



Technical note on long-term probe misalignment and proposed quality control using the heat pulse method for

3 transpiration estimations

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15 Abstract

16 Whole-plant transpiration is a crucial component in the hydrological cycle and a key parameter in many

- 17 disciplines like agriculture, forestry and ecology. Sap flow measurements are one of the most widely used
- 18 methods to estimate whole-plant transpiration in woody species due to its wide application range and its
- 19 ready automation for continuous data readings. Several different methods have been developed and
- 20 adjusted to different climatic conditions and wood properties. However, the scientific literature also
- 21 identifies several sources of error in the method that needs to be accounted for; misalignment of the
- 22 probes, wound to the xylem, thermal diffusivity and stem water content. This study aims to integrate
- 23 probe misalignment as a function of time to improve readings during long-term measurements (> 3
- 24 months). We conclude that even when geometrical misalignments errors are small, the introduced
- 25 corrections can imply an important shift in sap flow estimations. Additionally, we propose a new set of
- statistical record to be recorded during the measurement period to use as a quality control of the heat ratio
- 27 readings obtained from the sensors. By using standard deviation and slope as quality indicators we
- 28 concluded that no general time limit can be decided for all sensors but should rather be determined from
- 29 individual performance over time.
- 30

31 1. Introduction

32 Plant transpiration is a key process in the hydrological cycle and in forest ecosystems it is often the largest 33 component of total evapotranspiration (Jasechko et al., 2013). Accurate estimations of transpiration are still 34 difficult to obtain, and field assessments of transpiration estimations are therefore crucial in hydrological 35 planning as well as in forestry, ecophysiological research and climate forecasting. Sap flow measurement 36 is one of the most widely used techniques to estimate whole plant transpiration in woody species because 37 it is a readily automated for continuous readings and is not limited to single leaf measurements (Forster, 38 2017). Although some sap will go to stem and leaf storage, it is estimated that 99% is lost through 39 transpiration, and sap flow measurements can therefore be used to directly estimate transpiration values





- 40 (Forster, 2017). In addition, sap flow sensors estimate plant transpiration rates regardless of the orographic
 41 complexity and atmospheric conditions of stability or stratification, which can hinder the direct
 42 measurement of transpiration flows in forest environments.
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44 There are a range of different approaches to sap flow measurements, and methods vary between heat 45 dissipation (HD), steam heat balance (SHB), trunk segment heat balance (THB) and heat pulse velocity 46 (HPV). However, they are all based on tracing heat within the xylem (Burgess et al., 2001; Davis et al., 47 2012; Forster, 2017). Marshall (1958) developed a theoretical method to determine sap flow from the 48 thermal diffusion and dissipation theory of heat pulses in heterogeneous material. His theory relies on 49 calculating the heat ratios measured in two parallel thermocouples aligned vertically and symmetrically 50 with respect to a line heater along the direction of the xylem. Burgess et al. (2001) developed an improved 51 HPV technique, termed the heat ratio method (HRM), based on the Marshall (1958) methodology, which 52 is sensitive to the direction of sap flow and is capable of measuring low and reverse rates (Burgess et al., 53 2001), which is not possible using energy balance methods like eddy-covariance and which often is the 54 case for nightly values (Burgess et al., 2001; Novick et al., 2009). Burgess et al. (2001) developed two steps 55 of corrections for sap flow calculations by considering probe misalignment and wounding (caused by the 56 implementation of the sensors). By accounting for these sources of error and additionally estimating the 57 stem moisture content and radial variability, the heat ratio method (HRM) has been evaluated the heat pulse 58 velocity method (HPV) with highest accuracy, although with a tendency of underestimating transpiration 59 values (Forster, 2017).

