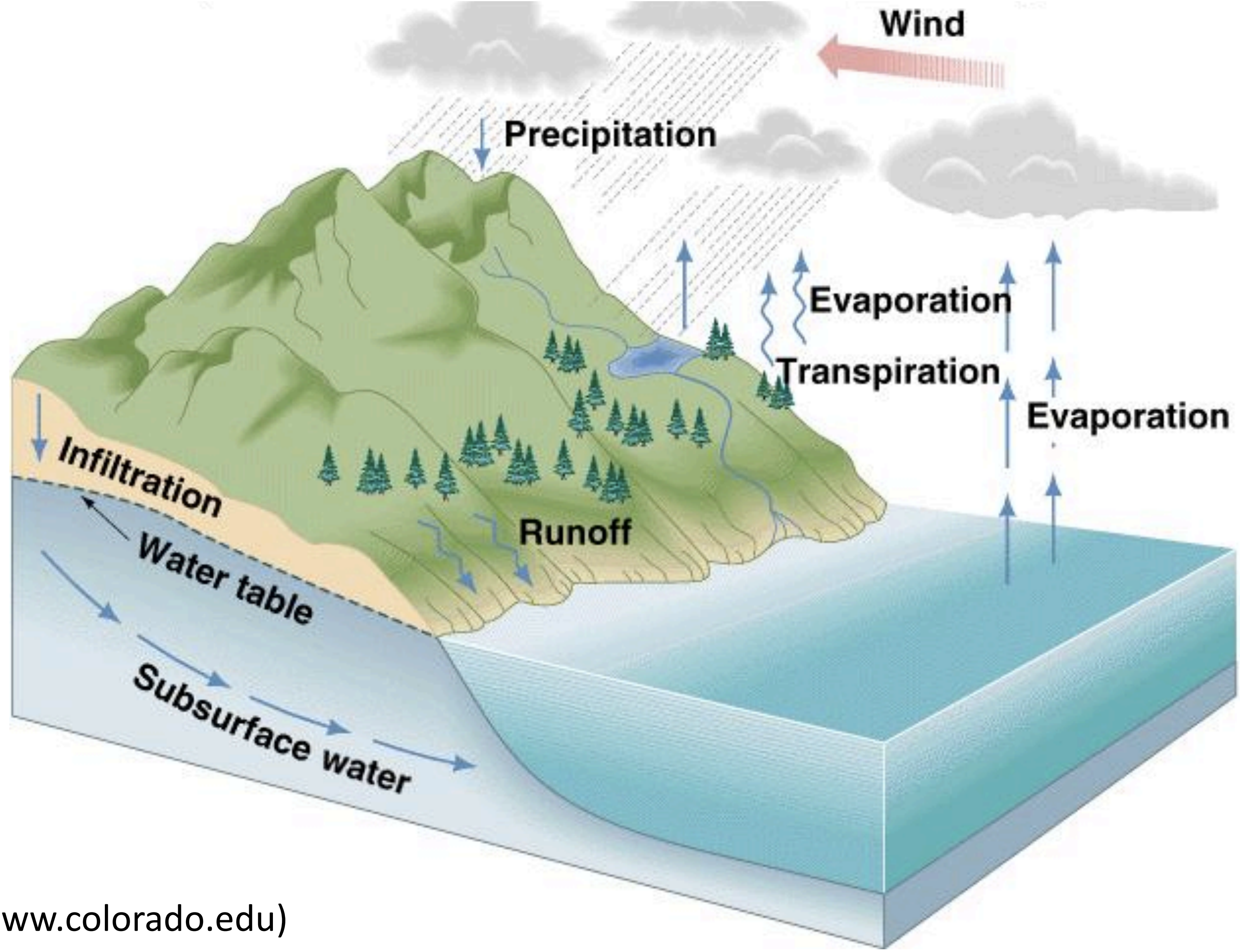
Supplementary Material File 1. Introductory lesson slides on stable isotope hydrology.

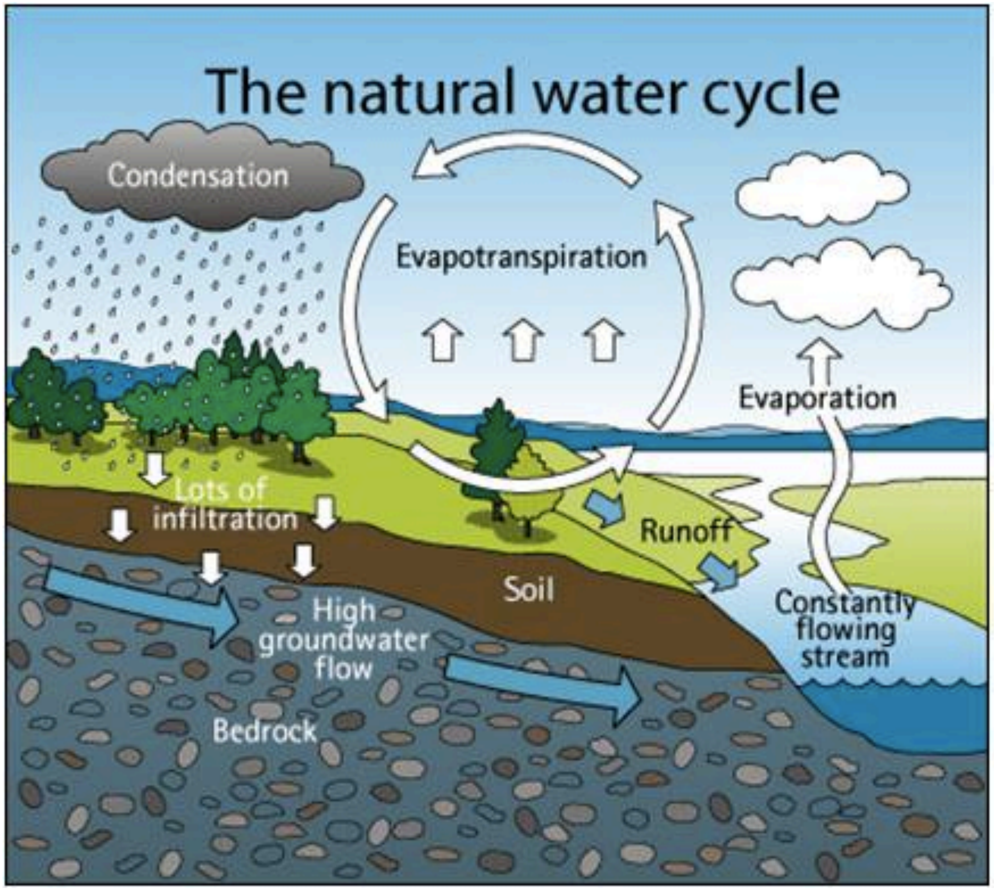
Introduction to Isotope Hydrology

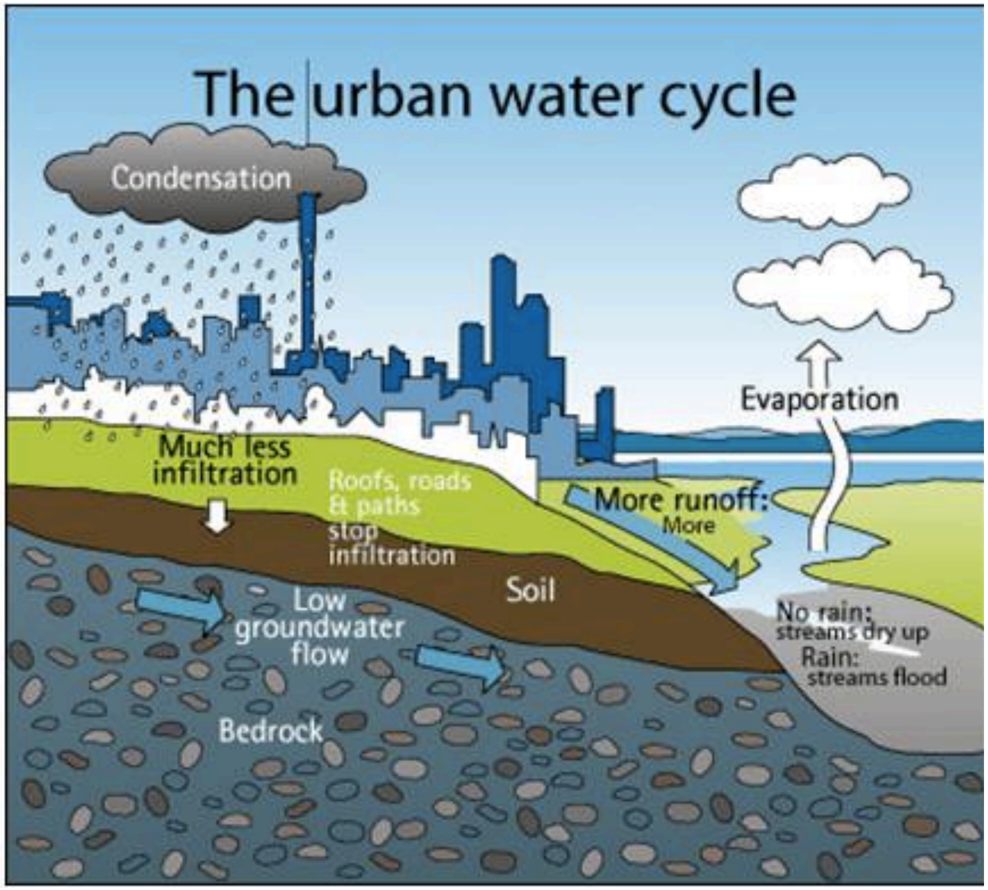
Water Cycle



(www.colorado.edu)

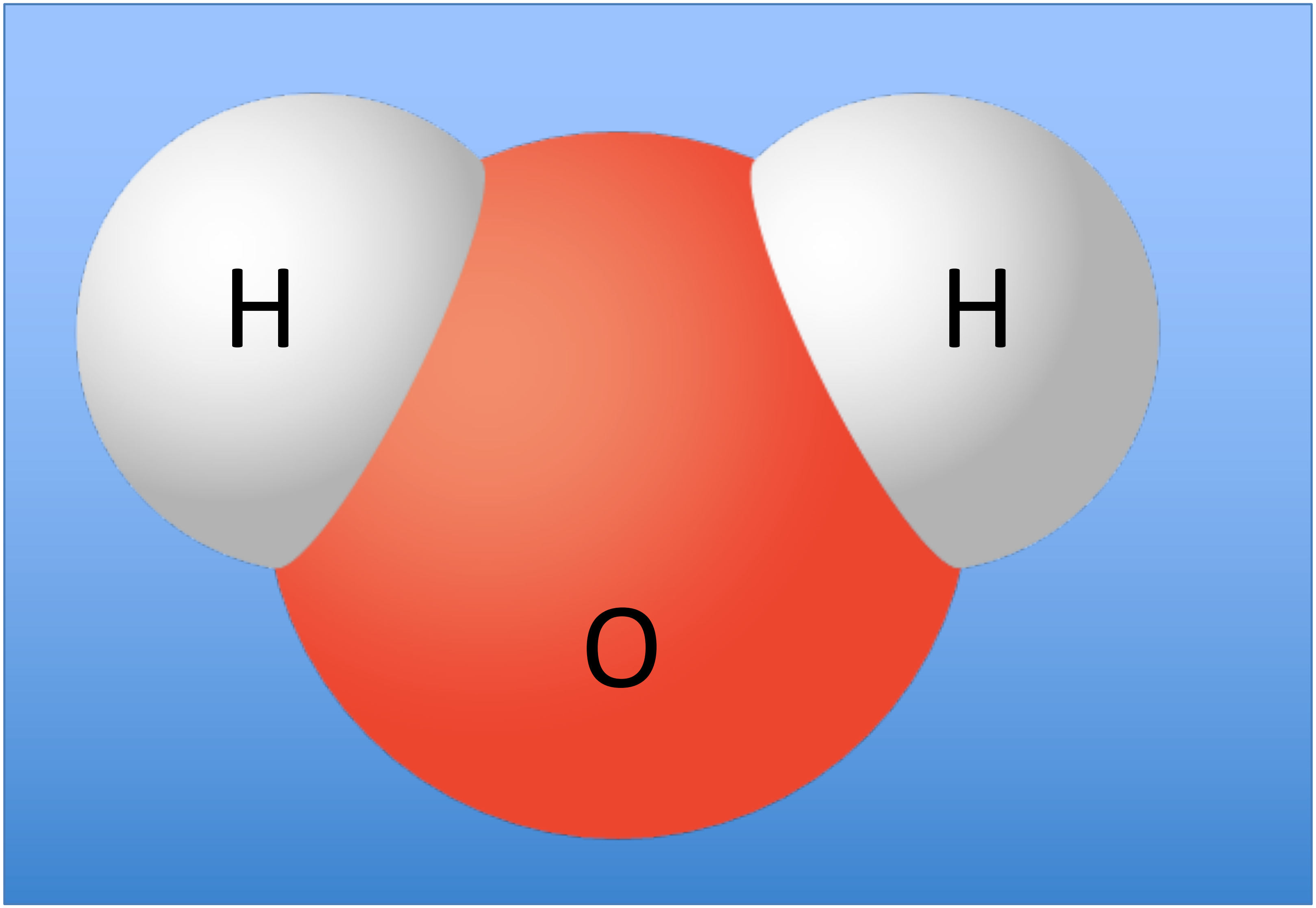
Urban Water Cycle



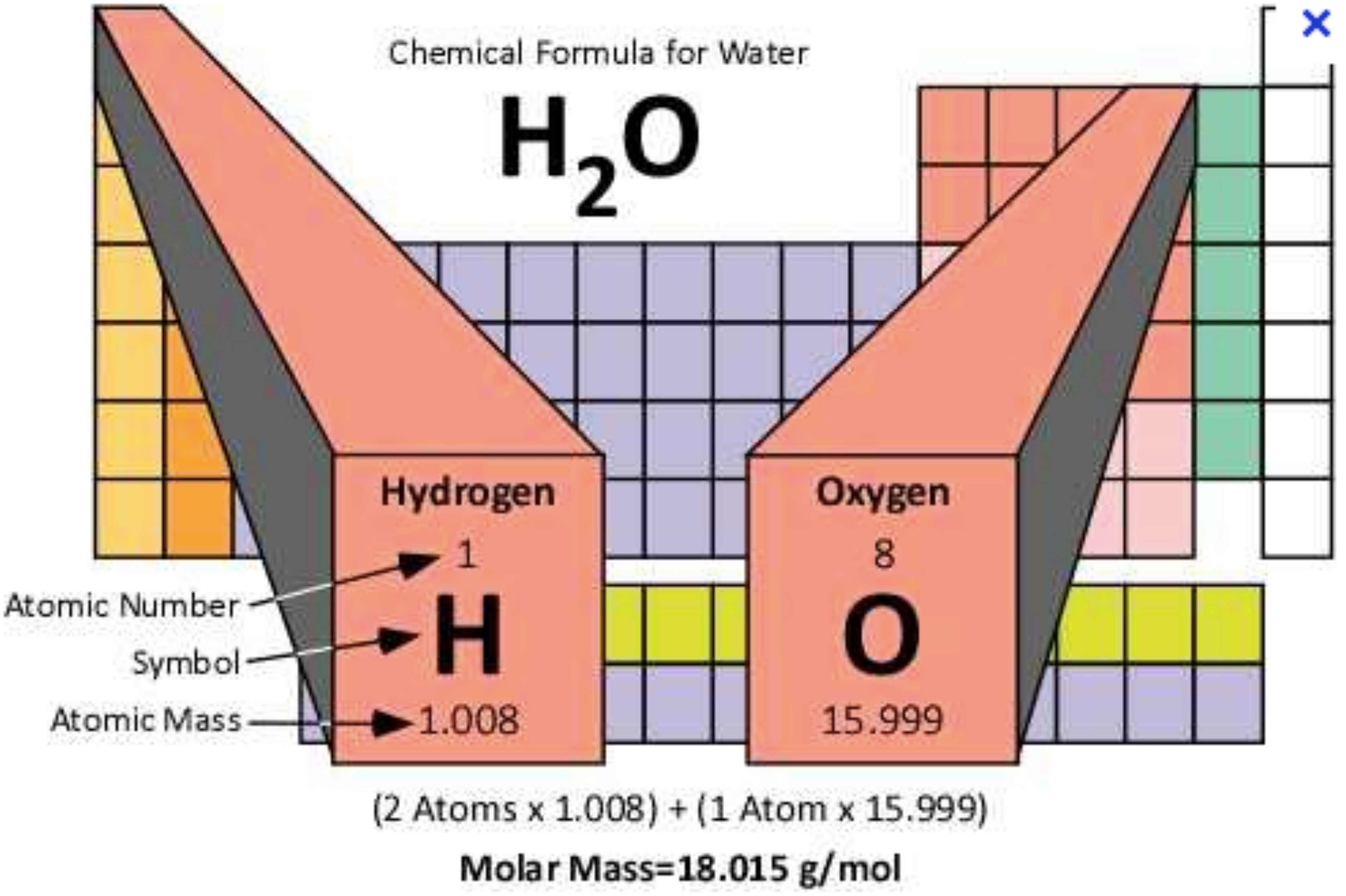


(www.clemson.edu)

Water Chemical Formula: H_2O

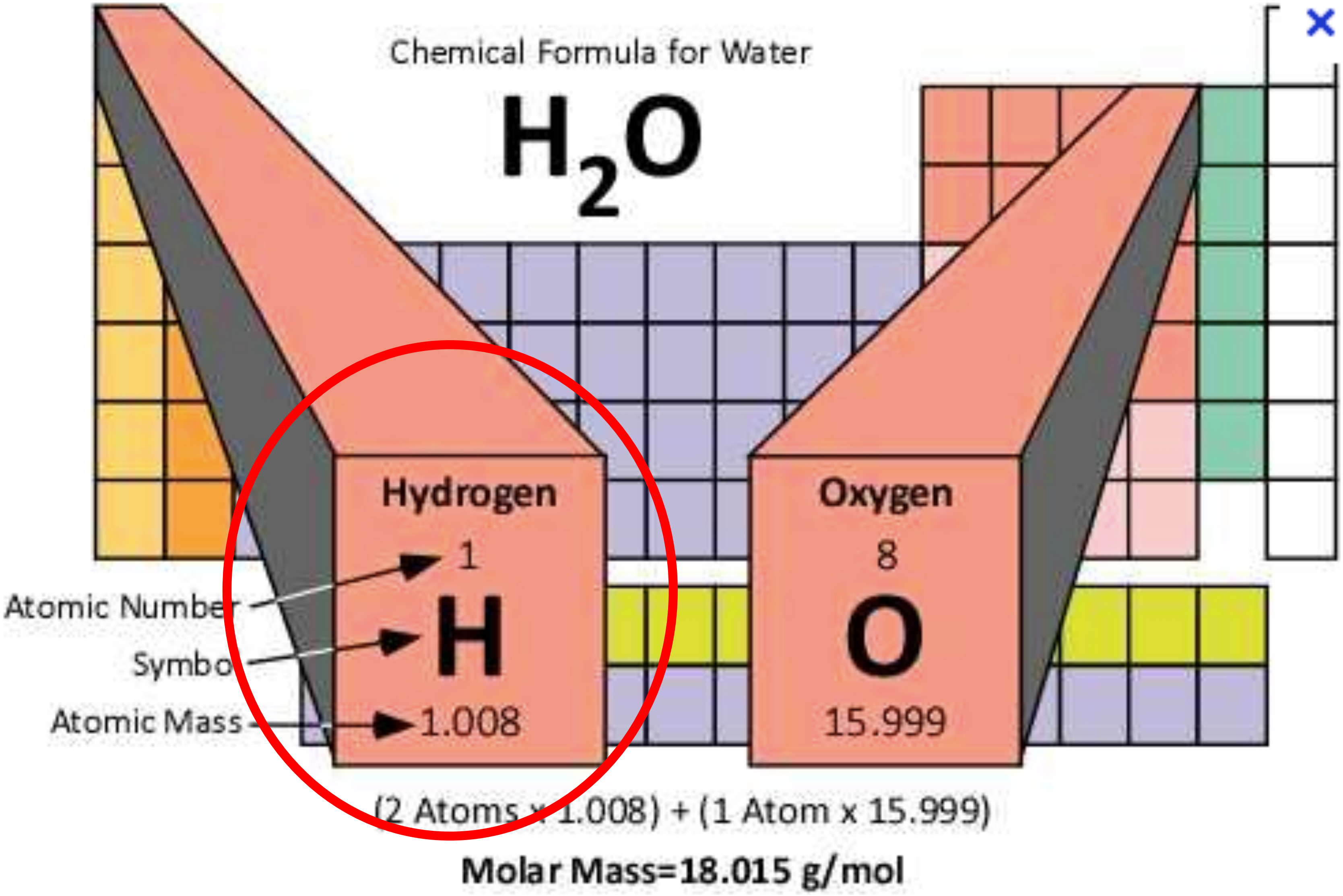


Water Chemical Formula: H_2O



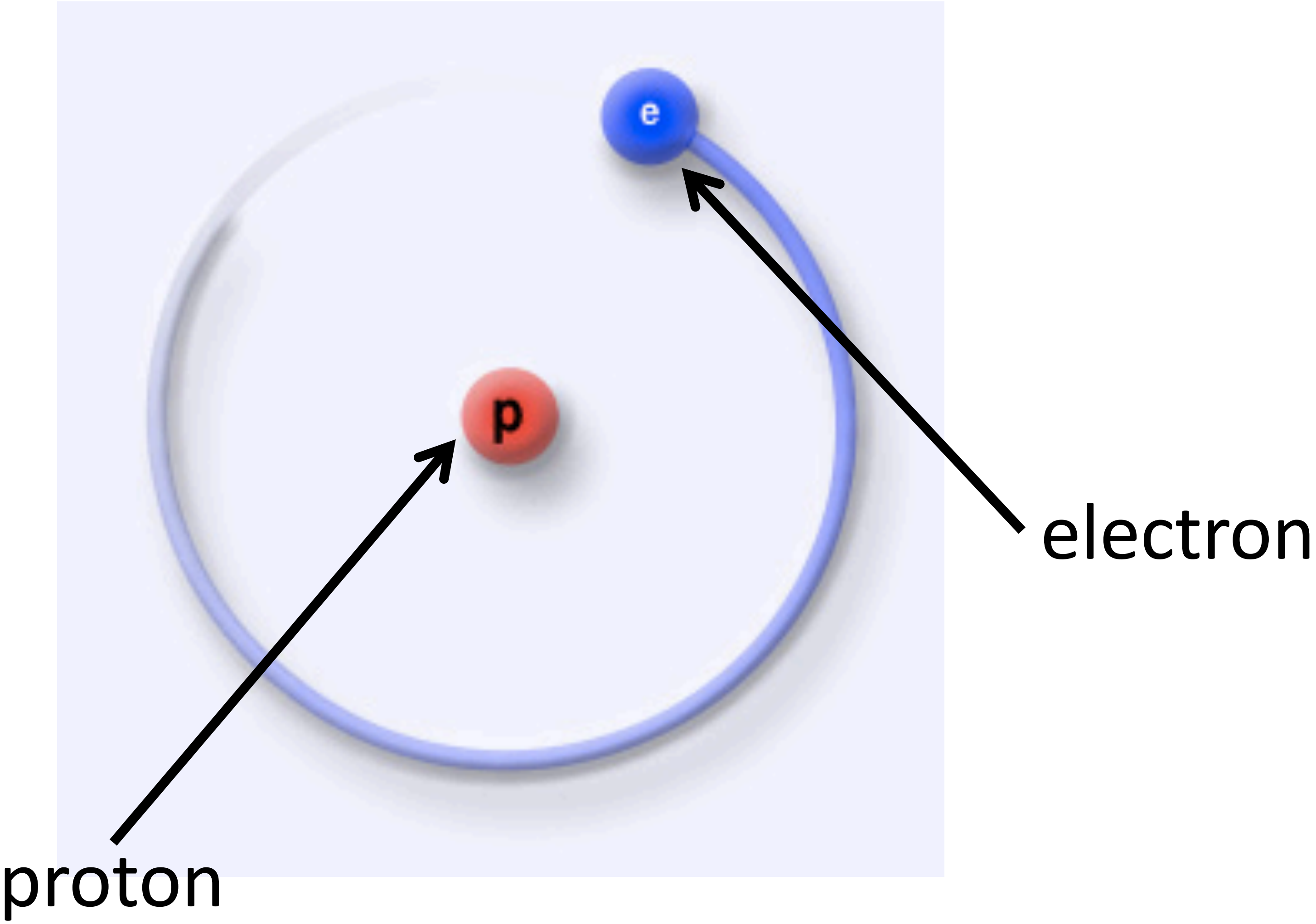
(www.herb03.jigsy.com)

