

1 **Technical Note: Large overestimation of pCO₂ calculated from pH and alkalinity in**
2 **acidic, organic-rich freshwaters**

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23 **Abstract**

24 Inland waters have been recognized as a significant source of carbon dioxide (CO₂) to the
25 atmosphere at the global scale. Fluxes of CO₂ between aquatic systems and the atmosphere
26 are calculated from the gas transfer velocity and the water-air gradient of the partial pressure
27 of CO₂ (pCO₂). Currently, direct measurements of water pCO₂ remain scarce in freshwaters,
28 and most published pCO₂ data are calculated from temperature, pH and total alkalinity (TA).
29 Here, we compare calculated (pH and TA) and measured (equilibrator and headspace) water
30 pCO₂ in a large array of temperate and tropical freshwaters. The 761 data points cover a wide
31 range of values for TA (0 to 14200 μmol L⁻¹), pH (3.94 to 9.17), measured pCO₂ (36 to 23000
32 ppmv), and dissolved organic carbon (DOC) (29 to 3970 μmol L⁻¹). Calculated pCO₂ were
33 >10% higher than measured pCO₂ in 60% of the samples (with a median overestimation of
34 calculated pCO₂ compared to measured pCO₂ of 2560 ppmv) and were >100% higher in the
35 25% most organic-rich and acidic samples (with a median overestimation of 9080 ppmv). We
36 suggest these large overestimations of calculated pCO₂ with respect to measured pCO₂ are
37 due to the combination of two cumulative effects: (1) a more significant contribution of
38 organic acids anions to TA in waters with low carbonate alkalinity and high DOC
39 concentrations; (2) a lower buffering capacity of the carbonate system at low pH, that
40 increases the sensitivity of calculated pCO₂ to TA in acidic and organic-rich waters. No
41 empirical relationship could be derived from our dataset in order to correct calculated pCO₂
42 for this bias. Owing to the widespread distribution of acidic, organic-rich freshwaters, we
43 conclude that regional and global estimates of CO₂ outgassing from freshwaters based on pH
44 and TA data only are most likely overestimated, although the magnitude of the overestimation
45 needs further quantitative analysis. Direct measurements of pCO₂ are recommended in inland
46 waters in general, and in particular in acidic, poorly buffered, freshwaters.

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48

49 **1 Introduction**

50 Inland waters (streams, rivers, lakes, reservoirs, wetlands) receive carbon from terrestrial
51 landscapes, usually have a net heterotrophic metabolism, and emit significant amounts of CO₂
52 to the atmosphere (Kempe 1984; Cole et al. 1994; Raymond et al. 2013). This terrestrial-
53 aquatic-atmosphere link in the global carbon cycle is controlled by complex biogeographical
54 drivers that generate strong spatial and temporal variations in the chemical composition of
55 freshwaters and the intensity of CO₂ outgassing at the water-air interface (e.g. Tamooh et al.
56 2013; Dinsmore et al. 2013; Abril et al. 2014; Borges et al. 2014). Hence, large datasets are
57 necessary in order to describe the environmental factors controlling these CO₂ emissions and
58 to quantify global CO₂ fluxes from inland waters (Sobek et al. 2005; Barros et al. 2011;
59 Raymond et al. 2013). Dissolved inorganic carbon (DIC) concentration and speciation in
60 freshwaters greatly depend on the lithological nature of watersheds (Meybeck 1987). For
61 instance, rivers draining watersheds rich in carbonate rocks have a high DIC concentration,
62 generally well above 1000 μmol L⁻¹. Bicarbonate ions contribute to the majority of the total
63 alkalinity (TA) in these waters, which have high conductivities and high pH. In these hard
64 waters, dissolved CO₂ represents a minor fraction (5-15%) of the DIC compared to
65 bicarbonates. In rivers draining organic-rich soils and non-carbonate rocks, DIC
66 concentrations are lower (typically a few hundred μmol L⁻¹) but dissolved organic carbon
67 (DOC) concentrations are higher, and commonly exceed the DIC concentrations. Organic
68 acid anions significantly contribute to TA of these soft waters (Driscoll et al. 1989; Hemond
69 1990), which have low conductivities and low pH. Dissolved CO₂ represents a large,
70 generally dominant, fraction of DIC in these acidic, organic-rich waters.

