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Organic carbon causes interference to nitrate and nitrite measurements by UV/Vis spectrometers : The importance of local calibration

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1 **Organic carbon causes interference with nitrate and nitrite measurements by UV/Vis spectrometers: The**
2 **importance of local calibration**

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27 **Abstract**
28

29 Compared with sporadic conventional water sampling, continuous water-quality monitoring with optical sensors has
30 improved our understanding of freshwater dynamics. The basic principle in photometric measurements is the incident
31 light at a given wavelength that is either reflected, scattered, or transmitted in the body of water. Here, we discuss the
32 transmittance measurements. The amount of transmittance is inversely proportional to the concentration of the
33 substance measured. However, the transmittance is subject to interference, because it can be affected by factors other
34 than the substance targeted in the water. In this study, interference with the UV/Vis sensor nitrate plus nitrite
35 measurements caused by organic carbon was evaluated. Total or dissolved organic carbon as well as nitrate plus nitrite
36 concentrations were measured in various boreal waters with two UV/Vis sensors (5-mm and 35-mm pathlengths), using
37 conventional laboratory analysis results as references. Organic carbon increased the sensor nitrate plus nitrite results,
38 not only in waters with high organic carbon concentrations, but also at the lower concentrations ($< 10 \text{ mg C L}^{-1}$) typical
39 of boreal stream, river, and lake waters. Our results demonstrated that local calibration with multiple linear regression,
40 including both nitrate plus nitrite and dissolved organic carbon, can correct the error caused by organic carbon.
41 However, high-frequency optical sensors continue to be excellent tools for environmental monitoring when they are
42 properly calibrated for the local water matrix.

43
44 **Keywords**

45 Boreal water monitoring, calibration, nitrate, high-frequency sensor, optical measurement, organic carbon
46

47 **Introduction**

48
49 Excess nitrogen (N) has become one of the world's main environmental challenges during recent decades (Rockström et
50 al. 2009). Humans have doubled the amount of reactive nitrogen (Nr) on Earth mainly due to food production,
51 wastewater, and fossil-fuel combustion (Gruber and Galloway 2008; Fowler et al. 2013), resulting in increased
52 eutrophication and greenhouse-gas emissions, acid rain, smog, and stratospheric ozone depletion (Galloway et al.
53 2013). In agricultural watersheds and other nonpoint Nr sources, continuous monitoring of Nr is crucial to measuring N
54 loading more accurately in dynamic water systems. Nutrient loading calculations based on traditional sporadic sampling
55 generate uncertainty and may lead to under- or overestimations, especially in small lotic systems where spatiotemporal
56 variation in water quality can be significant (e.g. Linjama et al. 2009; Koskiaho et al. 2010). Thus, high-frequency
57 measurements are needed to better understand the relationships between land use, weather, and water chemistry in
58 aquatic systems, especially in changing climates.

59
60 Optical sensors have a history spanning several decades in oceanographic and coastal studies, but their use in freshwater
61 systems has increased only during the last decade (Pellerin and Bergamaschi 2014). One of the *in-situ* photometric
62 sensors used is the ultraviolet/visible light (UV/Vis) scanning spectrometer manufactured by Messtechnik GmbH
63 (Klagenfurt am Wörthersee, Austria). It is a multiparameter instrument that records the complete absorbance spectrum
64 at wavelengths between 220 nm and 720 nm and is able to measure nitrate plus nitrite N (referred together as NO_x-N),
65 dissolved organic carbon (DOC), total organic carbon (TOC), and turbidity. Sensors with distinct measuring
66 pathlengths perform differently, because their precision and detection limits vary (spectrometer probe manual at
67 www.s-can.at). Increased use of optical *in-situ* sensors has raised questions about their reliability, compared with the
68 data obtained with conventional laboratory analyses.

69
70 For optical sensors with large absorbance spectra, it is not possible to differentiate the amounts of nitrate and nitrite, so
71 they indicate the sum by the use of NO_x-N. The absorbance range of NO_x-N and organic carbon (OC) partly overlap,
72 since they both absorb UV radiation, mainly at wavelengths between 200 nm and 250 nm (Van den Broeke et al. 2006).
73 For the NO_x-N results, the interference likely comes from elements that have absorbance properties similar to those of
74 OC, such as humic or fulvic acids, or inorganic elements such as bromide and hydrogen sulfide (Pellerin et al. 2013).
75 Turbidity influences the entire absorption spectrum by shading and scattering light from suspended particles (Huber and
76 Frost 1998). The effect of these substances can decrease the transmittance of light in the sample and result in
77 overestimation of the NO_x-N concentration.

