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Isotopic ( $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ ) Integrity of Water Samples Collected and Stored by Automatic Samplers

Mark R. Williams,\* Jessica L. Lartey, and Laura L. Sanders

## Core Ideas

- Isotopic integrity ( $\delta^{18}\text{O}/\delta^2\text{H}$ ) of water samples stored in autosamplers was assessed.
- Uncertainty in isotope values increased with increasing storage time and temperature.
- Addition of mineral oil to samples decreased evaporation and isotope fractionation.

**Abstract:** Stable water isotopes are increasingly becoming part of routine monitoring programs that use automatic samplers. The objectives of this study were to quantify the uncertainty in isotope signatures due to the length of sample storage (1–24 d) inside autosamplers over a range of air temperatures (5–35°C) and to evaluate the effectiveness of two evaporation reduction measures (mineral oil and high density polyethylene balls). Results of the laboratory study showed that up to 11.8% of the sample volume evaporated when samples were stored in an autosampler at 35°C for 24 d. To prevent significant water isotope fractionation, samples should be retrieved from autosamplers <7 d following sample collection when air temperatures are <22°C and <3 d following sample collection when the air temperature is 35°C. If samples need to be stored in an autosampler for longer periods of time, we found that mineral oil added to sample bottles effectively decreases evaporation and the potential for isotope fractionation.

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VARIATION in the stable isotopes of water ( $^1\text{H}/^2\text{H}$  and  $^{16}\text{O}/^{18}\text{O}$ ) has been widely used to examine hydrological processes across the soil–vegetation–atmosphere continuum. Use of stable water isotopes has led to new insights into hydrological response at the watershed (Vitvar et al., 2005; McGuire and McDonnell, 2007), field (Vidon and Cuadra, 2010; Williams et al., 2016), hillslope (McGuire and McDonnell, 2010; Klaus et al., 2013), and soil profile scales (Sprenger et al., 2016; Williams et al., 2018). Technical advancements in laser absorption spectroscopy have substantially decreased the time, energy, and expense of analyzing water samples for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  (e.g., Wassenaar et al., 2014). As a result, stable water isotope data collection and analysis are increasingly becoming part of routine monitoring programs. Dual isotope reporting combined with a greater frequency of observation has the potential not only for future breakthroughs in understanding of time–source components of flow (Klaus and McDonnell, 2013) but also for constraining uncertainty and improving estimates of boundary conditions in numerical models (Jensen et al., 2017).

Collection of water samples for analysis of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  is relatively easy compared with other solutes, such as nutrients and pesticides, which are often measured as part of routine monitoring programs (Kendall and Caldwell, 1998). Bottle rinsing, chilling, addition of preservatives, and, in many instances, filtering are unnecessary when collecting samples for stable water isotope analysis. A clean, dry bottle that is filled to the top and capped tightly to prevent evaporation and exchange with atmospheric vapor is all that is required. Many monitoring programs, however, use automatic water samplers to collect and store samples from hours to weeks, depending on research objectives and methodologies. While uncertainty associated with automated sampling and analysis of sediment, nutrients, and bacteria has been estimated (Kotlash and Chessman,

**Abbreviations:** *d*-excess, deuterium-excess; HDPE, high density polyethylene; IHS, isotope hydrograph separation.

1998; Jarvie et al., 2002; Harmel et al., 2006; 2009; 2016a; 2016b), uncertainty in stable water isotope signatures has not been quantified.

During the time between sample collection and sample retrieval from the autosampler, water samples are left open to the atmosphere and may be at risk for evaporative losses. Evaporation from an open-water surface results in kinetic fractionation of water isotopes in a manner that depends on several parameters, including humidity and air temperature (Craig and Gordon, 1965). Given the widespread use of autosamplers and increasing collection of samples for stable water isotope analysis, the objectives of the study were (i) to quantify the uncertainty in  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  signatures due to the length of sample storage (1–24 d) inside autosamplers over a range of air temperatures (5–35°C) and (ii) to evaluate the effectiveness of two evaporation reduction measures, mineral oil and high density polyethylene (HDPE) balls, that were added to autosampler bottles.