60

61 Previous studies have suggested additional solutions for probe misalignment (Ren et al., 2017), or for 62 determining thermal diffusivity (Vandegehuchte and Steppe, 2012), and correcting for heterogeneous heat 63 capacity in wood (Becker and Edwards, 1999). However, we suggest attention should be given to check the 64 accuracy of the heat pulse ratio itself, in which the rest of the methodology is built on. In addition, we found 65 it necessary to develop a dynamic probe misalignment correction method. One way to account for changes 66 over time has been to reinstall sensors throughout the study period (Moore et al. 2010), however there is 67 little information to be found on the exact interval of which this needs to happen (Vandegehuchte and 68 Steppe, 2013), and for continuous measurements this will interrupt the dataset. The adjustment proposed 69 here, built on the calculations of Burgess et al. (2001), is necessary when monitoring transpiration 70 continuously for more than 3 months because wood properties, the heterogeneity of the xylem and plant 71 tissue growth might further misplace the sensor after its implementation (Barrett et al., 1995). Burgess et 72 al. (2001) corrected for linear probe misalignment in situ. This correction must be effectuated during zero 73 flow. Actual probe placement is therefore suggested to be calculated one time, usually at the end of the 74 experiment when the root system can be severed to enforce zero flow (Burgess et al., 2001). However, this 75 solution is not suitable for long term measurements, as the misplacement will change over time (Ren et al., 76 2017), or when intrusive methods are not an option. Thus, the objective of this research is firstly, to develop 77 a statistical filtration method to ensure the quality and consistency of the measurements over time, and, 78 secondly, to implement a modified version of the probe misalignment calculation for sap flow series longer 79 than 3 months.





- 80 This methodological paper is structured in two parts: the first one, dealing with the statistical analysis of
- 81 long-term time series of heat pulse ratios to ensure the quality of data and their stability over time; the
- 82 second part, proposing an adaptation of the method developed by Burgess et al. (2001) to contemplate a
- 83 dynamic probe misalignment correction for the heat ratio method (HRM). The aim is to obtain a more
- 84 precise calculation of transpiration by parameterising the probe misalignment as a function of time to
- 85 correct for the effect of tree growth.
- 86

87 2. Materials and method

88

89 2.1 Field site

90 This study was carried out in the Turia river basin, Eastern Spain (39°57'45" N 1°8'31" W), in a

91 Mediterranean climate. Average annual rainfall is 475 mm, average annual maximum and minimum

92 temperature is 15.5 °C and 4.4 °C respectively. Sap flow sensors were installed in four pine trees (Pinus

93 halepensis Mill.) according to the heat ratio method (HRM, Burgess et al., 2001). Three needles, one

94 heater and two thermocouples (0.13 cm x 4 cm), were drilled into the uphill side of each tree trunk. Since

- 95 P. halepensis has a higher sap velocity average near the cambium with the velocity steady declining
- 96 nearer to the heartwood (Cohen et al., 2008), sensors were installed at 20 mm depth below the cambium
- 97 for average sap velocity rates, as estimated by Manrique-Alba (2017). A metal plate was used as guide to 98
- assure a 0.6 cm spacing between the drilling holes. P. halepensis selected had a mean diameter of 24.5 cm
- 99 at breast height (150cm). Continuous measurements were obtained from April 2017 to December 2018.
- 100

101 2.2 Construction of the sensors

102 The thermocouples were made after Davis et al. (2012), with a type E junction of chromium and

103 constantan. The E type has a higher accuracy, stronger signal and more stability than the type K (Davis et

- 104 al., 2012). The wires of the thermocouples were soldered together at temperatures not surpassing 200°C
- 105 and placed 2 cm inside a glass tube (0.1 cm x 4 cm) and into a needle (0.13 cm x 4 cm). The heater was
- 106 made of a constantan wire of 20 cm coiled around a 7 cm long aluminium wire before placed inside a
- 107 needle of 4 cm. The wire-ends were then soldered on to an electrical cable. Another resistance of 10 ohms
- 108 were soldered onto to the cable to get a total resistance of 20 ohms. The heater was connected to a 12 V

109 battery and delivered 7.0 W of power. All three sensors were connected to a CR800 datalogger

- 110 (Campbell Scientific Inc., USA).
- 111

112 2.3 Quality control of heat pulse ratios

113 Marshal (1958) parameterised the heat pulse velocity (V) in the HRM as a function of time following a

114 heat pulse, and the instantaneous ratio of the increase of temperature (from the temperature prior to the

115 release of the thermal pulse) at the downstream and upstream, v_1 and v_2 respectively, from a line heater: 116

117
$$V = \frac{4Kt \ln\left(\frac{v_1}{v_2}\right) - (x_2^2 + y_2^2) + (x_1^2 + y_1^2)}{2t(x_1 - x_2)}$$
[1]





