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Quantifying tropical peatland dissolved organic carbon (DOC) using UV-visible spectroscopy

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ABSTRACT

UV–visible spectroscopy has been shown to be a useful technique for determining dissolved organic carbon (DOC) concentrations. However, at present we are unaware of any studies in the literature that have investigated the suitability of this approach for tropical DOC water samples from any tropical peatlands, although some work has been performed in other tropical environments. We used water samples from two oil palm estates in Sarawak, Malaysia to: i) investigate the suitability of both single and two-wavelength proxies for tropical DOC determination; ii) develop a calibration dataset and set of parameters to calculate DOC concentrations indirectly; iii) provide tropical researchers with guidance on the best spectrophotometric approaches to use in future analyses of DOC. Both single and two-wavelength model approaches performed well with no one model significantly outperforming the other. The predictive ability of the models suggests that UV–visible spectroscopy is both a viable and low cost method for rapidly analyzing DOC in water samples immediately post-collection, which can be important when working at remote field sites with access to only basic laboratory facilities.

1. Introduction

Dissolved organic carbon (DOC) is derived from the solubilisation of organic matter, and can be leached from the terrestrial landscape into freshwater ecosystems (Thurman, 1985). It plays a crucial role in peatland carbon budgets (Cole et al., 2007; Hulatt et al., 2014; Abrams et al., 2015; Muller et al., 2015) because it represents a carbon loss from the peat itself and, once in the aquatic system can be degraded, both biologically and photo-chemically, liberating CO2 (carbon dioxide), CH4 (methane) and CO (carbon monoxide) into the atmosphere (Cole et al., 2007; Clark et al., 2010; Fellman et al., 2014).

Interest in DOC losses from tropical peatlands has increased in recent years, fuelled in part by the realization of how vulnerable this carbon loss pathway is to land-use related disturbance (Moore et al., 2011, 2013; Evans et al., 2014; Rixen et al., 2016). Furthermore, the controls governing DOC mobility and export, along with their wider local and international implications, in the context of the global carbon cycle, still remain uncertain (Evans et al., 2012, 2014).

Measuring DOC directly in the laboratory requires specialised analytical equipment (e.g. a TOC analyser), which may hinder researchers with limited funds and laboratory equipment or those working in remote locations. An alternative and cheaper method is UV–visible spectrometry and spectroscopy, which relies on establishing relationships between DOC quantity and quality (Weishaar et al., 2003), and absorbance values and ratios (Peuravuori and Pihlaja, 1997), along with the ability to derive DOC compositional information based upon spectral slopes and ratios (Helms et al., 2008; Spencer et al., 2012). As such, UV–visible spectroscopy has been shown to be effective for determining DOC concentrations in temperate freshwater systems (De Haan et al., 1982; Tipping et al., 2009; Carter et al., 2012; Peacock et al., 2014; Causse et al., 2016) as well as tropical catchments (Yamashita et al., 2010; Pereira et al., 2014). Spectrophotometric absorbance over a wide range of wavelengths has been used as a proxy for DOC, ranging from 250 nm (De Haan et al., 1982) to 562 nm (Carpenter and Smith, 2014).
Peacock et al. (2014) investigated the effectiveness of a range of single wavelengths between 230 and 800 nm as a proxy for DOC in waters draining two temperate upland catchments in the UK. The strongest correlations between absorbance and DOC were at 230 nm, 254 nm and 263 nm (Peacock et al., 2014). The correlation between absorbance and DOC was observed to decline with increasing wavelength, a finding also noted by Asmala et al. (2012) and Grayson and Holden (2016). A further spectrophotometric approach is to use an empirical model based on two or more wavelengths to calculate DOC concentrations (e.g. 270 nm and 350 nm; Tipping et al., 2009; Carter et al., 2012). This proxy technique is based on the ratio of optical absorbance of a DOC molecule at a given wavelength (nm) to DOC, referred to as the extinction coefficient (E; units g⁻¹ cm⁻¹; Tipping et al., 2009) otherwise known as SUVA (specific UV absorbance). Developing this further Tipping et al. (2009) describe a two-component model that can predict DOC based on the linear sum of two components A and B (Carter et al., 2012). Both components have different features giving them distinct spectra; component A absorbs UV light strongly whereas component B absorbs it weakly (Carter et al., 2012). The model uses these optical absorbance properties as well as differing E coefficients to estimate DOC concentrations using a number of steps (Carter et al., 2012). A more detailed description of the model and parameterization, as outlined by Carter et al. (2012), is presented in Section 2.2. A set of universal extinction coefficients (EA 31; EA 32; EB 31; EB 32) for the model, at wavelengths 270 and 350 nm, were generated by Carter et al. (2012) using a large number (n = 1700) of surface water samples collected from the UK and Canada. In principle, any pair of wavelengths can be used (Carter et al., 2012) but 270 and 350 nm have been found to provide particularly robust DOC estimations. For a higher degree of accuracy the universal extinction coefficients can be adjusted for individual sites to produce a calibrated dataset. Carter et al. (2012) found that their two-wavelength method improved the fit between modelled and measured DOC concentrations compared to a single wavelength approach. The practicality and wide applicability of this two-wavelength approach has also been demonstrated by Peacock et al. (2014) who used the universal parameters to measure DOC in surface water, but found that the model had to be re-parameterised to calculate DOC in pore water. It would be useful if the same parameterization could be used to calculate DOC concentrations for other systems, including samples from tropical peatlands. Due to the differing environmental conditions and peat chemistry experienced between temperate and tropical regions, the composition of DOC from these systems is likely to vary. It is, therefore, important that these models are validated on tropical samples, particularly given the increased interest in tropical peat dynamics in recent years.