78
79 Manufacturers of optical sensors may offer various calibration options, depending on the application, without the
80 absolute need for local calibration. The main purpose of these calibration options is to separate the NO_x-N signal from
81 that of the interfering substances that typically occur in the types of waters studied (Pellerin et al. 2013). However, if
82 differences with the reference method are observed, manufacturers recommend that local calibration be applied to
83 enhance the reliability of the sensor results. One of the methods most used in calibration is simple linear regression
84 (SLR); the results obtained from this reference method, usually in the laboratory, are plotted against the sensor results,
85 and the linear function obtained is then applied to correct the sensor results (Huotari and Ketola 2014).

86

87 In this study, OC interference with the NO_x-N results was investigated with UV/Vis scanning spectrolyzer sensors having
88 two different optical measuring pathlengths (35 mm and 5 mm). The TOC, DOC, and NO_x-N concentrations were
89 analyzed with both sensors, as well as with laboratory methods for reference. To obtain a thorough water matrix for
90 testing the performance of the sensors, we analyzed freshwaters ranging from spring water to a bog outlet and streams
91 draining from agriculturally influenced catchments. We also spiked the spring and bog water samples with potassium
92 nitrate (KNO₃) to obtain high NO_x-N concentrations. Finally, we applied the 5-mm pathlength sensor in an agricultural
93 stream for 6 months to compare with the weekly collected samples analyzed in the laboratory. This information was
94 used to consider the importance of OC in the local calibration method. This study provided much needed information
95 regarding the local calibration of the UV/Vis sensor used in the NO_x-N measurements in freshwaters with high OC
96 concentrations.

97

98 **Materials and Methods**

99

100 Experimental design

101

102 Various surface waters were collected from eight sites located in southern Finland for the two experiments. Samples for
103 Experiment I were collected in 2009 (DOC analysis) and 2011 (TOC analysis). For Experiment II, the waters were
104 collected in 2009. The water samples were kept in the dark and cold (+4 °C) until determined within a few hours of
105 sampling by standard analytical methods and by the UV/Vis optical sensors. The laboratory and sensor measurements
106 were performed simultaneously. To obtain a wide variety of combinations of NO_x-N and OC concentrations, the waters
107 were mixed and/or diluted with deionized water (MQ) (Millipore; EMD Millipore, Billerica, MA, USA). Before the
108 experiments, the accuracy of the OC measurements by the sensors was evaluated with laboratory TOC and DOC as
109 references.

110

111 Measurements done in the laboratory with standard analytical methods conducted at Lammi Biological Station,
112 University of Helsinki (see Arvola et al. 2015) were used as references for the sensor results. In this study, both TOC
113 and DOC were considered as corresponding to OC. TOC was measured from unfiltered and DOC from filtered (< 0.2
114 μm, Nuclepore; Whatman GE Healthcare, Chicago, IL, USA) sample water, using a high-temperature combustion
115 method with a TOC-5000 A analyzer (Shimadzu Corp., Kyoto, Japan). The detection limit of the TOC and DOC
116 analyses varied between 0.2 and 1.0 mg L⁻¹, depending on the type of sample (e.g. turbidity). NO_x-N was analyzed from
117 filtrates (< 0.2 μm, Nuclepore) with an automated flow-injection analyzer (Lachat QuikChem 8000 FIA; Lachat
118 Instruments, Hach Company, Loveland, CO, USA), using a nitrate-reducing method with a cadmium column. The
119 detection limit for the NO_x-N analysis was 0.01 mg L⁻¹.

120

121 The sensor NO_x-N measurements in the experiments were done with UV/Vis scanning spectrolyzers (Messtechnik) with
122 35-mm and 5-mm measuring pathlengths. The specifications state that the 35-mm pathlength sensor is capable of
123 measuring NO_x-N concentrations < 10.0 mg L⁻¹. For TOC and DOC, the measurement ranges are below 25 and 12 mg
124 C L⁻¹, respectively. At higher concentrations, the 5-mm pathlength sensor was more applicable, with measuring ranges
125 for NO_x-N < 70 mg L⁻¹, for TOC < 150 mg C L⁻¹, and for DOC < 90 mg C L⁻¹ (specifications at www.s-can.at). A
126 specific cuvette designed for the sensors was mounted, and the sample was poured into the cuvette. Four to five

127 sequential readings were recorded for each sample. The cuvette was rinsed with MQ and sample water between
 128 measurements.