- 118 where K is the thermal diffusivity, t is time from the release of thermal pulse, (x_1, y_1) and (x_2, y_2) are the 119 relative positions of the thermocouples to the line heater (considering x-axis along the xylem and y-axis 120 the perpendicular direction both to the xylem and to the heater line), and v_1, v_2 represents the temperature 121 increases following the heat release, in the downstream and upstream thermocouple respectively. 122 123 If probes are installed symmetrically above and below the heater line, $x = x_1 = -x_2$ and $y_1 = -y_2$, the 124 equation 1 simplifies into a function not dependent on time: $V = \phi \ln \left(\frac{v_1}{v_2}\right)$ $\phi = \frac{K}{x}$ 125 [2] 126 where ϕ is, a priori, a constant only depending on the placement of the probes and on the thermal diffusivity 127 128 of both the xylem and the material used in sensors. 129 130 The "perfect symmetry" assumption renders that HPR remains constant with time if sap flow velocity (V), 131 thermal diffusivity (K) and probe positions (in both, x and y directions) have negligible variations during 132 the time following each heat pulse (Marshall, 1958). However, Burgess et al. (2001) describe how empirical 133 results initially differ from the ideal approach described by equation 2, although they converge 134 asymptotically at least 60 seconds after the heat pulse release and, for at least, 40 seconds more (until 100 135 seconds after the heat pulse release). A visual inspection of heat pulse velocities (V in equations 1 and 2) 136 do not necessarily give enough information to decide if measured values are a good representation of the 137 sap velocity or not. On these premises, we have built a methodology utilising a quality check of systematic 138 sap flow measurements by means of a statistical analysis performed on the instantaneous values acquired 139 between 60 and 100 seconds. Hereafter we will denote the averaged instantaneous heat pulse ratio between 140 60 and 100 seconds as HPR. Threshold values were established for the relative standard deviation (RSD) 141 and the slope of the time evolution of instantaneous heat pulse ratios. 142 143 144 2.3.1 Logging specifications 145 The proposed analysis to ensure the reliability of long time series of sap flow measurements require the 146 storage of statistics that are not usually recorded (as it is considered unnecessary). The datalogger to be 147 used must have a minimum performance of the storage capacity (memory), the processing speed of the 148 algorithm implemented (especially in the routines related to the statistical calculations to be performed). In 149 this study a CR800 (Campbell Scientific, USA) was used. A flow chart of sampling and data log (Fig. S1) 150 was specifically designed, programmed and implemented in the datalogger to enable the calculus that are 151 presented in the next sections of this paper. 152 153 The RSD is statistically defined as the standard deviation divided by the mean. Therefore, we selected RSD 154 (%) and Slope (s⁻¹) of the instantaneous ratio versus time calculated for each of the periods from 60 to 100
- seconds and used for the quality control (Fig.1A and B). All HPR with relative standard deviation > 5%
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- 156and a $|slope median (slope)| < 0.003 s^{-1}$ were removed. The 5 % threshold was chosen to ensure that 95 %157of the dataset was considered in the data processing. The slope median was taken from all slope values158obtained during the measurement period. The magnitude of this slope threshold was chosen from modelled159output of instantaneous ratios performed by Burgess et al. (2001), were low heat pulse velocities (5 cm h⁻¹) are shown to display a slope of 0.001. The specific threshold of 0.003 was decided upon inspection of161the measurements and can be modified according to the sensor.
- 162

163 2.4 Correction for long-term probe misplacement

164 Under the assumption of "perfect symmetry", in periods when V = 0 cm h⁻¹, the ratio of the increase of 165 temperature at the downstream and upstream from a line heater would be equal to one ($v_1 = v_2$ in equation 166 2). However, this is not always the case with field measurements due to misalignment of the probe or 167 heterogeneous wood properties (Fig. 1A). Burgess et al. (2001) showed how probe placement can be 168 estimated in situ with the HRM. He proposed a methodology for probe misalignment which builds on the 169 assumption that errors arising from inaccurate probe spacing can be treated one-dimensionally. His 170 approach assumed that the total effect of probe misalignment (in both axis directions), observed in the ratio 171 of the increase of temperature, can be parameterised calculating an "effective" probe misalignment in only 172 one direction (x direction, parallel to the xylem). Thus, without the assumption of "perfect symmetry", in 173 periods when $V = 0 \text{ cm } h^{-1}$ equation 1 takes a more simplified form: 174