## Materials and Methods

In June 2017, 64 L of water was collected from the Matson Ditch located in northeast Indiana (41°27'23.98" N, 84°57'31.00" W). At the point of sample collection, the Matson Ditch drains 1934 ha dominated by row crop agriculture (77% of land use). Water was collected in 8-L glass bottles from the thalweg of the ditch during baseflow. Upon return to the laboratory, 273 ± 6 mL of ditch water was partitioned into 180 glass sample bottles (300 mL) used in Isco automatic samplers (Teledyne Isco). Isco bottles were randomly designated to receive one of three evaporation reduction treatments: control (i.e., open-water surface), mineral oil, and HDPE balls. For the mineral oil and HDPE ball treatments, 5 mL of mineral oil and 5 g of hollow HDPE balls (30 balls; 6.35 mm diam.) were added to each sample bottle, respectively. Both the mineral oil and the HDPE balls floated on the water surface inside the sample bottle and covered the surface.

Samples were placed in four insulated boxes with lids to replicate bottle storage in an autosampler. Each box contained 45 bottles (3 treatments × 15 samples). One box was placed in a walk-in cooler that was maintained at 5°C. One box was left at room temperature (22°C). A heating pad was added inside the third box to maintain an air temperature of 35°C. The fourth box was placed in a refrigerator set to be maintained at 10°C. The refrigerator did not maintain the desired air temperature of 10°C and resulted in an average air temperature over the study period of 7°C. Study results for the 5°C and 7°C treatments were identical; thus, we have not reported the data from the 7°C treatment herein.

Twenty-four replicates of ditch water were subsampled from the 8-L sample containers on the same day as water was added to the Isco bottles (i.e., Day 0) to establish baseline values of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ . On Days 1, 3, 7, 14, and 24 of the experiment, three replicates of each evaporation reduction treatment were removed from each box, weighed to determine the amount of evaporation, analyzed for stable water isotope ratios using a liquid water isotope analyzer (Los Gatos Research), and discarded. Samples were analyzed

against reference values calibrated to Vienna Standard Mean Ocean Water. Instrument precision for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  was ± 0.11‰ and ± 0.5‰, respectively.

Analysis of variance (ANOVA) was used to evaluate the effect of sample storage length and evaporation reduction treatment on the amount of evaporation at each air temperature. Deuterium-excess ( $d$ -excess =  $\delta^2\text{H} - 8 \cdot \delta^{18}\text{O}$ ), which is a measure of the deviation from the global meteoric water line, was used to evaluate changes in water isotope signatures resulting from kinetic fractionation during evaporation. Deuterium-excess values were compared with baseline (i.e., Day 0)  $d$ -excess using a two-sample  $t$  test. To evaluate the effect of sample storage length and evaporation reduction treatment on  $d$ -excess at each air temperature, ANOVA was used. To separate treatment means following both ANOVAs, the 'lsmeans' function with a 'tukey' adjustment and confidence level of 0.05 was used from the 'lsmeans' package in R statistical software (R Development Core Team, 2011).

## Results and Discussion

### Evaporation of Water Samples within Automatic Samplers

Evaporative losses from the control treatment tended to increase with an increase in sample storage length and air temperature (Fig. 1). After 1 d of storage, between 0.7 and 1.2 mL of water had evaporated from the control treatment across all air temperatures, which was equivalent to approximately 0.5% of the original sample volume. On average, 0.9, 3.3, and 11.8% of the original sample volume had evaporated from the control treatment by Day 24 for the 5, 22, and 35°C air temperatures, respectively (Fig. 1). Adding mineral oil to the sample bottle significantly decreased the amount of evaporation during sample storage (Fig. 1). Less than 0.3 mL (<0.1% of the original volume) of water was evaporated from the mineral oil treatment after 24 d in storage across all air temperatures. Evaporation from the HDPE ball treatment and control treatment was often not significantly different, suggesting that the addition of HDPE balls to sample bottles was not effective at reducing evaporative losses (Fig. 1).

The kinetic fractionation of water isotopes resulting from evaporation is discussed in the subsequent section, but it is also important to note the potential impact of evaporation on solute concentrations. Previous studies on sample storage in autosamplers have generally focused on chemical and biological processes that can alter the speciation of both dissolved and particulate solutes (e.g., Jarvie et al., 2002). Kotlash and Chessman (1998), for example, reported that 47% of filterable phosphorus was lost after 2 d of storage in an autosampler. The authors attributed the losses to adsorption associated with microbial uptake and chemical precipitation on internal container surfaces. Results from the current study suggest that in addition to chemical and biological processes, the physical loss of water from sample bottles during storage in autosamplers may increase uncertainty in solute concentrations.