129

130 **Table 1.** Sampling sites, coordinates, water type, and mean NO_x-N, DOC, and TOC concentrations measured in the
 131 laboratory. The standard deviations are shown in parentheses if available. Not determined = *nd*.

132

Site	Coordinates	Type	NO _x -N mg L ⁻¹	DOC mg L ⁻¹	TOC mg L ⁻¹
Laavionsuonoja Brook	61°2'N, 24°59'S	small peatland stream, high organic matter content	0.040 (±0.010, n=6)	74.0 (±0.38, n=2)	53.0 (±0.61, n=3)
Lake Ormajärvi	61°5'N, 24°57'S	medium-sized clear-water lake, mesotrophic	0.35 (±0.010, n=3)	<i>nd</i>	7.8 (±0.030, n=3)
Löytynlähde Spring	61°2'N, 24°58'S	spring, oligotrophic	0.41 (±0.010, n=4)	1.2 (±0.29, n=2)	2.3 (±0.060, n=3)
Luhtaanmäenjoki River	60°20'N, 24°47'S	small river, high turbidity, clay soils, agriculture	0.57	7.6 (±0.037, n=2)	<i>nd</i>
Lake Pääjärvi	61°5'N, 25°5'S	medium-sized humic lake, meso-oligotrophic	0.85	10.0 (±0.038, n=2)	<i>nd</i>
Teuronjoki River	61°5'N, 24°50'S	outflow river of Lake Pääjärvi	1.2	8.1 (±0.033, n=2)	<i>nd</i>
Vantaanjoki River	60°40'N, 24°56'S	small river, high turbidity, clay soils, agriculture	1.3 (±0.012, n=3)	<i>nd</i>	10.0 (±0.24, n=3)
Letkunoja Brook	61°3'N, 25°5'S	small stream, mineral soils, agriculture	2.5	7.9 (±0.028, n=2)	<i>nd</i>
Koiransuolenoja Brook	61°3'N, 25°4'S	small stream, mineral soils, agriculture	2.4 (±0.37, n=25)	10.6 (±4.5, n=25)	<i>nd</i>

133

134 *Experiment I:* In the first part of the experiment in 2009, water samples were collected from six sites (Laavionsuonoja,
 135 Löytynlähde, Teuronjoki, Letkunoja, Luhtaanmäenjoki, and Pääjärvi (Table 1). In addition, one mixed sample (1:1) of
 136 two streams (Laavionsuonoja : Luhtaanmäenjoki) was prepared. After analyzing the samples in the laboratory for NO_x-
 137 N and DOC, dilutions with these seven samples and MQ were done at ratios of 1:1, 1:3, 1:9, and 3:1. The diluted
 138 concentrations were calculated based on the laboratory-analyzed initial concentrations and compared with the sensor
 139 results. In the second part of the experiment, water samples were collected in 2011. Three to six replicate water samples
 140 from each of four sites (Laavionsuonoja, Löytynlähde, Ormajärvi, and Vantaanjoki) were analyzed in the laboratory for
 141 NO_x-N and TOC before making the dilutions and mixtures presented in Table 2. The concentrations were analyzed in
 142 the laboratory and compared with the sensor results.

143

144 **Table 2.** Mixtures of the waters examined in Exp. I from the 2011 samples (only TOC analyzed). MQ = deionized
145 water.

Sample	Mixing Ratios
Laavionsuonoja: Vantaanjoki	1:1
Löytynlähde: Vantaanjoki	2:1
Laavionsuonoja: Löytynlähde	1:2, 1:4
Vantaanjoki: Ormajärvi	1:1, 1:2
Ormajärvi: MQ	1:1, 1:2, 1:3, 1:4
Löytynlähde: MQ	1:1
Löytynlähde: Ormajärvi: MQ	1:1:1

146
147

148 *Experiment II* (only the 5-mm pathlength sensor): In this experiment, KNO₃ was added to waters collected from
149 Laavionsuonoja and Löytynlähde, to determine the lower and upper detection limits for NO_x-N in the presence of high
150 and low OC concentrations. First, the NO_x-N concentrations were analyzed in the laboratory from the original samples.
151 The sample waters were spiked with KNO₃ (1000 mg N L⁻¹), and the NO_x-N concentrations were: Laavionsuonoja 0.53,
152 2.03, 8.03, 16.0, and 32.0, and Löytynlähde 1.6, 2.6, 8.6, 16.6, and 32.6 mg N L⁻¹. Based on the known initial NO_x-N
153 concentrations, the calculated values of NO_x-N were compared with the corresponding sensor results.