At present we are unaware of any studies in the literature that have investigated the suitability of UV–vis spectroscopy methods for measuring DOC concentrations in water samples from tropical peatland catchments and, specifically, from oil palm plantations. While other research has explored tropical DOC concentrations and composition using this method (Johnson et al., 2006; Waterloo et al., 2006; Spencer et al., 2010; Yamashita et al., 2010; Pereira et al., 2014), previous studies have focused on mineral soil-dominated forest catchments, within the Congo (Spencer et al., 2010), Guiana Shield (Yamashita et al., 2010; Pereira et al., 2014) and Amazon basin (Johnson et al., 2006; Waterloo et al., 2006), with only one referencing the presence of peat within their study site (Yamashita et al., 2010). In addition, of these studies only one (Pereira et al., 2014) has applied the original Carter et al. (2012) model in the context of a tropical catchment. In view of the potentially wide applicability of this method, the cost-saving benefits and its potential to produce accurate results without the need for specialised laboratory facilities (particularly valuable for sample analyses at remote field sites), it is important to properly evaluate it. The aims of this investigation are, therefore, three-fold:

1) To investigate the suitability of different wavelength absorbance proxies for tropical DOC determination;
2) To develop a calibration dataset and a set of parameters that can be used to calculate tropical DOC concentrations indirectly;
3) To provide guidance for other tropical researchers on the best UV–vis spectrophotometric approaches to take when analyzing similar samples.

2. Methods

2.1. Site descriptions and sampling

Water samples for this investigation were collected from the Sebungan and Sabaju oil palm estates, located in the Malaysian province of Sarawak, northern Borneo (between 3°07.8’N and 3°14.9’N and 113°18.2’E and 113°32.1’E; Fig. 1). Both estates are established on tropical peat soils and cover a collective area of nearly 10,000 ha. Air temperatures within this region are high (mean 26 °C) and there is heavy rainfall throughout the year (~3000 mm received annually; Melling et al., 2005).

Water samples were collected during two field campaigns: (A) 18th to 30th April 2015 and (B) 3rd August to 6th October 2015. All samples were collected in 60 ml Nalgene® bottles and filtered through 0.45 μm cellulose nitrate membrane filters, using a hand-held vacuum pump within 24 h of collection. As there was no spectrophotometer present on site during Campaign A filtered samples were subsequently stored in a fridge at 4 °C for 6–12 weeks, until shipment back to the UK for analysis. However, an onsite Cole-Parmer UV/visible spectrophotometer was present during Campaign B allowing immediate sample analysis. All samples, regardless of campaign, were subjected to cold storage which has been shown to ensure reasonable preservation of DOC between sampling and analysis (Cook et al., 2016). Subsequently, significant alterations to DOC concentrations and spectrophotometric properties would not be expected in between sampling and DOC analysis back in to the UK.

