Authors' response to referee comment 2

Referee Comment:

I. Validity and claims:

The authors claim that their device prevents contact with the environment, including evaporation of the sample, after the sample is taken. They demonstrate in their fieldwork in the karst cave that their samples are statistically identical to manual samples. They furthermore show that their samples do not deteriorate over time by repeating the measurements. The question I have:

1. I would expect an autosampler to take measurements at regular intervals. However, in Figure 5 the samples seem to be taken at rather random times. Can the authors explain why this is?

Authors' response: The karst dripwater samples for which Fig. 5 shows the $\delta^{18}O$ values, have been collected automatically using the GUARD autosampler at (regular) hourly intervals from 13:30 o'clock on December 12, 2016 to 07:30 o'clock on December 13, 2016 and from 12:00 o'clock to 15:00 o'clock and at daily intervals from December 13, 2016 to January 14, 2017. However, not all of the collected samples were analysed for isotopic composition. This is why the samples seem to be unevenly spaced in time. As referee #1 deduced an incorrectly stated sampling interval, we will mention at the end of the caption to Fig. 5 that not all of the collected samples were also analysed.

Changes to the manuscript: Add at the end of the caption to Fig. 5: "Not all of the 33 samples were analysed for isotopic composition."

2. The authors substantiate their claim that the samples are kept airtight by placing them in a fridge for a considerable amount of time. However, there is no control to compare against, i.e. no open samples that are exposed to evaporation in that fridge. It is hard for the readership to judge the amount of expected evaporation had the samples not been properly sealed. I would find it unreasonable to ask the authors to redo their experiments, but would like to ask them to provide the readership with an estimate of expected evaporation in the setting of their fridge (8 °C, high humidity I guess?) based on literature values. This will help to show that indeed, their samples are sealed properly.

Authors' response:

We agree with referee #2 that we could have demonstrated the airtightness of the sample vials after sample injection even better had we implemented control samples that are not entirely sealed from the atmosphere and thus exposed to evaporation. To compensate for that caveat and to provide the readership with a notion of the effect of evaporation on the sample $\delta^{18}O$ values, we have calculated both evaporation and $\delta^{18}O$ change for the conditions prevalent in our fridge. Despite being set to 8 °C, the temperature in the fridge was measured to be 11.2 °C, relative humidity was 24 % according to measurements. Based on these conditions and assuming an opening of the sample vial of 5 % to imitate a minor lack of airtightness, evaporation was calculated using a formula that has proven adequate for inactive indoor swimming pools that are not influenced by direct sunlight or wind (Smith, Löf and Jones, 1994) using a water density of 1 g/cm^3:

$$\frac{\dot{m}}{A} = \frac{(30.6 + 32.1 * v_w)(P_w - P_a)}{\Delta H_v}$$

where $\dot{m}$/A is the evaporation rate [kg/(m^2 hr)], v_w is the air velocity over the water surface [m/s],

P_w is the saturation vapour pressure at the water temperature [mm Hg], P_a is the saturation vapour pressure at the air dew point [mm Hg] and ΔH_v is the latent heat of water at the pool temperature [kJ kg].

The $\delta^{18}O$ value of the residual water remaining at each given time was calculated on the basis of a fractionation factor α between water and vapour according to the following formula (e.g. Clark and Fritz, 1999):

$$1000\, ln \propto_{water-vapour} = 1.137\left(10^6/T_k^2\right) - 0.4156(10^3/T_k) - 2.0667$$

where T_k represents the temperature of the phase change [K] and on the following relationship (e.g. Hoefs, 2015):

$$\frac{R_w}{R_{w0}} = f^{(\frac{1}{\propto}-1)}$$

where R_w is the isotope ratio of the water at a given time [‰ V-SMOW], R_{w0} is the initial isotope ratio of the water [‰ V-SMOW], and f is the fraction of the residual water [-]. The results of these calculations (Fig. 1) demonstrate that even a small slit in a sample vial's rubber septum equalling only 5 % of the vial's inner cross section leads to a substantial shift towards higher $\delta^{18}O$ values in the residual water over time. After three months (90 days), for instance, $\delta^{18}O$ values have risen from -10.1 ‰ by about 1.3 ‰ to -8.8 ‰. The difference between the lowest and the highest $\delta^{18}O$ value in Fig. 6 of the manuscript is still below 0.3 ‰, while those data points span a longer period of six months. Most importantly, there is no positive trend in the $\delta^{18}O$ values in Fig. 6 of the manuscript which illustrates the sample vials are sealed properly, even after sample injection.