154

155 *Experiment III:* The 5-mm pathlength sensor measured NO_x-N and DOC in Koiransuolenoja for 6 months from early
156 May to late November in 2013. These data were compared with weekly collected samples analyzed in the laboratory.
157 The sensor data were calibrated in two different ways: simple linear regression (SLR) and multiple linear regression
158 (MLR). In SLR, the calibration was based on the regression function between the laboratory and sensor NO_x-N. In
159 MLR, the data calibration was based on the sensor NO_x-N and sensor DOC results in relation to the laboratory NO_x-N
160 results, and the sensor NO_x-N data were corrected, based on the resulting regression function. This information was
161 used to demonstrate the impact of OC on the local calibration procedure.

162

163 Data analysis

164

165 SLR was applied to compare the laboratory (x-axis) and sensor results (y-axis). The accuracy of the sensor results was
166 evaluated by the correlation coefficient (R²) of the regression function. Due to the rather small sample sizes and the
167 skewness of the data, statistical differences between the sensor and laboratory results were investigated with the
168 nonparametric Wilcoxon signed-ranks test. The level of statistical significance was 0.05. The data were analyzed in
169 their original form to maintain the relationship between the laboratory and sensor data. The effect of the increasing
170 DOC concentration on the NO_x-N sensor results was evaluated by the proportional difference between the laboratory
171 and sensor results (referred to as the NO_x-N error ratio). If the DOC concentration had not affected the NO_x-N results
172 by the sensor, the error ratio would have been steady. In Experiment III, we applied two different local calibration
173 methods: SLR and MLR, and analyzed their accuracy by comparing the laboratory results with the corrected sensor data
174 in linear regression and nonparametric Wilcoxon signed-ranks test. Data analyses were performed, using Microsoft

175 Excel for Mac (15.33; Microsoft Corp., Redmond, WA, USA) and IBM SPSS Statistics for Macintosh version 24.0
176 (IBM Corp., Armonk, NY, USA).

177

178 **Results**

179

180 The water quality varied between the sampling sites (Table 1.). The lowest NO_x-N concentration was detected in the
181 bog outlet (Laavionsuonoja) and the highest were found in the brook and river waters draining from the agriculturally
182 influenced catchments. Respectively, the lowest concentration of OC was measured in Löytynlähde and the highest in
183 Laavionsuonoja.

184

185 The laboratory-measured TOC and DOC concentrations in the experimental waters varied between 1.3-53.0 and 0.12-
186 74.0 mg C L⁻¹. Two TOC concentrations (31.0 and 53.0 mg C L⁻¹) and seven DOC concentrations (19-74.0 mg C L⁻¹)
187 were out of the measurement range of the 35-mm pathlength sensor (TOC < 25 mg C L⁻¹ and DOC < 12 mg C L⁻¹), and
188 these were omitted from the analysis. Additionally, the 35-mm pathlength sensor was unable to measure the TOC in one
189 sample from Laavionsuonoja, whereas the laboratory-analyzed TOC was 19.0 mg C L⁻¹. The one outlier seen in Fig. 1a
190 was a sample from Laavionsuonoja. All the DOC/TOC concentrations were within the measuring range of the 5-mm
191 pathlength sensor. A strong linear relationship ($p < 0.001$) was found between both sensors and the laboratory OC
192 results, but the sensor values were significantly (22-29%) higher than the laboratory results (**Fig. 1**) (35-mm: $Z = -$
193 3.269 , sig. = 0.001; 5-mm: $Z = -4.462$, sig. = 0.000).

194

195 **Fig. 1a, b** Laboratory TOC/DOC concentrations in relation to measured concentration by a) 35-mm pathlength sensor
196 and b) 5-mm pathlength sensor

197

198 Experiment I

199

200 The laboratory-analyzed and -calculated NO_x-N concentrations varied between 0.010 and 3.8 mg N L⁻¹ ($n = 63$). Thus,
201 the concentrations settled within the measurement range for both of the sensors given by the manufacturer in the
202 specifications.