175
$$4Kt \ln\left(\frac{v_1}{v_2}\right) = (x_2^2 + y_2^2) - (x_1^2 + y_1^2)$$
[3]

176

177 Which becomes equation (4) if $y_1 = -y_2$

178

179
$$4Kt \ln\left(\frac{v_1}{v_2}\right) = x_2^2 - x_1^2$$
 [4]

180

181 As it is unknown if the misalignment is in x_1 or in x_2 , the calculation is repeated twice, assuming first that 182 x_1 is correct when calculating actual x_2 placement and vice versa. Two different heat pulse velocities, V_1 183 and V_2 , are then derived (using equation 1 but with the assumption $y_1 = -y_2$) for both misalignments x_1 and 184 x_2 obtained; and the final V provided as their average.

185

186 Zero sap flow conditions can either be imposed artificially by severing the root or stem (Burgess et al., 187 2001), or assumed when atmospheric vapour pressure deficit (VPD) is close to zero, the soil is saturated, 188 usually after substantial precipitation, and values taken at predawn to avoid any biophysical driving force 189 (Forster 2017). Saturated soil is a necessary criterion due to the possibility of reverse flow at night-time. If 190 not considered, low HPR values representing reverse flows can be interpreted as zero flow. The latter 191 approach allows the parameterisation of misalignment as a function of time (equation 5), if several 192 calculations of x_1 and x_2 (equation 4) are performed. By applying equation 4 at varies throughout the 193 measurement period, it is possible to calculate a linear regression using the estimation of Burgess et al. 194 (2001) for misalignment for each of the thermocouples:





195	
196	$x_i = m_i d + n_i$; $i = 1, 2$ [5]
197	
198	where x_i are the relative positions of the thermocouples to the line heater in cm, d is the time along the
199	measuring campaign in days, m_i is the slope of the regression for thermocouple <i>i</i> , and n_i is the interception
200	coefficient.
201	
202	By introducing equation 5 in equation 1 and allowing the simplification assuming $y_1 = -y_2$, two equations
203	of the corrected heat pulse velocities are obtained as a function of the time in the measuring campaign
204	(equation 6).
205	
206	$V_1 = \frac{4Kt\ln\left(\frac{v_1}{v_2}\right) + (m_1 d + n_1)^2 - 0.6^2}{2t(m_1 d + n_2 + 0.6)} $ [6]

207
$$V_2 = \frac{4Kt \ln\left(\frac{v_1}{v_2}\right) + 0.6^2 - (m_2 d + n_2)^2}{2t(0.6 - m_2 d - n_2)}$$

208

In accordance to what Burgess et al. (2001) proposes, our approach averages the two estimates obtainedfrom equation 6 to obtain a corrected heat pulse velocity.

211

212 **3 Results**

3.1 Heat pulse ratios

215	The HPR obtained during the measurement period displays a clear positive shift away from the theoretical
216	ideal where the HPR would equal one at zero flow (Fig. 1A). This gives an indication of the necessity of
217	corrections, that being due to wound inflicted by the probe, misalignment, misestimation of thermal
218	diffusivity-or stem water content. However, the HPR data by itself does not give an indication of the
219	quality of each measurement, nor if it deteriorates over time. Therefore, the quality of the measurements
220	was indicated by calculating the RSD and slope for each HPR (Fig. 1B and 1C). All HPR with RSD
221	higher than 5% were eliminated. The data points eliminated corresponded to a 1 % of the total dataset.
222	Because the method is built on the theoretical assertion that the temperature in each of the thermocouples
223	is steady with time, specifically between 60 and 100 seconds after the release of a heat pulse, the slope of
224	the HPR should be close to zero. All HPR with $ slope - median (slope) < 0.003 s^{-1}$ were eliminated,
225	which corresponded to a 12 % of the original dataset (Fig. 1C).
226	







Figure 1. (A) Heat pulse ratios (HPR) throughout the measurement period in 30-minute intervals in tree number 1. Each HPR is an average of 41 instantaneous ratios corresponding to the temperature difference in two thermocouples at 0.6 cm up and down stream from a heater probe. (B) Relative standard deviation (%) for each HPR in tree number 1 for the whole measurement period. Red line indicates threshold used for the quality control were all HPR relative standard deviation > 5% were removed from the data analysis. (C) Slope (s⁻¹) for each HPR in tree number 1 for the whole measurement period. Red line indicates threshold used for the quality control for this particular sensor. HPR with |slope – median (slope)| < 0.003 s⁻¹ were removed from the data analysis.