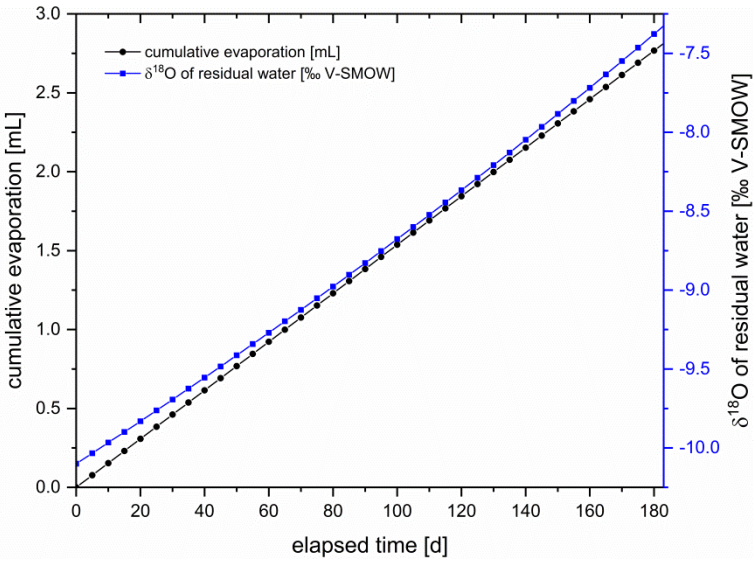

Fig. 1: Effect of evaporation on the $\delta^{18}O$ value of the residual water in a 12 mL sample vial at a temperature of 11.2 °C and a relative humidity of 24 %.

Changes to the manuscript: We will add Fig. 1 in the authors' response to the Supplementaries including the corresponding explanations as above. In the manuscript, we will a shortened version of these explanations at the end of Section 3.3 (page 7, line 3).

Referee Comment:

II. Open Science and reproducibility:

HESS is a fully Open Access journal and the editors also actively advocate for Open and Reproducible Science in general. In this spirit I think that although the article as it now stands informs the readership about the existence of the new autosampler, it does not allow hydrologists to start using it. The provided technical details are insufficient to rebuild the GUARD using just this article. If the authors intended this (because they maybe want to pursue manufacturing the GUARD commercially?) then I think that HESS might not be the ideal outlet to promote it, it is after all a non-for-profit Open Access Scientific journal, not a commercial advertisement leaflet.

I hope the authors did intend the GUARD to be re-buildable by other hydrologists, which would be completely in the spirit of Open Hardware, the movement spearheaded by the Arduino which the authors use as main CPU. By providing a flowchart of their code and their electrical circuitry the authors do hint that this is their intention. For the GUARD to be fully re-buildable I would ask the authors to add:

1. A detailed technical drawing of the physical device, including sizes of all components

Authors' response: Any user of the GUARD autosampler would benefit from it the most if the sampler's dimensions are precisely adapted to the user's specific requirements. Therefore, the exact dimensions of the GUARD device presented in the manuscript are not relevant as the device dimensions should be regarded flexible rather than fixed. The necessary minimum dimensions mainly depend on the number of sample vials needed. In the setup presented in the manuscript the GUARD autosampler comprises 48 sample vials, but can be equipped with up to 160 sample vials at the given casing dimensions by maximising the dimensions of the sample rack (to the left side in Fig. 1 in the manuscript) and by reducing the space between adjacent sample vials to zero. If a higher number of sample vials is needed, the sampler dimensions need to be adapted accordingly. Only few components of relevant size have fixed dimensions (Table 1). All x-profiles and guides that form the framework within which the x- and y-slides move have to be cut to fit into the chosen casing.

Table 1: Integral components of the GUARD autosampler of relevant size

COMPONENTS	Description	Dimensions
Mechanical		Length x Width x Height
Z-movement: servo	Reely® Standard RS-610 MG, operating voltage 6.6 V, attached to the Z-slide containing the double-cannula via an elongated hole in the servo's horn	40x20x42 mm
X-/Y- movement: motors	Sanyo Denki®, bipolar hybrid stepping motors, 1 A, 24 V, 1.8°/step, 0.265Nm, 4 wires	42x42x24 mm
Pump	Peristaltic (flexible-tube) pump, model AP-40; operating voltage 12 V,	55x50x42 mm

Changes to the manuscript: Add Table 1 in the autors' response to the Supplementaries.

2. A Bill of Materials akin to their Table 1, but with more detail. At least the price and an (online?) location where the parts can be bought at the time of publishing should be included.