71

72 Fluxes of CO₂ between aquatic systems and the atmosphere can be computed from the water-
73 air gradient of the concentration of CO₂ and the gas transfer velocity (Liss and Slater 1974) at
74 local (e.g. Raymond et al. 1997), regional (e.g. Teodoru et al. 2009), and global scales (e.g.

75 Cole et al. 1994; Raymond et al. 2013). The partial pressure of CO₂ (pCO₂) is relatively
76 constant in the atmosphere compared to surface freshwaters pCO₂ that can vary by more than
77 4 orders of magnitude spatially and temporally (Sobek et al. 2005; Abril et al. 2014).
78 Consequently, water pCO₂ controls the intensity of the air-water flux, together with the gas
79 transfer velocity. Presently, both measured and calculated water pCO₂ data are used to
80 compute CO₂ fluxes from freshwater systems, although calculated pCO₂ is overwhelmingly
81 more abundant than directly measured pCO₂ (e.g. Cole et al. 1994; Raymond et al. 2013).
82 pCO₂ can be calculated from the dissociation constants of carbonic acid (which are a function
83 of temperature) and any of the following couples of measured variables: pH/TA, pH/DIC,
84 DIC/TA (Park 1969). In a majority of cases, calculated pCO₂ is based on the measurements of
85 pH/TA and water temperature. These three parameters are routinely measured by many
86 environmental agencies, and constitute a very large database available for the scientific
87 community. Calculation of pCO₂ from pH and TA has been initiated in World Rivers in the
88 1970's (Kempe 1984) and relies on the dissociation constants of carbonic acid, and the
89 solubility of CO₂, all of which are temperature-dependent (Harned and Scholes 1941; Harned
90 and Davis 1943; Millero 1979; Stumm and Morgan 1996). Measured pCO₂ is based on water-
91 air phase equilibration either on discrete samples (headspace technique, e.g. Weiss 1981) or
92 continuously (equilibrator technique, e.g. Frankignoulle et al. 2001) using various systems
93 and devices, followed by direct, generally infrared (IR), detection of CO₂ in the equilibrated
94 gas. Commercial IR gas analysers are becoming cheaper and more accurate, stable and
95 compact, and provide a large range of linear response well adapted to variability of pCO₂
96 found in freshwaters.

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98 Only a limited number of studies have compared directly measured pCO₂ to computed pCO₂.
99 Earlier examples provided a comparison between pCO₂ measured by headspace equilibration
100 coupled to gas chromatography (GC), and pCO₂ calculated from pH and DIC (Kratz et al.

101 1997; Raymond et al. 1997). Reports by these authors in Wisconsin lakes and the Hudson
102 River show that the pCO₂ values were linearly correlated but showed a variability of ±500
103 ppmv around the 1:1 line, over a range of measured pCO₂ from 300 to 4000 ppmv. Later,
104 Frankignoulle and Borges (2001) reported the first comparison of pCO₂ calculated from pH
105 and TA and pCO₂ measured by equilibration coupled to an IR analyzer in an estuary in
106 Belgium. In this high TA (2500-4800 μmol L⁻¹) and high pH (>7.4) system, they found a
107 good agreement between both approaches, calculated pCO₂ being either overestimated or
108 underestimated, but always by less than 7%. In 2003, concomitant measurements of pH, TA
109 and pCO₂ were performed in acidic, humic-rich (“black” type) waters of the Sinnamary River
110 in French Guiana (Abril et al. 2005; 2006). Calculation of pCO₂ from pH (~5) and TA (~200
111 μmol L⁻¹) gave unrealistically high values compared to those measured directly with a
112 headspace technique (typically 30000 ppmv versus 5000 ppmv). Direct measurements of CO₂
113 and CH₄ outgassing fluxes with floating chambers and the computation of the respective gas
114 transfer velocities of these two gases (Guérin et al. 2007) confirmed that pCO₂ values
115 calculated from pH and TA were overestimated compared to direct measurements in the
116 Sinnamary River. More recently, Hunt et al. (2011) and Wang et al. (2013) provided evidence
117 that organic acid anions in DOC may significantly contribute to TA in some rivers and
118 generate an overestimation of calculated pCO₂. Butman and Raymond (2011) reported higher
119 calculated than measured pCO₂ in some US streams and rivers, but no information was
120 available on the potential role of organic acids on this overestimation. These authors
121 concluded that the low number of samples in their study reflected the need for more research
122 on this topic.