Water Chemical Formula: H_2O



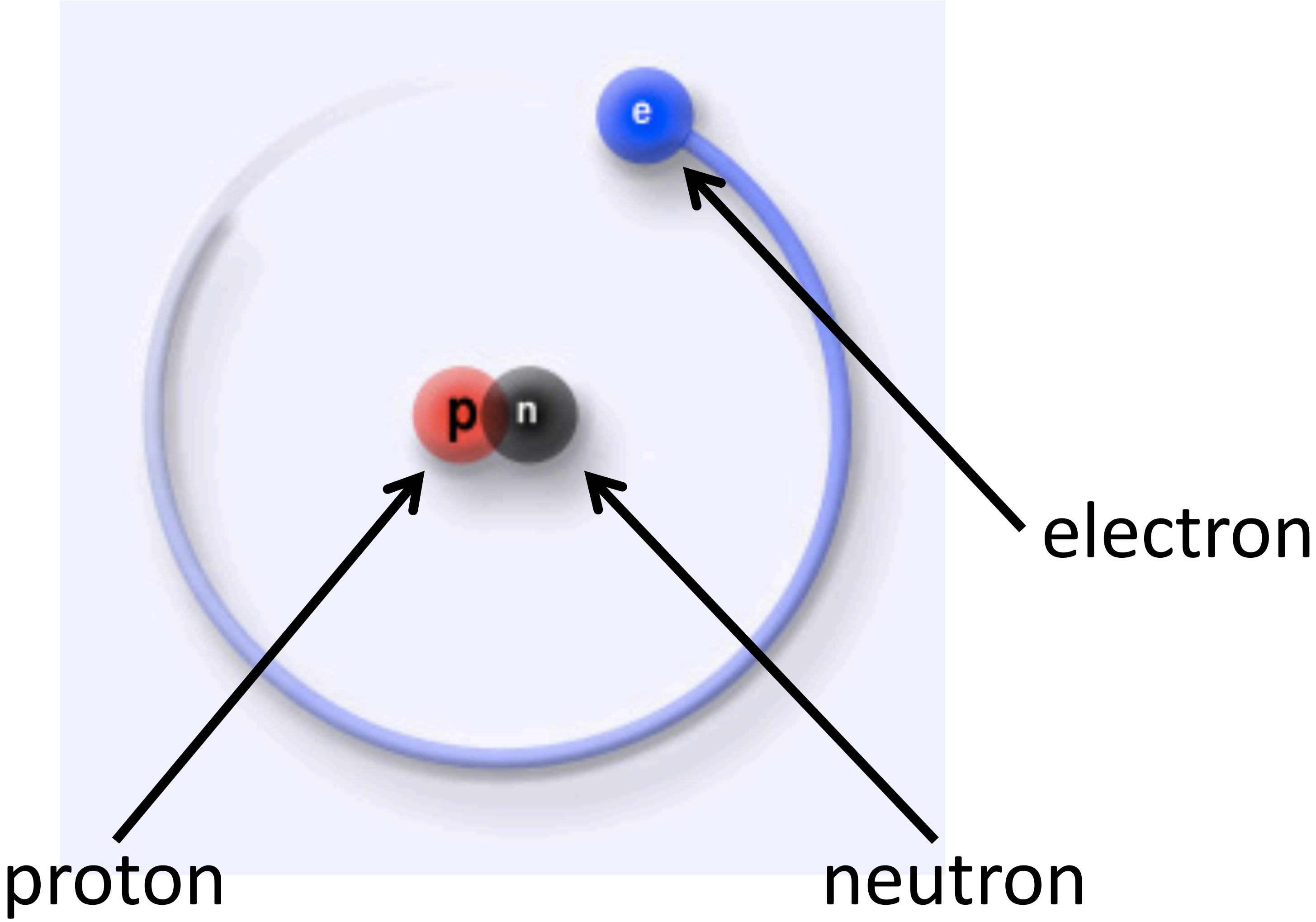
(www.herb03.jigsy.com)

“Normal” Hydrogen Atom



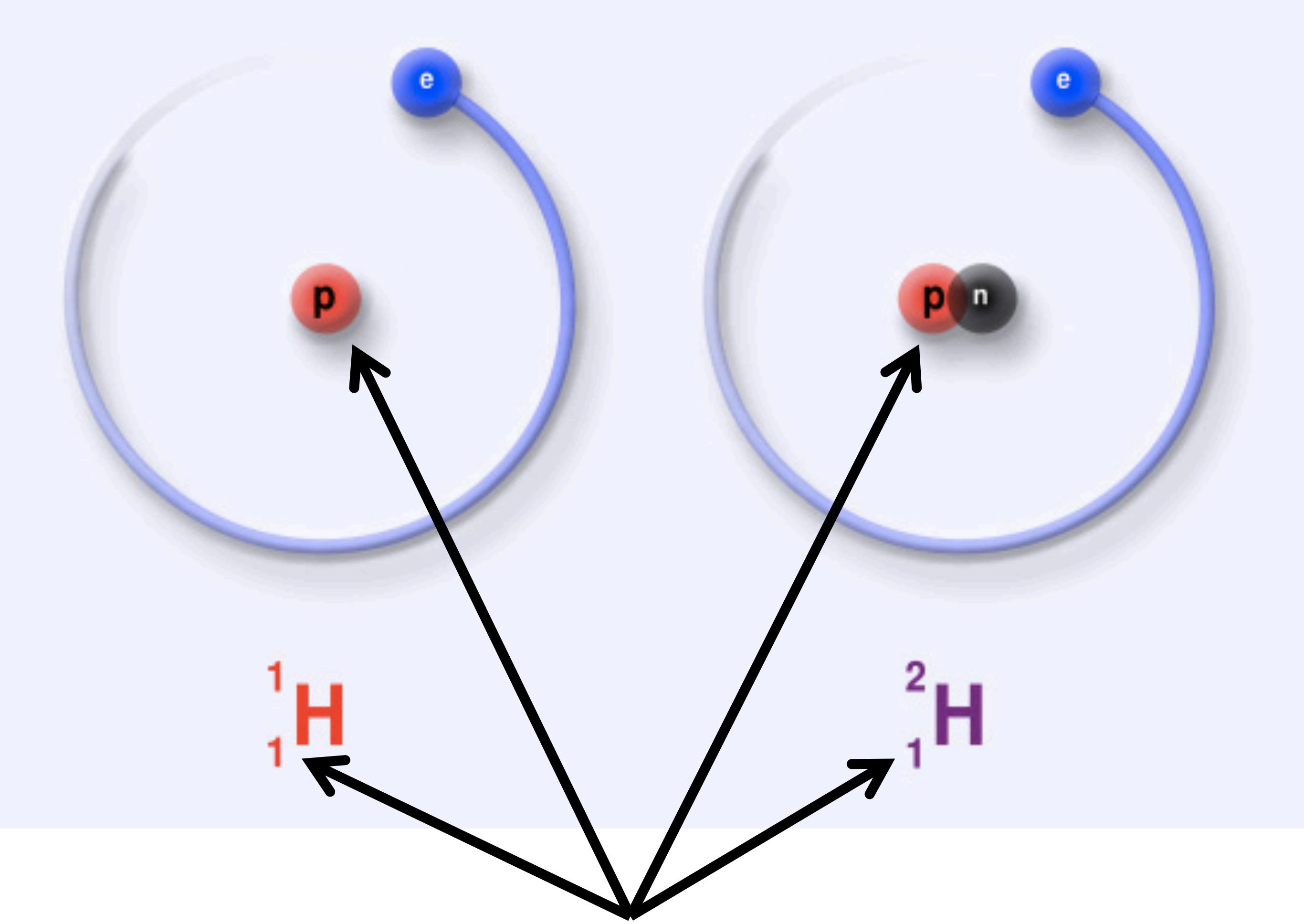
(en.wikipedia.org)

“Special” Hydrogen Atom: Isotope



(en.wikipedia.org)

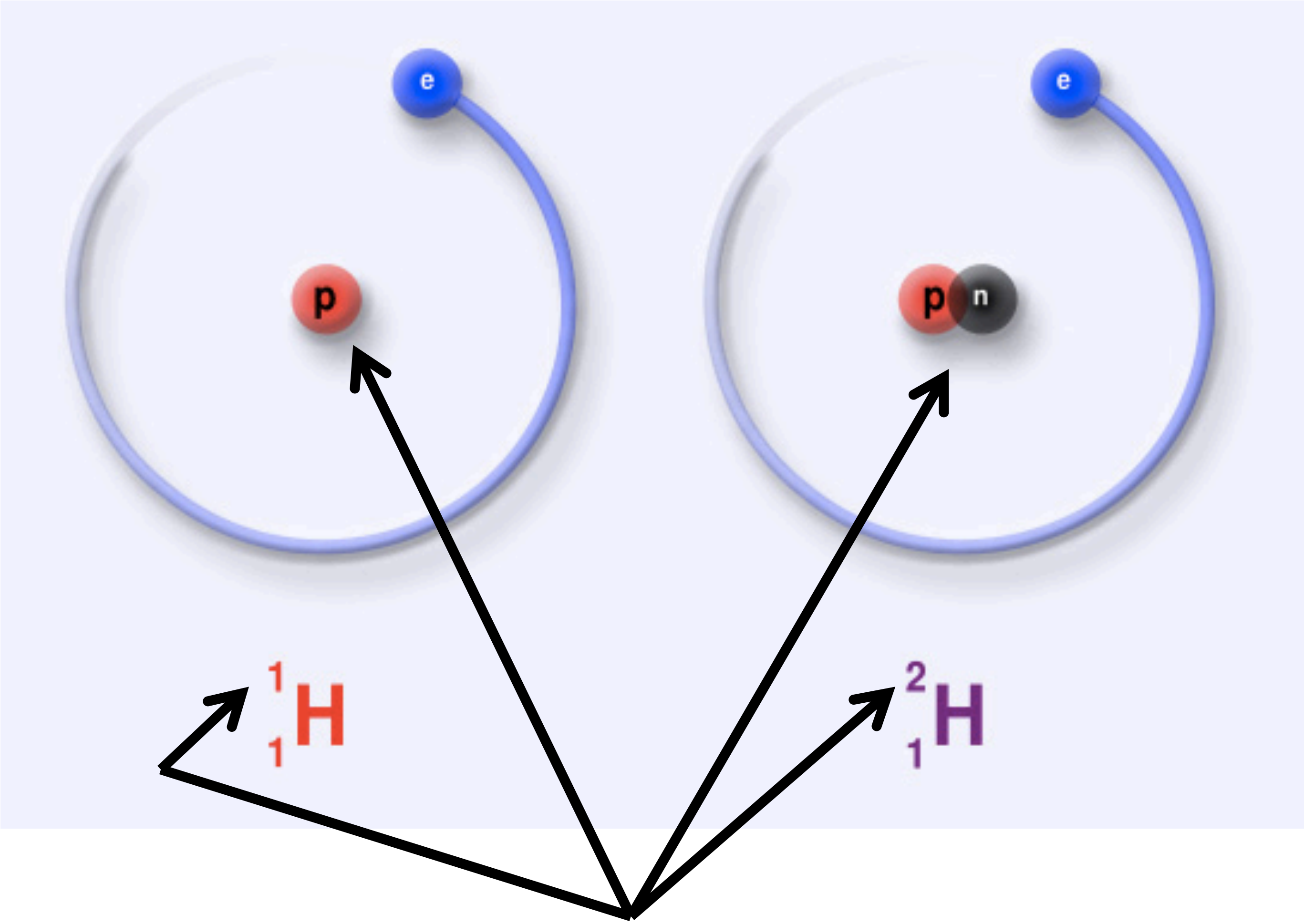
Stable Isotopes of Hydrogen



number of protons

(en.wikipedia.org)

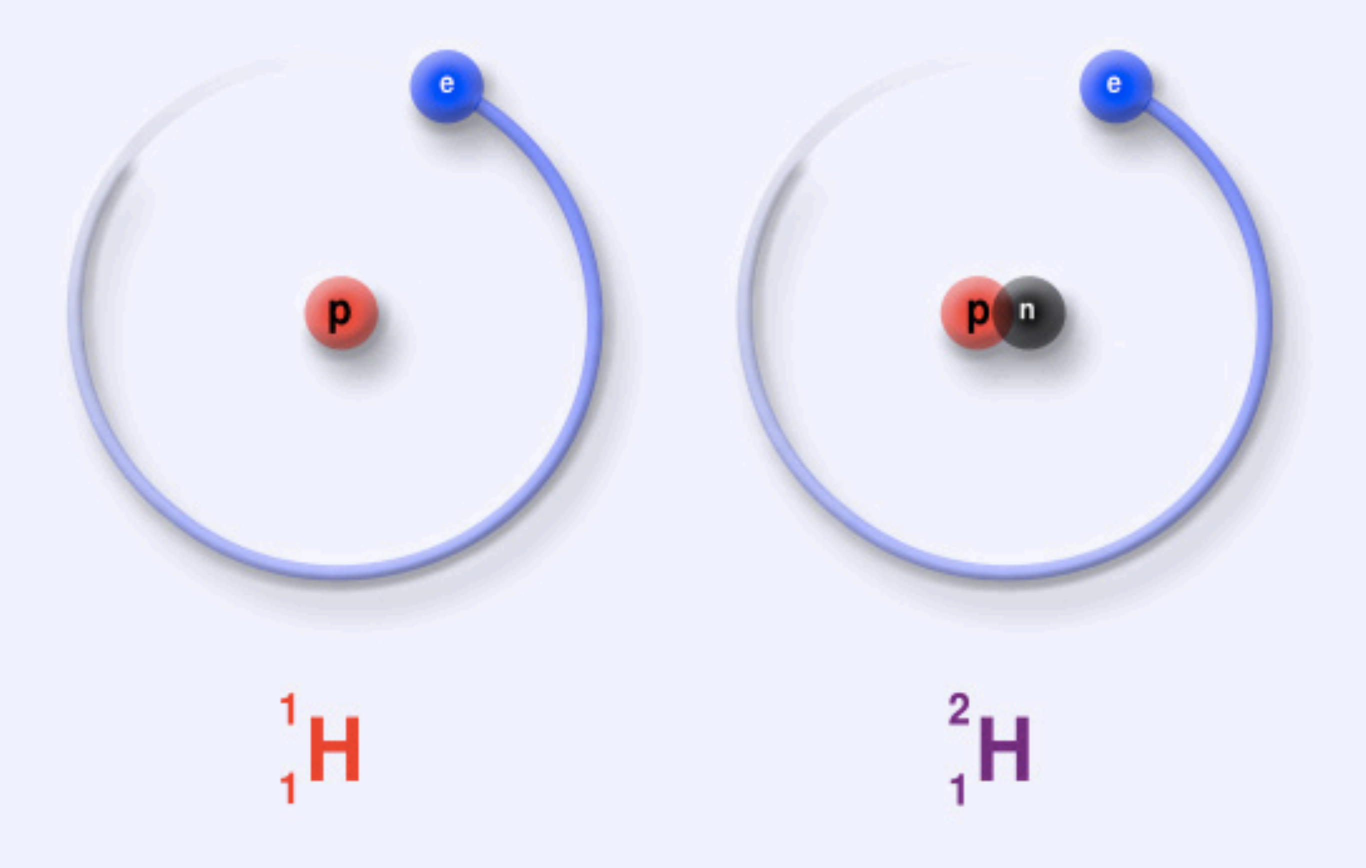
Stable Isotopes of Hydrogen



number of protons + neutrons

(en.wikipedia.org)

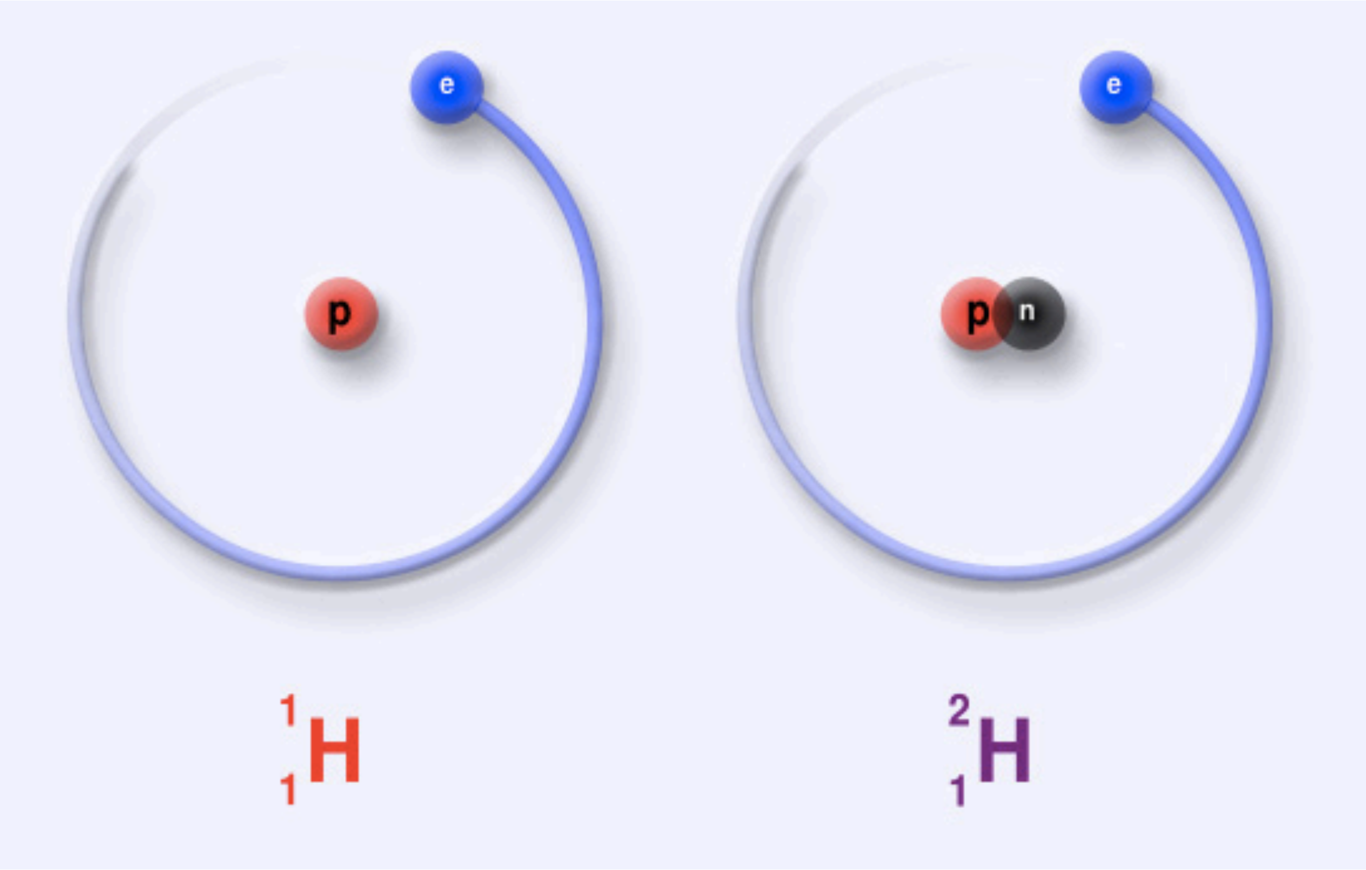
Stable Isotopes of Hydrogen



protons: 1 1

(en.wikipedia.org)