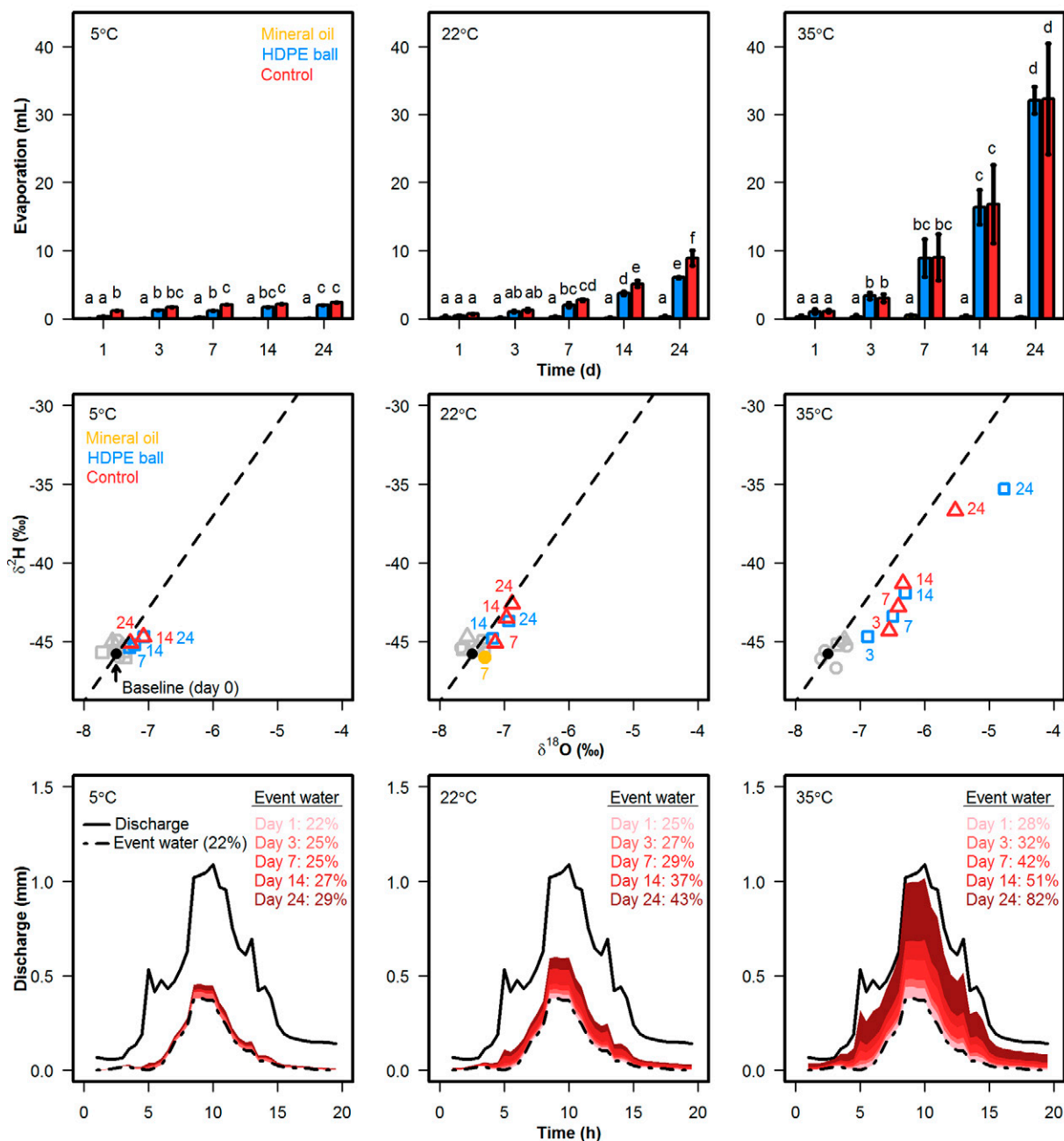


Fig. 1. (Top panels) Mean evaporative losses from sample bottles when stored in autosamplers at 5, 22, and 35°C. Error bars represent one standard deviation. Letters denote statistical differences ( $p < 0.05$ ) among evaporation reduction treatments (i.e., control, high density polyethylene [HDPE] balls, and mineral oil) within each air temperature. (Middle panels) Mean oxygen-18 ( $\delta^{18}\text{O}$ ) and deuterium ( $\delta^2\text{H}$ ) signatures of water samples throughout the experiment. Gray symbols indicate sampling days when deuterium-excess values were not significantly different from baseline values (i.e., Day 0). Colored symbols show deuterium-excess values significantly different from baseline values and adjacent numbers show the sampling day. The dashed line on each panel represents the local meteoric water line for river waters in Indiana ( $\delta^2\text{H} = 5.9 \cdot \delta^{18}\text{O} - 1.6$ ; Kendall and Coplen, 2001). (Bottom panels) Results of isotope hydrograph separation for a single storm event using  $\delta^2\text{H}$  signatures. Red shaded areas indicate the potential error in determining the amount of event water (i.e., new water) due to the length of sample storage in an autosampler. Calculations were based on the deviation from baseline values for the control treatment. Percentages listed show the amount of event water relative to total discharge.

### Isotope Fractionation during Storage

Baseline (Day 0)  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  signatures of water samples were  $-7.49 \pm 0.16\text{‰}$  and  $-45.8 \pm 0.4\text{‰}$ , respectively (Fig. 1). Deuterium-excess was calculated for water samples at baseline and averaged  $14.1 \pm 0.9$ . Values of  $d$ -excess tended to decrease as the length of storage and air temperature increased. For the control treatment,  $d$ -excess values were significantly different from baseline following 14, 7, and 3 d

in storage at air temperatures of 5, 22, and 35°C, respectively (Fig. 1). Deuterium-excess values were not significantly different between the control and HDPE ball treatment, except on Day 24 at 35°C, when isotope fractionation was greater for the HDPE ball treatment. Deuterium-excess for the mineral oil treatment remained similar to baseline values across all air temperatures (Fig. 1). Study results therefore suggest that to prevent significant water isotope fractionation, samples should generally be retrieved  $<7$  d following sample

collection if air temperatures are <22°C and <3 d following sample collection if air temperature is 35°C. If samples are to be stored in autosamplers for longer periods, then mineral oil should be added to sample bottles to limit evaporation and isotope fractionation. In warm climates or during warm periods of the year, study results also indicate that refrigerated autosamplers may help decrease sample evaporation and isotope fractionation.