Upon return to the UK, samples collected during Campaign A were analysed in June 2015 and those from Campaign B in November 2015 on a Total Organic Carbon (TOC; Shimadzu) analyser (precision – 2–5%; Granéli et al., 1996; Bjorkvall et al., 2008; Shafer et al., 2010) as non-purgeable organic carbon (NPOC), to generate measured DOC concentrations. Prior to analysis, samples were acidified (pH < 3) and sparged with purified air to remove inorganic carbon. Measured DOC concentrations were subsequently calculated using a calibration curve ranging from 0 to 100 mg L⁻¹. Additional standards with concentrations close to those expected in the samples were analysed to check for drift. In parallel, samples were also analysed on a Helios Gamma spectrophotometer to measure UV–vis absorbance at different wavelengths. A set of filtered blanks were analysed in the same manner as the water samples to ensure the suitability of the cellulose nitrate filters for SUVA analysis. Filters leached 0.008 absorbance at 254 nm, and 0.4 mg L⁻¹ DOC. However, considering the relatively high DOC concentrations and absorbance values for the majority of water samples, along with the precision of the TOC analyser, leaching was considered negligible.
2.2. Two-wavelength model description

The two wavelength model predicts DOC concentrations \( C_{\text{DOC}} \) on the basis of light absorption at two wavelengths (Tipping et al., 2009; Carter et al., 2012). Briefly,

\[
C_{\text{DOC}} = \frac{(\alpha_{270} - \alpha_{350})}{E_{270}} + C_{\text{MAC}} \tag{1}
\]

where \( \alpha_{270} \) is the absorbance at 270 nm, \( \alpha_{350} \) is the absorbance at 700 nm (used to account for instrumental drift, after Hernes et al., 2008), \( C_{\text{MAC}} \) is a constant concentration of DOC which does not absorb light (assumed here to be the same as the value reported by Carter et al., 2012 i.e. 0.8 mg L\(^{-1}\)) and \( E_{270} \) is an extinction coefficient (absorbance cm\(^{-1}\) \(C_{\text{DOC}}\)) of the light-absorbing DOC, given by

\[
E_{270} = (f_A \cdot E_{A,270}) + (f_B \cdot E_{B,270}) = (f_A \cdot E_{A,270}) + ((1 - f_A) \cdot E_{B,270}) \tag{2}
\]

where \( f_A \) and \( f_B \) are fractions of two components of DOC (A and B: each assumed to have different fixed absorbance spectra) and \( E_{A,270} \) and \( E_{B,270} \) are, respectively, empirically fitted extinction coefficients for components A and B at 270 nm. The fraction \( f_A \) is given by

\[
f_A = \frac{E_{B,270} - (R \cdot E_{B,350})}{(R \cdot E_{A,350}) - (R \cdot E_{B,350})} - E_{A,270} + E_{B,270} \tag{3}
\]

in which \( E_{A,350} \) and \( E_{B,350} \) are, respectively, empirically fitted extinction coefficients for components A and B at 350 nm and \( R \) is the measured absorbance ratio at 270 and 350 nm (\( \alpha_{270}/\alpha_{350} \)).

There are four empirically fitted extinction coefficients: \( E_{A,270} \), \( E_{B,270} \), \( E_{A,350} \) and \( E_{B,350} \) but only \( E_{A,270} \) and \( E_{B,270} \) were adjusted in our calibration with \( E_{A,350} \) and \( E_{B,350} \) unchanged from those reported by Carter et al. (2012).

2.3. Single-wavelength proxy assessment

The performance of a single wavelength (1\( \lambda \)) model for DOC was assessed using non-linear regression between absorbance at individual wavelengths (270 or 350 nm) and measured DOC concentrations in the samples collected in Campaign A. The resulting regression equations were then validated using the samples collected in Campaign B.