123

124 With the growing interest on pCO₂ determination in freshwaters globally, and given the
125 apparent simplicity and low cost of pH and TA measurements, the number of publications
126 that report calculated pCO₂ in freshwaters has increased dramatically in the last decade. Some

127 of these publications report extremely high and potentially biased $p\text{CO}_2$ values in low-
128 alkalinity and high DOC systems. It has thus become necessary to pay attention to this issue
129 and investigate the occurrence of such potential bias and its magnitude in the different types
130 of freshwaters. Here, we present a large dataset of concomitant measurements of temperature,
131 pH, TA, $p\text{CO}_2$, and DOC in freshwaters. This is the first comprehensive dataset to investigate
132 the magnitude of the bias between calculated and measured $p\text{CO}_2$, as it covers the entire range
133 of variation of most parameters of the carbonate system in freshwaters. The objective of this
134 paper is to alert the scientific community to the occurrence of a bias in $p\text{CO}_2$ calculation from
135 pH and TA in acidic, poorly buffered and organic-rich freshwaters, to briefly discuss its origin
136 in terms of water chemistry, and to provide the range of pH, TA and DOC values where $p\text{CO}_2$
137 calculation should be abandoned and the range where it still gives relatively accurate results.

138

139 **2 Material and Methods**

140 2.1 Sample collection

141 Our dataset consists of 761 concomitant measurements of temperature, pH, TA, water $p\text{CO}_2$,
142 and DOC in 12 contrasting tropical and temperate systems in Europe, Amazonia and Africa
143 (Fig. 1; Table 1). These samples were obtained in the Central Amazon River and floodplains
144 system in Brazil, the Athi-Galana-Sabaki River in Kenya, the Tana River (Kenya), small
145 rivers draining the Shimba Hills in southeastern Kenya, the Congo River and tributaries in the
146 Democratic Republic of the Congo (DRC), Lake Kivu in Rwanda and DRC, the Leyre River
147 and tributaries in France, the Loire River in France, the Meuse River in Belgium, the Rianila
148 and Betsiboka Rivers in Madagascar, the Sinnamary River downstream of the Petit Saut
149 Reservoir in French Guiana, and the Zambezi River in Zambia and Mozambique (Fig. 1).
150 Details on some of the sampling sites can be found in Abril et al. (2005; 2014), Borges et al.
151 (2012 ; 2014), Marwick et al. (2014a,b), Polsenaere et al. (2013), Tamooh et al. (2013),
152 Teodoru et al. (2014). These watersheds span a range of climates and are occupied by

153 different types of land cover, which include tropical rainforest (Amazon, Congo, Rianila), dry
154 savannah (Tana, Athi-Galana-Sabaki, Betsiboka, Zambezi), temperate pine forest growing on
155 podzols (Leyre), mixed temperate forest, grassland and cropland (Meuse) and cropland
156 (Loire). Lithology is also extremely contrasted as it includes for instance carbonate-rocks
157 dominated watershed as for the Meuse, sandstone dominated silicates (Leyre), and
158 precambrian crystalline magmatic and metamorphic rocks with a small proportion of
159 carbonate and evaporite rocks for the Congo river.

160

161 2.2 Field and laboratory measurements

162 Although pH measurements might seem almost trivial, highly accurate and precise pH data
163 are in fact not easy to obtain, especially in low-ionic strength waters, where electrode readings
164 are generally less stable. Even though pH measurements in the laboratory might be more
165 accurate, it is crucial to measure pH *in situ* or immediately after sampling, as pH
166 determination several hours or days after sampling will be affected by CO₂ degassing and/or
167 microbial respiration (Frankignoulle and Borges 2001). In this work, water temperature and
168 pH were measured in the field with different probes depending on the origin of the dataset.
169 However, all the pH data were obtained with glass electrodes and rely on daily calibration
170 with two-point United States National Bureau of Standards (NBS) standards (4 and 7).
171 Measurements were performed directly in the surface water, or in collected water
172 immediately after sampling.