203

204 For the 35-mm pathlength sensor, a strong linear correlation ($R^2 = 0.88$, $p < 0.001$, $n = 40$) was evident between the
205 sensor and laboratory NO_x-N results in samples with OC concentrations of 1.3-10.0 mg C L⁻¹ (TOC) and 0.12-10.0 mg
206 C L⁻¹ (DOC). The linear relationship strengthened when the bog water samples ($n = 3$) were removed from the
207 regression analysis ($R^2 = 0.93$) (**Fig. 2a**). However, the sensor indicated 26% higher NO_x-N concentrations than did the
208 laboratory results with statistical significance ($Z = -1.989$, sig. = 0.047). The sensor was not able to measure NO_x-N in
209 two bog water samples and gave 'NaN' (Not a Number) indications. The NO_x-N and TOC concentrations in these
210 samples were 0.23, 0.28 mg N L⁻¹ and 19.0, 13.0 mg C L⁻¹, respectively.

211

212 For the 5-mm pathlength sensor, the OC concentration in all the samples ($n = 51$) fell inside the measurement range of
213 the sensor (TOC < 150 and DOC < 90 mg C L⁻¹.) However, the linear dependency between the NO_x-N sensor and
214 laboratory results was weak ($R^2 = 0.024$, $p < 0.28$). Use of the sensor resulted in significant overestimation, compared

215 with the laboratory results ($Z = -2.925$, sig. = 0.003), due to the bog outlet waters from Laavionsuonoja having high OC
216 concentrations (TOC 13.0-53.0 mg C L⁻¹, DOC 4.0-74.0 mg C L⁻¹) and low NO_x-N concentrations. Omission of these
217 bog water samples from the analysis significantly increased the linear dependency ($R^2 = 0.96$, $p < 0.001$, $n = 37$). Yet,
218 the sensor resulted in overestimation of the NO_x-N concentration by 32% compared with the laboratory results, but the
219 difference was not verified statistically ($Z = -0.228$, sig. = 0.820).

220

221 **Fig. 2a, b** Relationships of NO_x-N between the sensor results and laboratory measurements at DOC/TOC ranges of
222 0.12-10.0 mg C L⁻¹. The bog outlet waters from Laavionsuonoja (marked with empty circles) were excluded from the
223 regression lines. The detection limit for the laboratory NO_x-N was 0.010 mg N L⁻¹
224

225 Low NO_x-N concentrations

226

227 The results showed that both sensors experienced difficulty when samples contained low levels of NO_x-N (0.010-0.43
228 mg NO_x-N L⁻¹). As shown in **Fig. 3a**, the NO_x-N concentrations in samples containing Laavionsuonoja water ($n = 3$,
229 DOC 4.03-10.0 mg C L⁻¹) were highly overestimated (10-50 times higher than the laboratory result), using the 35-mm
230 pathlength sensor. No strong linear dependency between the sensor and laboratory results could be observed ($R^2 = 0.59$,
231 $p = 0.000$). In addition, eight samples with NO_x-N concentrations between 0.056 and 0.28 mg N L⁻¹ resulted in readings
232 of zero by the sensor. The OC concentrations in these samples were low (TOC 1.8-2.1 and DOC 0.12-0.99 mg C L⁻¹).
233 However, the inability to measure NO_x-N concentrations < 0.28 mg N L⁻¹ was not consistent, since the sensor was able
234 to measure 10 other samples < 0.28 mg N L⁻¹ (TOC 1.3-4.0, DOC 0.79-7.4 mg C L⁻¹).

235

236 The 5-mm pathlength sensor showed a higher measurement range for OC. Thus, it was able to measure more samples
237 containing bog water within the range 0.010-0.43 mg NO_x-N L⁻¹ (**Fig. 3b**). However, the NO_x-N concentrations
238 measured by the sensor were overestimated by a greater extent (5-130 times higher than the laboratory result). No
239 strong linear dependency could be observed between the laboratory and sensor results, even without the bog water
240 samples ($R^2 = 0.55$, $p = 0.000$). Similarly, difficulty in measuring low NO_x-N concentrations was observed, since the
241 sensor recorded no results in 12 samples with NO_x-N concentrations of 0.056-0.28 mg L⁻¹. The OC concentrations in
242 these samples were 1.3-2.7 mg C L⁻¹ as TOC and 0.12-1.9 mg C L⁻¹ as DOC. Yet, the sensor was able to measure 16
243 other samples with NO_x-N concentrations < 0.28 mg L⁻¹, of which 13 were bog water samples (TOC 3.3-53.0, DOC
244 2.5-74.0 mg C L⁻¹).