2.4. Two-wavelength proxy assessment

Absorbance data (at 270 and 350 nm) were combined with the measured DOC concentrations to generate a calibration data set for the two-wavelength model (2\( \lambda \): Tipping et al., 2009; Carter et al., 2012). Model parameters (extinction coefficients at each wavelength) were adjusted by trial and error so as to maximize the R\(^2\) value and minimize the sum of squared residuals between absorbance-derived DOC concentration and DOC concentrations measured by the TOC analyser. The calibrated extinction coefficients are displayed in Table 1 alongside the universal extinction coefficients proposed by Carter et al. (2012).

The empirical model was tested on an independent validation data set (water samples collected during Campaign B). These samples were analysed immediately after filtration (to minimize storage losses of DOC) on a Cole-Parmer UV/visible spectrophotometer, in Malaysia, at wavelengths of 270 nm and 350 nm. These UV–vis absorbance values were subsequently used in the calibrated model to calculate DOC concentrations. DOC concentrations were measured on a TOC analyser, using the method previously described. As well as the coefficients derived from the calibration using the samples collected during Campaign A, ‘universal calibration coefficients’ proposed by Carter et al. (2012) were also used to generate DOC concentrations (Table 1). This allowed the general validity of the universal coefficients in the 2\( \lambda \) model to be evaluated. It should also be noted that a subset of five of the DOC water samples were chosen to cross-check for consistency between the absorbances produced on the UK-based and Malaysian-based spectrophotometers. This comparison showed an average difference in absorbance values of only 0.003 ± 0.004 cm\(^{-1}\).

2.5. Comparisons between approaches

In summary, a total of four approaches were used to estimate DOC concentrations using UV–vis spectrophotometry:

1) 1\( \lambda \) approach using absorbance values at 270 nm (1\( \lambda_{270} \))
2) 1\( \lambda \) approach using absorbance values at 350 nm (1\( \lambda_{350} \))
3) 2\( \lambda \) approach calibrated on the April 2015 dataset/ Campaign A (2\( \lambda_{\text{calibrated}} \))
4) 2λ approach using the ‘universal calibration coefficients’ (Carter et al., 2012) (2λnon-calibrated)

The performance of the four models was assessed using the following metrics:

a) Actual differences between measured and estimated DOC concentrations (mg L\(^{-1}\));

b) The coefficient of determination (R\(^2\)) for the regression between measured and modelled DOC concentrations;

c) The root mean squared error (RMSE);

d) The Nash–Sutcliffe efficiency (Nash and Sutcliffe, 1970) which is a measure of goodness of fit between the modelled and actual DOC concentrations i.e.:

\[
\text{NSE} = 1 - \frac{\sum (C_{\text{meas}} - C_{\text{est}})^2}{\sum (C_{\text{meas}} - C_{\text{mean}})^2}
\]

where \(C_{\text{meas}}\) is the measured DOC concentration (TOC analyser), \(C_{\text{est}}\) is the DOC concentration estimated using the various wavelength proxies and \(C_{\text{mean}}\) is the mean measured DOC concentration. The closer the NSE is to +1 the stronger the model fit. A value of 0 or lower indicates that the model performs no better than the mean of the data (Nash and Sutcliffe, 1970).

2.6. Statistical analyses

Quantitative data analysis was performed using parametric statistical tests when appropriate (GraphPad Prism, version 6; Microsoft Excel 2013). Normality was tested using the Shapiro–Wilk test.

3. Results

3.1. Single-wavelength approach

3.1.1. Single wavelength model development

Fig. 2 shows the results of a series of regression analyses of modelled and measured DOC concentrations using a single wavelength proxy approach. Measured DOC concentrations for Campaign A data are plotted against modelled DOC concentration length proxy approach. Measured DOC concentrations for modelled and measured DOC concentrations using a single wave-

\[
\text{3.2. Two-wavelength approach}
\]