173

174 Several techniques were used to measure water pCO₂. Water-gas equilibration was performed
175 with a marbles type equilibrator (Frankignoulle et al. 2001) for the Amazon, Loire, Leyre,
176 Sinnamary and Congo Rivers (December 2013) as well for Lake Kivu, or with a Liqui-Cel
177 MiniModule membrane contactor equilibrator (see Teodoru et al. 2009; 2014) for the
178 Zambezi and some sites within the Congo basin (December 2012): water was pumped either

179 continuously from a ship, or on an ad hoc basis from the bank of the rivers after waiting ~15
180 minutes for complete equilibration; air was continuously pumped from the equilibrator to the
181 gas analyser (see *e.g.* Abril et al. 2014 for a more detailed description of the system). A
182 syringe-headspace technique (Kratz et al. 1997; Teodoru et al. 2009) was used in the field in
183 all African rivers and in the Meuse River: 30 mL volume of atmospheric air was equilibrated
184 with 30 mL volume of river water by vigorously shaking during 5-10 min in four replicate
185 gas-tight syringes. The four replicates 30 mL of equilibrated gas and a sample of atmospheric
186 air were injected in an IR gas analyser (Li-Cor® models 820 or 840, or PP systems® model
187 EGM-4); the first gas injection served as a purge for the air circuit and cell and the three other
188 injections were used as triplicate pCO₂ determination (average repeatability of ±1%). The
189 pCO₂ in the river water was deduced from that measured in the headspace accounting for the
190 initial pCO₂ in the air used for equilibration, water temperature in the river and in the water at
191 equilibrium in the syringe, and based on Henry's law. Comparison between syringe-
192 headspace and marbles or membrane equilibrator was made during two cruises on the Congo
193 River and three cruises in the Zambezi basin and gave very consistent results, deviation from
194 the 1:1 line being always less than 15% (see Fig. 2). This highlights the consistency of the
195 present data-set of direct pCO₂ measurements although different techniques were used. A
196 serum bottle-headspace technique (Hope et al. 1995) was also used on the Sinnamary River;
197 surface water was sampled in 120 mL serum bottles that were poisoned with HgCl₂ and
198 sealed excluding air bubbles. Back in the laboratory, a 40 mL headspace was created with
199 pure N₂ (Abril et al. 2005). The CO₂ concentration of equilibrated gas in the headspace was
200 analysed by injecting small volumes (0.5 mL) of gas in a GC calibrated with certified gas
201 mixtures.
202
203 Immediately after water-gas phase equilibration, CO₂ was detected and quantified in most
204 samples with non-dispersive IR gas analysers (Frankignoulle et al. 2001; Abril et al. 2014).

205 The gas analysers were calibrated before each field cruise, with air circulating through soda
206 lime or pure N₂ for zero and with a certified gas standard for the span. Depending on the
207 cruises and expected pCO₂ ranges, we used gas standard concentration of 1000 - 2000 ppmv,
208 or a set of calibration gases at 400, 800, 4000 and 8000 ppmv. Stability of the instrument was
209 checked after the cruise, and deviation of the signal was always less than 5%. These
210 instruments offer a large range of linear response, depending on manufacturers and model: 0-
211 20000 ppmv or 0-60000 ppmv. The linearity of a Li-COR® Li-820 gas analyser was verified
212 by connecting it to a closed circuit of gas equipped with a rubber septum to allow injection of
213 pure CO₂ with a syringe. Linearity was check by injecting increasing volumes of CO₂ in order
214 to cover the whole range of measurement and was excellent between zero and ~20000 ppmv.
215 In addition to the IR analysers generally used in this work, in the Sinnamary River, pCO₂ was
216 also measured with an INNOVA® 1312 optical filter IR photoacoustic gas analyser (range 0
217 – 25000 ppmv) connected to an equilibrator and with a Hewlett Packard® 5890 GC equipped
218 with a thermal conductivity detector (TCD); both analysers were calibrated with a gas mixture
219 of 5000 ppmv of CO₂. Both methods gave results consistent at ± 15% in the 0 – 13000 ppmv
220 range (Abril et al. 2006). Sinnamary data reported here are from headspace and GC
221 determination.