234

235 3.2 Heat pulse velocities

236 A linear regression was obtained from misalignment calculations for each sensor performed during zero-

- 237 flow conditions. Because of the dry climate, only five events fulfilled these criteria (field capacity, low
- 238 VPD, predawn), during the measurement period of 20 months. The outputs indicate a clear shift of
- 239 placement in each of the sensors over time, here denoted as x_1 and x_2 in the case of tree number 1, with a
- 240 greater shift in x_2 than x_1 (Fig. 2). On average, the eight sensors (two per tree) showed a shift of 0.04 cm
- 241 in placement after twenty months of measurement. The equation obtained from the linear regression was
- 242 then implemented in equation 6, and corrections to the heat pulse velocity data was done using an average





- 243 of V_1 and V_2 (Fig. 3,4). Outputs were compared with one-time misalignment correction calculated in the
- 244 beginning of the measurement period to demonstrate the evolution of the probe misalignment. The one-
- 245 time correction demonstrated a steady decline in accuracy over time (Fig. 3, 4). The difference between
- 246 the two correction methods showed significance after three months of employment (Fig. 3).



247

- Figure 2. Sensor misalignment positions calculated once (solid lines) compared to probe misplacement over time in
- 248 249 250 (solid circles with dotted lines). Each point represents the probe misalignment position calculated during its respective
- zero flow event.



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252 253 254 255 Figure 3. Heat pulse velocities for tree number 1 throughout the measurement period. Dark green represents velocities with only one-time misalignment correction applied at the beginning of the campaign. Light green represents velocities with time- dependent probe misalignment corrections. Each circle represents a measurements every 30 minutes.









256 257 258 259 Figure 4. Heat pulse velocities during one week of measurements in the beginning (A), halfway (B) and at the end (C) of the measurement period. Dark green lines represent velocities with probe misalignment corrected for once at the beginning of the experiment. Light green lines represent velocities with the time- dependent probe misalignment corrections.

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261 3.3 Sap velocities

262 Heat pulse velocities (both corrections) were converted into sap velocities according to Burgess et al. 263 (2001). Our data demonstrated that by not correcting for changes in probe misalignment under continuous 264 measurement for more than 3 months, the errors corresponded to an averaged difference of 0.29 cm h⁻¹ in 265 sap velocity per quartile for the four trees. At the end of the 20-months period, this corresponded to an 266 averaged difference of 0.53 cm h⁻¹ (Table 1, Fig. 5) In terms of transpiration values, this corresponded to a mean difference of 0.7 L tree $^{-1}$ day $^{-1}$ (sapwood area = 170 cm²), which would correspond to a difference of 267 268 542 L ha ⁻¹ day ⁻¹ (775 tree ha ⁻¹) assuming 8 hours of daylight. It is relevant to note our conversion did not consider the differences in stem moisture content, which can also affect the output values (Vandegehuchte 269 270 and Steppe, 2013). The outputs obtained should be considered as relative differences as the one-time 271 correction was applied in the beginning of the experiment.

272 273 274 275 Table 1. Seasonal averages of sap velocity for 4 different trees. All sap velocities are expressed in cm h⁻¹. Sap velocities corrected for with time-dependent misalignment calculations are compared with sap velocities corrected for once in the beginning of the measurement period. Averages were taken from daily values. Abbreviations Sp, Su, Fa, and Wi $\bar{2}76$ indicates Spring, Summer, Fall and Winter respectively, each with the corresponding year.

Pine number	Correction method	Sp-17	Su-17	Fa-17	Wi-17/18	Sp-18	Su-18	Fa-18
1	One-time correction Time-dependent correction	0.98 1.08	0.23 0.44	-0.31 0.04	-0.37 0.10	0.12 0.64	0.05 0.70	-0.16 0.67
2	One-time correction Time-dependent correction	1.33 1.40	0.49 0.66	-0.28 -0.02	-0.25 0.09	0.06 0.36	0.04 0.42	-0.05 0.45
	One-time correction	0.82	0.73	-0.02	-0.24	0.57	0.75	0.53









Figure 5. Seasonal averages of sap velocities (cm h⁻¹) calculated using the two misalignment corrections throughout the 20-month measurement period. Dotted lines represent probe misalignment corrected for once. Solid lines represent sap velocity corrected for with the time-dependent probe misalignment method. Abbreviations Sp, Su, Fa and Wi indicates Spring, Summer, Fall and Winter respectively, each with the corresponding year. 1, 2, 3, 4 corresponds to different trees.