3.2.1. Two-wavelength model development and validation

Fig. 3 shows the results of several regression analyses of modelled and measured DOC concentrations that were used to calibrate the model and validate it. The original calibration (2λoriginal-calibrated model: Fig. 3a) displays the measured DOC concentrations for the April 2015 data set against the modelled DOC concentrations, generated by adjusting the extinction coefficients to maximize the goodness of fit. DOC concentrations ranged from 9.3 to 52.0 mg L\(^{-1}\) and 9.3–52.8 mg L\(^{-1}\) for the measured and modelled techniques, respectively. This calibrated model was then validated by testing it on the independent data set (2λcalibrated model: collected during Campaign B), the results of which are displayed in Fig. 2b. The DOC concentrations from the same data set (Campaign B) were then modelled using the universal extinction coefficients suggested by Carter et al. (2012), as shown in Fig. 3c (2λnon-calibrated model). Respective mean pH and electrical conductivity values were 3.7 and 177 μS cm\(^{-1}\) for Campaign B samples. Goodness of fit metrics between the three models are displayed in Table 3. Predicted DOC concentrations ranged from 5.5 to 71.7 mg L\(^{-1}\) and from 6.7 to 82.8 mg L\(^{-1}\) for the 2λcalibrated and 2λnon-calibrated models, respectively. While the DOC concentration ranges within the validation data set (Fig. 3b; c) were greater than those observed for the calibrated data (Fig. 3a), 92% of the validation data (190 samples out of 206 samples) fell within the broad range encompassed by the DOC calibration (0–60 mg L\(^{-1}\)). Accordingly, the majority of the DOC concentration data set was represented.

![Fig. 3](image-url)
3.3. Overall assessment of models

The overall effectiveness of the four different models to predict DOC concentrations are summarized in Table 2, displaying the concentration and percentage differences between the modelled and measured DOC. The goodness of fit metrics for all four model approaches are present in Table 3; validation data.

The extinction coefficients for A and B were adjusted to optimize the fit between modelled DOC concentrations and the respective measured values ($R^2 = 0.98; p < 0.05$) (Fig. 3 a). Lower and upper 95% confidence intervals were 0.746 and 1.344, respectively. The optimal extinction coefficients for the 2λ model were:

- $E_{233,270} = 74.32 \text{ L g}^{-1}$;
- $E_{\lambda,21} = 30 \text{ L g}^{-1}$;
- $E_{270,350} = 15 \text{ L g}^{-1}$;
- $E_{350,350} = 0 \text{ L g}^{-1}$.

Table 2

<table>
<thead>
<tr>
<th>Model development</th>
<th>$2\lambda_{\text{original-calibrated}}$</th>
<th>$1\lambda_{\text{original-270}}$</th>
<th>$1\lambda_{\text{original-350}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.98</td>
<td>0.98</td>
<td>0.95</td>
</tr>
<tr>
<td>NSE</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>RMSE</td>
<td>1.45</td>
<td>1.51</td>
<td>2.27</td>
</tr>
</tbody>
</table>

Validation data

<table>
<thead>
<tr>
<th>Validation data</th>
<th>$2\lambda_{\text{calibrated}}$</th>
<th>$2\lambda_{\text{non-calibrated}}$</th>
<th>$1\lambda_{270}$</th>
<th>$1\lambda_{350}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>206</td>
<td>206</td>
<td>206</td>
<td>206</td>
</tr>
<tr>
<td>Difference between modelled and measured DOC (mg L$^{-1}$)</td>
<td>$-4.1 \pm 0.4$</td>
<td>$1.2 \pm 0.3$</td>
<td>$-3.7 \pm 0.3$</td>
<td>$-3.4 \pm 0.4$</td>
</tr>
<tr>
<td>% Difference between modelled and measured DOC</td>
<td>$14.4 \pm 0.8$</td>
<td>$9.7 \pm 0.8$</td>
<td>$11.9 \pm 0.5$</td>
<td>$15.5 \pm 1.0$</td>
</tr>
</tbody>
</table>