222

223 TA was analysed by automated electro-titration on 50 ml filtered samples with 0.1N HCl as
224 titrant. Equivalence point was determined with a Gran method from pH between 4 and 3
225 (Gran 1952). Precision based on replicate analyses was better than ±5 µmol L⁻¹. TA
226 measurements should be done on filtered samples; otherwise some overestimation would
227 occur in turbid samples, which may content significant amount of acid-neutralizing particles
228 (*e.g.* calcium carbonate). In contrast to TA measurements based on titration to an endpoint of
229 5.6 (*e.g.* Wallin et al. 2014), the Gran titration method allows the determination of TA values
230 in samples with situ pH down to ~4.5, *i.e.* very close to the dissociation constant of HCO₃⁻

231 /H₂CO₃. In most acidic samples with low TA, reproducibility was improved by slightly
232 increasing the pH by up to 0.2 units by vigorously stirring during ~15min in order to degas as
233 much CO₂ as possible before starting the titration. DOC was measured on samples filtered
234 through pre-combusted (490°C) glass fibre filter with a porosity of 0.7 µm and stored
235 acidified with ultrapure H₃PO₄ in borosilicate vials capped with polytetrafluoroethylene
236 stoppers. Analysis was performed with a Shimadzu TOC5000 analyser based on high
237 temperature catalytic oxidation, after removal of dissolved CO₂ for samples from Amazon,
238 Loire, Leyre, and Sinnamary Rivers. DOC concentrations were measured with a customized
239 wet oxidation TOC analyzer (Thermo HiperTOC, or IO Analytical Aurora 1030W) coupled to
240 a Delta+XL or Delta V IRMS..

241

242 2.3 pCO₂ calculation from pH and TA

243 We calculated pCO₂ from TA, pH and temperature measurements using carbonic acid
244 dissociation constants of Millero (1979) (based on those of Harned and Scholes (1941) and
245 Harned and Davis (1943)) and the CO₂ solubility from Weiss (1974) as implemented in the
246 CO2SYS program. Hunt et al. (2011) reported discrepancy lower than 2% for pCO₂ computed
247 this way with those obtained with the PHREEQC program (Parkhurst and Appelo, 1999).
248 Differences in software or dissociation constants cannot account for the large bias in
249 calculated pCO₂ compared to measured pCO₂ we report in this paper.

250

251 **3 Results**

252 3.1 Data ranges and patterns in the entire dataset

253 Measured pCO₂ varied between 36 ppmv in a floodplain of the Amazon River and 23000
254 ppmv in a first order stream of the Leyre River (Table 1). Minimum values of pH and TA
255 occurred in the Congo River (pH = 3.94 and TA = 0) and maximum values in Lake Kivu (pH
256 = 9.16 and TA = 14200 µmol L⁻¹). Highest DOC concentrations (>3000 µmol L⁻¹) were

257 observed in small streams in the Congo basin and in first order streams draining podzolized
258 soils in the Leyre basin. Lowest DOC concentrations ($<40 \mu\text{mol L}^{-1}$) occurred in some
259 tributaries of the Athi-Galana-Sabaki, in the Rianila and Betsiboka Rivers, and in the Shimba
260 Hills streams. When considering the whole dataset, measured pCO_2 and DOC were negatively
261 correlated with pH, whereas TA was positively correlated with pH (Fig. 3, $p < 0.0001$ for the
262 three variables). This illustrates the large contrast in acid-base properties between acidic,
263 organic-rich and poorly buffered samples on the one hand, and basic, carbonate-buffered
264 samples on the other hand.

265

266 3.2 Comparison between measured and calculated pCO_2

267 Calculated pCO_2 was more than 10% lower than measured pCO_2 in 16% of the samples; both
268 methods were consistent at $\pm 10\%$ in 24% of the samples; calculated pCO_2 was more than
269 10% higher than measured pCO_2 in 60% of the samples and more than 100% higher in 26%
270 of the samples. Absolute values, as expressed in ppmv, were largely shifted toward
271 overestimation, calculated *versus* measured pCO_2 data being well above the 1:1 line, and
272 calculated minus measured pCO_2 values ranging between -6180 ppmv and +882022 ppmv
273 (Fig. 4). The largest overestimation of calculated pCO_2 occurred in the most acidic samples,
274 whereas underestimations of calculated pCO_2 occurred in neutral or slightly basic samples
275 (Fig. 4b). Ranking the data according to the pH, TA and DOC reveal that overestimation of
276 calculated pCO_2 compared to measured pCO_2 increased in acidic, poorly buffered waters in
277 parallel with an increase in the DOC concentration (Table 2). Discrepancies between
278 calculated and measured pCO_2 were very different from one system to another, depending on
279 the chemical status of the waters. On average at each sampled site, the relative overestimation
280 of calculated pCO_2 decreased with pH and TA and increased with DOC (Fig. 5).
281 Overestimation of calculated pCO_2 was on average $<10\%$ in the Kivu Lake, and the Meuse,
282 Loire, Shimba Hills and Tana Rivers, which all have neutral or basic pH, $\text{TA} > 1000 \mu\text{mol L}^{-1}$