The current laboratory study evaluated evaporative losses and resulting isotope fractionation at constant air temperatures. When autosamplers are deployed in the field, collected water samples may be subject to large air temperature fluctuations during storage. While not explicitly evaluated, study results provide an estimate of sample collection frequency for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  under fluctuating air temperatures (Fig. 2). Deuterium-excess for the control treatment was significantly different from baseline values after ~2 mL of water (0.7% of the original sample volume) was evaporated from sample bottles. Since evaporation is a continuous process, we postulate that forecasted mean air temperature could therefore be used to estimate the risk of exceeding this evaporation threshold (Fig. 2). For example, if the 7-d weather forecast is predicting an average air temperature of 15°C, then samples should be retrieved from the autosampler before the end of the 7-d period to limit the risk of significant isotope fractionation (Fig. 2). Additional research exploring the effect of air temperature fluctuations on evaporation and isotope fractionation are needed to provide further sampling guidelines for stable water isotopes.

The amount of water isotope fractionation as the result of evaporation is largely controlled by humidity (Kendall and Caldwell, 1998), with lower humidity resulting in a greater change in  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  signatures. Differences in isotope fractionation resulting from evaporation at varying levels of humidity (0–95%) become pronounced once >20% of the sample volume has been evaporated (Gat and Gonfiantini, 1981). Although humidity within the storage boxes used in the current study was not measured, it is unlikely that any slight difference in humidity among storage boxes had an impact on isotope fractionation results given the amount of evaporation from sample bottles (i.e., maximum of ~12% of the original sample volume evaporated for the control treatment stored at 35°C for 24 d). Many studies have used isotope fractionation as a method to predict evaporation from surface waters (e.g., Gat et al., 1994). However, accurate estimates of isotope fractionation based on evaporative losses from sample bottles are likely not possible due to poor autosampler precision in terms of sample volume. Sample volume collected typically varies by  $\pm 5\%$  for autosamplers, which would equate to ~14-mL difference for an autosampler programmed to collect a 275-mL sample.