Fig. 3. a) Original model calibration (constructed from April 2015 data/Campaign A). Regression of modelled DOC concentrations ($n = 47$) against respective measured DOC concentrations. Extinction coefficients were derived independently from the same data used for calibration (calibrated extinction coefficients): $E_{270,350} = 74.32, E_{2,31} = 30, E_{270,350} = 15, E_{350,350} = 0$. b) Regression of DOC concentrations from the independent data set ($2\lambda_{\text{calibrated model}}$) (collected from Campaign B), against respective measured DOC concentrations. Model DOC concentrations generated using the calibrated extinction coefficients c) Regression of 2 λ modelled DOC concentrations for validation samples against respective measured DOC concentrations using ‘universal calibration coefficients’ (Carter et al., 2012). Dashed lines show 1:1 relationships.
models ranged from 9.7% to 15.5% (Table 2), with the \(2\Lambda_{\text{non-calibrated}}\) approach producing the smallest % difference. This trend was also observed for the mean absolute differences (modelled \(\pm\) measured) in DOC concentration (Table 2). The highest NSE was produced by the \(2\Lambda_{\text{calibrated}}\) approach (NSE 0.91). The \(2\Lambda_{\text{calibrated}}\) approach produced the lowest NSE (0.80). The intercept of the 270 nm proxy (1.2720) model was closest to zero (0.20 ± 0.74 mg L\(^{-1}\), \(p < 0.05\)) and the intercept of the 350 nm proxy (1.3530) was furthest away from zero (5.06 ± 1.04 mg L\(^{-1}\), \(p < 0.05\)). However, the slope of the 1.3530 regression was closest to unity (0.95 ± 0.03 mg L\(^{-1}\), \(p < 0.0001\)) and that of the 1.2720 regression was furthest away from 1 (1.12 ± 0.02 mg L\(^{-1}\), \(p < 0.0001\)). Of the \(2\Lambda\) approaches, the \(2\Lambda_{\text{non-calibrated}}\) model had a closer intercept to zero (1.61 ± 0.77 mg L\(^{-1}\), \(p < 0.05\)) than the \(2\Lambda_{\text{calibrated}}\) model (2.09 ± 0.97 mg L\(^{-1}\), \(p < 0.05\)) and also had a slope which was closest to unity (0.92 ± 0.02 mg L\(^{-1}\), \(p < 0.0001\)).

4. Discussion

All four models performed well statistically suggesting that tropical DOC concentrations in surface waters can be estimated accurately using UV–vis spectroscopy. Both the two-wavelength and single-wavelength approaches exhibited similar statistical performance and were both suitable as DOC concentration proxies, reinforcing findings reported for temperate peatland waters by Peacock et al. (2014).

Carter et al. (2012) found that a two-wavelength model improved \(R^2\) values by 0.02 and 0.05 compared to 270 and 350 nm UV proxies, respectively. However, our data suggest that the single-wavelength model at 270 nm produced the strongest \(R^2\) value and the second highest NSE, suggesting that it is as robust as a two-wavelength proxy. This is in agreement with other previous studies (Asmala et al., 2012; Peacock et al., 2013, 2014) and is also reinforced by both Johnson et al. (2006) and Pereira et al. (2014) who noted distinct seasonal differences in the composition of tropical DOC. In addition, data used to derive the original calibrated model (Campaign A) were applied to a data set analysed much more rapidly after collection (Campaign B). Therefore, some of the overestimations made by the \(2\Lambda_{\text{calibrated}}\) model could be due to small DOC losses during storage, although an independent assessment of such cold storage losses suggested that they are modest (Cook et al., 2016). As such, this offers further opportunities to improve upon our existing model and the locally-calibrated model may be improved as sampling continues.

The performance of the universal calibration coefficients (Carter et al., 2012) in this tropical surface water system is encouraging. From a practical perspective, this suggests that other tropical researchers may also be able to use these parameters, in the absence of their own calibration data set. This would allow DOC concentrations to be determined soon after sampling without having to ship samples from remote field locations to the laboratory for site-specific calibrations (although this is always the preferred practice).

There will always be a need for quality control checks on proxy DOC determinations, but the fact that UV–vis spectroscopy is able to predict tropical DOC concentrations accurately and rapidly is extremely promising because it offers the ability to generate in-situ data which may improve both the spatial and temporal range of DOC measurements. This may be particularly important for research groups working in remote locations which lack immediate access to specialised (and often expensive), analytical equipment. DOC concentrations and quality (absorbance and fluorescence properties) can change in stored water samples over time even after acidification and or freezing (Spencer et al., 2007; Fellman et al., 2008; Peacock et al., 2015; Cook et al., 2016), so the possibility of immediate post-collection analysis is attractive.

5. Conclusions

The concentrations of DOC in tropical water samples collected from peat-dominated catchments can be determined accurately using both single- and two-wavelength spectrophotometric techniques. This offers researchers the potential to analyse samples rapidly post-collection using an inexpensive method and could be invaluable when working in remote tropical field sites.

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