283 and low to moderate DOC concentrations ($< 400 \mu\text{mol L}^{-1}$) (Fig. 5). In contrast, calculated
284 pCO_2 was overestimated by $>200\%$ on average in the Congo, Leyre, Sinnamary and Amazon
285 Rivers, which have acidic pH, $\text{TA} < 500 \mu\text{mol L}^{-1}$ and highest DOC concentration, reaching
286 $1000 \mu\text{mol L}^{-1}$ on average in the Congo. The cases of Athi-Galana-Sabaki, Rianila, Betsiboka,
287 and Zambezi Rivers were intermediate in pH, TA and DOC, and with average
288 overestimations of calculated pCO_2 of 50-90% (Fig. 5).

289

290 **4 Discussion**

291 4.1 Origin of overestimation of calculated pCO_2

292 Our dataset (Fig. 3; Table 1) probably covers the full range of conditions of carbon speciation
293 that can be encountered in continental surface waters. A pCO_2 overestimation negatively
294 correlated with pH ($p = 0.001$) and TA ($p = 0.005$) and positively correlated with DOC ($p <$
295 0.001) (Fig. 5) is consistent with the observations of Cai et al. (1998) in the freshwater end-
296 members of some estuaries in Georgia, USA, and of Hunt et al. (2011) in rivers in New
297 England (USA) and New Brunswick (CA). These authors performed NaOH back-titration in
298 order to measure non-carbonate alkalinity (NCA). They found that NCA accounted for a large
299 fraction (in some cases the majority) of TA; in addition, the contribution of inorganic species
300 other than carbonate was assumed negligible and most of the NCA was attributed to organic
301 acid anions. Hunt et al. (2011) also showed that in the absence of direct titration of NCA,
302 which is labour-intensive and whose precision may be poor, this parameter could be
303 calculated as the difference between the measured TA and the alkalinity calculated from
304 measurements of pH and DIC and the dissociation constants of carbonic acid. Using the latter
305 approach, Wang et al. (2013) obtained a positive correlation between NCA and DOC
306 concentrations in the Congo River, evidencing the predominant role of organic acids in DIC
307 speciation and pH in such acidic system. Because we did not directly measure DIC in this
308 study, we could not calculate NCA with the same procedure as these studies. We attempted to

309 calculate TA from our measured pH and pCO₂ with the CO2SYS program. However, TA
310 values calculated this way were inconsistent with other measured variables (with sometimes
311 negative values). Indeed, because pH and pCO₂ are too interdependent in the carbonate
312 system, very small analytical errors on these variables lead to large uncertainties in the
313 calculated TA (Cullison Gray et al. 2011). A second attempt to correct our TA data from
314 NCA consisted in calculating organic alkalinity using pH and DOC as input parameters. We
315 compared the model of Driscoll et al. (1989), which assumes a single pK value for all organic
316 acids, and the triprotic model of Hruska et al. (2003), which assumes three apparent pK values
317 for organic acids. These two models applied to our pH and DOC gave very similar organic
318 alkalinity values, which could be subtracted from the measured TA. In the most acidic
319 samples (*e.g.* some sites from the Congo basin), modelled organic alkalinities were larger than
320 measured TA and the difference was thus negative. Nevertheless, we then recalculated pCO₂
321 from the measured pH and the TA corrected from organic alkalinity. Calculated pCO₂
322 corrected with that method were, however, still very different from those measured in the
323 field, being sometimes higher and sometimes lower than the measured pCO₂, without any
324 meaningful pattern (indeed, corrected pCO₂ was negatively correlated ($p < 0.001$) with
325 measured pCO₂). Consequently, we were unable to derive any empirical relationship to
326 correct for the bias in pCO₂ calculation from pH and TA. Nevertheless, the negative
327 correlation between pH and DOC and positive correlation between pH and TA (Fig. 3)
328 confirm a strong control of organic acids on pH and DIC speciation across the entire dataset.