245

246 **Fig. 3a, b** Relationship for the NO_x-N concentrations 0.010 – 0.43 mg L⁻¹ by the sensors, compared with the laboratory
247 results: a) 35-mm pathlength sensor, b) 5-mm pathlength sensor. The bog outlet waters from Laavionsuonoja (empty
248 circles) are omitted from the regression lines. The detection limit (0.010 mg N L⁻¹) of the laboratory NO_x-N is indicated
249 by the dashed line. Note the different scales

250

251 Experiment II

252

253 The initial laboratory-analyzed NO_x-N concentrations in Laavionsuonoja and Löytynlähde were 0.031 and 0.56 mg
254 NO_x-N L⁻¹, respectively. The DOC concentration in Löytynlähde was 0.12 mg C L⁻¹ and in the bog outlet water of
255 Laavionsuonoja 74.0 mg C L⁻¹. All the concentrations were within the measurement range given by the manufacturer of
256 the 5-mm pathlength sensor (NO_x-N < 70.0 mg N L⁻¹ and for DOC < 90.0 mg C L⁻¹).

257

258 The 5-mm pathlength sensor failed to measure NO_x-N in the two highest concentrations, 16.0 and 32.0 mg NO_x-N L⁻¹
259 (**Fig. 4**) and gave 'NaN' indications in the high-OC waters of Laavionsuonoja. As a result, the measurement range
260 observed was 0.031-8.03 mg NO_x-N L⁻¹, having a strong linear correlation ($R^2 = 0.99$, $p < 0.01$). In the spring water
261 with low OC, the sensor measured all the samples accurately ($R^2 = 1.0$, $p < 0.001$) within the concentration range 0.56-
262 33.0 mg NO_x-N L⁻¹.

263

264 **Fig. 4** NO_x-N concentrations of Laavionsuonoja (empty circles) and Löytynlähde (filled circles) measured with the 5-
265 mm pathlength sensor, compared with the laboratory results. The detection limit for the laboratory NO_x-N was 0.010
266 mg N L⁻¹

267

268 NO_x-N error ratio

269

270 The proportional difference between the laboratory and sensor results is referred to as the NO_x-N error ratio. This ratio
271 was plotted against the corresponding DOC concentration to demonstrate the effects of OC on the NO_x-N results by the
272 sensors. Only study sites with at least four data points were included. Based on the results, the NO_x-N error ratio for the
273 35-mm pathlength sensor mostly increased along with the corresponding DOC concentration (DOC < 10.0 mg C L⁻¹),
274 yet remained below 2.0 (**Fig. 5**). The OC strongly influenced the NO_x-N results by the 5-mm pathlength sensor in the
275 bog waters, resulting in error ratios of 40.0-66.0 and 3.7-15.0 (**Fig. 6a**). The first data points in these lines (error ratios
276 40.0 and 3.7) were observed in DOC concentrations of 7.4 and 4.03 mg C L⁻¹. In samples with no bog waters (DOC
277 below 10.0 mg C L⁻¹), the NO_x-N error ratio remained under 2.0 (**Fig. 6b**).

278

279 **Fig. 5** NO_x-N error ratios for the 35-mm pathlength sensor with corresponding DOC concentrations (< 10.0 mg C L⁻¹)
280 in Letkunoja, Teuronjoki, Pääjärvi, and Luhtaanmäenjoki

281

282 **Fig. 6a, b** NO_x-N error ratios for the 5-mm pathlength sensor with corresponding DOC concentrations, including a) bog
283 outlet waters with high levels of OC (Laavionsuonoja and mixture of Laavionsuonoja and Luhtaanmäenjoki) and b)
284 Letkunoja, Teuronjoki, and Pääjärvi. Note the different scales

285

286 Experiment III

287

288 Sensor data collected from Koiransuolenoja was corrected with two different methods. In SLR, the correlation
289 coefficient of the regression function between the sensor and laboratory NO_x-N was weak ($R^2 = 0.32$, $n = 25$). In MLR,
290 the sensor NO_x-N data were corrected, based on the resulting regression function (laboratory NO_x-N = 781.087 -
291 (74.994 * scan-DOC) + (0.935 * scan-NO_x-N), and the model was significant ($R^2 = 0.92$, $p = 0.000$). Yet, both sensor
292 results showed the dynamic nature of the NO_x-N concentration in the agriculturally influenced stream. As can be seen
293 in **Fig. 7a** and **b**, the sensor data corrected by MLR were more accurate ($R^2 = 0.93$, $p = 0.000$) than the sensor data with
294 higher variability corrected by SLR ($R^2 = 0.33$, $p < 0.010$). The mean NO_x-N concentrations were similar with both
295 correction methods (2.4 mg L⁻¹), and no statistical differences were found in the medians with the Wilcoxon signed-
296 ranks test.

