducibility and systematic bias.

381 28 **Figure 3-** The mean ΣNO_x concentration ($\pm 1\text{ S.D}$) for CRM KANSO-CD determined using gas segmented
382 29 flow analysis (GSF) and Lab-on-Chip analysers (LoC). The certified value is $5.63 \pm 0.0031\text{ }\mu\text{M}$.

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Table 1

		Mean value determined (μM)	Standard deviation (μM)	<i>n</i>	Certified value (μM)	Standard deviation (μM)
Kanso CD	ΣNO_x	5.56	0.05	31	5.63	0.0031
	Phosphate	0.47	0.01	31	0.446	0.0004
	Silicic acid	14.57	0.26	31	14.27	0.0089
Kanso CJ	ΣNO_x	16.59	0.13	34	16.59	0.0080
	Phosphate	1.26	0.02	34	1.22	0.0001
	Silicic acid	39.88	0.71	34	39.63	0.0200
Kanso CB	ΣNO_x	36.95	0.25	30	36.66	0.0180
	Phosphate	2.66	0.04	30	2.58	0.0038
	Silicic acid	112.11	2.07	30	111.85	0.0520

Table 2

		ΣNO_x	Phosphate	Silicic acid
Gas Segmented Flow (Shipboard)	u(Rw) (%)	0.83	2.03	1.79
	u(bias) (%)	0.84	2.74	1.27
	u_c (%)	1.2	3.4	2.2
	Conc Range (μM)	5.63-36.66	0.446-2.58	14.27-111.85
Gas Segmented Flow	u(Rw) (%)	3.33 (7.57)	3.36 (6.84)	n.d
	u(bias) (%)	1.50 (2.80)	1.69 (2.37)	n.d
	u_c (%)	3.6 (8.1)	3.8 (7.2)	n.d
	Conc Range (μM)	5.63-44.43	0.446-3.13	n.d
Lab-on-Chip Analyser	u(Rw) (%)	5.24 (3.73)	1.55*	n.d.
	u(bias) (%)	2.36 (3.19)	4.50*	n.d.
	u_c (%)	4.9 (5.7)	4.8*	n.d.
	Conc Range (μM)	5.63-44.43	0.446-1.541	n.d

Table 3

		Mean value determined (μM)	Standard deviation (μM)	<i>n</i>	Certified value (μM)	Standard deviation (μM)
Kanso CD	ΣNOx	5.57 (5.80)	0.26 (0.61)	5 (6)	5.63	0.0031
	Phosphate	0.45 (0.47)	0.02 (0.04)	5 (6)	0.446	0.0004
Kanso BZ	ΣNOx	45.20 (45.56)	0.23 (0.91)	5 (6)	44.43	0.0350
	Phosphate	3.13 (3.14)	0.05 (0.06)	5 (6)	3.13	0.034

Table 4

		Mean value determined (μM)	Standard deviation (μM)	<i>n</i>	Certified value (μM)	Standard deviation (μM)
Kanso CD	ΣNOx	5.48	0.22	10	5.63	0.0031
	Phosphate [*]	0.42	0.01	5	0.446	0.0004
Kanso BZ	ΣNOx	42.85 (43.59)	1.49 (2.73)	9 (10)	44.43	0.0350
	Phosphate	n.d.	n.d.	n.d.		
Kanso BW	ΣNOx	n.d	n.d	n.d		
	Phosphate [*]	1.57	0.06	15	1.541	0.0027