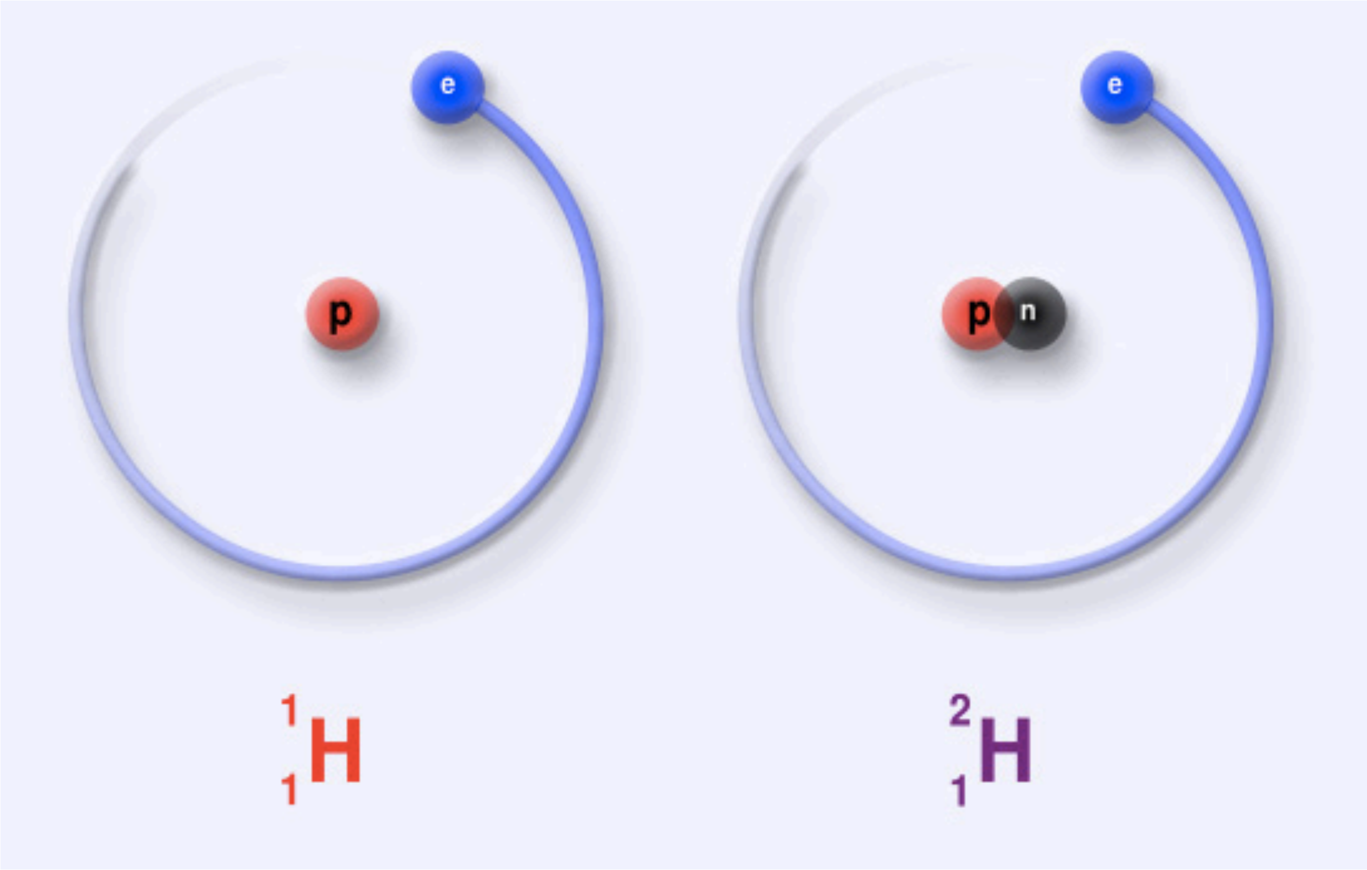
Stable Isotopes of Hydrogen



protons:	1	1
neutrons:	0	1

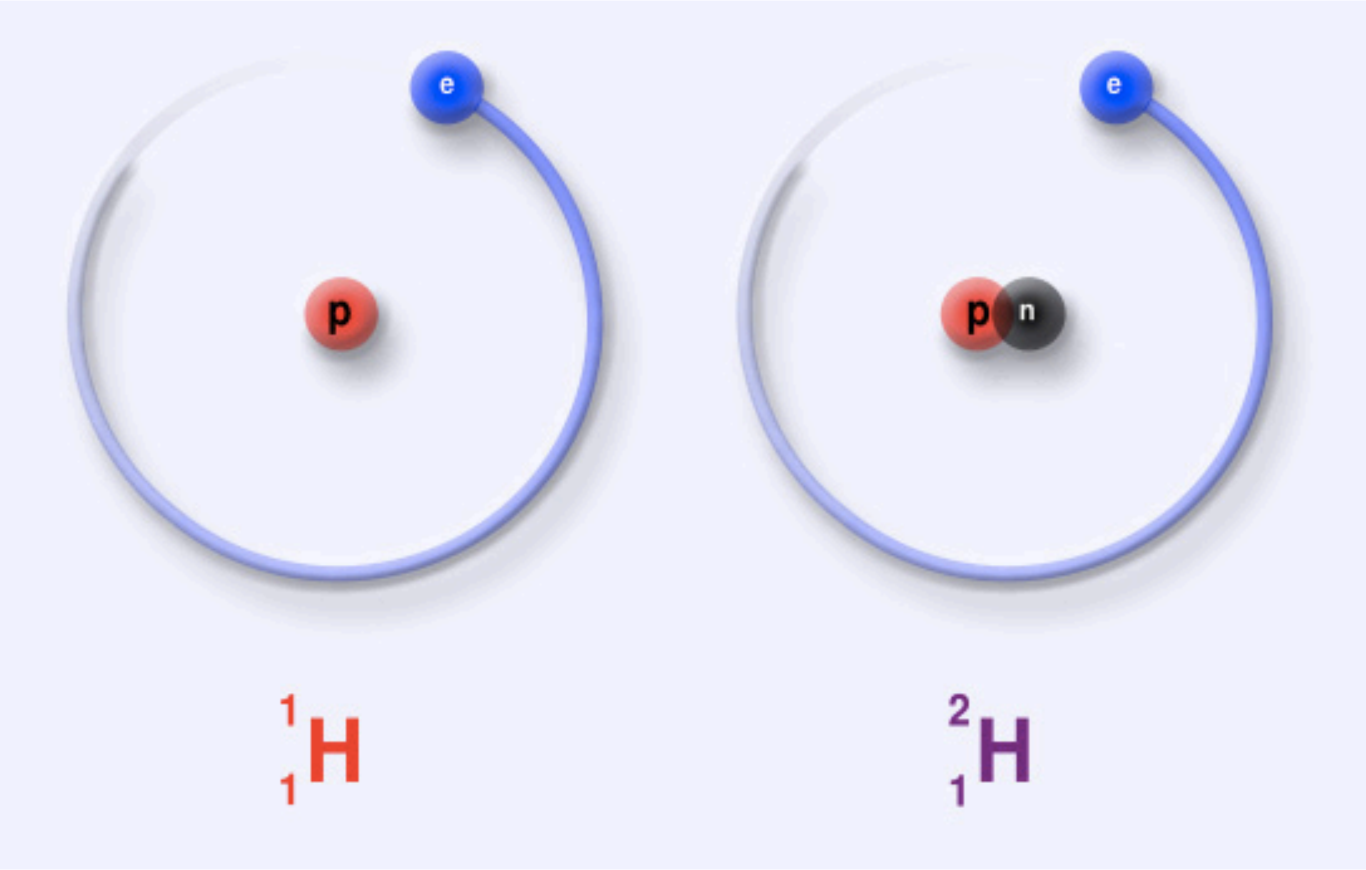
(en.wikipedia.org)

Stable Isotopes of Hydrogen



protons:	1	1
neutrons:	+ 0	+ 1
total:	1	2

Stable Isotopes of Hydrogen



mass:

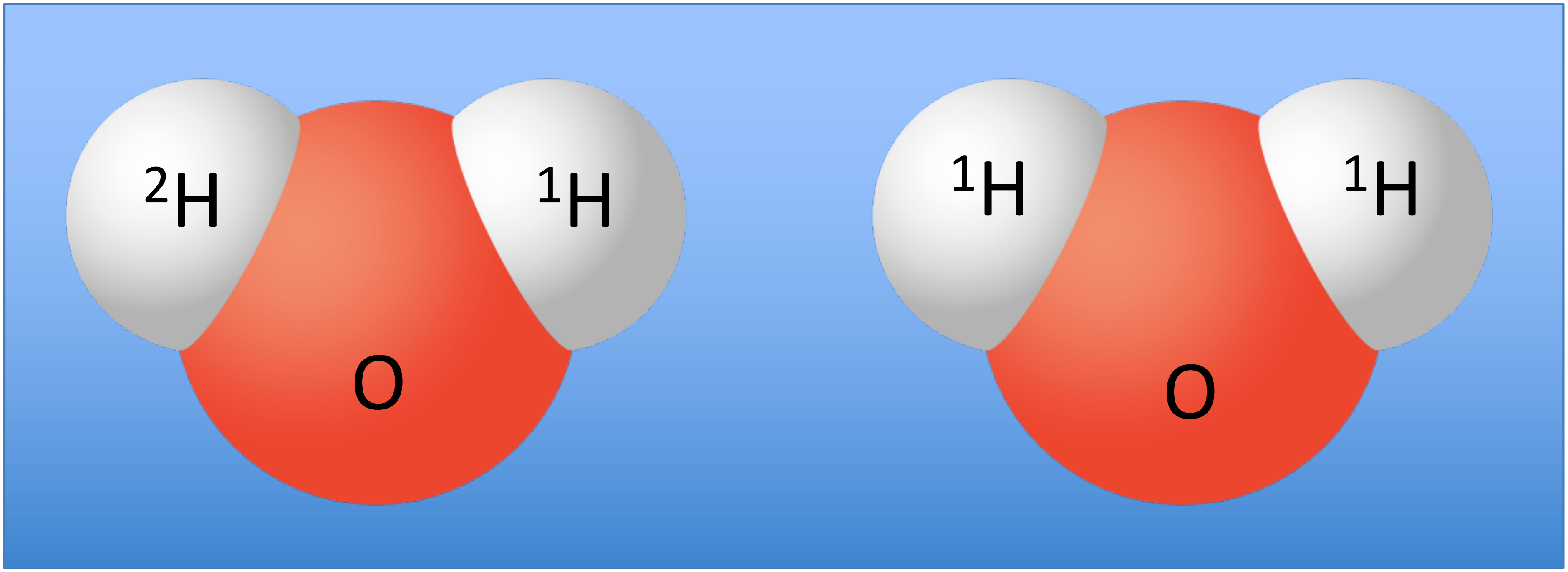
protons:	1 a.m.u.	1 a.m.u.
neutrons:	+ 0 a.m.u.	+ 1 a.m.u.
total mass:	1 a.m.u.	2 a.m.u.

Stable Isotopes of Hydrogen

^{2}H is twice as heavy as ^{1}H

	^{1}H	^{2}H
protons:	1 a.m.u.	1 a.m.u.
neutrons:	+ 0 a.m.u.	+ 1 a.m.u.
total mass:	1 a.m.u.	2 a.m.u.

Stable Isotopes of H in H_2O



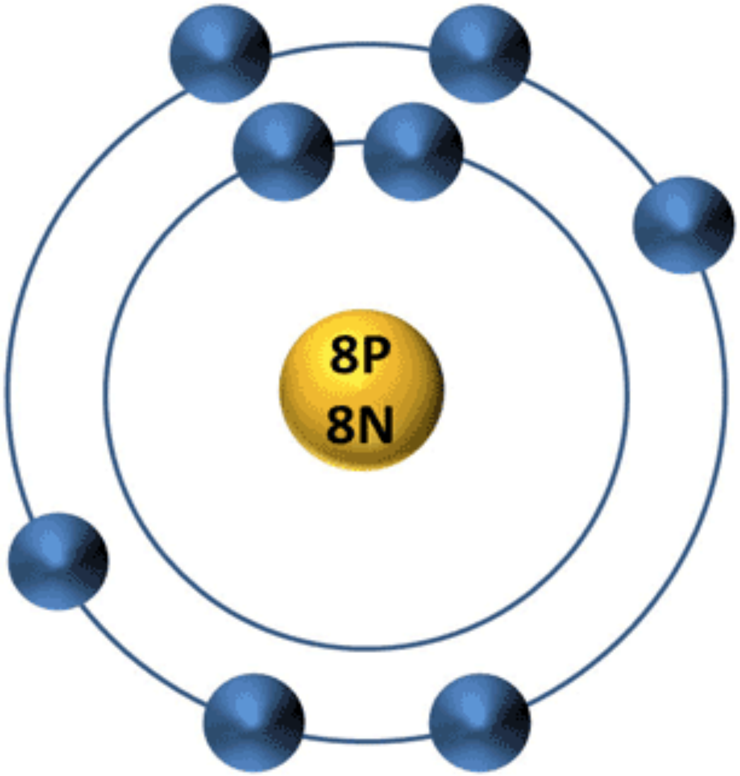
H_2O: $^2H^1HO$

heavier

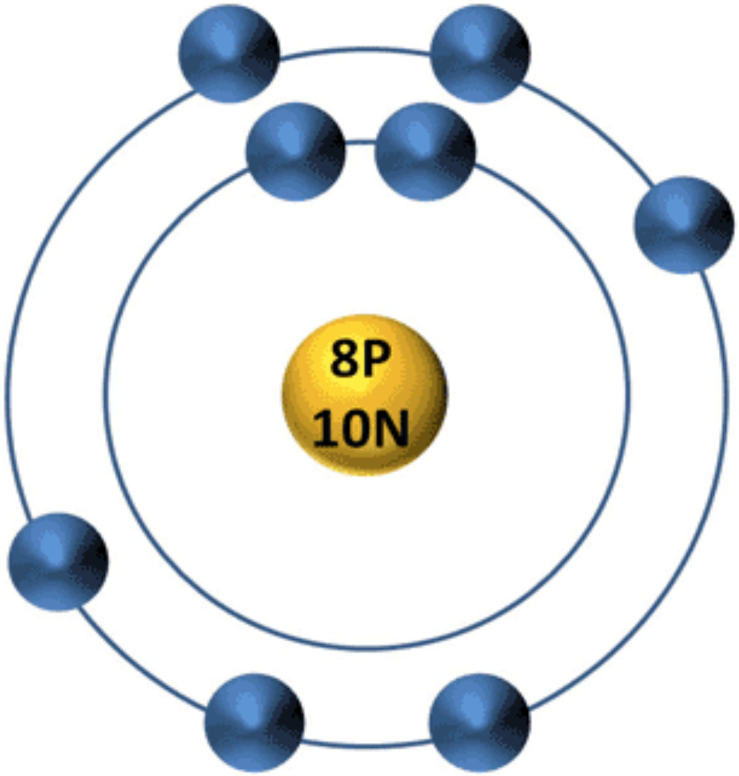
H_2O: $^1H^1HO$

lighter

Oxygen Stable Isotopes



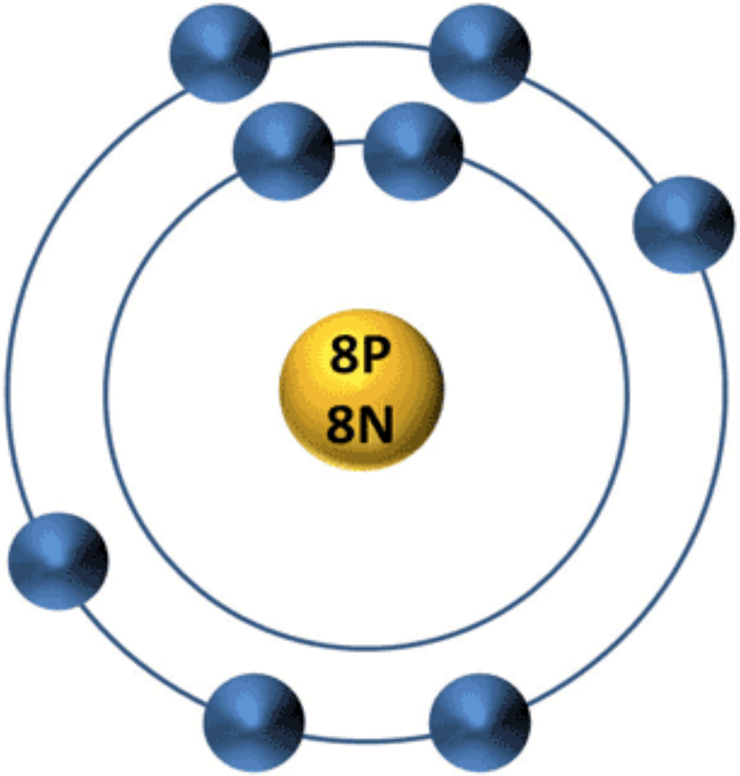
^{16}O Isotope



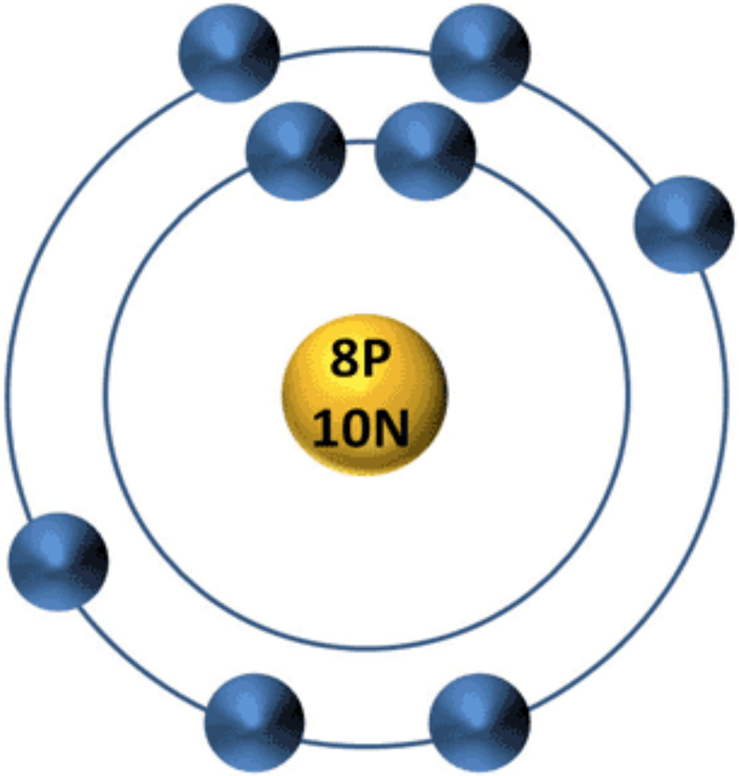
^{18}O Isotope

(www.ces.fau.edu)

Oxygen Stable Isotopes



^{16}O Isotope



^{18}O Isotope

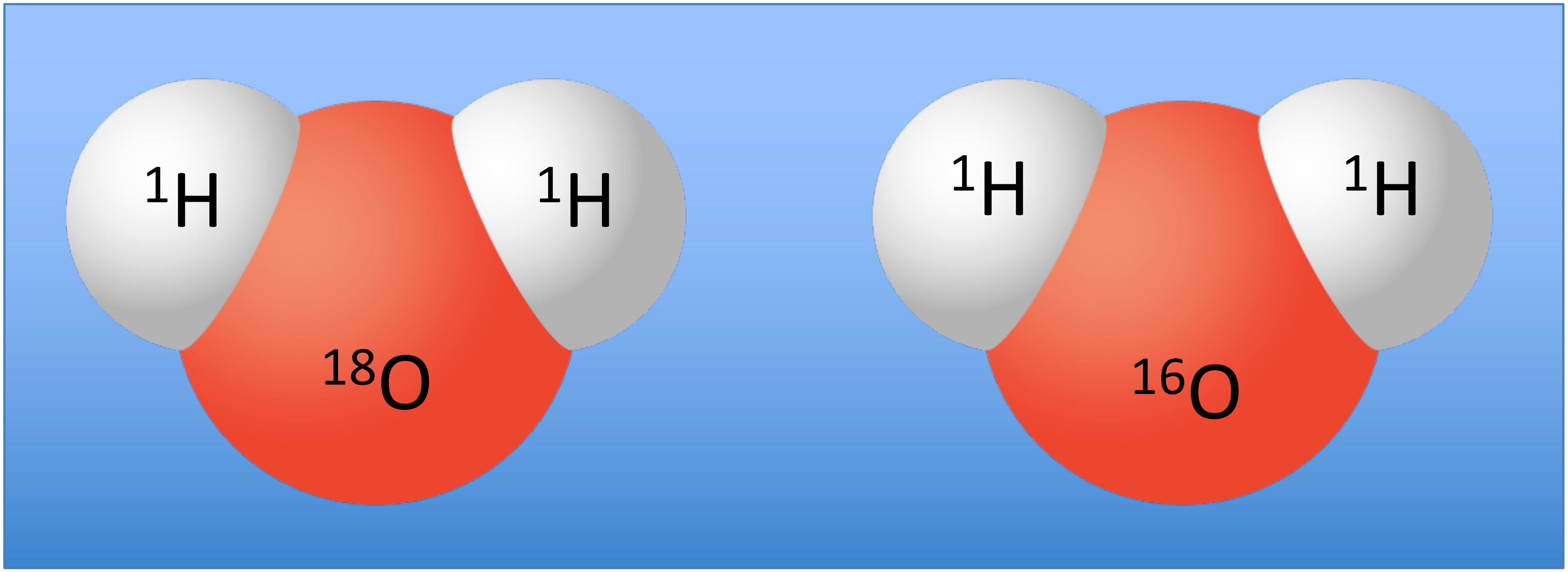
protons:	8 a.m.u.	8 a.m.u.
neutrons:	+ 8 a.m.u.	+ 10 a.m.u.
total mass:	16 a.m.u.	18 a.m.u.

Oxygen Stable Isotopes

$^{18}\mathrm{O}$ is heavier than $^{16}\mathrm{O}$

	$^{16}\mathrm{O}$	$^{18}\mathrm{O}$
protons:	8 a.m.u.	8 a.m.u.
neutrons:	+ 8 a.m.u.	+ 10 a.m.u.
total mass:	16 a.m.u.	18 a.m.u.