To illustrate the potential impact of isotope fractionation during sample storage in an autosampler, isotope hydrograph separation (IHS) using  $\delta^2\text{H}$  signatures was conducted for a single storm event (Fig. 1). Precipitation and ditch baseflow  $\delta^2\text{H}$  signatures were  $-33.3$  and  $-48.6\text{‰}$ , respectively, with water samples collected every hour from the ditch during the storm event. Using a mass balance approach (e.g., Williams et al., 2016), we separated the storm hydrograph

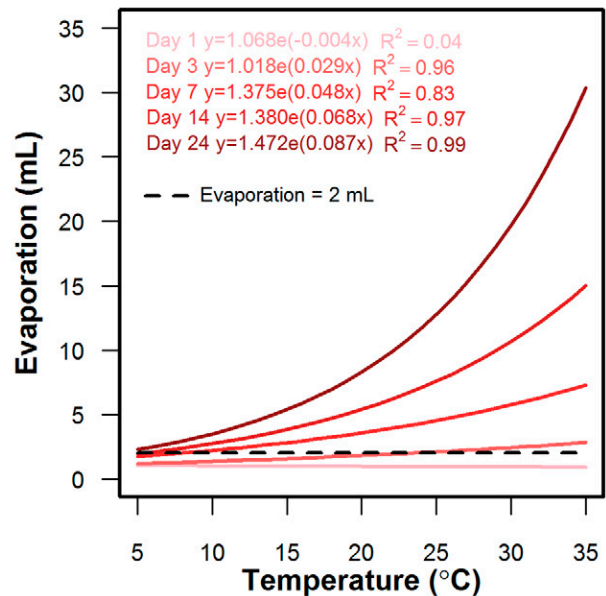


Fig. 2. Relationship between air temperature and evaporative losses for the control treatment during sample storage. Deuterium-excess for the control treatment was significantly different from baseline values after ~2 mL of water (0.7% of the original sample volume) was evaporated from sample bottles. Given forecasted average air temperature over a specified period (e.g., 1, 3, 7, 14, 24 d), the potential for isotope fractionation based on this evaporation threshold can be estimated and provide additional guidance for determining how quickly samples should be retrieved from the field. For example, if the 7-d weather forecast is predicting an average air temperature of 15°C, then samples should be retrieved from the autosampler before the end of the 7-d period to limit the risk of significant isotope fractionation.

into pre-event (i.e., old water) and event (i.e., new water) water components. Results showed that event water comprised 22% of storm discharge (Fig. 1). For each air temperature, IHS was repeated using the deviation from baseline  $\delta^2\text{H}$  signatures for the control treatment based on the length of sample storage. The event water fraction of the storm hydrograph ranged from 22 to 29%, 25 to 43%, and 28 to 82%, for the 5, 22, and 35°C temperatures, respectively, if water samples were potentially stored in an autosampler for 1 to 24 d. These findings indicate that isotopic fractionation during storage in an autosampler has the potential to lead to large errors, which have significant implications for data interpretation. It should be noted that in this example, the potential for isotope fractionation in end-member samples (i.e., precipitation and baseflow) was not considered, which would result in additional uncertainty in IHS results.

## Conclusions

Evaporation of water samples occurs during storage in autosamplers, which results in isotope fractionation. Retrieving samples from the autosampler soon after sample collection (<7 d if air temperature is <22°C; <3 d if air temperature is 35°C) is recommended to prevent evaporative losses. If samples need to be stored in an autosampler for longer periods of time, mineral oil added to the sample bottles effectively decreases evaporation and the potential for isotope fractionation. In contrast, addition of HDPE balls to sample bottles was determined to be ineffective at

decreasing evaporation and isotope fractionation. Before adding mineral oil to sample bottles, consideration should be given to the interaction between the mineral oil and other potential analytes (e.g., nutrients, pesticides, heavy metals). Refrigerated automatic samplers may also help limit evaporation, as evaporative losses were less for the 5°C air temperature compared with the 22 and 35°C air temperatures. Anecdotal experience operating autosamplers in the upper Midwest, however, suggests that substantial condensation can form in samplers when large temperature gradients exist between the inside and outside of the autosampler. While not examined in the current study (i.e., no condensation was observed inside of the 5°C box because the air temperature was the same inside and outside of the box), condensation may drip into sample bottles, potentially altering the isotope signature.

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