329

330 As discussed by Hunt et al. (2011), a significant contribution of organic acids to TA leads to
331 an overestimation of calculated pCO₂ with the CO2SYS program, or with any program that
332 accounts only for the inorganic species that contribute to TA. It is thus obvious that the
333 observed increase in pCO₂ overestimation when pH decreases (Fig. 4b & 5; Table 2) is due to
334 an increasing contribution of organic acid anions to TA. However, this effect is not the only

335 driver of the observed overestimation of pCO₂, which is also due to a decrease in the
336 buffering capacity of the carbonate system at acidic pH. To investigate the magnitude of this
337 second effect, we calculated the factor dpCO₂/dTA (in ppmv mol⁻¹), which describes the
338 change in calculated pCO₂ induced by a change in TA. This factor, which is the opposite of a
339 buffer factor as it reflects the sensitivity of pCO₂ calculation to the TA, increases
340 exponentially when pH decreases (Fig. 6a), i.e. it is proportional to the H⁺ concentration. To
341 go further in this theoretical analysis, we computed the difference between the pCO₂
342 calculated at a given TA value and the one calculated at a slightly higher TA value (TA+ X
343 μmol L⁻¹). These calculations reveal an extreme sensitivity of calculated pCO₂ to TA at acidic
344 pH (Figure 6b). For instance, increasing TA by 5 μmol L⁻¹ (a value close to the precision of
345 TA titrations) increases the calculated pCO₂ by 31 ppmv at pH 7, by 307 ppmv at pH 6 and by
346 3070 at pH 5. Increasing TA by 100 μmol L⁻¹ (a typical value of NCA found in freshwaters,
347 Driscoll et al. 1994; Cai et al. 1998; Hunt et al. 2011), increases the calculated pCO₂ by 615
348 ppmv at pH 7, by 6156 ppmv at pH 6 and by 61560 ppmv at pH 5. Note that this increase in
349 calculated pCO₂ is independent of the chosen initial TA value. The difference between
350 calculated and measured pCO₂ from our data-set, shows that a NCA contribution around 100
351 μmol L⁻¹ is sufficient to explain the overestimation of calculated pCO₂ of most samples at
352 pH<6, whereas a NCA contribution higher than 500 μmol L⁻¹ would be necessary for several
353 samples at circumneutral and slightly basic pH (Fig. 5b). Samples requiring this high NCA
354 contribution are from the Athi-Galana-Sabaki and Zambezi watersheds, and correspond to TA
355 values well above 1000 μmol L⁻¹. A NCA value of 500 μmol L⁻¹ in these samples is thus
356 plausible.

357

358 We have no definitive explanation for lower calculated than measured pCO₂, which is
359 observed mainly at neutral to slightly basic pH, e.g., in the Zambezi River (Fig. 4). In most of
360 these samples, owing to the relatively high TA value, an overestimation of pH of less than 0.2

361 units is sufficient to account for the low calculated pCO₂ compared to measured values. In
362 general, it is not easy to judge how accurate are pH measurements, and even less when data
363 come from environmental agencies. Thus, one factor of variability throughout the dataset as
364 well as in literature data is the accuracy of pH measurements – despite the care taken (e.g,
365 calibrations with NBS buffers for each day of measurements), we cannot rule out that drift or
366 malfunction of pH electrodes contribute to the observed variability, constituting an additional
367 disadvantage compared to direct pCO₂ measurements with very stable gas analysers.

368

369 4.2 Impact on estimates of CO₂ emissions from freshwaters

370 According to our analysis, overestimation of calculated pCO₂ is largest in acidic, poorly
371 buffered and organic-rich waters. Consequently, the overestimation of regional and global
372 CO₂ emissions computed from calculated pCO₂ depends on the relative contribution of these
373 types of waters worldwide. In their analysis, Raymond et al. (2013) have discarded all
374 calculated pCO₂ values with a pH value of less than 5.4, as well as all pCO₂ values above
375 100000 ppmv. These criteria would exclude only 8% of samples from our dataset. Indeed,
376 from our analysis, it appears that overestimation of calculated pCO₂ occurs at pH much higher
377 than 5.4 (Fig. 4,5&6; Table 2). Both techniques were consistent at ±10% on average in only
378 five of the twelve studied systems, which combine a circumneutral to basic pH with a TA
379 concentration well above 1000 μmol L⁻¹ (Fig. 5). Although it would not be sufficient for the
380 cases of the Zambezi and Athi-Galana-Sabaki Rivers where overestimation is still significant,
381 a TA value above 1000 μmol L⁻¹ appears as a more robust criteria, rather than a pH threshold,
382 to separate calculated pCO₂ affected by bias from those consistent with measured pCO₂
383 (Table 2). In fact, pCO₂ calculation from pH and TA in freshwaters historically relies on
384 theoretical background and validation data in high alkalinity waters (Neal et al. 1998),
385 including karstic waters (Kempe 1975). At the global scale, high TA typically occurs in rivers
386 draining watersheds with a significant proportion of carbonate rocks, typically >30% of their