Stable Isotopes of O in H_2O



H_2O: $^{1}H^{1}H^{18}O$

heavier

evaporates less easily

0.2% of all water on Earth

H_2O: $^{1}H^{1}H^{16}O$

lighter

evaporates more easily

99.8% of all water on Earth

Stable Isotope Measurement Units

$H_2{}^{16}O$ is very common
99.8% of all water on Earth

$H_2{}^{18}O$ is very rare
0.2% of all water on Earth

$$\delta^{18}O\ (‰) = \left[\frac{\left(\frac{{}^{18}O}{{}^{16}O}\right)_{\text{sample}}}{\left(\frac{{}^{18}O}{{}^{16}O}\right)_{\text{standard}}} - 1 \right] \times 1{,}000$$

Higher values of $\delta^{18}O$ = more ^{18}O (heavier)
Lower values of $\delta^{18}O$ = less ^{18}O (lighter)
same for $\delta^{2}H$

Stable Isotope Measurement Units

Examples

$\delta^{18}O = -10‰$

$\delta^{18}O = -25‰$

$\delta^{2}H = -100‰$

$\delta^{2}H = -20‰$

Higher values of $\delta^{18}O$ = more ^{18}O (heavier)

Lower values of $\delta^{18}O$ = less ^{18}O (lighter)

same for $\delta^{2}H$

Stable Isotope Measurement Units

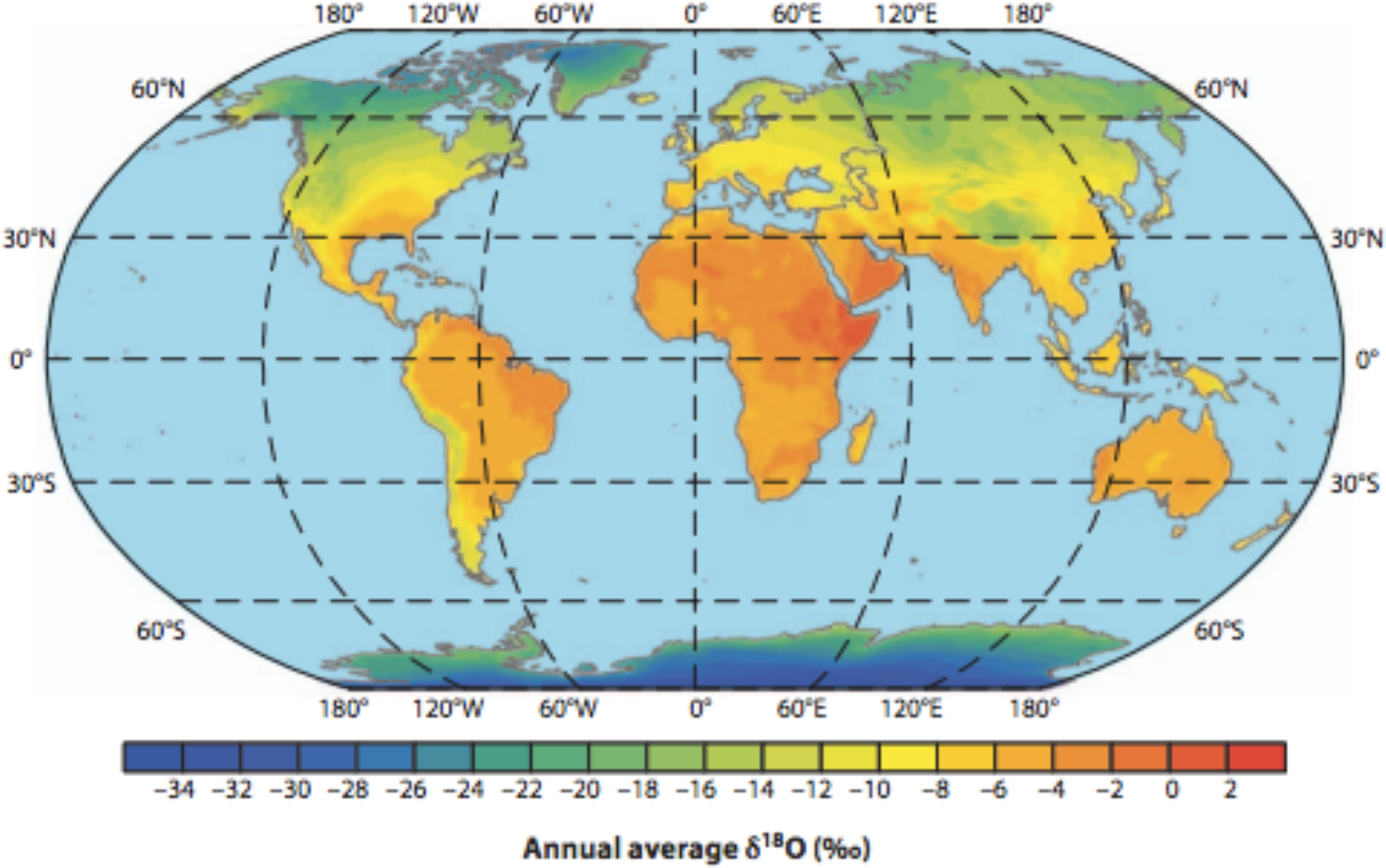
Examples

$\delta^{18}O$ = -10‰ (higher value)
$\delta^{18}O$ = -25‰ (lower value)

$\delta^{2}H$ = -100‰ (lower value)
$\delta^{2}H$ = -20‰ (higher value)

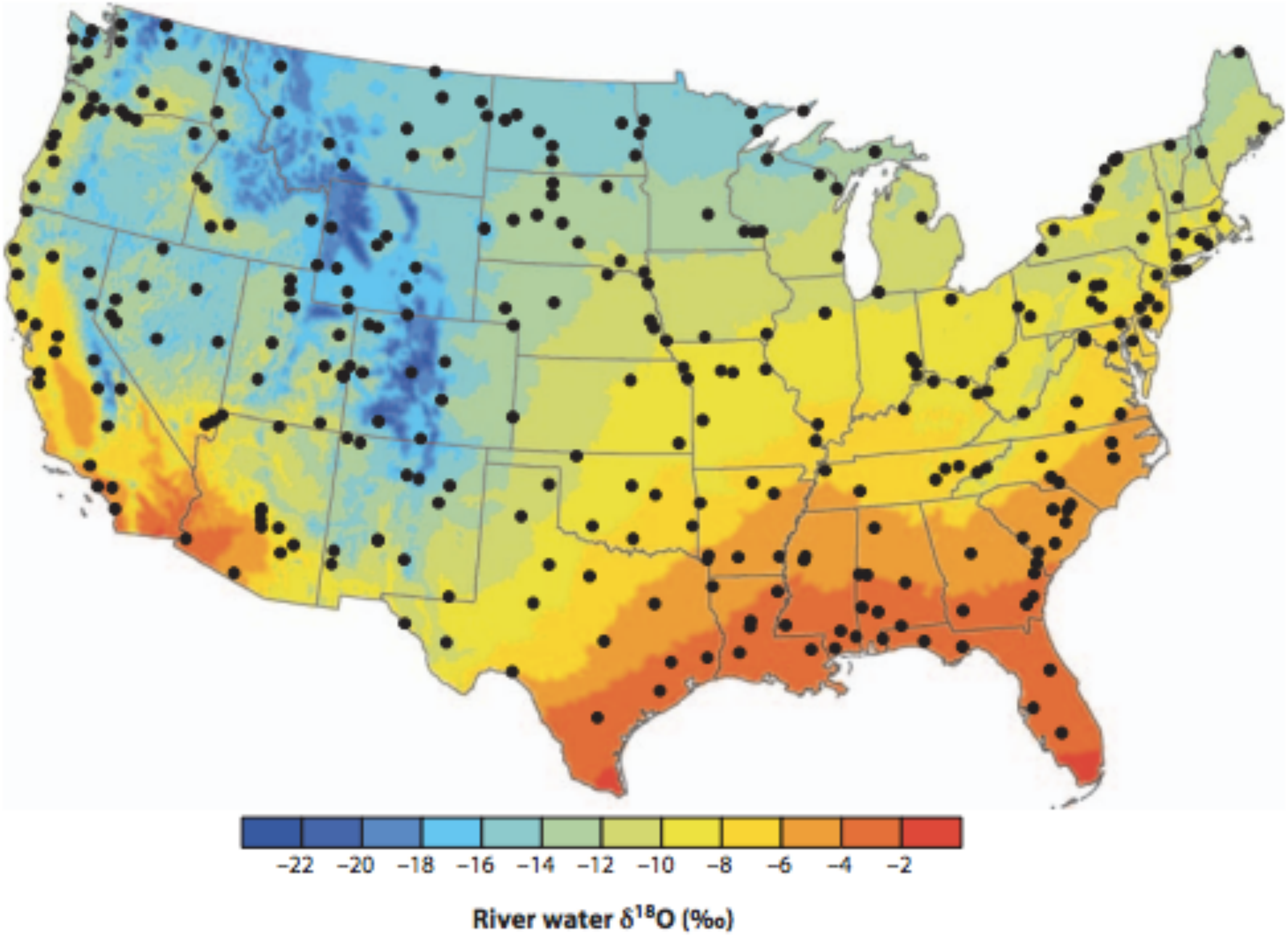
Higher values of $\delta^{18}O$ = more ^{18}O (heavier)
Lower values of $\delta^{18}O$ = less ^{18}O (lighter)
same for $\delta^{2}H$

Map of $\delta^{18}O$ in H_2O



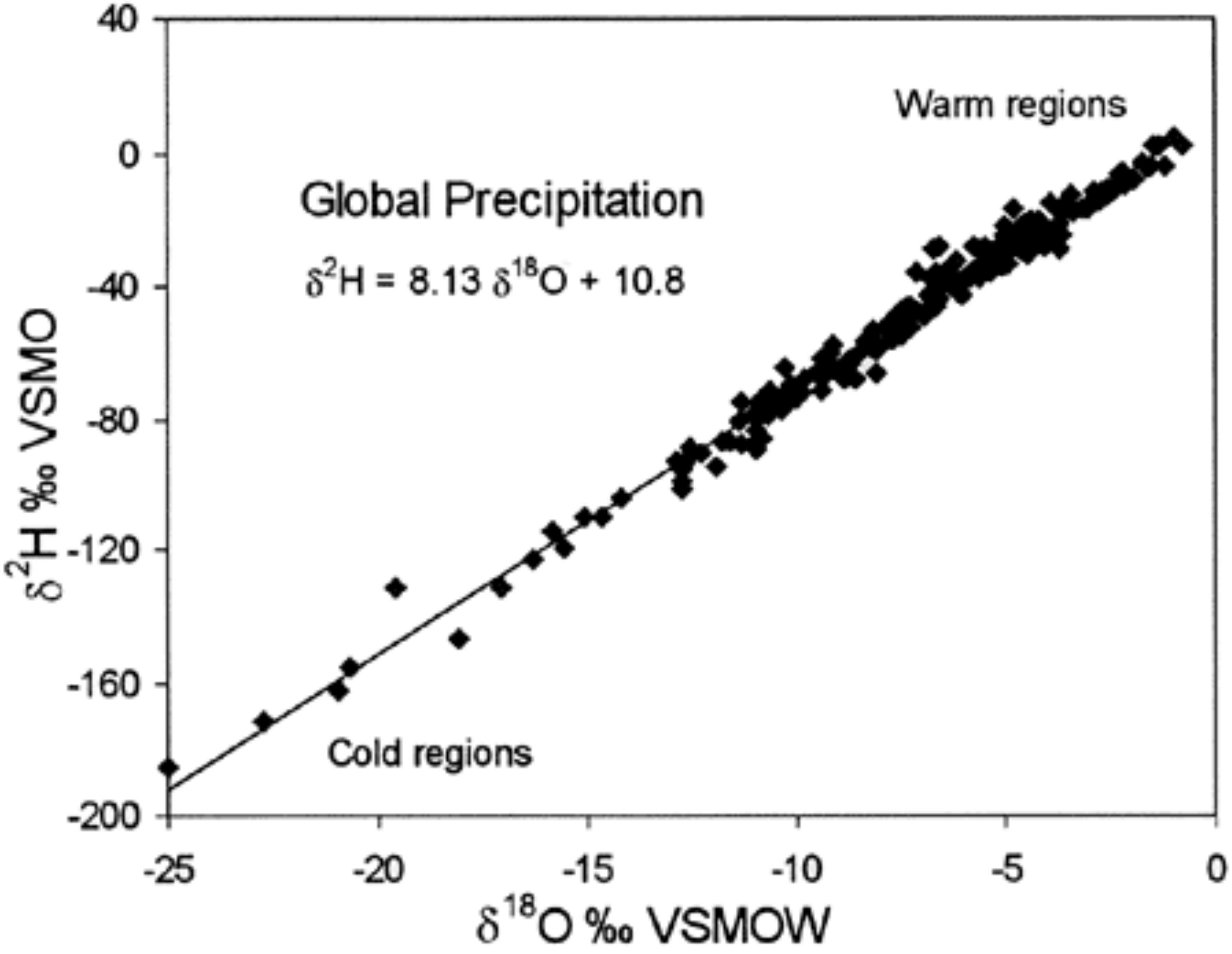
(Bowen, 2010, *Ann. Rev. EPS*)

Map of $\delta^{18}O$ in H_2O



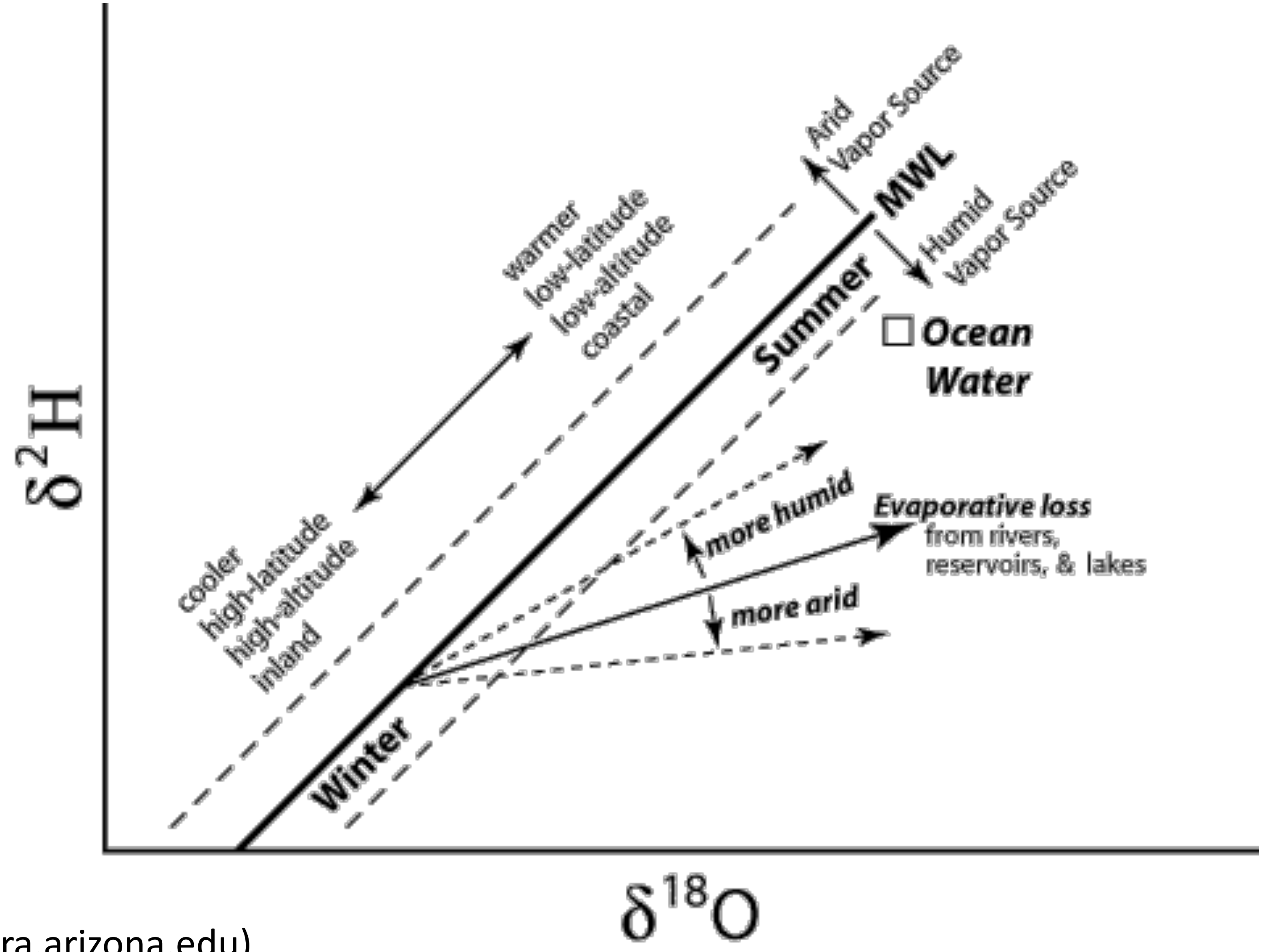
(Bowen, 2010, *Ann. Rev. EPS*)

Relationship of δ^2H and $\delta^{18}O$



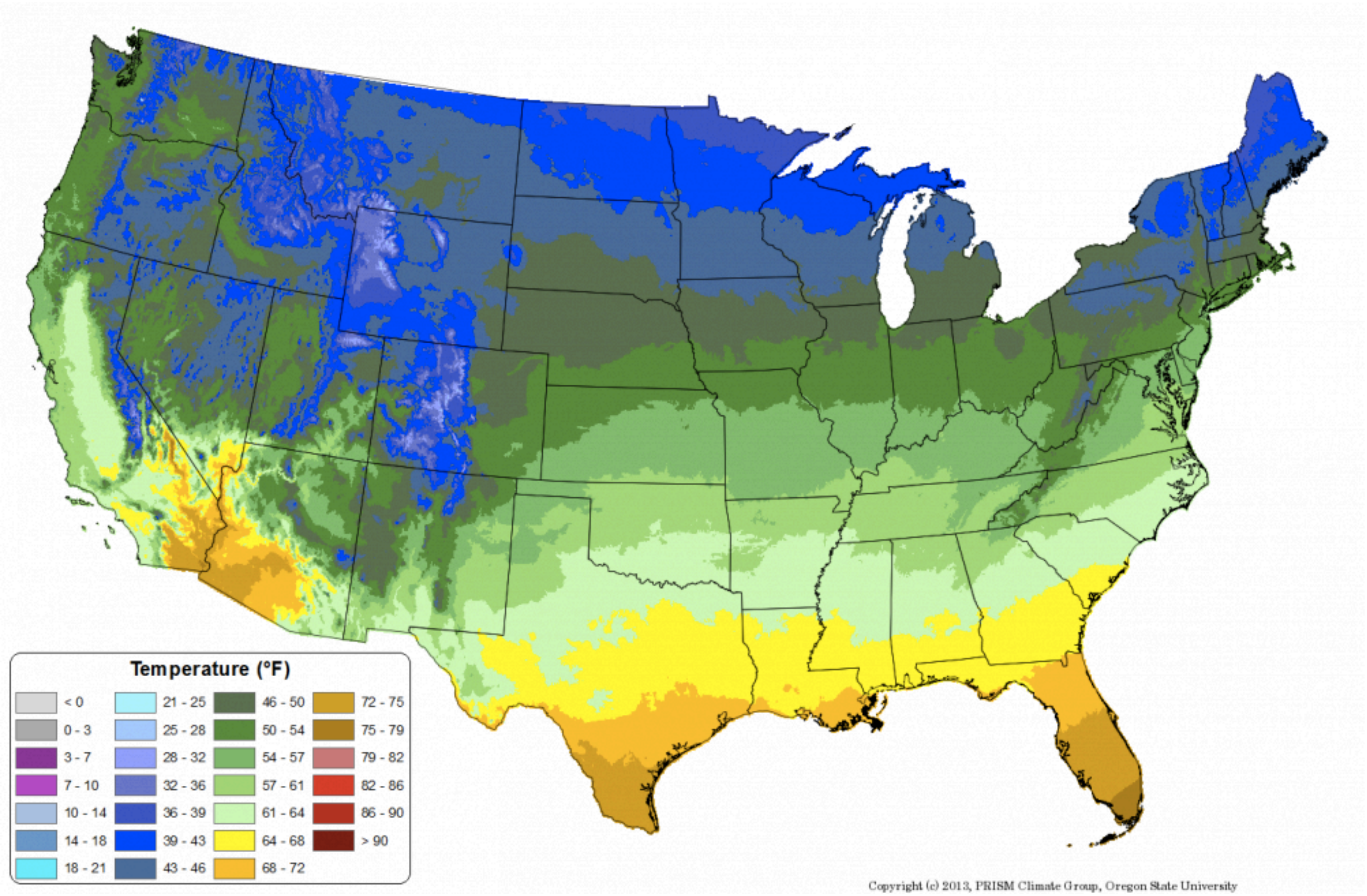
(sahra.arizona.edu)

Relationship of δ^2H and $\delta^{18}O$



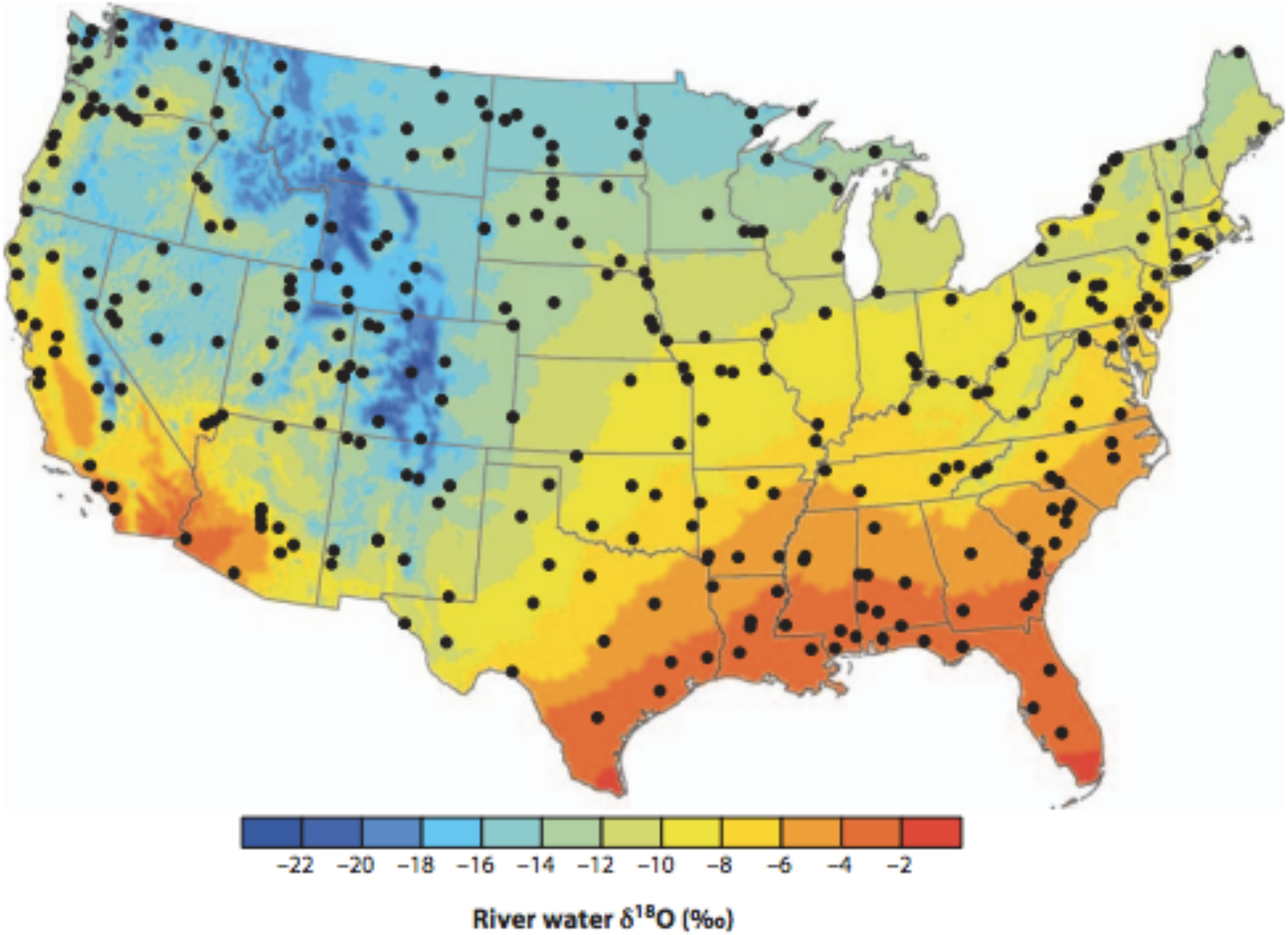
(sahra.arizona.edu)