Figure 1
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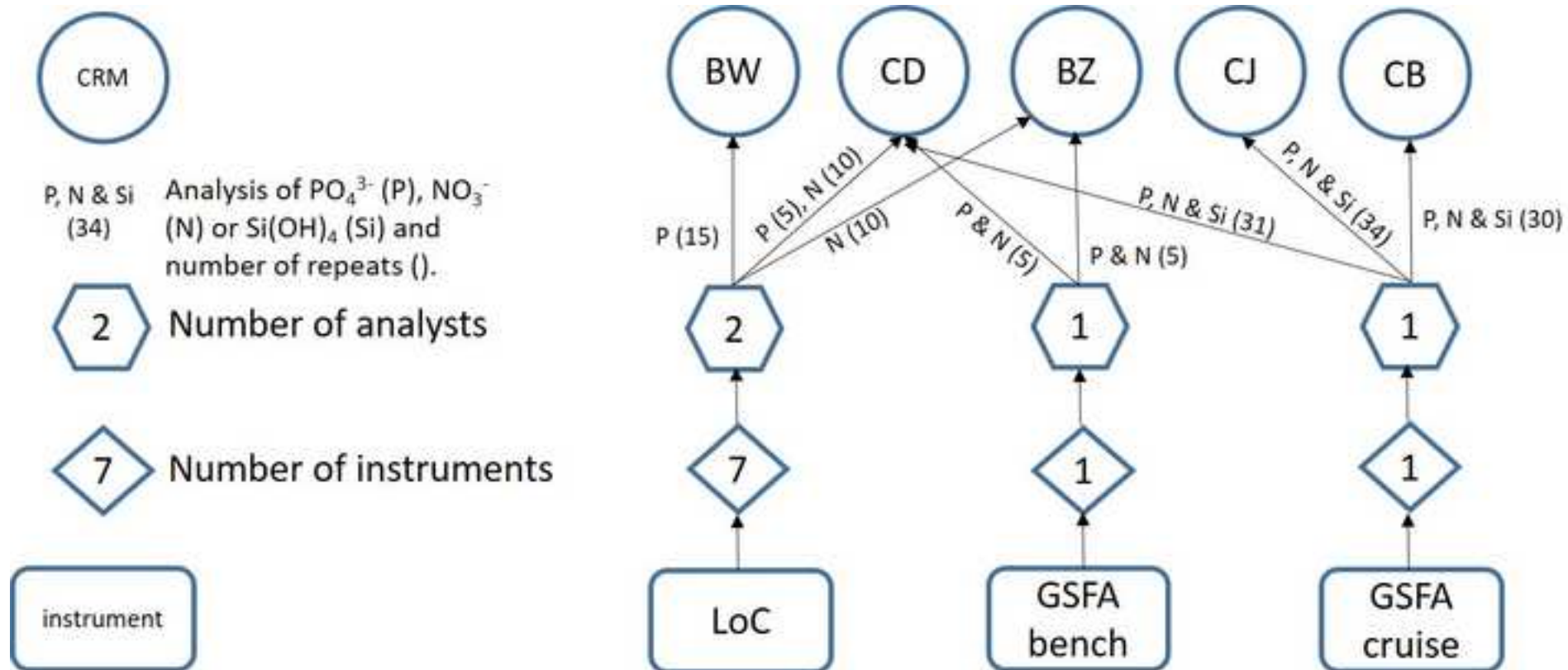