387 surface area if the criteria of $TA > 1000 \mu\text{mol L}^{-1}$ is chosen and the normalized weathering
388 rates of Meybeck (1987) are applied. According to Meybeck (1987), the average and
389 discharge-weighted TA is around $900 \mu\text{mol L}^{-1}$ for world rivers and around $600 \mu\text{mol L}^{-1}$ for
390 tropical rivers. Among the 25 largest rivers in the world, 15 have a $TA > 1000 \mu\text{mol L}^{-1}$
391 according to Cai et al. (2008). The two largest rivers in the world in terms of discharge, the
392 Amazon and the Congo, are also well below this limit of $1000 \mu\text{mol L}^{-1}$ and have large
393 overestimation in calculated $p\text{CO}_2$ (on average 200% and 360%, respectively). Very low TA
394 and pH and high DOC values have also been reported in boreal streams and rivers (Humborg
395 et al. 2010; Dinsmore et al. 2012; Wallin et al. 2014).

396

397 In lakes, the highest $p\text{CO}_2$ values in the literature come from tropical black water lakes and
398 were also calculated rather than directly measured (Sobek et al. 2005). Calculated $p\text{CO}_2$ was
399 65250 ppmv in Lago Tupé in the Brazilian Amazon, a Ria Lake connected to the Rio Negro,
400 where, according to our own dataset, pH is below 5 and TA is around $70 \mu\text{mol L}^{-1}$. It was
401 18950 ppmv in Kambanain Lake in Papua New Guinea corresponding to a pH value of 6.1
402 and a TA value of $350 \mu\text{mol L}^{-1}$ (Vyverman 1994). This suggests a widespread overestimation
403 of calculated $p\text{CO}_2$ that significantly impacts the estimation of global CO_2 emissions from
404 inland waters. However, a precise analysis based on exact quantitative information on the
405 relative contribution of acidic and high and low alkalinity waters to the total surface area of
406 inland waters is necessary in order to evaluate the exact magnitude of the overestimation.

407

408 **5 Conclusion**

409 From our analysis, it appears that the validity of calculating $p\text{CO}_2$ from pH, TA, and
410 temperature is most robust in freshwaters with circumneutral to basic pH and with TA
411 exceeding $1000 \mu\text{mol L}^{-1}$. At lower TA and pH, however, calculated $p\text{CO}_2$ (and hence, CO_2
412 degassing rates) are overestimated by 50 to 300% relative to direct, *in situ* $p\text{CO}_2$

413 measurements. Since a large majority of freshwater systems globally have characteristics
414 outside of the range of applicability of pCO₂ calculation, it appears reasonable to assume that
415 recent estimates of global CO₂ emission from lakes and rivers, which are based exclusively on
416 calculated pCO₂ data, are overestimated. We propose that while TA and pH measurements
417 remain useful to describe the aquatic chemistry, data on pCO₂ should in the future rely on
418 direct measurements of pCO₂. Even if some studies report relatively robust calculation of
419 pCO₂ from pH and DIC measurements (Raymond et al. 1997; Kratz et al. 1997; Aberg and
420 Wallin 2014), direct pCO₂ in the field are stable, precise, and straightforward and do not
421 depend on the quality of pH measurements, which are often uncertain. Further, high-quality
422 DIC measurements are very time consuming, fairly complicated to set-up and do not allow to
423 carry out continuous measurements in a simple and straightforward fashion. Although there
424 are some practical limitations to their use in the field, submerged IR sensors, which allow
425 high temporal resolution, are also promising (Johnson et al. 2010). The long-term instrument
426 stability and accuracy based on newly developed Off-Axis Integrated Cavity Output
427 Spectroscopy and Cavity Ring-Down Spectroscopy technologies seems to improve in
428 comparison to traditional IR instruments, although the latter are more affordable, compact and
429 with lower power requirements. Joint international efforts are necessary to define most
430 appropriate protocols for the measurements of dissolved inorganic carbon parameters in
431 freshwaters.

432

433

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