Map of Average Annual Temperature



(www.ucar.edu)

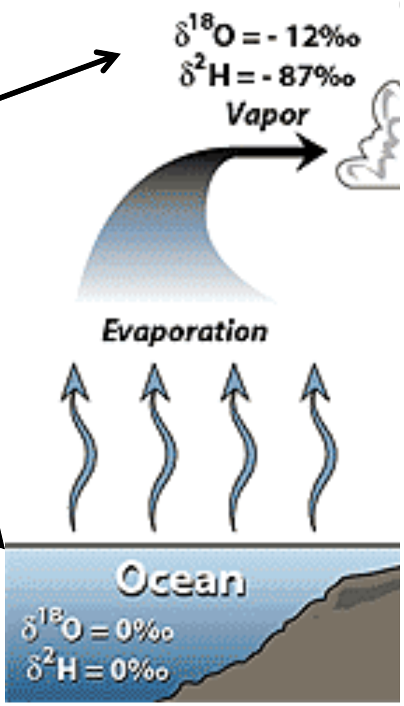
Map of $\delta^{18}O$ in H_2O



(Bowen, 2010, *Ann. Rev. EPS*)

Effects of Temperature

For $\delta^{18}O$: difference between liquid and vapor of water is -12‰ at 1° C (35° F),
and is -8.3‰ at 38° C (100° F)

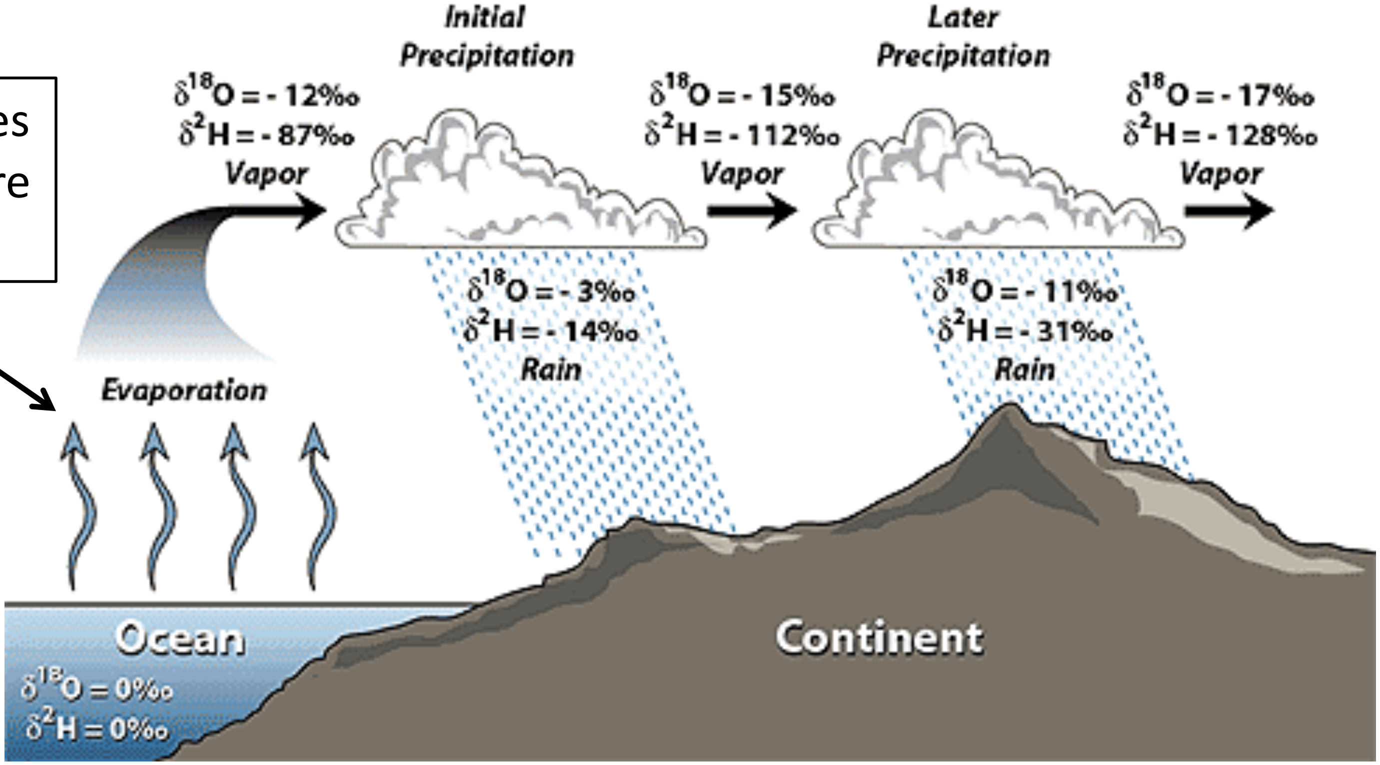


Bigger isotope effect at colder temperatures

(sahra.arizona.edu)

Effects of Temperature

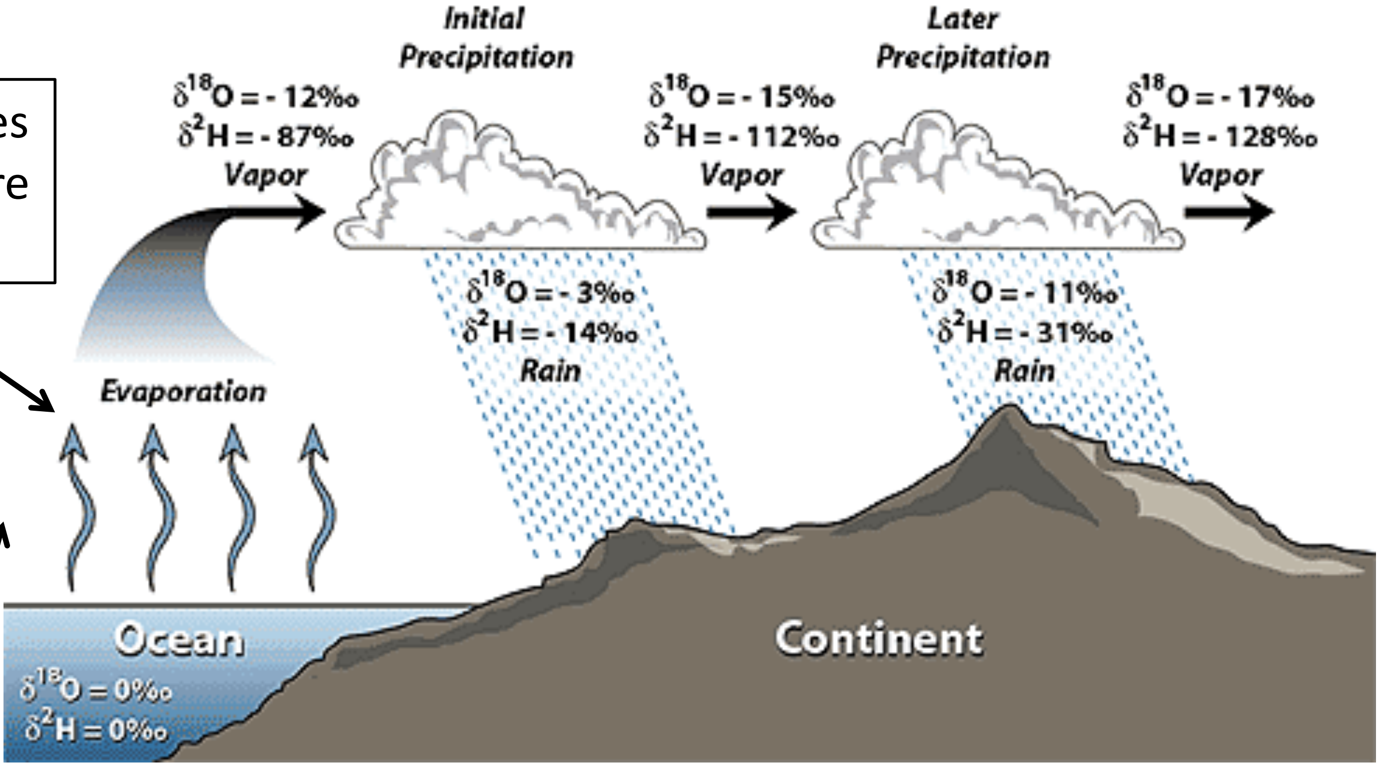
Lighter isotopes evaporate more easily



(sahra.arizona.edu)

Effects of Temperature

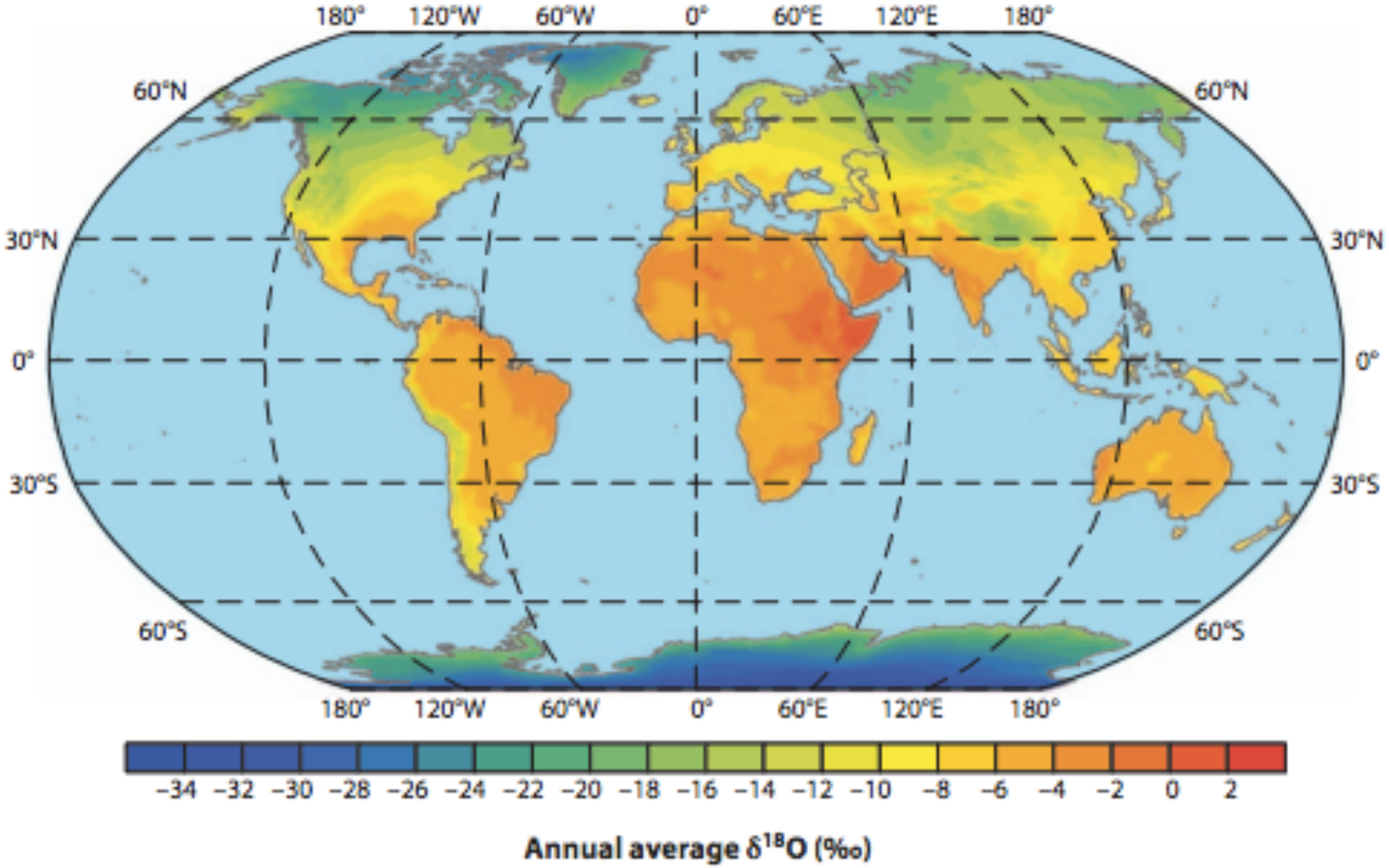
Lighter isotopes evaporate more easily



In cold regions, there is much less energy to drive evaporation, so the lighter isotopes are really at an advantage

(sahra.arizona.edu)

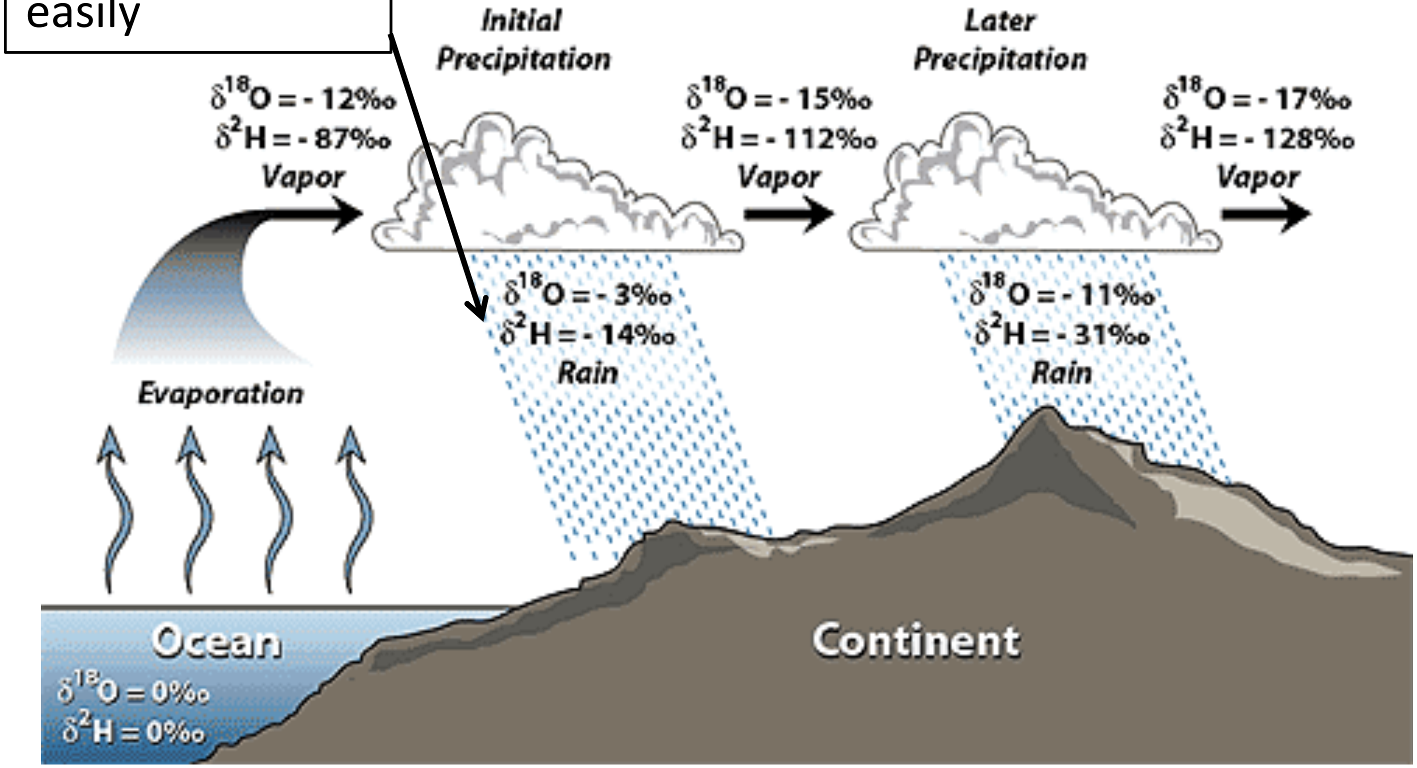
Map of $\delta^{18}O$ in H_2O



(Bowen, 2010, *Ann. Rev. EPS*)

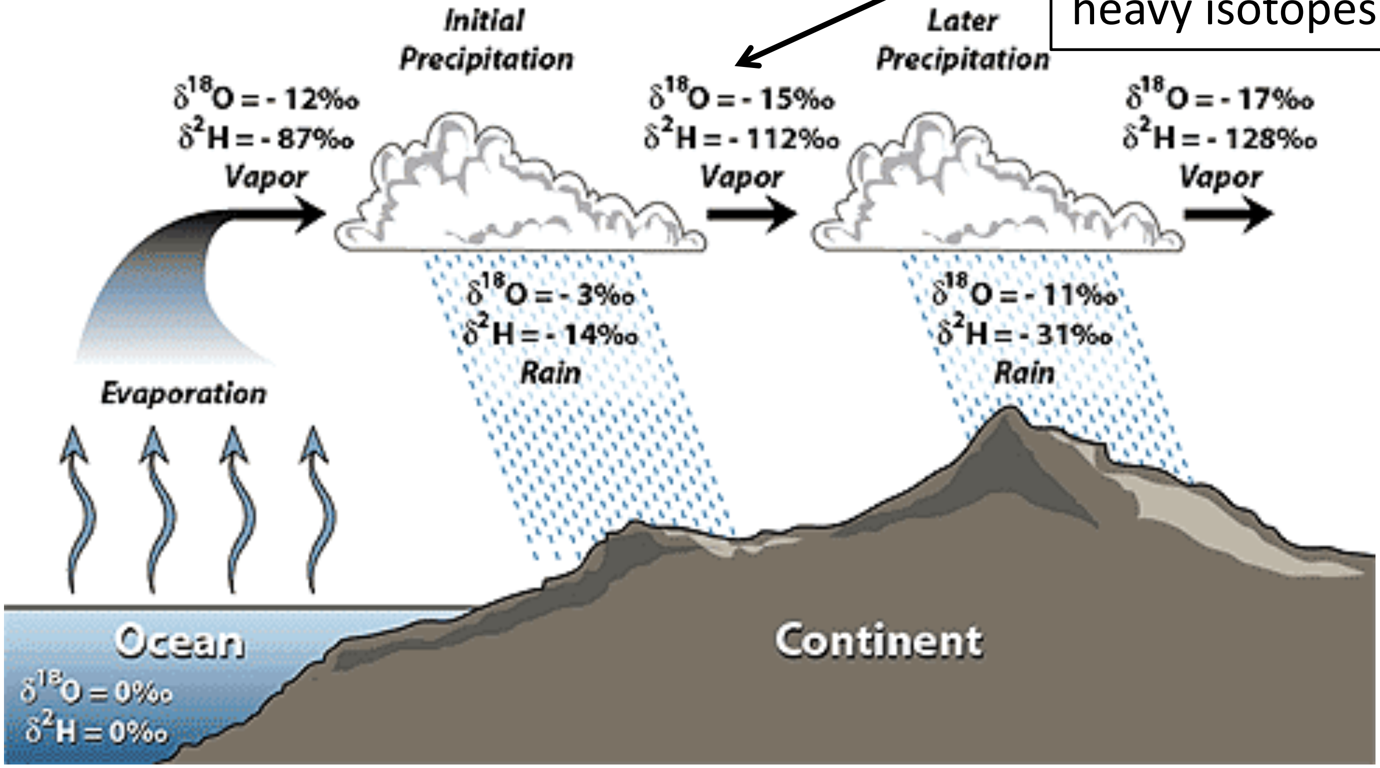
Effects of Topography and Distance

Heavier isotopes condense more easily



(sahra.arizona.edu)