Authors' response / Changes to the manuscript: We will add the following Bill of Materials to the Supplementaries

Components	Description	quantity	cost/unit	total cost	company	purchase order no.
Mechanical						
Casing	Peli®, model 1610, heavy-duty, water-tight and airtight, including a valve for automatic pressure purge	1	252.35 €	252.35 €	Waterproof-Cases	-
Z-movement: servo	Reely® Standard RS-610 MG, operating voltage 6.6 V, attached to the Z-slide containing the double-cannula via an elongated hole in the servo's horn	1	12.60 €	12.60 €	Conrad Electronic	1365925 - 05
X-/Y- movement: motors	Sanyo Denki®, bipolar hybrid stepping motors, 1 A, 24 V, 1.8°/step, 0.265Nm, 4 wires	2	38.95 €	77.90 €	RS Components	829-3499
Pump	Peristaltic (flexible-tube) pump, model AP-40; operating voltage 12 V,	1	19.90 €	19.90 €	Gemke Technik GmbH	APE40CD12V
Sample vials	Labco Exetainer® 738W, soda glass, 12 mL, flat bottom, height (vial + cap) ≤ 101 mm; external ø ≤ 15.5 mm; internal ø ≥ 13.2 mm; including rubber septa with a thickness ≥ 3 mm; 48 vials of 300 in a packaging unit	1	22.28	22.28 €	IVA	IVA738W
Tubing	Deutsch & Neumann®, FKM (synthetic rubber, "Viton"), Shore hardness 75, external ø ≤ 6.2 mm, internal ø 4 mm	3	12.90 €	38.70 €	häberle Shop	9.205 765
Double cannula	Braun Sterican®, metal, external ø 0.60 mm; length excluding Luer-Lock connector 30 mm	2	3.40 €	6.80 €	häberle Shop	7.079 505
Framework for slide movement	Makeblock XY Printer	1	269.95 €	269.95 €	Eckstein	MB90014
Electronic						
Battery	Panasonic®, valve regulated Pb-acid battery 12 V, 20 Ah, maintenance-free, non-spillable, low self-discharge, 5.8 kg, 76 x 167 x 181 mm; the sampler can also run on 12 V Li-ion batteries if weight is an important constraint	1	75.03 €	75.03 €	Voelkner	S167901
Microcontroller board	Arduino® Mega 2560 including an Atmel ATmega 2560 microcontroller with 54 digital I/O pins, 16 analogue inputs, 6 interrupt inputs, 4 serial interfaces, 1 I^2C interface and 4 KB EEPROM memory (non-volatile); hibernation mode-enabled	1	21.99 €	21.99 €	Conrad	1409778 - 05
Real-time clock	RTC PCF8563 powered by a separate 3V lithium button cell battery as a buffer battery	1	10.91 €	10.91 €	Conrad	1195070 - 05
Display	Liquid crystal display (LCD) with 2 lines à 16 characters	1	9.87 €	9.87 €	Conrad	183045 - 05
Other electronic components:	relay module	1	8.52 €	8.52 €	Exptech	EXP-R25-187
	drivers for stepping motors	2	7.95 €	15.90 €	Exptech	EXP-R25-001
	casing for control panel	1	5.28 €	5.28 €	Conrad	522641-99
	DC/ DC converter 12V	1	12.00 €	12.00 €	Conrad	154170-05
	DC/ DC converter 5V	1	2.65 €	2.65 €	Conrad	157954-05
	DC/ DC converter 6,5V	1	5.82 €	5.82 €	Conrad	156674-05
	CR2032 3V lithium button cell battery as a buffer battery	1	2.26 €	2.26 €	Conrad	1086225-05
	USB service interface FrontCom® Micro IE-FCM-USB-A Weidmüller	1	20.35 €	20.35 €	Conrad	746885-05
	Membrane keypad Matrix 1 x 12 SU709948	1	11.11 €	11.11 €	Conrad	1341283-62
	3D print-outs (sample rack, connectors, double-canula adapter)	1	15.00 €	15.00 €	-	-
	Aluminium slot profiles 20x20 mm Slot 5 (m)	1	2.94 €	2.94 €	Motedis	19586
	Sliding nuts Slot 5 100 pieces	1	21.42 €	21.42 €	Motedis	96214
	Screw DIN 7984 M4x10 Slot 5	100	0.12 €	12.00 €	Motedis	-
	Bracket 20x40 I-type Slot 5 10 pieces	3	7.50 €	22.50 €	Motedis	093W202N05
	Swivel Feet. Series 10 PA; foot 40, threaded rod 5x60 4 pieces	4	1.00 €	4.00 €	Motedis	-
	Miniature sliding rail IGUS drylin TK-04	1	10.16 €	10.16 €	IGUS	TS-04-07
	CNC Aluminium Servo Horn 60mm for Futaba servos 25 teeth	1	6.90 €	6.90 €	Ebay	251439671553
	Cable gland PG7 Polyamide black (RAL 9005) KSS EGRWW7 water-tight	1	0.34 €	0.34 €	Conrad	533738-05
	zip ties different sizes 200 pieces	1	3.80 €	3.80 €	Conrad	541665-62
	USB cable PC/Sampler	1	4.29 €	4.29 €	Conrad	1592198-62
	Merck® silicone grease for sealing 100gr.	1	68.70 €	68.70 €	häberle Shop	1.07746.0100
	Hose fitting, straight, 4040	10	2.15 €	21.50 €	häberle Shop	9.207 801
	Total			**1,095.72 €**		