297

298 **Fig. 7a, b** Laboratory NO_x-N results indicated with circles (n = 25) in relation to the continuous 5-mm pathlength
299 sensor results from Koiransuolenoja Brook on 3 May to 22 November 2013 corrected by a) simple linear regression
300 (laboratory NO_x-N result as the only explanatory variable) and b) multiple linear regression (explanatory variables scan-
301 DOC, scan-NO_x-N) (unpublished data, Uusheimo et al.)
302

303 **Discussion**

304
305 Originally, optical nitrate sensors were developed for ocean environments with low turbidity and color (Pellerin et al.
306 2013). Thus, their use in various aquatic environments, such as rivers and lakes with high turbidity and color, calls for
307 careful planning in application procedure. Use of these sensors resulted in increasing overestimation of the NO_x-N
308 concentrations under ascending OC, which was not a result of inaccurate measurement of OC, but rather due to the
309 partial absorption of light at the same wavelengths. The 35-mm pathlength sensor, recommended for lower NO_x-N and
310 OC concentrations, performed according to the manufacturer's specifications, excluding the samples of bog outlet
311 waters, in which the TOC concentrations fell within the measuring range given by the manufacturer. This result is in
312 agreement with the findings of Drolc and Vrtovšek (2010), who observed that the NO_x-N values obtained from the
313 sensor were higher than those from the reference method, due to interference from other substances in the local water
314 matrix. Additional support for cross-sensitivity between OC and NO_x-N was found in the KNO₃ amendment
315 experiment, in which the measuring range of NO_x-N by the 5-mm pathlength sensor was clearly decreased under the
316 influence of the high OC levels in the bog outlet water. However, a matter of OC quality was also seemingly involved,
317 because the NO_x-N error ratio was higher in the bog outlet waters than in the river, stream, or lake water. It should be
318 noted that the error ratio was not linear. In addition, both sensors were unreliable at measuring low NO_x-N
319 concentrations (< 0.28 mg N L⁻¹) often resulting in readings of zero in the spring, lake and stream waters. This should
320 be considered, especially in working with waters typically low in nitrates, such as forest areas, oligotrophic lakes,
321 headwaters, and other more pristine areas.
322

323 As the manufacturer states, the accuracy of the measurements can be improved by local calibration, i.e. correcting the
324 sensor results with sufficient manual sampling. Caradot et al. (2015) suggested that calibration should be based on at
325 least 15-20 samples, covering for the most part the variation in concentration of the substance measured. There are
326 several procedures for calibration; Lepot et al. (2016) used SLR, which has proven a robust and applicable tool. Our
327 results show that if sufficient accuracy in sensor NO_x-N results is not achieved by simple correction, an MLR including
328 OC data can be applied to achieve a more accurate correction.

329
330 Based on the results of this study, local calibration in NO_x-N measurements with optical sensors should always be
331 carried out in dealing with waters containing some organic matter. Streams, lakes, and other natural boreal waters can
332 contain high amounts of OC. In Finland, due to the large areas of coniferous forest and marshland, the average TOC of
333 surface waters ranges from 0.50 to 47.0 mg L⁻¹ (median 12.0 mg L⁻¹) (Kortelainen 1999). In boreal latitudes, seasonality
334 may also result in changes in freshwater OC quantity and quality (e.g. Erlandsson et al. 2012). Interference of organic
335 matter in NO_x-N measurements with optical sensors may become even more relevant, due to future changes in climate
336 and their effects on OC.

337

338 In conclusion, neither sporadic manual sampling nor continuous sensor monitoring can ensure excellent data quality
339 alone. Our results demonstrate that sensor data need to be verified with samples analyzed in the laboratory. When the
340 data quality of photometric sensors has been assured with care, high-frequency measurements by sensors can provide
341 extremely useful data and improve our knowledge of water quality and nutrient loading, especially in highly dynamic
342 aquatic environments.

343

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345

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