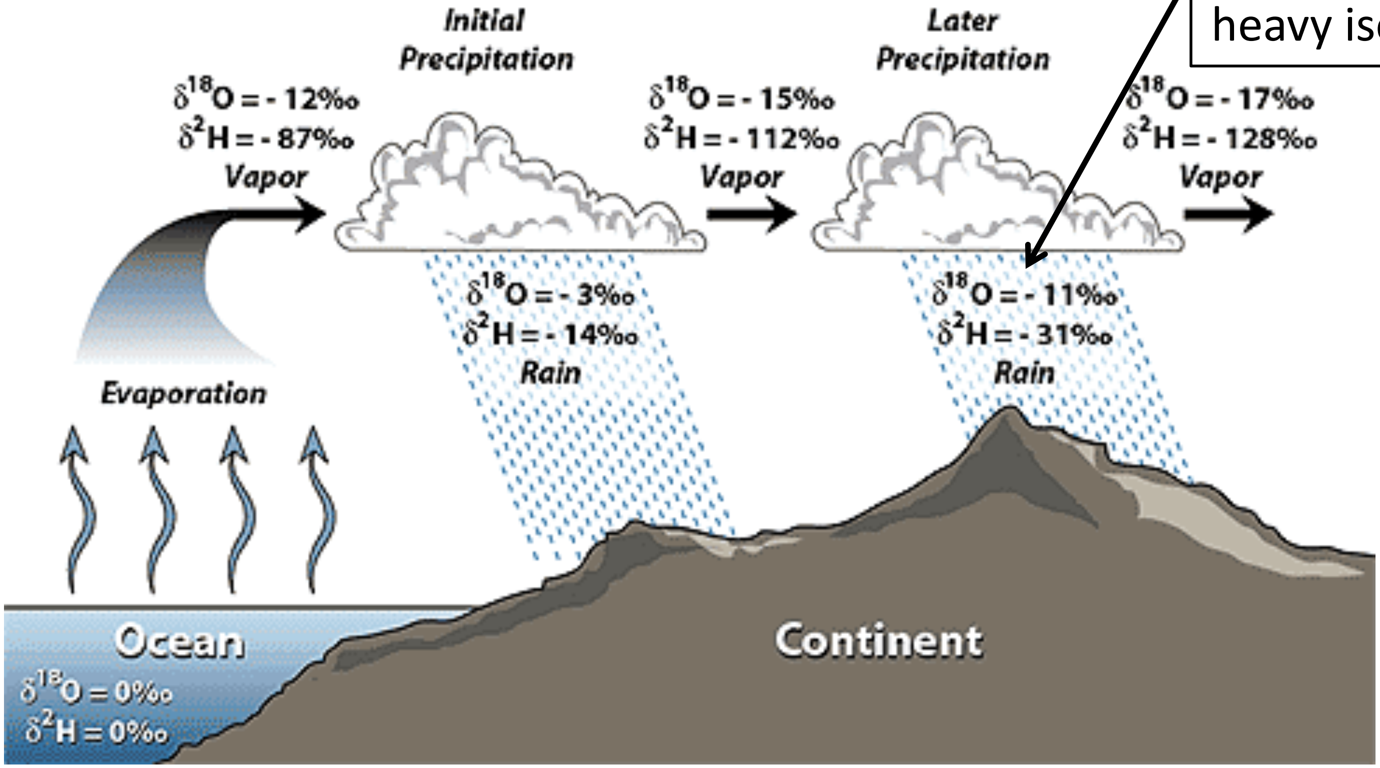
Effects of Topography and Distance



Resulting clouds have less of the heavy isotopes left

(sahra.arizona.edu)

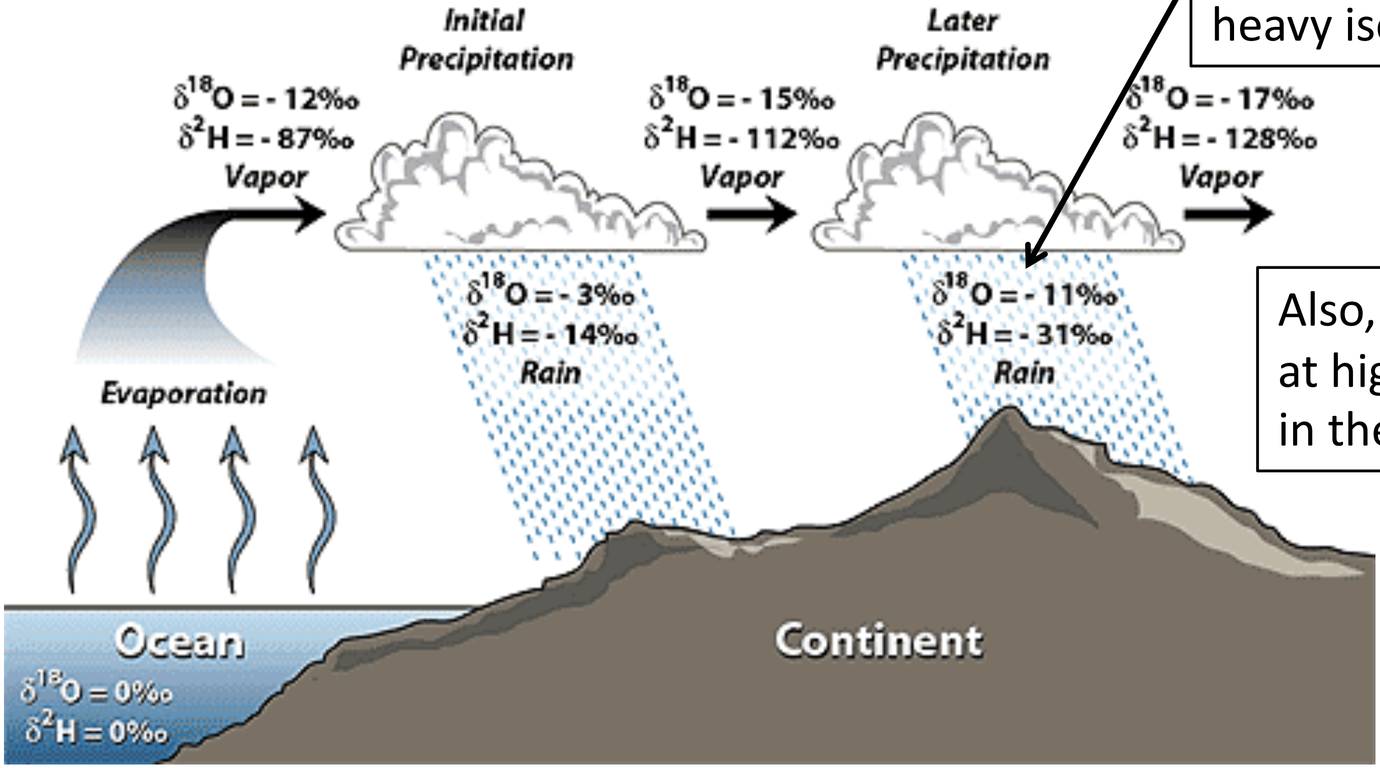
Effects of Topography and Distance



Resulting rain has less of the heavy isotopes

(sahra.arizona.edu)

Effects of Topography and Distance

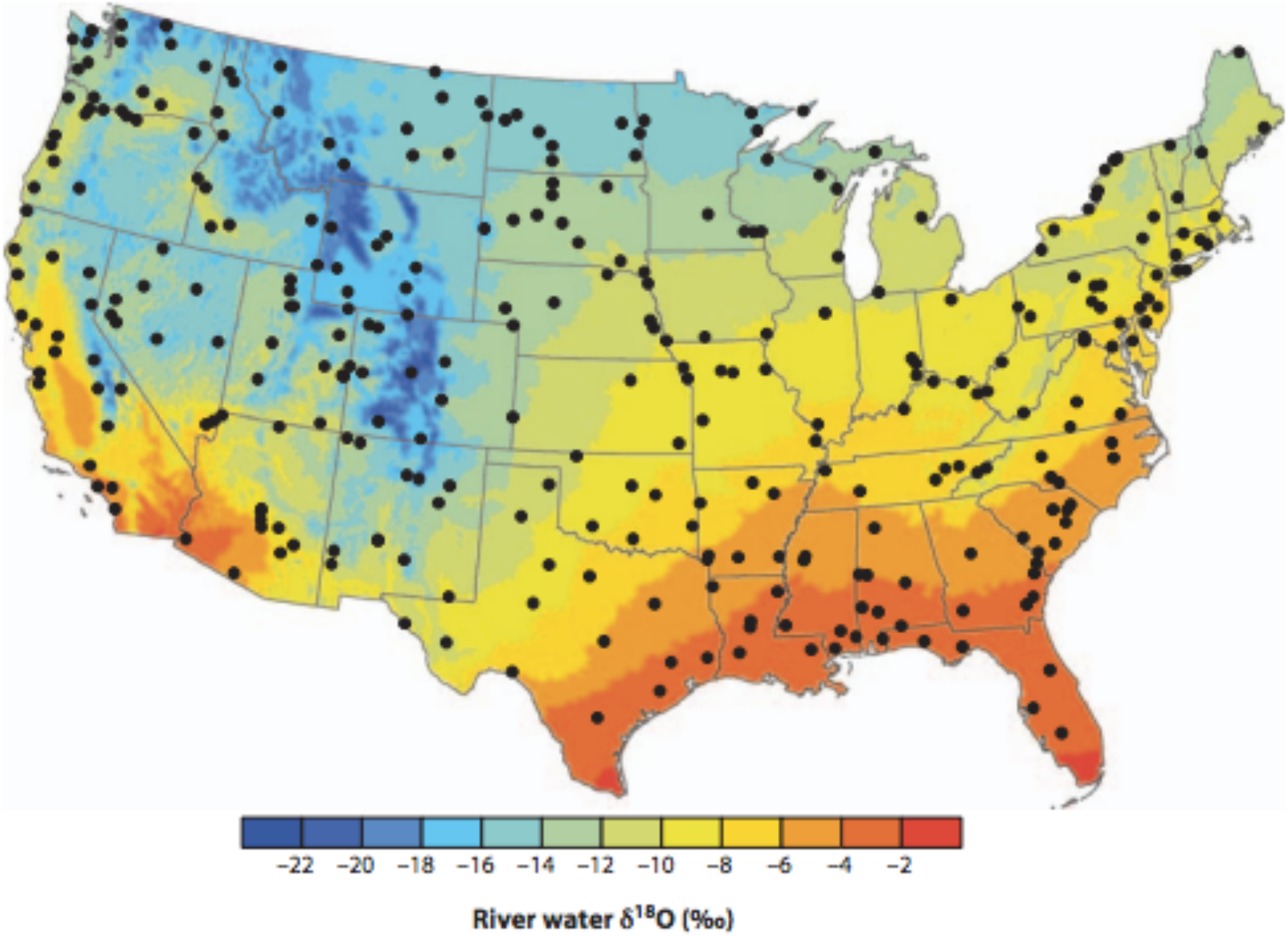


Resulting rain has less of the heavy isotopes

Also, it is colder at high elevations in the mountains

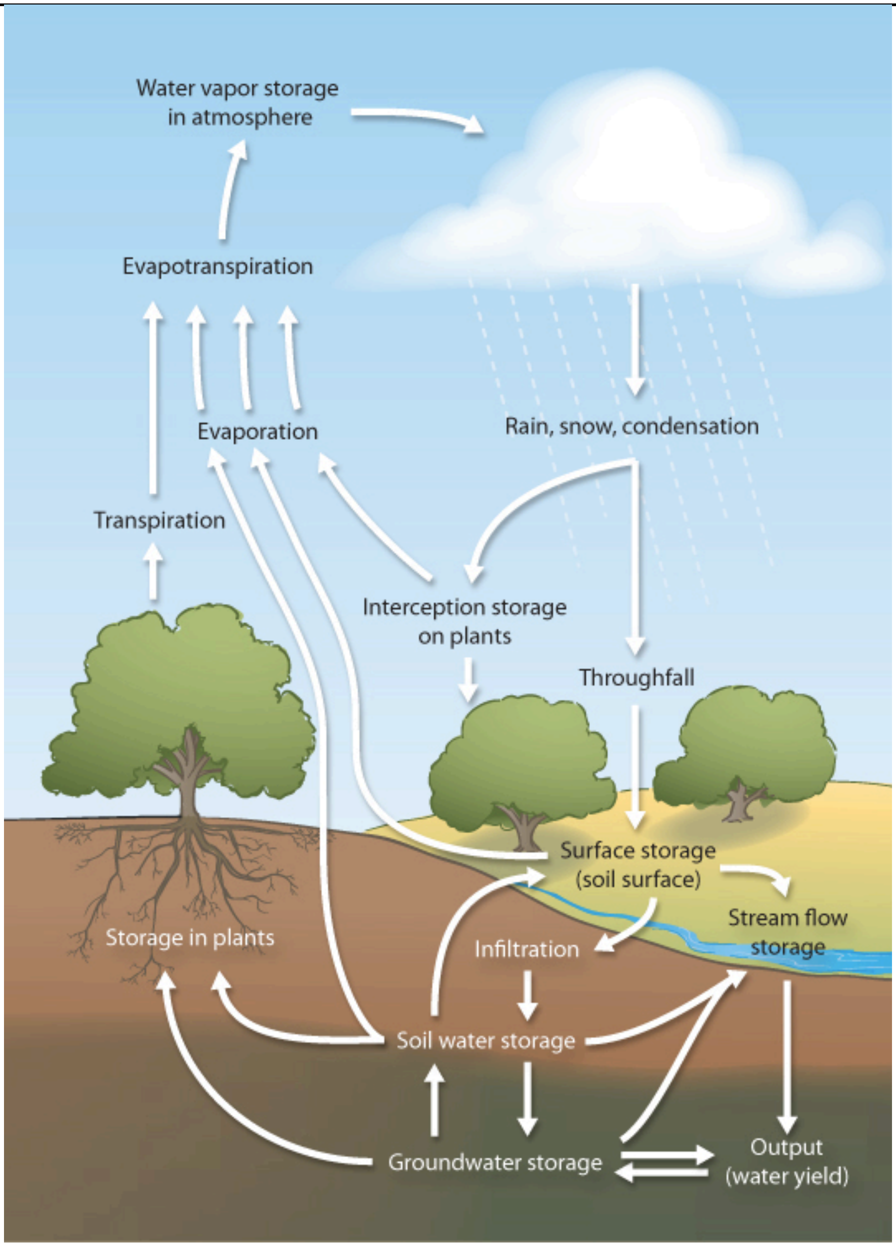
(sahra.arizona.edu)

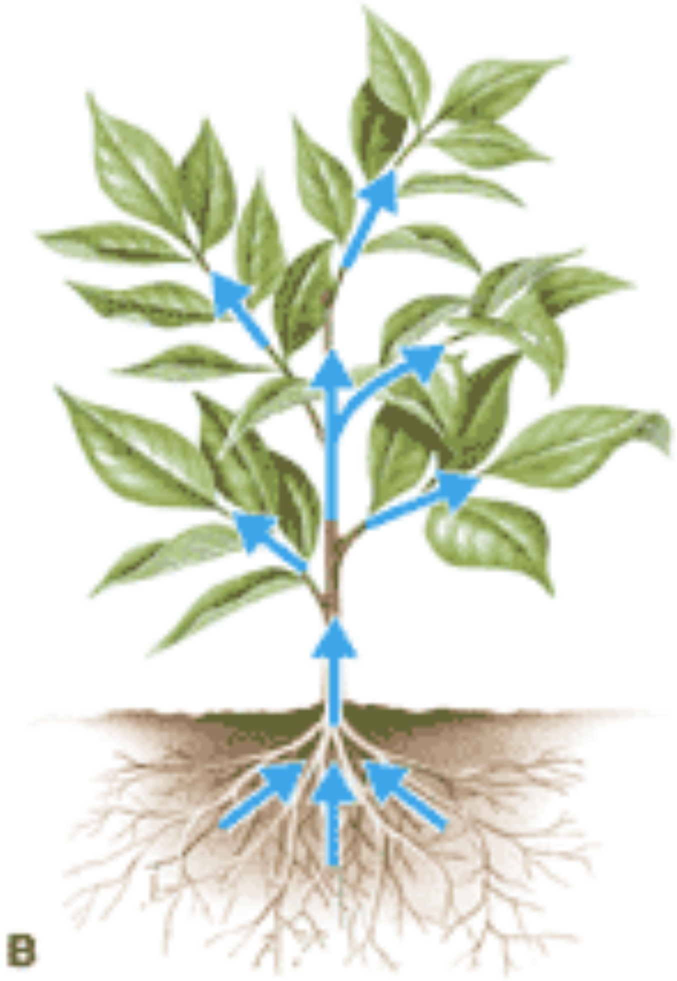
Map of $\delta^{18}O$ in H_2O



(Bowen, 2010, *Ann. Rev. EPS*)

Water Cycle: Soil → Plant → Fruit





(www.ucanr.edu)

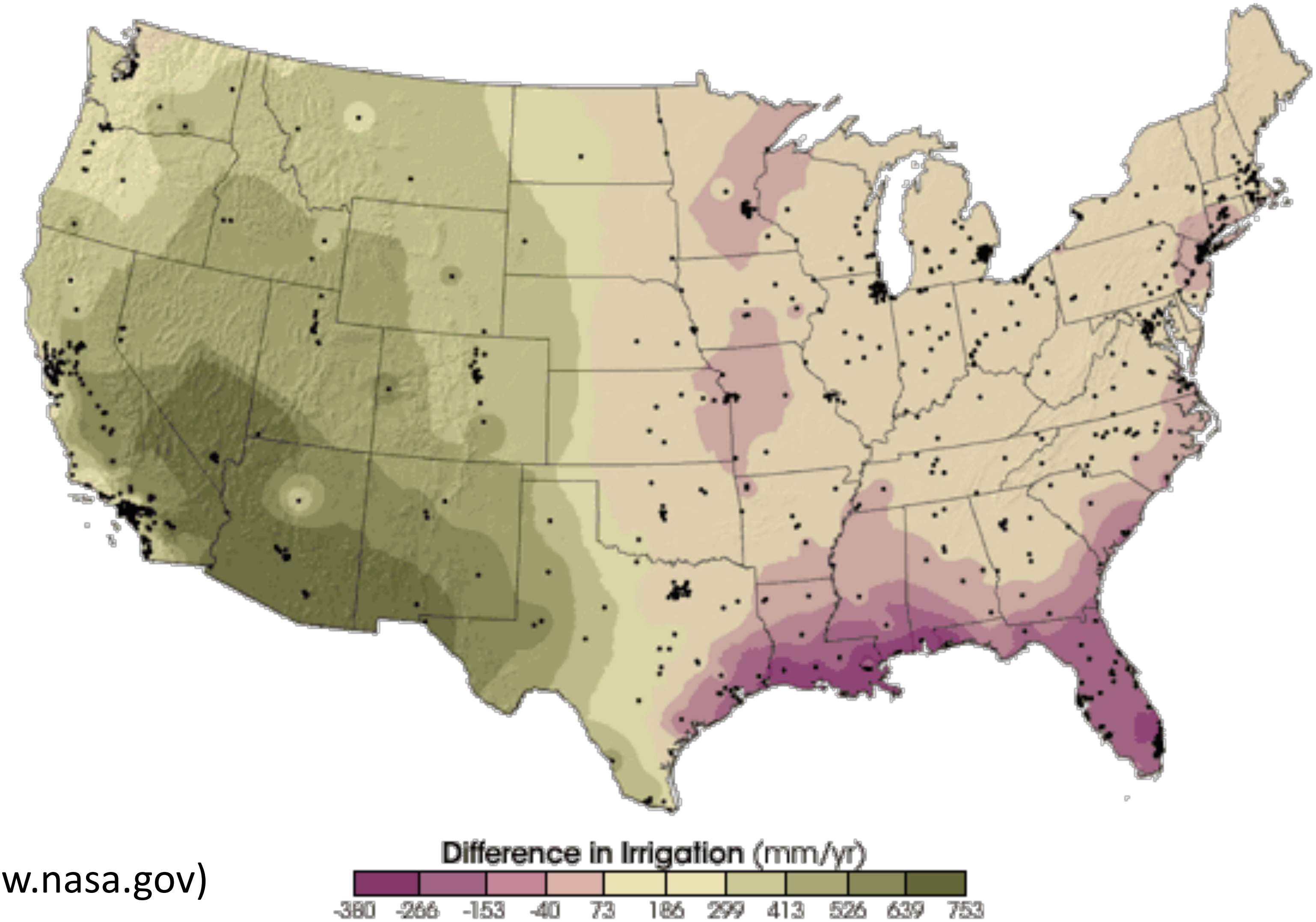
Water in Fruits and Vegetables

Fruit or Vegetable	Water Content
cucumber and lettuce	96%
zucchini and celery	95%
tomato	94%
watermelon	92%
grapefruit and cantaloupe	91%
apple and pear	84%
potato	79%
banana	74%

(Bowes and Church, 1994)

Water for Crops: Irrigation

This map shows the difference between precipitation and irrigation



(www.nasa.gov)

Research Question:

Can we use the stable isotopes of hydrogen and oxygen in water as "fingerprints" to determine where food comes from?

Food Origin: Compare Food to Rain

1) Measure food water isotopes and temperature
2) Convert the vapor isotope value to liquid isotope value with the temperature-dependent fractionation factor
3) Plot the liquid isotope values on a graph of $\delta^{2}H$ vs. $\delta^{18}O$
4) Fit a line through the food water isotope data
5) Find the intersection of the food line with the GMWL
   This intersection is the water the food was grown with
6) Compare the source water to maps of rain water
7) Places where the two match are likely source areas where the food was grown

Supplementary Material File 2. Stable isotope hydrology and fruit water sourcing computer laboratory exercise.

Stable Isotopes in the Water Cycle and in Our Food

Introduction

The Water Cycle is the idea that all of the water on Earth is continuously moving from various storage areas ("reservoirs") through different pathways ("fluxes") in a continuous cycle. Examples of reservoirs are the oceans and the water that is in the atmosphere (think of clouds), and examples of fluxes are water that is evaporating from the ocean surface or water that is falling as rain from clouds. The total amount of water on Earth stays the same, but the particular amount of water in each flux or reservoir changes through time.

One way that we can track and measure how water is moving through the water cycle is by using the stable isotopes of hydrogen and oxygen in the water molecule as a tracer. Stable isotopes are atoms of the same element that have a different number of neutrons in their nucleus, but the same number of protons. For example: oxygen-16 (^{16}O) has 8 protons and 8 neutrons in its nucleus, while oxygen-18 (^{18}O) has 8 protons and 10 neutrons in its nucleus. As a result, ^{18}O is a little bit heavier than ^{16}O and so it behaves differently. Likewise, a water molecule (H_2O) with an ^{18}O atom is heavier than another water molecule with a ^{16}O. The hydrogen in H_2O also occurs as different stable isotopes (^{1}H and ^{2}H), but let's concentrate on the oxygen isotopes for now.

As a result of these differences in weight, or mass, the water molecule with the ^{18}O will be a little bit harder to evaporate from the ocean or a lake, as compared to a water molecule with a ^{16}O. This difference in evaporation behavior is largest at colder temperatures (because there is less energy to drive the process of evaporation) than at warmer temperatures (warmer = more energy). These temperature effects mean that in cold regions (like closer to the north and south poles and at high altitudes in the mountains) the clouds that form from this evaporation of ocean water, and the resulting rain and snow that falls from these clouds will have less ^{18}O. In contrast, warmer areas (near the equator or at lower altitudes) precipitation will have more ^{18}O.

Because of these geographic differences in the stable isotopes of oxygen and hydrogen in rain, we could take a sample of water from an unknown location (perhaps from a bottle of water we bought at the store), analyze its O and H stable isotope composition on an instrument called a mass spectrometer (which measures the weight of each molecule), and compare the result to maps of the O and H stable isotope composition of rain. If we found a place where the stable isotope results match, we could say that the water sample likely came from that location.