3. A step by step build guide. This could be hosted on an external website like instructables.com and linked to in the article, it could also be provided as supplementary material

Authors' response: We find the idea of referee #2 of a step-by-step build guide highly intriguing and would like to provide such a guide in the near future, being aware of the potential benefit it might have for researchers and other users who want to build their own GUARD autosampler. However, considering the complex process required building the GUARD autosampler with its many steps and including multiple custom adaptions involving 3D-printouts, we hope that the referees and the editors of HESS understand that a complete step-by-step build guide is clearly beyond the scope of this journal article, even for the Supplementaries. As we certainly do intend to make the GUARD autosampler available to potential users in research and other fields and bearing in mind the complexity of the building process, however, we would like to offer these users our advice during their building process until a step-by-step guide can be provided. As another way of making the GUARD autosampler accessible for the scientific community and other groups, we might lend our device to interested users, of course free of charge. We hope that the referees and the editors of HESS can accept one or both of the offered solutions.

Minor points:

Referee Comment: The opens lab at OSU is also working on an autosampler, with a completely different setup. Might be worth citing their work: http://www.open-sensing.org/opensampler/. They have a paper forthcoming, but did present it at the AGU fall meeting (where I spotted it). Maybe that abstract can be cited.

Authors' response: We will mention the OPEnSampler and cite the conference abstract, as suggested.

Changes to the manuscript: Insert at the end of page 2: "(Note that similar types of autosamplers are currently worked on, for instance, the "OPEnSampler" developed at Oregon State University (Nelke, Selker and Udell, 2017)".

Referee Comment: On line 3 of page 3 the terms "high frequency, long term monitoring" etc. are used. What constitutes high frequency of long term is very dependent on the field of science one is in. Please make this more specific to the GUARD.

Authors' response: We will specify the time scale referred to in the manuscript.

Changes to the manuscript: On page 3 in line 3 replace line by "either high-frequency sampling (e.g. every minute), long-term monitoring (e.g. 6 months), or medium-term monitoring at medium sampling frequency (e.g. daily sampling for 48 days)."

Referee Comment: On page 3, line 6: I had to look up what "septa" is. Maybe this is because I'm not a native English speaker. If septa is considered a technical term, please explain it once you introduce it for the first time.

Authors' response: We will define the term upon its first appearance in the manuscript.

Changes to the manuscript: On page 3, in line 6 insert after "septa": "(engineered membranes that permit the transfer of fluids without air contact, usually using a double-canula)"

Referee Comment: On page 4, line 24: Future work might be better placed in the discussion, although mentioning it at both places is also fine.

Authors' response: Connection multiple batteries in parallel, replacing discharged batteries using an electrical bypass and operating the GUARD autosampler on mains power using an appropriate rectifier are options viable for any battery-powered autosampler. Therefore, these options mentioned on page 4, in line 24, do not distinguish the GUARD autosampler from any other autosampler such as the 3700C Compact from Teledyne Isco. For that reason, we think that mentioning these options again in the discussion (Section 5) would be redundant and inappropriate.

Referee Comment: On page 6, line 4: "effectively prevented" assumes certain demands from applications. I suggest replacing it with something like: "prevented for most common use cases".

Authors' response: As there might be applications we might not have considered, we agree to change the passage.

Changes to the manuscript: On page 6, in line 4 delete "effectively".

Referee Comment: On page 18, table one: Sentences like "the sampler can also run … … important constraint" are more suited in the discussion.

Authors' response: We agree with referee #2 and will move the indicated sentence.

Changes to the manuscript: On page 18, in Table 1 in line "Battery" remove the remark "the sampler can also run on 12 V Li-ion batteries if weight is an important constraint" and insert it on page 4 in line 29 at the end of the paragraph.

References

Clark, Ian D.; Fritz, Peter (1999): Environmental isotopes in hydrogeology. [2. print., corr.]. Boca Raton: Lewis Publ.

Hoefs, Jochen (2015): Stable isotope geochemistry. 7. ed. Cham: Springer (Earth Sciences).

Nelke, Mitch; Selker, John, S.; Udell, Chet (2017), The OPEnSampler: A Low-Cost, Low-Weight, Customizable and Modular Open Source 24-Unit Automatic Water Sampler, Abstract H41J-1596 presented at 2017 Fall Meeting, AGU, New Orleans, LA, 11-15 Dec.

Smith, Charles, C.; Löf, George; Jones, Randy (1994): Measurement and analysis of evaporation from an inactive outdoor swimming pool. In: Pergamon 1994, pp. 3-7.