Figure 2
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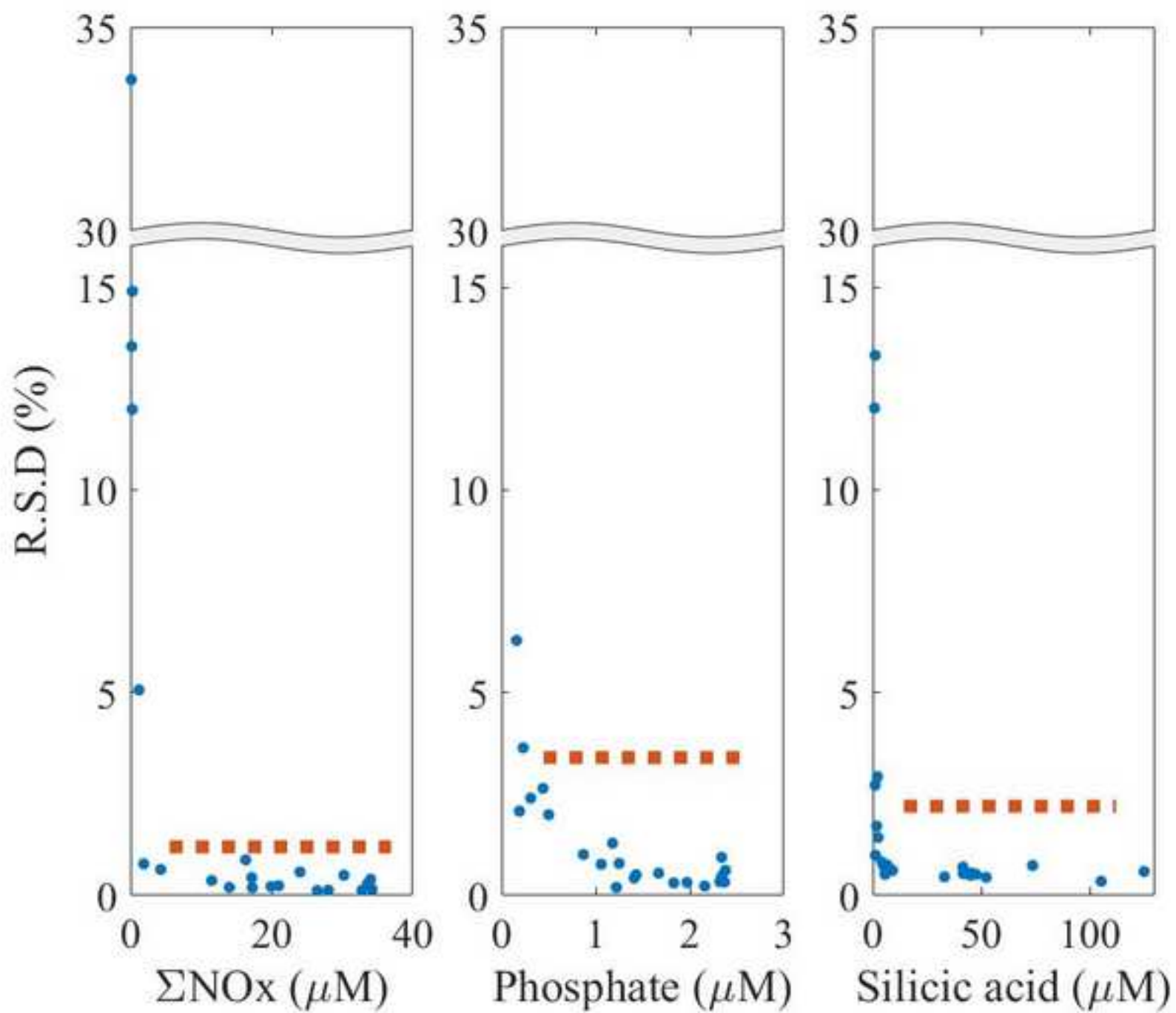
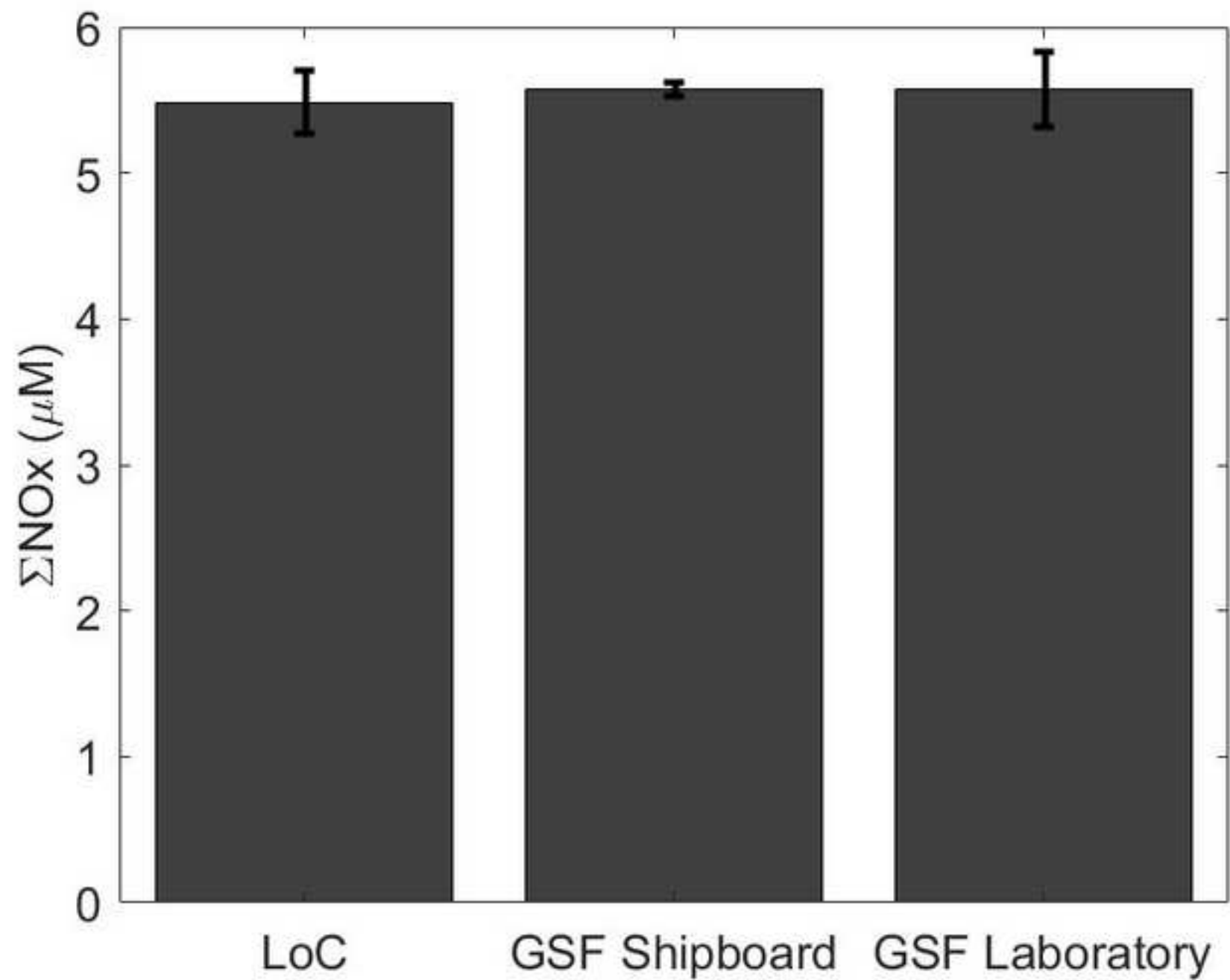


Figure 3
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