We can extend this concept of using the stable isotopes of O and H as a water tracer to determining the origin of fruits (and vegetables) because these crops incorporate the rain that falls on their soil (or irrigation water sprayed on them) into the fruit as it grows. Similarly to the example of the bottled water sample, we could determine the geographic area of where a fruit was grown by comparing the stable isotopes of O and H in the water inside the fruit to maps of rain water O and H stable isotopes.

Step 1) Determine the rain or irrigation water used to grow the food

Earlier today, we demonstrated how the stable O and H isotope composition of fruit water can be measured by using water vapor probes attached to a mass spectrometer. To review, the food item is placed in a plastic bag and cut in half with a knife. The vapor probe is placed between the two halves of the food item so that there is good contact between the food and the probe. A thermometer probe is inserted into an accessible part of the food item and the bag is closed around the probes as well as possible with a flexible tie. After approximately one minute, the water vapor from the food item has travelled through the probe, is received at the mass spectrometer, and data for the O and H stable isotope values begin to read out on the display screen. Once the data values have become constant for approximately two minutes, the values are written down along with the food temperature reading. The vapor probe is then removed from the food item and replaced with a fresh vapor probe for the next measurement.

Remember that the effect of temperature on the O and H stable isotope values ($\delta^{18}O$ and $\delta^{2}H$) is important, so the next step is to use the temperature of the food to convert the data that we measured (which was from water vapor) to the equivalent stable isotope value of the liquid water that produced the vapor. We do this by adding the temperature dependent fractionation factor to the measured $\delta^{18}O$ and $\delta^{2}H$ vapor values. Below is an example for a fruit measured at 21° Celsius (room temperature) using oxygen stable isotope values ($\delta^{18}O$):

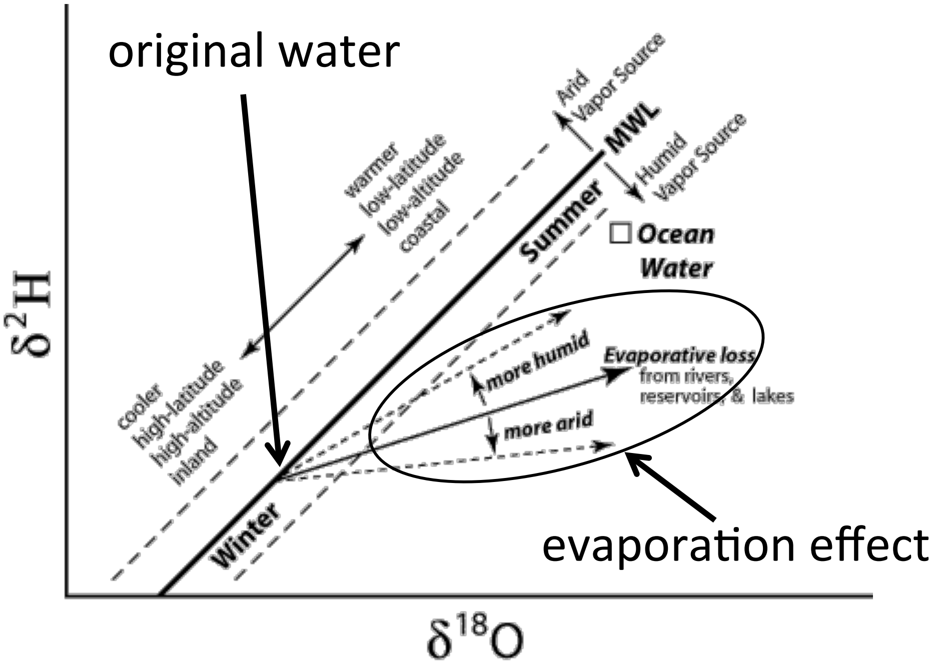
$$\delta^{18}O_{vapor} + \text{fraction factor} = \delta^{18}O_{liquid}$$

$$-14.6‰ + 9.6‰ = -5‰$$

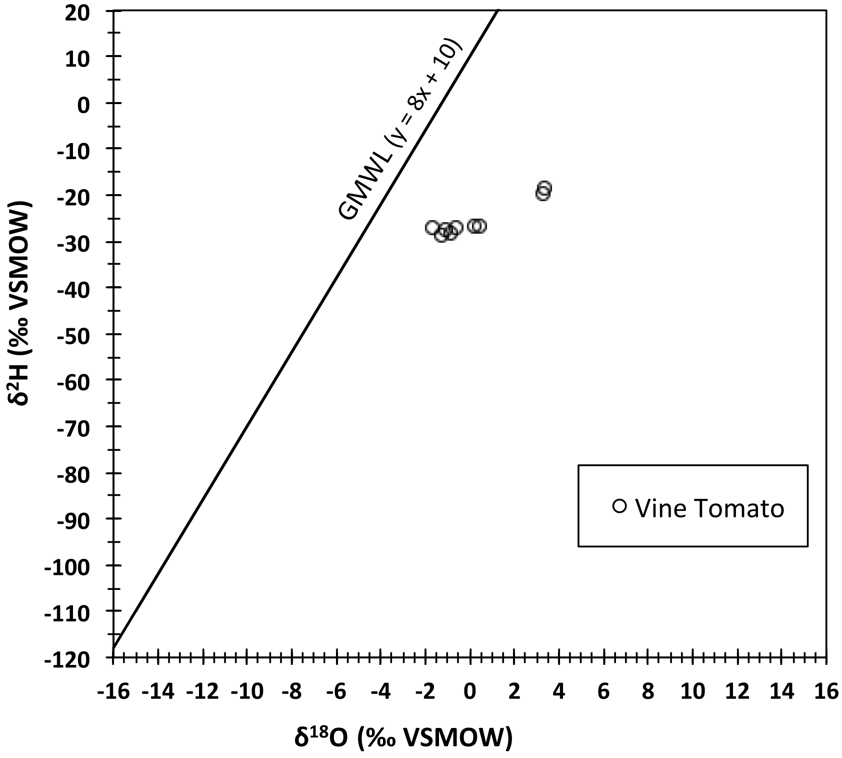
So, in the above example, we measured the $\delta^{18}O$ value of the vapor inside the fruit to be -14.6‰ and converted it to the liquid water $\delta^{18}O$ value using the fraction factor of 9.6‰.

It is important that we measure several (at least three) separate pieces of the same type of food item. This is because the water inside fruits and vegetables will always be a little bit evaporated compared to the rain or irrigation water that fell on them due to several reasons:

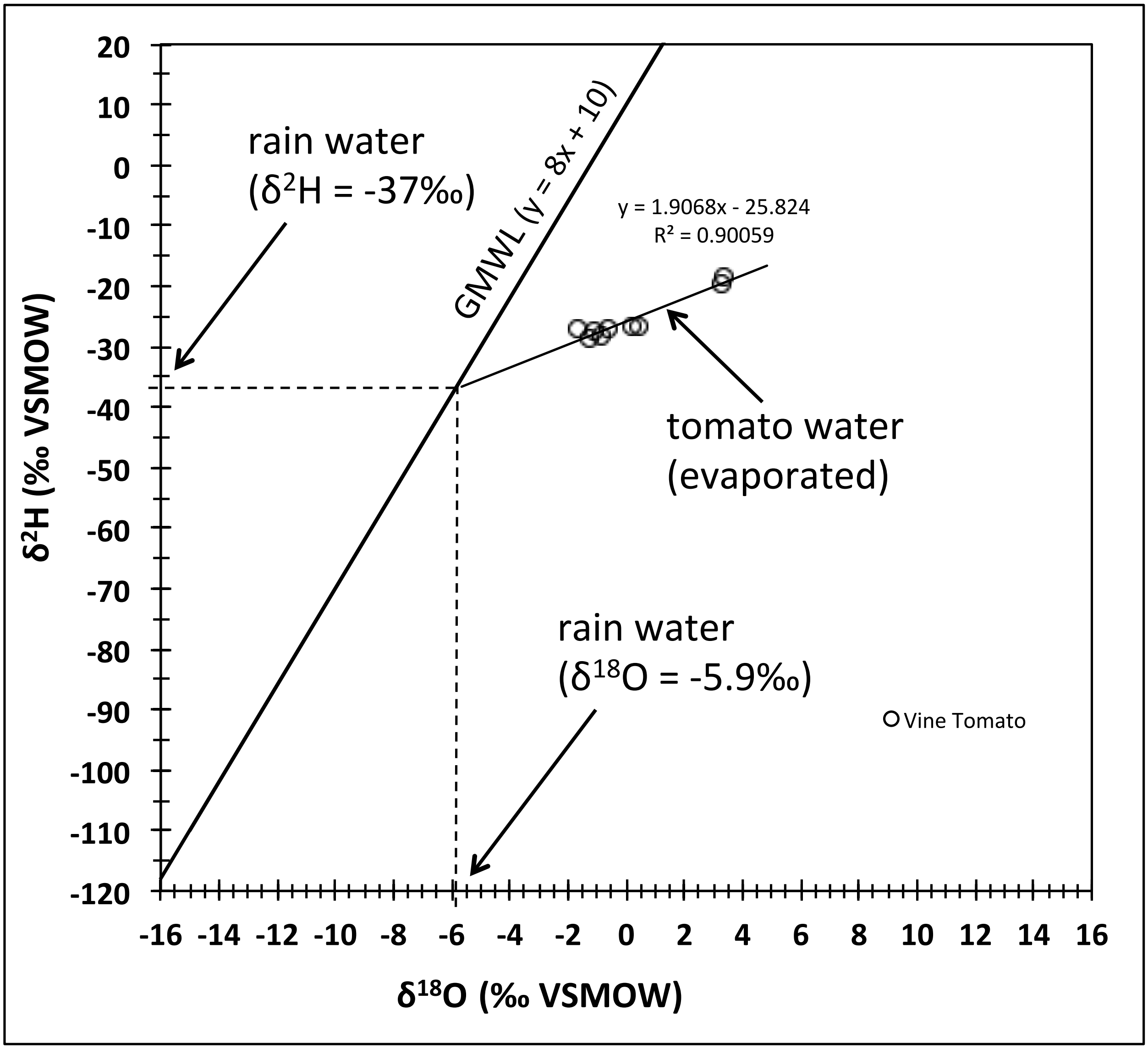
1) The water that plants use is really from the soil, not from the rain that falls on the leaves. Although soil water is sourced from rain or irrigation, it undergoes evaporation as it percolates into the soil and before the plant can use it all up (in between rain storms, for example). As water evaporates, the oxygen stable isotopes are affected differently than the hydrogen, and so the relationship between $\delta^{2}H$ and $\delta^{18}O$ values in the soil water are different than in the original rain water (see figure below).



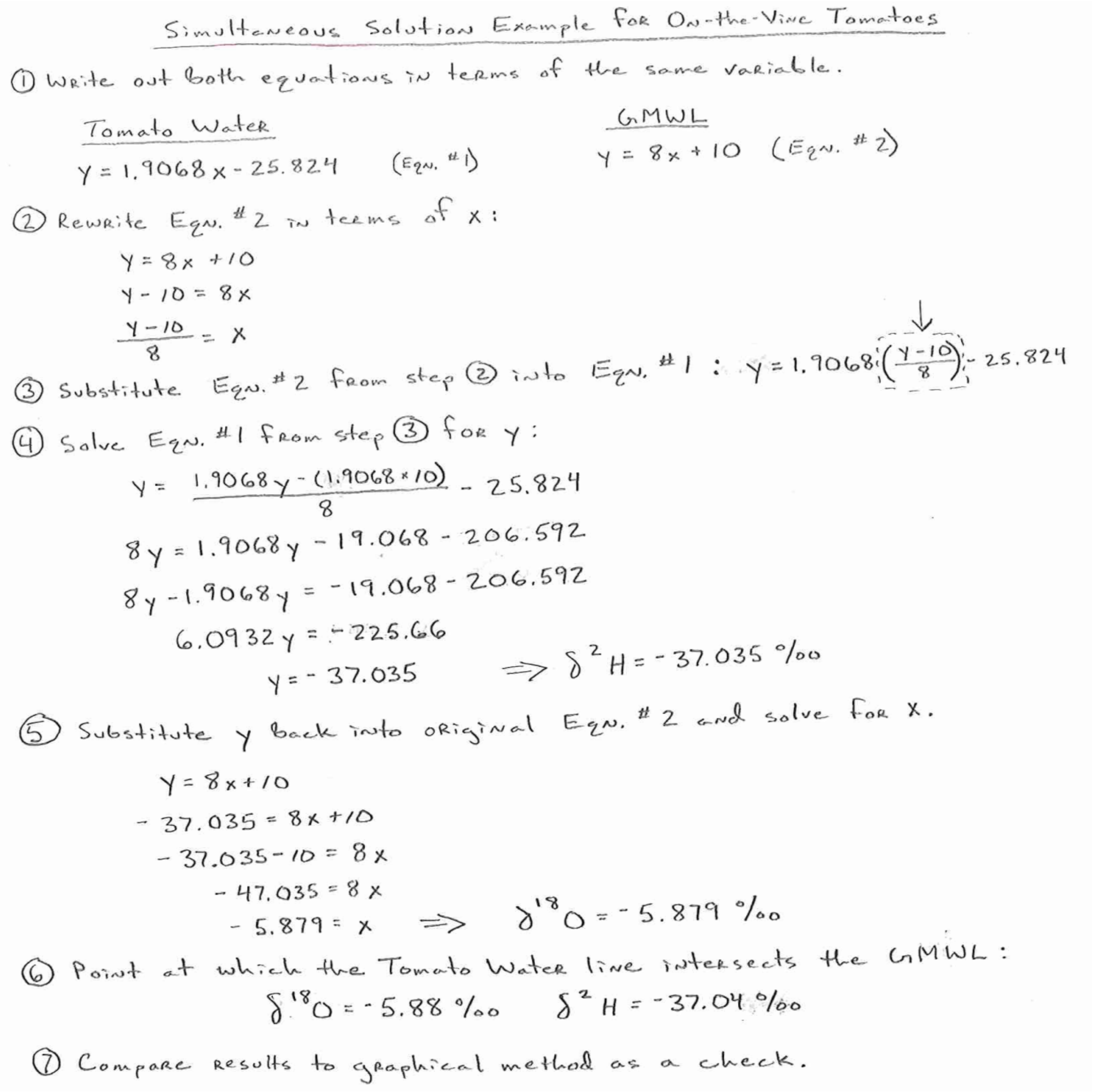
2) By measuring several pieces of fruit, we can get independent values of δ^{18}O and δ^{2}H for each piece, and plot the data on a graph that allows us to compare the fruit water to rain water. In the figure below, δ^{18}O and δ^{2}H values for 9 different On-the-Vine Tomatoes are plotted, as well as the Global Meteoric Water Line (GMWL), which shows the relationship between δ^{18}O and δ^{2}H values in rainwater worldwide.



Notice how the tomato water plots to the right of the GMWL. First, we fit a line through the tomato data (using a linear regression method that is found in many spreadsheet software programs) that shows the trend of the relationship between $\delta^{18}O$ and $\delta^{2}H$ values of the tomato water. Then, we simply extend the regression line back to where it intersects the GMWL, and we can see that the tomato water evaporated from an original rainwater composition of $\delta^{18}O$ = 5.9‰ and $\delta^{2}H$ = 37‰.



This way of determining the original water stable isotope composition is called a graphical method. This graphical method is quick and easy, and gives a fairly good idea of the original water $\delta^{18}O$ and $\delta^{2}H$ values. A more precise method is to use an algebra technique called Simultaneous Solution of Equations, where the exact intersection of the fruit water (evaporation) line with the GMWL is calculated. Here is an example of the Simultaneous Solution method:



Simultaneous Solution Example for On-the-Vine Tomatoes

① Write out both equations in terms of the same variable.

Tomato Water

$y = 1.9068x - 25.824$ (Eqn. #1)

GMWL

$y = 8x + 10$ (Eqn. #2)

② Rewrite Eqn. #2 in terms of x:

$$y = 8x + 10$$
$$y - 10 = 8x$$
$$\frac{y-10}{8} = x$$

③ Substitute Eqn. #2 from step ② into Eqn. #1 : $y = 1.9068\left(\frac{y-10}{8}\right) - 25.824$

④ Solve Eqn. #1 from step ③ for y:

$$y = \frac{1.9068y - (1.9068 \times 10)}{8} - 25.824$$
$$8y = 1.9068y - 19.068 - 206.592$$
$$8y - 1.9068y = -19.068 - 206.592$$
$$6.0932y = -225.66$$
$$y = -37.035 \Rightarrow \delta^2H = -37.035‰$$

⑤ Substitute y back into original Eqn. #2 and solve for x.

$$y = 8x + 10$$
$$-37.035 = 8x + 10$$
$$-37.035 - 10 = 8x$$
$$-47.035 = 8x$$
$$-5.879 = x \Rightarrow \delta^{18}O = -5.879‰$$

⑥ Point at which the Tomato Water line intersects the GMWL:

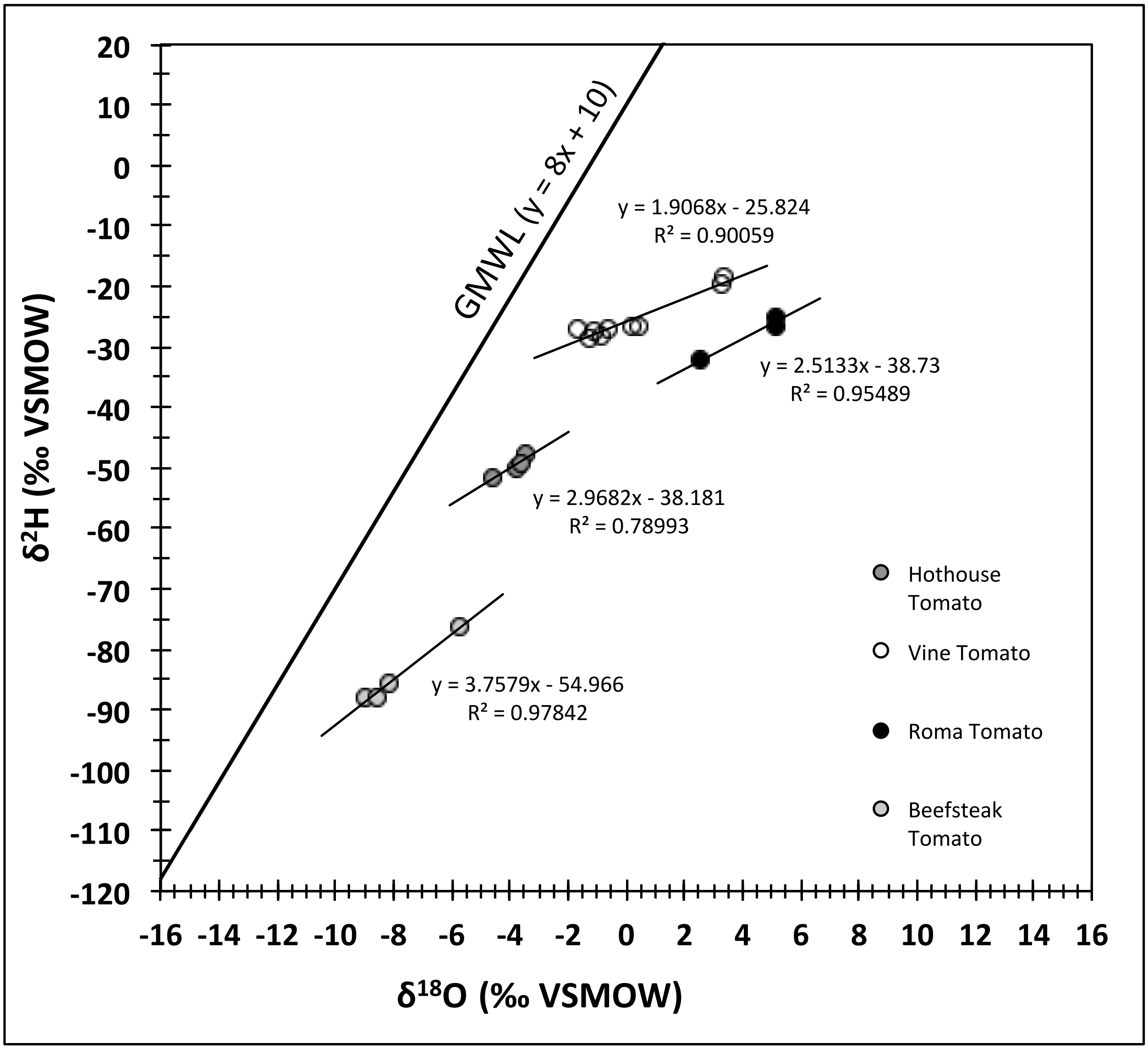
$\delta^{18}O = -5.88‰$ $\delta^2H = -37.04‰$

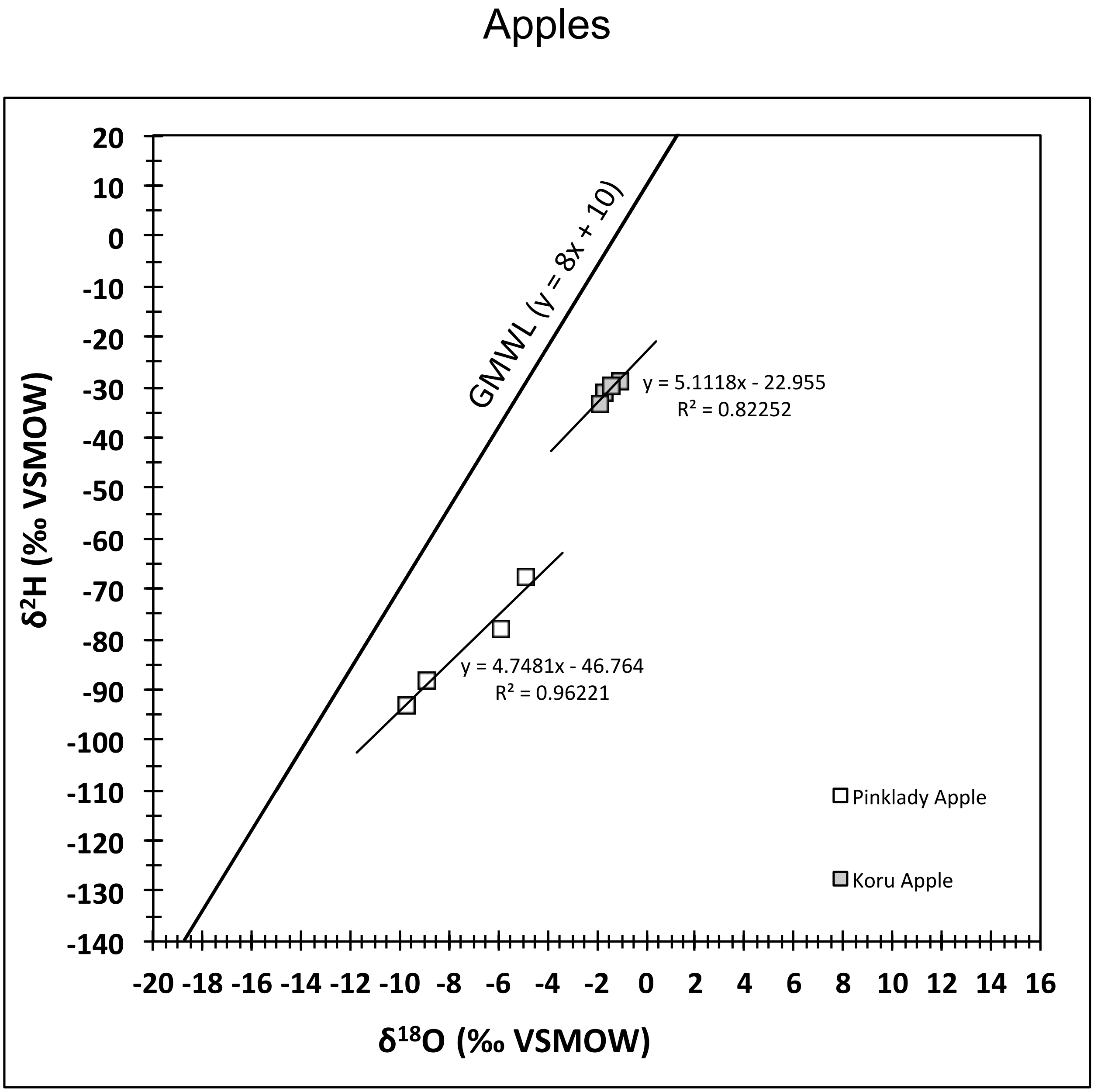
⑦ Compare results to graphical method as a check.