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286 4.1 Filtration of heat pulse ratios

Discussion

287 Because the HPR is an average of instantaneous ratios it is difficult to ensure its accuracy only by visual 288 inspection of the averaged output, and without further statistical information. We suggest RSD and slope 289 to filter out random ratios, and to ensure the quality of the data. A clear tendency was seen of higher RSD 290 and slope values during periods of higher flow rates when compared to the HPR data (Fig. 1A, 1B and 291 1C), indicating more noise and less trustworthy data during higher flow rates. The HRM is known for 292 being limited at higher rates (> 45 cm h⁻¹, Forster, 2017), but our dataset showed no sap velocity values 293 higher than 6 cm h⁻¹, and therefore was not initially considered as a limitation. 294 295 During twenty months of field measurements the thermocouples showed no visible sign of deterioration 296 with time. However, it is still important to note that this does not consider the possible diminishing 297 amplitude of the ratios over time, which can also lead to underestimations of actual flow (Barett et al.,





298	1995; Green et al., 2003; Forster, 2017). Therefore, this should be observed separately, comparing sap
299	flow ratios using data obtained under similar climatic conditions (Moore et al., 2010).
300	
301	4.2 Long term variation in probe placement
302	When applying the HRM for longer than a few weeks it is relevant to quantify how ϕ in equation (2) and
303	the misalignment term in equation (4) evolve during the measuring period. The predicted variation of x_1
304	and x_2 is due to the growth of the tree and, on the other hand, periodical variations of K due to annual and
305	seasonal variations of the physiological properties of the tree (Green et al., 2003; Vandegehuchte and
306	Steppe, 2012; Ren et al., 2017). In the HRM the probe misalignment calculations can be corrected using
307	the methodology proposed here, considering that as an average, each sensor displayed a 0.04 cm
308	displacement within the tree after twenty months of measurements. The correction would also be more
309	rigorous with more assumed zero flow events which would be easier to obtain in humid environments.
310	
311	By going back to the original assumption of "perfect symmetry" we investigated the original premises the
312	method is built on. Even though Burgess et al. (2001) elaborated a correction method for sensor
313	misalignment we saw that changes in sensor misplacement was detectable after each season. Therefore,
314	multiple corrections should be carried out throughout the measurement period. The proposed modified
315	method coincides with the one-time correction method (Burgess et al., 2001) for short time periods (< 3
316	months) but differs progressively over time. We found that this shift in placement is significant already
317	after 3 months, and therefore dynamically misplacement calculations should be carried out or sensors
318	should be reinstalled at this frequency.
319	
320	5 Conclusion
321	Continuous measurements for 20 months in Pinus halepensis under semi-arid Mediterranean conditions
322	confirmed the theory of steady temperature difference between the sensors with time (60-100 s) for 87
323	% of the obtained values (every 30 min over 20 months), when using 5 % relative standard deviation and
324	slope - median (slope) < 0.003 s ⁻¹ as quality filter. High quality long-term measurements can be obtained
325	with sap flow sensors if proper data filtering is carried out and the time-dependent misalignment
326	technique applied. Long-term measurements can therefore be performed without reinstallation of the
327	sensors to obtain a continuous dataset, if the species allows for the sensor to be removed again after long-
328	term implementation. In conclusion, the HRM method can be used in long-term studies if probe
329	misplacement is corrected for at varies stages throughout the measurement period when exceeding 3
330	months of sampling.
331	
332	Author contributions
333 334 335 336 337 338	JLP and EC conceptualized the study. EKL made the sensors with the supervision of JB. EKL implemented the sensors and had the responsibility of the data processing and field site. JLP, EKL and JAV all worked on the data analysis. JAV wrote the script for the datalogger and had the technical responsibility. EKL and JLP prepared the manuscript with inputs from all the co-authors. JLP and JAV designed the flow chart to be programmed in any data logger system.

- 337 338 339
- The authors declare that they have no conflict of interest





340	
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