The following pages have $\delta^{18}O$ and δ^2H data from several different types of fruits and vegetables that have been converted into liquid water $\delta^{18}O$ and δ^2H values (using the food temperature measured during analysis). The data have been plotted for you along with linear regression best-fit lines. Choose one set of data (or several) and determine the $\delta^{18}O$ and δ^2H values of the original rainwater by either the graphical method, or by the Simultaneous Solution method, or both to be sure.

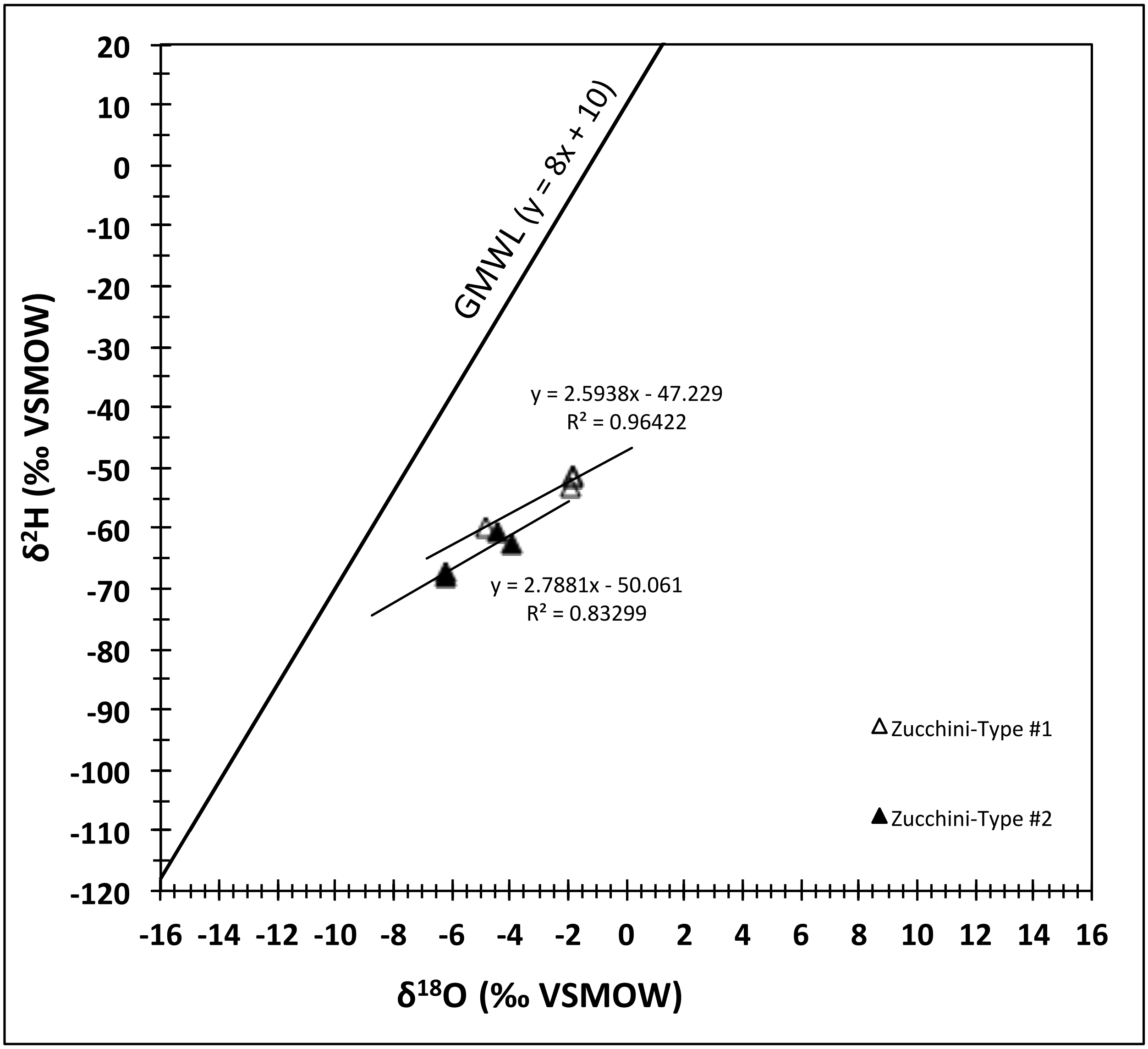
After you have determined the $\delta^{18}O$ and δ^2H values of the water used to grow the food items, add your values to the group list. In the next section, we will use these $\delta^{18}O$ and δ^2H data to evaluate where the food was grown.

Tomatoes

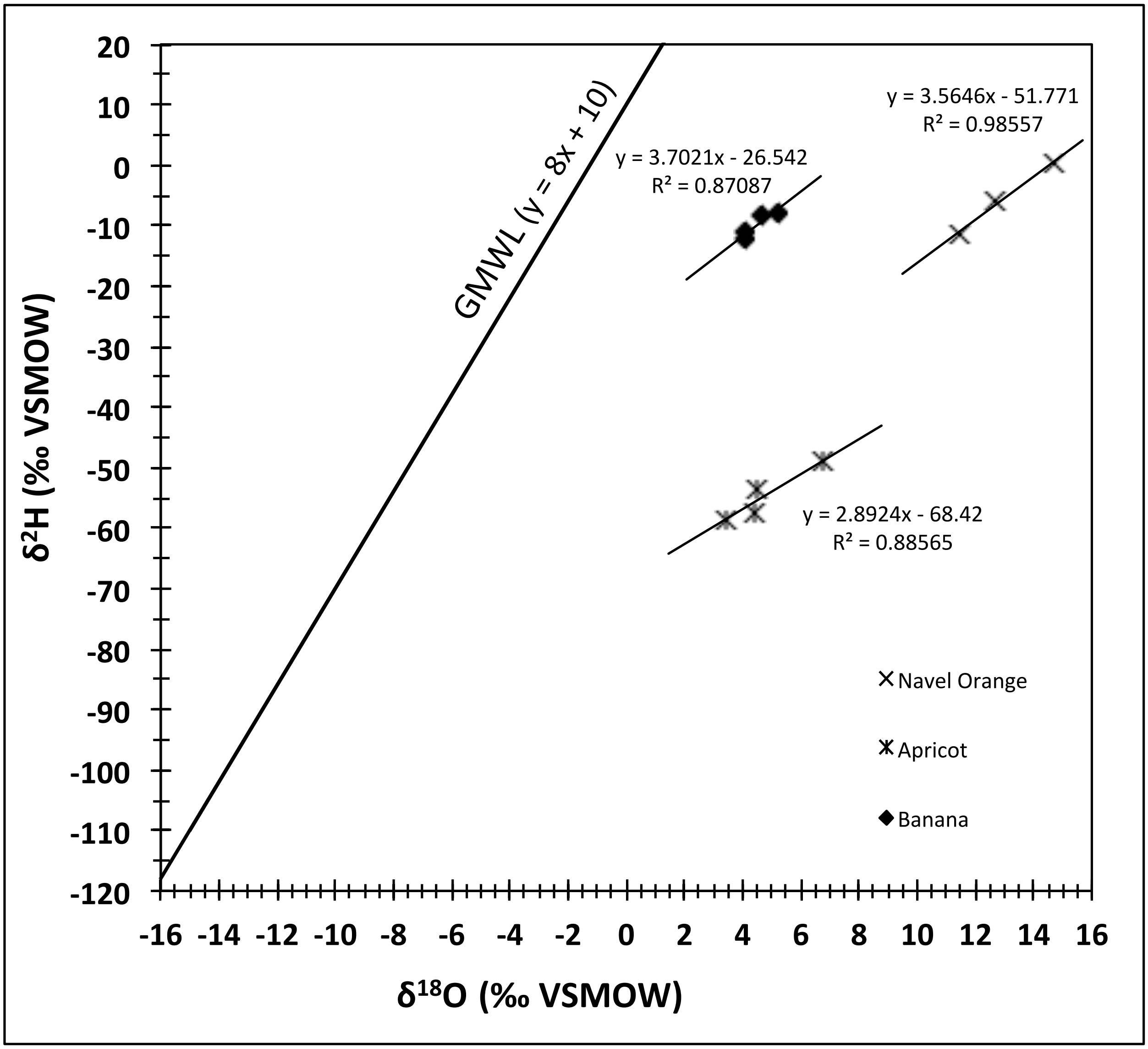

GMWL (y = 8x + 10)
y = 1.9068x - 25.824
R² = 0.90059
y = 2.5133x - 38.73
R² = 0.95489
y = 2.9682x - 38.181
R² = 0.78993
y = 3.7579x - 54.966
R² = 0.97842
Hothouse Tomato
Vine Tomato
Roma Tomato
Beefsteak Tomato
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-16
-14
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
δ2H (‰ VSMOW)
δ18O (‰ VSMOW)


Apples
GMWL (y = 8x + 10)
y = 5.1118x - 22.955
R² = 0.82252
y = 4.7481x - 46.764
R² = 0.96221
☐ Pinklady Apple
▨ Koru Apple
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-20
-18
-16
-14
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
δ²H (‰ VSMOW)
δ¹⁸O (‰ VSMOW)

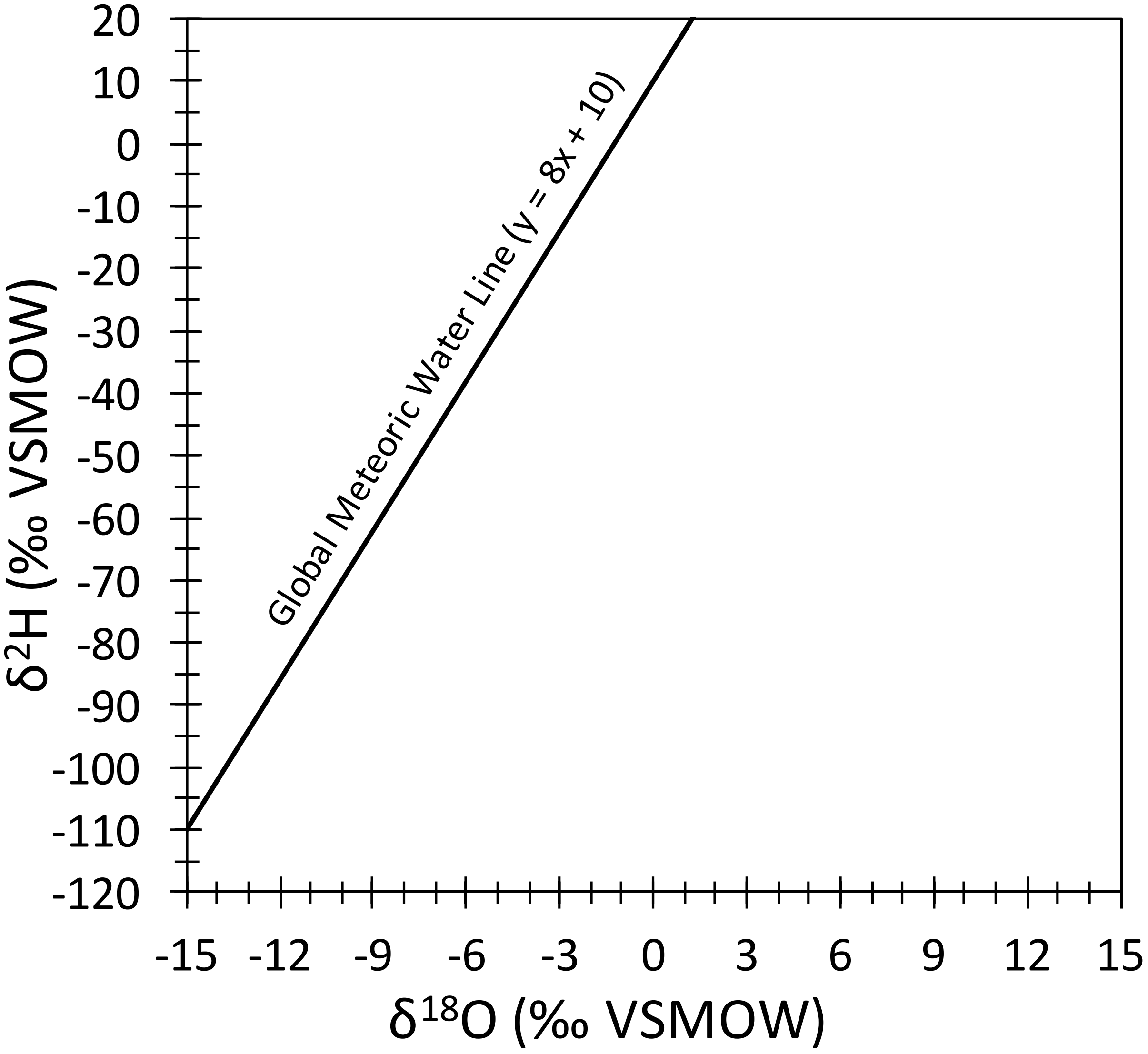
Zucchini


20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
δ2H (‰ VSMOW)
-16
-14
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
δ18O (‰ VSMOW)
GMWL (y = 8x + 10)
y = 2.5938x - 47.229
R² = 0.96422
y = 2.7881x - 50.061
R² = 0.83299
△ Zucchini-Type #1
▲ Zucchini-Type #2

Assorted Fruits



Plot Your Own Data



Step 2) Compare the fruit water O and H isotopes to maps of rain O and H isotopes to determine the possible geographic areas where the food could have been grown

Now that you have determined the $\delta^{18}O$ and $\delta^{2}H$ values of the water used to grow your food items, the next step is to compare those values to maps of $\delta^{18}O$ and $\delta^{2}H$ values in precipitation.

To better visualize and explore the spatial organization of O and H stable isotopes in precipitation, go to the website: http://www.erikoerter.org/isotopehydrology.html and download the file ”d18o_hover_global_mjj.html”. When you click on this file a map of $\delta^{18}O$ values of precipitation show open. When you drag your cursor over each pixel on the map, its $\delta^{18}O$ value should show. You can do the same for the other .html files, which show $\delta^{2}H$ values worldwide and in North America. Notice how both $\delta^{18}O$ and $\delta^{2}H$ values of rain get lower further to the north and south (away from the equator), and further into the interior of the continents. Using these maps alone you could make some well-educated guesses about the origin of the food items.

To get a more quantitative estimate of the food’s origin:

1) Go to the website: http://isomap.rcac.purdue.edu:8080/gridsphere/gridsphere

2) Click on “Create New Account”

3) It should be sufficient to create just one account per group, so fill in someone’s info and click “Save”. Now check your email account for the info on how to activate your account (the email may take a minute or two to go through).

4) Once your account is activated, go back to:
http://isomap.rcac.purdue.edu:8080/gridsphere/gridsphere

5) Go to the “Precipitation Model” tab, and then to “Display Map”. This is a list of maps of H or O stable isotope values that people have created with the IsoMAP system and have published for the public to use. The maps that have been created for this food sourcing experiment are:

Job Key #	Case Name	Description
57014	World_O_Summer_Map	Map of worldwide summer $\delta^{18}O$ values in rain
57015	World_O_Winter_Map	Map of worldwide winter $\delta^{18}O$ values in rain
57016	World_H_Summer_Map	Map of worldwide summer $\delta^{2}H$ values in rain
57018	World_H_Winter_Map	Map of worldwide winter $\delta^{2}H$ values in rain

6) To view one of these maps:
    1) Click on the map in the list
    2) Click on the green arrow in the Search box at the top of the screen

3) The map should appear and you can zoom or navigate by using the symbols in the left toolbars. The "i" tool allows you to click on a spot on the map and the isotope value for that pixel will be displayed. The "?" symbol on the lower right gives more info about the map.
HINT) To switch between maps (or if IsoMAP gets stuck and wont respond) go to the "About IsoMAP" tab (or another tab) to reset IsoMAP and then go back to "Precipitation Model" to continue working and display another map.
4) Compare the summer time maps to those made with data from winter months only. Notice how values are generally higher in the summer than in the winter, but there are still some low values in the mountains and far north during the summer.
5) Compare the source water $\delta^{18}O$ or $\delta^{2}H$ data from your fruits to locate areas that you think are realistic possibilities for where your fruit grew.

7) Now let's make a map that can help you further pinpoint where your food grew.
1) Go to "Assignment Model"
2) Click on one of the maps in the list that corresponds to whether you want to use O or H isotope data, and whether you want to use summer or winter data.
3) Click on the Green Arrow that appears in the upper red box.
4) Use the Square-and-Arrow tool that appears to select a geographic region that you want to concentrate your search on. HINT: Use the Hand tool to drag the map so that you can select a geographic region more easily.
5) Click on the Green Arrow that appears in the upper orange box.
6) In the "Parameter Entry" box that appears, enter the $\delta^{18}O$ or $\delta^{2}H$ value (round to one decimal place) of your food item in the "Observed Value" box, and enter 1.0 (with the decimal point) in the "Standard Deviation" box (this is an estimate of the uncertainty of your isotope data, and we can discuss this further if there is interest).
HINT: After you enter values in each box, you have to click in the other box to get the error message to disappear, or press the Enter key.
7) Click on the Green Arrow that appears in the upper yellow box.
8) Enter a name in the "Run Name" box. Try to pick something that is descriptive and will help you keep track of the various runs that you create, e.g. "RomaTom_O_yearly".
9) Click on the Green Arrow that appears in the upper green box.
10) You should now get a message that says that your job has been sent to the computer servers at Purdue University in Indiana. It will take a few minutes to process your job, especially if there are many users on IsoMAP at the time.
11) To check on your job's progress, go to the "My Jobs" tab. Press "Refresh Jobs" to update this page.
12) When your job says "Done" under the Job Status column, go to "Assignment Model" and then go to "Display Assign".
13) Click on your job from the list, then on the Green Arrow in the brown box.
14) The colors on this map denote the relative likelihood that your water in your food item came from each of the gridcells (pixels) on the map, with red being more likely and blue being less likely. HINT: This map may take a little while to display.
15) Make a few more Assignment Models and maps with different food items and with different O or H, and winter or summer data for comparison.

A few things to consider:
- Most places in the western U.S. use irrigation water to grow crops, and that irrigation water may reflect precipitation received during the winter as snow and stored in reservoirs to be released during the summer. A winter isotope map may be useful for comparison to summer, and may actually give more useful information.
- Many places use groundwater for irrigation and that water may look isotopically more like winter precipitation.
- However, at lower latitudes and at low elevations summer rainwater may be the water used for irrigation. This may be important for "tropical" foods like banana or pineapple, or for foods grown in areas at low elevations that are far from mountains.

Supplementary Material File 3. Interactive isotope maps (.html files)

http://www.erikoerter.org/isotopehydrology.html

Supplementary Material File 4. Isotope hydrology evaluation test.

NOTES TO EDUCATOR / EVALUATOR:

1) The learning objectives are in grey italics above each test question.
2) The correct answer to each test question is in bold.

Isotope Hydrology Evaluation

Last 4 digits of your phone number: ______________________

Circle the correct answer

Students should be able to describe how water moves through the water cycle from precipitation to soil to incorporation in plants (and fruits and vegetables).

1. The water that plants take up from the soil and use for growth comes from:
    a. Oceans and lakes
    b. Precipitation and irrigation
    c. Evaporation and transpiration
    d. Streams and rivers

Students should be able to define what a stable isotope is.

2. Stable isotopes are atoms of the same element that:
    a. Have different numbers of neutrons and protons in the nucleus.
    b. Have different numbers of neutrons, but the same number of protons in the nucleus.
    c. Have the same numbers of neutrons and protons in the nucleus.
    d. Have the same numbers of neutrons and different number of protons.

Students should be able to identify the main factor that controls how stable isotopes of water are distributed around the globe.

3. The main factor that controls how stable isotopes of hydrogen and oxygen in precipitation are distributed around the globe is:
    a. Wind
    b. UV radiation
    c. Temperature
    d. Ocean currents

Students should be able to explain why/how the stable isotopes in fruit and vegetable water can yield information on the geographic origin of the food itself.

4. The stable isotopes of Hydrogen and Oxygen in fruit water give information about the region in which the fruit is grown because:
    a. The stable isotopes from sunny regions and cloudy regions are known.
    b. The stable isotopes of different types of fertilizer used on fruit crops in different regions are known.
    c. The stable isotopes of the types of soil in different regions in which fruit crops are grown in are known.

NOTES TO EDUCATOR / EVALUATOR:

1) The learning objectives are in grey italics above each test question.
2) The correct answer to each test question is in bold.

d. The stable isotopes in precipitation and irrigation water in many regions are known.